# HYPOXIC RESPONSES OF SYMPATHETIC PREGANGLIONIC NEURONS IN THE ACUTE SPINAL CAT

BY

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ABSTRACT

The relation between $PaO_2$ and the firing rate of sympathetic preganglionic neurons (SPN) of the cervical sympathetic trunk was studied during graded isocapnic hypoxia and hyperoxia in unanaesthetized acute C-1 spinal cats. Between 40 and 400 mmHg $PaO_2$ there was no relation between the two variables. Below 40 mmHg firing rate increased as $PaO_2$ decreased, reaching an average peak value of ten times control at 20 mmHg $PaO_2$. A similar response was observed in CNS intact, anaesthetized, and peripherally chemodenervated preparations. Mean arterial pressure (MAP) was also independent of $PaO_2$ between 40 and 400 mmHg and increased by an average of 25% below 40 mmHg $PaO_2$. Hexamethonium or phentolamine (i.v.) abolished the MAP response but not the SPN response to hypoxia. Pentobarbital (5-60 mg/kg i.v.) did not modify the SPN response to hypoxia although normoxic SPN background firing was considerably depressed. The excitatory effect of hypoxia seems independent of excitatory afferent input and appears to be a general property of SPNs.

RESUME

La relation entre $PaO_2$ et la fréquence de décharge des neurones préganglionaires sympathiques (NPS) du nerf cervical sympathique a été étudiée durant l'hypoxie isocapnique graduée et l'hyperoxie dans les chats non anesthésiés avec moëlle épinière sectionnée au niveau de C-1. Entre 40 et 400 mmHg $PaO_2$, il n'y avait aucune relation entre les variables. Au-dessous de 40 mmHg, la fréquence de décharge a augmenté pendant que $PaO_2$ a diminué, atteignant une valeur moyenne maximale dix fois plus grande à 20 mmHg $PaO_2$ qu'en normoxie. Une réaction similaire a été observée dans des préparations anesthésiées, au système nerveux central intact, dont les chémorécepteurs périphériques fut denervés. La pression artérielle moyenne (PAM) était également indépendante de $PaO_2$ entre 40 et 400 mmHg et augmentait en moyenne de 25% au-dessous de 40 mmHg. Hexamethonium ou phentolamine (i.v.) a aboli la réaction de la PAM à l'hypoxie mais non celle des NPS à l'hypoxie. Pentobarbital (5-60 mg/kg i.v.) n'a pas modifié la réaction des NPS à l'hypoxie même si la décharge des NPS à normoxie était considérablement basse. L'effet stimulant de l'hypoxie semble indépendant des afférents excitatoires et apparaît être une propriété générale des NPS.

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# I. INTRODUCTION

The autonomic nervous system (ANS) plays a key role in the maintenance of a relatively constant and adequate internal environment in the homeothermic vertebrate organism. To this end it exerts considerable control over a number of aspects of circulatory and visceral function. This is achieved through a continuous outflow of neural information to the various effector mechanisms (Polosa et al., 1979). The existence of such tonic efferent activity raises a basic question concerning the functioning of the ANS; that is how is this tonic activity generated?

In studying this question it has been demonstrated that a significant portion of the sympathetic preganglionic neuron (SPN) population, with axons travelling in the cervical sympathetic trunk (CST), is tonically active in the anaesthetized animal preparation with an intact central nervous system (CNS) (Polosa, 1968; Janig & Schmidt, 1970). More extensive examination by Mannard and Polosa (1973) confirmed this observation and showed that this was also the case in the unanaesthetized decerebrate animal preparation. In addition, it was found that spinal cord transection at C-1 reduced the amount of tonic SPN activity and that section of the dorsal roots further attenuated tonic SPN firing. Neverthless, some tonic SPN activity has been seen to persist in the isolated spinal cord fragment prepared by section of the spinal cord at C-8 and T-5 and section of the dorsal roots between these two levels (Polosa, 1968; Mannard & Polosa, 1973). Similar

results have been obtained by DeGroat and Krier (1979) while recording from the postganglionic lumbar colonic nerves innervating the large intestine. In the latter study tonic sympathetic activity persisted in the isolated upper lumbar spinal cord, following spinal cord transection at T-13 and L-5, even after section of the lumbar dorsal roots. This postganglionic activity was not generated peripherally in the sympathetic ganglia since it was eliminated by section of the lumbar ventral roots.

On the basis of these observations it would appear that spinal as well as supra-spinal afferent connections play an important role in the generation of tonic SPN activity. However, the persistence of tonic SPN activity in the "isolated" spinal cord has led to the suggestion that reverberating neural circuits, putative pacemaker properties of SPNs, or physico-chemical factors such as temperature or $PO_2$ of the neuronal environment may also have a role in the generation of tonic SPN activity (Polosa, 1968; Koizumi & Brooks, 1972). None of these possibilities have been clearly demonstrated. The study presented here deals with the possibility that the oxygen tension of the neuronal environment plays a role in the generation of tonic SPN activity.

It was known at the turn of the century that asphyxiation caused an increase in arterial blood pressure in the CNS intact preparation as well as in animals in which supra-spinal sympathetic influences had been eliminated (see Mathison 1910 for references). The pressor effects of asphyxia in the acute C-1 spinal animal were shown to be neurogenic and due largely to $O_2$ lack in the blood by Kaya and

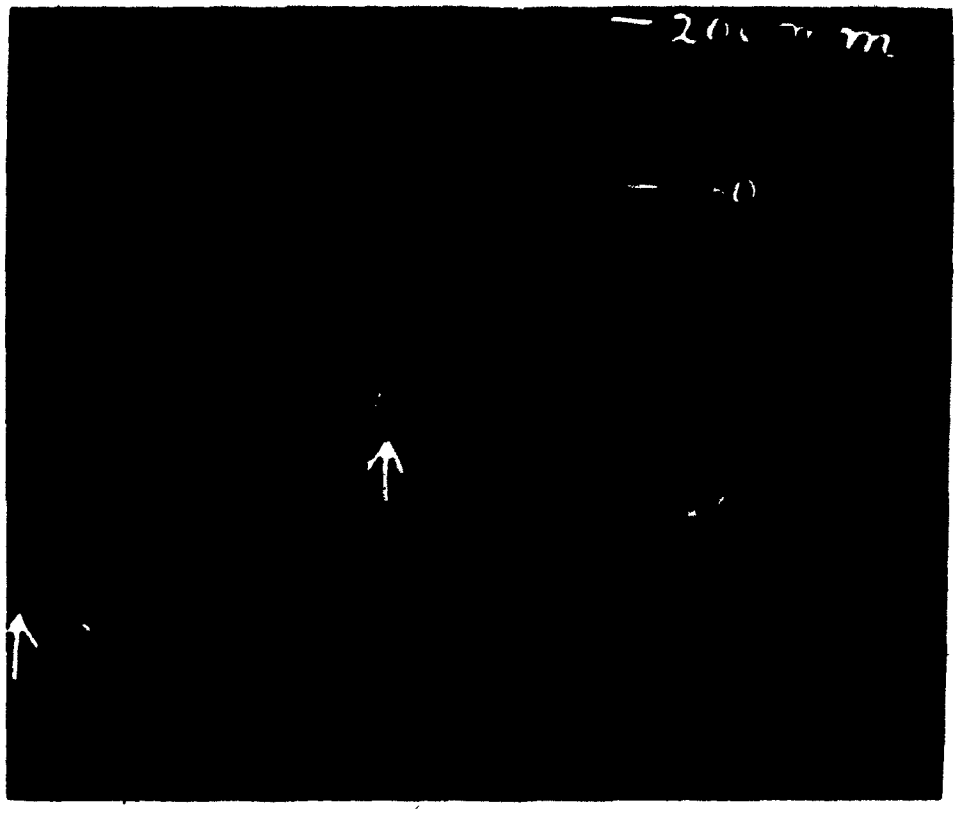

FIGURE 1. The pressor effects of ventilation with hypoxic gas (from Mathison, 1910). The results were obtained from an acute C-1 spinalized and curarized cat. Arterial blood pressure is indicated in mmHg. Gas containing 2% $O_2$ and 98% $N_2$ was administered between the arrows. Time calibration: 10 sec.

Starling (1909) and by Mathison (1910). These workers found that asphyxia or ventilation with hypoxic or anoxic gas consistently led to a significant rise in arterial blood pressure (Figure 1). There was no pressor response to asphyxia or hypoxia following destruction of the spinal cord in these animals. Ventilation with hypercapnic gas or elevation of arterial $H^+$ produced inconsistent results and significant pressor responses were only seen at levels of inspired $CO_2$ greater than 25%. It was therefore suggested that the pressor responses seen during asphyxia were mainly due to a marked sensitivity of the neurons of the so-called spinal vaso-motor centers to hypoxia.

Later observations suggested a sensitivity of spinal sympathetic structures to changes in the neuronal environment caused by alterations in arterial blood pressure and thus presumably in nervous tissue perfusion. Working with chronic spinalized (C-6) cats Brooks (1935) found that this preparation could compensate for a decrease in arterial blood pressure caused by acute hemorrhage of between 15% and 25% of its total blood volume. This response was found to be unaffected by removal of the adrenal glands or by further section of the spinal cord at L-5 and section of the dorsal roots between C-6 and L-5. However, section of the ventral roots between C-6 and L-5 or complete removal of the sympathetic chains abolished the compensatory response to hemorrhage. A role of the sympathetic system in this response was further suggested by the observation of nictitating membrane contraction and of increased resistance to flow in a vascularly isolated, innervated, perfused limb following acute hemorrhage in chronic C-6 spinal cats.

It was thus concluded that the spinal sympathetic neurons themselves possessed an intrinsic sensitivity to decreases in arterial blood pressure caused by hemorrhage possibly as a result of decreases in nervous tissue perfusion which may accompany large falls in arterial blood pressure. An influence of nervous tissue perfusion on sympathetic activity was also suggested by Alexander (1945) on the basis of experiments upon CNS intact, baroreceptor denervated, cats as well as acute C-1 spinal cats. He found that the amounts of efferent electrical activity in the inferior cardiac nerve of these preparations decreased as arterial blood pressure was increased by the intravenous administration of pressor drugs or by occlusion of the abdominal aorta. The same type of response to increases in arterial blood pressure was seen following the elimination of sensory afferents by section of the dorsal roots. The explanation put forward for these findings was that spinal sympathetic neurons possessed an intrinsic sensitivity to the increase in arterial blood pressure or to some consequence of it. On the basis of the observations by Brooks (1935) and Alexander (1945) a relation between some perfusion dependent variable and sympathetic activity seemed possible. The experiments of Kaya and Starling (1909) and Mathison (1910) had already suggested that such a factor was not tissue $H^+$ or $CO_2$ levels but more likely $O_2$ levels. Therefore, it appeared that there might exist a sensitivity to nervous tissue oxygen tension over a considerable range of values.

Further experiments supported the possibility that the

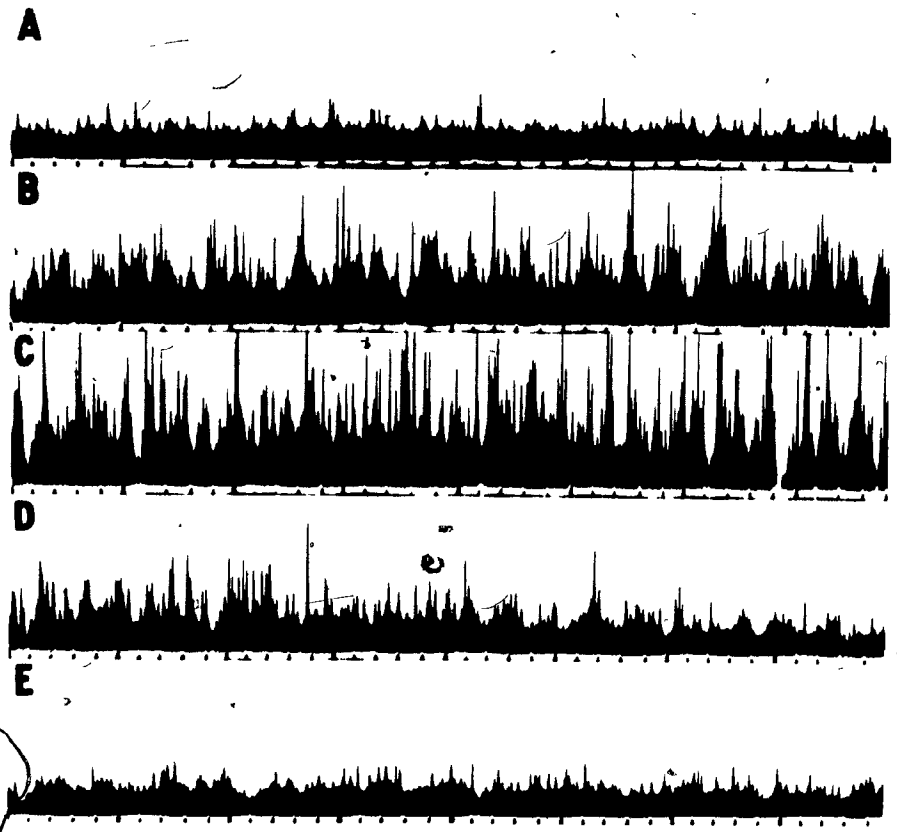


FIGURE 2. Effects of anoxia upon inferior cardiac nerve activity in the deafferented spinal preparation (from Alexander, 1945). All the records are from the same preparation at the same amplification. Time calibration: 200 msec. The spinal cord was sectioned at C-5 and T-6 and all the dorsal roots between these two segments were cut. A) Ventilation at a moderate rate with room air. B) Activity after 90 sec ventilation with $N_2$. C) Further increase in activity with anoxia, 5 sec of recording omitted between B and C. D) A mixture of 90% $O_2$ and 10% $CO_2$ substituted for $N_2$. Record shows decrease in activity as oxygen reaches tissues. E) Return to original level while being ventilated with $O_2$ - $CO_2$ mixture, 5 sec of recording omitted between D and E.

excitability of spinal sympathetic structures was increased as oxygen delivery to nervous tissue was decreased. Gellhorn et al (1942) found in the cat that electrical stimulation of the dorsal surface of the thoracic spinal cord during systemic hypoxia caused a greater pilomotor activity of the back and tail and a greater nictitating membrane contraction than stimulation during normoxia. Since these increased sympathetic responses were restricted to the stimulated spinal cord segments and since the nictitating membrane contraction evoked by electrical stimulation of the distal part of the sectioned CST was not modified by hypoxia these observations were taken to indicate an increase in the excitability of spinal sympathetic neurons during hypoxia. Alexander (1945) came to a similar conclusion on the basis of somewhat more direct evidence. He found that the electrical activity of the inferior cardiac nerve in the acute C-1 spinal cat increased during asphyxia or ventilation with 100% $N_2$ and decreased during hyperventilation with air or ventilation with hyperoxic gas mixtures. Similar results were seen following dorsal root section (Figure 2). On the basis of these observations and of those previously mentioned concerning a decrease in sympathetic activity upon increases in arterial blood pressure in CNS intact, baroreceptor denervated or C-1 spinal, deafferented cats he suggested that there was an inverse relationship between sympathetic activity and spinal cord $PO_2$.

A variety of cardiovascular phenomena have been attributed to a CNS action of $PO_2$ upon sympathetic neurons. As was outlined above an action of nervous tissue $PO_2$ upon spinal sympathetic neurons may be a

major factor in the generation of tonic SPN activity in the "isolated" spinal cord. In addition, it has been suggested that in the C-1 spinal preparation slow spontaneous oscillations of arterial blood pressure, known as Mayer waves, and the hypertensive response to elevated intradural pressure, similar to the Cushing response of the CNS intact preparation, are the result of changes in nervous tissue perfusion (Kaminski et al., 1970; Meyer & Winter, 1970). In addition, systemic hypoxia in the CNS intact preparation without functional peripheral chemoreceptors has been shown to initiate neurogenic cardiac responses, such as increases in ventricular contractility, heart rate and cardiac output (Achtel & Downing, 1972; Krasney et al., 1973, 1977), as well as increases in sympathetic activity (Bower, 1975; Gregor & Janig, 1977). At least part of these responses may be due to a spinal action of systemic hypoxia. However, the experiments of Downing et al (1963) on peripherally chemodenervated dogs in which the brain was perfused with hypoxic blood while most of the spinal cord remained normoxic showed that significant neurogenic cardiovascular responses also occurred in this preparation. This suggests that supra-spinal neurons, antecedent to the SPNs may also be excited by oxygen lack.

From the literature reviewed above it is evident that a number of experiments over the past 70 years have suggested that there may be a spinal or even supra-spinal action of $PaO_2$ upon the ANS independent of peripheral chemoreceptor input. Most of the evidence is somewhat indirect and qualitative in showing that rather extreme levels of hypoxia or ischemia excite sympathetic neurons. However, if tissue

of arterial $PO_2$ play a role in the generation of tonic SPN activity then there should exist a relationship between $PaO_2$ and sympathetic activity over the whole range of physiological values. None of the previous studies have clearly demonstrated that such a relationship in fact exists. Much of the earlier work (Kaya & Starling, 1909; Mathison 1910; Brooks, 1935) was based upon the observation of changes in arterial blood pressure in the spinal animal during asphyxia, ventilation with anoxic gases or hemorrhage. However, due to the direct effects of systemic hypoxia upon the heart and vascular smooth muscle it is very difficult to infer changes in sympathetic activity from the changes in arterial blood pressure observed during such experiments. In addition, the exact extent to which arterial oxygen tension and/or oxygen delivery to the various tissues decreases during asphyxia, ventilation with anoxic gases or hemorrhage is unknown. Thus, the characteristics of any relationship between sympathetic activity and $PaO_2$ cannot be described on the basis of such data. Neither can this be done using the data obtained in the later work by Alexander (1945). He recorded the efferent electrical activity of the inferior cardiac nerve. However, the recordings do not lend themselves to quantitation. As with earlier investigators he also depended upon asphyxiation and ventilation with anoxic or hypoxic gases to achieve changes in arterial gas tensions without actually measuring blood gas tensions.

The question posed in the series of experiments presented here concerns the nature of the supposed relationship between arterial oxygen tension and tonic SPN firing in the acute C-1 spinal cat. This

was investigated by observing changes in tonic SPN firing as arterial oxygen tension was slowly decreased from normoxia to extreme hypoxia ($PaO_2$ < 25 mmHg) or increased to hyperoxic values ($PaO_2$ > 400 mmHg). Since nervous tissue $PO_2$ has been shown to follow quite closely such changes in $PaO_2$ (Leniger-Folbert et al., 1975) it was felt that this type of data would indicate to what extent tonic SPN firing is influenced by the $PO_2$ of its environment.

## II. METHODS

Experiments were conducted on 15 adult cats of either sex weighing between 3 kg and 4 kg. Ten of these animals were spinalized at C-1 and anaemically decerebrated by occlusion of the carotid and vertebral arteries while anaesthetized with ethyl ether. The completeness of the spinal section was confirmed by visual inspection. The success of the decerebration was assessed by the loss of the corneal and light reflexes and by appearance of rigor mortis of the head. After the completion of the spinal section and decerebration ether anaesthesia was discontinued. The remaining five cats had an intact CNS and were anaesthetized with pentobarbital (35 mg/kg) administered intraperitoneally. Anaesthesia was maintained in these preparations with supplemental doses of pentobarbital administered intravenously. All the cats were tracheostomized. Following muscular paralysis with pancuronium bromide (0.5 mg/kg i.v.) the cats were ventilated with positive pressure. Ventilation was adjusted to maintain end-tidal $PCO_2$ between 25 mmHg and 35 mmHg. Gases were supplied to the respirator from a 1 litre anaesthesia rebreathing bag. The contents of this bag could be regulated with needle valves controlling the flow of $O_2$, air, $CO_2$, and $N_2$. Rectal temperature was measured and maintained at between 36°C and 38°C by means of infrared lamps.

In all the preparations a CST was exposed in the neck by a ventral approach, desheathed by removing the surrounding connective tissue, sectioned peripherally, and kept under paraffin oil in a pool made of

the skin flaps of the neck incision. Fine strands were dissected from the CST with glass probes and placed on bipolar silver recording electrodes. In two of the spinalized animals an indwelling stimulating electrode was placed around one of the radial nerves in order to evoke SPN activity reflexly. In all the CNS intact preparations the vago-sympathetic trunks, including the aortic nerves, and the carotid sinus nerves were sectioned in order to eliminate the influence of peripheral chemoreceptors. To assess the completeness of the chemodenervation in these preparations one phrenic nerve was exposed and desheathed and its electrical activity recorded and integrated. Prior to chemodenervation the integrated electrical activity of the phrenic nerve was characteristically increased in amplitude upon the intravenous administration of KCN (50 µg/kg). Such doses of KCN have been shown to cause a large increase in efferent discharge in the carotid sinus nerve (Eyzaguirre & Nishi, 1974). After chemodenervation this dose, or greater doses, of KCN had no effect on phrenic nerve activity.

The firing of SPNs was recorded in all the preparations from single unit or multi-unit strands of the CST. The electrical activity of the strands was amplified with an AC coupled GRASS P511 preamplifier (bandpass 10 Hz-10 k Hz) and displayed on a storage oscilloscope. Firing frequency of the units was measured with an amplitude discriminator and ratemeter.

In addition to sympathetic activity a number of other physiological variables were also monitored. Inspired and expired $CO_2$ and $O_2$ concentrations were monitored with a BECKMAN LB-2 and OM-11 gas analyzer respectively. Arterial blood pressure was monitored from a

cannula in the femoral artery using a STATHAM pressure transducer. Arterial $PCO_2$, $PO_2$, and pH were periodically determined with a RADIOMETER PHM72 Mk-2 digital acid base analyzer and a BMS-3 Mk-2 blood micro-system, in duplicate, from 1 ml samples taken anaerobically from the femoral artery. Inspired and expired $CO_2$ and $O_2$, arterial blood pressure, and sympathetic unit firing frequency were displayed on a GRASS model 7 polygraph. Sympathetic activity, inspired and expired $O_2$, and arterial blood pressure were also stored on magnetic tape for further analysis.

Changes in sympathetic activity during arterial hypoxia were observed in five single unit strands and five multi-unit strands in eight acute spinal cats. The results of radial nerve stimulation during arterial hypoxia were observed in one single unit strand and one multi-unit strand in two acute spinal cats. In addition the effect of arterial hypoxia upon tonic SPN activity in the CNS intact anaesthetized preparation was observed following peripheral chemodenervation in 1 single unit strand and 4 multi-unit strands in 5 cats. In three single unit strands in three spinal cats the effects of ventilation with 100% $O_2$ were also observed. The effects of asphyxia upon tonic SPN firing in acute spinal cats were observed in two animals. Asphyxiation was accomplished by turning the respirator off.

In all the preparations which were made hypoxic arterial $PO_2$ was gradually decreased from a mean normoxic level of 90 mmHg to a mean maximally hypoxic level of 20 mmHg at a mean rate of 4 mmHg/min.

This was accomplished by gradually decreasing the flow of air and increasing the flow of $N_2$ to the anaesthesia bag supplying gases to the respiration pump. End-tidal $PCO_2$ was maintained at a relatively constant level during the period of hypoxia (see Figure 7) by compensating for small changes in end-tidal $PCO_2$ with adjustments in the rate and depth of ventilation. In those preparations which were made hypoxic more than once (e.g. the experiments illustrated in Figure 14) a period of at least twenty minutes was allowed to elapse between hypoxic runs. During this period the arterial acid-base balance was monitored and acidosis, if present, corrected by the intravenous infusion of a $NaHCO_3$ solution (1 mEq/ml).

Arterial blood gases could only be determined periodically. Three or four arterial blood samples were taken during each hypoxic run and $PaO_2$ values occurring between these determinations were estimated. Measurement of arterial blood gases was performed in duplicate on samples taken at the beginning and at the end of each hypoxic run as well as at intermediate points. $PaO_2$ values which were not measured directly were estimated by interpolation with respect to time between the measured values. The information was used to plot sympathetic unit firing frequency as a function of $PaO_2$ (i.e., Figures 7,10,14,15). This method of estimating $PaO_2$ was compared with values obtained by a second method. End tidal $PO_2$ was measured in order to estimate $P_AO_2$. This end-tidal $PO_2$ was plotted against simultaneously measured $PaO_2$ values (Figure 3). These points were also fitted with an equation

using least squares linear regression (Figure 3). Correlation coefficients were 0.90 or greater in all cases. Thus this method gave a good estimate of $PaO_2$ as a function of the measured end-tidal $PO_2$. When the $PaO_2$ values estimated by interpolation were plotted on the same axes they were clustered in a narrow band around the fitted line (Figure 3). This demonstrated that similar results could be obtained with two different methods of estimating $PaO_2$. Since the first method, using interpolation, depended on the measurement of one variable while the second, using linear regression, depended on the measurement of two variables the former method of estimating intermediate $PaO_2$ values was used in these experiments.

Where differences between values were tested for statistical significance the Wilcoxon two sample test was used (Rumke & DeJonge, 1964). P values of less than 0.05 were considered to be significant. Non-parametric statistical analysis was used due to the small sample sizes involved and because of the uncertainty concerning the nature of the distribution of the values obtained.

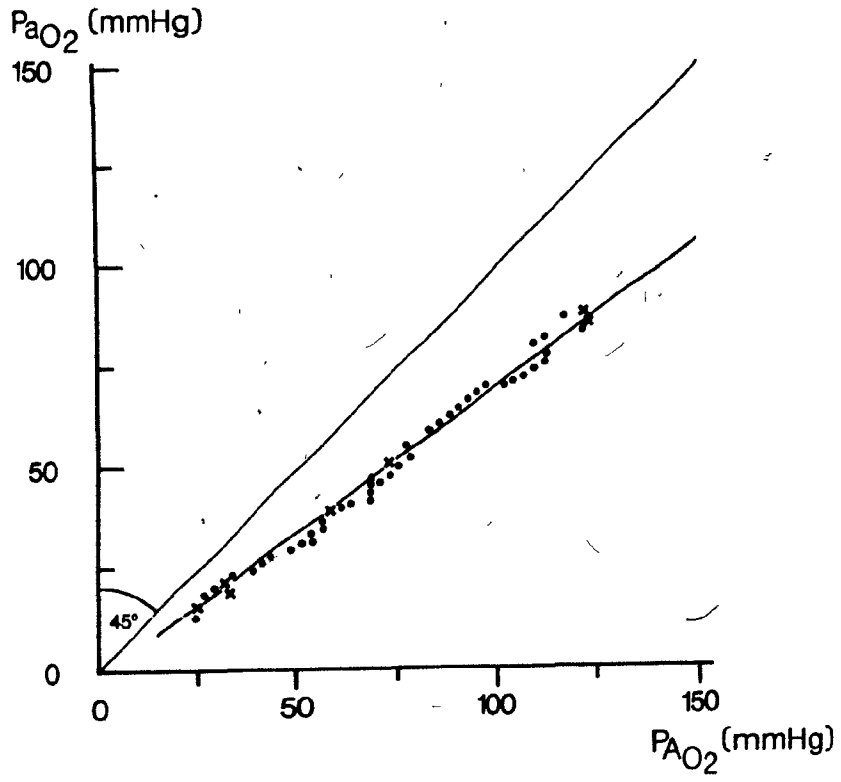


FIGURE 3. Comparison of two methods of estimating $PaO_2$ values not measured directly in a single experiment. One method was to fit a straight line (—) by least squares linear regression to measured $PaO_2$ values (X) plotted against $P_AO_2$ values. A second method was to estimate $PaO_2$ values not measured directly by interpolation with respect to time between the measured values (•). The line of identity is also indicated.

## III. RESULTS

### a) Asphyxia in the Spinal Preparation

Initial experiments showed that asphyxiation of the spinal animal produced a large increase in SPN firing frequency. This observation was made during 6 trials in one single unit strand and one multi-unit strand in two spinal cats. An example of the response of the single SPN to asphyxia after ventilation in room air is illustrated in Figure 4. The respirator was turned off between the arrows. SPN firing frequency did not change for about one minute following the arrest of the pump but started to increase after this latent period reaching a peak level of seven times the control value. Arterial blood gases were not measured in this case but in similar experiments arterial $PO_2$, $PCO_2$, and pH values of 16 mmHg, 60 mmHg, and 7.100 respectively were obtained at the time when the SPN firing rate reached a peak. Thus, hypoxia, hypercapnia or acidosis, singly or cooperatively, might have been the cause of this SPN excitation in asphyxia. Further trials conducted on this unit showed that a period of hyperventilation in air prior to asphyxia, which reduced $P_ACO_2$ to approximately 15 mmHg, did not appreciably change the latency or magnitude of the SPN response (Figure 5). However, ventilation with 95% $O_2$ plus 5% $CO_2$ for several minutes prior to the arrest of ventilation prolonged the latency of the response by about 15 minutes (Figure 6). Reduction of the alveolar and arterial $PCO_2$ prior to the apnea should have delayed the onset of the increase in SPN firing rate if accumulation of $CO_2$ was the critical

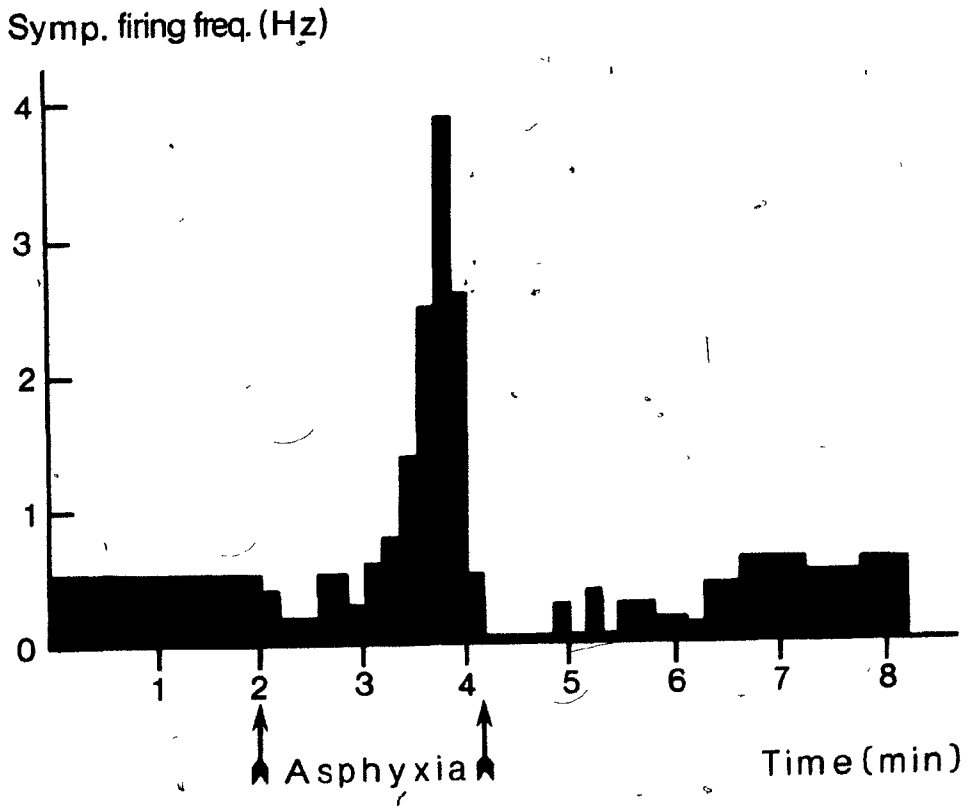


FIGURE 4. C-1 spinal cat. SPN firing frequency response to asphyxia. The preparation was ventilated with room air and end-tidal $CO_2$ was 35 mmHg prior to turning the respirator off for the period between the arrows.

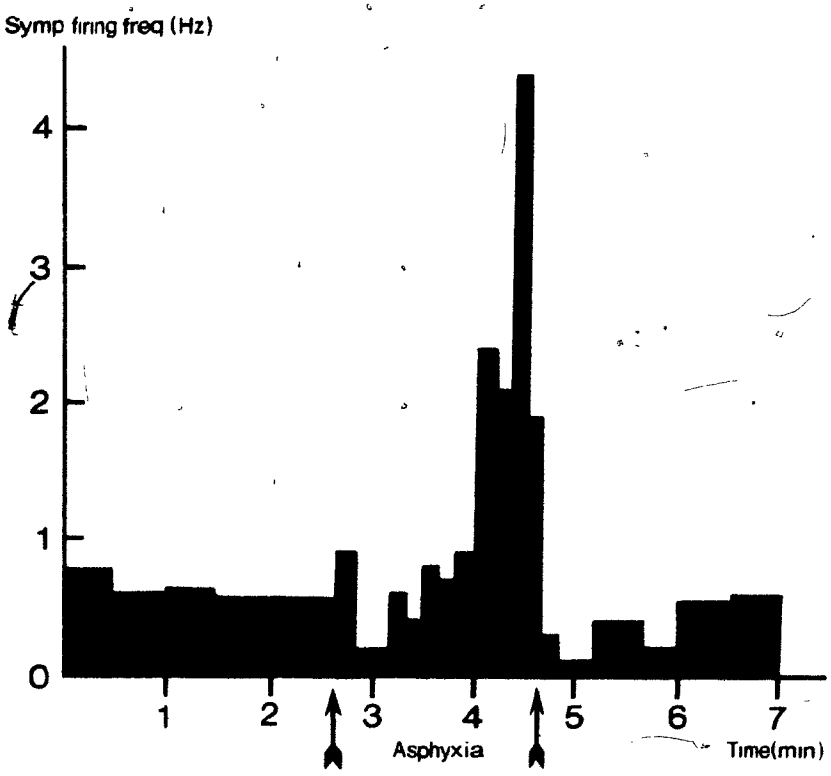


FIGURE 5. C-1 spinal cat. SPN firing frequency response to asphyxia. The preparation was hyperventilated in room air and end-tidal $CO_2$ was 15 mmHg prior to turning the respirator off for the period between the arrows.

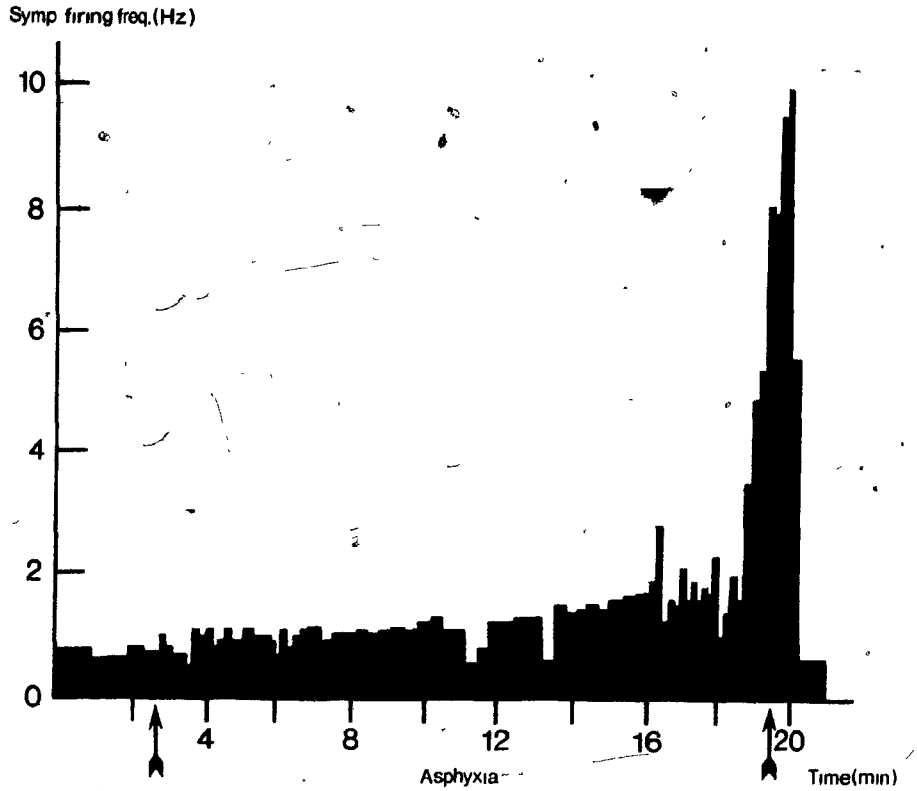


FIGURE 6. C-1 spinal cat. SPN firing frequency response to asphyxia. The preparation was ventilated with a gas mixture containing 95% $O_2$ plus 5% $CO_2$ prior to turning the respirator off for the period between the arrows.

factor. Increase of alveolar $PO_2$ should have had a similar effect if a decrease in $O_2$ was the critical factor. Since the former procedure did not influence the latency of the SPN excitation in asphyxia, whereas the latter did, $O_2$ lack is probably the major factor involved in these SPN responses to asphyxia. Therefore, these data confirm previous inferences made on the basis of arterial blood pressure data (Kaya & Starling, 1909; Mathison, 1910) and on the basis of postganglionic recording (Alexander, 1945). In addition, since the SPN firing rate does not increase continuously with time during apnea but abruptly after a long latent period, these data suggest the existence of a critical low $PaO_2$ at which the response begins.

b) Hypoxia in the Spinal Preparation

The relation between $PaO_2$ and SPN firing rate was explored in eight experiments in acute C-1 spinal animals, in which arterial $PO_2$ was gradually decreased from normoxia to extreme hypoxia. A total of 13 trials on 5 single unit strands and 5 multi-unit strands was performed. The pooled data from all 13 trials is presented in Figure 7 where sympathetic unit firing frequency, expressed as a percentage of the normoxic level, is plotted against $PaO_2$ . On the average no statistically significant change in SPN firing frequency was evident for $PaO_2$s above 40 mmHg. Below this value SPN firing frequency increased steeply reaching a mean value of nearly ten times the normoxic firing frequency at a mean $PaO_2$ of 20 mmHg.

The results of a typical experiment on a unit can be seen in

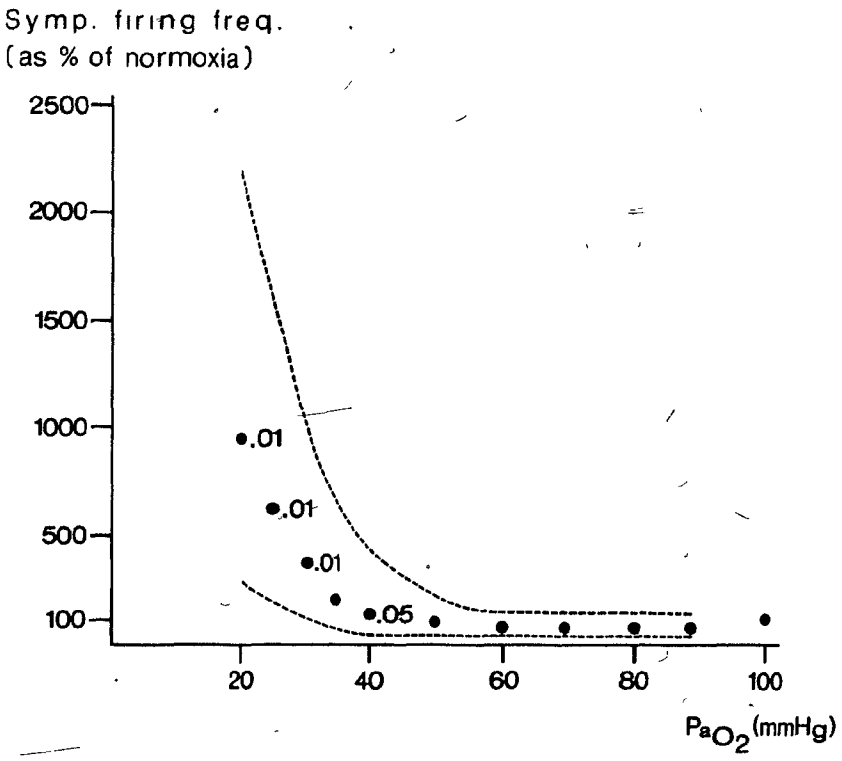


FIGURE 7. Average SPN response to graded isocapnic arterial hypoxia. Points are averages based upon 13 trials in 8 acute spinal cats. The dashed lines indicate the range of the responses. Numerals indicate levels of significant difference from the point at the next greater $PaO_2$.

Figure 8 where firing frequency, end-tidal and arterial $PO_2$ and $PCO_2$, arterial pH and mean arterial blood pressure are plotted against time. Samples of the firing of this unit at $PaO_2$s of 71 and 14 mmHg are shown in Figure 9. The relation between unit firing and $PaO_2$ is illustrated in Figure 10. As is shown in Figure 8 this unit fired steadily in normoxia over a period of more than ten minutes. As alveolar and arterial oxygen tensions decreased over a period of several minutes very little change in firing frequency took place until a very low level of oxygen tension was reached. At this point (approximately 30 mmHg) the SPN firing rate started to increase as $PaO_2$ fell further until a nearly six-fold rise in firing rate took place. As hypoxia was terminated and the animal reoxygenated the frequency of SPN firing fell close to the normoxic level within 30 seconds. Arterial $PCO_2$ and pH showed some fluctuations during hypoxia but no trends to which changes in the SPN firing rate could be related were evident. An increase in mean arterial pressure was apparent as $PaO_2$ decreased.

The effect of low $PaO_2$ was not only to increase the firing rate of already active SPNs but also to recruit units which were silent in normoxia. Recruitment of new units at low $PaO_2$ was observed in 5 of the 10 strands. An example of such recruitment is shown in Figure 11 where samples of the firing of a strand at normoxia ($PaO_2$ = 75 mmHg), when only a single unit was active, and in extreme hypoxia ($PaO_2$ = 35 mmHg), after additional units had been recruited are shown. It was impossible to assess, in a number of multi-unit strands, the extent

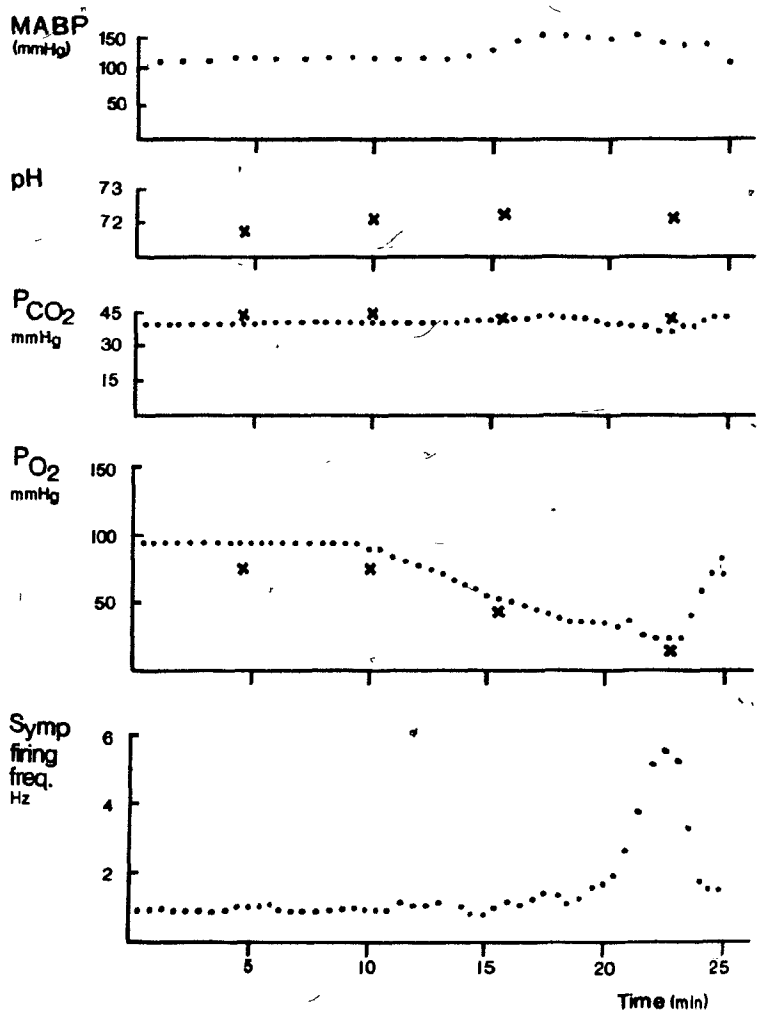


FIGURE 8. C-1 spinal cat. Inspired oxygen tension was gradually decreased from normoxia to extreme hypoxia. From top to bottom: mean arterial blood pressure, arterial pH, arterial (X) and alveolar (.) $PCO_2$, arterial (X) and alveolar (.) $PO_2$, and mean firing frequency of a sympathetic unit.

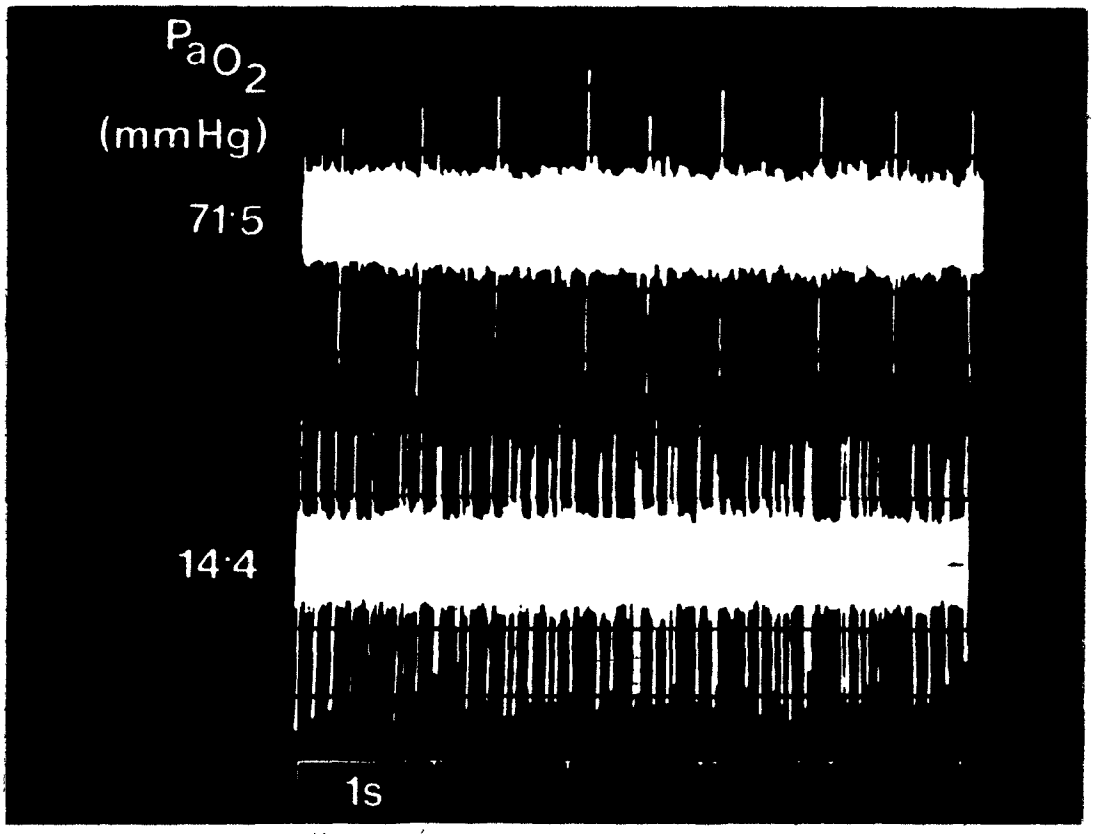


FIGURE 9. Samples of the activity of the sympathetic unit from the experiment described in Figure 8 at $PaO_2$s of 71.5 mmHg and 14.4 mmHg.

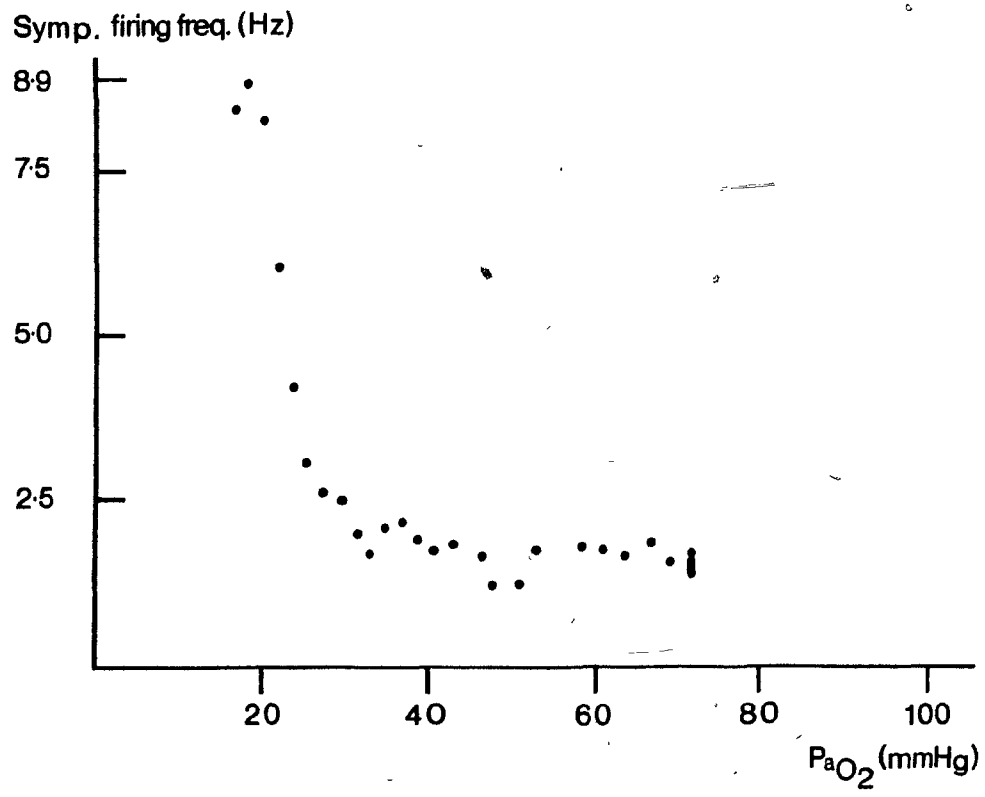


FIGURE 10. Relationship between SPN firing frequency and $PaO_2$ in the experiment described in Figure 8.

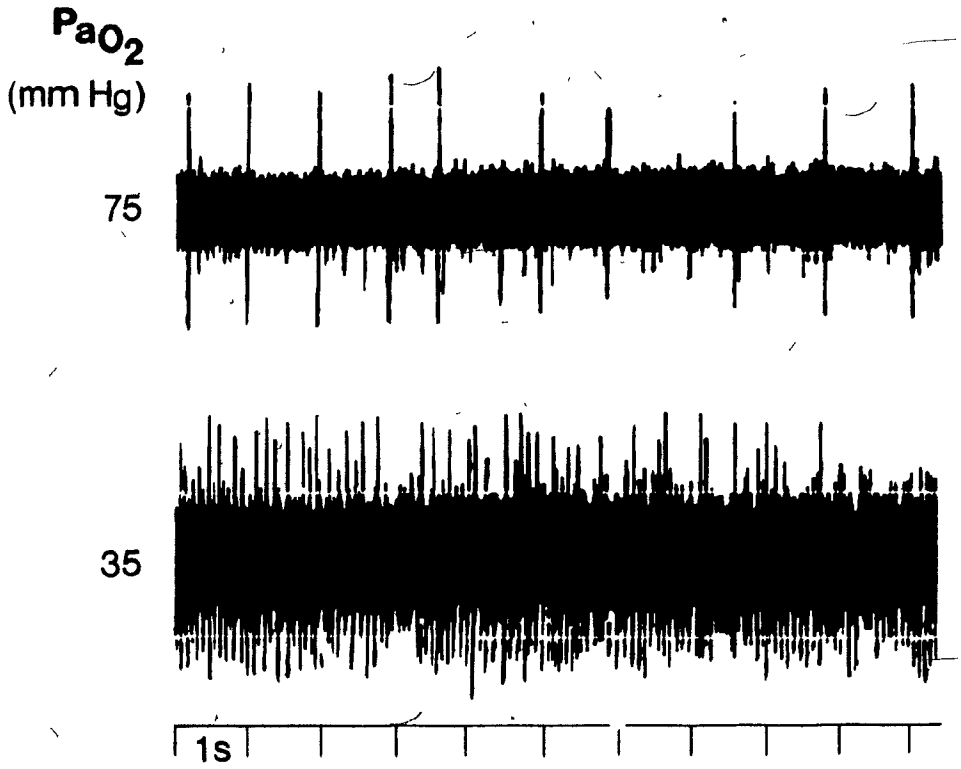


FIGURE 11. Recruitment of sympathetic units during arterial hypoxia. The activity of the same strand of the CST is shown in both traces. In the top trace ($PaO_2$ = 75 mmHg) only one unit is active. In the lower trace ($PaO_2$ = 35 mmHg) several additional units are active.

to which the increase in firing frequency of the strands was due to increases in firing rate of units that were already active or to the recruitment of further units.

The hypoxic increase in SPN excitability was also reflected in an enhanced synaptic efficacy of afferent input. In two experiments SPN unit activity was evoked in the spinal preparation by electrical stimulation of myelinated afferents in the radial nerve. In both cases the probability of evoking a response by stimulation of the radial nerve increased markedly (≈50%) when $PaO_2$ was reduced to less than 40 mmHg. An example of the evoked activity of a single SPN at a $PaO_2$ of 105 mmHg and 34 mmHg is shown in Figure 12.

Systemic hypoxia was accompanied by an increase in mean arterial blood pressure, as in the experiment shown in Figure 8, in all the trials except two in which mean arterial pressure decreased. Figure 13 shows the pooled data for all the experiments in which mean arterial blood pressure increased, expressed as a percentage of the normoxic values, plotted on the vertical axis against $PaO_2$ on the horizontal axis. In normoxia the mean arterial pressure was 77 $\pm$ 4 (SEM) mmHg. No significant change in mean arterial blood pressure was seen in these trials as $PaO_2$ was reduced to 40 mmHg. At 40 mmHg $PaO_2$ an average, statistically significant, increase of nearly 25% occurred. Mean arterial blood pressure showed a tendency in some trials to decrease, after the initial increase, as $PaO_2$ was further reduced. This may have been due to the dilator effects of hypoxia upon peripheral resistance

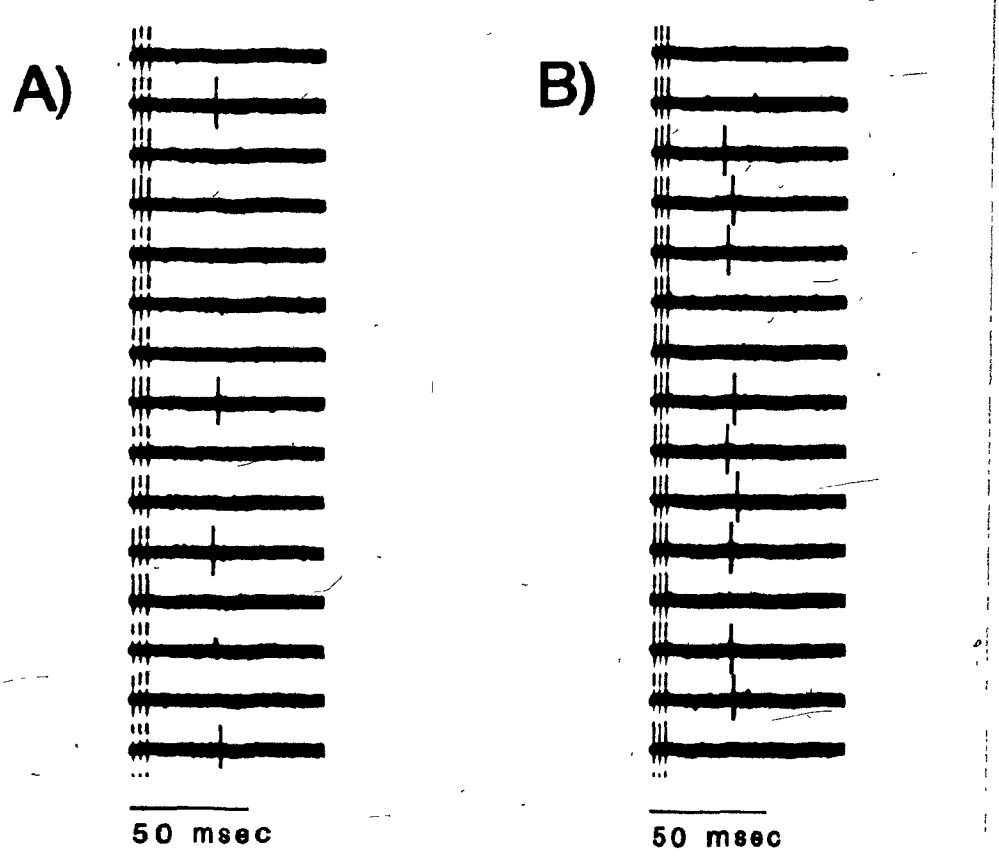


FIGURE 12. Effect of hypoxia on SPN activity evoked by electrical stimulation of myelinated afferents in the radial nerve. (Train of 3 square pulses, 4V and 0.2 msec each, at 500 Hz every 4 sec). A) Evoked activity in normoxia ($PaO_2$ = 105 mmHg): response probability = 0.26. B) Evoked activity in hypoxia ($PaO_2$ = 32 mmHg): response probability = 0.60.

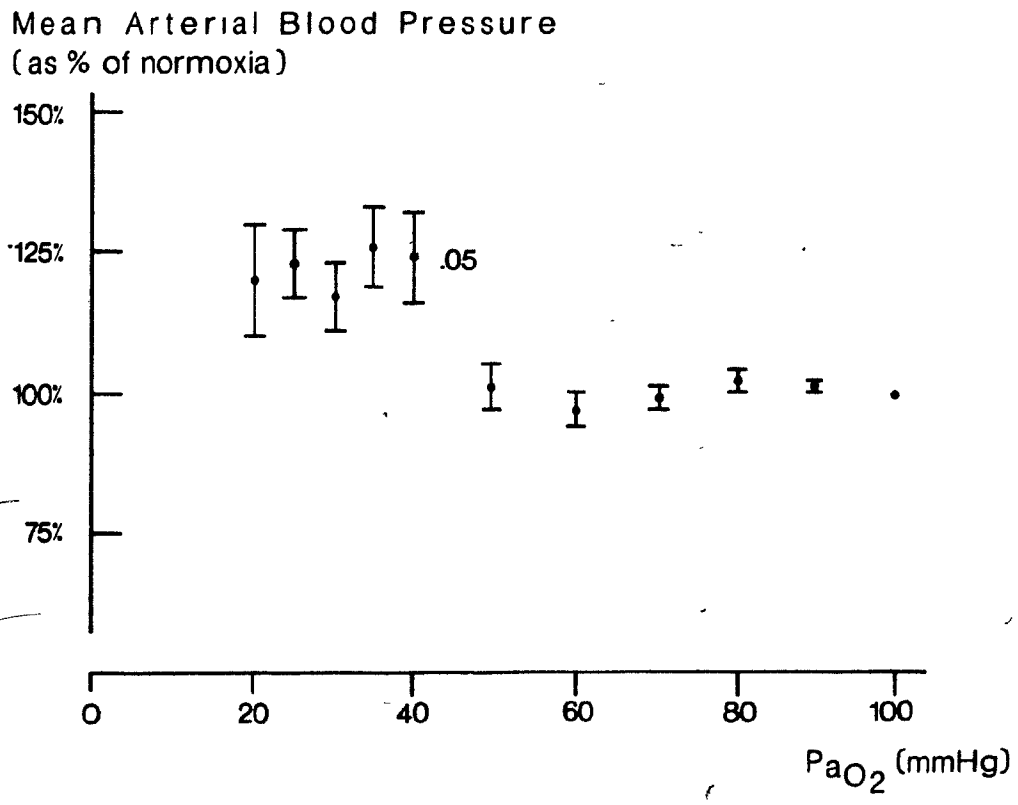


FIGURE 13. Mean arterial blood pressure response to graded isocapnic arterial hypoxia. Points are averages based upon 8 trials in 7 acute spinal cats. Bars indicate ± standard error of the mean. Numerals indicate levels of significant difference from the point at the next greater $PaO_2$.

vessels and the depressant effects of hypoxia upon the heart (Mountcastle, 1980). Nevertheless the group average arterial blood pressure (Figure 13) remained elevated down to the lowest $PaO_2$ studied. The increase in mean arterial blood pressure was apparently neurogenic as it was almost completely abolished upon alpha-adrenergic and/or ganglionic blockade with i.v. phentolamine (2 mg/kg) and/or hexamethonium (10 mg/kg). The response of SPN firing frequency to arterial hypoxia was unaltered following such blockade showing that it was not secondary to the increase in mean arterial blood pressure.

The hypoxic excitation of SPNs could result from an action of hypoxia on the SPN itself or on its synaptic input. Since barbiturates are known to depress excitatory synaptic transmission (Weakly, 1969) the effect of pentobarbital on the hypoxic response was tested on 7 strands in 5 cats. The response of SPNs to arterial hypoxia was found to be resistant to barbiturate. The administration of pentobarbital in doses ranging from 5 mg/kg to 60 mg/kg intravenously did not appreciably alter the response. The frequency of firing of SPNs in normoxia was most often greatly decreased (5 of 7 trials in 5 cats) by the administration of pentobarbital. In one case SPN firing remained unchanged and in the other SPN firing increased somewhat upon the administration of pentobarbital. However, both the threshold of the hypoxic response and the maximal rate of firing were comparable, at all dosages, to those observed in the unanaesthetized state. The response of a single unit to decreasing $PaO_2$ in the unanaesthetized state,

after the i.v. administration of 20 mg/kg of pentobarbital, and after the i.v. administration of a further 40 mg/kg of pentobarbital is shown in Figure 14. At the highest dosage of pentobarbital this unit was silent at $PaO_2$s greater than about 35 mmHg. However, the response to arterial hypoxia below 35 mmHg $PaO_2$ in the three states was almost identical.

As was already mentioned, upon the termination of hypoxia SPN activity returned, within thirty seconds, to or near the normoxic level in most cases. However, in 5 trials in 4 acute spinal animals SPNs became silent for periods ranging from 30 seconds to 7 minutes upon the termination of hypoxia and the reoxygenation of the animal. A reduction in SPN firing rate below the control level was also noted immediately following the termination of asphyxia (i.e., Figure 4).

c) Hyperoxia in the Spinal Preparation

Hyperoxia obtained by ventilation with 100% $O_2$ for several minutes ($PaO_2$ > 400 mmHg) was found to be without effect upon SPN firing frequency in three strands in three animals.

d) Hypoxia in the CNS Intact Anaesthetized and Peripherally Chemodenervated Preparation

The relation between $PaO_2$ and SPN firing rate was also investigated in 5 CNS intact anaesthetized preparations following peripheral chemoreceptor denervation in order to ascertain whether or not excitation of SPNs during hypoxia, as in the spinal preparation, takes place in this preparation. A total of 5 trials in 1 single

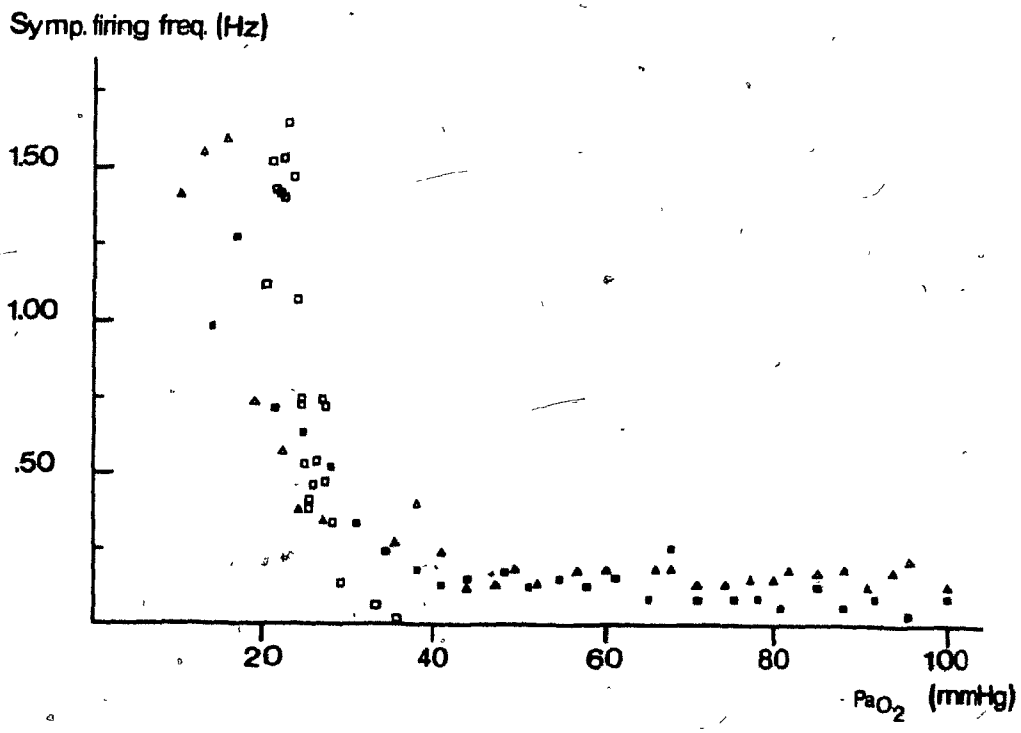


FIGURE 14. Response of a single SPN to graded isocapnic arterial hypoxia in the unanaesthetized state ( △ ), following the administration of 20 mg/kg i.v. of pentobarbital ( ■ ), and following a cummulative dose of 60 mg/kg i.v. of pentobarbital ( □ ) given over a period of 60 minutes.

unit strand and 4 multi-unit strands were performed. Similar results as in the spinal preparation were obtained. No significant change in SPN firing rate occurred until a $PaO_2$ of 35 mmHg was attained. Below this value SPN firing rate increased rapidly reaching a mean level of approximately four times the normoxic level at a mean minimum $PaO_2$ of 25 mmHg. An example of the response of a multi-unit strand to graded hypoxia in the CNS intact and peripherally chemodenervated preparation is shown in Figure 15.

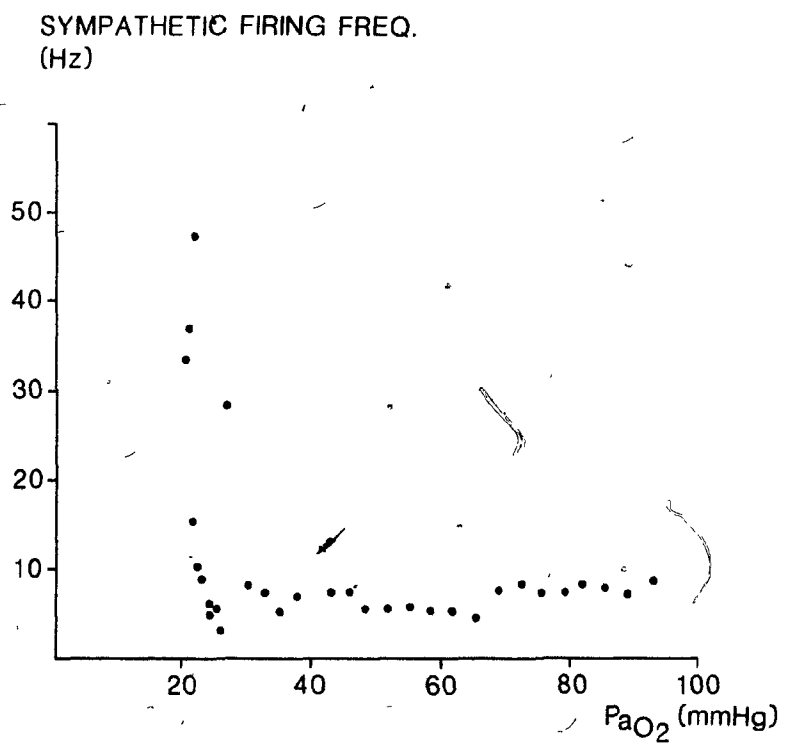


**FIGURE 15.** **CNS intact, peripherally chemodenervated, anaesthetized preparation. Response of a multi-unit strand of the CST to graded isocapnic arterial hypoxia.**

## IV. DISCUSSION

Previous work has suggested that nervous tissue $PO_2$ may have a tonic influence upon sympathetic discharge in the spinal animal (Kaya & Starling, 1909; Mathison, 1910; Brooks, 1934; Alexander, 1945). Such a purported influence might play a role in the generation of the tonic SPN activity which is present after the elimination of a large portion of the afferent input to the spinal cord. However, the existence of a continuous relationship between $PaO_2$ and sympathetic discharge in the physiological $PaO_2$ range has never been substantiated. In order to establish whether or not such a relationship exists SPN activity was studied in a situation of graded isocapnic hypoxia and isocapnic hyperoxia in acute C-1 spinal cats.

In the range of $PaO_2$s between 400 mmHg and 40 mmHg there was no relationship between $PaO_2$ and SPN firing. As $PaO_2$ decreased below 40 mmHg there was a marked increase in the firing frequency of spontaneously active SPNs as well as recruitment of previously inactive ones. All spontaneously active units studied showed this pattern of response. This suggests that such a response to graded hypoxia is a general property of the population studied. Experiments conducted upon CNS intact, anaesthetized and peripherally chemodenervated preparations showed that a very similar relationship existed between $PaO_2$ and SPN firing. Thus, SPN firing rate is not a continuous function of $PaO_2$ in either the acute spinal or the CNS intact, peripherally chemodenervated preparation. However, extreme hypoxemia ($PaO_2$ < 40 mmHg) seems to be

a strong excitatory stimulus in both preparations.

Nervous tissue $PO_2$ was not measured in the present experiments. However, measurement of nervous tissue oxygen tension from the surface of the cerebral cortex (Leniger-Folbert, 1975) as well as from within the cerebral cortex with microelectrodes (Nair et al., 1975; Metzger & Heuber, 1977) has shown that nervous tissue oxygen tension decreases rapidly during ventilation with hypoxic or anoxic gases. Recent work has also shown a uniformity of oxygen consumption within different regions of the CNS (Buchweitz et al., 1980). Thus, although nervous tissue oxygen tension was not measured in the present experiments it appears very likely that nervous tissue oxygen tension decreased as the oxygen content of the arterial blood was gradually reduced. Therefore, a relationship similar to that described for $PaO_2$ and tonic SPN firing probably also exists for nervous tissue $PO_2$ and SPN activity.

A discontinuous relation was also found between $PaO_2$ and mean arterial blood pressure. Arterial blood pressure was independent of arterial $PO_2$ in the $PaO_2$ range from 400 mmHg to 40 mmHg. Below 40 mmHg an average, statistically significant, increase of 25% occurred. This increase was neurogenic because it was abolished by i.v. phentolamine or hexamethonium. The increase in mean arterial pressure was not likely due to a significant release of catecholamines by the adrenal medulla of itself since neither hypoxia nor asphyxia have been shown to have such an effect in the denervated adrenal gland (Comline & Silver, 1961, 1966; Bloom et al., 1976, 1977). The similarity between

cervical trunk SPN and arterial blood pressure responses to hypoxia suggests that the findings in this small sample of units from the CST must apply to a large part or the whole of the cardiovascular SPN population in order to account for the increases in arterial blood pressure. The discrepancy between the ten fold increase in SPN firing and the more modest increase in mean arterial blood pressure of only 25% may well be due to the effects of hypoxia upon the peripheral circulation and the myocardium (Mountcastle, 1980).

The present observations also indicate that while the administration of anaesthetic doses of pentobarbital (Barnes & Eltherington, 1966) drastically reduces or abolishes tonic SPN activity in normoxia, in most cases, it does not change the response to arterial hypoxia. Comparable doses of pentobarbital have also been shown to abolish the somato-sympathetic reflex mediated by spinal and supra-spinal pathways (Sato et al., 1965). Studies of a number of anaesthetic agents, including pentobarbital, have shown that their depressant effect upon the monosynaptic reflex of lumbar motoneurons is mediated presynaptically through a decrease in excitatory transmitter release (Weakly, 1969; Zorychta, 1974; Zorychta & Capek, 1978). If the same mechanism applies to the SPN, the reduction by pentobarbital of tonic SPN activity in normoxia is likely due to a reduction of excitatory afferent input to the SPN's. Therefore, the persistence without change of the SPN response to arterial hypoxia under pentobarbital suggests that this response is not dependent on excitatory afferent input.

In two strands of the CST it was found that the probability of evoking a response by radial nerve stimulation was markedly increased during extreme hypoxia ($PaO_2$ < 40 mmHg). This increase occurred in the $PaO_2$ range in which an increase in tonic SPN firing was observed. Such an increase in synaptic efficacy may be a reflection of an increase in excitability of the soma-dendritic region.

Intracellular recordings from various mammalian neurons has shown that during asphyxia or anoxia a gradual depolarization occurs at a rate of 2 to 6 mV/min (Kolmodin & Skoglund, 1959; Collewijn & Van Harreveld, 1966). Experiments conducted upon autoactive invertebrate neurons by Chalazonitis (1963) indicate that a decrease of intracellular $PO_2$ from 10 to 3 mmHg is sufficient to cause a large depolarization of the membrane as well as a large increase in firing frequency. As was reviewed by Ritchie (1973) the main source of energy for active $Na^+ - K^+$ pumping in nerve is oxidative phosphorylation. Inhibition of this pumping by hypoxia may explain, at least in part, the neuronal depolarization although assay of intracellular $Na^+$ and $K^+$ provides evidence also of a generalized increase in membrane permeability (Segal, 1970). Thus, a possible mechanism for the observed hypoxic excitation of SPNs could be a sustained depolarization of the neuron membrane resulting in increased excitability. This would be reflected in enhanced efficacy of synaptic input. If this maintained depolarization exceeded threshold repetitive firing of the SPN, that was relatively independent of synaptic input, might occur.

On termination of hypoxia and reoxygenation SPN firing returned to or near to normoxic control level. This ready reversability of the response as well as its repeatability for a particular strand indicates that the high levels of activity seen are not due to irreversible neuronal damage.

The silence seen in some strands following asphyxia or hypoxia is reminiscent of the decrease in sympathetic activity which has been noted following asphyxia in the CNS intact, anaesthetized and in the acute spinal animal (von Kehrel et al., 1963) as well as following periods of hypoxia in the CNS intact anaesthetized and peripherally chemodenervated animal (Gregor & Janig, 1977). A transient hyperpolarization of neurons immediately following the termination of asphyxia or hypoxia has been reported both in vivo (Kolmodin & Skoglund, 1959) as well as in vitro (Lorente de No, 1947; Lundberg & Oscarsson, 1953; Segal, 1970). The results of Lundberg (1953) indicate that this hyperpolarization is a result of electrogenic $Na^+$ pump activity due to the accumulation of $Na^+$ intracellularly during anoxia. Thus, the observed decreases in sympathetic activity might well be due to a hyperpolarization of the neuronal membrane, leading to a decreased neuronal excitability, caused by an electrogenic activity of the $Na^+$ pump consequent to the intracellular load produced by a period of hypoxia. Hyperpolarization due to electrogenic $Na^+$ extrusion following repetitive firing has also been hypothesized as a possible mechanism for the silent period of SPNs following antidromic driving (Mannard

et al., 1977). Alternatively, it is possible that a large increase in extracellular $K^+$, consequent to a period of hypoxia and repetitive firing, might cause sufficient depolarization of afferent terminals synapsing onto the SPNs to significantly reduce their transmitter release thus reducing tonic SPN firing. Depolarization of afferent terminals due to increases in extracellular $K^+$ has been suggested as a significant component of presynaptic inhibition (Krnjevic & Morris, 1972; ten Bruggencate et al., 1974).

Graded isocapnic hypoxia was seen to have qualitatively similar effects upon tonic SPN firing in the CNS intact, anaesthetized and peripherally chemodenervated preparation as in the acute spinal preparation. The similarity of the responses to hypoxia of SPNs in the spinal preparation and the CNS intact peripherally chemodenervated preparation suggests the possibility that the spinal actions of hypoxia may form an important part of the overall sympathetic response in the CNS intact and peripherally chemodenervated preparation.

The material presented in the RESULTS section of this thesis is original.

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