DISSERTATION

CARDIOPULMONARY HEMODYNAMIC CONSEQUENCES OF MOTOR SEIZURE ACTIVITY IN THE KAINIC ACID MODEL OF TEMPORAL LOBE EPILEPSY

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JANN RHODES ENTITLED CARDIOPULMONARY HEMODYNAMIC CONSEQUENCES OF MOTOR SEIZURE ACTIVITY IN THE KAINIC ACID MODEL OF TEMPORAL LOBE EPILEPSY BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

CARDIOPULMONARY HEMODYNAMIC CONSEQUENCES OF MOTOR SEIZURE ACTIVITY IN THE KAINIC ACIDINIC ACID MODEL OF TEMPORAL LOBE EPILEPSY

Epilepsy is a generic term applied to a group of chronic conditions characterized by recurrent epileptic seizures; however, few animal models have been successful in modeling the chronic, spontaneous nature of human epilepsy. Individuals with epilepsy also have a higher death rate than the population at large, with 10-17 % of these deaths occurring for unexplained reasons. Studies in which cardiopulmonary and seizure activity have been monitored simultaneously are limited and, in the case of animals models, are restricted to the period of time immediately following chemically-induced seizures often in animals that are anesthetized, intubated, and paralyzed. Thus, the relevance of these studies to spontaneous motor seizure activity in humans is questionable. These studies were undertaken to characterize the cardiopulmonary hemodynamic consequences of acute and chronic kainic acid-induced spontaneous motor seizure activity in the kainic acid model of temporal lobe epilepsy. In addition, the effect of mild altitude exposure (10,000 ft., P_B = 525 mm Hg) on seizure frequency was evaluated to determine if

a mild, stress (e.g. hypoxia) alters seizure occurrence via a decrease in overall activity.

The first study evaluated the acute effect of multiple, systemic, low-dose injections of kainic acid and multiple, generalized motor seizures on systemic and pulmonary hemodynamics, and addressed the potential application of this model in the study of sudden unexplained death and neurogenic pulmonary edema. We hypothesized that mean systemic (mSAP) and pulmonary (mPpa) pressure, and heart rate would increase during acute, hourly intraperitoneal injections of kainic acid as well as during kainic acid-induced motor seizures. At the end of 4 h, mSAP, mPpa, and heart rate remained relatively unchanged or were slightly lower than baseline values in the control rats. Marked systemic hypotension and bradycardia was observed in all the kainate-treated animals after the first injection. One of two distinct mPpa responses was provoked by the initial kainic acid bolus. mPpa increased in half of the animals within minutes of the 1st injection while mPpa decreased in the other half of the group. With the onset of seizure activity, interictal mSAP and mPpa increased gradually with spikes in pressure accompanying each motor seizure. Heart rate also increased gradually; however, ictal responses were more variable with both tachycardic and bradycardic responses. With the α receptor antagonist phentolamine, mSAP fell, heart rate increased, and ictal mSAP spikes were attenuated. Ictal mPpa spikes were slightly attenuated with phentolamine; however, overall mPpa in the kainic acid-treated group was no different than that recorded prior to the phentolamine injection. Histologically, there was no evidence of pulmonary edema in either

group. Simultaneous monitoring of neurological activity via *in vivo* electrophysiological implants and mSAP, mPpa, and heart rate are needed to assess the mechanism(s) regulating the initial cardiopulmonary responses noted in this study. The initial mPpa response was inconsistent and the increase in mPpa that accompanied overt seizure activity was unaffected by phentolamine suggesting the involvement of non-adrenergic vasoactive mediators. Therefore, additional systematic pharmacological studies are needed to delineate the mechanism(s) regulating pulmonary vascular reactivity in this model.

The majority of data to date regarding the cardiopulmonary effects of seizure activity has been collected during acute administration of chemical convulsants. Therefore, we used the protocol described in the first study to induce chronic seizure activity in a group of rats and subsequently evaluated the cardiopulmonary effect of spontaneous motor seizure activity and the effect of hypoxia on these responses. We hypothesized that mSAP, mPpa, and heart rate would increase during spontaneous seizure activity in kainic acid-treated animals and, that these responses would be exacerbated under acute hypoxia (5 min, 10% O_2), moderate hypoxia (2 h, 12-14% O_2), and attenuated by the α-antagonist phentolamine. Systemic and pulmonary pressures were monitored continuously for 8 h to record pressure changes under normoxic and hypoxic conditions and during spontaneous seizures that occurred during the protocol. Baseline hemodynamic parameters were not significantly different between control and kainic acid rats; however, under the physiological stress of hypoxia, significant differences between the two groups with regard to stroke volume,

mPpa, and pulmonary vascular resistance were evident. With phentolamine, mPpa and were exacerbated under normoxia and both hypoxic challenges. With seizure onset under normoxic conditions, mSAP and mPpa increased and heart rate decreased immediately. With seizure onset under hypoxic conditions and normoxic and hypoxic conditions with phentolamine, the relative increase in mPpa was exacerbated while mSAP was attenuated. Overall, the disparity between the control and kainic acid-treated rats in response to hypoxia suggests some degree of autonomic or myocardial dysfunction in the kainic acid animals. The systemic vascular responses noted in this study were mediated primarily via altered adrenergic activity as mSAP was attenuated by phentolamine. The exacerbation of the mPpa and pulmonary vascular resistance with phentolamine suggests the involvement of other, non-adrenergic mediators such as, nitric oxide and endothelin or, reflects permanent vascular damage incurred during the initial kainic acid exposure. A carefully planned pharmacological study is needed to further evaluate the pulmonary vascular responses noted in this model.

Endogenous circadian rhythms, such as the light-dark and sleep-wake cycles and behavioral circumstances affect some forms of epilepsy by inhibiting neuronal excitability. In the kainic acid model of temporal lobe epilepsy, seizures appear to coincide with the activity or degree of inactivity rather than with the light-dark cycle. We hypothesized that seizure occurrence (i.e. frequency) would increase in kainic acid-treated rats during exposure to moderate hypobaric hypoxia and that the increase in seizure frequency would be inversely related to activity level. As anticipated, all rats were more active at night than during

daylight hours; however, the kainic acid-treated rats were significantly more active than the control rats at both time points and under all conditions. Seizure frequency in the kainic acid-treated rats with previous documented seizure activity increased hypoxia and remained elevated during the normoxic recovery. However, the increase in seizure frequency was not associated with a hypoxia-induced decrease in animal activity. There was a shift toward less 'severe' seizures during hypoxia with 2-3 times as many class III seizures recorded during hypoxia than at any other period. The association between behavioral states and seizure activity in this study was clear-cut with the majority of seizures occurring when the animals were inactive despite the fact that the kainic acid-treated animals were significantly more active than control the majority of the time. Hypoxia, however, had a minimal impact on behavior. The greatest increase in seizure frequency in those rats with previous seizure activity occurred during the light phase of the hypoxic period that could perhaps be attributed to an altitude-induced disturbance in the normal sleep cycle. While it is possible that seizure activity in humans is unaffected by mild altitude exposure, the lack of incidence data may be due to the failure of people with seizure conditions to consider the event unusual especially if the seizure was considered relatively 'mild'. A more systematic review of altitude-induced changes in seizure patterns is warranted.

In conclusion, this is the first study to document the acute effects of repeated, low-dose injections of kainic acid and kainic acid-induced generalized seizure activity as well as the long-term effect of recurrent spontaneous motor seizure activity in the chronic kainic acid model of temporal lobe epilepsy on the

cardiopulmonary system. For the most part, the systemic cardiovascular responses observed in both the acute and chronic kainic acid models are consistent with the responses documented for other acute and chronic seizure models. However, the pulmonary vascular responses recorded here were not consistent and suggests the involvement of additional mediators or perhaps a direct effect of kainic acid on the pulmonary vasculature. Therefore, systematic pharmacological evaluation of the pulmonary vascular responses noted in both the acute and chronic models is warranted. Furthermore, continued evaluation of altitude-induced changes in seizure patterns, possibly as part of the overall acute mountain sickness syndrome, is suggested, especially in people with a documented history of seizures.

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To my partner,
whose support and unwavering faith,
carried my through the darkest hours.

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LIST OF ABBREVIATIONS

CON	control
HX	hypoxia (10% O_2)
KA	kainic acid
MHX	moderate hypoxia (12-14% O_2)
mPpa	mean pulmonary arterial pressure
mSAP	mean systemic arterial pressure
NX	normoxia (21% O_2)
P_B	barometric pressure
P_aCO_2	partial pressure of carbon dioxide
P_aO_2	partial pressure of oxygen
SUD	sudden unexpected or unexplained death

CHAPTER I

INTRODUCTION

Approximately 2.5 million Americans have recurring epileptic seizures; with 1 in every 10 Americans experiencing a seizure in their lifetime (1) Approximately 125,000 new cases are reported yearly, 50% of which are classified as partial seizures (2). In addition, individuals with epilepsy have a higher death rate than the population at large, with 10-17% of these deaths occurring for an unexplained reason (1). The phenomena of sudden unexplained death (SUD) in epileptics occurs most commonly in individuals between the ages of 20 and 40 and is often attributed to cardiovascular dysfunction stemming from massive sympathetic activation (3).

Kainic acid, an agent extracted from seaweed, has been used for centuries in Japanese folk medicine as an antihelmintic agent (4). Over the last 20 years, kainic acid has been used extensively in neurobiology to examine the mechanisms governing acute seizure activity as well as the mechanisms of neuronal reorganization thought to occur as the result of recurrent epileptic seizure activity (4). The development of a chronic kainic acid model of temporal lobe epilepsy (5) provides an excellent opportunity to examine the

physiological impact of prolonged seizure activity on the cardiovascular and pulmonary systems, as well as the effect of pharmacological agents and physical stresses, on this response.

Studies in which cardiovascular parameters have been monitored during seizures are limited and, in the case of animal models, restricted to the period of time immediately following chemically induced seizure activity. Ictal, as well as interictal, epileptiform activity impairs cardiovascular function due to the variability of sympathetic and parasympathetic neuronal discharge (6,7). Systemic arterial pressure increase dramatically with the onset of seizure activity (8-10) accompanied by an increase in pulmonary arterial pressure (11). A variety of cardiac also arrhythmias frequently occur (3,6,12,13), the severity of which are dependent upon the origin or epileptic focus (14). In addition to systemic complications, neurogenic pulmonary edema (NPE) is also seen in cases of significant neurological trauma including epileptic seizure activity (15). In these instances however, edema is not thought to be the cause of death, but rather presumed to occur as a result of large, transient increases in pulmonary pressures (9,16,17-19). However, reports of pulmonary vascular pressures during spontaneous, generalized seizures are lacking.

The relevance of many of these studies is questionable, as the vast majority of data has been collected during acute administration of chemical convulsants to anesthetized, paralyzed, and intubated animals. In humans, these measurements have been obtained either during status epilepticus or after overt seizure activity has subsided (20). The cardiopulmonary consequence(s) of

spontaneous seizure activity in the chronic kainic acid model of temporal lobe epilepsy has not been documented. It is possible that chronic epileptic activity alters autonomic nervous stimulation of the myocardium and/or the systemic and pulmonary vasculature, exacerbating normal hemodynamic and cardiac responses to physiological challenges, such as mild hypoxia (e.g. altitude exposure).

Behavioral circumstances, such as stress, can increase the probability of seizure (18). Many people with epilepsy experience some of the same sleep disturbances visitors to moderate altitude (10,000 to 14,000 ft.) experience such as, prolonged non-REM sleep cycles and an increase in the number of arousals, during the first 48-72 hours at moderate altitude (21). In addition, nocturnal temporal lobe complex partial seizures occur most frequently in both light and deep stages of NREM sleep, and rarely occur during REM sleep (21). Therefore, for the first 48-72 hours at altitude, a person with epilepsy is potentially spending more time in sleep stages with the highest incidence of seizure occurrence with the potential for generalization. However, the relationship between exposure to a mild hypoxic stress and seizure activity as a function of disrupted sleep patterns has not been examined. Given the prevalence of epilepsy in the general population and the large number of people who either live at moderate altitude or travel to altitude for business or recreation (13 million in Colorado alone in 1990) (22), it seems prudent to investigate the impact of a mild hypoxic stress on seizure frequency.

Statement of the Problem

In the following experiments, we characterized the cardiopulmonary effect of multiple low-dose injections of kainic acid and acute, kainic acid-induced, generalized motor seizures to evaluate the impact of seizure activity on both systemic and pulmonary circulations. Furthermore, we evaluated the cardiopulmonary responses of kainic acid-treated rats with chronic, spontaneous seizure activity during exposure to acute hypoxia (5 min, 10% O_2), and short-term, moderate hypoxia (2 h, 12% O_2) to determine if chronic seizure activity alters the normal compensatory cardiovascular responses associated with hypoxic exposure. Lastly, we monitored the effect of mild altitude exposure (10,000 ft., P_B = 525 mm Hg) on seizure frequency to determine if exposure to a mild, exogenous stress (e.g. hypoxia) alters seizure occurrence via a decrease in level of activity (i.e. volitional movement).

Specific Hypothesis

The specific hypotheses tested were as follows:

Study 1: Acute systemic and pulmonary vascular effect of repeated low-dose injections of kainic acid

1. Systemic and pulmonary arterial pressure, and heart rate will increase significantly after acute, intraperitoneal administration of kainic acid.
2. Systemic and pulmonary arterial pressure will increase during generalized motor seizure activity. The pressor responses will be attenuated by phentolamine.

3. Evidence of alveolar hemorrhage and/or moderate pulmonary edema will be observed in kainic acid-treated animals.

Study 2: Cardiopulmonary hemodynamics during spontaneous seizure activity in a chronically epileptic kainic acid model of temporal lobe epilepsy

1. Cardiopulmonary responses (systemic and pulmonary arterial pressures, heart rate, and cardiac output) to either acute hypoxic (5 min, 10% O_2) or short-term, moderate hypoxia (2 h, 12-14% O_2) from the kainic acid-treated rats will be significantly higher than the cardiopulmonary responses recorded from age-matched control rats.
2. Systemic and pulmonary arterial pressures, cardiac output, and heart rate will increase during spontaneous seizure activity in kainic-treated animals.
3. Systemic and pulmonary arterial pressures, cardiac output, and heart rate will be significantly greater during spontaneous seizures under acute (5 min, 10% O_2) and short-term moderate hypoxic (2 h 12-14% O_2) conditions than those measured under normoxic conditions.
4. Administration of an α-antagonist will attenuate the systemic and pulmonary arterial pressures responses measured under both normoxic and hypoxic in both groups as well as during seizure activity in kainic acid-treated rats under these conditions.

5. Evidence of alveolar hemorrhage and/or moderate pulmonary edema will be observed in kainic acid-treated animals.

Study 3: Influence of moderate hypobaric hypoxia on seizure frequency in the chronically epileptic kainic acid model of temporal lobe epilepsy

1. Spontaneous seizure frequency in kainic acid-treated animals will increase during exposure to 48 h of moderate hypobaric hypoxia (simulated altitude of 10,000 ft) as activity levels decrease.

Specific aims

The specific aims of the project were as follows:

Study 1:

1. Measure systemic and pulmonary arterial pressures, heart rate, arterial blood gases, and pH in unanesthetized, freely moving rats undergoing acute, intraperitoneal administration of either kainic acid or saline.
2. Measure systemic and pulmonary arterial pressure and heart rate in unanesthetized, freely moving control and kainic acid-treated rats with and without phentolamine.
3. Measure systemic and pulmonary arterial pressure and heart rate in unanesthetized, freely moving kainic acid-treated rats during generalized motor seizure activity with and without phentolamine.

4. Examine the pulmonary vasculature for morphological evidence of pulmonary edema, alveolar hemorrhage and/or altered vascular structure in both saline and kainic acid-treated rats.

Study 2:

1. Measure systemic and pulmonary arterial pressures, heart rate, cardiac output, arterial blood gases, and pH in unanesthetized, freely-moving saline and kainic acid-treated rats under normoxic, acute hypoxic (5 minutes, 10% O_2), and short-term, moderate hypoxic (2 hours, 12-14% O_2) conditions with and without phentolamine.
2. Measure systemic and pulmonary arterial pressures, heart rate, cardiac output, arterial blood gases, and pH in unanesthetized, freely-moving kainic acid-treated rats during overt seizure activity under normoxic, acute hypoxic (5 minutes, 10% O_2), and short-term, moderate hypoxic (2 hours, 12-14% O_2) conditions with and without phentolamine.
3. Examine the pulmonary vasculature for morphological evidence of pulmonary edema, alveolar hemorrhage and/or altered vascular structure saline and kainic acid-treated rats.

Study 3:

1. Video-monitor control and kainic acid-treated rats for 4-8 h during each light-dark cycle under ambient (5140 ft. P_B ~ 640 mm Hg),

normoxic conditions for 24 h, mild hypoxic (10,000 ft. P_B ~ 523 mm Hg) conditions for 48 h, and ambient conditions for 24 h.

2. Document activity in control rats and activity and seizure frequency kainic acid-treated rats

CHAPTER II

REVIEW OF LITERATURE

Epilepsy is a generic term applied to a group of chronic conditions characterized by recurrent epileptic seizures. Approximately 25 million Americans (45 million worldwide), have some type of seizure disorder (1). The risk for epilepsy varies with age and has a bimodal incidence distribution. The greatest risk is in children ranging in age from 1 to 4 (1,23,24) and in the elderly. Of all new cases of epilepsy reported, 75% occur in individuals under the age of 20.

Epileptic seizures occur with a variety of diseases and disorders. An epileptic disorder is "a chronic neurological condition characterized by recurrent epileptic seizures" (14). Seizure disorders can be classified into 3 broad categories based on electrophysiological characteristics and behavioral features that are displayed during ictal activity (25). Primary or idiopathic seizure disorders are genetically transmitted disorders with no identifiable structural lesion (i.e. benign childhood epilepsies). Secondary or symptomatic disorders arise as a direct result of some recognizable cause or trauma (e.g. stroke, subarachnoid hemorrhage). Cryptogenic seizure disorders are those for which no etiology or identifiable cause can be determined (25).

Clinically, seizures can also be classified according to the degree of cerebral involvement (25). Partial seizures are those with onset limited to one part of the cerebral hemisphere while generalized seizures begin diffusely and involve both cerebral hemispheres from the onset. There are more than 20 seizure disorders that vary in terms of clinical presentation and duration. Most often a cause is not identifiable (approximately 70% of all cases have no identifiable cause (25)), although the focal nature of many epileptic disorders implies some type of cerebral injury or lesion. How a lesion or area of injury becomes epileptogenic is poorly understood.

Sudden unexpected death

The segment of the population with epilepsy has a mortality rate of up to 3 times greater than that of the general population (26,27). Epilepsy-related deaths can be subdivided into traumatic deaths directly related to overt seizure activity, death due to prolonged seizure activity (status epilepticus) and non-traumatic, sudden unexpected deaths (SUD), also known as sudden unexpected death in epileptic patients (SUDEP) (1,28). Epilepsy-associated deaths account for 10-17% of all SUDs reported (1,28).

The World Health Organization designates 'unexpected' as relating to the surprising nature of the death in light of the non-existence or mildness of the disease (26,29). Documentation of SUDEP and the potential role of the cardiovascular and pulmonary systems in this phenomenon began in the early 1900s, although its occurrence was recognized and anecdotally reported much earlier (23,30). Data used in early studies was gathered from institutionalized

populations, as individuals with epilepsy were often kept in epileptic 'colonies' in the early part of the 20th century. Based on these populations, it was reported that approximately 4-17% of deaths in individuals with epilepsy could be characterized as sudden, unexpected deaths (23,30). Retrospective studies conducted in the 1980s estimated the SUD risk factor for individuals with epilepsy to be approximately 1:1000 (26) to 1:1100 (31). The most current SUD statistics estimate the SUD risk factor for this segment of the general population to still be about 1:1000, or approximately 10-17% of all epilepsy-related deaths (1,28). Some have suggested that the largest percentage of these deaths involve individuals with the most frequent and most severe seizure activity (32) while others report that seizure frequency is not an important risk factor (33). Of the fatal seizures witnessed, the majority is described as generalized tonic-clonic seizures; however, most SUDEPs are not witnessed (32). Although antiepileptic drug therapy has improved overall seizure control over the last 80+ years, the morbidity and mortality of SUDEP has remained fairly constant (1,28,31).

The occurrence of fatal cardiac arrhythmias (tachyarrhythmias or bradyarrhythmias) is most frequently hypothesized as the cause of death in SUDEP (26,31-33). Other proposed mechanisms include myocardial infarction, catecholamine toxicity, pulmonary edema, and respiratory depression (apnea) (6,34,35). It has been hypothesized that repeated exposure to catecholamines released during a seizure could cause permanent myocardial lesions or scarring from the release of free radicals, with the potential to disrupt the conduction of electrical activity in the heart (6,35-37). Stress cardiomyopathy, associated with

excessive catecholamine exposure, has been documented in animal models (37) as well as in humans who were victims of violent acts (36). Plasma catecholamines increase abruptly during seizure activity (35,38,39) and induce myocardial necrosis in animals (40). In addition, prolonged, bilateral stimulation of the hypothalamus has been shown to cause myofibrillar degeneration that is indistinguishable from that caused by catecholamine toxicity and stress (41).

Clinically, it is important to recognize that some systemic symptoms arising from aberrant electrical activity are identical to those seen in patients with cardiovascular disease cardiovascular disease (6,42) and vice versa (13,43,44). Experimentally, lesions placed in the temporal lobe reproduced ECG changes similar to those recorded from patients with ischemic heart disease (26). Cardiovascular misdiagnosis, in instances where epileptic manifestations were mistaken for cardiovascular disease, has been reported (42). Several investigators have reported that during complex partial seizures, the primary visceral aura were cardiac symptoms ranging from angina and sinus tachycardia to syncope (12,13,44). These reports are evidence of the importance of understanding the physiological impact of overt seizure activity on the cardiopulmonary system.

Overall, the chances of dying of SUD are fairly remote; however; subgroups or segments within the population of epileptics with specific, common physiological characteristics may be at higher risk. The characteristics of the subgroup thought to be most at risk are young males who abuse alcohol and are non-compliant with antiepileptic drug therapy (AED)(33). However, one group

overlooked and often not considered for inclusion in SUDEP studies is the elderly. Stroke or cerebral vascular accidents are the most commonly identified cause of epilepsy in the elderly (2). In a recent study, five vascular risk factors were found to be twice as common among elderly subjects with epilepsy compared to those without (2). When the effect of stroke was removed statistically, other vascular risk factors (e.g. systemic hypertension and peripheral vascular disease) were significantly correlated with the incidence of epilepsy. These investigators suggest that diffuse cerebral damage associated with other vascular diseases may make the brain more vulnerable and increase the chance of neural tissue become unstable (i.e. epileptogenic). In addition, this segment of the population has a much higher incidence of chronic myocardial and cardiovascular disease. Therefore, the elderly with recurrent seizure activity might constitute a second cluster of people with a fairly high risk of SUD that has not been recognized.

In a review of SUD published several years ago, the authors noted that SUDEP occurs in relatively young individuals between 30 and 40 years of age, "an age group that would not normally be expected to have coronary artery disease or other obvious myocardial damage"(26). While this might have been a valid hypothesis then, the same may not hold true today. Atherosclerotic disease is not a disease process restricted to the elderly (29,45). In the United States alone, 1 out of every 5 males and females have some form of cardiovascular disease with 41.4% of all deaths in the United States in 1996 caused by one or more types of cardiovascular disease (46). A very recent study found evidence

of atherosclerosis in individuals 15-34 years of age, an age group spanning the transition from high to low risk of epilepsy (45). It may now be more important than ever, to recognize that many people, both young and old, with seizure disorders may also have some form of CVD, increasing the likelihood that aberrant autonomic discharge during a seizure could trigger a fatal cardiovascular event. The role of CVD as a contributing factor in SUDEP needs to be addressed.

The central autonomic network (CAN) and the cardiovascular system

The central autonomic network is an intricate network of ascending, descending, lateral, and parallel interconnecting neuronal pathways regulating visceromotor, neuroendocrine, pain, and behavioral responses (6,47-50). The central autonomic network encompasses specific areas of the telencephalon, diencephalon, and brain stem and includes the insular cortex, amygdala, hypothalamus, nucleus of the tractus solitarius (NTS) and ventrolateral medulla (47). Forebrain nuclei, such as the central nucleus of the amygdala, the hypothalamus (paraventricular and lateral nuclei), and the bed nucleus of the stria terminalis, are crucial in modulating cardiovascular and respiratory responses (47,49). These areas, in an integrated fashion, modulate autonomic output indirectly via reciprocal pathways with the NTS and the parabrachial nucleus, as well as via direct projections to sympathetic and parasympathetic preganglionic neurons found in the intermediolateral cell column of the spinal cord, rostral ventrolateral medulla, dorsal vagal nucleus, and nucleus ambiguus of the ventrolateral medulla (47-49).

Within the reticular formation of the medulla, there are centers or clusters of neurons that mediate reflexes with distinct systemic effects characterized by hypertension, bradycardia, apnea, and pulmonary edema formation (51). The systemic effects initiated by these neurons, the Cushing and cerebral ischemic reflexes, mimic the reflex response induced by hypoxia, but are induced by stimuli such as, distortion of the brain stem (Cushing reflex) (52,53), and a reduction in cerebral blood flow (54), rather than hypoxia. However, these are not the only cerebral areas that are associated with systemic hypertension and pulmonary edema.

Autonomic regulation during epileptiform activity

Cardiovascular and respiratory responses

Many studies in humans and animals have documented the cardiovascular and respiratory changes that occur during generalized tonic-clonic seizures (7,55-58), direct cortical stimulation (59-65), direct hypothalamic stimulation (66-68), direct limbic stimulation and spontaneous temporal lobe seizures (12,14,32,42,43,55,59,60,69-72), and with direct brainstem stimulation (50,73,74). The physiological impact of seizure activity on the cardiovascular and respiratory systems is variable and dependent upon the anatomic location of the epileptogenic focus, the synchronization and spread of electrical activity within the brain, and the duration of ictal activity. Cardiopulmonary responses to aberrant autonomic activity include a wide variety of arrhythmias, severe systemic and/or pulmonary hypertension or hypotension, altered cardiac output, the formation of pulmonary edema, as well as variable changes in ventilation

such as apnea, hypoventilation, and hyperventilation. Generalized autonomic discharge, as a result of widespread seizure activity, can uncouple normal homeostatic mechanisms regulating heart rate, systemic and pulmonary pressures, and neutralize the normal antagonistic effects of the sympathetic and parasympathetic branches of the nervous system (6,7,56,69,71;75). Preferential discharge of either branch of the autonomic nervous system during ictal activity can significantly alter ventricular excitability (7,56,76) with changes becoming more pronounced as ictal activity increases (56). Adrenergic blockade in some instances, has been shown to reduce the occurrence of arrhythmias upon central nervous system stimulation (59,77), while atropine administration has eliminated seizure-related bradycardia (12,55,59,71) Sympathetically-mediated changes in the cardiovascular system have also been recorded during interictal or subconvulsant activity (7,35,56,61,64,65). In addition, an increase in sympathetic activity triggered by abrupt changes in environment, such as changes in light intensity have been reported to initiate seizure activity in some instances (53,78).

Cardiac vulnerability

Cardiac rhythm disturbances may be secondary to undetected cardiac damage caused by repeated, massive neural stimulation (6). Of concern is the potential for incipient cardiac damage caused by aberrant neural activity that does not have overt motor manifestations. Direct stimulation of the RVM and the lateral hypothalamus causes a decrease in coronary blood flow via intense coronary vasoconstriction that could lead to centrally-mediated transient

myocardial ischemic episodes (67,68). A reduction in left ventricular contractility has been shown to follow intense sympathetic nervous system activation and central nervous system insults (79-81). However, a reduction in left ventricular contractility is only observed after a prolonged (i.e. over 2 hours) elevation in systemic pressure and plasma catecholamines (79,80) and therefore, probably does not play a significant role in short-lived seizure activity but may be significant in cases of status epilepticus (SE).

Clinically, it is critical to understand the intricate central nervous system network that modulates peripheral autonomic activity and to recognize the potential for aberrant central nervous system discharges to have serious, and sometimes lethal, consequences. However, it is also important to recognize that some antiepileptic drugs may predispose an individual to autonomic instability, thus increasing their risk of death during a seizure (27). Antiepileptic drugs and other pharmacological agents have been successfully used in tandem to reduce some of the autonomic disturbances that occur during seizure activity (26). For example, the left stellate ganglion is generally dominant and can increase the likelihood of ventricular fibrillation by reducing the ventricular refractory period (6,76). Phenytoin and carbamazepine, when given together have been shown to suppress the hyperactivity of the left stellate ganglion and decrease the occurrence of an arrhythmia (42). Lown et al. (76) have successfully raised the ventricular refractory period by the concurrent administration of Carbidopa, a dopamine analogue, and phenelzine, a monoamine oxidase inhibitor. This effect is attributed to the suppression of serotonin formation in the periphery by

Carbidopa and the accumulation of serotonin in the central nervous system via the action of phenelzine. Accumulation of serotonin in the central nervous system increases the ventricular refractory and reduces the occurrence of ventricular fibrillation. Drugs with both central nervous system and cardiovascular effects might be appropriate in some instances were the individual presents with specific, documented cardiovascular risk factors such as previous myocardial injury, or previous arrhythmic episodes. For example, Vezzani et al. (82) have used the Ca^{++} channel blockers nifedipine and verapamil, in 3 different epilepsy models, for their anticonvulsant properties. This class of drugs, as well as other cardiovascular-specific drugs, might warrant further evaluation, especially in light of the every-growing population at risk for developing both CV disease and epilepsy.

Cerebral blood flow

For many years, it was assumed that neuronal damage associated with seizure activity was due to cerebral anoxia or hypoxic/ischemic episodes caused by the attenuation of cerebral blood flow during seizure activity. It was even suggested at one time that changes in cerebral blood gas tensions (a fall in P_aO_2 and an increase in P_aCO_2) during seizure activity were responsible for seizure termination (83). However, an increase in cerebral blood flow with epileptic seizures is now well established, as well as the fact that neuronal necrosis occurs independently of seizure type, in spite of adequate oxygenation meeting or even exceeding metabolic demands (84-91).

Epileptic seizures may occasionally be associated with a measurable transient elevation in intracranial pressure and cerebral spinal fluid pressure. These pressures are thought to reflect a change in intracranial volume originating in the vascular system as cerebral blood flow increases concomitant with a general vasodilation of cerebral vessels (87). Blood-brain barrier integrity can be disrupted during seizure activity when a marked increase in mean systemic arterial pressure occurs during the ictal phase (92,93). However, in order for the blood-brain barrier to be compromised, mean systemic arterial pressure must increase, as abolishing systemic arterial pressure changes prevents blood-brain barrier disruption (93). Furthermore, it is not the magnitude of mSAP that is the critical element but the rate at which blood pressure increases and the loss of cerebral autoregulation that markedly increase regional CBF.

Pulmonary circulatory response and edema formation

Neurogenic pulmonary edema (NPE) or non-cardiogenic pulmonary edema, is a protein-rich exudate that develops after neurological insult (94) whereas, cardiogenic pulmonary edema is characterized by a protein-poor transudate that develops from the failure or impairment of cardiac function (41,95). NPE is most commonly associated with severe, traumatic, head injuries but has been reported to occur in conjunction with severe, spontaneous grand mal seizures (96) but not grand mal seizures as a result of electroconvulsive shock therapy (97). Shanahan (98) was one of the first to document pulmonary edema as a complication of grand mal seizures, although Spratling (99) had alluded to it much earlier. Munson (30) documented an inordinate number of

pulmonary-related complications from lung tissue of SUDEP victims and concluded that many of the pathological changes were subacute rather than instantaneous seizure or post-seizure events and therefore could not have been the primary cause of death. More recent pathology reports from postmortem examinations of SUDEP victims have reported that various internal organs, particularly the lungs, have some degree of edema, although, like Munson, not considered to be extensive enough to cause death (27,31). However, as suggested by the autopsy results of Jay and Leetsma (26), pulmonary edema might occur more frequently in SUD than suggested by anecdotal reports alone.

The mechanisms regulating NPE remain elusive. However, it is agreed that NPE results from either a sharp, substantial increase in pulmonary vascular pressure, an increase in vascular permeability, or both, all of which occur as a result of massive central sympathetic outflow or 'sympathetic storm' (94,100). During a seizure, pulmonary vascular pressure increases as a result of sudden, pulmonary arterial vasoconstriction and venoconstriction exacerbated by the rapid displacement of systemic blood volume to the pulmonary circulation via sympathetic α-adrenergic mediated systemic vasoconstriction (11,19,96,100,101). Early studies suggested that the movement of systemic blood volume to the compliant pulmonary bed is critical in determining extent of pulmonary pressure increase. However, an increase in pulmonary venous pressure with subsequent edema formation can occur independent of an increase in left atrial pressure and/or systemic hypertension (95).

Pulmonary arterial constriction is not a necessary part of the NPE sequelae; however, if pulmonary arterial constriction plays a role in NPE, it may be due to a non-uniform constriction response shunting blood away from some areas with subsequent overloading of other regions (94). A very recent study reported that edema formation in high-altitude pulmonary edema-susceptible individuals was associated with an abnormal cephalad redistribution of pulmonary blood flow in response to hypoxia as a result of an exaggerated vasoconstriction response in the basal lung (102).

The greatest disagreement regarding the mechanisms of NPE is the issue of capillary permeability. It is agreed that pulmonary pressures do increase during a seizure; however, it is debatable as to whether this is absolutely necessary for edema to occur. There are several reports of recurrent pulmonary edema in patients with grand mal seizures (20,96), one of which recorded normal pulmonary wedge pressures following the seizures (20), lending credence to the idea that edema can occur following a transient increase in pressure. However, this seizure occurred post-operatively following the removal of a brain tumor and therefore is not representative of other seizure disorders. Others have suggested that a combination of an increase in pulmonary pressures, above 50 mm Hg, and impaired lymphatic flow leads to edema formation suggesting that fluid accumulation is a result of impaired fluid removal via norepinephrine-mediated lymphatic constriction (103). These results have been replicated in a different animal model with massive sympathetic activation and an extreme degree of pulmonary hypertension before edema occurred (104); however, the

increase in lung lymph flow persisted for several hours following the seizure event further supporting the idea of a transient pressure insult (9).

In 1954, Cassen and Kistler (105) proposed that elevated pulmonary pressure could stretch the "intracellular processes holding endothelial cells together" allowing fluid to move through the respiratory barrier and into the alveoli. Whether this process was reversible or caused permanent, irreversible damage was dependent upon the magnitude of the pressure response. This hypothesis is now known as the 'stretched-pore' hypothesis implying that pores or elastic structures can withstand fairly high pressure then recoil back to their original size once the pressure diminishes, restoring normal permeability (31,106). It is also possible that sympathetic activity may actually cause a physical opening of tight junctions in the pulmonary capillaries, permitting the movement of fluid into the alveoli (107) or endothelial cell cytoskeleton contraction (108). Mechanical failure or stress failure of the blood-gas barrier in the face of extreme pulmonary hydrostatic pressures is also possible (109).

It has been shown that an increase in left atrial pressure alone induces a pattern of pulmonary edema that is identical to that formed during convulsive seizures (9). It has also been demonstrated that with an increase in left atrial pressure (identical to that observed during a seizure) causes an increase in lymph flow similar to the lymph flow observed during a seizure (16). However, these results are more indicative of cardiogenic pulmonary edema formation rather than NPE.

Experimentally, the conditions experienced during a spontaneous epileptic seizure are very difficult to recreate. Very little experimental data regarding pulmonary vascular response(s) to seizure activity is available and what data is available has been collected in a variety of models during acute seizure activity from anesthetized, intubated, and often paralyzed animals (8,9,11,35,96,103,104,110-112). The relevance of this information to 'real-life' seizure events is not known as experimental data regarding pulmonary vascular pressure responses during overt, spontaneous, motor seizures has not been reported.

While the mechanisms responsible for NPE are debatable, it is agreed that all or part of the mechanism of NPE must include a change in pulmonary capillary permeability (94). It is most likely however, that NPE is the result one or more of the conditions discussed. The most likely scenario is that milder (i.e. subclinical) forms of NPE commonly occur with severe generalized seizures, whereas full-blown NPE is rare, requiring both an severe increase in pulmonary venoconstriction as well as an increase in lung vascular permeability (95,113,114). It is possible that inflammatory mediators with the potential to change the 'normal' permeability of the lung could be upregulated as a result of previous subclinical edema episodes. Their relevance or possible role in subsequent, potentially more serious, episodes has never been addressed.

Circulating catecholamines can also mediate some of the mechanisms discussed above. Circulating catecholamines and vasoactive hormone levels increase during a seizure and remain elevated during the interictal phase

(38,115). It is thought that catecholamines play a minor role in mediating the vascular changes that occur during a seizure (96); however, they may have a significant impact on ventricular function and arrhythmia generation.

An increase in intracranial pressure can also cause the rapid formation of full-blown NPE via the induction of widespread sympathetic discharge (116). This response occurs most often in association with traumatic head injury and is mediated by the medullary and hypothalamic regions of the brain as well as via the release of adrenal catecholamines (107,117-119). However, while intracranial pressure has been shown to increase transiently during generalized seizures (87,120), it is generally believed that intracranial pressure does not play a significant role in mediating cardiovascular and pulmonary function regardless of seizure type (120). The effect of an increase in intracranial pressure with regard to traumatic brain injury has been reviewed (107).

Temporal lobe epilepsy

Temporal lobe epilepsy (TLE) is the most common epileptic syndrome accounting for at least 40% of all cases of epilepsy (121). Synchronous, aberrant electrical activity arises most often from mesial temporal limbic structures and is thought to be due to a selective loss of neurons in the hippocampal subfields (CA1, CA3) and dentate hilar cells with concomitant degeneration of axons projecting from cells of the hilar region of CA4 (4,122,123). As a result, the remaining viable hippocampal neurons respond with abnormal, synchronous bursts of electrical activity in response to cortical stimuli (124).

There is probably no single synchronizing or epileptogenic mechanism; however, many 'epileptic' neurons have a number of common characteristics (125,126). Many of these neurons have a recurring high-voltage, long-duration depolarization with superimposed high-frequency bursts of action potentials. The action potential bursts and paroxysmal depolarization shifts result in the characteristic 'spike and wave' discharge pattern characteristic of TLE (126). Paroxysmal depolarization shifts are generated by an excitatory postsynaptic potential that is enhanced, possibly through a reduction in inhibition, and amplified through regenerative dendritic action potentials (126). The spike and wave discharge pattern signifies a susceptibility to seizure activity and presumably any modification that increases neuronal excitability and favors burst potentials can lead to recruitment and synchronization of neurons (125). As the clinical behavioral characteristics of a TLE seizure progress electrophysiologically, the numbers of spikes in each burst increase, while the duration of each depolarization shift decreases.

Seizures, in general, are dynamic and evolving with the variability in seizure pattern dependent upon the spread of electrical discharge. Human TLE is most frequently associated with complex partial seizure activity that can potentially evolve into secondarily generalized seizures and motor convulsions. Only patients with TLE demonstrate an amygdala response to hippocampal stimulation, whereas all patients with epilepsy display a hippocampal response to amygdala stimulation (4). Facilitation of the hippocampal-amygdala connection is unique in the pathology of TLE and direct projections from the hippocampus

and amygdala to the hypothalamus, NTS subnuclei, and other brain stem regions, are critical in determining the cardiovascular autonomic consequences of epileptogenic activity originating in the limbic system.

Kainic acid model of temporal lobe epilepsy

Temporal lobe epilepsy is a very complex disorder and as such, a single animal model cannot be expected to reproduce all electrophysiological, physiological and behaviorally relevant characteristics. Experimentally, generalized motor and partial seizure activity has been induced by direct stimulation of specific cortical and subcortical areas (e.g. kindling), direct chemical application to specific subcortical or cortical structures, or indirect chemical insult via intraperitoneal or intravenous introduction of agents with epileptogenic properties (124,127). Historically, the substances most often used to induce generalized tonic-clonic seizures have been penicillin, pilocarpine, alumina, bicuculline, and pentylenetetrazol applied either directly to exposed cortical regions or introduced intravenously (126,128,129). However, these substances, in addition to the high mortality rate associated with their use, have unwanted secondary effects such as widespread lesions in non-limbic structures. In addition, these agents frequently do not produce spontaneous seizure activity. While these models have yielded valuable information regarding the electrophysiological sequelae of seizure activity, most are not appropriate as models for studying the physiological and behavioral components of TLE. There are very few genetic animal models available for the study of spontaneous seizure activity (100,128). Kainic acid, a glutamate agonist, has been used

successfully to model the effect of chronic, spontaneous temporal lobe seizure activity and comes very close to representing a 'true' model of human TLE (4)

Kainic acid is one agent in a family of toxic ligands similar in chemical structure to glutamate, an excitatory neurotransmitter, and is used extensively in neurobiology to investigate the pathological consequences of recurrent epileptic activity in the limbic system (4,50,130). Shinozake and Konishi (131) were the first to recognize the neuroexcitatory properties of kainic acid and its potential utility in neurological research. Using rat cortical neurons, these investigators began characterizing the excitatory effects of kainic acid and its capacity to potentiate the excitatory effect of exogenously applied L-glutamate. Olney et al. (130), intrigued by the neurotoxic properties of select amino acids such as glutamate (132), and the neuroexcitatory effects of kainic, linked the two observations together and suggested that kainic acid acted neurophysiologically as an 'excitotoxin'; implying, that the toxic nature of kainic acid is tied to its inherent excitatory properties. Nadler et al. (133) recognized the morphological similarities between kainic acid-induced lesions and hippocampal sclerosis in human TLE, thus paving the way for the experimental use of kainic acid to study TLE.

The utility of kainic acid in neurobiology lies in its 'axon-sparing' properties and the characteristic hippocampal lesions produced from either local intracerebral or peripheral administration of kainic acid (i.e. intravenous or intraperitoneal) (4,51,122,130,133-138). Unlike lesions induced electrolytically or by other chemical agents, kainic acid lesions are characterized as 'axon-sparing',

implying that axons and afferent nerves passing through a kainic acid-induced lesion, are unaffected (139). Kainic acid can thus be used to selectively destroy neurons in a given region.

Within the hippocampal formation, kainic acid causes select neuronal damage and neuronal death in the hilus, including inhibitory interneurons, and in the pyramidal cell layers (CA1, CA3 and CA4 subfields) (140). Autoradiographic experiments, with the concurrent administration of the radiolabeled metabolic marker 2-deoxyglucose and kainic acid, have mapped the structures with the highest metabolic rates during kainic acid-induced seizures (141). As expected, the most prominent changes in metabolism occurred in the areas most susceptible to kainic acid (i.e. limbic structures) and in structures with direct axonal connection, such as the hippocampus, amygdala, and mediodorsal thalamus (122,141). In the weeks following the initial kainic acid insult, mossy fiber collaterals of the dentate granule cells begin to appear in the granule cell layer and in the inner molecular layer of the dentate gyrus (4,133,140,142). The functional consequence of this 'sprouting' of axon collaterals and the overall synaptic reorganization that occurs within the hippocampus and dentate gyrus is a matter of debate.

In general, there are two schools of thought as to the consequence of synaptic reorganization in the hippocampus (124, 127). The first suggests that the hyperexcitability observed in the hippocampus following kainic acid administration is due to mossy fiber sprouting and the formation of recurrent excitatory circuits or positive feedback loops within and between granule cells.

The second suggests that if granule cell sprouting has functional significance, it is primarily inhibitory or restorative in nature and results in the formation of recurrent inhibitory circuits. The new recurrent inhibitory circuits serve to repair and temper the general hyperexcitability induced by the neuronal insult. There are numerous *in vitro* and *in vivo* experiments using human as well as animal tissues, with results to support each of these hypothesis (4,140,142-146).

Chronic focal epileptogenic activity can cause distant areas to become unstable or hyperexcitable and capable, in and of themselves, of generating epileptiform electrical discharge (122,134,139). These secondary foci develop presumably as a result of continuous epileptiform bombardment along specific fiber tracts. Experimentally, neuronal degeneration within the limbic system occurs away from the primary site of injection, and lesions that disrupt seizure propagation within the limbic system reduce 'distant' damage (134,138,147). In addition, ontological studies with rat pups have shown that the expression of KA receptors in the hippocampus is not sufficient to cause Ammon's horn sclerosis (148). A fully mature mossy fiber system must be intact before limbic seizures can even be induced and neuronal damage only occurs after kainic acid receptor expression in the amygdala and pyriform cortex is complete and these areas can be activated (137,148).

Peripheral administration of kainic acid is capable of producing extensive brain lesions in adult animals; however, the integrity of the blood-brain barrier prevents systemically administered kainic acid from moving freely into cerebral tissue (4,149). So, how much kainic acid actually reaches the brain? Studies

have shown that the appearance of kainic acid in the brain is a gradual process, with the concentration increasing in susceptible areas over time. However, even after 1 hour, concentrations are still in the 100nM range in the hippocampus and related regions (149). It is estimated that the actual brain concentration of kainic acid sufficient to produce paroxysmal discharge of pyramidal cells in the CA3 subfield is two orders of magnitude lower that the concentration required to directly produce damage by *in situ* injections (150). Therefore, with peripheral injections of kainic acid, indirect mechanisms must be involved in causing neuronal degeneration in order for such a minuscule amount of kainic acid to cause such specific structural changes.

The direct effect of kainic acid on the cerebral circulation in general, and in the hippocampus specifically, has received little attention. Long-term morphological studies have documented hypertrophic changes in the vessels of the frontoparietal cortex in kainic acid-treated rats without evidence of pathological alteration of neurons or glial cells in the area adjacent to the vessels (51). Acute changes such as small perivascular hemorrhages around drainage veins in the hippocampus and infarct scars have been noted (135). The physiological consequences of these vascular changes have not been addressed.

Perhaps one of the more striking morphological observations noted in many studies is related to the asymmetrical nature of the lesions and the observation that both mild and severe changes can occur in the same animal (151). In addition, in studies using systemically administered kainic acid; there

are instances in which the experimental animals fail to have limbic motor seizures (5,51,142). However, in these cases, clear-cut pathological alterations are found but are restricted to the hippocampal formation in contrast to the damage found in animals with spontaneous limbic motor seizures. While this may seem to be a liability or source of error in many studies, considering the tremendous amount of neurological variability in human temporal lobe epilepsy, this variability may actually be an asset precisely because it more closely represents the physiological and electrophysiological variability seen in the general population.

Epilepsy, hypoxia, and sleep

The influence of hypoxia on acute, induced, seizure activity is somewhat controversial, while the effect of hypoxia in a chronic, spontaneous model of epilepsy has not been examined. The cardiovascular and pulmonary responses to both acute and chronic hypoxia (e.g. altitude) have been extensively reviewed (152) however, the cardiopulmonary impact of a seizure or a sudden, 'sympathetic storm' under mildly hypoxic conditions, has yet to be addressed. The incidence of seizures at mild to moderate altitude has not been reported. It may be that seizure activity (i.e. frequency) is not affected by a change in barometric pressure as proposed by Dr. Clark (152) "...in view of the effect of hypoxemia and respiratory alkalosis upon the central nervous system, one might expect exposure to high altitude to provoke seizures in epileptics, but this is apparently not the case". There is no evidence that epileptic seizures are more frequent at high altitude than at sea level. Epileptics who are well controlled at

sea level are not at particular risk at high altitude. However, while the risk of having a seizure may not change with altitude, seizures can and do happen at altitude. Under these conditions, the cardiovascular and pulmonary hemodynamic changes triggered by a seizure could potentially be much more serious. Massive pulmonary venoconstriction coupled with hypoxic pulmonary arterial vasoconstriction and a shift of blood from the systemic circulation to the pulmonary circulation during a seizure could result in a significant increase pulmonary vascular pressure as well as capillary hydrostatic pressure; conditions favorable for pulmonary edema formation. Pulmonary vascular pressures under these conditions have not been reported.

From a model perspective, several investigators have shown that exposure to hypoxia (F_IO_2 of 0.08) prior to or during the administration of kainic acid is neuroprotective, either suppressing seizure activity (153,154) or attenuating the characteristic morphological changes that occur in the hippocampus in this model (155-157). The primary mechanism mediating the response in this acute model is thought to be the endogenous release of adenosine. Extracellular levels of adenosine in the brain increase significantly during hypoxia (84), acting as a vasodilator to maintain cerebral blood flow, and, during epileptiform activity (158,159) acting as an 'endogenous anticonvulsant' mediating seizure arrest (156,158). In addition, GABA-transaminase, which is responsible for the catabolism of GABA (gamma butyric acid), an endogenous neuronal inhibitory mediator requires oxygen. Therefore, under hypoxic conditions, the activity of the enzyme would be suppressed resulting in an

increase in extracellular levels of GABA, thereby inhibiting synaptic transmission. Hypoxia can inhibit or prevent seizures by depressing neuronal excitability, decreasing free radical production, stimulating the release of adenosine, depressing release of neural transmitters, and by suppressing excitability at post-synaptic sites (84,154,160). Thus, one could hypothesize that seizure frequency would decrease under hypoxic conditions via the hypoxia-induced release of adenosine.

While hypoxia alone may not be epileptogenic in adult rats, it is possible however, that hypoxia could exacerbate a nervous system predisposed to epileptic activity. For example, a reduction in O_2 has been shown to impair Na^+-K^+ pump activity thereby causing hyperexcitability (161). Furthermore, hypoxia-induced hyperventilation can result in cerebral hypocapnea and subsequent alkalosis thereby altering neuronal excitability (161). Other mechanisms such aggravating neuronal metabolic disturbances, reducing extracellular cerebral space, or an increase in the activity of excitatory amino acids have been proposed (162,163). Adrenergic activity increases in response to the stress of either acute or chronic hypoxic exposure (164) and one could hypothesize that enhanced sympathetic discharge could exacerbate a nervous system predisposed to epileptic activity (24,61). Individuals prone to high-altitude pulmonary edema have an exaggerated sympathetic response to short-term hypoxic challenges as well as moderate altitude exposure (165). It is also possible that hypoxia could indirectly affect seizure occurrence through the disruption of biological rhythms that are intrinsically tied to seizure activity. It is

not known whether an abrupt change in environmental conditions alters seizure susceptibility via changes in behavioral state.

Contrary to common perception, epileptic seizures are not entirely unpredictable. Seizures arising from many forms of epilepsy tend to cluster in daily patterns. Endogenous circadian rhythms, such as the light-dark cycle, as well as the sleep-wake cycle, affect some forms of epilepsy by inhibiting neuronal excitability; thereby altering seizure threshold and interictal discharges (166). Numerous clinical studies have linked spontaneous seizure activity, in both partial and generalized forms of epilepsy, to the sleep-wake cycle (167,168). However, while the sleep-wake cycle is the most 'recognized' biological rhythm, time of day (i.e. light-dark) is a biological cycle that also exerts a strong influence on seizure expression. More than 100 years ago, Gowers (169) noted the tendency of seizures to occur in temporal patterns outside the classical sleep-wakefulness characterizations. He classified patients based on the daily occurrence of seizures into three general groups: diurnal (daytime onset); nocturnal (nighttime onset); and, diffuse (random onset). These early observations were later confirmed and expanded as peak occurrences within each group were identified. 'Diurnal' seizures peak upon awakening and at late afternoon, whereas 'nocturnal' seizures cluster at bedtime and in early morning before awakening (170). Patients with mesial temporal lobe epilepsy have a cyclical seizure pattern with peak occurrence falling in the late portion of the light phase of the day (171). Nocturnal rats and diurnal humans have circadian rest cycles that are out-of-phase with each other; however, both species have

predominantly diurnal seizures despite the fact that rats are nocturnal. Therefore, the light-dark daily pattern in limbic seizures is a true endogenously mediated circadian pattern, as it is not disrupted when the animals are placed in constant darkness (166).

Behavioral circumstances can also alter the incidence of seizures. For example, many patients with epilepsy are less likely to have a seizure when they engage in strenuous exercise (172,173). However, many times, a seizure will occur in the relaxation period immediately following the stress (78). In addition, it has been hypothesized that an increase in stress increases the probability of seizures (18). Recently, it was reported that in the kainic acid model of limbic seizures, seizures appear to coincide with the activity state or degree of inactivity rather than with the light-dark phase, suggesting that behavioral states modulate seizure susceptibility in the kainate-induced epileptic rat (174).

The association between sleep and epileptiform neural activity is well documented. Nocturnal temporal lobe complex partial seizures generalize 37% of the time, compared to complex partial seizures during wakefulness (17%), occur most frequently in light sleep (Stage 1 and 2) or deep sleep (Stage 3 and 4) of NREM sleep, and rarely occur during REM sleep (175,176). In addition to circadian influences, epilepsy is associated with other sleep abnormalities, such as prolonged sleep latency, an increase in the number and duration of awakenings, and reduced or fragmented REM sleep (177). Sleep at altitude, is also punctuated with many of the same sleep abnormalities. Eighty-three percent of newcomers exposed to even moderate altitudes of 10,000 and 14,000 ft

frequently complain of disturbed sleep (178). Malkin et al. (179) have identified specific changes in the sleep-cycle that account for the anecdotal reports of poor sleep. For the first 48-72 h at moderate altitude (14,000 ft), subjects experience an increase in the number of awakenings, a significant increase in the duration of stage 1 and/or stage 2 sleep, and a significant decrease in stage 3 and 4, slow wave, and REM sleep stages. Therefore, at altitude, a person with epilepsy is potentially spending more time in the sleep phases in which the probability of seizure occurrence and generalization is the highest. In the study conducted by Malkin et al. (179), the greatest increase in seizure frequency did occur during the light phase of the HX exposure period, the primary sleep period for these animals. Thus, these results could be explained by an altitude-induced disturbance in the normal sleep cycle of these nocturnal animals.

The relationship between disrupted sleep patterns at altitude and seizure activity has not been reported. Several surveys have identified numerous factors that are related to the occurrence of acute mountain sickness in travelers to moderate altitude (22,178,180). A very recent report examining the occurrence of high altitude cerebral edema at moderate altitude did note the occurrence of seizures, one in a patient without a previous seizure history (181). Interestingly, only 2 of 11 EEGs from 9 patients mentioned in this study were normal; however, no further diagnostic information was provided.

Additional information might be provided again by examining the effect of endogenous adenosine on sleep patterns. Adenosine may be the major sleep-promoting substance in the brain (182). Adenosine antagonists cause prolonged

wakefulness, much like the effect of caffeine and theophylline (183). Adenosine agonists increase slow-wave sleep and paradoxical sleep in a dose-dependent manner (184). In ventilation studies, adenosine infusion increases ventilation and produces periodic breathing in normal subjects (185). All three conditions, hypoxia, sleep, and seizures can be linked to a single, common mediator, endogenous adenosine. This relationship has yet to be explored.

While it is possible that seizure activity does not change with mild altitude exposure, the lack of data may be due to the failure of people with seizure conditions to consider the event unusual and necessary of medical attention. It is not unusual for occasional seizures to occur even if the disorder is pharmacologically controlled. Therefore, an episode is less likely to come to the attention of medical personnel unless it is complicated by other medical conditions or the individual is if at altitude, complicated by other symptoms of acute mountain sickness, and therefore overlooked. The potential underlying mechanism(s) responsible for the results described in this report need to be examined in greater detail. A more systematic review of altitude-induced changes in seizure patterns, possibly as part of the overall acute mountain sickness syndrome, is warranted, especially in people with a documented history of seizures.

CHAPTER III

Acute systemic and pulmonary vascular effects of repeated low-dose injections of kainic acid

Abstract

By definition, epilepsy implies the recurrence of spontaneous seizures over time; however, few experimental models meet this criterion. Systemic administration of multiple, low-dose injections of kainic acid (KA) have been shown to reliably produce chronic, recurrent, spontaneous motor seizures in rats. However, the cardiopulmonary impact of acute KA administration has not been evaluated. The objective of this study was document the acute effect of multiple, low-dose injections of KA on systemic and pulmonary arterial pressure and heart rate in unanesthetized, freely moving rats. Eight control (CON) and 10 KA rats completed the protocol. Prior to the 1st injection, there were no hemodynamic differences between the 2 groups. Systemic hypotension and bradycardia occurred immediately after the 1st KA injection and was sustained for a minimum of 1 h. The mPpa response was less consistent as mPpa increased in half of the KA-treated rats and decreased in the other half. After the 1st motor seizure, interictal mSAP gradually increased with spikes in mSAP occurring with each seizure. Heart rate responses were more variable with periods of tachycardia and bradycardia. Interictal mPpa also increased gradually following the 1st motor seizure and spiked with each seizure. Interictal and ictal mSAP was attenuated

and heart rate was exacerbated following phentolamine (1 mg/kg). Overall, mPpa was unaffected by phentolamine. This study has documented the cardiopulmonary effect of multiple, low-dose injections of KA as well as the hemodynamic responses to acute, KA-induced seizure activity. The initial systemic, pulmonary, and heart rate responses were probably centrally mediated events; however, simultaneous monitoring of cardiopulmonary and hippocampal/amygdala activity will be needed to completely assess these responses. The increase in mSAP and heart rate with seizure onset and the attenuation of mSAP with phentolamine is consistent with that reported for other acute seizure models. Of concern was the ineffectiveness of phentolamine on mPpa and suggests the involvement of other vasoactive mediators; however, the direct effect of KA cannot be ruled out. Therefore, the pulmonary vascular responses in this acute model merit further investigation.

Introduction

Complex partial seizures, especially those originating in the temporal lobe, are the single most common seizure type and one of the more difficult seizure syndromes to control with anticonvulsant drugs (1). Only 25% of adults with complex partial seizures experience complete seizure control with anticonvulsants; thus, an understanding of the underlying mechanisms of epileptogenesis is of tremendous clinical importance. However, the development of valid animal models of temporal lobe epilepsy, as well as other seizure syndromes, has been challenging. By definition, epilepsy implies the recurrence of spontaneous ictal or aberrant electrical activity over time; however, few experimental epilepsy models meet this criterion. Furthermore, in many instances, temporal lobe seizures in humans occur spontaneously following a quiescent or silent period after an acute, precipitating event. Thus, the optimum experimental model for this form of temporal lobe epilepsy should included an acute neurological insult that is followed by a quiescent or latent period that is spontaneously broken by the development of chronic, recurrent ictal seizure activity.

While in general, the primary objective of epilepsy models is to identify the mechanisms that cause and sustain aberrant electrical activity, these models are also well suited for evaluating the mechanisms regulating other seizure-related phenomena. It is well recognized that the mortality for people with epilepsy is significantly higher than that for the general population and is related to the phenomena of sudden unexpected death (SUD) and neurogenic pulmonary

edema (2-4). SUD victims are often found alone and with no pathological evidence for the cause of death although pulmonary edema is often present. SUD is not restricted to that segment of the epilepsy population with the more 'severe' seizure syndromes. SUD and neurogenic pulmonary edema are thought to be the direct result of the disruption (i.e. dysfunction) of the cardiovascular and/or pulmonary systems during overt seizure activity, although the mechanism(s) remains unknown. There are numerous models that have been developed to investigate SUD and neurogenic pulmonary edema in which acute, generalized seizures or status epilepticus has been induced using neurotoxic chemicals such as alumina, pilocarpine, pentylenetetrazol, and penicillin. However, while many of these studies have provided valuable information and details about autonomic nervous system function, or dysfunction, under these conditions, the direct application of the results from many of these studies to chronic seizure conditions is inherently limited by use of anesthetics and paralytics.

Acute, generalized seizure activity can be induced suddenly by a variety of agents or by widespread direct electrical stimulation (i.e. electroconvulsive shock therapy); however, very few methods have been successful in modeling the chronic, spontaneous nature of human epilepsy. The systemic administration of pilocarpine (5) and alumina (6) has been used to successfully induce spontaneous generalized seizures following a brief period of status epilepticus. In either model, spontaneous seizures have been recorded between 2 weeks and 6-8 months following the initial insult. Unfortunately, the systemic administration

of either of these agents is associated with a high mortality and inconsistent results (i.e. few animals with spontaneous seizures). The self-sustaining "limbic-status-epilepticus" (7) and the "kindling" models (1) do not rely upon neurotoxin exposure for the initial neurological insult nor do they use status epilepticus as the precipitating insult. The kindling model is the most common seizure model of this type. In this model, repeated, subconvulsant electrical current is delivered over a period of time (days) directly to the region of interest (e.g. amygdala). Eventually, permanent changes occur such that each time the stimulus is presented, a generalized seizure is triggered (8). This model has been used to examine cardiovascular changes during the kindling acquisition process as well as during provoked (i.e. kindled) seizures (9). Although the kindling process does allow partial as well as generalized seizures to be examined in the same animal, kindled animals only develop spontaneous (i.e. non stimulus-provoked) seizures after hundreds of stimulus-provoked seizures, thus requiring a substantial amount of time to develop (1,10).

Systemic administration of multiple, low dose injections of kainic acid (KA) has also been shown to reliably produce chronic, recurrent, spontaneous complex partial and motor seizures in rats approximately 77 days after the initial KA insult (11). However, unlike other neurotoxin models, the mortality associated with this model is very low and almost all animals (97%) develop spontaneous seizures. The hippocampi of the KA-treated animals develop a significant degree of hippocampal sclerosis and also undergo a synaptic reorganization or remodeling process that is remarkably similar to that found in

people with temporal lobe epilepsy. In addition, the seizure frequency associated with KA-treated animals progresses overtime, much like that exhibited in people with more severe syndromes. Therefore, in many ways, the KA model more accurately reflects some of the pertinent characteristics of chronic seizure disorders that are lacking in other epilepsy models.

In considering the use of a neurotoxin-based seizure model as a valid experimental model for epilepsy-associated phenomena such as SUD and neurogenic pulmonary edema, it is imperative to evaluate the acute systemic impact of the convulsant agent itself, as well as the impact of the barrage of motor seizures provoked during the acute insult. Therefore, using the initial induction protocol for the chronic KA model described by Hellier et al. (11), we have evaluated the acute systemic and pulmonary vascular effects of repeated, low-dose intraperitoneal injections of KA. The purpose of this study was to: 1) assess the immediate acute effect(s) of KA introduced via intraperitoneal injection on mean systemic arterial pressure (mSAP), mean pulmonary artery pressure (mPpa), and heart rate; 2) measure mSAP, mPpa, and heart rate during the overt motor seizures induced as a consequence of multiple IP doses of KA; and 3) evaluate the role of α-adrenergic mechanisms in regulating the cardiopulmonary responses (i.e. mSAP, mPpa) during KA-induced motor seizures. We hypothesized that: 1) following the administration of KA, mSAP, mPpa, and heart rate will increase in a linearly manner throughout the protocol as motor seizures develop; 2) the gradual increase in mSAP, mPpa, and heart rate will be punctuated by transient pressure and heart rate peaks coinciding with

each motor seizure; and 3) mSAP and mPpa during ictal, as well as interictal periods, will be attenuated by phentolamine, an α-adrenergic antagonist.

Methods

In vivo catheterization: Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed in the Laboratory Animals Resource facility, an Association for the Assessment and Accreditation of Laboratory Animals Care International-approved facility, with access to food and water *ad libitum* for a minimum of 1 week prior to the surgery protocol. Forty-eight hours prior to surgery, the animals were given water supplemented with TMS and AC (0.127 mg trimethoprim, 0.634 mg sulfamethoxazole, 0.50 mg acetaminophen, and 0.050 mg codeine/ml H_2O). On the day of surgery, the rats were taken to a surgery suite at the Laboratory Animal Resource facility where food and water was withheld until they had recovered from surgery. Postoperatively, animals were housed under standard husbandry conditions in individual cages on a 12 h light/dark cycle with access to food and water. All procedures were reviewed and approved by the Animal Care and Use Committee of Colorado State University.

The rat was placed in an induction chamber that was flooded with a mixture (Aerane, Clover Medical Equipment Services, Buffalo, NY) of gas anesthesia (IsoFlo, Abbott Laboratories, North Chicago, IL) and oxygen. Once unconscious, the rat was removed from the induction chamber and placed on a covered heating pad on the surgery table. An appropriate anesthetic plane was maintained by masking the animal and administering Isoflurane at a concentration of 1-3% with an oxygen flow rate of 200 ml/kg/min for the duration

of the procedure. Fur covering the dorsal and ventral neck regions was removed using a scalpel blade and the area cleansed and disinfected (Hibiclens, Zeneca Pharmaceuticals, Wilmington, DE). Immediately prior to the first incision, the rat was given a 3 ml subcutaneous injection of warmed (37°C) lactated Ringers and placed in a supine position and covered with sterile drapes so that only the neck region was visible. A small, midline incision was made above the right clavicle followed by gentle dissection to isolate the right jugular vein jugular vein and carotid artery carotid artery. The carotid artery was ligated rostrally with 3-0 silk suture and cannulated with a polyethylene (PE-50, 0.58mm ID) catheter. The jugular vein was also ligated rostrally with 3-0 silk suture and cannulated with a polyvinyl catheter (PV-1, 0.28 mm ID) with a shallow bend at its tip. This catheter was guided through the jugular into the right ventricle and placed in the main pulmonary artery while monitoring the pressure tracing on a Gilson Duograph recorder (Model ICT-2H, Gilson Medical Electronics, Middleton, WI). The catheter was secured with sutures once the characteristic pulmonary artery pressure tracing was consistent and stable. Following placement of the pulmonary artery catheter, PE-50 and PV-1 straight-tipped catheters were introduced into the jugular vein, advanced to the superior vena cava, flushed with heparinized saline (1:100 vol/vol) and chloramphenicol (1 mg/ml) solution, and ligated. A third PE-50 catheter was introduced into the intraperitoneal cavity via a small midline incision approximately 5 cm below the xyphoid process. The intraperitoneal catheter was secured using a Chinese finger-trap suture (4-0 silk) and tunneled subcutaneously to the right of the sternum joining the other

catheters at the open neck incision The catheters were tunneled subcutaneously from the ventral neck to the crown of the head and exteriorized. The catheters were coiled and placed in a plastic housing sutured to the skin and covered with a threaded plastic cap. The animal was then given a second 3 ml subcutaneous injection of lactated Ringers and placed in a clean, partially warmed cage for recovery. All rats were given a post-operative injection of Banamine (2.5 mg/kg, IM) and Diazepam (3mg/kg) for analgesia and kept on water supplemented with TMS and AC for 48 hours. All instruments were autoclaved and catheters sanitized by cold sterilization (Amersol, ConvaTec, St. Louis, MO.) prior to each surgical procedure.

Hemodynamic measurements: Forty-eight hours after cannulation, the rats were transported from the LAR facility to the laboratory. The cap from the plastic housing was removed and the rat was placed in a small rectangular plastic chamber. The catheters were passed through an opening at the top of the chamber, opened, and irrigated with heparinized saline. The intraperitoneal catheter was not flushed. The catheters were connected to P23 Db transducers (Statham, Oxnard, CA) for measurement of heart rate, systemic arterial, and pulmonary arterial pressures. Each transducer was positioned at midthorax for all pressure measurements and was calibrated daily with a mercury manometer. The remaining catheters provided intravascular access for indocyanine green dye injection for cardiac output measurements and for intravenous administration of the α-antagonist, phentolamine (1 mg/kg). An inlet at one end of the plastic chamber was connected via a flow meter to a gas cylinder containing

compressed air (21% O_2).

Mean systemic (mSAP) and pulmonary arterial pressures (mPpa) were calculated as (P_s + 2*P_d)/3). Heart rate (beats/min) was determined from the systemic arterial pressure tracing. Systemic and pulmonary vascular resistances (mm Hg/ml/min) were calculated as mean pressure (e.g. mSAP, mPpa) divided by cardiac output. Cardiac output was determined by the standard indocyanine green dye-dilution technique (12). A bolus of dye (0.1 mg) was injected into a jugular catheter while arterial blood was simultaneously withdrawn via the carotid artery catheter by a rotary circulatory pump (Cole-Parmer Instrument Company, Vernon Hills, IL) and passed thorough a densitometer cuvette (D-402A Densitometer, Waters Instruments, NY) at a rate of 4 ml/min. Arterial blood was returned to the rat via the second jugular catheter. The signal from the densitometer was recorded on a dual channel recorder (Kipp-Zonen, Delft, Holland). The area under the inscribed dye-curve tracing was calculated as described by Williams et al. (13): Cardiac output (ml/min) = (amt of dye injected (mg) * paper speed (cm/min)) / (area (cm_2) * calibration factor (mg/L·cm)). The reported cardiac output measurements are an average of 3 dye curves for each condition per animal. Baseline arterial blood samples (0.2 ml) were withdrawn via the carotid artery catheter immediately following the cardiac output measurements and analyzed for arterial pH, P_AO_2, P_ACO_2, and other calculated values (Radiometer, ABL 300, Copenhagen, Sweden).

Acute induction protocol: The rats were transported in covered cages from LAR to the laboratory at least 30 minutes prior to the start of the study. The rats

were allowed to acclimate for an additional 30 min in the plastic chamber prior to the initial baseline measurements. Following this acclimation period, baseline measurements for all cardiopulmonary parameters and arterial blood gas values were obtained.

The KA-induced epileptic rat model of temporal lobe epilepsy has been well-documented (14,15) and the induction protocol used in this study has been described elsewhere in detail (11). Following cannulation, male Sprague-Dawley rats (150-200 g, Harlan, Indianapolis, IN) were randomly divided into KA and control (CON) groups. Each rat in the KA group was given an hourly bolus of KA (5 mg/kg) via the intraperitoneal catheter while CON rats received an equal amount of saline. After 3-4 boluses, most of the KA-treated animals began having overt motor seizures. Seizure severity was scored using a modified Racine scale (16), with severity defined as follows: Class III, rats displayed forelimb clonus with a lordotic posture; Class IV, rats reared with simultaneous forelimb clonus; and Class V, rearing with forelimb clonus accompanied by a loss of balance. KA treatment continued with a progression in seizure severity for a minimum of 3 h. The total dose of KA for each rat varied between 20-50 mg/kg. If an animal exhibited excessive activity (continuous circling, jumping) or became catatonic, the subsequent injection was reduced to 2.5 mg/kg, as continued treatment at the initial dose has proven fatal. Injections were suspended when the animal began having continuous motor seizures.

Systemic and pulmonary pressures were recorded continuously during the induction protocol in an attempt to precisely record pressure and heart rate

responses to each KA bolus as well as during overt seizure activity. Arterial blood gas samples were withdrawn from the carotid artery catheter prior to the 1st KA injection, immediately following the first recorded seizure, and at the end of the protocol prior to euthanasia. A subgroup of rats from both CON and KA groups were given an intravenous bolus of phentolamine (1mg/kg) and monitored an additional 1-2 hours. Animals were not given additional KA injections during the phentolamine challenge. All rats were euthanized with an overdose of sodium pentobarbital (IV, 100mg/kg) at the end of the protocol.

Cardiopulmonary measurements during seizure activity: A 'time profile' for mSAP, mPpa, and heart rate responses during each seizure was constructed from the pressure tracings. The first sign of aberrant motor activity was defined as time zero (0) and was used as the reference point from which all subsequent time points were determined. The final pressure profile for each motor seizure encompassed a time period extending from 2 min prior (-120 sec) to 2 minutes after (120 sec) the reference point. For each time point, the percent change for mSAP, mPpa, and heart rate was calculated using the measurement recorded 2 min prior to the start of the seizure as the baseline measurement. All statistical comparisons for each time point were contrasted against the measurements recorded at one minute (-60 sec) prior to the beginning of aberrant motor behavior.

Histology: The trachea was isolated and cannulated for subsequent intratracheal formalin instillation. The chest cavity was opened and retracted and a bolus of heparin (0.3 ml, 1000 U/ml) was injected directly into the right

ventricle. A small incision was made in the apex of the left ventricle followed by the cannulation of the main pulmonary artery via the right ventricle. Approximately 10-15 ml of heparinized saline was slowly flushed through the pulmonary artery followed by buffered formalin (10%) until partial fixation was apparent. The heart/lung block was then excised and formalin slowly instilled into the trachea until the lungs were inflated. The trachea was then ligated with 3-0 silk and the lung was submerged in a specimen cup filled with 10% formalin.

Following a minimum of 24 h in fixative, the heart-lung block was removed from the formalin solution and prepared for paraffin embedding and sectioning. For the lungs, the lower 2-3 mm of the right lung was cut away and discarded. A central section (~3 mm thick) was then obtained from the remaining mid-lung portion, placed in a cassette, and returned to 10% formalin solution. A central section of the heart was taken in a similar manner. The initial cut was made approximately 2-3 mm above the apex. A central section was then obtained, placed in a cassette, and the remaining specimen returned to formalin solution. All sections were taken to the Histopathology Laboratory, Pathology Department, at Colorado State University for paraffin embedding, sectioning, and hematoxylin-eosin staining. A Board-certified veterinary pathologist evaluated slides of lung tissue from both groups.

Statistical analysis: A 2-way repeated measures analysis of variance was used to assess changes in the hemodynamic parameters between and within the KA and CON groups at 3 time points (baseline, post-induction, post-induction with phentolamine). For the statistical analysis of mSAP, mPpa, and heart rate

responses during seizure activity, a repeated measures analysis of variance with a contrast variable (-60 sec) was used to compare the value recorded at each time point against the initial baseline measurement. Where appropriate, significance was assessed by post hoc least square difference pairwise comparisons. The data presented in tabular and graphic form represent raw (i.e. non-transformed) means ± standard error of the mean. A P value less than 0.05 ($p < 0.05$) was accepted as significant. The number of data points for each measurement is displayed on the bars of the graphs. The number of data points from parameter to parameter vary since not all measurements could be made on each animal due to technical problems inherent with experiments conducted in conscious cannulated animals.

Results

A total of 18 animals (CON = 8; KA = 10) were instrumented for the induction protocol. A summary of baseline hemodynamic measurements for each group is presented in Tables III-1 and III-2. With the exception of body weight, there were no significant differences between the two groups prior to the induction protocol. On average, the KA animals received a total of 2.5 injections of KA eliciting an average of 21 seizures/rat (2 Class III, 7 Class IV, and 12 Class V). Each CON rat received 4 saline injections over the course of 4 hours. All KA-treated rats progressed through the behavioral stages previously described for the KA model such as initial staring spells, multiple wet-dog shakes, facial clonus, rearing, forepaw clonus, and generalized motor seizures (15,17,18).

Table III-1. Baseline weight, mSAP, mPpa, and heart rate (HR) for acute CON and KA groups.

	Wt (g)	mPpa (mmHg)
	383±17 (8)	19±3 (2)
	457±24 (10)*	14±2 (6)

*significantly different from CON

Table III-2. Baseline cardiac output (CO), stroke volume (SV), and systemic and pulmonary vascular resistance (SVR, PVR) for acute CON and KA groups.

	CO (ml/min)	SVR mmHg ml/min
	142±24.0 (4)	0.93±0.19 (4)
	121±25.4 (6)	1.20±0.21 (6)

At the end of the induction period, mSAP, mPpa, and heart rate for the CON rats were unchanged while all were significantly higher than baseline measurements in the KA group (Table III-3). With phentolamine, mSAP returned to baseline levels; however, mPpa and heart rate remained significantly higher than baseline.

Table III-3. mSAP, mPpa, and heart rate (HR) responses following saline and KA injections with and without phentolamine.

	mSAP (mmHg)	HR (beats/min)
	121±2(8)	398±14(8)
	110±6(5)	381±22(5)
	125±3(10)	405±13(10)
	152±5(10)*‡	442±18(7); p=0.069
	129±5(6)‡+	480±16(6)*+ p=0.062

* significantly different from baseline
‡ significantly different from end-induction
+ significantly different from CON

Arterial blood gases remained constant throughout the induction protocol (Table III-4). Blood gas measurements taken after the first documented seizure and at the end of the protocol were not different from baseline measurements. Phentolamine had no effect on arterial blood gases.

Table III-4. Arterial blood gas values at baseline, during the induction protocol, and following phentolamine.

	P_aO_2 mm Hg	P_aCO_2 mm Hg	pH
CONTROL			
	73.1±1.3(6)		7.42±0.0(4)
	72.0±1.0(4)		7.42±0.0(4)
	73.2±2.5(8)		7.44±0.0(5)
	77.3±1.3(8)*		7.45±0.0(8)
	73.4±1.3(5)		7.46±0.0(5)

* significantly different from baseline

Over the course of 4 hours, mSAP, mPpa, and heart rate remained relatively unchanged or were slightly lower than baseline values in the CON

animals (Figures III-1 and III-2). Marked systemic hypotension and bradycardia were observed in all of the KA rats after to the 1st KA bolus (Figures III-3 and III-4). In most cases, the hypotensive and bradycardic responses were sustained for over an hour. There were two distinct mPpa responses provoked by the initial KA injection (Figures III-5 and III-6). mPpa increased in 50% of the animals within minutes of the 1st bolus while mPpa fell in the other half of the group. Figures III-3 (animal 308) and III-4 (animal 314) are representative of the systemic hypotensive and bradycardic responses recorded after the 1st injection, while Figures III-5 (animal 308) and III-6 (animal 314) illustrate each mPpa response. mSAP, mPpa, and heart rate graphs for all other animals can be found in Appendix A.

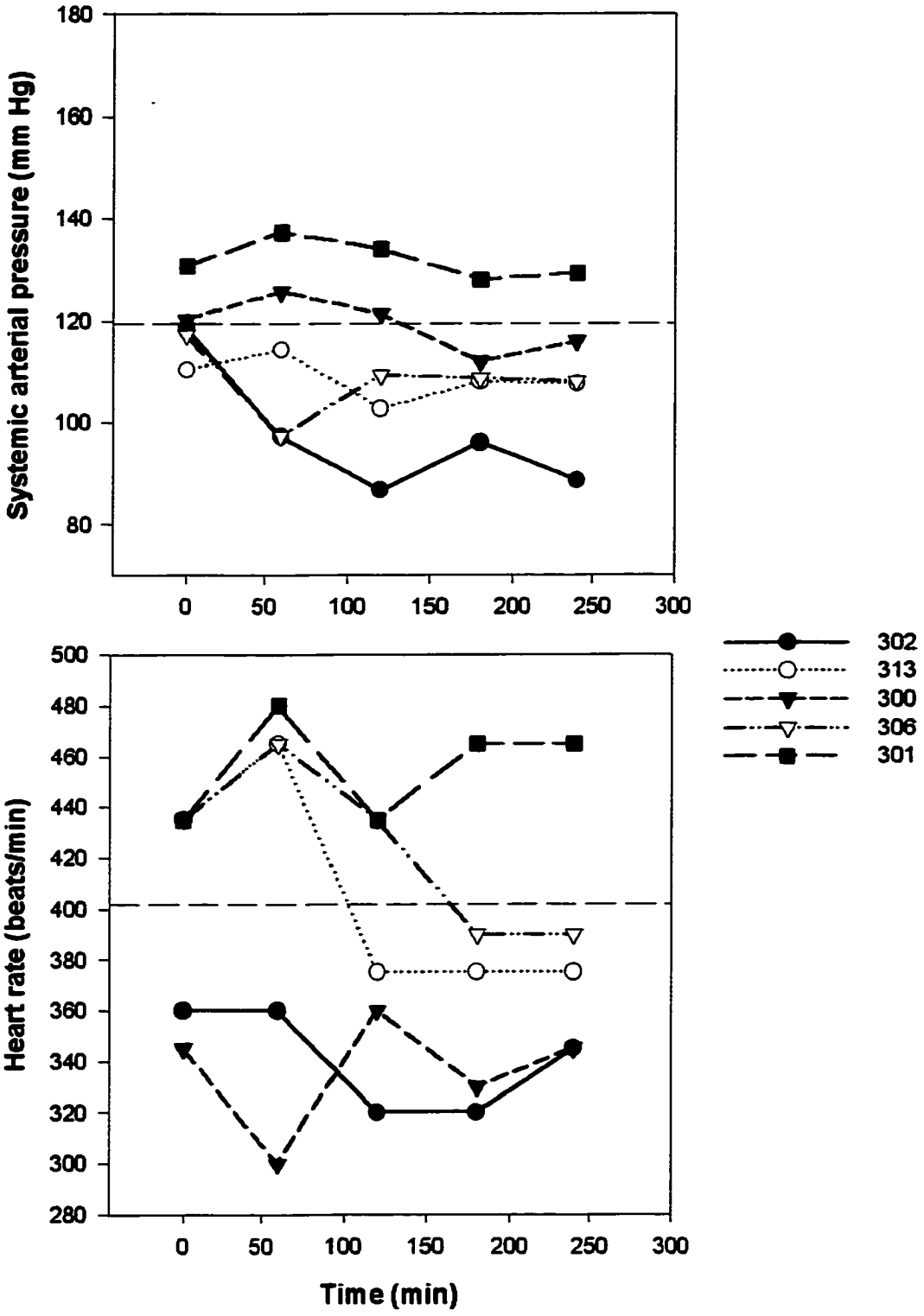

Figure III-1. Time course of mSAP and HR responses in CON animals during the induction protocol. mSAP and HR fell over the course of the induction protocol.

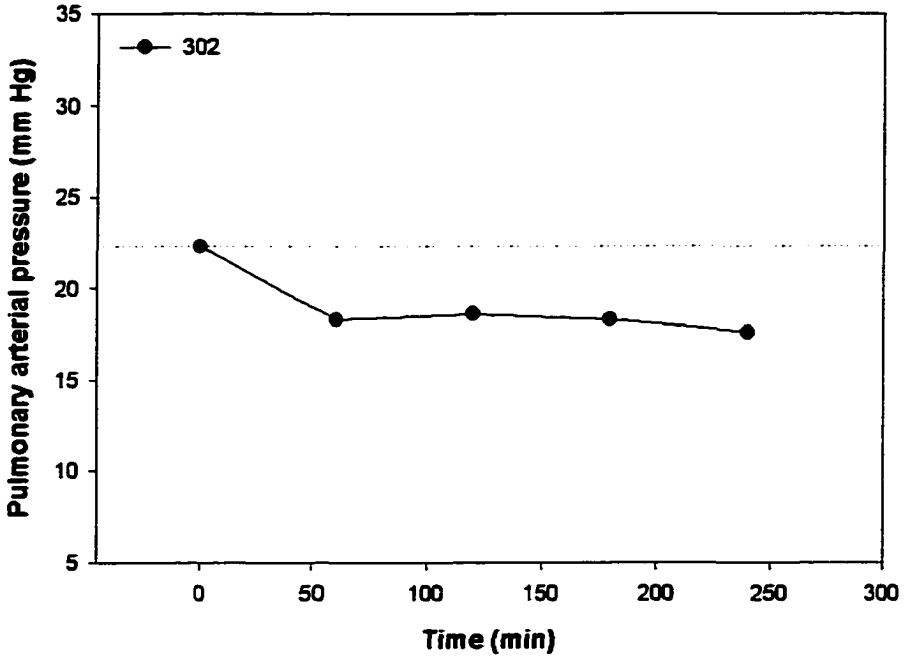

Figure III-2. Time course of mPpa response in CON group during the induction protocol. mPpa remained relatively stable throughout the the induction protocol.

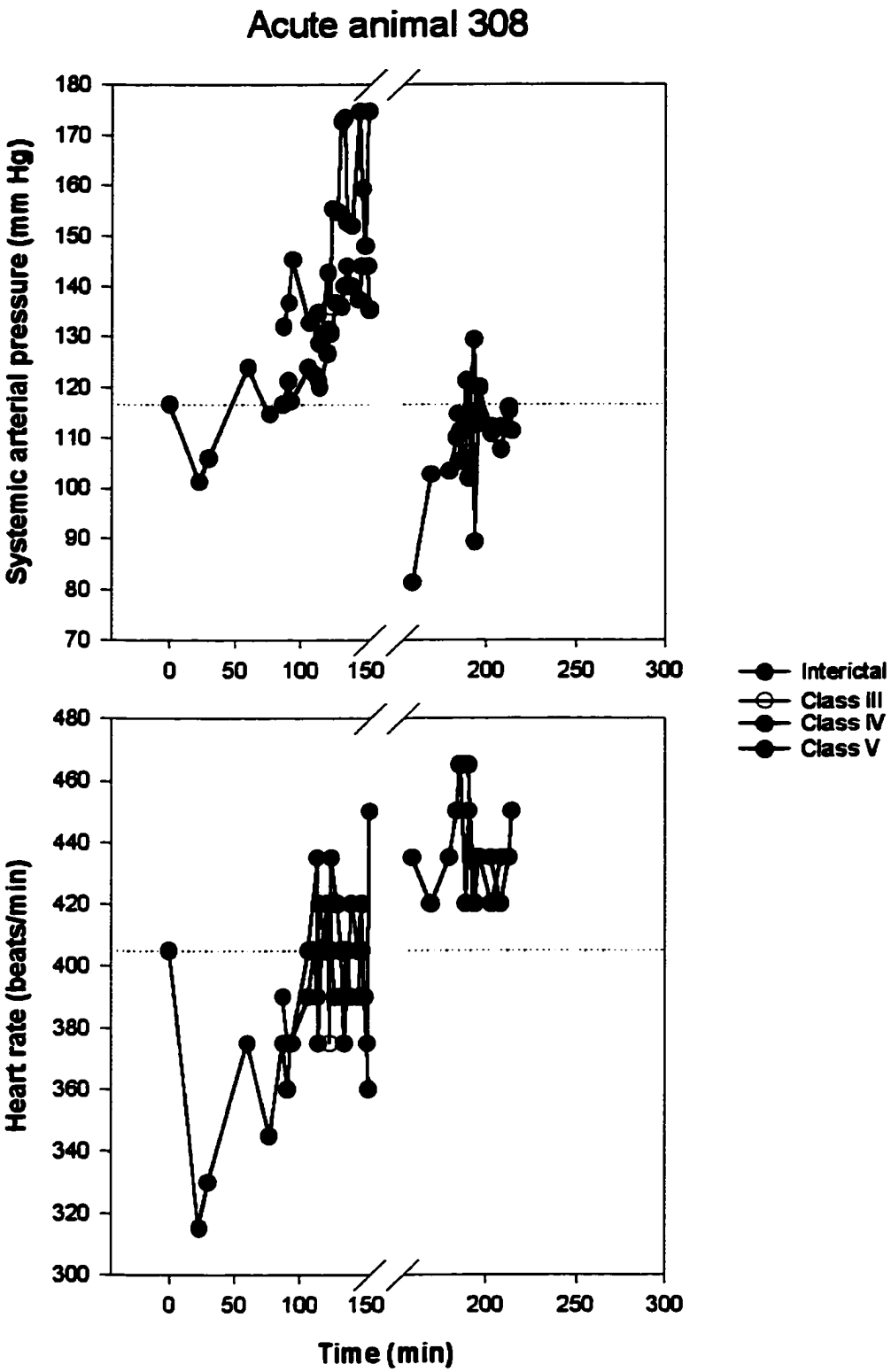

Figure III.3. Time course of mSAP and heart rate (HR) responses for KA-treated rat 308 during the induction protocol. Marked systemic hypotensions and bradycardia characterized the initial response to KA with subsequent systemic hypertension and tachycardia. mSAP decreased and HR increased in response to phentolamine.

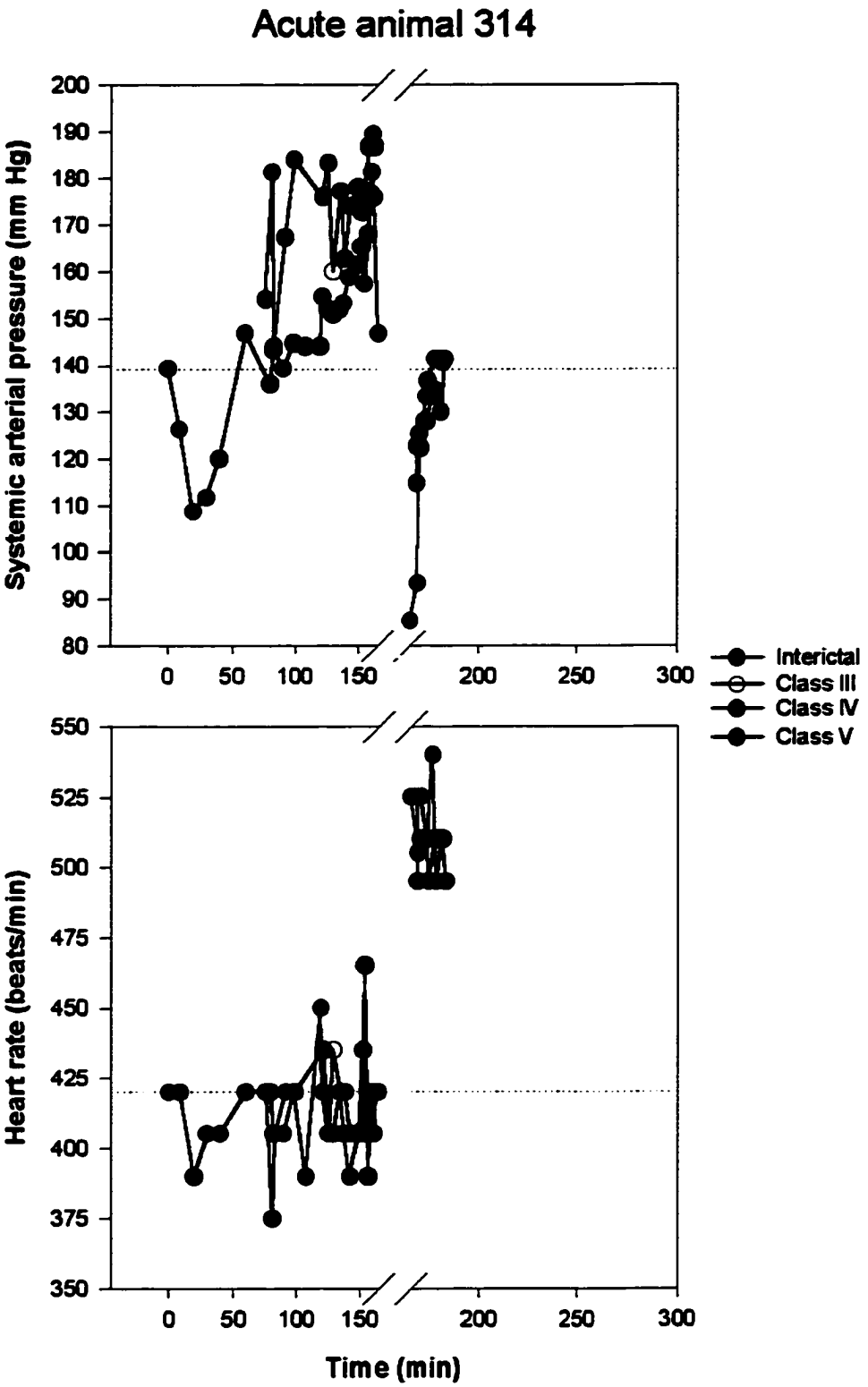

Figure III.4. Time course of mSAP and heart rate (HR) response for KA-treated rat 314 during the induction protocol. Marked systemic hypertension and bradycardia characterized the initial response to KA with subsequent systemic hypertension and tachycardia. mSAP decreased and HR increased with phentolamine

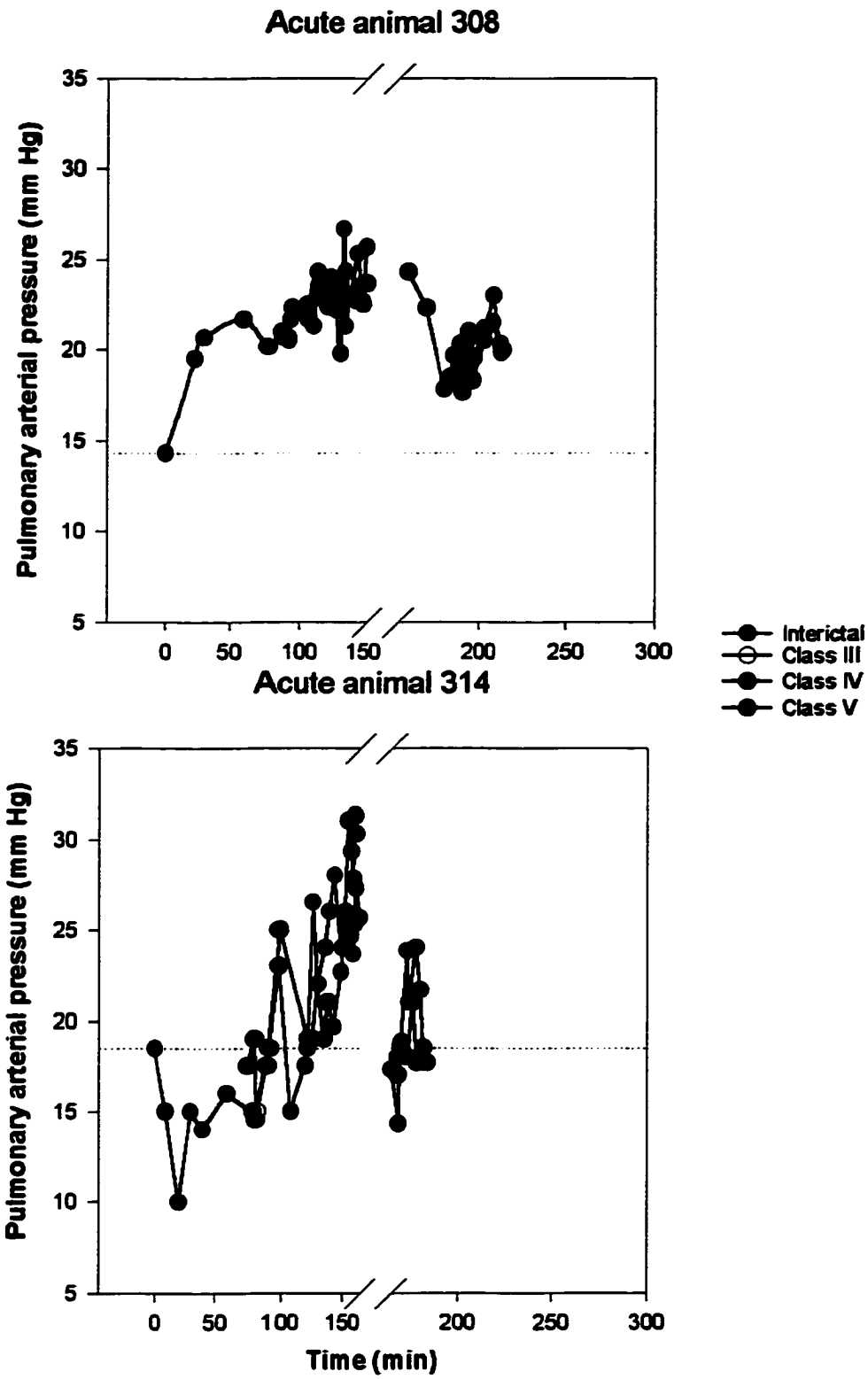

Figure III.5. Time course of mPpa response for KA-treated rats 308 and 314 during the induction protocol. mPpa increased in 308 and decreased in 314 following the initial KA injection. As motor seizures developed, mPpa increased in both animals. Phentolamine attenuated mPpa more effectively in rat 314.

In general, mSAP increased transiently with each seizure independent of seizure severity (Figures III-3 and III-4). Heart rate was more variable with both tachycardic and bradycardic responses. mPpa also increased transiently during seizures although in animal 308 (Figure III-5) the pressure responses were clustered while the pressure changes during seizures in animal 314 (figure III-5) exceeded each interictal measurement. Interictal mSAP fell significantly and heart rate increased immediately following the intravenous bolus of phentolamine (Table III-3, Figures III-3 and III-4). Ictal mSAP pressure peaks were also attenuated by phentolamine. Ictal mPpa spikes were also attenuated slightly by phentolamine (Figures III-5 and III-6); however, the final interictal mPpa for the KA group was no different than that recorded immediately prior to the phentolamine injection (Table III-3).

A *composite profile of* mSAP, mPpa, and heart rate responses during Class IV and V seizures for 7 KA rats with the most complete hemodynamic data is shown in Figure III-6. mSAP and mPpa increased significantly 30 seconds before aberrant motor activity was apparent. There was a gradual decrease in mSAP after time 0 although pressure was still significantly higher than baseline 2 min later. In contrast, mPpa did not peak until 15 sec after the start of the seizure after which the pressure returned to baseline relatively quickly. Heart rate remained relatively constant throughout the motor seizure but dipped significantly at time 0 and increased significantly 30 sec after the start of the seizure.

Lung tissue from 5 CON and 6 KA rats was evaluated for evidence of pulmonary edema. There was no evidence of pulmonary edema in either group. Two animals from the CON group and 1 animal from the KA group had some evidence of mild patchy alveolar hemorrhage. Lungs from the remaining rats were normal.

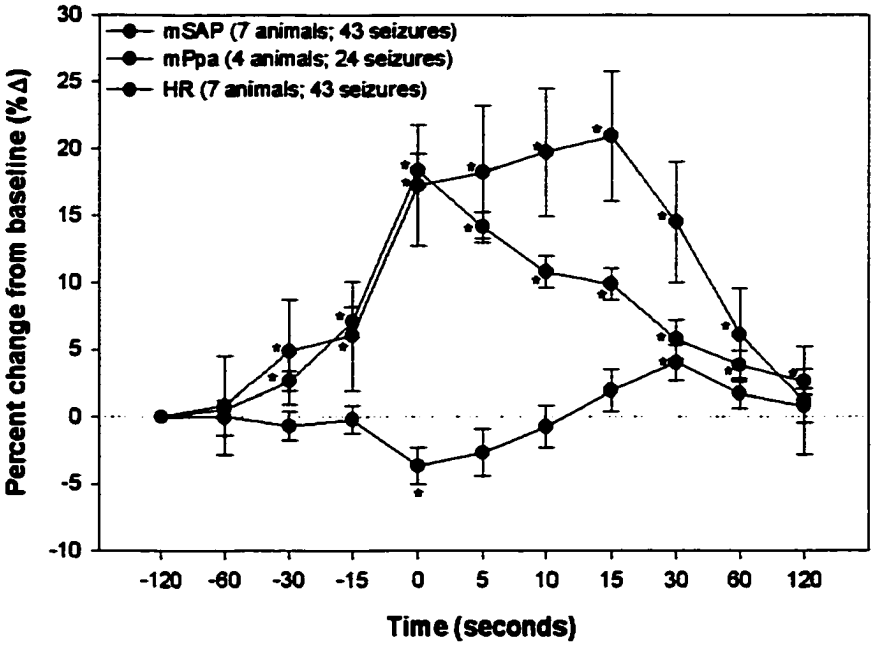

Figure III-6. mSAP, mPpa, and heart rate (HR) for motor seizures recorded in 7 KA-treated rats during the induction protocol. mSAP and mPpa increased significantly 30 seconds prior to overt motor behavior. mPpa peaked 15 seconds after peak mSAP. *significantly different from baseline (-60 sec).

Discussion

The present study demonstrates that repeated, low-dose intraperitoneal injections of KA have a dramatic impact on the cardiopulmonary system. Unexpectedly, mSAP and heart rate decreased markedly immediately following the first injection of KA. In most animals, this period of hypotension and

bradycardia was relatively long, extending well past the 2nd injection. However, as hypothesized, mSAP and heart rate increased coinciding with the start of motor seizures and continued to increase as the animal approached status epilepticus. Phentolamine significantly reduced the systemic pressor response accompanied by a significant increase in heart rate. mPpa, on the other hand, followed one of two distinct patterns. There was either an immediate increase or decrease of approximately 10 mmHg immediately following the 1st injection that, as with mSAP and heart rate, was sustained until the 1st motor seizure occurred, after which time mPpa gradually increased. Overall, phentolamine did not significantly attenuate the pulmonary pressor responses.

The initial systemic hypotension and bradycardia immediately following the 1st injection has been observed in other acute models. Lathers and Schrader (19) and Schrader and Lathers (20) have reported that autonomic dysfunction (i.e. disruption of the normal homeostatic relationship between heart rate and blood pressure) can occur with subconvulsant doses of pentylenetetrazol such that a fall in blood pressure does not provoke an increase in heart rate. They suggest that an imbalance between discharges from sympathetic and parasympathetic cardiac nerves during ictal as well as interictal activity results in the disruption or dysfunction of the autonomic system and thus may be at the root of SUD. However, over concerns associated with the non-specific nature of pentylenetetrazol, these investigators later reported that when penicillin was injected directly into the right hippocampus, mSAP did not change (21). mSAP and heart rate did not change until the first occurrence of epileptiform activity had

spread from the right to the left hippocampus at which time mSAP and heart rate both increased. This suggests the possibility of a direct vascular effect of pentylenetetrazol and therefore, could explain the responses we observed. However, it is also possible that the early systemic (i.e. mSAP and heart rate) responses observed in this study can be explained by the unique neurochemical binding properties of KA.

KA is a naturally occurring glutamate agonist that preferentially binds to limbic structures independent of the route of administration (e.g. intravenous, intraperitoneal). The pyramidal cells of the CA1, CA3 and dentate hilus regions of the hippocampus are most vulnerable to KA due to the presence of numerous high-density binding sites (15,22). Thus, experiments using focal electrical and chemical stimulation of the hippocampus offer the best speculations as to source of the initial hypotensive and bradycardiac responses observed in this model. Ruit and Neatsey reported (23) that direct electrical stimulation of the hippocampus caused a marked decrease in mSAP and heart rate, as well as respiratory depression. These responses could be attenuated by ablation of the medial frontal cortex, which projects to and is comprised, in part, of direct projections from the CA1 and subicular regions of the ventral hippocampus. Furthermore, Talman et al. (24) have shown that KA injected directly into the nucleus tractus solitarius causes systemic hypotension, bradycardia, and apnea. Thus, the initial systemic hypotension and bradycardia observed in the KA-treated animals could be the result of discharges from the nucleus tractus solitarius triggered by hippocampal activity in response to KA that, in turn, was

relayed to the nucleus tractus solitarius via the medial frontal cortex. It is also possible that the mSAP and heart rate responses could have been initiated from the amygdala once the electrical activity propagated beyond the hippocampus; however, the relationship between cardiovascular function and amygdala activity is less clear. A mild pressor response (≤ 20 mm Hg increase), a depressor response, bradycardia, and tachycardia have all been recorded during systematic electrical stimulation of the amygdala (25-27). However, given the consistency of the responses and the preferential binding of KA within the hippocampus, it is more probable that the initial systemic responses were initiated by activity within the hippocampus. Thus, studies incorporating the simultaneous monitoring of cardiovascular parameters (e.g. mSAP and heart rate) and hippocampal/amygdala activity during the initial phase of this induction protocol will be needed to fully understand this relationship.

The immediate changes in mPpa following the 1st KA injection were less consistent. In half of the KA-treated rats, a sharp increase in mPpa occurred simultaneously with a dramatic fall in mSAP and heart rate suggesting that the increase in pulmonary vascular resistance was more substantial than that reflected by the percent change in pressure alone. It is possible that the increase in mPpa could be due to hypoxia as Ruit et al. (23) and Talman et al. (24) have noted that apnea and respiratory depression occurred during electrical stimulation of the hippocampus. However, others have noted that hypoxia does not accompany KA-induced limbic status (17). Arterial blood gas measurements were not made during the initial phase of this study and are needed to completely

address the possibility of hypoxic pulmonary vasoconstriction. However, mPpa did not increase in all animals following the 1st KA injection. While this protocol reliably produces chronically epileptic rats, there is a notable degree of variability in seizure onset and frequency between the animals (11). In addition, there are anecdotal observations that during this protocol described here, some animals begin having motor seizures almost immediately while others, do not, even after several KA injections. Thus, it is possible that the variability in the immediate mPpa responses noted in this study were due to differences in susceptibility to KA. However, the mSAP and heart rate responses were consistent in both groups, thus suggesting the involvement of other variables specific to the pulmonary vasculature. While there is a great deal of information regarding systemic (i.e. mSAP and heart rate) responses during direct electrical stimulation of the amygdala, hippocampus, hypothalamus, and nucleus tractus solitarius, information regarding the interrelationship of these regions and the pulmonary vasculature is lacking. The possibility of a direct effect of KA on the pulmonary vasculature must also be considered and merits further investigation.

As expected with the onset of motor seizures, mSAP and heart increased dramatically. This is consistent with responses documented in a variety of seizure models using systemic convulsants such as pentylenetetrazol (19,20,28,29), bicuculline (30), and penicillin (21), as well as during the initial kindling acquisition (9,31) and electroconvulsive shock therapy (29). Although Lothman et al. (17) reported that "blood pressure did not fluctuate from control values even during severe limbic convulsions" following a single IV bolus (12

mg/kg) of KA. This is in stark contrast to the responses recorded here. Interictal mSAP and heart rate increased gradually from the point of the 2nd injection until the experiment was terminated. The uncoupling of the baroreceptor reflex as the experiment progressed was most likely the result a gradual increase in systemic KA. A complete override of the baroreceptor reflex (i.e. sustained systemic hypertension and tachycardia) has been reported following the administration of a single high dose of KA (24,32).

It has been shown that the increase in mSAP and heart rate accompanying generalized motor seizures are primarily sympathetically-mediated responses that can be attenuated with α-adrenergic antagonists (29) (as verified here with phentolamine), chemical sympathectomy (28), or blocked by cervical transection (29). It is well known that plasma levels of neuroendocrine mediators increase during a seizure and remain elevated even after the EEG has returned to normal (33). Thus, it is conceivable that adrenal catecholamines and/or other potential vasoactive neuroendocrine mediators could have contributed to the pressor response noted in the present study. However, mSAP following phentolamine was not significantly different from baseline suggesting that if other vasoactive mediators were involved, they did not contribute significantly to the pressor response under these conditions.

Interictal, as well as peak ictal mPpa, gradually increased as motor seizures developed; however, unlike mSAP, the responses were somewhat variable attributable to the compliant nature of the pulmonary vasculature. While mSAP and heart rate measurements have been made repeatedly in a variety of

epilepsy models, very few investigators have included measurements of pulmonary vascular pressures in models other than severe, status epilepticus models. The primary objectives of these models have focused on the mechanisms regulating neurogenic pulmonary edema and therefore, have used convulsive agents to induce a continuous period of status epilepticus in anesthetized, paralyzed, and ventilated in order to assess mPpa, lymph flow, and composition over time (34-38). There are significant differences between the acute status epilepticus models and the model discussed here; therefore, generalizations between the two are difficult to make. However, one pertinent difference between the two is the fact the pulmonary pressor response in the acute status epilepticus models could be blocked by α-antagonists and sympatholytics (39,40); whereas, in this model, phentolamine caused a slight downward shift in mPpa in most animals, although overall, the change in mPpa at the end of the procedure was negligible. It has been presumed (41,42) and demonstrated (43), that mPpa increases during generalized seizures are primarily the result of sympathetically-mediated venoconstriction and in part by systemic arterial vasoconstriction. In this study, contrary to its effect on mSAP, phentolamine had little effect of mPpa suggesting the primary involvement of vasoactive agents other than norepinephrine. The possibility of a direct effect of KA however, cannot be ruled out. Additional, systematic pharmacological studies will need to be conducted to delineate the mechanism(s) sustaining pulmonary vascular resistance under these conditions.

The purpose of this study was to determine the acute effect of multiple, systemic, low-dose injections of KA and multiple generalized motor seizures on systemic and pulmonary hemodynamics and, to evaluate the potential application of this model in the study of SUD and neurogenic pulmonary edema. The initial systemic hypotensive and bradycardiac responses have been noted in other models and were probably a consequence of the direct effect of KA on limbic structures. The systemic hypertensive and tachycardic responses recorded during overt motor seizure activity mirror those previously reported for other seizure models and are the result of widespread central, sympathetic activity. The mPpa responses recorded immediately following the injection of KA were not consistent suggesting the involvement of additional variables. The pulmonary hypertensive response noted during seizures was consistent with that previously reported in other models; however, unlike previous reports, the phentolamine did not attenuate the pulmonary pressure response again suggesting the involvement of additional mediators. Thus, the pulmonary vascular responses in this model merit further evaluation.

A great deal of information has been obtained relative to the mechanisms regulating SUD and neurogenic pulmonary edema from many different models; however, the models themselves inherently limit the applicability of the results. This is especially true for many of the acute models, like the one described here, in which it is difficult to separate the initial neurotoxic effects from true epileptogenic processes. While acute status epilepticus models do address neurogenic pulmonary edema as it might occur following massive cerebral insult

or prolonged status epilepticus, neurogenic pulmonary edema, at least in the context of SUD, is most frequently associated with non-status conditions or deaths that occur as a consequence of a single generalized seizure. Therefore, this phenomenon needs to be addressed in a model characterized by spontaneous, recurrent partial and generalized seizures. The multiple, low-dose KA model successfully meets this criterion and, along with many other characteristics, makes it an attractive model for this purpose. However, at present, additional studies need to be conducted to fully characterize the immediate direct effect of KA injections, as well as the effect of the ensuing period of multiple, generalized motor seizures, on pulmonary vascular hemodynamics.

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Chapter IV

Cardiopulmonary hemodynamics during spontaneous seizure activity in a chronically epileptic kainic acid model of temporal lobe epilepsy

Abstract

The majority of data to date regarding the cardiovascular effects of seizure activity has been collected during acute administration of chemical convulsants. In humans, the measurements that have been recorded have been obtained either during status epilepticus or after overt seizure activity has subsided and therefore, do not represent the cardiovascular impact of 'normal' spontaneous seizures experienced by thousands of individuals on a daily basis. The objective of this study was to assess specific cardiopulmonary hemodynamic parameters such as mean systemic arterial pressure (mSAP), mean arterial pulmonary pressure (mPpa), and heart rate in the kainic acid (KA) model of temporal lobe epilepsy. The specific aims were to determine: 1) if chronic, spontaneous motor seizure activity has significant long-term effects on the cardiopulmonary system under normoxic and hypoxic conditions; 2) the extent of systemic and pulmonary pressure changes during motor seizure activity in this model; and, 3) the contribution of α-adrenergic mechanisms to the hemodynamic profiles recorded during seizures. We hypothesized that mSAP, mPpa, and heart rate would: 1) increase during spontaneous seizure activity; 2) be exacerbated during acute (3

min, 10% O_2) and moderate (2 h, 12-14% O_2) hypoxic challenges; and, 3) be attenuated by the α-antagonist phentolamine in the KA-treated rats. Forty-one male Sprague Dawley rats (CON = 20, KA = 21) were anesthetized with Isoflurane and catheters were inserted into the carotid and pulmonary arteries and the jugular vein. The catheters were tunneled subcutaneously from the ventral neck to the crown of the head and exteriorized. Forty-eight hours later, cardiopulmonary function was assessed under normoxic (NX), acute hypoxic (HX, 10% O_2), and during short-term moderate hypoxic (MHX, 12-14% O_2) conditions as well as during seizures under each condition. Baseline NX hemodynamic parameters were not significantly different between CON and KA rats; however, under the physiological stress of hypoxia, significant differences between the two groups with regard to stroke volume, mPpa, and pulmonary vascular resistance were evident. With phentolamine, mPpa and pulmonary vascular resistance was exacerbated under NX, HX, and MHX conditions. With seizure onset, mSAP and mPpa increased immediately while heart rate decreased. Under HX conditions and following phentolamine, the relative change in mPpa was exacerbated and mSAP was attenuated. Overall, the disparity between the CON and KA animals in response to HX suggests some degree of autonomic or myocardial dysfunction in the KA animals. The systemic vascular responses noted in this study were mediated primarily by adrenergic activity. The exacerbation of mPpa and pulmonary vascular resistance with phentolamine suggests the involvement of other, non-adrenergic mediators such as nitric oxide and endothelin, or could be the result of a permanent, direct effect of KA.

Introduction

Individuals with epilepsy have a mortality rate 2-3 times higher than that of the general population at large (2). The cause of death for 10-15% of these individuals will never be known and are classified as sudden unexplained or unexpected deaths (SUD) (3,4). The phenomenon of sudden unexplained death in epileptics occurs most commonly in individuals between the ages of 20 and 40 years and is often attributed to cardiovascular dysfunction stemming from massive sympathetic activation (5). However, while the occurrence of fatal cardiac arrhythmias (tachyarrhythmias or bradyarrhythmias) is most frequently hypothesized as the cause of death in SUD in epileptic patients (6-9), the physiological impact of repeated, spontaneous seizure activity on the overall function of the cardiovascular system, and its relationship to SUD in epileptic patients, remains unknown.

Innumerable studies in both humans and animals have documented the cardiovascular and respiratory changes that occur during acute seizure activity. In general, the overall effect of epileptogenic activity on the cardiovascular and respiratory systems is variable and dependent upon the anatomic location of the epileptogenic focus, the synchronization and spread of electrical activity within the brain, and the duration of ictal activity (6,10-18). Systemic arterial pressure has been shown to change dramatically with seizure onset (19-21) accompanied by an increase in plasma catecholamine levels (22-24) and a wide variety of cardiac arrhythmias (5,9,25,26), the severity of which are dependent upon the origin or epileptic focus (27).

Neurogenic pulmonary edema often occurs secondary to significant neurological traumas, including epileptic seizure activity (28,29), and is often seen at autopsy in cases of SUD in epileptic patients (6,9,30). However, very little information is available regarding pulmonary vascular pressure changes during overt, spontaneous seizure activity. In humans, changes in pulmonary arterial pressure or pulmonary capillary pressure during a seizure have been inferred from radiographic evidence of edema formation (29,31) or the lack thereof (16,31). Pulmonary arterial wedge pressures have been measured in several patients following neurogenic pulmonary edema formation (32); however, in each case, wedge pressures were normal and remained normal until death, suggesting that elevated pulmonary vascular pressures are not a critical factor in sustaining edema formation once it begins.

In acute animals models, Maron et al. (33,34) and Minnear et al. (35) have shown that severe edema can develop rapidly after massive sympathetic activation, such as that experienced during a seizure. However, an extreme, transient increase in pulmonary vascular pressure (i.e. > 70 mm Hg increase) does not mean that pulmonary edema will inevitably follow and suggests that a combination of factors, such as high pulmonary capillary pressure and impaired lymphatic clearance, must be present. Simon et al. (36) in this regard have conducted the most convincing studies (19,37). These investigators have repeatedly shown that during bicuculline-induced generalized seizures there is a dramatic increase in pulmonary capillary pressures followed by an immediate rise in lymphatic flow that continues to increase more than 3 hours after seizure

cessation. The nature of the lymphatic fluid also changes over time from a low lymph-to-plasma protein ratio to a high lymph-to-plasma protein ratio (36). These data suggest that a dramatic spike in pulmonary capillary pressure not only increases the immediate transcapillary fluid flow but also damages the endothelium, thus accounting for the increase in the protein content of the lymphatic fluid over time. Furthermore, these studies suggest that it is the magnitude of the pressure spike and resultant endothelial damage that determines the severity of postictal pulmonary edema formation.

While these studies have provided a great deal of information regarding cardiopulmonary responses to massive sympathetic discharge, the relevance of most to spontaneous epileptic seizures is questionable, as the vast majority of data have been collected during acute administration of chemical convulsants to anesthetized, paralyzed, and ventilated animals. In humans, the measurements that have been recorded have been obtained either during status epilepticus or after overt seizure activity has subsided and therefore do not represent the cardiovascular impact of 'normal' spontaneous seizures experienced by thousands of individuals on a daily basis. The cardiopulmonary consequence(s) of repeated, short-lived, spontaneous motor seizure activity in a chronic model of temporal lobe epilepsy has not been documented. In addition, it is possible that chronic, repeated massive sympathetic stimulation, as occurs with each epileptic seizure, alters the responsiveness of the systemic and pulmonary vasculature over time, such that normal hemodynamic and cardiac responses to physiological challenges, such as mild hypoxia (e.g. altitude exposure), are

exacerbated. This possibility has not been examined. Therefore, the purpose of this study was three-fold: 1) to assess specific cardiopulmonary hemodynamic parameters (systemic arterial pressure (SAP), pulmonary artery pressure (Ppa), cardiac output, heart rate, stroke volume, systemic vascular resistance, and pulmonary vascular resistance), in the kainic acid (KA) model of chronic temporal lobe epilepsy, as well as in saline-treated control (CON) rats, to determine if chronic, spontaneous motor seizure activity has significant, long-term effects on the cardiopulmonary response to: (i) normoxia (NX); (ii) acute, severe hypoxia (HX, 10%); and (iii) short-term moderate hypoxia (MHX, 12-14%); 2) to document both systemic and pulmonary hemodynamic changes during overt seizure activity under NX, HX, and MHX conditions, in KA-treated rats; and 3) to assess the contribution of α-adrenergic mechanisms to the hemodynamic profiles recorded during seizure activity under baseline NX, HX, and MHX conditions in KA-treated rats. We hypothesized that: a) there will not be a significant difference in measured parameters between KA and CON rats under NX conditions; b) there will be a significant difference in measured hemodynamic parameters between KA and CON rats in response to HX and MHX; c) there will be a significant increase in SAP, Ppa, and heart rate during seizure activity that will be greatest during HX seizures; and, d) the α-adrenergic antagonist, phentolamine, will reduce the increase in SAP, Ppa, and heart rate during seizures under both NX and HX conditions.

Methods

Model: The KA-induced chronically epileptic rat model of temporal lobe epilepsy has been well-documented (38) and the induction protocol used in this study has been described elsewhere in detail (1). Briefly, male Sprague-Dawley rats (150-200 g, Harlan, Indianapolis, IN) were randomly divided into KA and CON groups. Each animal in the treatment group (KA) was given hourly injections of kainic acid (5 mg/kg, IP) while control (CON) animals received an equal volume of saline. After 3-4 injections, most of the KA-treated animals began having overt motor seizures. Motor seizure severity was scored using a modified Racine scale (39,40), with severity defined as follows: Class III, rats displayed forelimb clonus with a lordotic posture; Class IV, rats reared with simultaneous forelimb clonus; and Class V, rearing with forelimb clonus accompanied by a loss of balance. Kainate treatment continued with a steady progression in seizure severity for a minimum of 3 h. The total dose of KA for each animal varied between 20-50 mg/kg. If an animal exhibited either excessive activity (i.e. excessive running and/or jumping) or became catatonic, the subsequent injection was reduced to 2.5 mg/kg, as continued treatment with the initial dose has proven to be fatal under these circumstances. Mortality using this protocol is approximately 16% (1). All surviving rats received a subcutaneous injection of lactated Ringer's and were provided moistened rat chow for the first week following treatment.

The animals were housed in the Laboratory Animal Resource facility, an Association for the Assessment and Accreditation of Laboratory Animals Care International-approved facility, with food and water provided *ad libitum*, and were subjected to a 12-12 h light-dark cycle. Following the induction protocol, KA and matched CON animals were monitored at the Laboratory Animal Resource facility over the course of the next 5 months for a minimum of 10 hrs per week to document spontaneous seizure activity. KA-treated animals began displaying spontaneous seizure activity approximately 77 days post-induction (1). All procedures were reviewed and approved by the Animal Care and Use Committee of Colorado State University.

In vivo catheterization: Approximately 3 months after the induction protocol, KA rats and matched CON rats were chosen for the cannulation study. Previous seizure activity had been documented in most of the KA-treated rats. Forty-eight hours prior to surgery, the animals were given water supplemented with TMS and AC (0.127 mg trimethoprim, 0.634 mg sulfamethoxazole and 0.50 mg acetaminophen, 0.050 mg codeine/ml H_2O). Food and water were withheld on the morning of surgery.

The rat was placed in an induction chamber that was flooded with a mixture (Aerane, Clover Medical Equipment Services, Buffalo, NY) of gas anesthesia (IsoFlo, Abbott Laboratories, North Chicago, IL) and oxygen. Once unconscious, the rat was removed from the induction chamber and placed on a covered heating pad on the surgery table. An appropriate anesthetic plane was maintained by masking the animal and administering isoflurane at a

concentration of 1-3% with an oxygen flow rate of 200 ml/kg/min for the duration of the procedure. Fur covering the dorsal and ventral neck region was removed using a scalpel blade and the area cleansed and disinfected (Hibiclens, Zeneca Pharmaceuticals, Wilmington, DE). Immediately prior to the first incision, animals were given a 3 ml subcutaneous injection of warmed (37°C) lactated Ringers. The rat was placed in a supine position and covered with sterile drapes so that only the neck region was visible. A small, midline incision was made above the right clavicle and the right jugular vein and carotid artery were isolated. The carotid artery was ligated rostrally with 3-0 silk sutures and cannulated with a polyethylene (PE-50, 0.58mm ID) catheter. The jugular vein was also ligated rostrally with 3-0 silk sutures and cannulated with a polyvinyl catheter (PV-1, 0.28 mm ID) with a shallow bend at its tip. This catheter was guided through the jugular vein into the right ventricle and placed in main pulmonary artery while monitoring the pressure tracing on a Gilson Duograph (Model ICT-2H, Gilson Medical Electronics, Middleton, WI) recorder. The catheter was secured once the characteristic pulmonary arterial pressure tracing was consistent and stable. Following placement of the pulmonary arterial catheter, PE-50 and PV-1 straight-tipped catheters were introduced into the jugular vein and advanced toward the superior vena cava. Following placement of all catheters, the catheters were flushed with heparinized saline (1:100 vol/vol) and chloramphenicol (1 mg/ml) solution and sealed. The catheters were tunneled subcutaneously from the crown of the head and exteriorized. The catheters were coiled and placed in a plastic housing sutured to the skin and covered with a threaded plastic cap. The

rat was then given a second 3 ml subcutaneous injection of lactated Ringers and placed in a clean, warmed cage for recovery. All rats were given a post-operative injection of Banamine (2.5 mg/kg, intramuscular) and Diazepam (3mg/kg, intravenous) for analgesia and kept on water supplemented with TMS and AC for 48 hours. All instruments were autoclaved and catheters sanitized by cold sterilization (Amerse, ConvaTec, St. Louis, MO.) prior to each surgical procedure.

Hemodynamic measurements: Forty-eight hours after cannulation, the rats were transported from the LAR facility to the laboratory. The cap on the plastic housing was removed and the rat was placed in a small rectangular Plexiglas chamber. The catheters were passed through an opening at the top of the chamber, opened, and irrigated with heparinized saline. The catheters were connected to P23 Db transducers (Statham, Oxnard, CA) for measurement of systemic and pulmonary arterial pressures and heart rate. Each transducer was positioned at midthorax for all pressure measurements and was calibrated daily with a mercury manometer. The remaining catheters provided intravascular access for indocyanine green dye injection for determination of cardiac output and for intravenous administration of the α-antagonist, phentolamine (1mg/kg). An inlet at one end of the plastic chamber was connected via a flow meter to gas cylinders containing either compressed air for the normoxic (NX, 21% O_2,), acute hypoxic (HX, 10% O_2), or short-term, moderate hypoxic (MHX, 12-14% O_2) conditions. A clamp was used to control the flow from each tank to the flow meter

so that the animal was not disturbed by the change from one gas cylinder to another, as it is possible to induce seizures in the KA animals by sudden noises.

Mean systemic (mSAP) and pulmonary arterial pressures (mPpa) were calculated as $(P_s + 2*P_d)/3$. Heart rate (beats/min) was determined from either the SAP or Ppa pressure tracing. Pulmonary vascular (mmHg/ml/min) and systemic vascular resistance (mmHg/ml/min) was calculated as mean pressure (e.g. mSAP, mPpa) divided by cardiac output. Cardiac output was determined by indocyanine green dye-dilution (41). A bolus of dye (0.1 mg) was injected into a jugular vein catheter while arterial blood was simultaneously withdrawn via the carotid artery catheter by a Masterflex variable speed pump (Model 7013-21, Cole-Parmer, Chicago, IL) and passed through a densitometer cuvette (D-402A densitometer, Waters Instruments, NY). Arterial blood was returned to the rat via one of the PE-50 jugular vein catheters. The signal from the densitometer was interfaced with a dual channel recorder (Kipp-Zonen, Delft, Holland). The area under the inscribed dye-curve tracing was calculated as described by Williams et al. (42): Cardiac output (ml/min) = (amt of dye injected (mg) * paper speed (cm/min)) / (area (cm^2) * calibration factor (mg/L·cm)). The reported cardiac output measurements are an average of 3 dye curves per animal for each condition. Cardiac index, defined as cardiac output divided by body weight (kg), was used to normalize cardiac output measurements by weight for comparison. Arterial blood samples (0.2 ml) were withdrawn via the carotid catheter immediately following the cardiac output measurements and analyzed for arterial

PO_2 (P_aO_2), PCO_2 (P_aCO2), and pH (Radiometer, ABL 300, Copenhagen, Sweden).

General protocol: The rats were transported in covered cages from Laboratory Animal Resource facility to the laboratory at least 30 min prior to the start of the study. The rats were then allowed to acclimate for an additional 30 min in the Plexiglas chamber prior to the initial baseline measurements. Following this acclimation period, the initial 2 hr NX period was begun. A 5 min acute hypoxic challenge (10% O_2) was imposed at the beginning of each hour of the NX protocol. mSAP, mPpa, and heart rate values immediately prior to each HX challenge were averaged and used as NX baseline measurements. Cardiac output measurements were made during the 2 h NX period at 30 and 90 min into the protocol, as well as during the acute HX challenges, and then averaged. Pressure tracings were recorded continuously.

At the end of the 2 h NX period, the animals were exposed to 12-14% O_2 (MHX) for 2 h. Acute HX challenges were not done during MHX exposure. mSAP, mPpa, heart rate, and cardiac output measurements were made at the same time points described above. Following the 2 h MHX period, the α-adrenergic antagonist phentolamine (1 mg/kg) was administered via a jugular vein catheter and the entire protocol was repeated. An attempt was made to study the animals on two consecutive days; however, due to the aggressive nature of the KA animals, it was very difficult to re-secure the catheters for the next day. Therefore, the decision was made to complete both protocols on the same day.

Cardiopulmonary measurements during seizure activity: mSAP and mPpa were measured continuously in an attempt to precisely record pressure and heart rate changes during overt seizure activity. In order to construct a 'time profile' of the hemodynamic changes that occurred during a seizure, the first sign of aberrant motor activity was noted and the SAP and Ppa at this time were defined as time zero (0) and used as the reference point from which all subsequent time points were compared. The final pressure profile encompassed a time period extending from 2 min prior (-120 sec) to 2 minutes after (120 sec) the reference point. All statistical comparisons for each time point were made with reference to baseline (-60 sec) pressures. Due to the limited number of seizures recorded during the protocol described above, seizures occurring under HX and MHX conditions were pooled for analysis.

Histology: The rats were euthanized with an overdose of sodium pentobarbital (100 mg/kg, iv) administered slowly via a jugular vein catheter. The trachea was isolated and cannulated for subsequent intratracheal formalin instillation. The chest cavity was opened, retracted, and a bolus of heparin (0.3 ml, 1000 U/ml) was injected directly into the right ventricle. A small incision was made in the apex of the left ventricle followed by the cannulation of the main pulmonary artery. Approximately 10-15 ml of heparinized saline was slowly flushed through the pulmonary artery followed by buffered formalin (10%) until partial fixation was apparent. The heart/lung block was then excised and formalin slowly instilled into the trachea until the lungs were inflated. The trachea

was then ligated with 3-0 silk and the lung was submerged in a specimen cup filled with 10% formalin.

Following a minimum of 24 h in fixative, the heart-lung block was removed from the formalin solution and prepared for paraffin embedding and sectioning. For the lungs, the lower 2-3 mm of the right lung was cut away and discarded. A central section (~3 mm thick) was then obtained from the remaining mid-lung portion, placed in a cassette, and returned to 10% formalin solution. A central section of the heart was taken in a similar manner. The initial cut was made approximately 2-3 mm above the apex. A central section was then obtained, placed in a cassette, and the remaining specimen returned to formalin solution. The heart and lungs from a second group of CON and KA animals that did not undergo the cannulation procedure were also fixed and prepared for analysis to remove potential confounding variables (i.e. acute inflammatory changes) that could be related to catheter placement. These tissues were fixed by whole body perfusion with 4% paraformaldehyde. All sections were taken to the Histopathology Laboratory in the Department of Pathology at Colorado State University for paraffin embedding, sectioning, and hematoxylin-eosin staining. A Board-certified pathologist evaluated all slides.

Statistical analysis: A 2-factor repeated measures analysis of variance was used to assess changes in the hemodynamic parameters between and within the KA and CON groups for all treatment conditions (NX, HX, MHX), with and without phentolamine. For the purpose of comparing relative changes in the hemodynamic variables of interest between NX, HX, and MHX, a subgroup of

both KA and CON animals were selected based on the availability of complete or paired data. Relative changes in any of the hemodynamic parameters, expressed as a percent change from baseline measurements made immediately prior to the challenge, are reported from subgroups or paired data only. For the statistical analysis of mSAP, mPpa, and heart rate responses during seizure activity, a repeated measures analysis of variance with a contrast variable (-60 sec) was used to compare the value recorded at each time point against the initial baseline. Where appropriate, significance was assessed by *post hoc* least square difference pairwise comparisons. The data presented in tabular and graphic form represent raw (i.e. non-transformed) means ± standard error of the mean. A P value less than 0.05 ($p < 0.05$) was accepted as significant. For the MHX challenge, a subgroup consisting of only the animals with both NX and MHX measurements was used to assess the effect of MHX as well as the relative changes in hemodynamic parameters. For all groups, the number of data points for each measurement is displayed on the bars of each graph. The number of data points from parameter to parameter vary since not all measurements could be made on each animal due to technical problems inherent with *in vivo* cannulation experiments. Due to the length of the protocol and the serial design, there is often a progressive decrease in the number of measurement across time due to the loss of catheter patency, catheter placement, and in the case if the KA rats, the complete loss of the catheter following severe seizures.

Results

A total of 41 male Sprague-Dawley rats (21 KA, age 11.9 ± 0.8 mo, wt. 519 ± 14.7 g; 20 CON, age 15.9 ± 1.5 mo, wt. 562 ± 6.0 g) were cannulated and studied.

NX and acute HX challenges: Baseline NX values for mSAP, mPpa, heart rate, cardiac index, systemic vascular resistance, and pulmonary vascular resistance were not significantly different between CON and KA rats (Tables IV-1 and IV-2). However, cardiac output was significantly lower in the KA group as compared to the CON group under both NX and HX conditions. In response to acute HX, mSAP was unchanged in both groups while mPpa was significantly higher than the NX baseline measurement. The mPpa response of the KA rats was significantly greater than that of the CON animals (Figure IV-1). Heart rate was significantly higher in both groups in response to acute HX with the heart rate response for KA rats significantly different from that recorded in the CON rats.

Table IV-1. mSAP and mPpa for CON and KA groups during NX and HX.

	mPpa (mmHg)
NX	15±1 (16)
HX	21±2 (15)*
NX	15±1 (18)
HX	25±2 (18)*‡

*significantly different from comparable NX
‡ significantly different from comparable CON

Table IV-2. Cardiac output (CO), stroke volume (SV), and heart rate (HR) for CON and KA groups during NX and HX.

	SV (mL/beat)
NX	0.38 ± 0.02 (13)
HX	0.40 ± 0.03 (7)
NX	0.33 ‡ 0.02 (16)
HX	0.32 ± 0.02 (11) ‡

*significantly different from comparable NX
‡ significantly different from comparable CON

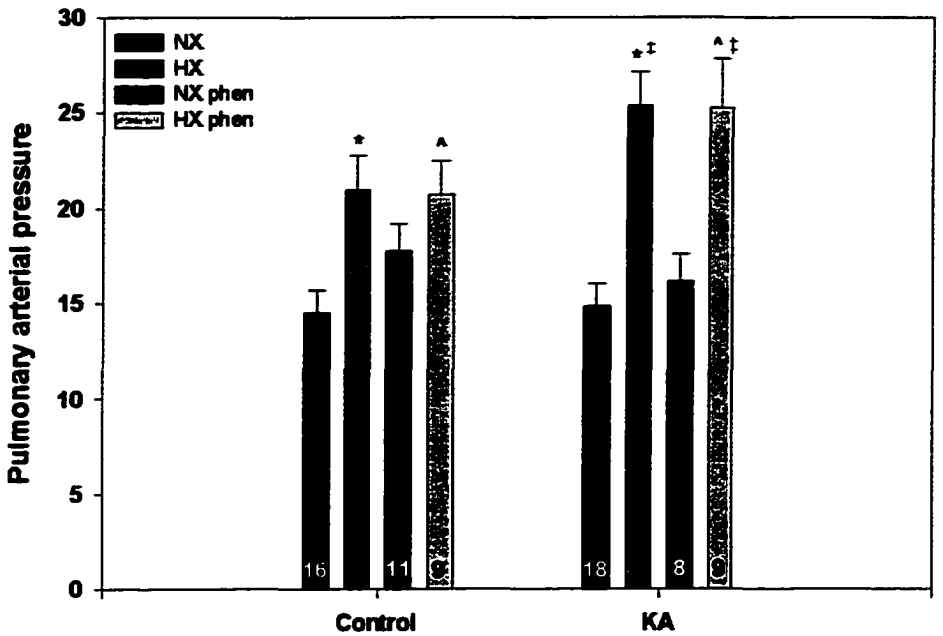


Figure IV-1. Changes in mPpa during acute HX challenge with and without phentolamine. KA animals have a greater pressor HX response to HX than CON.*significantly different from NX baseline; ‡signficantly different from control; ^ significantly different from NX phen baseline. phen = phentolamine. $p < 0.05$.

In response to acute HX, cardiac output was significantly higher than baseline in the CON group and, while cardiac output also increased slightly in the KA group, it remained significantly lower than that of the CON animals (Table IV-2). Stroke volume for the CON animals increased slightly in response to acute HX while stroke volume for KA animals decreased slightly, becoming significantly

lower than that of the CON rats (Figure IV-2). In contrast to cardiac output, cardiac index was significantly higher in both groups in response to acute HX and there were no differences between the two groups (Table IV-3). Baseline NX systemic vascular resistance was higher in the KA group than in the CON group; however, the difference was not significant (Table IV-3). In response to HX, systemic vascular resistance decreased in both groups; however, overall, systemic vascular resistance for the KA group remained higher than that recorded in the CON group under both NX and HX conditions. Pulmonary vascular resistance was significantly higher in both groups in response to the acute HX challenge, with the response of the KA animals significantly higher than that of the CON animals (Figure IV-3).

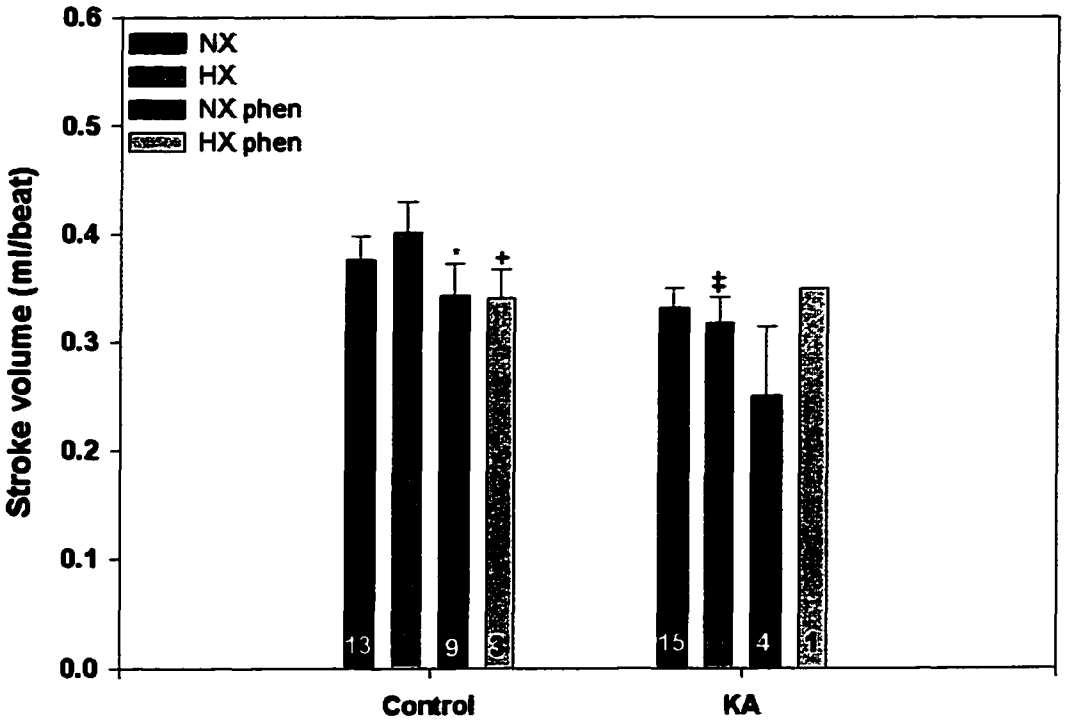


Figure IV- 2. Changes in stroke volume (SV) during acute HX challenge with and without phentolamine. SV increased in CON group and decreased in KA group in response to acute HX. SV fell significantly in the CON group following phentolamine. *significantly different from NX baseline; ‡ signficantly different from CON; +significantly different from acute HX baseline. phen = phentolmaine. $p<0.05$.

Table IV-3. Cardiac index (CI) and systemic vascular resistance (SVR) for CON and KA groups during NX and HX.

	SVR (mm Hg · min/ml)
NX	0.85 ± 0.06 (13)
HX	0.71 ± 0.03 (7)
NX	1.00 ± 0.07 (14)
HX	0.86 ± 0.05 (11)

*significantly different from comparable NX

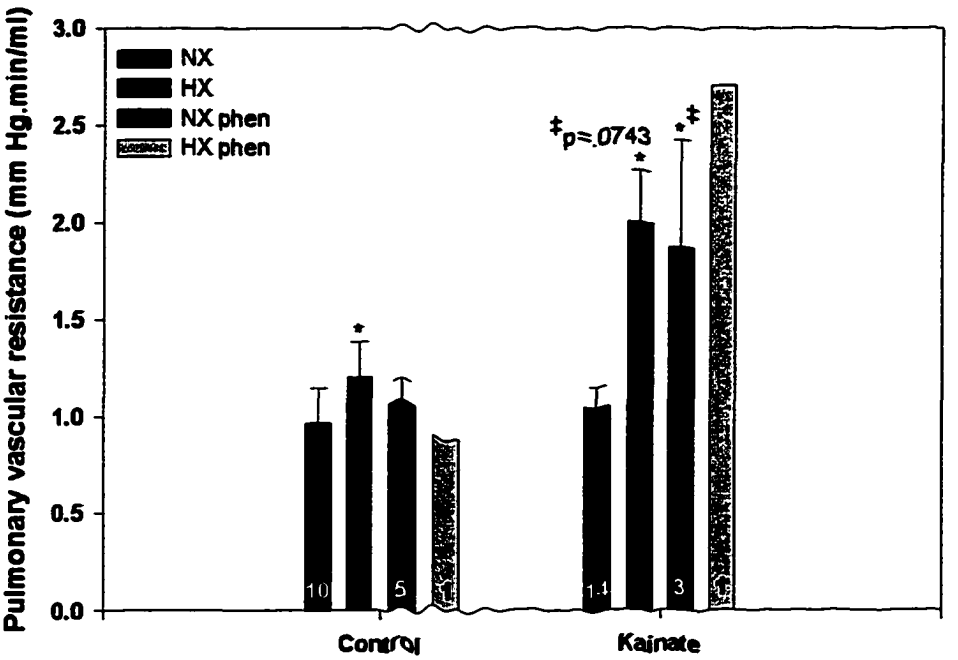


Figure IV- 3. Changes in pulmonary vascular resistance during acute HX with and without phentolamine. Pulmonary vascular resistance in the KA group was significantly greater than that of the CON group under all conditions. * significantly different from NX baseline; ‡ significantly different from CON; ^ significantly different from NX phen baseline; + significantly different from acute HX baseline. phen = phentolamine. $p < 0.05$.

A summary of the hemodynamic responses of both CON and KA animals to the acute HX challenge is presented in Figure IV-4. Systemic vascular resistance decreased and cardiac index increased in the CON group during

acute HX as stroke volume and heart rate increased. In the KA rats, cardiac index and systemic vascular resistance increased slightly; however, stroke volume fell and was significantly lower than that of the CON group. Heart rate also increased in the KA group during the acute HX challenge and was higher than that of the CON group. Pulmonary vascular resistance increased in both groups; however, the KA response was significantly greater than that of the CON group.

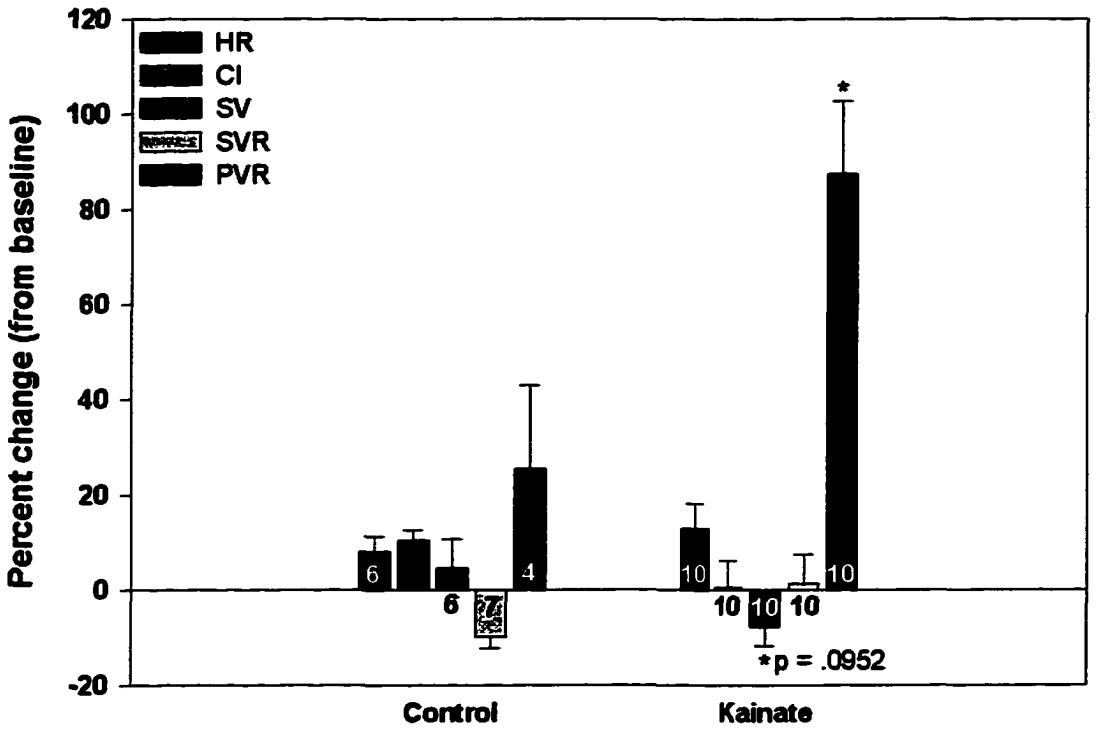


Figure IV-4 Summary graph of CON and KA hemodynamic responses to acute HX challenge. SVP = systemic vascular resistance. PVR = Pulmonary vascular resistance. *significantly different from CON. $p < 0.05$.

NX and acute HX responses with phentolamine: For both KA and CON animals, NX mSAP and systemic vascular resistance with phentolamine were significantly

lower than that recorded under baseline NX, while heart rate was significantly higher (Tables IV-4 and IV-5). Baseline NX mPpa in both groups increased slightly with phentolamine; however, the changes were not significant (Table IV-4 and Figure IV-1). In response to phentolamine, pulmonary vascular resistance fell slightly in the CON group but was significantly higher in the KA group (Figure IV-3). Stroke volume was significantly lower for both groups during the NX period with phentolamine; however, while cardiac output increased slightly in the CON group and fell in the KA group (Table IV-5 and Figure IV-2), they were not significantly different. Cardiac index values are reported in Table IV-6 for comparison.

Table IV-4. mSAP, mPpa, and heart rate (HR) for CON and KA groups during NX, NX with phentolamine, and HX with phentolamine.

	mPpa (mmHg)
NX	15±1(16)
NX phen	18±2(11)
HX phen	24±2(9)^
NX	15±1(16)
NX phen	16±1(8)
HX phen	25±3(9)^‡

^significantly different from comparable NX
*significantly different from comparable NX phen
‡ significantly different from CON

Table IV-5. Cardiac output (CO), stroke volume (SV), and heart rate (HR) for CON and KA groups during NX, NX with phentolamine, and HX with phentolamine.

	SV (ml/beat)
NX	0.36 ± 0.02 (13)
NX phen	0.34 ± 0.03 (9) ^
HX phen	0.34 ± 0.03 (3)
NX	0.33 ± 0.02 (16)
NX phen	0.25 ± 0.06 (4) ^
HX phen	0.35 (1)

^significantly different from comparable NX
*significantly different from comparable NX phen
‡ significantly different from comparable CON

Table IV-6. Cardiac index (CI) and systemic vascular resistance (SVR) for CON and KA groups during NX, NX with phentolamine, and HX with phentolamine.

	SVR (mm Hg · min/ml)
NX	0.88 ± 0.06 (13) ‡
NX phen	0.49 ± 0.04 (9) ^*
HX phen	0.56 ± 0.07 (3)
NX	1.01 ± 0.70 (14)
NX phen	0.90 ± 0.19 (4) ^
HX phen	0.51 (1) *

^significantly different from comparable NX

In response to the acute HX challenge with phentolamine, mSAP and heart rate in both groups was unchanged from NX baseline (Table IV-4). mPpa increased significantly in both groups; however, the response of the KA animals was significantly greater than that recorded in the CON group (Figure IV-1 and Table IV-4). In the CON group, heart rate, cardiac output, stroke volume, cardiac index, and systemic vascular resistance did not change significantly during the

acute HX challenge (Tables IV-4 thru IV-6). For the KA group, heart rate, cardiac output, stroke volume, cardiac index, and systemic vascular resistance data is incomplete and therefore cannot be compared. Statistical comparisons also could not be made for the pulmonary vascular resistance parameter for either group.

Arterial blood gas measurements: At rest, KA animals had a significantly lower P_aO_2 and slightly higher P_aCO_2 than CON rats (Table IV-7). Blood pH was not significantly different between the two groups. As expected, P_aO_2 levels fell significantly for both groups in response to acute HX; however, the P_aO_2 for KA rats was significantly lower than that of CON rats. P_aCO_2 levels were significantly lower in both groups; however, the changes were not significant. Blood pH increased slightly for both groups during the HX challenge.

Table IV-7. Arterial blood gas values for CON and KA groups during NX and HX.

	P_aCO_2 (mm Hg)
NX	28.3 ± 1.2 (16)
NX phen	27.2 ± 1.8 (11)
HX	20.3 ± 1.2 (6)*
HX phen	27.6 (1)
NX	30.7 ± 2.7 (10)
NX phen	26.2 ± 1.6 (7)
HX	20.2 ± 1.3 (5)*
HX phen	23.6 ± 1.4 (2) (0.0699)

*significantly different from comparable NX
‡ significantly different from CON
^ significantly different from comparable HX
# significantly different from comparable NX phen

NX and responses to short-term moderate HX (MHX) challenge: The cardiovascular responses of CON and KA rats during the 2 h exposure to MHX mimicked those recorded during the acute HX exposure. In response to MHX, mSAP and heart rate were not different from baseline NX levels in the CON group (Table IV-8). In the KA group, mSAP was unchanged while heart rate was significantly higher (Table IV-8). In response to MHX, mPpa increased significantly in both groups but were not significantly different (Table IV-8 and Figure IV-5). Pulmonary vascular resistance increased significantly in both groups; however, unlike the acute HX challenge, the responses were not significantly different between the 2 groups (Figures IV-5 and IV-6).

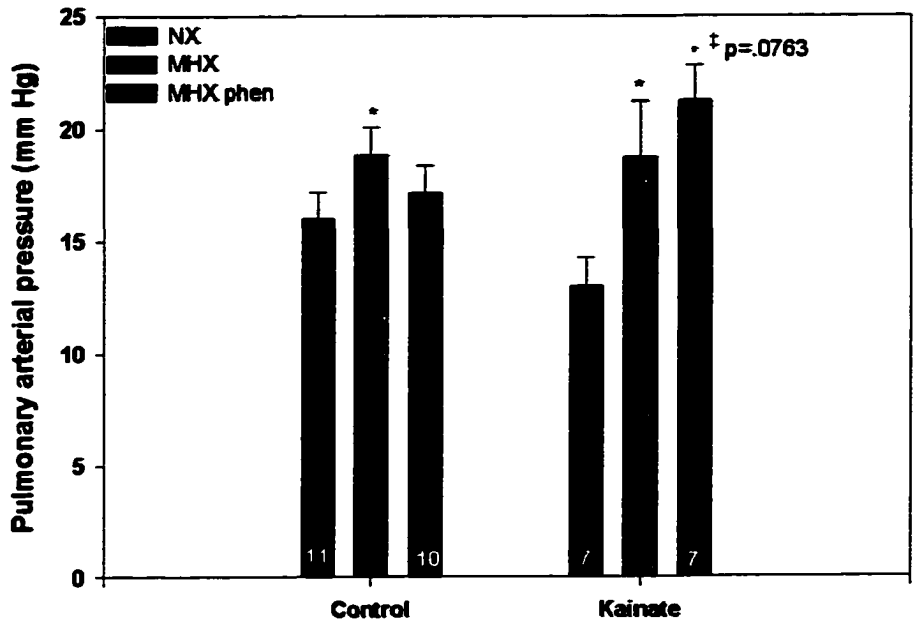


Figure IV-5. Changes in mPpa during MHX challenge with and without phentolamine. mPpa for both CON and KA groups increased significantly during the MHX challenge. *significantly different from NX; + significantly different from MHX; ‡ significantly different from CON. phen = phentolamine. $p < 0.05$.

Table IV-8. mSAP, mPpa, and heart rate (HR) for CON and KA subgroups during NX, MHX, and MHX with phentolamine.

	mPpa* (mmHg)	HR
NX	16±1 (11)	
MHX	19±1 (10)*	
MHX-phen	17±1 (10)*	
NX	13±1 (7)	
MHX	19±3 (7)*	
MHX-phen	21±2 (7)*	

* significantly different from comparable NX
+ significantly different from comparable MHX
‡ significantly different from comparable CON

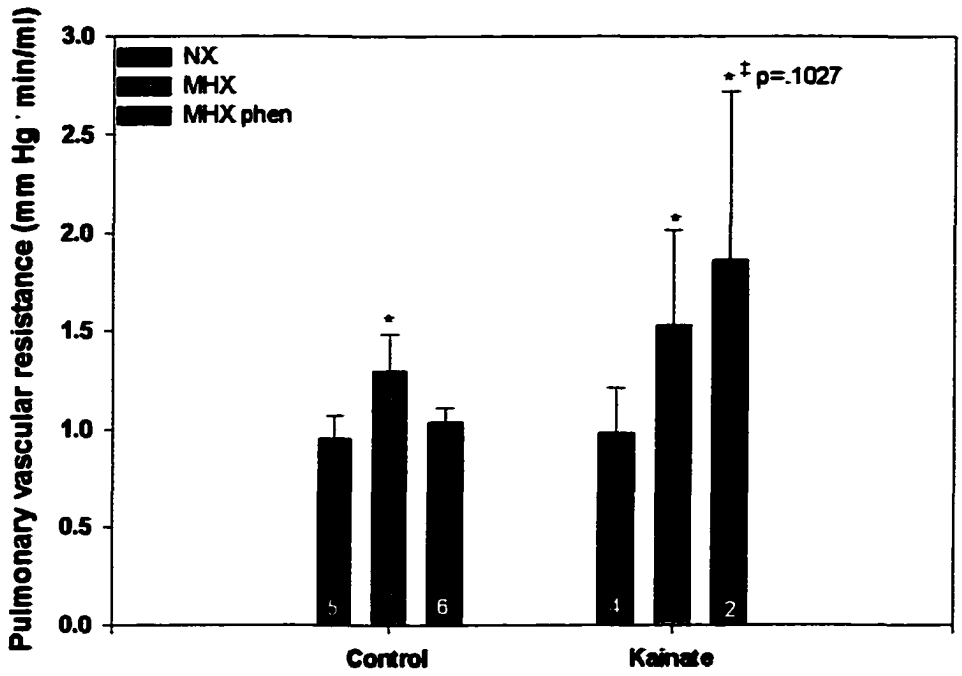


Figure IV-6. Changes in pulmonary vascular resistance (PVR) during MHX with and without phentolamine. PVR increased significantly in CON and KA groups during MHX. With phen, PVR decreased in the CON group and increased in the KA group. *significantly different from NX; $^{+}$ significantly different from MHX; ‡signficantly different from control. phen = phentolamine. $p < 0.05$.

Cardiac output and stroke volume were significantly lower than that of the CON animals (Table IV-9). After 2 h of MHX, cardiac output, cardiac index, and stroke volume were significantly lower in the CON subgroup compared to baseline NX (Table IV-9 and Figure IV-7). In the KA group during MHX, stroke volume also fell significantly from NX baseline and was significantly lower than that of the CON group (Tables IV-9 and IV-10, Figure IV-7). cardiac output and cardiac index for the KA rats were unchanged; however, cardiac output remained significantly lower than that observed in the CON group (Tables IV-9 and IV-10). systemic vascular resistance in the KA animals was significantly higher than that recorded for the CON group under both conditions (Table IV-10).

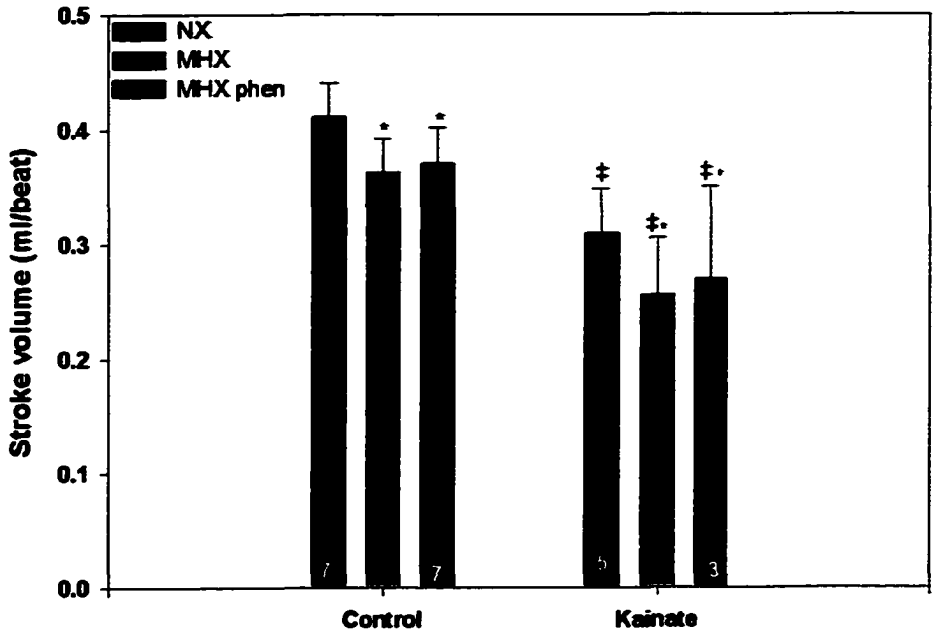


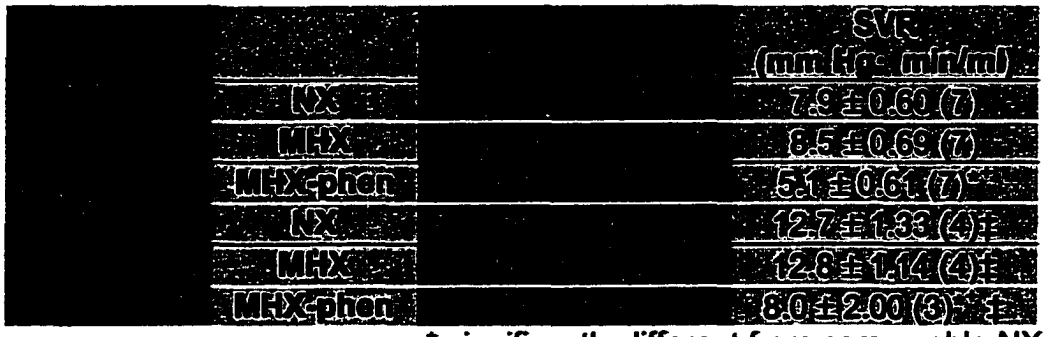
Figure IV-7. Changes in stroke volume during MHX challenge with and without phentolamine. Stroke volume decreased significantly in both CON and KA during MHX. Stroke volume in the KA group was significantly lower than CON. Subgroup statistical comparisons are made with matched data. *significantly different from NX; +significantly different from MHX; ‡ signficantly different from CON. phen = phentolamine. $p < 0.05$.

Table IV-9. Cardiac output (CO), stroke volume (SV), and heart rate (HR) for CON and KA subgroups during NX, MHX, and MHX with phentolamine.

	CO (ml/min/kg)	SV (ml/beat)	HR (beats/min)
NX		0.411 ± 0.03 (7)	
MHX		0.363 ± 0.03 (7)*	
MHX-phen		0.370 ± 0.03 (7)*	
NX		0.310 ± 0.03 (5)‡	
MHX		0.257 ± 0.05 (4)‡	
MHX-phen		0.270 ± 0.08 (3)‡	

* significantly different from comparable NX
\+ significantly different from comparable MHX
‡ significantly different from comparable CON

Table IV-10. Cardiac index (CI) and systemic vascular resistance (SVR) for CON and KA subgroups during NX, MHX, and MHX with phentolamine.



	SVR (mm Hg · min/ml)
NX	7.9 ± 0.60 (7)
MHX	8.5 ± 0.69 (7)
MHX-phen	5.1 ± 0.61 (7)*
NX	12.7 ± 1.33 (4)‡
MHX	12.8 ± 1.14 (4)‡
MHX-phen	8.0 ± 2.00 (3)*‡

* significantly different from comparable NX
\+ significantly different from comparable MHX
‡ significantly different from comparable CON

MHX responses with phentolamine: With phentolamine, mSAP and systemic vascular resistance decreased significantly in both CON and KA subgroups; however, in the KA rats, while systemic vascular resistance fell significantly, it remained significantly higher than in the CON rats (Tables IV- 8 and IV-10). While heart rate increased in both subgroups, only the CON rats had a significantly higher heart rate than that recorded during MHX (Table IV-8). With phentolamine, mPpa and pulmonary vascular resistance increased significantly in the KA subgroup; however, mPpa and pulmonary vascular resistance in the CON subgroup returned to baseline NX values (Figures IV-5 and IV-6). This increase should be interpreted with caution as the sample size for the KA group was very small and with a considerable amount of variability. cardiac output increased significantly in both subgroups while with phentolamine, stroke volume remained unchanged in the CON animals and was significantly higher than that of the KA animals (Table IV-9 and Figure IV-6).

Arterial blood gas measurements during MHX: The disparity in P_aO_2 between the two groups observed during the initial baseline measurements, as well as during the acute HX challenge, were absent during MHX. With phentolamine, P_aO_2 increased slightly in the CON subgroup and increased significantly in the KA subgroup. There were no differences in P_aCO_2 or pH between the two subgroups during either the MHX challenge or with the addition of phentolamine.

Table IV-11. Arterial blood gas values for CON and KA subgroups during MHX and MHX with phentolamine.

	P_aCO_2 (mmHg)
MHX	22.5±1.3 (11)
MHX phen	21.7±0.8 (11)
MHX	22.4±1.8 (3)
MHX phen	19.5±1.6 (3)

*significantly different from CON

A summary of the hemodynamic responses to MHX relative to NX baseline conditions is presented in Figure IV-8. Overall, systemic vascular resistance increased while cardiac index and stroke volume fell in both groups in response to MHX. Heart rate increased for both groups; however, the increase recorded in the KA group was significantly higher than that of the CON group. Pulmonary vascular resistance increased in each subgroup but was not significantly different between the two subgroups.

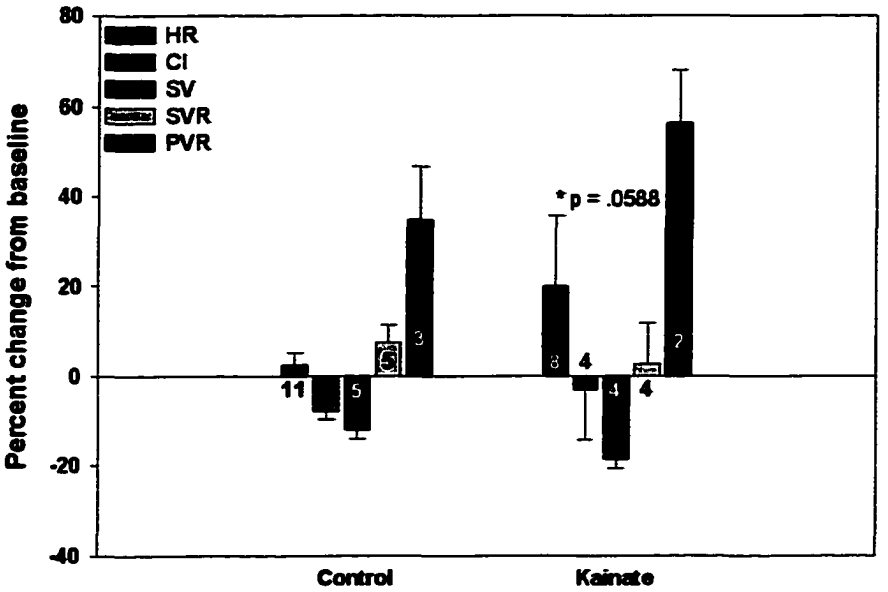


Figure IV-8. Summary graph of CON and KA hemodynamic responses to MHX. SVR = systemic vascular resistance. PVR = pulmonary vascular resistance. * significantly different from CON. $p < 0.05$.

Seizure activity: Of the 21 KA animals studied, all had at least 1 observed seizure recorded at the LAR facility. Nine of these animals (43%) had a seizure(s) at some point during the protocol described above (Table IV-11). There was no relationship between the SF recorded at LAR and the occurrence of seizure(s) during the study. The animal(s) with the highest SF at LAR did not necessarily have a seizure(s) when studied.

Table IV-12. Seizure distribution across conditions.

Condition	# seizures	# animals
NX	18	7
HX	6	3
MHX	5	2
NX + phen	2	1
HX + phen	2	1
MHX + phen	2	2

Cardiopulmonary hemodynamic response profile during motor seizure activity:

The impact of generalized motor seizure activity on the hemodynamic parameters mSAP, mPpa, and heart rate, under NX conditions, are illustrated in Fig. IV-9. At seizure onset, mSAP increased significantly and was accompanied by a significant decrease in heart rate. The effect of ictal activity on mPpa was variable, peaking roughly at the same time as systemic pressure. However, unlike mSAP, mPpa remained elevated as mSAP returned to baseline.

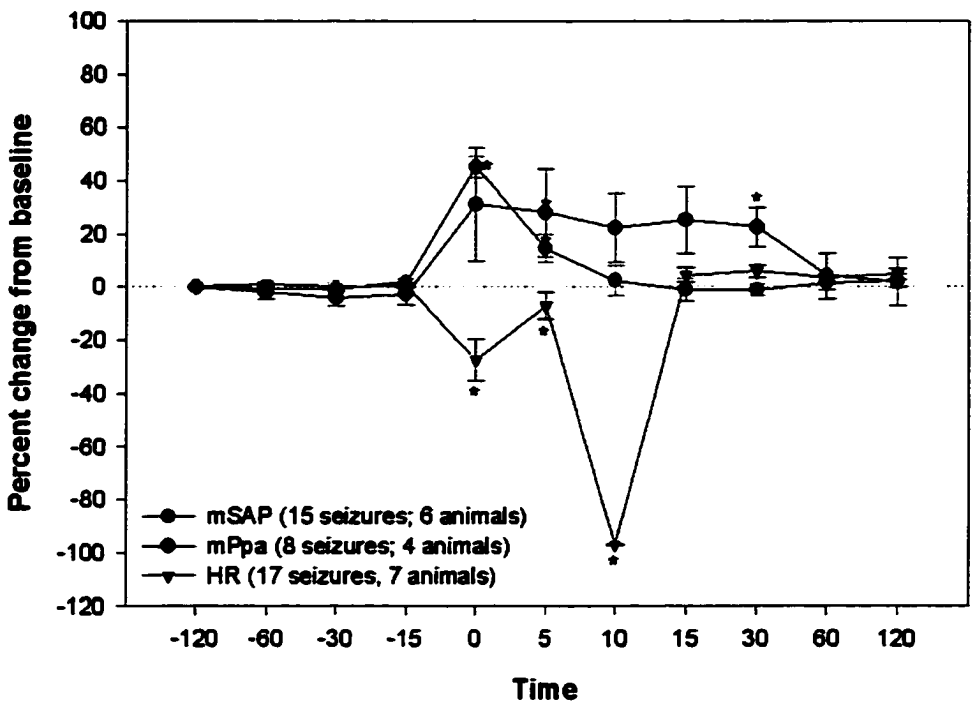


Figure IV-8. Hemodynamic profile for seizures occurring under NX conditions. *significantly different rom baseline. $p < 0.05$.

Under HX conditions, the same trend for mSAP and heart rate were evident; however, the peak mSAP was somewhat blunted relative to the response noted under NX conditions, becoming significantly lower than baseline 30 sec after the peak pressure response (Figure IV-10). Under HX, mPpa

increased significantly 30 sec prior to any visible motor signs of seizure activity. From this point, mPpa continued to increase, falling immediately after seizure onset and then rebounding, becoming significantly higher than baseline within 10 sec after the peak pressure response and remained significantly higher than baseline until 1 min after the peak pressure response.

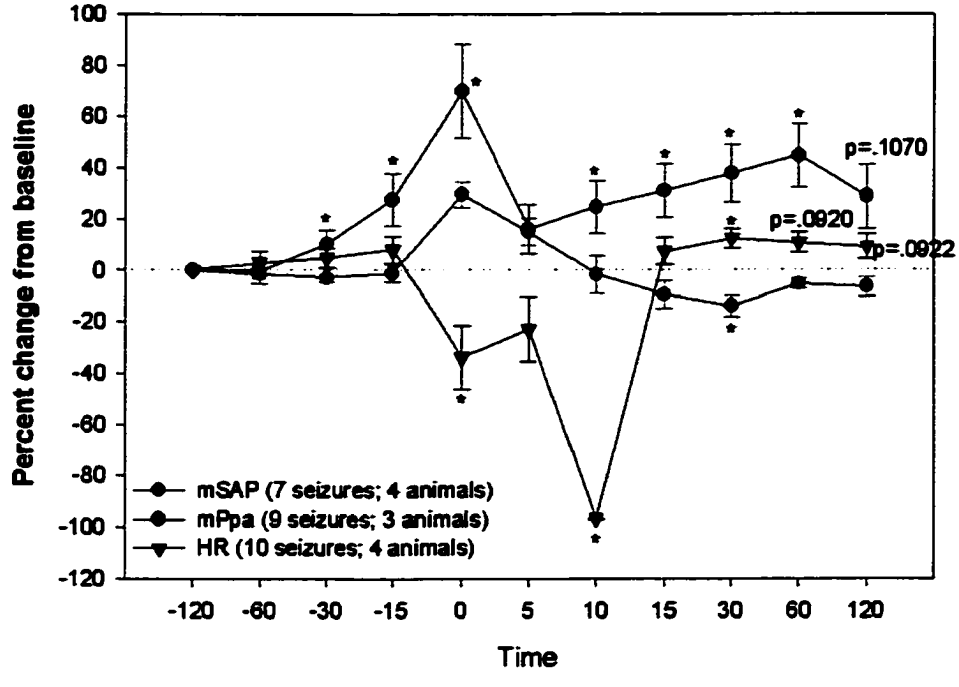


Figure IV-10. Hemodynamic profile for seizures occurring under HX conditions. *significantly different from baseline. $p < 0.05$.

A limited number of seizures were recorded under NX and HX conditions following phentolamine administration; therefore, statistical comparisons were not possible. Under NX conditions, data for mSAP with phentolamine were not available; however, the peak mPpa response exceeded that recorded under NX

and acute HX without phentolamine and remained elevated 2 min after the peak response (Figure IV-11). The heart rate response followed the same pattern as that observed during seizures under other conditions.

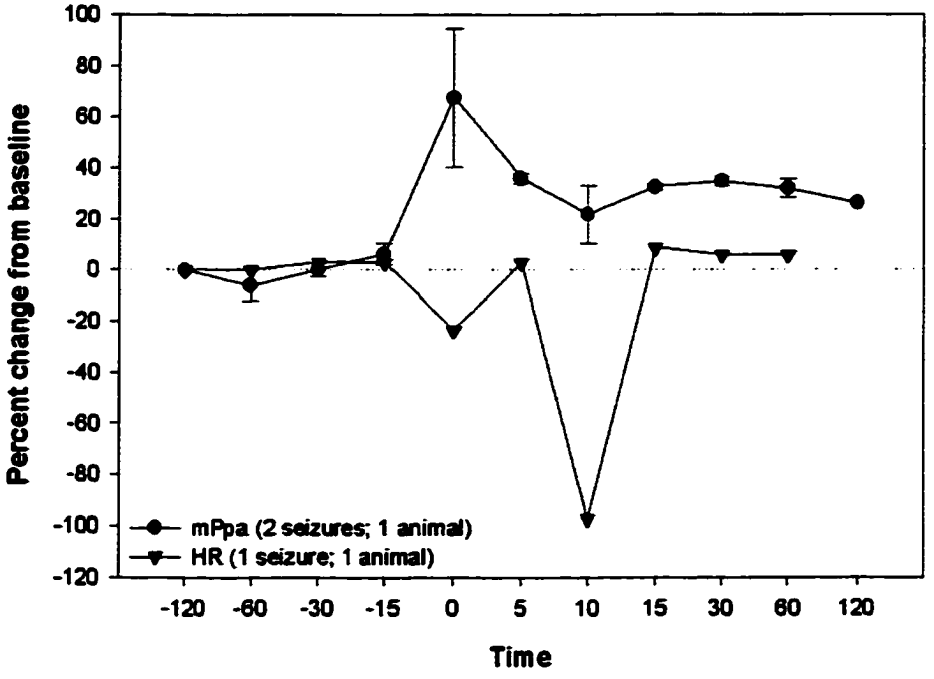


Figure IV-11. Hemodynamic profile for seizures occurring under NX conditions following phentolamine. Sample size was too small for statistical comparison.

Under HX, mSAP increased slightly as motor involvement became apparent; however, the large increase in mSAP was absent and, compared to NX seizures, remained attenuated throughout the 2 min period (Figure IV-12). The increase in mPpa was greater under HX conditions with phentolamine than that recorded under any other condition. The initial bradycardic response noted under all other conditions was absent; however, heart rate fell significantly approximately 10 sec after the peak mPpa response. The seizure profiles

recorded following phentolamine must be interpreted cautiously as the sample size was fairly small with regard to all of the variables. Other seizure profiles, as well as comparisons between NX and HX responses for each parameter, and seizure profiles by seizure class can be found in Appendix B.

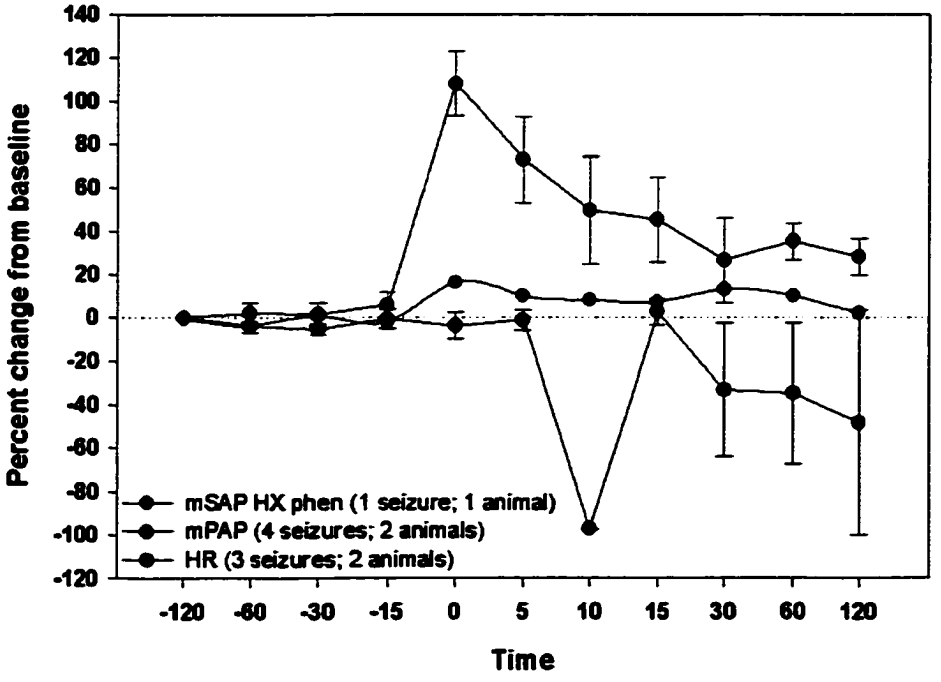


Fig. IV-12. Hemodynamic profile for seizures occurring under HX conditions following phentolamine. Sample size was too small for statistical comparison.

Histology: There were no significant pathological findings in either the heart or lungs of either the cannulated or non-cannulated KA-treated and CON animals that could be distinguished from normal pathological changes associated with heart and lung tissues of older rats.

Discussion

The primary findings of this study are three-fold. First, under NX resting conditions, there were no significant differences between CON and KA rats for any of the hemodynamic parameters with the exception of cardiac output. However, under the physiological stress of hypoxia, significant differences between the two groups became readily apparent, especially with regard to stroke volume, mPpa, and pulmonary vascular resistance. Second, mSAP and mPpa increased concurrently with overt seizure activity and were accompanied by profound bradycardia, and, the relative change in mPpa was exacerbated and mSAP was attenuated during the seizures occurring under HX conditions. Third, mPpa was exacerbated by α-adrenergic blockade under NX and HX conditions, and during seizures occurring under these conditions.

The most significant hemodynamic disparity between the two groups at rest was found in the stroke volume parameter. While cardiac output at rest was not different between the two groups, stroke volume was significantly lower in the KA-treated rats. When moderately stressed, the KA animals relied solely upon an increase in heart rate to sustain cardiac output (or cardiac index). During postmortem examination of many SUD victims, it is not uncommon to find significant myocardial damage (e.g. myofibrillar degeneration and diffuse necrotic areas) in the absence of coronary artery disease (6,43). It has also been shown that repeated, massive sympathetic activation and exposure to excessive levels of catecholamines impairs ventricular contractility for hours following the insult as a direct result of excessive activation of α- and β-adrenergic receptors (44-46).

Although we did not measure plasma catecholamines, it is well documented that the plasma concentration of both norepinephrine and epinephrine increase significantly during seizures (23). Thus, the repeated exposure of these KA animals to extreme levels of catecholamines, both during the initial induction period and later, as spontaneous seizures developed, could have damaged the myocardium. This, perhaps, was reflected in this study as the inability of the KA animals to maintain an adequate stroke volume especially under the stress of hypoxia where right ventricular afterload increased suddenly. However, postmortem analysis of the hearts from both CON and KA groups was unremarkable. Thus, there was no anatomical evidence of myocardial impairment, although the decrease in stroke volume due to a stress-induced arrhythmia would not be evident upon histological examination.

It is also possible that rather than an inability to increase or maintain stroke volume, an imbalance existed between sympathetic and parasympathetic input to the heart, thereby prohibiting the normal sympathetically-mediated increase in stroke volume. It is well known that autonomic dysfunction occurs in association with ictal events, even with minimal epileptogenic activity (6,11). It is not known if these relationships extend into the 'normal' interictal period, or what the impact of this imbalance is over time. Trimarchi et al. (48) have shown that genetically epilepsy-prone rats have an increased responsiveness to central cholinergic stimulation as the result of enhanced central pre-synaptic activity at synapses that participate in cardiovascular regulation. In addition, several clinical studies have indicated that autonomic control of the heart in epilepsy patients is

altered and manifests as a decrease in sympathetic tone (49) and impaired CV response to certain stimuli (2). However, it is not known if these are inherent central changes secondary to the epileptic condition or a side-effect of the medications used to control the seizures (2,50). In addition, Karson et al. (51) have shown that WAG/Rij rats with absence seizures have a potentiated systemic pressor response when GABAergic inhibition is blocked supporting the presence of excessive GABA-mediated inhibition within the amygdaloid nuclei in this model. It is not known if, or to what extent, synaptic reorganization (i.e. mossy fiber sprouting) occurring within the limbic (i.e. hippocampus) structures in the KA model might impact centrally mediated cardiac and vasomotor responses.

The more puzzling observation in the KA-treated animals was the significant increase in mPpa during MHX, and pulmonary vascular resistance during NX, acute HX, and MHX, following phentolamine administration. In general, the mPpa and pulmonary vascular resistance responses in the KA group under all conditions with phentolamine are confusing. It is only under NX conditions that mPpa in the KA group did not increase significantly with phentolamine although pulmonary vascular resistance was significantly higher. While these data must be interpreted with caution due to the small sample size, especially the acute HX data, this response in the KA group was consistent across all conditions and statistically significant at both ambient and MHX conditions. The mechanism(s) contributing to this response are unknown; however, there are several potential explanations that merit consideration.

It is a well-accepted tenet of vascular physiology that the primary determinant of resistance to flow within a vessel is inversely related to the caliber or diameter of the vessel and, the diameter of the vessel, is directly related to transluminal pressure (52). In intact animals, systemic vasodilation occurs passively in response to a sympathetically-mediated increase in cardiac output, as occurs during hypoxia (53), and it has been shown in an intact animal model that flow (i.e. cardiac output) is inversely related to pulmonary vascular reactivity to hypoxia (54,55). Thus, an increase in flow should be accompanied by a reduction in pulmonary vascular reactivity. Therefore, given that cardiac output (i.e. flow) was significantly lower in the KA animals during MHX and sharply lower during NX with phentolamine, we would predict that the KA rats would have a much greater mPpa and pulmonary vascular resistance response than that of the CON animals under these conditions. A much greater pulmonary vascular resistance response was observed in the KA group. However, this relationship did not hold when cardiac output was normalized to body weight (i.e. cardiac index). Therefore, it seems unlikely that the magnitude of the pulmonary vascular resistance response to phentolamine in the KA group could be explained entirely by this relationship unless the pulsatile (i.e. stroke volume) component of cardiac output (or cardiac index) rather than total volume is the most critical factor defining this relationship. This rationale would also explain, in part, the significantly higher acute HX pulmonary vascular resistance response in the KA group as cardiac output increased slightly while stroke volume decreased significantly, as well as the responses during MHX and MHX with phentolamine

where stroke volume remained significantly lower than the NX baseline. This is in contrast to the CON group in that stroke volume, which fell initially during MHX, returned to baseline, as did mPpa and pulmonary vascular resistance, during MHX with phentolamine.

A brisk or marked increase in mPpa and pulmonary vascular resistance in response to hypoxia is more commonly associated with permanent, irreversible structural changes within the pulmonary vessels (56). However, there was no indication under NX conditions to suggest that the KA and CON groups were markedly different, as mPpa and pulmonary vascular resistance at rest were statistically similar for both groups. In addition, there was no histological evidence of vascular changes within the lungs of the KA-treated animals that could have contributed to the significantly higher hypoxic pressor response in this group. There was, however, a significant difference in P_aO_2 between the two groups at rest as well as during the acute HX challenge. The ventilatory response (i.e. ventilatory rate and tidal volume) to the hypoxic challenges was not monitored; therefore, it is not known whether the KA animals had an abnormal or altered hypoxic ventilatory response. However, P_aCO_2 was not different under NX conditions between the KA and CON groups and, while P_aCO_2 levels, as expected, were significantly lower in both groups in response to acute HX, there was no difference between the two groups. Therefore, there is little evidence from a ventilatory perspective to support the possibility that KA animals were not able to respond appropriately to the acute HX challenge.

The relationship between P_aO_2 and mPpa is an inverse curvilinear relationship with a sharp upward increase in mPpa occurring at an approximate P_aO_2 of 40 mm Hg (57). Thus, given this relationship, the direct effect of the significantly greater level of hypoxemia observed in the KA animals (P_aO_2 = 29.46 ± 2.30 mm Hg), could account for the significantly higher hypoxic pulmonary vasoconstriction response in this group. During the MHX challenge; however, there was no difference in mPpa and pulmonary vascular resistance between the two groups even though the CON group had a slightly lower P_aO_2. Therefore, during the milder MHX challenge, the differences between the two groups regarding mPpa and pulmonary vascular resistance were negligible. Under acute HX with phentolamine, mPpa in both groups appeared to be equivalent to that recorded during the acute HX challenge without phentolamine. However, in the KA group, P_aO_2 during this challenge was considerably higher than that recorded during acute HX. Again, the sample size relative to this parameter is small; however, it is possible that given the higher P_aO_2, the mPpa response in the KA group during the acute HX challenge with phentolamine may represent a potentiated hypoxic pulmonary vasoconstriction response that is coincidentally the same in magnitude as the acute HX response. If this were the case, then the response to phentolamine in the KA group regarding mPpa and pulmonary vascular resistance would be consistent across all conditions in that blocking α-adrenergic receptors led to an increase in mPpa and pulmonary vascular resistance despite a less severe degree of hypoxemia. This suggests the potential involvement of other non-adrenergic pulmonary vasoactive peptides

(i.e. vasoconstrictors) such as endothelin, or the inhibition of vasodilatory mediators, such as nitric oxide, that are known to play a role in hypoxic pulmonary vasoconstriction, or other endogenous vasoactive mediators.

Nitric oxide is a pulmonary vasodilatory mediator that is released from the vascular endothelium during hypoxia and acts to modulate the HPV response. It is well known that injury to the vascular endothelium will impair or eliminate nitric oxide release and potentiated the HPV response (56,58). It is possible that KA directly damaged the pulmonary vasculature during the initial KA induction or, as speculated by Theodore and Robin (59) 25 years ago, that the extreme transient swings in pulmonary vascular pressures during overt seizure activity injures the endothelium thereby altering vascular reactivity. Neither the direct effect of KA on vascular reactivity in general, nor the effect of repeated, transient pressure spikes, as occurs during epileptic seizures, on pulmonary vascular reactivity, has been addressed.

Extensive reciprocal connections exist between limbic structures and the hypothalamus and, it is well known that plasma serum levels of specific hormones change in concentration as ictal activity generalizes and seizure intensity progresses (22,60). Persistent endocrine abnormalities occur frequently in people with epilepsy and the release of endogenous hormones such as norepinephrine, epinephrine, vasopressin, oxytocin, prolactin, and cortisol, during seizures has been documented in both humans and animal models (22,23,60-63). It is also well known that plasma levels of some hormones such as vasopressin stay elevated for hours after seizure cessation (22). In the KA

model, as well as in the amygdala kindling model, vasopressin mRNA and oxytocin mRNA expression in the paraventricular and supraoptic nuclei of the posterior pituitary, as well as plasma vasopressin levels do change over time (64). It is also well known that vasopressin, in addition to playing a role in regulating blood volume, causes systemic vasoconstriction as does oxytocin but to a lesser extent. However, both vasopressin and oxytocin act as vasodilators in the pulmonary vasculature (65). Thus, vasopressin and/or oxytocin cannot account for the responses noted here.

Endothelin, a potent vasoconstrictor peptide released locally from pulmonary arterial endothelial cells under hypoxic conditions (66-68), has been co-localized with vasopressin and oxytocin in the paraventricular and supraoptic nuclei of the posterior pituitary and acts within the pituitary to further modulate the release of vasopressin (69). In addition, while in low doses endothelin causes pulmonary vasodilation, it is well known that endothelin in higher concentrations is a potent vasoconstrictor and can potentiate the effects of other vasoactive hormones, such as norepinephrine (66-68). Although the identification of endothelin as a posterior pituitary hormone has been known for over a decade, it is not known whether plasma endothelin levels change during seizure activity as occurs with vasopressin and oxytocin. We could speculate that if endothelin, like vasopressin and oxytocin, is released from the posterior pituitary during seizures at levels sufficient to upregulate vasoregulatory mechanism(s) (i.e. receptor density), then, the subsequent release of endothelin under HX conditions, could have enhance mPpa and pulmonary vascular resistance responses under both

acute HX and MHX challenges with phentolamine. The potential long-term physiological consequences of elevated levels of endogenous vasoactive hormones, like endothelin, on vascular reactivity secondary to seizure disorders, have not been addressed, nor is it known if vascular receptor density for vasoactive hormones change over time in response to dramatic seizure-related swings in bioavailability. It is feasible that, in cases of poorly controlled or intractable epilepsy, physiologically significant changes in receptor density could occur and could significantly impact vascular responses in innumerable scenarios.

Finally, focal epileptic discharges in the brain can cause either central inhibition or facilitation of breathing depending upon the region(s) of the brain involved. Apnea frequently accompanies overt seizure activity and its occurrence is not restricted to the more 'severe' secondarily generalized seizures, but frequently accompanies complex partial seizures (8,70). This could have a significant impact in terms of arterial oxygen saturation and could become critical during tonic-clonic seizures when oxygen demand increases dramatically. This brief, spontaneous, apneic period could also be considered somewhat analogous to the intermittent periods of hypoxia that accompany obstructive sleep apnea. Studies have shown, although not conclusively, those patients with obstructive sleep apnea have a greater risk of developing systemic hypertension (71). Furthermore, repeated bouts of hypoxia have been shown to alter vascular reactivity (72), potentiate the pulmonary vasoconstrictor response to hypoxia (73,74), as well as cause an increase in right heart mass (75). Of greater interest

is the fact that clinical sleep apnea studies have documented that plasma endothelin levels are elevated immediately after an apneic episode (76) and, experimentally, it has been shown that plasma endothelin levels increased progressively with intermittent periods of hypoxia (5-10%) accompanied by an increase in systemic blood pressure (77). Furthermore, it has been shown that with recurrent episodes of hypoxia there is a significant loss of resting nitric oxide release in systemic vessels (72). In obstructive sleep apnea models, intermittent bouts of hypoxia can vary from a few episodes a day to many episodes per hour thus some of our animals would fall within the lower end of this spectrum. Due to the random nature of seizures and the constraints of our system, we were unable to obtain arterial blood samples during overt seizure activity thus, we could not evaluate whether the animals became hypoxic during seizures. However, this scenario would provide a mechanism by which nitric oxide release would be impaired and the response to endothelin would be potentiated, and, it could explain the difference in vascular reactivity between the two groups and in the KA-treated animals during seizures.

Seizure profiles

The other major findings of this study are with regard to the hemodynamic changes, specifically mSAP, mPpa, and heart rate, that were recorded during spontaneous seizure activity. It is well known that seizures are accompanied by a massive 'sympathetic storm' initiated by the simultaneous stimulation of sympathetic and parasympathetic preganglionic fibers resulting in the release of neuronal norepinephrine as well as epinephrine from the adrenal medulla

(10,11). The rapid ascent to peak mSAP and its sharp attenuation by phentolamine in this experiment indicates that this response was mediated primarily by alpha-adrenergic mechanisms (i.e. norepinephrine) and also suggests that the involvement of other vasoactive mediators was minimal. Goodman et al. (18) also noted a reported significant attenuation of the systemic pressor response during kindled Class 5 seizures with phentolamine.

Numerous papers have addressed the development of neurogenic pulmonary edema using a variety of epileptogenic agents with various animal models and have demonstrated that mPpa does increase during prolonged ictal activity (i.e. status epilepticus), primarily as a result of systemic α-adrenergic vasoconstriction with a small contribution from direct pulmonary venoconstriction (34,35,37,78). From these acute models, it has been speculated that for the most part, the immediate, severe, transient increase in pulmonary vascular pressure during spontaneous seizures plays a direct role in the development of neurogenic pulmonary edema. However, this is the first report of pulmonary pressure changes during spontaneous seizures in a chronic seizure model and the results reported here do not support the predictions of the earlier studies. During seizures under NX conditions, mPpa is elevated in concert with mSAP; however, unlike mSAP, mPpa does not return to baseline until approximately 1 min after seizure onset. Thus, if a portion of the mPpa response was dependent upon the displacement of systemic blood volume via systemic vasoconstriction as suggested by Sarnoff (16), then mPpa should have decreased shortly after mSAP returned to baseline. This was not the case, and suggests the

involvement of other vasoactive mechanisms. What is striking is the potentiation, not the attenuation, of the mPpa NX and HX profiles with the addition of phentolamine. This is consistent with the significant increase in mPpa and pulmonary vascular resistance responses reported for the KA-treated animals discussed above. This again stresses that the pulmonary pressor response that accompanied seizure activity was not dependent upon the displacement of blood volume in response to systemic vasoconstriction. The extreme degree of variability in the mPpa seizure profiles is indicative of the compliant nature of the pulmonary vascular bed therefore, statistical comparisons are not significant. However, it does not appear that α-adrenergic mechanisms play a role in mediating mPpa during overt seizure activity under NX or HX conditions in this model.

The mPpa profile was exacerbated during seizures under HX conditions with the peak pressor response approximately doubling that recorded during NX. There were also other significant differences of note between the NX and HX seizures. In contrast to the NX seizure profile, during the HX seizures, mPpa began to increase significantly 30 sec prior to the peak response. It is possible that with the added stress of HX, and the prerequisite lower P_aO_2, that the early gradual upslope in mPpa represents the early phases of impaired ventilation or apnea that were amplified under HX conditions. A more severe hypoxemia could also account for the potentiated mPpa profile in general. However, the other critical feature of the HX profile is the sustained elevation of mPpa that was still considerably higher than baseline almost 2 minutes after the peak response. The

combined action of HPV and other seizure-dependent vasoactive hormones such as endothelin cannot be ruled out.

As suggested by other investigators, these data show that there is a transient elevation in mPpa during spontaneous seizure activity that remains elevated for a short time after the peak hemodynamic response. What is lacking here is evidence of pulmonary edema formation. Pulmonary edema formation in the KA model has not been reported nor was there any indication of edema formation here; therefore, this may not be an appropriate model to use to investigate this phenomenon. In addition, if a transient elevation in mPpa is a critical factor for edema formation, then the magnitude of pressure swings must be greater than those recorded here to disrupt the integrity of the alveolar-capillary barrier. What is most likely the case is that it is impossible to predict with absolute confidence when or why pulmonary edema formation will accompany a seizure; and, if transient mPpa spike(s) do play a role in either clinical or subclinical neurogenic pulmonary edema, it is a very remote possibility that they will ever be recorded.

Minimal epileptogenic activity has been shown to alter both sympathetic and parasympathetic neural discharges to the heart (10,11). Furthermore, bradyarrhythmias are considered relatively rare and tachycardia most often dominates the cardiac response to aberrant seizure activity (11). Bradycardia accompanied each spontaneous seizure recorded during this study. In other acute models, as well as our own acute KA study (Chapter III), this is true. Although we did not have the capacity to record ECG, it is evident from the

tracings shown in Appendix B that arrhythmias were present. Irregularities or arrhythmias occur more commonly after central vagal stimulation when there is a rise in blood pressure than when there is a fall (79,80), as occurred during the seizures recorded during this study. The most distinguishing characteristic of the heart rate response observed here was the bimodal dip in heart rate. The first dip occurred concurrently with the peak in systemic arterial pressure while the second dip was recorded approximately 10 sec after the peak systemic pressor response. Our method of recording did not have the resolution to determine if the bradycardic response occurred immediately after the systemic pressure peak; however, the initial fall in heart rate was most likely the result of a baroreceptor-mediated reflex triggered by the sharp increase in systemic arterial pressure as it was eliminated when the systemic arterial pressor response was attenuated with phentolamine. An atropine-sensitive bradycardia has been documented during Class V kindled seizures (18); however, there was only a single dip in heart rate that accompanied the systemic pressor response in this model. It was also verified in this model that the initial bradycardia was eliminated with phentolamine as shown here. The second bradycardic dip in heart rate occurred as mSAP returned toward baseline and persisted even after phentolamine. This response could be the result of a neurally mediated bradycardia driven by strong vagal outflow as medullary nuclei were recruited. However, a reduction in pulmonary stretch receptor input, as occurs during breath-holding or apnea, can also result in, and/or potentiate, bradycardia (8,81). Thus, the second bradycardic dip could be due to cardiopulmonary reflex triggered by seizure-

related apnea rather than secondary to the propagation of epileptic activity. Careful evaluation of ventilatory parameters during seizure activity in this model would help to clarify this response as well as the mPpa profile.

There is a second, more serious implication of the 2nd bradycardic dip noted in all seizure profiles. Carotid artery cannulation has been used for years in many animal models to assess systemic arterial pressure responses under a variety of circumstances without seriously compromising cerebral circulation. However, carotid occlusion (i.e. ligation) is also used in models of focal cerebral ischemia (82). It is possible in the KA model in which 'normal' neurological function had been compromised, that ligation of the carotid artery significantly impaired autonomic regulation, as suggested in the previous discussion, and caused a brief period of brainstem ischemia during seizures, as reflected in the 2nd bradycardic dip (i.e. Cushing reflex (83)). It is also possible that the ligation of the carotid unloaded the baroreceptors in the carotid sinus and subsequently interfered with baroreceptor-mediated responses (82) such as those seen during acute HX and MHX. Attenuation of cardiovascular reflexes, including baroreceptors and chemoreceptors, has been shown following middle cerebral artery occlusion in the rat (82). Thus, these possibilities need to be evaluated; however, it must also be noted that the CON animals in these experiments responded as anticipated. The potential application of this model as an epileptic stroke model also deserves additional attention as stroke is the most commonly identified cause of epilepsy (84) and silent strokes are presumed to be the root of most seizure disorders in older persons (80)

Overall, the cardiovascular consequences of chronic, spontaneous motor seizure activity has historically been investigated using acute status epilepticus models, and, while the release of hormones during overt seizure activity has been recognized for decades, the role of endothelin and the long-term impact of intermittent elevated levels of vasoactive hormones on vascular reactivity, specifically in the pulmonary vasculature, has not been addressed. A carefully planned pharmacological study is needed to evaluate the cardiopulmonary responses noted in this model and extended to other models of temporal lobe epilepsy. Furthermore, of concern, is the systemic impact and potential for incipient cardiac and vascular damage that could result from aberrant neural activity that does not have overt motor manifestations. Fairly significant swings in pulmonary and systemic pressures occurred in this study, even during the less severe P3 seizures. However, we do not know if pressure changes occurred in the absence of overt motor activity. Transient elevations in mSAP have been reported with subconvulsive stimuli during kindling acquisition (17). Simultaneous monitoring of central activity via *in vivo* electrophysiological implants and cardiopulmonary pressures would help to address this concern. Finally, what was evident from these data is the potential for severe and sustained elevated mPpa during ictal activity under moderately HX conditions, no more severe than that experienced while exercising at many Colorado resorts, especially in non-acclimated individuals. This could be especially critical as heart disease and other cardiovascular problems are no longer restricted to the older segment of the general population. However, further evaluation of the model itself from a

cardiovascular perspective is needed before clinically relevant cardiopulmonary issues can be addressed using this model.

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CHAPTER V

Influence of moderate hypobaric hypoxia on seizure frequency in the chronically epileptic kainic acid model of temporal lobe epilepsy

Abstract

Endogenous circadian rhythms, such as the light-dark and sleep-wake cycles, and behavioral circumstances affect some forms of epilepsy by inhibiting neuronal excitability. In the kainic acid (KA) model of temporal lobe epilepsy, seizures appear to coincide with the activity or degree of inactivity rather than with the light-dark cycle. Thus, conditions that disrupt behavioral patters (e.g. inactivity) may directly affect seizure frequency and, while hypoxia alone is not epileptogenic in adult rats, may alter the level of activity. Therefore, the objective of this study was to assess the effect of mild, hypobaric hypoxia on seizure frequency and activity in KA-treated rats with and without previously documented spontaneous seizure activity. We hypothesized that the activity level of KA-treated rats would be suppressed during 48 h of moderate hypobaric hypoxia resulting in an increase in seizure frequency. As anticipated, all rats were more active at night than during daylight hours; however, the KA-treated animals were significantly more active than CON at both time points and under all conditions. Seizure frequency, in a subgroup

of KA-treated rats with a documented history of seizures, increased under hypoxic conditions and remained elevated during the recovery phase. However, the increase in seizure frequency was not associated with a hypoxia-induced decrease in animal activity. There was a slight shift toward less 'severe' seizures during hypoxia with 2-3 times as many class III seizure recorded under hypoxic conditions than at any other period. The association between behavioral state and seizure frequency in this study was clear-cut with the majority of seizures occurring when the KA-treated rats were inactive despite the fact that they were significantly more active than the CON rats the majority of the time. Hypoxia, however, had a minimal impact on behavior. The greatest increase in seizure frequency occurred in the KA-subgroup with a previous history of seizure activity. This increase in seizure activity occurred during the light phase of the hypoxic period that could perhaps be attributed to an altitude-induced disturbance in the normal sleep cycle. While it is possible that seizure activity does not change with mild altitude exposure, the lack of data may be due to the failure of people with seizure conditions to consider the event unusual especially if the seizure was considered relative 'mild'. A more systematic review of altitude-induced changes in seizure patterns is warranted.

Introduction

Contrary to common perception, epileptic seizures are not entirely unpredictable. Seizures arising from many forms of epilepsy tend to cluster in daily patterns. Endogenous circadian rhythms, such as the light-dark cycle, as well as the sleep-wake cycle, affect some forms of epilepsy by inhibiting neuronal excitability; thereby altering seizure threshold and interictal discharges (1). Temporal lobe epilepsy is one form of epilepsy that is susceptible to the modulatory effect of these endogenous cycles (2). Therefore, identifying and understanding these patterns is of clinical relevance and could help alleviate the anxiety associated with the seeming randomness of overt seizure activity. In addition, while temporal lobe seizures are not typically triggered or induced by specific exogenous stimuli, it is of benefit to identify common, exogenous conditions known to disrupt biological rhythms and identify their effects on temporal seizure patterns.

Numerous clinical studies have linked spontaneous seizure activity, in both partial and generalized forms of epilepsy, to the sleep-wake cycle (3-5). However, while the sleep-wake cycle is the most 'recognized' biological rhythm, time of day (i.e. light-dark) is a biological cycle that also exerts a strong influence on seizure expression. More than 100 years ago, Gowers (6) noted the tendency of seizures to occur in temporal patterns outside the classical sleep-wakefulness characterizations. He classified patients based on the daily occurrence of seizures into three general groups: diurnal (daytime onset); nocturnal (nighttime onset); and, diffuse (random onset).

These early observations were later confirmed and expanded as peak occurrences within each group were identified. 'Diurnal' seizures peak upon awakening and at late afternoon, whereas 'nocturnal' seizures cluster at bedtime and in early morning before awakening (7). Patients with mesial temporal lobe epilepsy have a cyclical seizure pattern with peak occurrence falling in the late portion of the light phase of the day (8). Nocturnal rats and diurnal humans have circadian rest cycles that are out-of-phase with each other; however, both species have predominantly diurnal seizures despite the fact that rats are nocturnal. Therefore, the light-dark daily pattern in limbic seizures is a true endogenously mediated circadian patten, as it is not disrupted when the animals are placed in constant darkness (1).

Behavioral circumstances can also alter the incidence of seizures. For example, an increase in stress increases the probability of seizures (12) while many patients with epilepsy are less likely to have seizures if they engage in strenuous exercise (9-11). Seizures also appear to coincide with the activity state or degree of inactivity rather than with the light-dark phase in the chronically epileptic KA model, suggesting that activity level modulates seizure (13).

The influence of hypoxia on seizure activity is controversial. Hypoxia alone is not epileptogenic in adult rats; however, it is possible that hypoxia could indirectly exacerbate a nervous system predisposed to epileptic activity. Disruption of cerebral water, pH, and electrolyte balance secondary to hypoxia can significantly affect neuronal excitability. For example, a reduction

in O_2 has been shown to impair neuronal Na^+-K^+ pump activity thereby causing hyperexcitability (14). Furthermore, hypoxia-induced hyperventilation can result in cerebral hypocapnea and subsequent alkalosis thereby precipitating seizure activity (14). On the other hand, hypoxia can inhibit or prevent seizures by depressing neuronal excitability, decreasing free radical production, stimulating the release of adenosine, depressing release of neural transmitters, and by suppressing excitability at post-synaptic sites (15-20). While the direct effect of hypoxia on seizure generation is debatable, hypoxia may also indirectly affect seizure occurrence through the disruption of biological rhythms that are intrinsically tied to seizure activity. It is not known whether an abrupt change in environmental conditions alters seizure susceptibility via changes in behavioral state. Therefore, we hypothesized that seizure frequency, in KA-treated rats with documented spontaneous seizure activity, would increase during exposure to moderate hypoxia, and that the increase in seizure frequency would be inversely related to activity.

Methods

Model: The kainic acid chronic epileptic rat model of temporal lobe epilepsy has been well documented (21). The treatment protocol for the animals used in this study has been previously described in detail (22). Male Sprague-Dawley rats (150-200 g, Harlan) were given hourly intraperitoneal injections of kainic acid (5 mg/kg) while control (CON) rats received an equal amount of intraperitoneal saline. After 3-4 injections, most of the KA-treated rats began having overt motor seizures. Seizure severity was scored using a

modified Racine scale (21,23) with severity defined as follows: Class III, rats displayed forelimb clonus with a lordotic posture; Class IV, rats reared with simultaneous forelimb clonus; and Class V, rearing with forelimb clonus accompanied by a loss of balance. KA treatment continued with a steady progression in seizure severity for a minimum of 3 h. The total dose of KA for each animal varied between 20-50 mg/kg. If an animal exhibited either excessive activity (i.e. excessive running and/or jumping) or became catatonic, the subsequent injection was reduced to 2.5 mg/kg as continued treatment at the initial dose has proven to be fatal. Mortality using this protocol is approximately 16%. All surviving rats received a subcutaneous injection of lactated Ringer's and moistened rat chow for the first week following treatment. The rats were paired and housed in the Laboratory Animal Resource facility, an Association for the Assessment and Accreditation of Laboratory Animals Care International-approved facility, with access to food and water *ad libitum* and were subjected to a 12-12 h light-dark cycle. All procedures were reviewed and approved by the Animal Care and Use Committee of Colorado State University.

Protocol: Following induction, KA-treated and matched CON rats were monitored at the Laboratory Animal Resource facility over the course of the next 5 mos for a minimum of 10 h a week to document spontaneous seizure activity. On average, KA-treated animals began displaying spontaneous seizure activity approximately 77 days post-induction (22). KA and matched CON rats were then chosen at random, transported from the resource facility

resource facility to the hypo/hyper baric chamber. Rats were monitored in the chamber via videotape for 4-8 hours during ach light-dark cycle for the next 4 days. The source of illumination at night for taping was by darkroom red safe lights. On Day 1 of normoxia, the animals were monitored at an ambient altitude of 5,140 ft (1500 m, P_B ~ 640) for 24 h. For Days 2 and 3, the chamber was decompressed and maintained at a simulated altitude of 10,000 ft (3,050 m, P_B ~ 523) where the animals remained for 48 h. Day 4 was a 'recovery' day spent in the chamber at ambient conditions for 24 h. Videotapes were reviewed for documentation of level of activity, seizure type, and seizure frequency (number of seizures/hour) for each animal.

Activity: The behavioral state for each animal was determined at 10 min intervals for all videotapes. The behavior exhibited at the beginning of each interval was used as a sample or snapshot of the behavior-state for that rat and coded as either active or inactive. 'Inactive' was defined as no overt, volitional movement by the animal while 'active' encompassed such behaviors as grooming, exploring, and eating. The percent of time the animal spent in an active state was calculated as the ratio of the number of 'active' intervals to the total number of intervals reviewed for that animal. Most animals in this experiment were exposed to 48 h of hypoxia on more than one occasion however, each bout was separated by a minimum of 3 days of normoxia. Animals were evaluated during both light (AM) and dark (PM) periods for all 3 conditions.

Seizure frequency: For the evaluation of the effect of hypoxia on seizure frequency, a subpopulation of KA-treated rats were selected and divided into one of two groups, hot or quiescent, based on the presence or absence of previously documented seizure activity at the Laboratory Animal Resource facility. These rats were videotaped under the conditions stated above. The tapes were reviewed at half-speed to score overt motor seizure activity using the Racine scale described above. The animal, time, condition, and seizure classification were noted for each seizure. Rats used in this experiment had not been used previously for the collection of activity data. In addition, the segment of time immediately preceding the recorded seizure was reviewed to access the behavioral state of the animal at seizure onset. Seizure frequency, defined as the ratio of the number of seizures to the length of the observation period, was then calculated for each animal.

Statistical analysis: A 3-way repeated measures analysis of variance was used to assess changes in behavioral states (i.e. activity) between and within the KA and CON groups while housed in the hypobaric chamber. A repeated one-way analysis of variance was used to assess changes in seizure frequency in the KA-treated animals during the 4 days in the hypobaric chamber. Data for activity were evaluated by log transformation to correct for a skewed variance distribution while the seizure frequency data were evaluated by square root transformation. Significance was assessed by *post hoc* least square difference pairwise comparisons. A P value less than 0.05 ($p < 0.05$) was accepted as significant. The data presented in tabular

form or in graphs represent raw (i.e. non-transformed) means ± standard error of the mean (SEM), while significance was assessed using the transformed values.

Results

Activity: Approximately 5 mo after induction, a total of 37 animals (CON = 16 KA = 21) were placed in the hypobaric chamber for four days to monitor behavioral changes during hypobaric hypoxia. Animals were evaluated during both light (AM) and dark (PM) periods for normoxia (i.e. ambient), hypoxia, and recovery. For each graph, the number of data points used for data analysis is indicated on each bar. The numbers vary due to the loss of potential data points from either bad videotapes or the inability to clearly observe the animal. This was occasionally a problem under red light conditions. A total of 3791 10 min segments during light conditions and 3888 10 min segments during dark conditions were evaluated for assessment of animal activity. Overall, both CON and KA-treated rats were significantly more active at night than during daylight hours (31.4 ± 1.59 and 10.8 ± 0.66 %, respectively). However, KA-treated animals were significantly more active than CON at both time points (Figure V-1).

Overall activity during hypoxic and recovery was not different from that recorded during normoxia (Table V-1); however, KA-treated rats were more active than CON rats under normoxic conditions and significantly more active under both hypoxic and recovery conditions (Figure V-2). However, while KA-treated animals were significantly more active than CON animals with regard

to time and condition, activity for KA group was only significantly different from CON animals at night during normoxic and during the day for the recovery period (Figure V-3).

Table V-1: Percent of time spent active by condition and time.

	Normoxia	Hypoxia	Recovery	Mean
AM	10.8 ± 0.87(49)	11.0 ± 1.2 (50)	10.5 ± 1.5 (28)	**10.7**
PM	31.0 ± 3.3 (25) *	31.2 + 2.1 (74) *	31.2 + 2.1 (74) *	**31.13***

*significantly different from AM

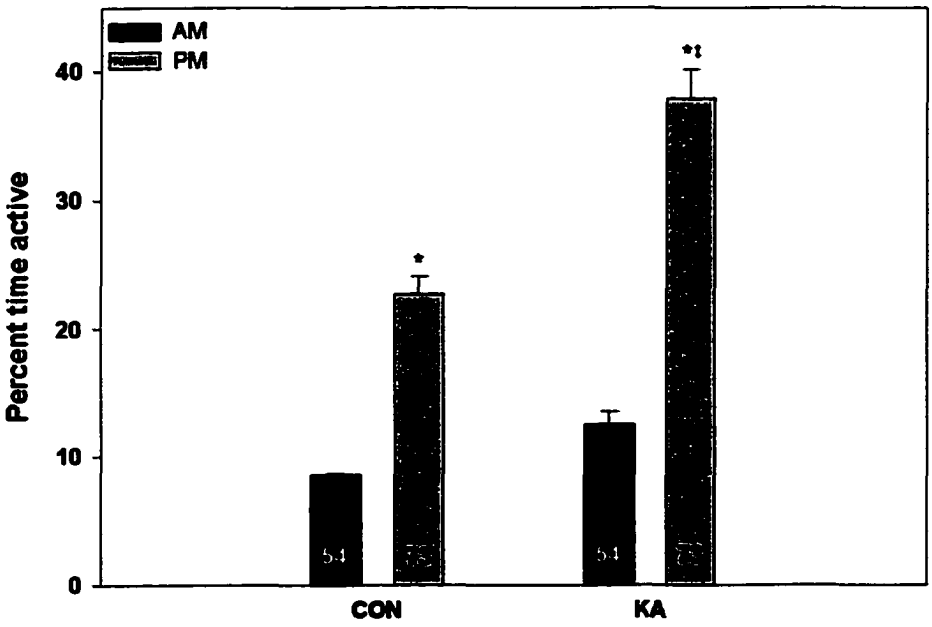

Figure V-1. Activity of CON and KA-treated rats during AM and PM periods. KA-treated rats were significantly more active than CON rats during both AM and PM periods. *significantly different from AM ‡significantly different from CON. $p < 0.05$

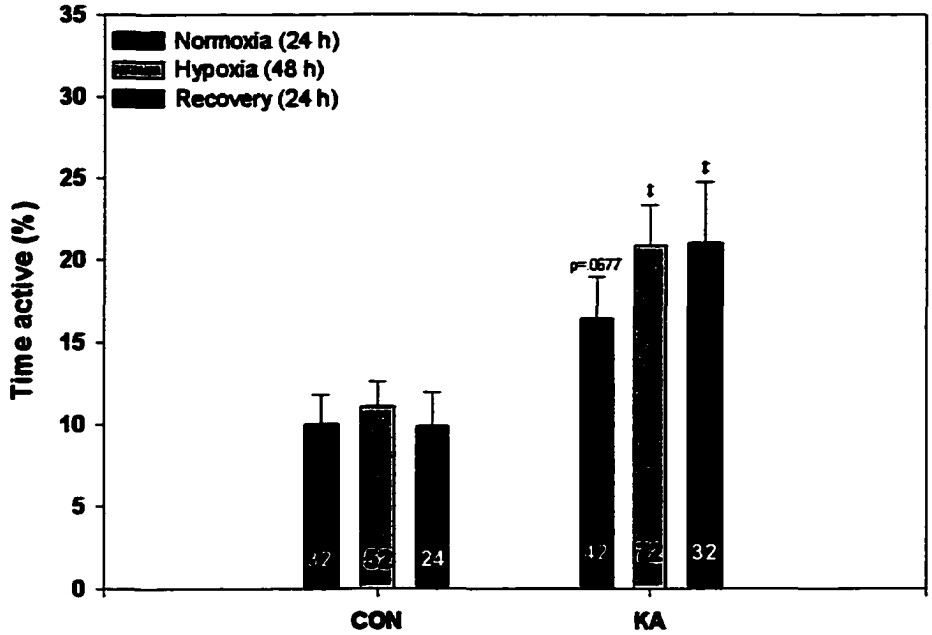

Figure V-2. Activity of CON and KA-treated rats during NX, HX, and RC. KA-treated rats were significantly more active than CON rats under all conditions. ‡significantly different from CON. $p < 0.05$.

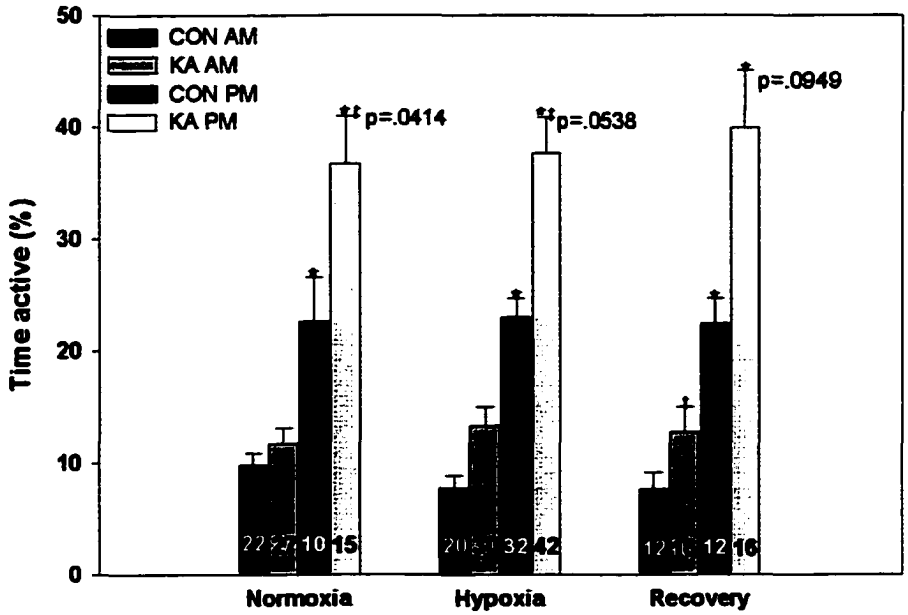

Figure V- 3. Activity of CON and KA-treated rats by time and condition. KA-treated rats were more active than CON rats under all conditions. ‡significantly different from CON. *significantly different from AM period. $p < 0.05$.

Seizure frequency: Eighteen KA-treated animals plus age-matched CON animals were evaluated to determine the effect of hypoxia on seizure frequency. A total of 207 seizures were observed in the KA rats during the 4 days the animals spent in the chamber (Table V-2). Most seizures occurred when the animals were inactive (72% vs. 28%) with the majority of the seizures identified as Class V seizures; however, there was a slight increase in the less severe Class III and Class IV seizures (Figure V-4). One KA animal was removed from the analysis as the observed seizure frequency for this animal was greater than 1 seizure/hour and therefore, more representative of status epilepticus than spontaneous seizure activity. The relationship between seizure type and condition is illustration in Figure V-5. On a percentage basis, the majority of seizures were Class V seizures regardless of the condition. There was a slight shift toward less 'severe' Class III seizures during hypoxia with almost twice as many Class III seizures recorded during this period than under any other condition. Class IV and V seizures were proportionately the same for all conditions.

Table V-2: Distribution of recorded seizures by time and condition.

Time	Normoxia (24 h)	Hypoxia (48 h)	Recovery (24 h)	Total
AM	22	51	33	106
PM	26	60	15	101
Total	48	111	48	207

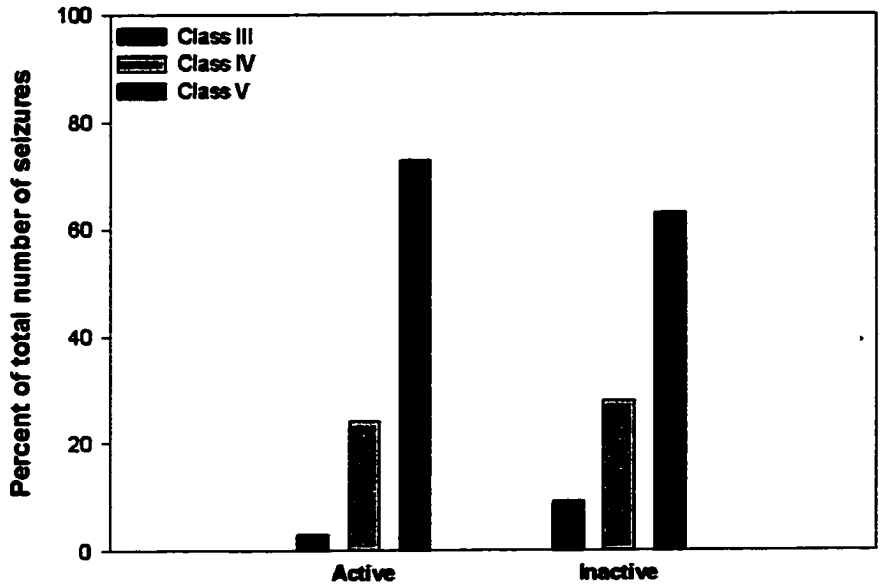

Figure V-4. Seizure severity for activity and inactive states. The majority of seizures occurred when the KA-treated animals were inactive. There was a slight shift toward the less severe seizures when the animal was inactive.

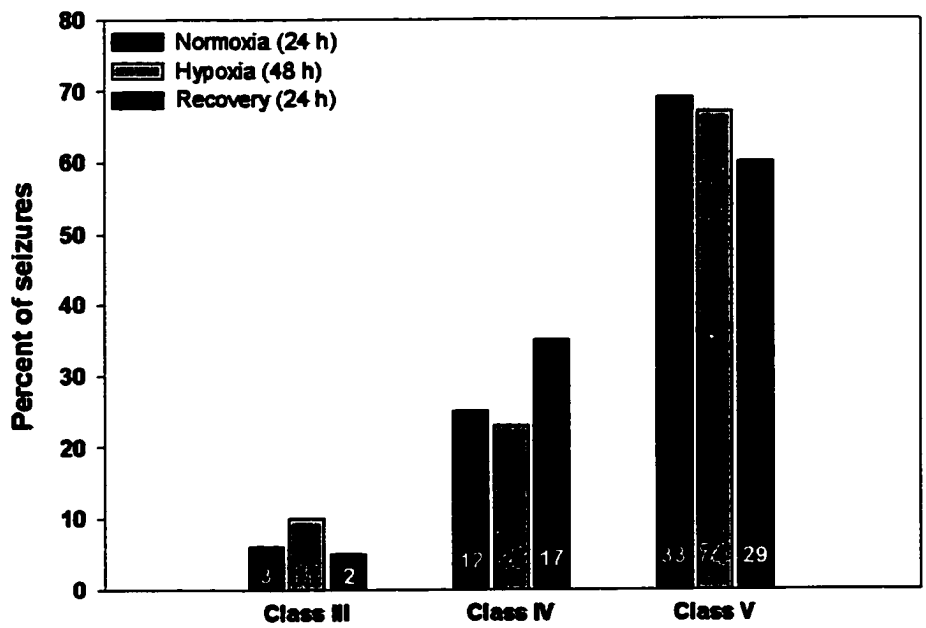

Figure V- 5. Distribution of seizures by severity for all conditions. Distribution of seizures by class for all conditions. The number of seizures recorded for each class is indicated on the bar.

With regard to time, seizure frequency increased under hypoxic conditions, with a significant increase during the light period (Figure V-6). As anticipated, seizure frequency was significantly greater in the hot group as compared to the quiescent group for both AM and PM time periods (Table V-3). In addition, seizure frequency for the hot group increased during hypoxia and was significantly greater than the quiescent group during recovery (Figure V-7). There was no correlation between seizure frequency and level of activity.

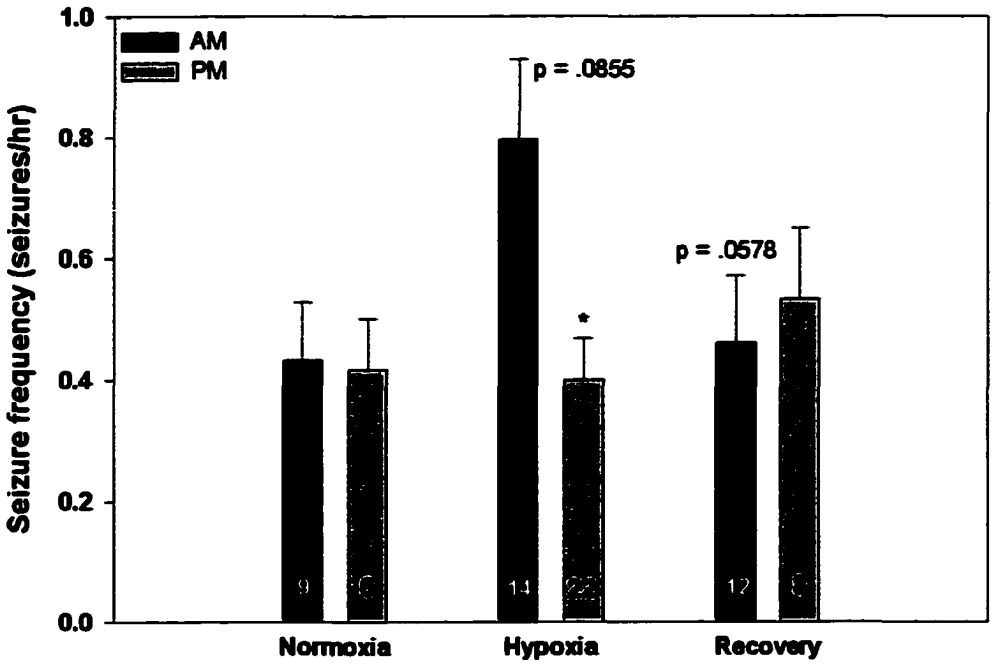

Figure V- 6. Seizure frequency during normoxia, hypoxia, and recovery. Seizure frequency increased during the light cycle under HX. More seizures occurred during the AM than the PM period. *significantly different from AM. $p < 0.05$.

Table V-3: Seizure frequency for quiescent and hot subgroups.

Time	Quiescent	Hot
AM	0.328 ± 0.068	0.739 ± 0 .089 *
PM	0.312 ± 0.047	0.540 ± 0.079 ‡
Total	**0.320 ± 0.058**	**0.640 ± 0.084**

significantly different from Q
‡p = .0669

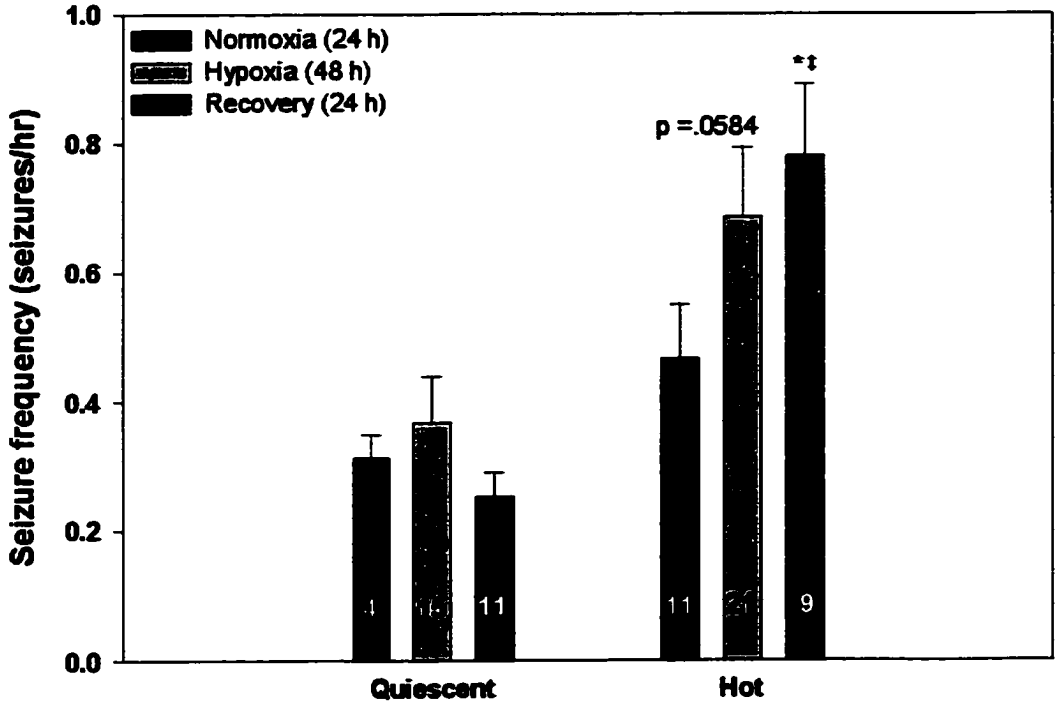

Fig V-7. SF increased under hypoxic conditions in both groups and increased significantly during recovery in the H group. *significantly different from normoxia. ‡significantly different from CON. p<.05. The 'n' for each condition is indicated on each bar.

Discussion

The principal finding of this study is that seizure frequency, in KA-treated animals with a history of spontaneous seizure activity, increased during short-term (i.e. 48 h) exposure to moderate hypoxia and seizure frequency remained elevated in the immediate recovery period. The majority of seizures occurred when the animals were inactive. However contrary to our hypothesis, the increase in seizure frequency was not associated with a hypoxia-induced decrease in animal activity. Thus, hypoxia had a minimal impact on behavior. KA-treated animals were at all times, significantly more active than CON animals.

This is the first study addressing the effect of a sustained moderate hypoxic challenge on spontaneous seizure activity in the chronically epileptic. Several investigators have shown that exposure to moderate hypoxia prior to or during the acute administration of KA was neuroprotective, either suppressing seizure activity (15,18) or attenuating the characteristic morphological changes that occur in the hippocampus in this model (24-26). The primary mechanism mediating the response in the acute model is thought to be the endogenous release of adenosine. It is well known that extracellular levels of adenosine in the brain increase significantly during hypoxia (17), acting as a vasodilator to maintain cerebral blood flow during epileptiform activity (27,28) or acting as an 'endogenous anticonvulsant' mediating seizure arrest (25,27). In addition, GABA-transaminase, which is responsible for the catabolism of GABA (gamma butyric acid), an endogenous neuronal inhibitory

mediator, requires oxygen. Therefore, under hypoxic conditions, the activity of the enzyme would be suppressed resulting in an increase in extracellular levels of GABA, thereby inhibiting synaptic transmission. Thus, one could hypothesize that seizure frequency would decrease under hypoxic conditions via the hypoxia-induced release of adenosine. However, this was not the case in our study.

One of the primary differences between our study and the acute studies mentioned above, is the discrepancy between the definitions of moderately hypoxic conditions. Moderate hypoxia, in the acute studies, was defined as an O_2 concentration of 8-9%, which is equivalent to an altitude of 20-22,000 ft (6000-7000m) for 8 h. Using this definition, our animals were subjected to a fairly mild hypoxic challenge at 10,000 ft (equivalent to an F_IO_2 of 0.14); which is comparable in altitude to most of the ski resorts in the Colorado Rocky Mountain region. Based on the work of Winn et al. (17), under these conditions, brain adenosine levels would not be much different from those measured at sea level. Brain adenosine levels do not increase significantly until P_AO_2 (PaO_2) approaches 50mm Hg. This would occur at an altitude of approximately 12,000 ft (3660m). Therefore, in our study, the hypoxic challenge was probably not severe enough to increase adenosine release in the brain and subsequently suppress seizure activity.

It is also possible that the increase in seizure frequency seen in the 'hot' group is the result of the underlying synaptic reorganization that occurs within the hippocampus in the KA model. In the chronic KA model,

spontaneous seizures develop gradually over time as morphological changes (i.e. axonal sprouting, and/or synaptic reorganization) (29) within the hippocampus occur in response to the acute KA insult and resultant convulsive seizures. However, the nature of this synaptic 'rewiring' is hotly debated. It is generally accepted that either recurrent excitatory currents form as surviving neurons establish new synapses or that there is a loss of GABAergic-inhibition via the loss of inhibitory interneurons (21,29). It is well known that hypoxia is an excitatory stimulus and that many physiological responses to hypoxia are driven by an overall increase in sympathetic nervous system activity. If recurrent excitatory circuits are predominant in the newly reorganized hippocampus, it is possible that hypoxia, as an excitatory stimulus, could trigger a seizure via these recurrent circuits. If GABAergic-inhibition is lost via the loss of inhibitory interneurons, then it is possible that some of the mechanisms responsible for ensuring that hypoxia does not trigger aberrant activity will no longer be present. In either case, this remodeling could establish conditions in which a relatively mild innocuous stimulus, such as hypoxia, becomes a trigger initiating aberrant epileptiform activity. Whether the most reactive animals under hypoxia also had the most extensive remodeling of the hippocampus is not known.

It is also possible that the shift away from Class IV and V seizures to the less severe Class III seizures recorded during hypoxia is in some way related to the suppression of GABA-transaminase activity. The reduced availability of the enzymatic cofactor O_2 would result in an increase in

endogenous extracellular levels of GABA that in turn could attenuate or inhibit neuronal synchronization required for the more severe convulsive seizures. A more in-depth analysis of changes in seizure patterns under mild hypoxia needs to be explored.

The association between behavioral states and seizure activity in our study is very clear, with the majority of seizures occurring when the animals were inactive despite the fact that the KA animals were significantly more active than CON animals the majority of the time. W. G. Lennox (30), in a 1941 article, stated: "Physical and mental activity seems to be an antagonist of seizures. Enemy Epilepsy prefers to attack when the patient is off guard, sleeping, resting, or idling". The results reported here cannot refute that claim and support the hypothesis that conditions that alter the quality and quantity of time spent in a specific behavioral state can have a significant impact on the probability of seizure activity.

The association between sleep and epileptiform neural activity is well documented. Nocturnal temporal lobe complex partial seizures generalize 37% of the time, compared to complex partial seizures during wakefulness (17%), occur most frequently in light sleep (Stage 1 and 2) or deep sleep (Stage 3 and 4) of NREM sleep, and rarely occur during REM sleep (31,32). In addition to circadian influences, epilepsy is associated with other sleep abnormalities, such as prolonged sleep latency, an increase in the number and duration of awakenings, and reduced or fragmented REM sleep (33). Sleep at altitude, is also punctuated with many of the same sleep

abnormalities. Eighty-three percent of newcomers exposed to even moderate altitudes of 10,000 and 14,000 ft frequently complain of disturbed sleep (34). Malkin et al (35) have identified specific changes in the sleep-cycle that account for the anecdotal reports of poor sleep. For the first 48-72 h at moderate altitude (14,000 ft), subjects experience an increase in the number of awakenings, a significant increase in the duration of stage 1 and/or stage 2 sleep, and a significant decrease in stage 3 and 4, slow wave, and REM sleep stages (35-37). Therefore, at altitude, a person with epilepsy is potentially spending more time in the sleep phases in which the probability of seizure occurrence and generalization is the highest. In this study, the greatest increase in seizure frequency did occur during the light phase of the hypoxic exposure period, the primary sleep period for these animals. Thus, these results could be explained by an altitude-induced disturbance in the normal sleep cycle of these nocturnal animals.

The relationship between disrupted sleep patterns at altitude and seizure activity has not been reported. Several surveys have identified numerous factors that are related to the occurrence of acute mountain sickness in travelers to moderate altitude (38-40). A very recent report examining the occurrence of high altitude cerebral edema at moderate altitude did note the occurrence of seizures, one in a patient without a previous seizure history (41). Interestingly, only 2 of 11 EEGs from 9 patients mentioned in this study were normal; however, no further diagnostic information was provided.

Additional information might be provided again by examining the effect of endogenous adenosine on sleep patterns. Adenosine may be the major sleep-promoting substance in the brain (42) as adenosine antagonists cause prolonged wakefulness, much like the effect of caffeine and theophylline (43) while adenosine agonists increase slow-wave sleep and paradoxical sleep in a dose-dependent manner (44). In ventilation studies, adenosine infusion increases ventilation and produces periodic breathing in normal subjects (45). Thus, there is a hypothetical common endogenous denominator between seizures, hypoxia, and sleep – adenosine. This potential relationship has yet to be explored.

While it is possible that seizure activity does not change with mild altitude exposure, the lack of data may be due to the failure of people with seizure conditions to consider the event unusual and necessary of medical attention. It is not unusual for occasional seizures to occur even if the disorder is pharmacologically controlled. Therefore, an episode is less likely to come to the attention of medical personnel especially if it is considered to be relatively 'mild' or if the individual is at altitude, complicated by other symptoms of acute mountain sickness, and therefore overlooked. The potential underlying mechanism(s) responsible (e.g. adenosine) for the results described in this report need to be examined in greater detail. A more systematic review of altitude-induced changes in seizure patterns is warranted, especially in people with a documented history of seizures.

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CHAPTER VI

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The objectives of the present set of studies were to 1) characterize the acute cardiopulmonary hemodynamic effects of kainic acid (KA) and KA-induced motor seizure activity; 2) characterize the cardiopulmonary hemodynamic effects of recurrent, spontaneous motor seizure activity in the chronically epileptic KA model of temporal lobe epilepsy under baseline normoxic conditions and under the physiological stress of acute hypoxia; and, 3) determine if short-term (48 h) mild to moderate hypoxia (10,000 ft., P_B ~ 523 mm Hg) alters the behavioral state of KA-treated rats and subsequently results in a change in seizure frequency. In addressing the specific aims and hypotheses set forth in this study, the following conclusions were drawn.

Study 1: Acute systemic and pulmonary vascular effect of repeated low-dose injections of kainic acid

Summary: The first study demonstrated that, while KA acts as a 'neuroexcitotoxic' agent, the systemic response to the initial bolus of KA was severe systemic hypotension, bradycardia, and a variable mean pulmonary arterial pressure (mPpa) response. Once generalized motor seizures began, systemic hypertension dominated the interictal period and spikes in mean

systemic arterial pressure (mSAP) accompanied each motor seizure. Interictal and ictal heart rate was more variable with both tachycardic and bradycardic responses. Interictal mPpa also increased with spikes during each motor seizure. Interictal mSAP fell significantly, heart rate increased, and mPpa remained relatively unchanged immediately following phentolamine while ictal mSAP spikes were attenuated and mPpa spikes were unaffected. There was no morphological evidence of either alveolar hemorrhage and/or acute pulmonary edema formation.

I. **Specific hypothesis and findings:** We hypothesized that both systemic and pulmonary vascular pressure and heart rate would increase following the initial intraperitoneal KA injections. Systemic hypotension and bradycardia occurred immediately after the 1st KA injection and were sustained until the 1st motor seizure. The acute effect of KA on mPpa was variable eliciting both an increase and decrease in pressure.

A. Conclusions:

1. The initial systemic hypotension, bradycardia, and variable mPpa responses were not anticipated; however, the hypotensive and bradycardic responses have been observed in other acute models. The initial cardiopulmonary responses could be the result of KA-induced discharges with the hippocampus and amygdala relayed to central cardiovascular

centers (e.g. NTS). The mechanism(s) mediating the mPpa responses are not known.

B. Recommendations:

1. Additional studies with simultaneous monitoring of cardiovascular parameters (e.g. mSAP, mPpa, heart rate) and hippocampal/amygdala activity are needed to evaluate the role central mechanisms play in mediating the initial systemic and pulmonary responses observed in this model.
2. Systematic pharmacological evaluation of pulmonary vascular reactivity following acute KA administration is needed to determine if KA directly damages the pulmonary vascular endothelium and/or the role of local mediators in the responses documented in this study.
3. Serum levels of vasoactive posterior pituitary hormones (e.g. vasopressin, oxytocin, endothelin) need to be assessed during the initial KA insult.

II. **Specific hypothesis and finding:** We hypothesized that the interictal and ictal systemic and pulmonary pressure and heart rate would increase with each motor seizure via sympathetically mediated activity. After the 1st motor seizure, a gradual increase in interictal and ictal mSAP and mPpa occurred over the course of the induction period. Spikes in both pressure parameters also accompanied each motor seizure. The heart rate response was more variable with both

tachycardic and bradycardic responses. Interictal and ictal mSAP were significantly attenuated with phentolamine; however, the interictal and ictal pulmonary pressor response was unaffected by phentolamine. heart rate increased significantly following phentolamine administration.

A. Conclusions:

1. The systemic responses were mediated primarily via increased α-adrenergic activity. The increase in heart rate was triggered by the fall in mSAP mediated via baroreceptor reflex.
2. The pulmonary pressor response was mediated by non-adrenergic mechanisms. The possibility of a direct effect of KA on the pulmonary vasculature cannot be ruled out.

B. Recommendations:

1. The identity of and role of vasoactive hormones such as endothelin, and other local vasoactive agents such as endothelin and nitric oxide, in mediating the mPpa and pulmonary vascular resistance observed during KA-induced motor seizures needs to be assessed.
2. The potential for direct damage to the pulmonary endothelium (i.e. shear stress) from repeated, transient swings in mPpa needs to be addressed.

III. **Specific hypothesis and finding:** We hypothesized that evidence of alveolar hemorrhage and/or pulmonary edema would be present in the

lungs of the KA-treated animals. There was no evidence of alveolar hemorrhage or pulmonary edema formation in any of the KA-treated or CON animals.

A. Conclusions:

1. This may not be an appropriate model for the study of acute neurogenic pulmonary edema.

Study 2: Cardiopulmonary hemodynamics during spontaneous seizure activity in a chronically epileptic kainic acid model of temporal lobe epilepsy

Summary: The second study demonstrated that, although there were no differences under baseline normoxic conditions between control (CON) and KA-treated rats, there were significant hemodynamic differences between the two groups when stressed. Under the physiological stress of acute hypoxia, significant differences between the two groups with regard to stroke volume, mPpa, and pulmonary vascular resistance were evident. With phentolamine, mPpa were exacerbated under normoxia, hypoxia, and moderate hypoxia in KA-treated rats. With seizure onset under normoxic conditions, mSAP and mPpa increased and heart rate decreased immediately. With seizure onset under hypoxic conditions, and, during normoxia and hypoxia with phentolamine, the relative increase in mPpa was exacerbated while mSAP was attenuated.

I. **Specific hypothesis and findings:** We hypothesized that cardiopulmonary responses (e.g. mSAP, mPpa, heart rate) to either acute hypoxia (3 min, 10% O_2) or short-term, moderate hypoxia (2 h,

12-14% O_2) would be significantly higher in the KA-treated rats than responses recorded in the CON rats due primarily to an increase in α-adrenergic activity. Under normoxic conditions, there were no significant differences between the CON and KA-treated rats; however, in both the acute and moderate hypoxia challenges, there were significant differences between the two groups regarding stroke volume, mPpa, and pulmonary vascular resistance. These differences were not due to α-adrenergic activity as stroke volume fell significantly while mPpa and pulmonary vascular resistance increased in response to phentolamine in the KA-treated rats under normoxia, acute, and moderate hypoxia.

A. Conclusions:

1. The KA-treated rats were able to maintain cardiac output despite the fall in stroke volume by relying solely upon an increase heart rate. The disparity in stroke volume responses between the CON and KA-treated rats for both hypoxic challenges suggests impaired myocardial function, the source of which could be an autonomic imbalance (e.g. reduction in sympathetic input), arrhythmias, or myocardial (i.e. contractility) dysfunction. It is also possible that hippocampal synaptic reorganization (i.e. loss of GABAergic inhibition) that occurs in response to the acute KA insult altered centrally mediated cardiac and vasomotor reflexes.

2. The mPpa and pulmonary vascular resistance responses in the KA-treated rats under normoxia, acute hypoxia, and moderate hypoxia following phentolamine were unexpected. It is possible that the increase in mPpa and pulmonary vascular resistance during hypoxia might be related to the fall in stroke volume. However, the potentiated mPpa and pulmonary vascular resistance response under normoxia and hypoxia following phentolamine were also unanticipated. The potentiated mPpa responses observed in the KA-treated rats suggests the involvement of or inhibition of other non-adrenergic vasoactive mediators.
3. Autonomic dysfunction as a direct result of carotid cannulation could have confounded the observations documented in the present study.

Recommendations:

1. Evaluations of sympathetic and parasympathetic pathways in both the acute and chronically epileptic KA models are warranted. The potential problem with the carotid cannula needs to be addressed first by eliminating the carotid cannula and using a femoral cannula to record systemic pressure responses.
2. Concurrent monitoring of ECG is needed to document and identify cardiac arrhythmias in this model. Assessment of cardiac enzyme (e.g. creatine kinase myocardial band (Ck-MB))

levels would provide additional information regarding the presence of neurogenic electrocardiographic abnormalities.

3. The effect of synaptic reorganization and remodeling within the hippocampus on cardiac and vasomotor reflexes, especially those relayed through the nucleus tractus solitarius, needs to be addressed in this model.
4. Systematic pharmacological studies of potential non-adrenergic mediators, such as nitric oxide and endothelin, are needed to fully delineate the mechanism(s) regulating the exacerbated mPpa response in the KA-treated rats with phentolamine. Evaluation of endothelin, both as an endogenous pituitary hormone and as a local pulmonary vasoactive mediator, in mediating pulmonary vascular responses in this model is recommended.
5. A profile of the interictal and ictal serum levels of vasoactive hormones is also needed to identify other potential mediators of the pulmonary pressor response. The long-term effect of transient elevated levels of vasoactive hormones on pulmonary vascular reactivity as well as effects such as changes in receptor density needs to be addressed in this model.
6. Ventilatory responses and the role of intermittent hypoxia and release of endothelin, as a potential mechanism mediating the mPpa response in this model should be further examined.

Intermittent hypoxia within the context of generalized seizure disorders has not been addressed.

II. **Specific hypothesis and finding:** We hypothesized that mSAP, mPpa, and heart rate would increase during spontaneous seizure activity, and, that mSAP, mPpa, and heart rate responses recorded during spontaneous seizure activity under hypoxia conditions, would be exacerbated. Furthermore, we hypothesized that the systemic and pulmonary pressure changes observed during seizures, under either condition, would be attenuated following the administration of phentolamine. mSAP and mPpa increased while heart rate decreased immediately upon seizure onset. During seizures under normoxic conditions, mSAP returned to baseline within 10 sec of seizure onset. The initial bradycardic response was followed by a second fall in heart rate at approximately the same time as mSAP returned to baseline. mPpa remained elevated after motor seizure activity had ceased and mSAP and heart rate had returned to baseline. During hypoxia, mSAP, mPpa, and heart rate responses were consistent with those recorded under normoxic conditions except that the mPpa response was exacerbated. With phentolamine, mSAP and the 1st bradycardic dip were attenuated; however, mPpa was unaffected during normoxia and potentiated during hypoxia.

A. Conclusions:

1. With the exception of the 2nd fall in heart rate, the mSAP and heart rate profiles recorded under both normoxic and hypoxia conditions are consistent with those reported for Class V amygdaloid "kindled" seizures. The increase in mSAP was mediated via α-adrenergic activity.
2. The initial fall in heart rate was probably mediated via reflex vagal activity as part of the baroreceptor response triggered by the immediate increase in systemic hypertension.
3. mPpa remained elevated after mSAP had returned to baseline thus suggesting that the mPpa response was not solely the result of the displacement of systemic blood volume to the pulmonary circulation.
4. The increase in mPpa during seizures was not mediated via α-adrenergic mechanisms.
5. The increase in mPpa noted during seizures under hypoxia conditions probably reflects the potentiation of the 'normal' mPpa seizure response via hypoxic pulmonary vasoconstriction.

B. Recommendations:

1. mPpa changes during spontaneous, generalized seizures, in awake and freely moving rats have not been previously reported and need to be defined in other chronic seizure models (i.e. "kindling" model).

2. As addressed above, a thorough investigation into other potential vasoactive mediators is needed in this model to fully understand the pulmonary vascular responses documented during motor seizure activity.

Study 3: Influence of moderate hypobaric hypoxia on seizure frequency in the chronically epileptic kainic acid model of temporal lobe epilepsy

Summary: The third study evaluated the association between mild to moderate, long-term hypoxia, activity, and seizure frequency in KA-treated rats. KA-treated rats were significantly more activity than CON rats for both light and dark periods and under all conditions. The majority of seizures occurred when the KA-treated rats were inactive; however, hypoxia had a minimal impact on activity and seizure frequency. Of those seizures occurring while the animal was inactive, a greater proportion of the seizures were less 'severe' (i.e. Class III and Class IV seizures). There was a slight shift toward less 'severe' seizures during hypoxia. The greatest increase in seizure frequency occurred in the group of rats with previously documented seizure activity during the light phase, perhaps suggesting an altitude-induced disturbance in the normal sleep cycle.

I. **Specific hypothesis and findings:** We hypothesized that spontaneous seizure frequency would increase during exposure to moderate hypobaric hypoxia secondary to a decrease in activity level.

A. Conclusions:

1. As previously reported, the majority of seizures in the KA-treated animals occurred when the animals were inactive

2. Hypoxia did not alter activity or affect seizure frequency in the KA-treated rats; however, seizure frequency did increase in those animals with a history of spontaneous seizure activity.
3. There was a shift toward the less 'severe' seizures in the KA-treated rats when they were inactive and hypoxic.

B. Recommendations:

1. Additional studies are needed to define the relationship between moderate hypoxia, equivalent to that experienced at ski resorts, sleep patterns, and seizure occurrence. These studies would be most easily accomplished in humans.
2. Further evaluation of the increase in the less 'severe' Class III seizures during hypoxia, perhaps by extending the period of time spent at altitude or with concurrent EEG monitoring, would be helpful in identifying altitude-induced changes in seizure severity and patterns.

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APPENDIX A

Additional graphs for Chapter III

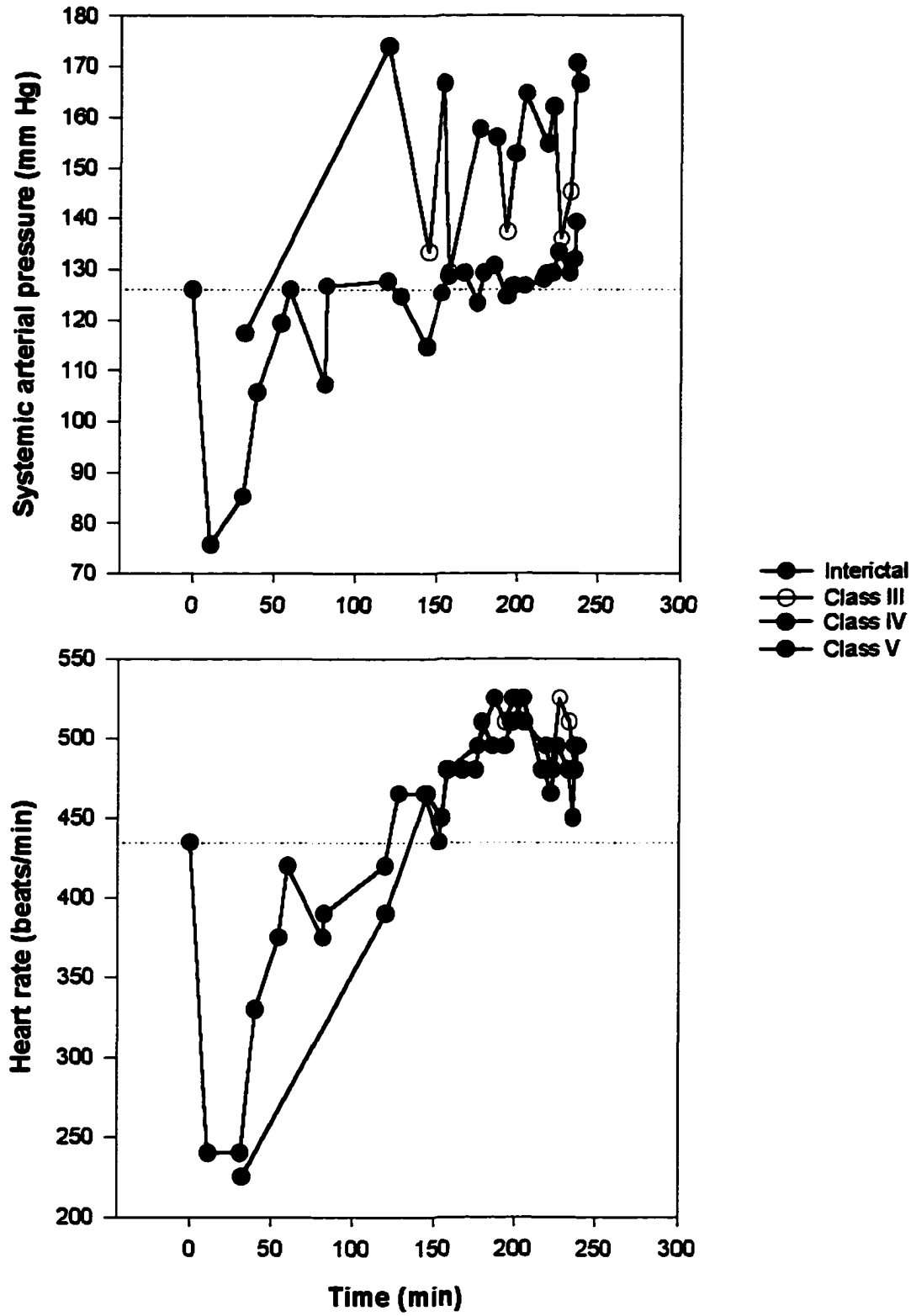


Figure A-1. Time course of mSAP and HR responses for KA-treated rat 307 during the induction protocol.

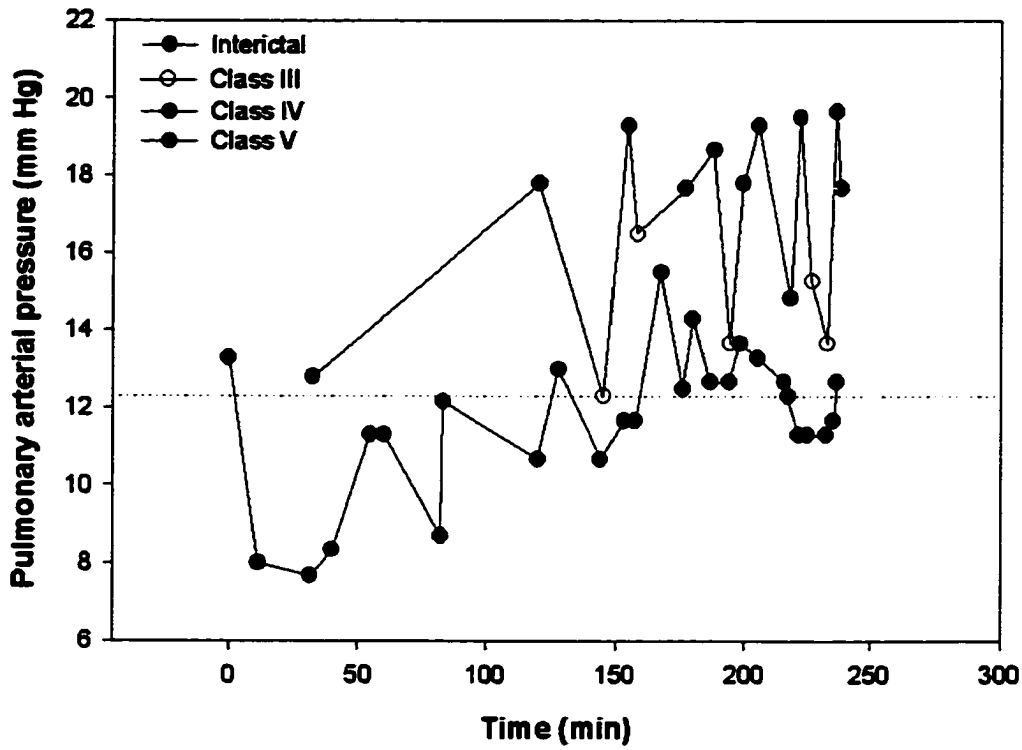


Figure A-2. Time course of mPpa response in KA-treated rat 307 during the induction protocol.

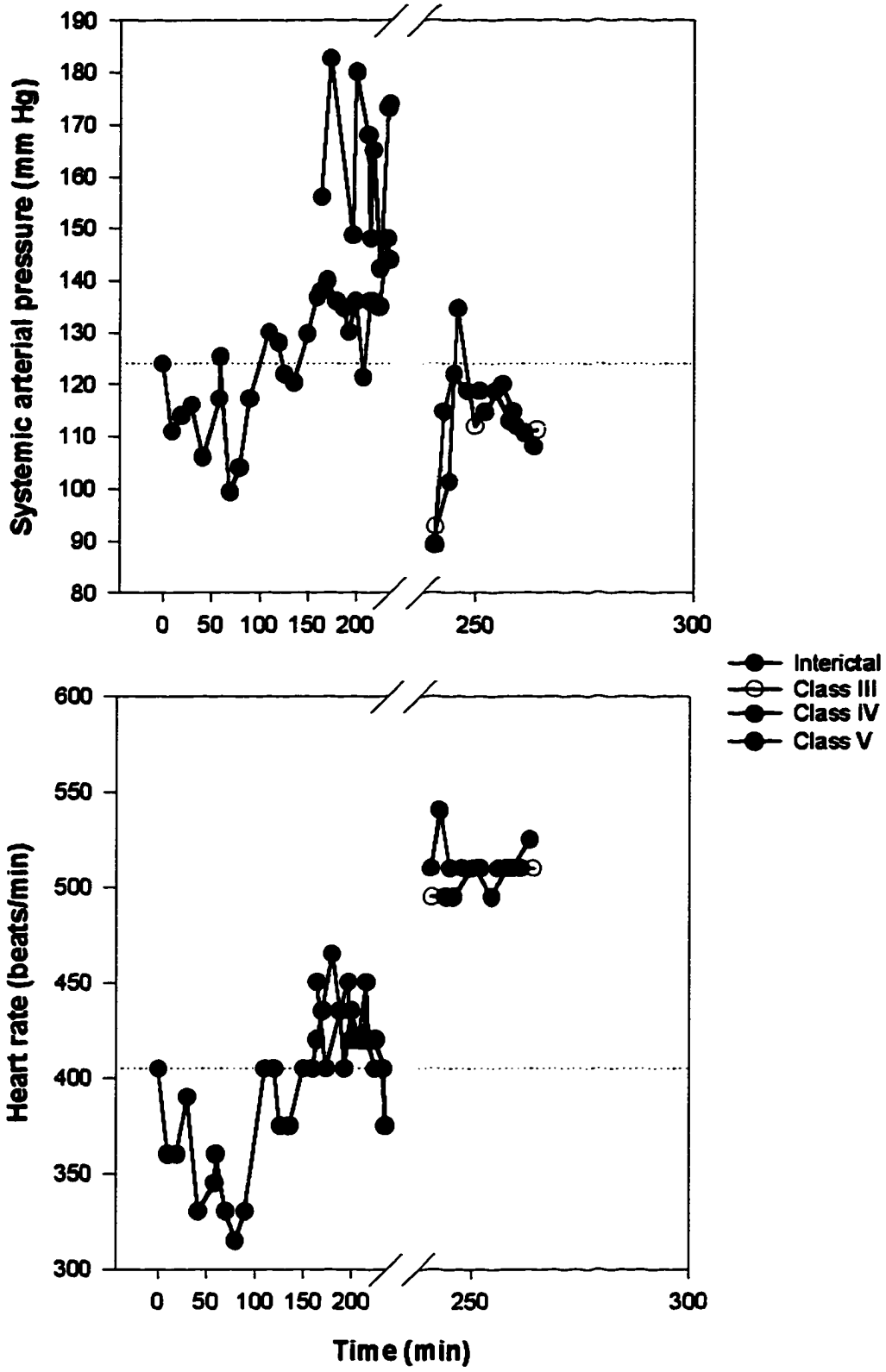


Figure A-3. Time course of mSAP and HR responses for KA-treated rat 321 during the induction protocol.

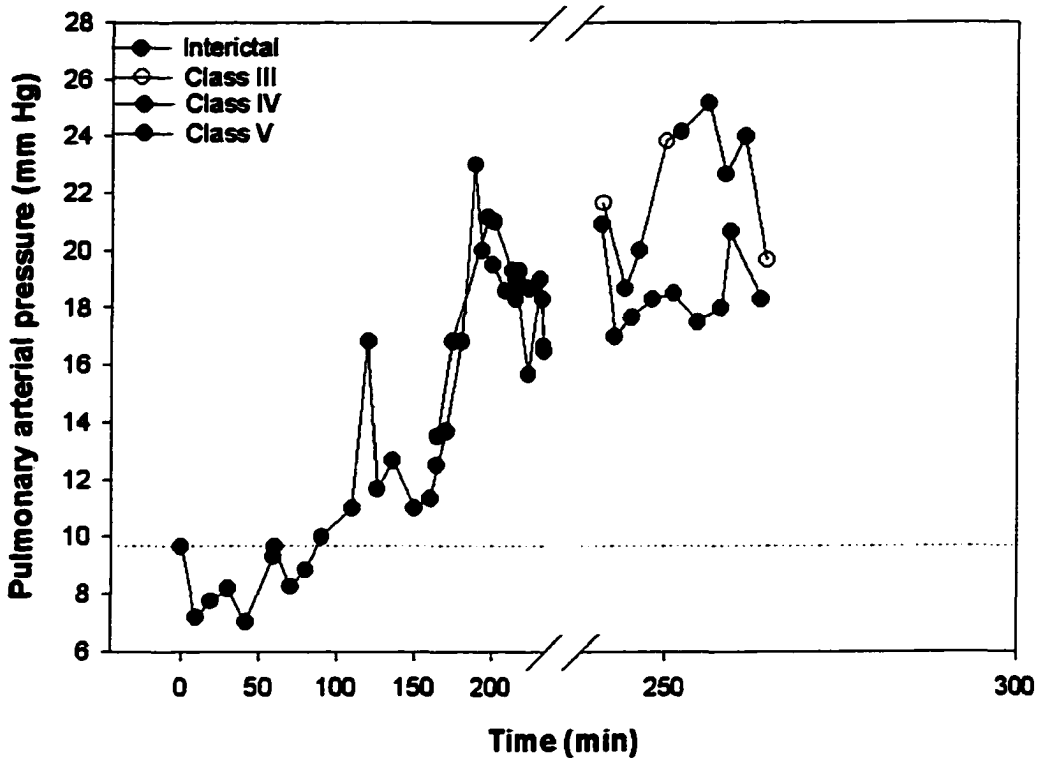


Figure A-4. Time course of mPpa response for KA-treated rat 321 during the induction protocol.

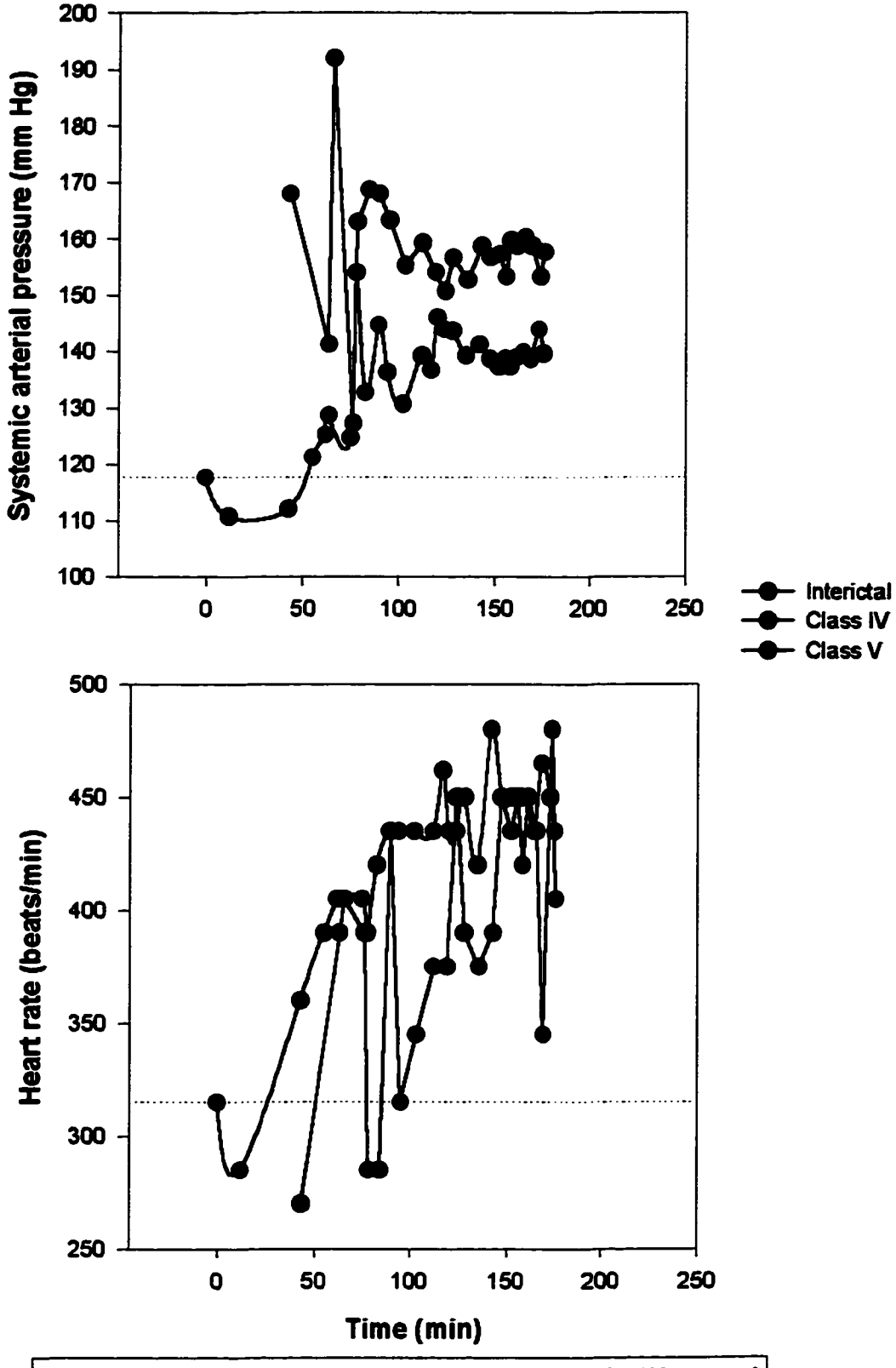


Figure A-5. Time course of mSAP and HR response for KA-treated rat 312 during the induction protocol.

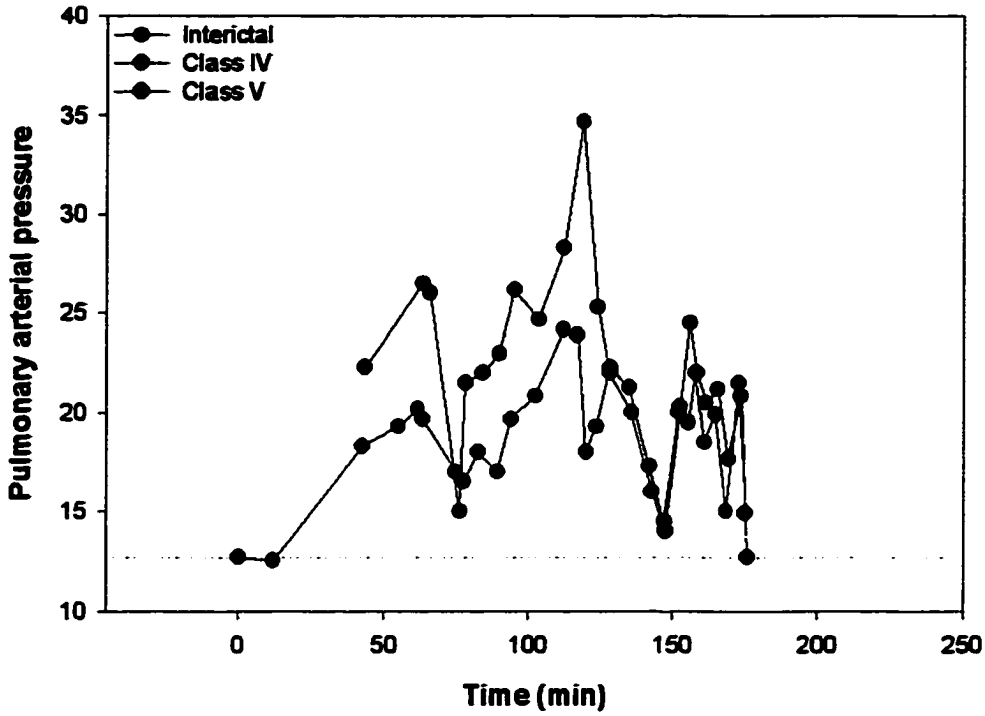


Figure A-6. Time course of mPpa response for KA-treated rat 312 during the induction protocol.

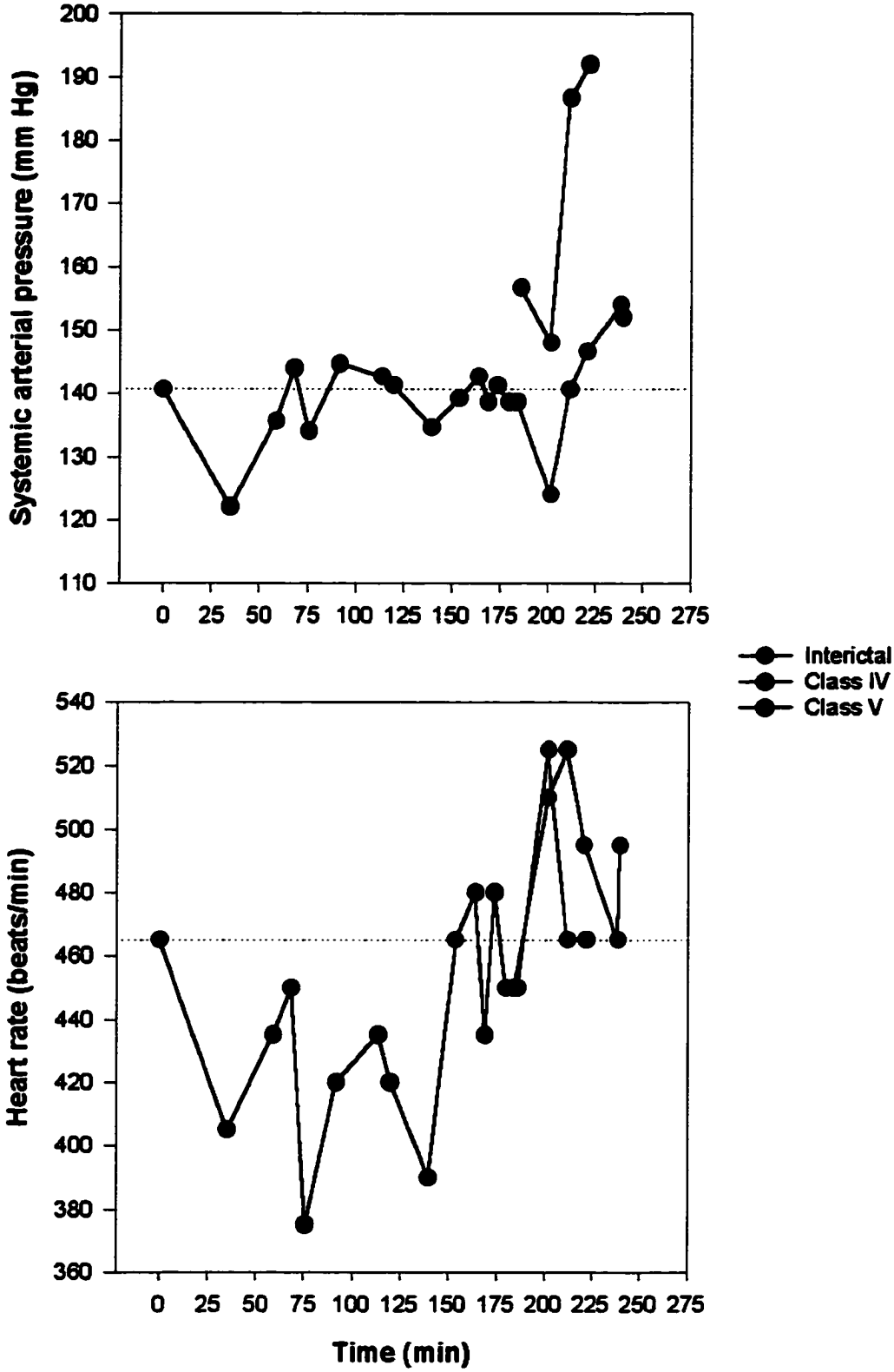


Figure A-7. Time course of mSAP and HR response for KA-treated rat 315 during the induction protocol.

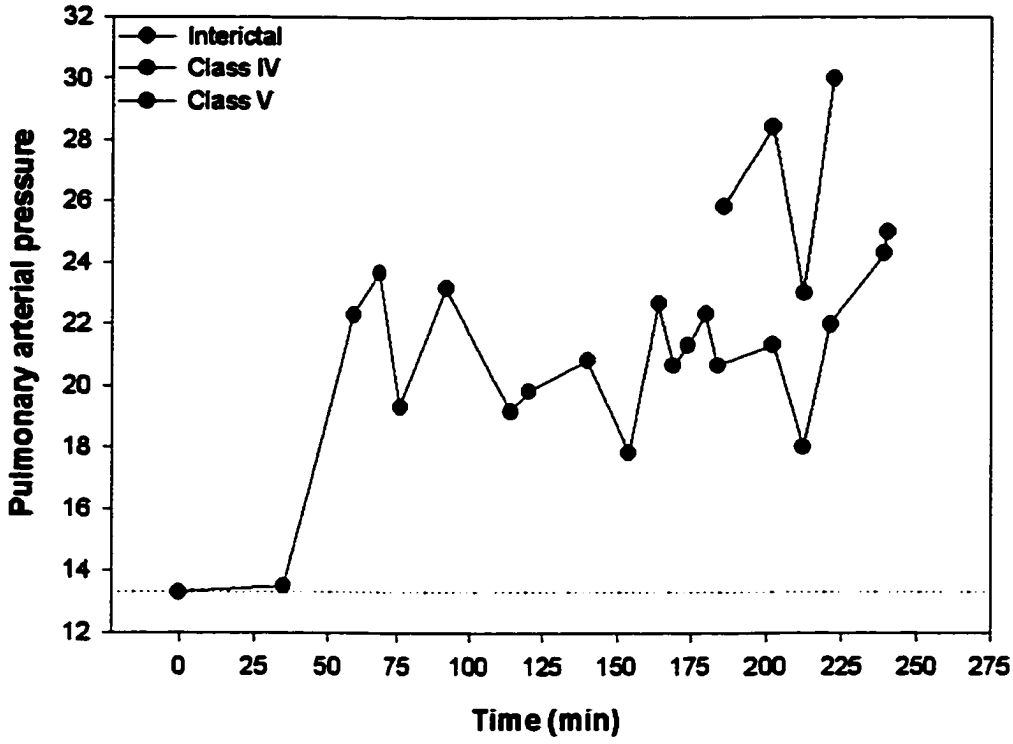


Figure A-8. Time course of mPpa response for KA-treated rat 315 during the induction protocol.

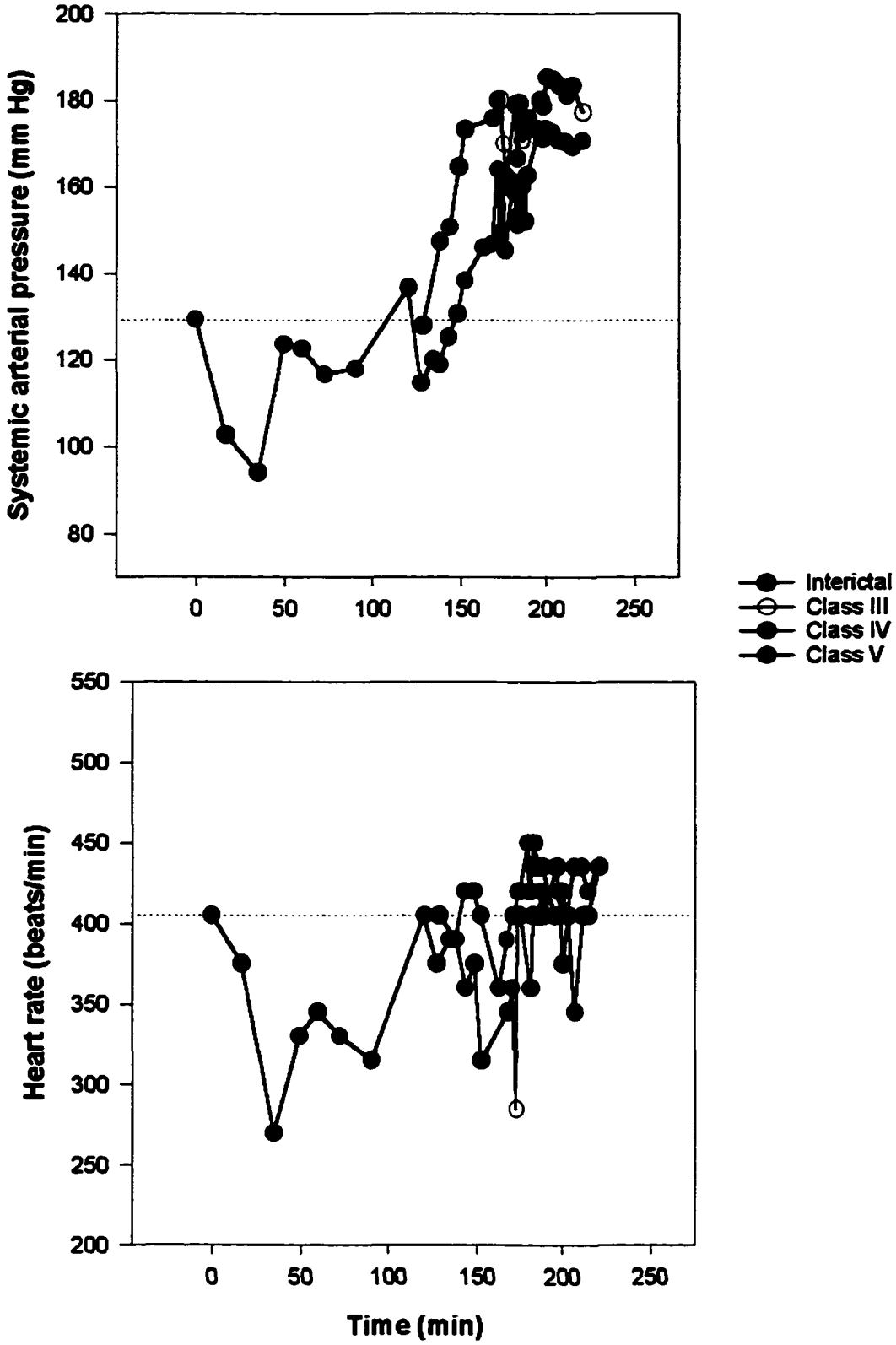


Figure A-9. Time course of mSAP and HR responses for KA-treated rat 304.

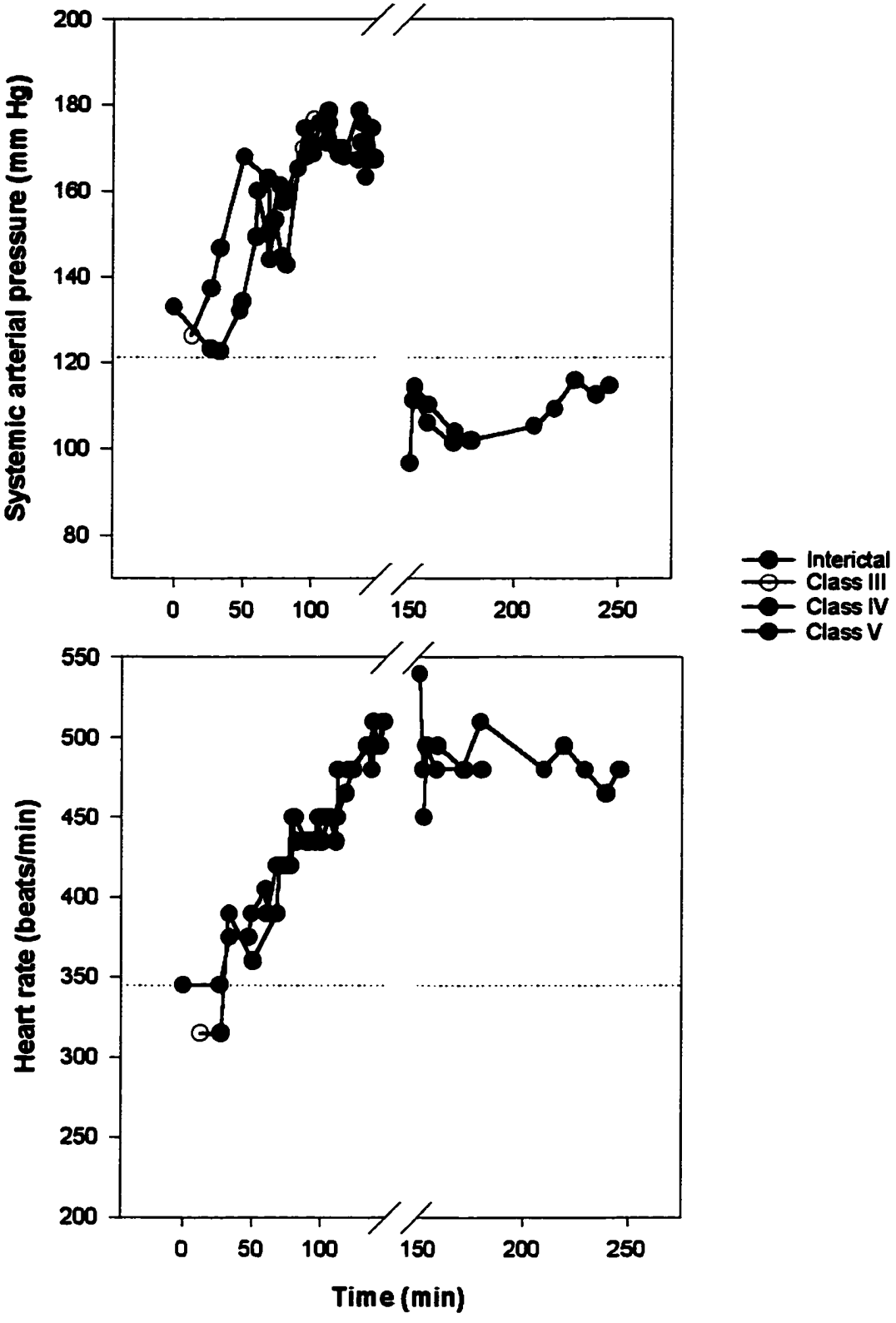


Figure A-10. Time course of mSAP and HR responses for KA-treated rat 314a

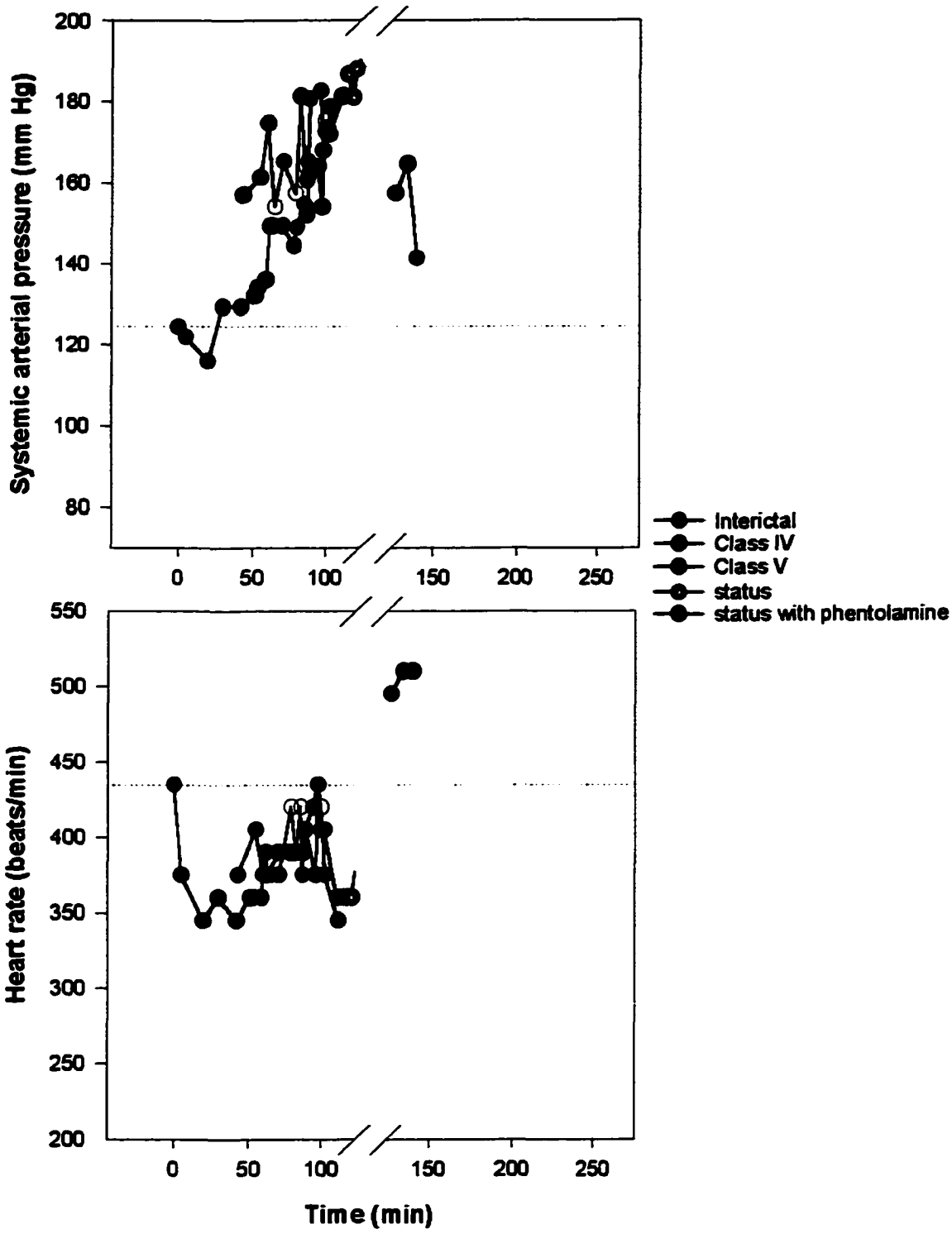


Figure A-11. Time course of mSAP and HR responses for KA-treated rat 320.

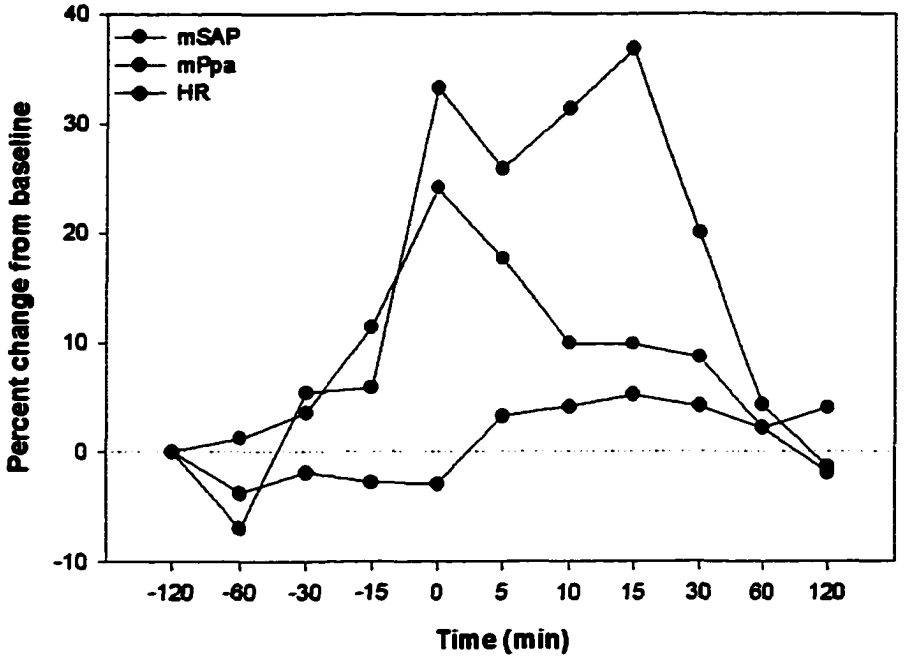


Figure A-12. Seizure profile for KA-treated rat 307. Each time point represents the average of 5 class V seizures.

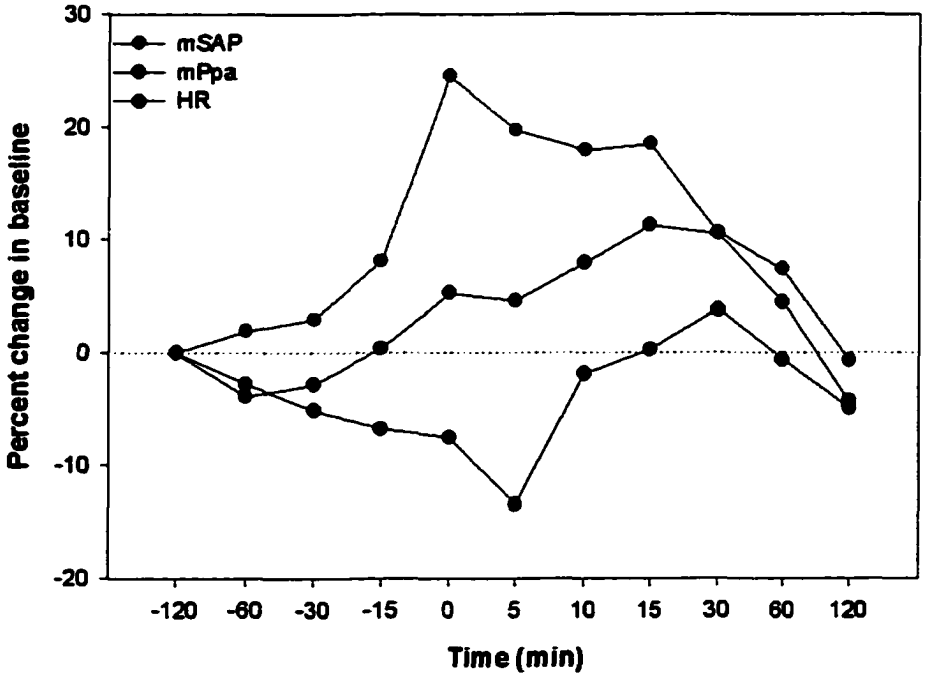


Figure A-13. Seizure profile for KA-treated rat 321. Each time point represents the average of 4 Class IV and 4 Class V seizures.

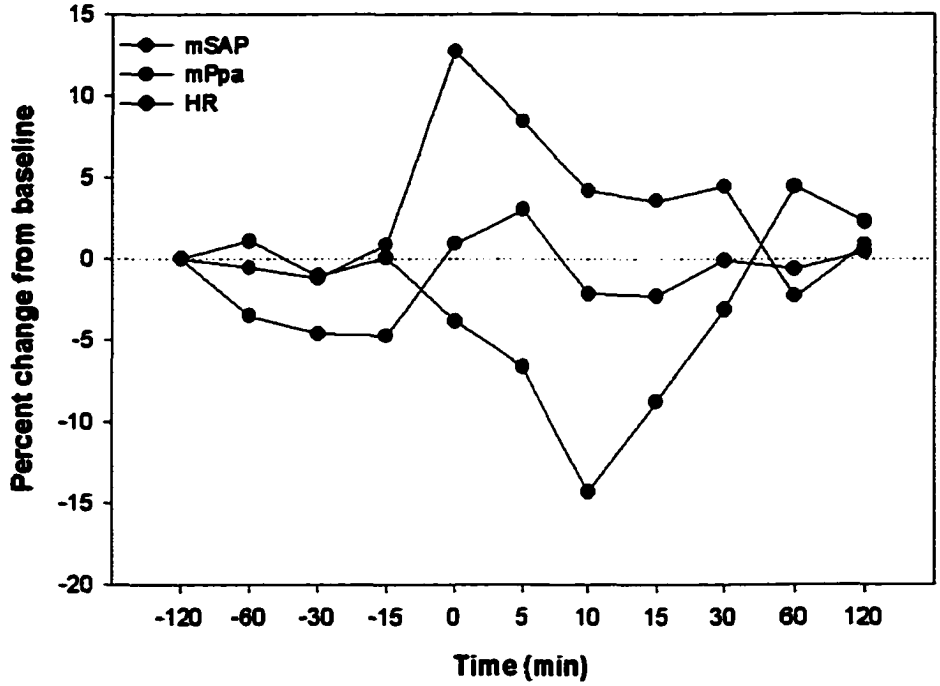


Figure A-14. Seizure profile for acute KA-treated rat 312. Each time point represents the average of 5 Class V seizures.

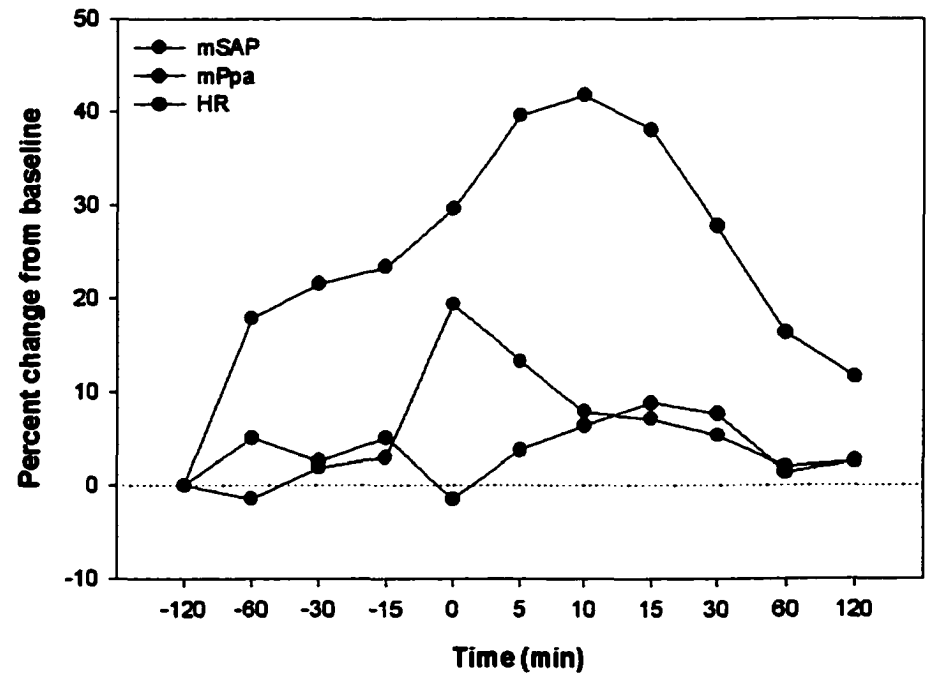


Figure A-15. Seizure profile for KA-treated rat 314. Each time point represents the average of 2 Class IV and 5 Class V seizures.

APPENDIX B

Additional graphs for Chapter IV

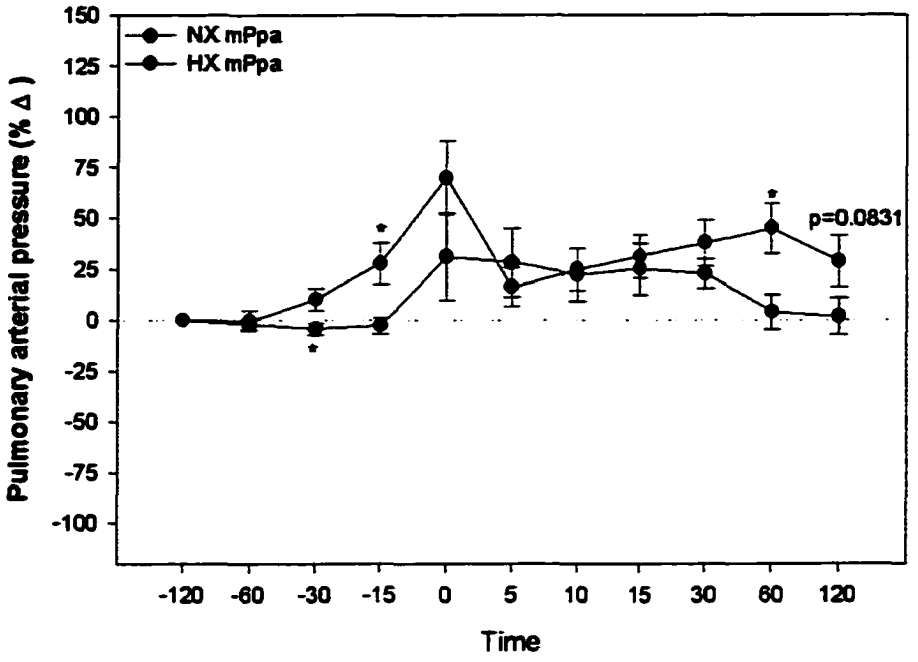


Figure B-1. Comparison of mPpa profiles for seizures recorded during NX and HX. NX seizures (8 seizures; 4 animals) HX seizures (9 seizures; 3 animals).p<0.05.

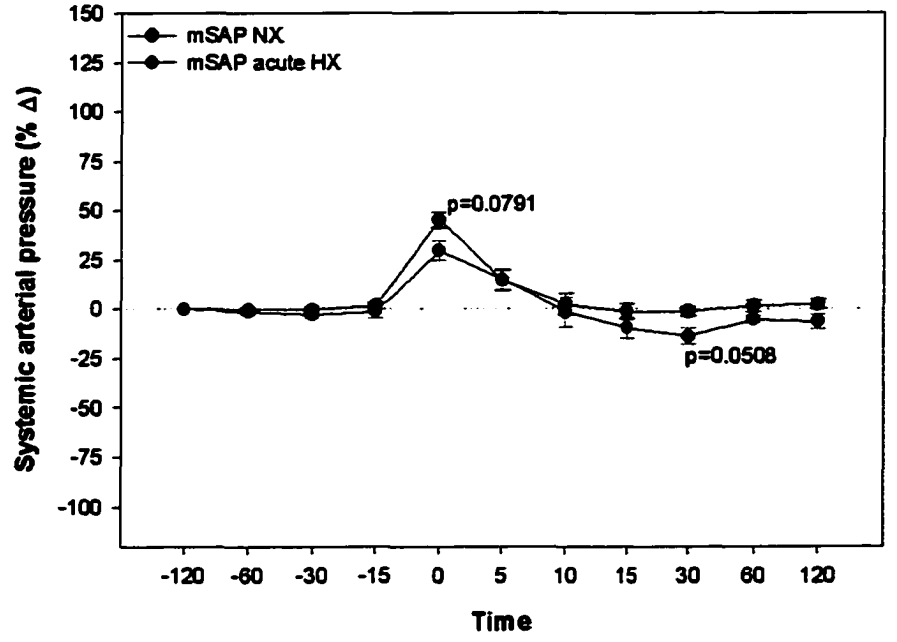


Figure B-2. Comparison of mSAP responses during seizures under NX and HX. NX (15 seizures; 6 animals), HX (7 seizures; 4 animals).p<0.05.

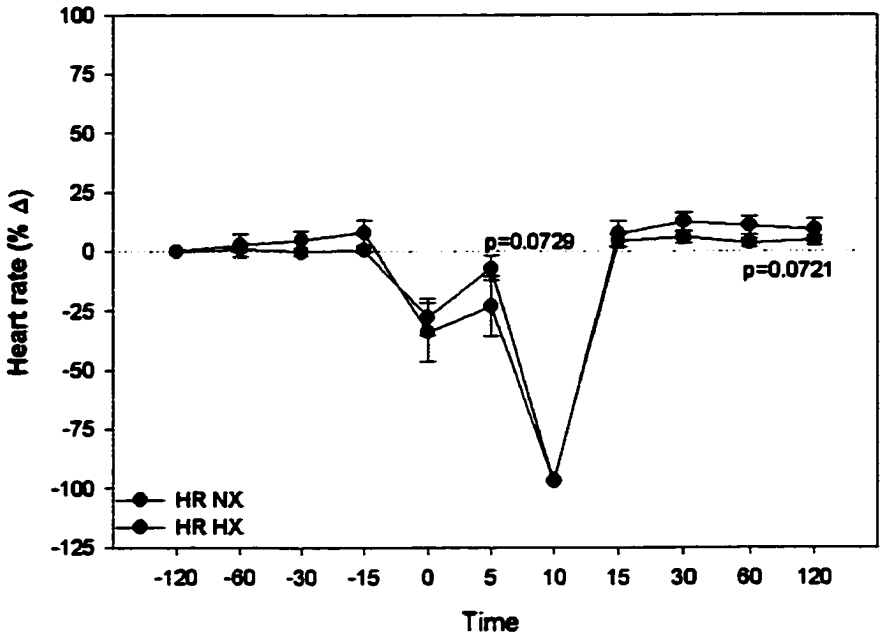


Figure B-3. Comparison of HR responses during seizures under NX and HX. NX (17 seizures; 7 animals), HX (10 seizures, 4animals). p<0.05.

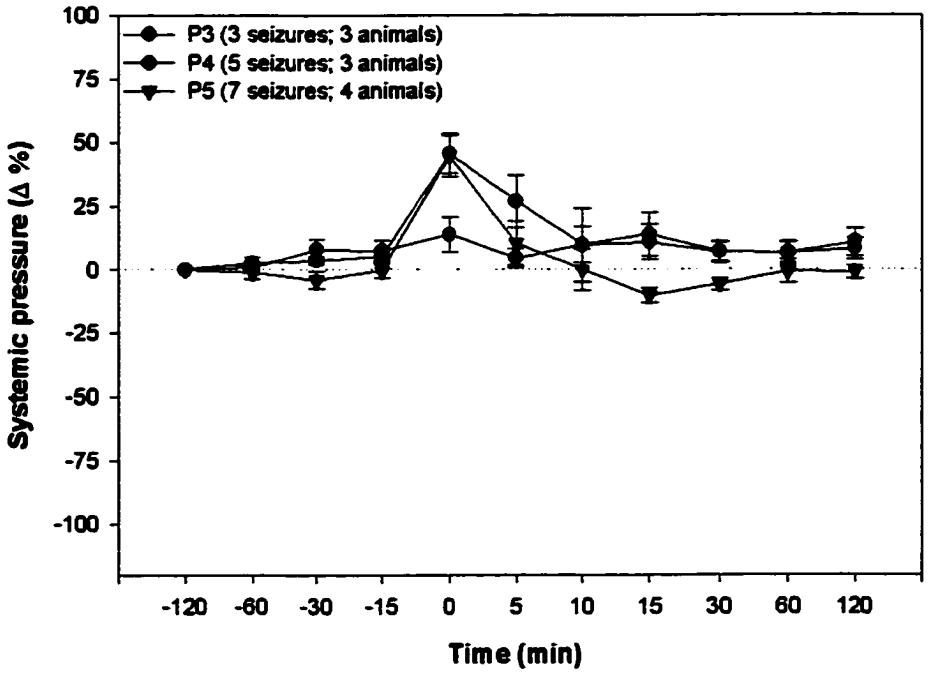


Figure B-4. mSAP profile for seizures occurring during NX by seizure severity.

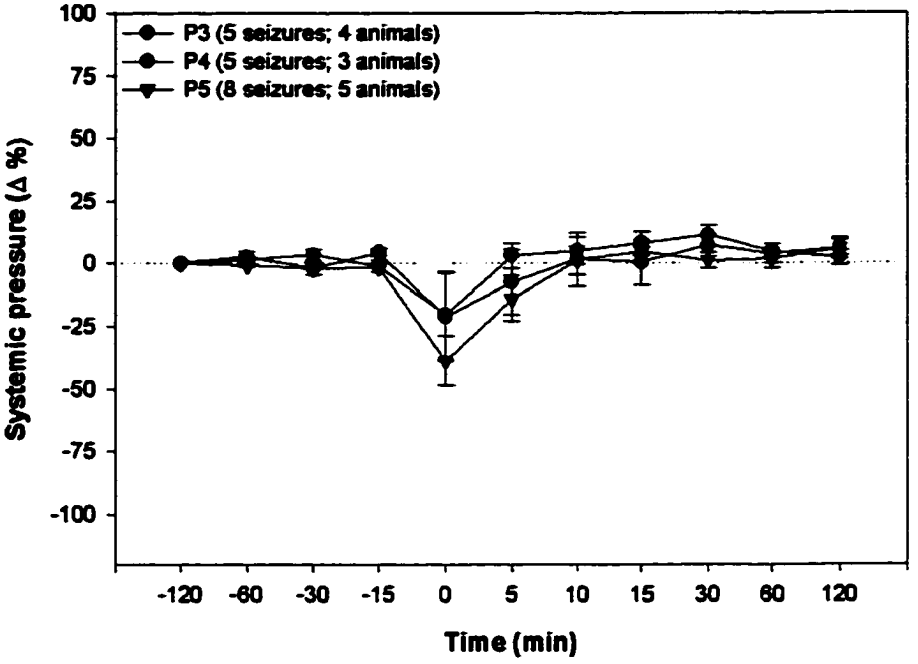


Figure B-6. HR profile for seizures occurring during NX by seizure severity.

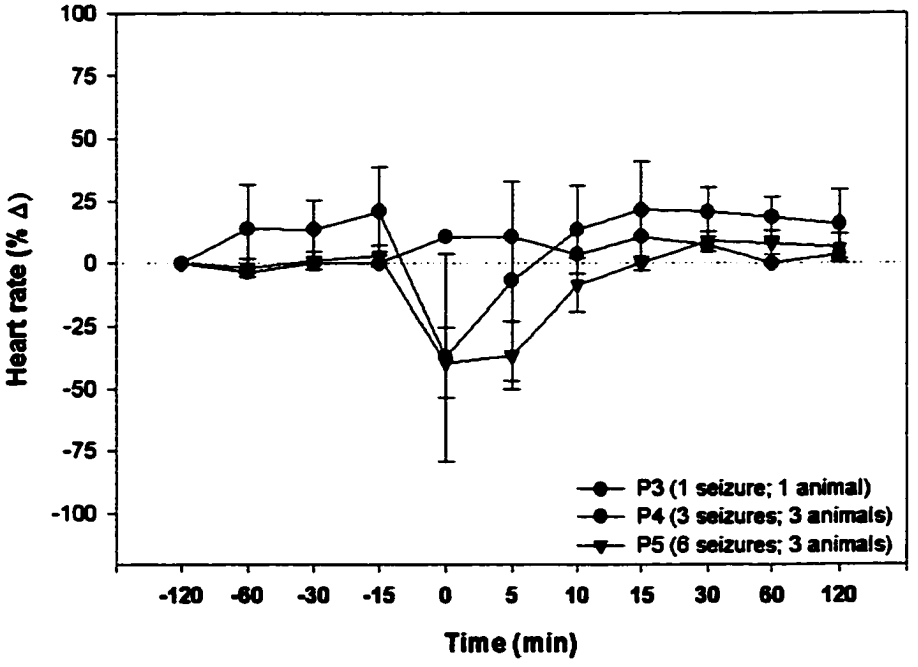
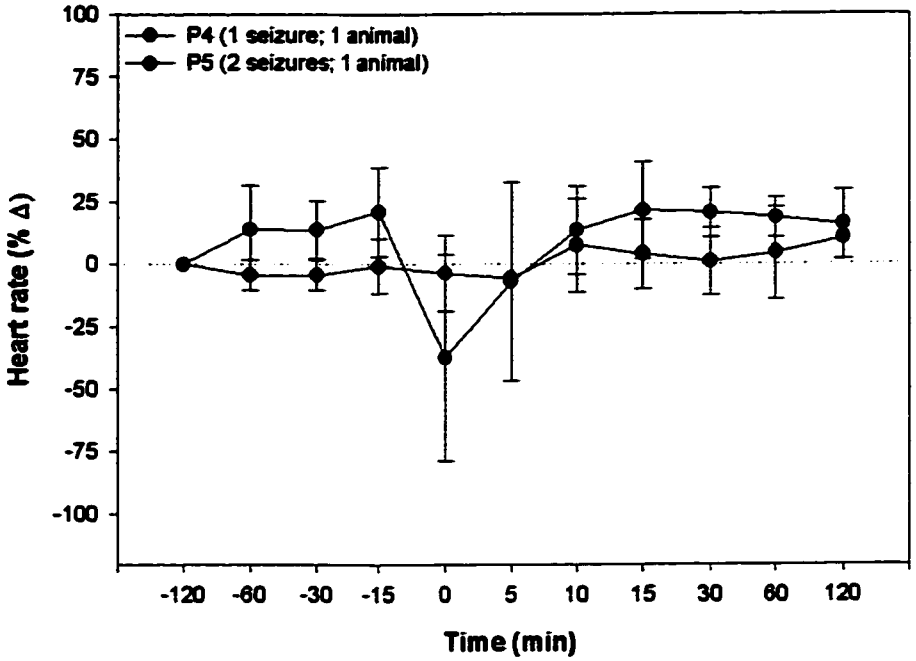


Figure B-7. HR profile for seizures occurring during HX by seizure severity.



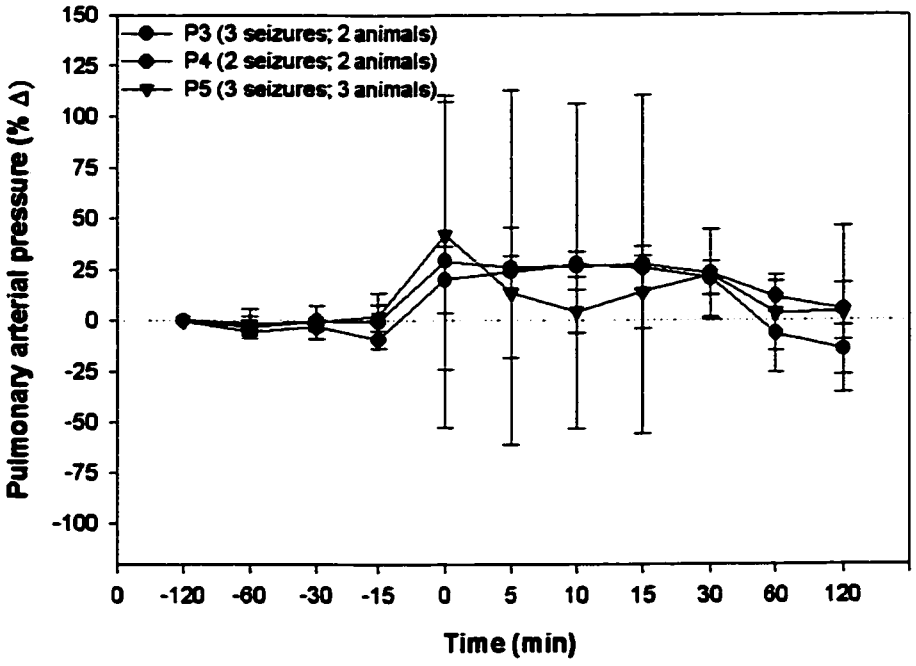


Figure B-9. mPpa profiles for seizures occurring during NX by seizure severity.

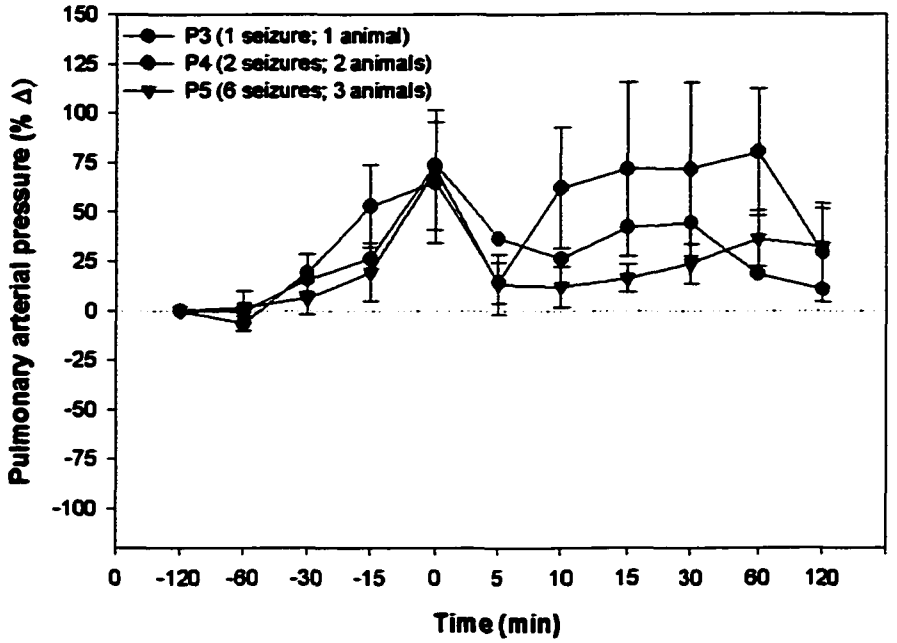


Figure B-10. mPpa profile for seizures occurring during HX by seizure severity.

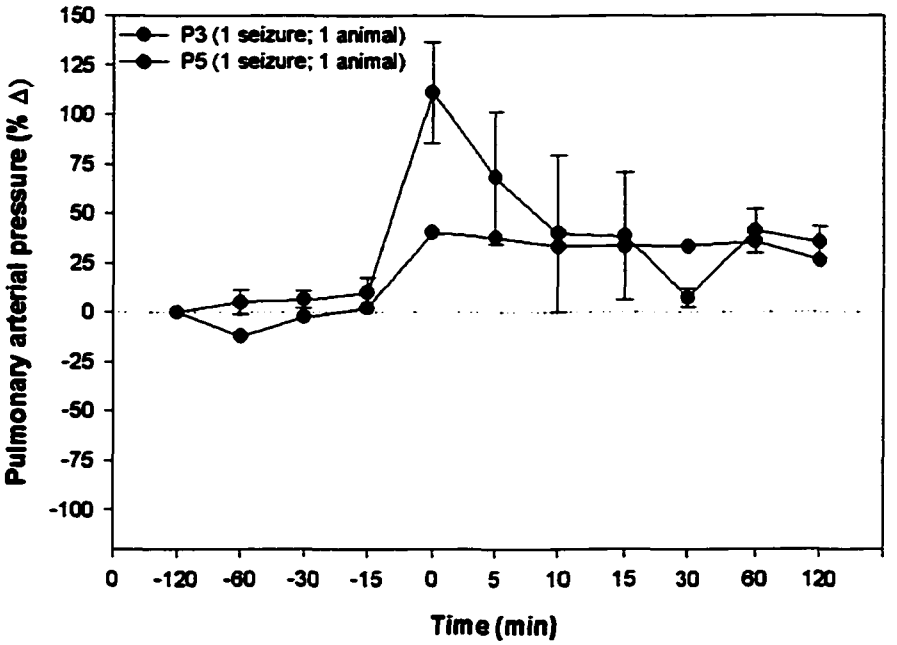


Figure B-11. mPpa profile for seizures occurring during NX following phentolamine.

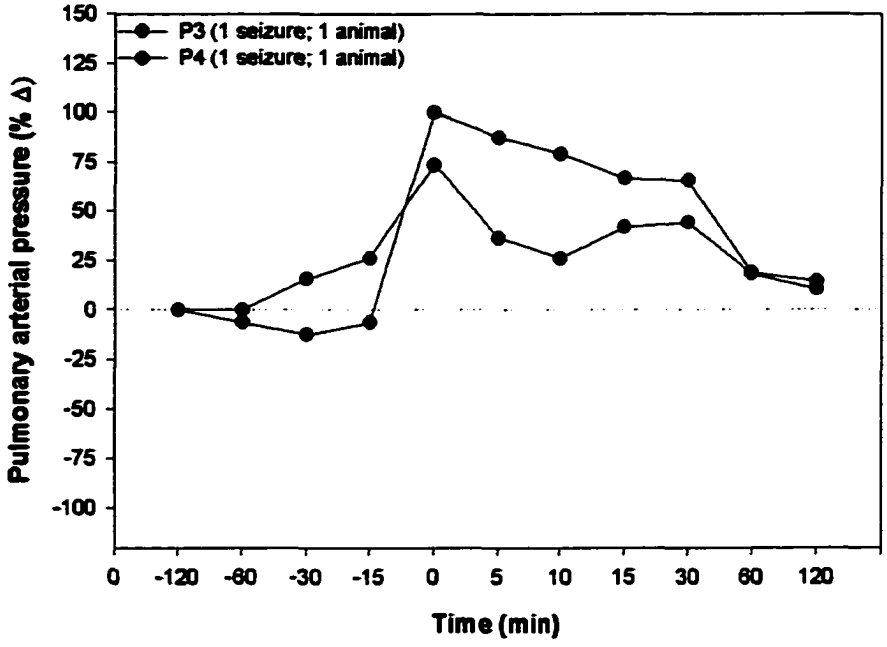
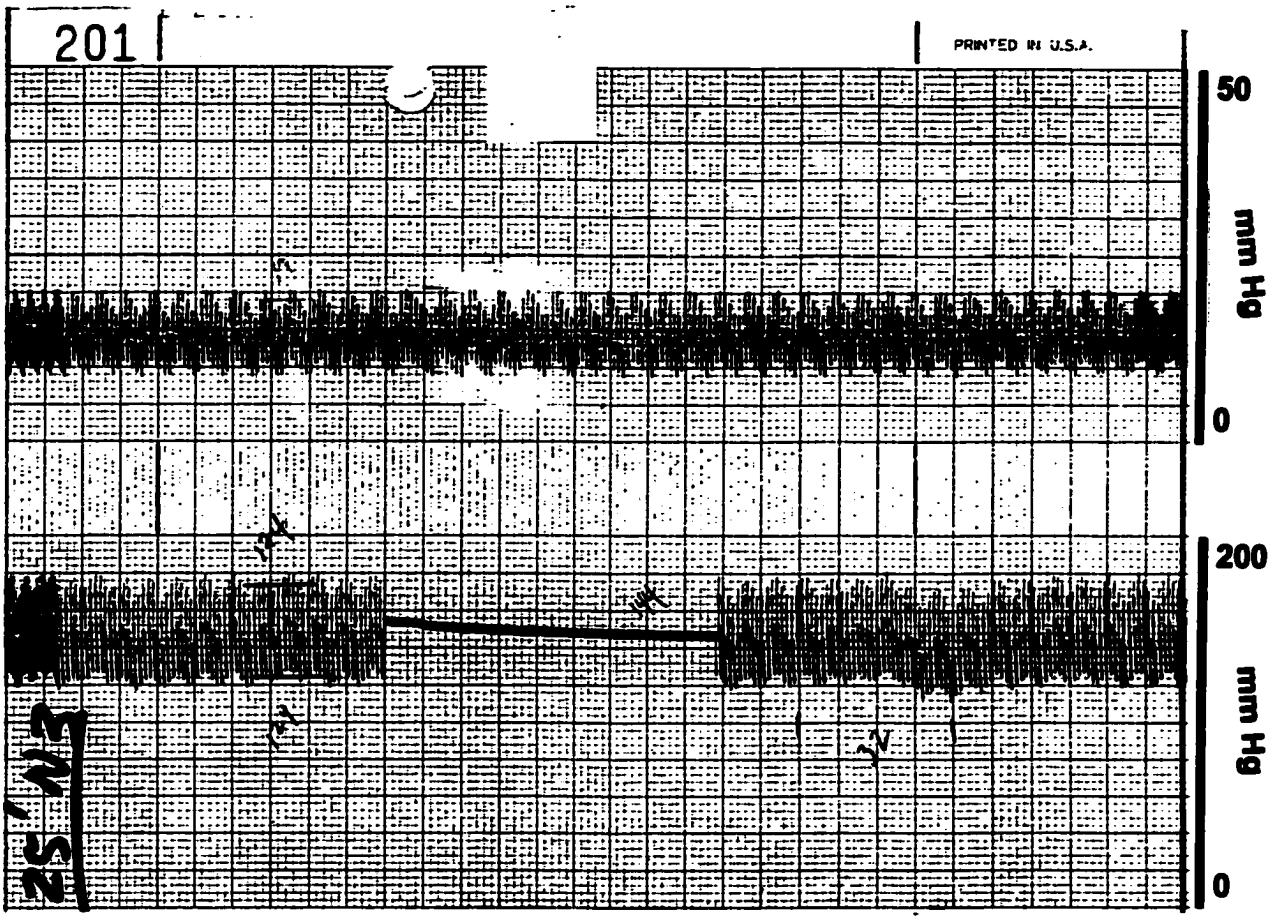
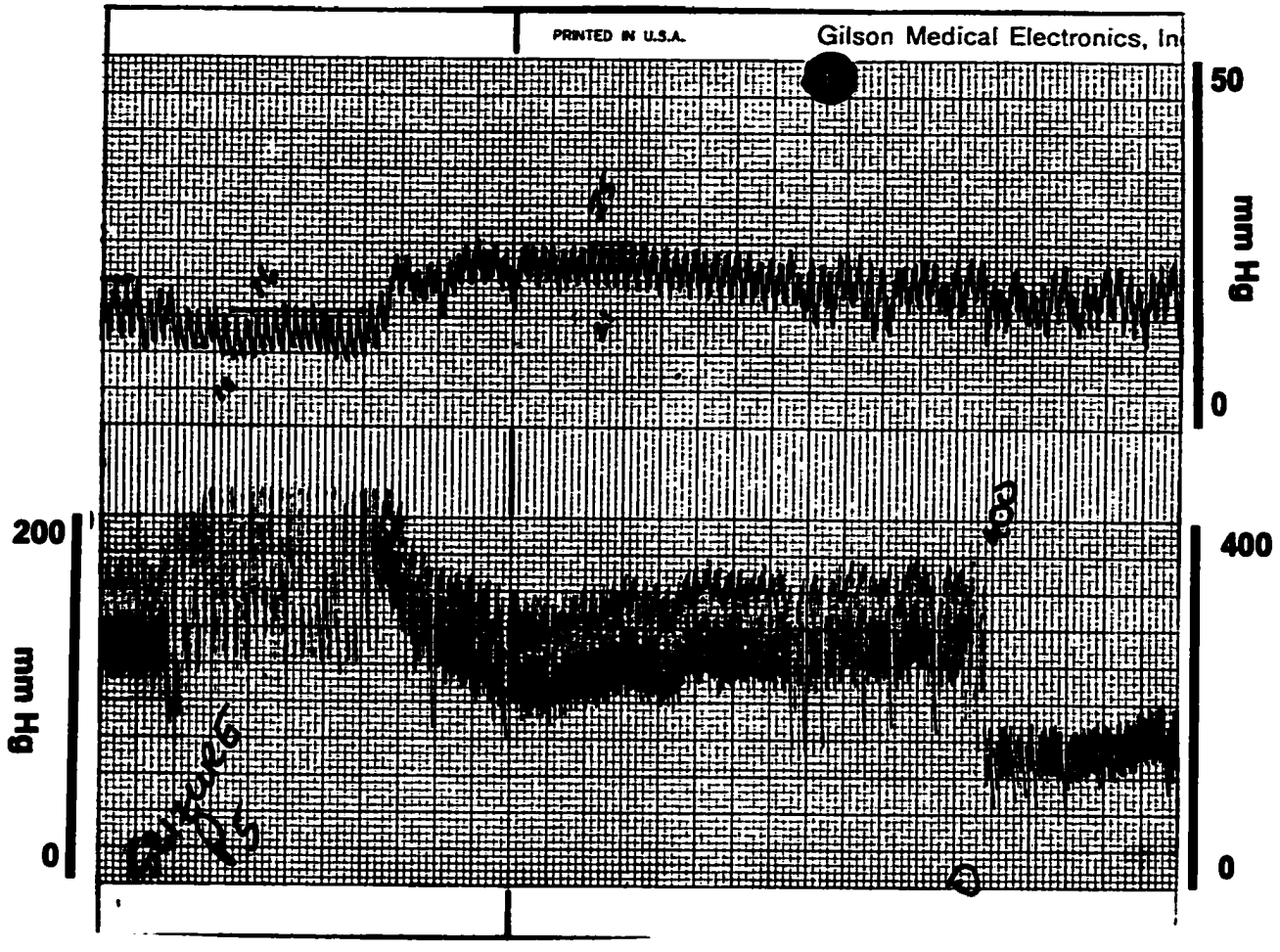


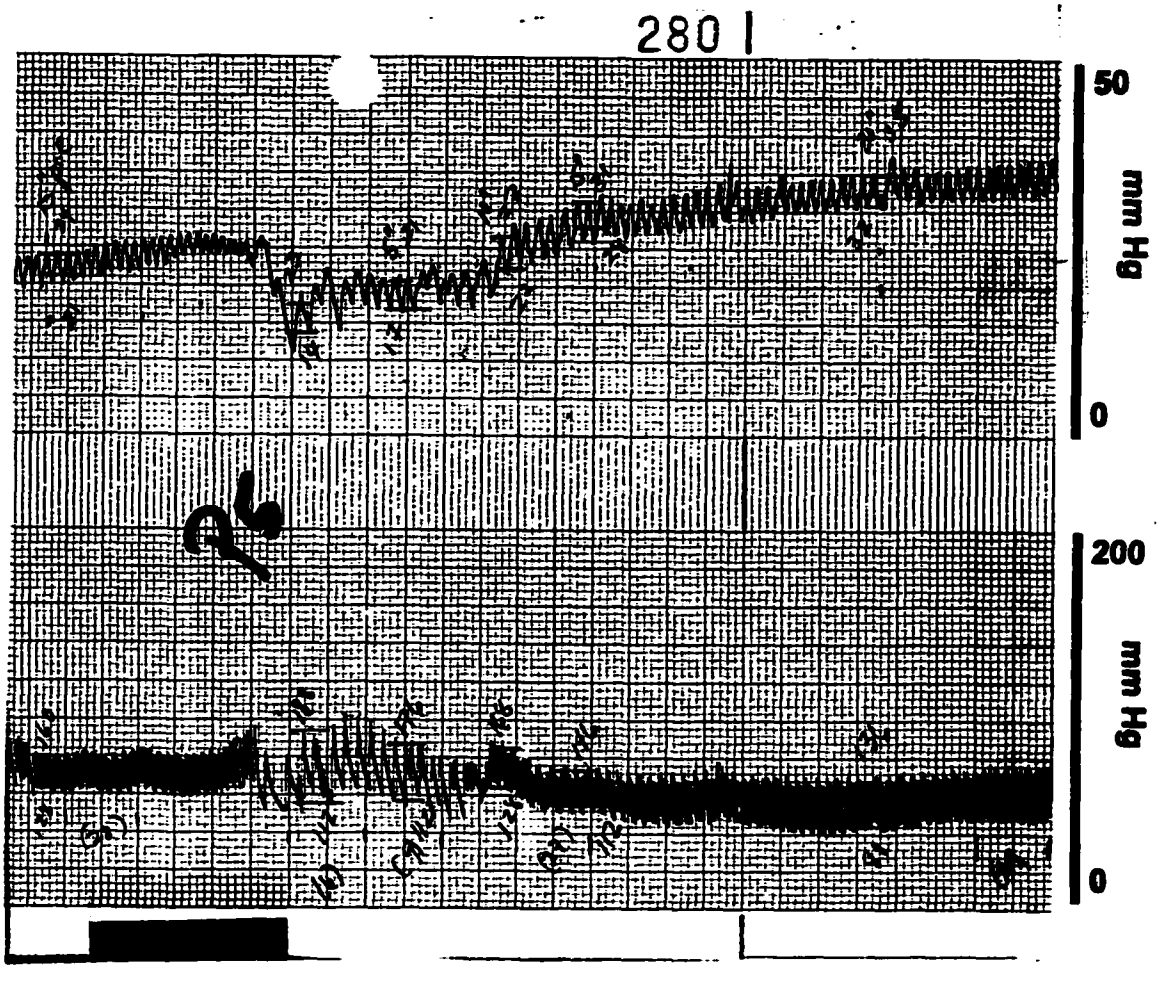
Figure B-12. mPpa profiles for seizures occurring during HX following phentolamine.



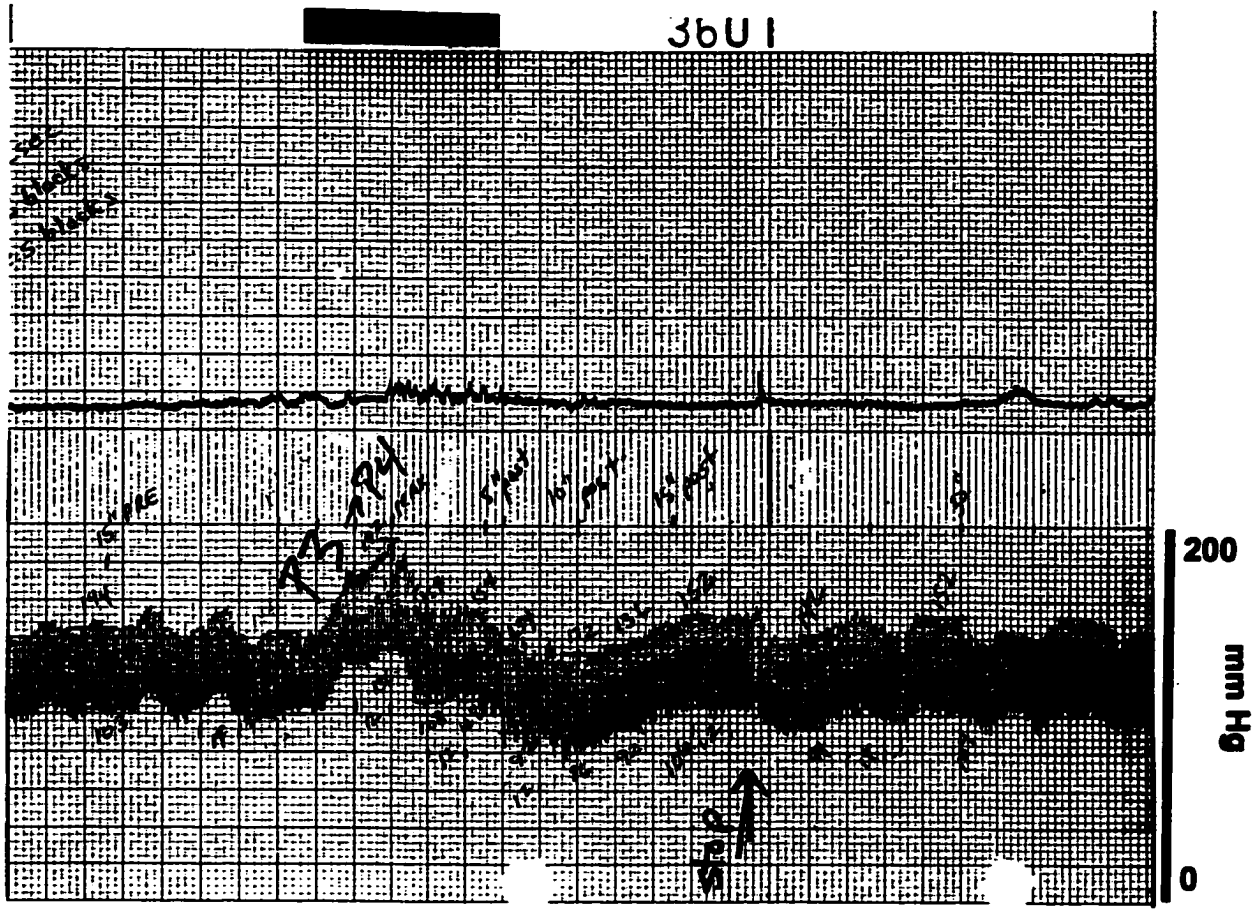
Systemic and pulmonary pressure tracing recorded during NX baseline period.



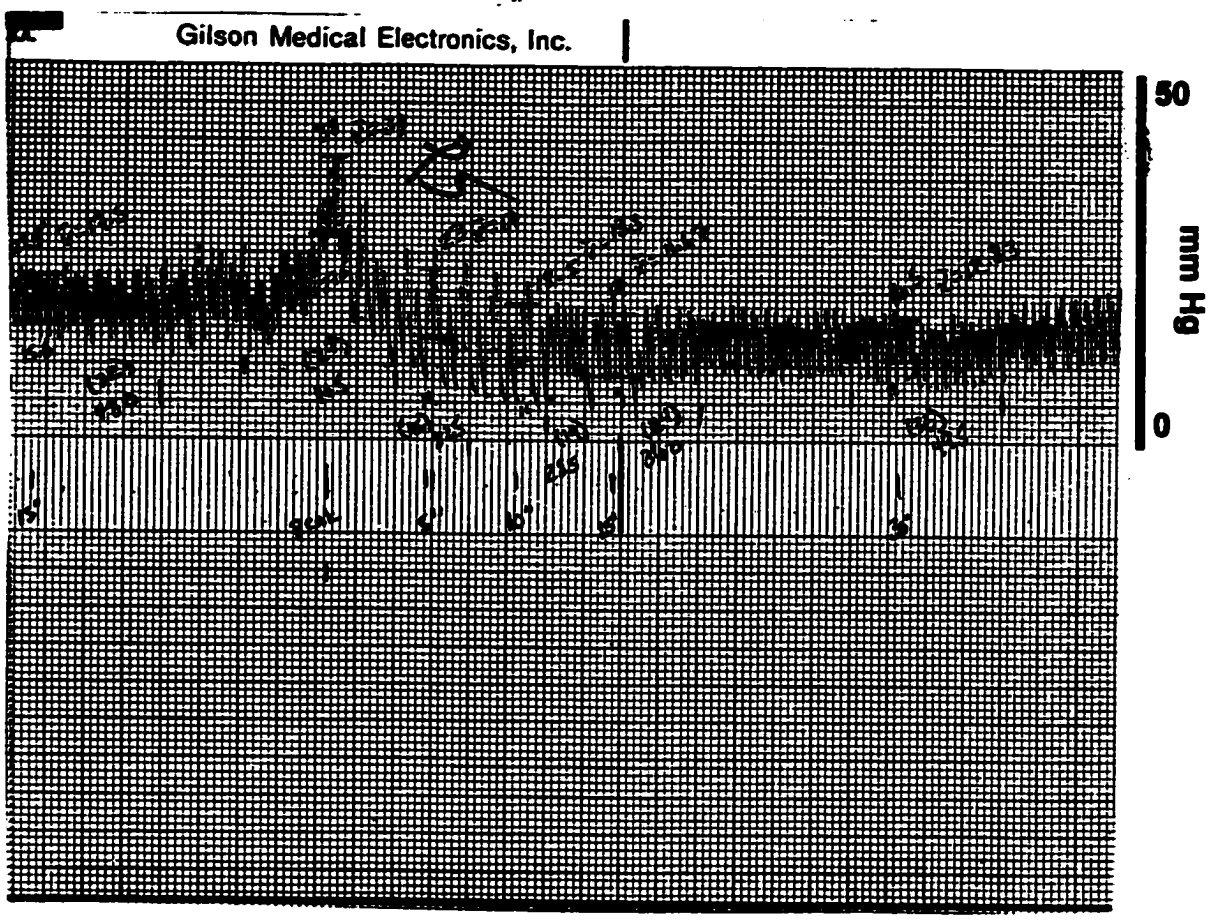
Systemic and pulmonary pressure tracing recorded during a Class V motor seizure under NX conditions.



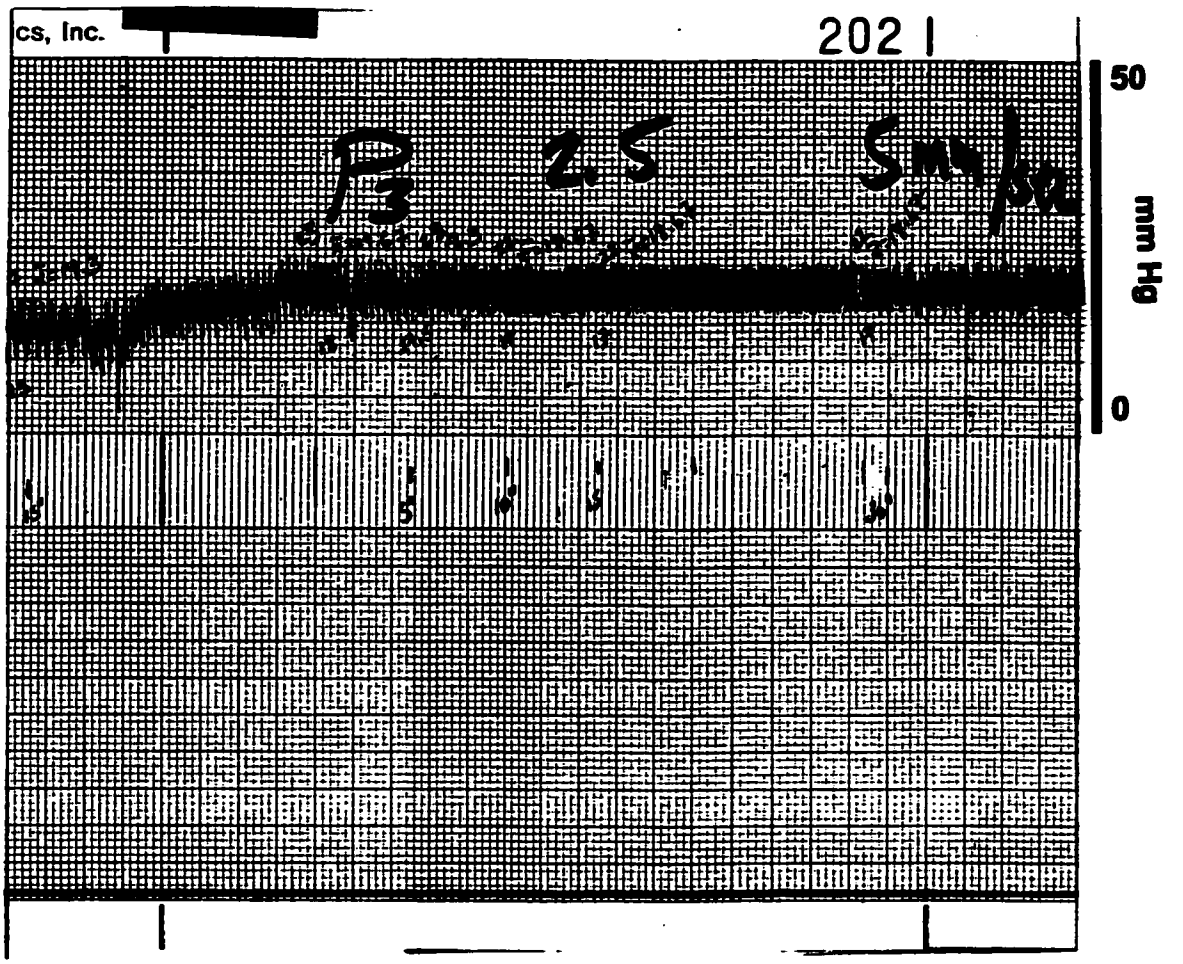
Systemic and pulmonary pressure tracing recorded during a Class V motor seizure during an acute HX challenge.



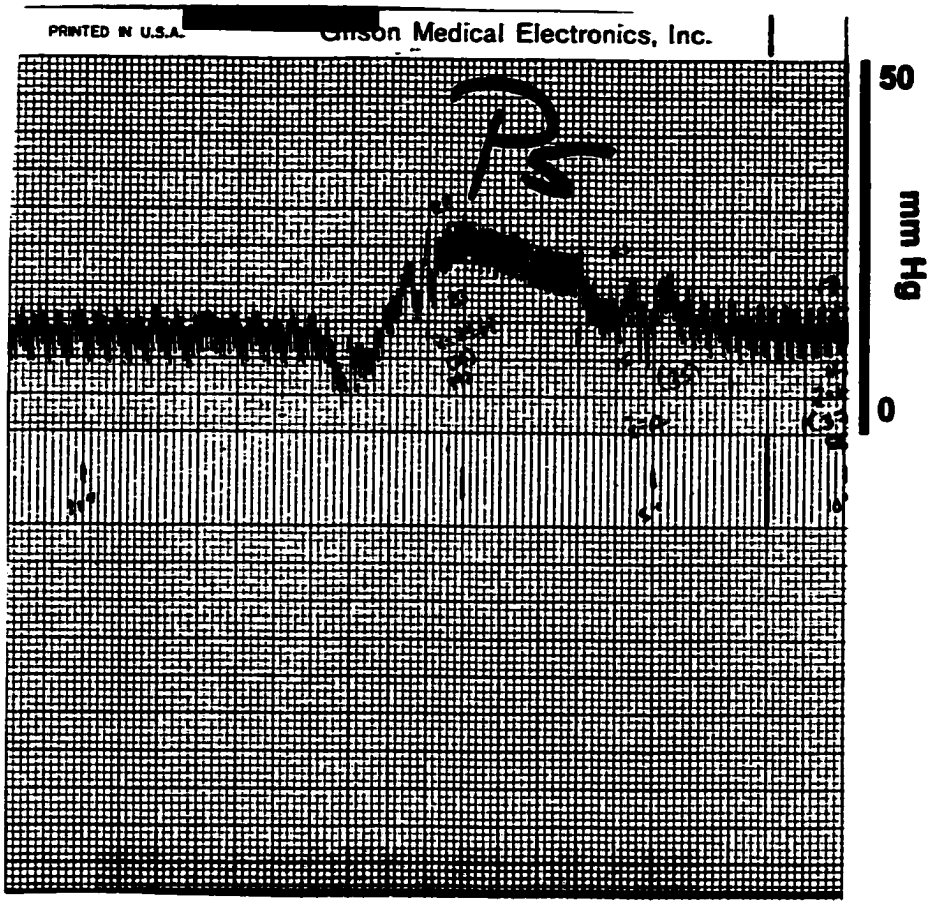
Systemic pressure tracing recorded during a Class III – IV motor seizure during initial NX period.



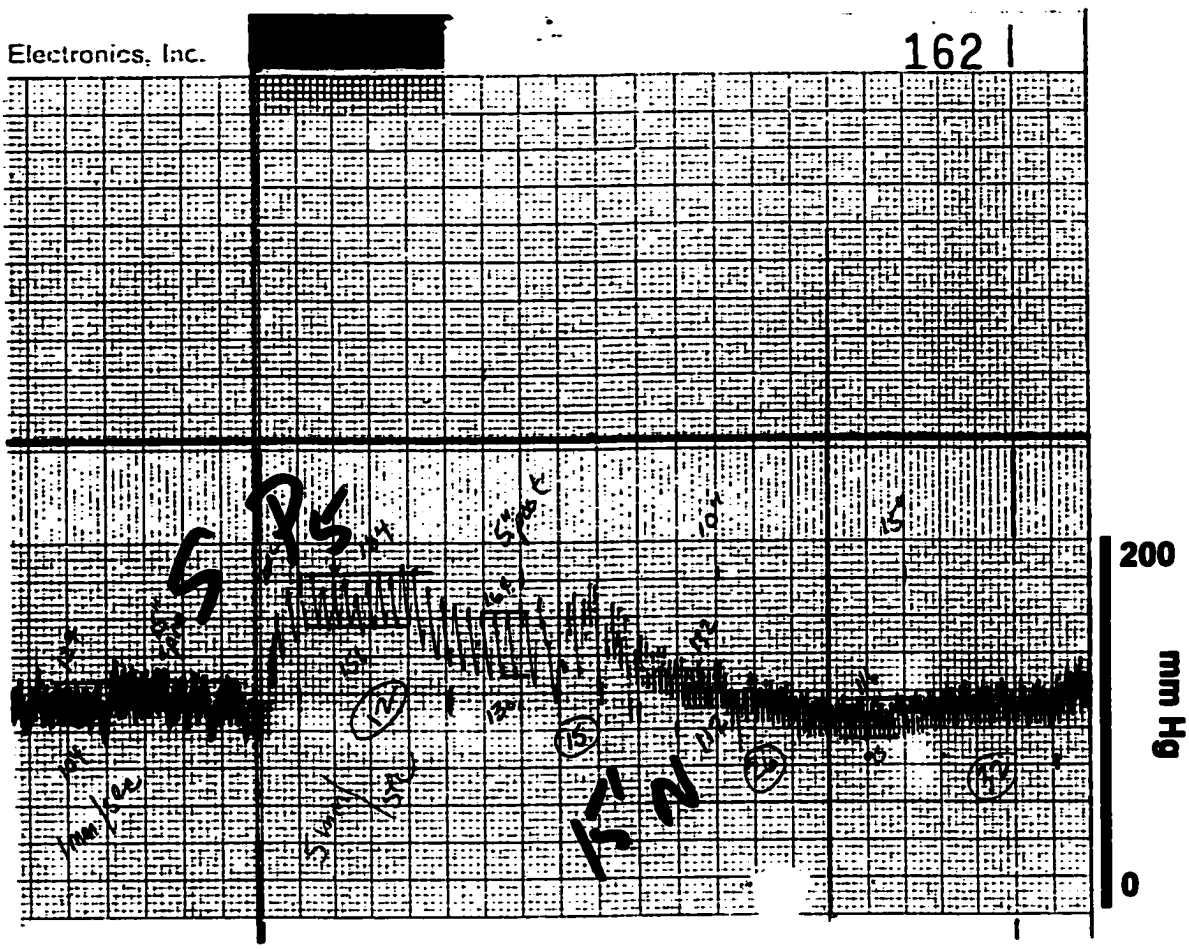
Pulmonary pressure tracing recorded during a Class V motor seizure during initial NX period.



Pulmonary pressure tracing recorded during a Class III motor seizure under NX conditions following phentolamine



Pulmonary pressure tracing recorded during a Class V motor seizure under NX conditions following phentolamine



Systemic pressure tracing recorded during a Class V seizure during NX.