BRAIN NEUROTRANSMITTER AND HIGH ENERGY PHOSPHATE CONCENTRATION AFTER COMBINED HYPOXIA AND HYPOTENSION


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**Title:** Brain Neurotransmitter and High Energy Phosphate Concentration After Combined Hypoxia and Hypotension

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**Abstract:** See enclosed manuscript.
ABSTRACT

Previous work by the authors has established decreased brain ATP concentration after a combined hypoxic-hypotensive episode. This study was undertaken to determine what changes, if any, occur in brain norepinephrine (NE), dopamine (DA) and serotonin (5HT) concentration in association with hypoxia and hypotension, and to correlate any observed changes with simultaneously measured ATP concentrations. Rats were subjected to a 30 minute period of hypoxia (FIo2 = 0.075) and hemorrhagic hypotension (MAP = 30 mm Hg) and then resuscitated. Significant increases (p < .05) in cortical 5HT and DA concentrations were observed, at a time when ATP concentration was significantly (p < .005) reduced.

Additional experiments were conducted on rats depleted of 5HT by prior treatment with 5,7-dihydroxytryptamine. Equal decreases in ATP concentration were measured, and the cardio-vascular response to hypoxic-hypotension in 5HT depleted rats was similar to 5HT intact rats. We conclude that the increased 5HT after hypoxic-hypotension does not cause the decreased ATP concentration, nor does 5HT play a major role in cardio-vascular homeostasis under the conditions of this experiment.

KEY WORDS

shock, hypoxia, ATP, norepinephrine, dopamine, serotonin, brain
INTRODUCTION

Any temporary or permanent deficit in CNS neurotransmitter production and release can be followed by a failure to either appropriately excite or depress a neuronal system. Such altered neuronal function could have ominous significance if such deficits occurred in vital centers which control the heart, blood vessels, or respiration. Moreover, changes in cerebral perfusion or permeability can be provoked by biogenic amines. Thus, Osterholm(10) found that the monomine transmitters norepinephrine (NE) and 5-hydroxytryptamine (5HT) can cause major disruption of vascular endothelial cell function, and may contribute to the development or maintenance of vasospasm. Osterholm conceived neurotransmitter dysfunction as being triggered by trauma, then becoming self-sustaining, and possibly releasing toxic materials in excessive, abnormal, or unbalanced quantities.

Cerebral dysfunction after hypoxia and hypotension continues to be a major problem confronting trauma surgeons. We have reported previously a decreased brain ATP concentration after combined hypoxia and hypotension in an experimental shock model in rats(15). Since neurotransmitter synthesis, release, and synaptic uptake are dependent upon availability, the present study sought to clarify the role of neurotransmitters in the cerebral dysfunction following hypoxia and hypotension. We have thus attempted to correlate brain high energy phosphate concentration with concentrations of NE, 5HT, and dopamine (DA) in the brain stem, caudate nucleus, and frontal cerebral cortex.
of rats during and after a 30 minute period of combined hypoxia and hypotension.

Since these initial studies suggested that hypoxic hypotension was associated with an increase in regional serotonin content, additional experiments were designed to examine if the altered serotonin neuronal function was involved in the coincident decrease in cortical ATP concentration. Our results suggest that the changes in ATP are not simply a consequence of altered serotonin availability.

MATERIALS AND METHODS

Male albino rats (Sprague-Dawley) three to four months old were used without fasting. The animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.), a tracheostomy was performed, and they were ventilated using a Harvard Rodent Respirator. Both femoral arteries and one femoral vein were cannulated with Intramedic PE 50 polyethylene tubing for recording arterial pressure, arterial withdrawal of blood, and intravenous reinfusion of blood and saline. The animals were ventilated with a mixture of 30% $O_2$ - 70% $N_2$ while the respirator was adjusted to give an arterial $pCO_2$ of 35-40 mm of Hg. Animals with a baseline arterial $pO_2$ of less than 95 mm Hg or a mean arterial pressure of less than 110 mm Hg were discarded. After a steady state had been achieved, some animals were sacrificed as described below to determine baseline concentrations of 5HT, NE, DA, ATP, and lactate. The remaining animals were ventilated with a gas mixture containing 7.5% $O_2$ - 92.5% $N_2$ while the mean arterial pressure was lowered to 30 mm Hg.
over a five minute period by withdrawal of arterial blood into a heparinized syringe. All animals were maintained at this level of hypoxia and hypotension for 30 minutes at which time arterial parameters were again measured. In one group of animals (shock) the experiment was terminated at this point and the brain was frozen with liquid nitrogen. Other animals were resuscitated by ventilation with 30% O₂ - 70% N₂ and intravenous reinfusion of shed blood plus an equal volume of saline. There were two recovery groups in which brains were frozen at 20 (S + 20) and 120 (S + 120) minutes post-resuscitation. Freezing of the brain was accomplished by pouring liquid nitrogen on the exposed skull through a plastic funnel for four minutes, followed by whole body submersion in liquid nitrogen for about five minutes as described by Ponten (14). The parietal cortex and subjacent white matter were removed from one half of the skull at -25°C. These frozen samples were rapidly weighted and extracted. The concentrations of lactate and ATP were subsequently measured using the enzymatic, fluorometric methods of Lowry and Passoneau (9).

Additional samples of frontal cortex, brain stem, and caudate nucleus were obtained from the other half of the brain and the levels of 5HT, DA, and NE were estimated with fluorometric procedures described by Bogdanski (3) and Breese and Traylor (4). As employed in our laboratory 50 ng of serotonin, 20 ng of dopamine and 10 ng of NE give fluorescent intensities of about twice reagent blanks.
Tests of statistical significance were performed with the student t-test or an analysis of variance using Tukey's procedure to declare significance of differences between means (Steele and Torrie(19)).

RESULTS

Experiment I:

The mean arterial pressure, arterial pO₂, pCO₂, and pH, and brain lactate concentrations are depicted in Figure 1 and 2 to document the severity of the hypoxic-hypotensive insult. Note that despite the failure of the arterial pressure to return to baseline values, adequate perfusion of the brain was present to restore aerobic metabolism as evidenced by the return of brain lactate to near normal concentrations. We have also previously documented normal and greater than normal cerebral perfusion post-resuscitation employing the same experimental procedure(16).

As seen in Figure 3, brain ATP concentration was decreased as a result of the 30 minute period of combined hypoxia and hypotension, and failed to return to baseline values after resuscitation (p < .005), a finding we have also noted previously(15).

Figure 4 depicts the concentrations of 5HT, DA, and NE for the areas of brain sampled. A significant elevation in cortex and caudate 5HT concentration was noted during the shock period and in the brain stem at S + 20 (p < .05). However, by 120 minutes post-resuscitation the concentrations had returned toward control values.
and were no longer significantly different from baseline. Dopamine content was also significantly increased in the caudate regions during the shock period, (p < .05), with recovery of normal levels achieved by S + 120. No changes in brain stem or cortical NE were noted at any period.

Experiment II:

Having noted the increase in cortical 5HT immediately after hypoxia and hypotension, a second experiment was conducted to assess if the change in 5HT neurons function suggested by the increase in content of 5HT was responsible for the decrease in cortical ATP concentration.

Ten to fourteen days prior to the shock experiment, 5,7-dihydroxytryptamine (5,7-DHT) was injected intracisternally 30 minutes after 50 mg/kg pargyline (i.p.) to selectively destroy 75% of cortical 5HT producing fibers as described by Breese and Mueller(5). The pargyline pretreatment is required in order to prevent the toxic effect of 5,7-DHT on norepinephrine neurons. Pargyline's effect on monoamine oxidase is no longer present 10-14 days after administration to rats of this size according to Planz(13). A second group of rats (control for this experiment) were treated with pargyline alone.

On the day of the experiment, both groups of rats were subjected to identical preparation, experimental protocol, and assay technique as described above with the exception that data
were collected only at the baseline, end of hypoxia-hypotension, and 20 minutes post-resuscitation. Only a limited number of animals were sacrificed after baseline stabilization to document 5HT depletion. The 5HT depletion noted in these baseline values agrees with our previous results (5), and baseline values of NE and DA were unaltered by the 5,7-DHT treatment, as expected.

There were no significant differences (Figure 5 and 6) in arterial pressure pO₂, pCO₂, pH, or brain lactate concentrations, either between 5,7-DHT plus pargyline and pargyline-alone treated animals, or between these two groups compared with those animals studied in Experiment 1. The decrease in concentrations of 5HT produced by 5,7-DHT are depicted in Figure 7, and indicate a dramatic baseline depletion of 5HT. Although a similar relative increase in 5HT concentration was again noted during shock (p < .05), the concentration attained was well below normal values of cortical 5HT, and again the 5HT content decreased promptly toward baseline values by 20 minutes after resuscitation.

Of most importance is the fact that there were no significant differences in the concentrations of ATP produced by the shock paradigm when 5HT depleted animals were compared with control or Experiment I animals (Figure 8). In fact, the relative change was slightly greater in the 5,7-DHT treated rats.

DISCUSSION

The data presented confirm that a 30 minute hypoxic-hypotensive period causes severe interference with brain energy metabolism.
It is also demonstrated that the biochemical lesion produced by this event is only partially reversible by restoration of $F_1O_2$ to 30% and infusion of shed blood plus saline, despite cerebral perfusion adequate to restore aerobic metabolism.

Although Saavedra(18), Osterholm(11, 12) and Kidman(7) have measured changes in 5HT, DA, and NE in neuronal tissue following direct trauma, only scattered and conflicting reports, occasionally from the same laboratory(1), are available concerning neurotransmitter concentrations associated with ischemia. Zervas(22) found a depression of dopamine concentration after ligation of the gerbil common carotid artery. Kogure et. al.(8) found the opposite effect after regional embolization. Welch et. al.(20) found an increase in cerebral 5HT in the baboon in conjunction with ischemia. We found an increase in 5HT and DA concentration after ischemia as described above. These results do not necessarily contradict the earlier studies inasmuch as our model measures acute changes secondary to combined hypoxia and partial ischemia. Over and above the difference in the experimental model, both the quoted studies and our own concern themselves only with transmitter concentrations in a given tissue. Since concentrations of amine do not reflect such factors as rate of synthesis, storage, release, uptake and metabolic degradation, interpretation of the data must be guarded.

The results of our initial study in which the content of all three amines was measured during the course of shock and resuscitation
was predicated on the assumption that a large change in any of the above dynamic parameters might be reflected by changes in amine content. This assumption and our results of Experiment I thus indicated that the importance of 5HT fibers in producing the metabolic alterations was worthy of further investigation in this shock model. The changes noted in DA concentration have not yet been pursued further. The increase in content of 5HT in intact (Experiment I) and 5,7-DHT treated rats (Experiment II) could reflect an increased synthesis, storage, and reuptake or a decrease in release rate and metabolic degradation. Since Davis and Carlsson\(^6\) have demonstrated that monamine synthesis is directly related to brain oxygen tension, which must be low in our model, enhanced synthesis is an unlikely explanation. Since storage granules in monoaminergic neurons are made in the cell bodies and require many hours or days to arrive in the nerve terminal region (such as frontal cortex), an increase in storage capacity would also seem unlikely. Roth\(^17\) has recently demonstrated that the 5HT metabolizing enzyme activity MAO-A of human frontal cortex varies directly with oxygen tension, with a Km for oxygen of 170 \(\mu\)M. Since normal brain oxygen tensions are in range of 40 mm Hg, or about 50 \(\mu\)M in cytoplasm, a transient decrease in \(\text{PaO}_2\) could dramatically decrease the destruction of 5HT and thus increase tissue levels. Thus, if release and reuptake were constant during shock, post-synaptic serotonin availability would be increased. A similar mechanism might be postulated to
account for the observed changes in DA concentration.

Welch\(^{(20, 21)}\) and Bell\(^{(2)}\) have shown that increased concentrations of 5HT exist in ischemic areas of brain, and that vessels in the ischemic area are abnormally sensitive to 5HT administration, leading to vasospasm, worsening of the ischemia, cell death, increased capillary permeability, and brain edema. Our data on the cortex 5HT immediately after the hypoxic-hypotensive period tend to confirm this work. However since the increase promptly returned toward normal with resuscitation, we doubt that the increased 5HT was the cause of the persistent decrease in ATP concentration, although a subsequent enhanced release of 5HT could be the mechanism by which amine values return to control levels after reinfusion and increase in \(F_{102}\).

To test the hypothesis that increased 5HT release might be responsible for the low post-resuscitation ATP values, Experiment II was undertaken. Rats with decreased numbers of cortical 5HT nerve terminals as a result of 5,7-DHT pretreatment demonstrated no difference in their ability to tolerate the hypoxic-hypotensive stress in terms of arterial pressure or acid base response. Thus, CNS 5HT containing neurons do not appear to play a major role in cardiovascular homeostasis in response to the stress of hypoxia and hypotension as employed in this study. Moreover, since cortical ATP was decreased as sharply in the 5,7-DHT pretreated rats as in controls and as in the rats in Experiment I, the 5HT increase noted
by ourselves and by others (see above) probably does not play an important role in causing the cerebral metabolic derangement produced in this shock model.

SUMMARY

In anesthetized rats DA and 5HT concentration were significantly increased after a 30 minute period of hypoxia and hypotension, and returned to baseline concentrations with resuscitation. These increases are postulated to be on the basis of decreased destruction due to decreased MAO-A activity resulting from hypoxia. Based on results obtained from a second series of shock in rats with drug-induced destruction of 5HT neurons, 5HT did not appear to play a major role in cardiovascular or metabolic homeostasis as evidenced by mean arterial pressure, brain lactate, and brain ATP concentrations.
REFERENCES cont’d.


REFERENCES


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LEGEND - FIGURE 1

Mean and standard error of the mean of arterial pressure before and during the time course of combined hypoxia and hypotension (shock) and 20 and 120 minutes after resuscitation (S + 20; S + 120).
LEGEND - FIGURE 2

Changes in mean and standard error of the mean of arterial 
$pO_2$, $pCO_2$, pH, and brain lactate concentrations during and after 
combined hypoxia and hypotension.
LEGEND - FIGURE 3

Changes in mean and standard error of the mean in brain ATP concentration before, during, and after hypoxia and hypotension.
LEGEND - FIGURE 4

Changes in mean and standard errors of the mean of the concentration of 5HT, DA and NE in the brain stem, cortex, and caudate nucleus during and after hypoxia and hypotension. Concentrations of NE were determined in brain stem and cortex only.
LEGEND - FIGURE 5

Changes in mean and standard error of the mean of arterial pressure before and as a result of hypoxia and hypotension (shock) and 20 minutes after resuscitation (S + 20) comparing control and SHT depleted animals.
LEGEND - FIGURE 6

Changes in mean and standard error of the mean arterial pO$_2$, pCO$_2$, pH, and brain lactate concentration comparing control and 5HT depleted animals.
LEGEND - FIGURE 7

Changes in mean and standard error of the mean of 5HT concentration in control and 5HT depleted cortices during and after hypoxia and hypotension.
LEGEND - FIGURE 8

Changes in mean and standard error of the mean ATP concentrations during and after hypoxia and hypotension, comparing the results from Experiment I with control and 5HT depleted animals in Experiment II.
ARTERIAL PRESSURE