Pharmacology Biochemistry & Behavior, Vol. 18, pp. 909–916, 1983. © Ankho International. Printed in the U.S.A.

Cholinergic Drugs and 4-Aminopyridine Alter Hypoxic-Induced Behavioral Deficits

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Received 8 July 1982

GIBSON, G. E., C. J. PELMAS AND C. PETERSON. *Cholinergic drugs and 4-aminopyridine alter hypoxic-induced behavioral deficits.* PHARMACOL BIOCHEM BEHAV **18**(6) 909–916, 1983.—To test the hypothesis that decreased acetylcholine (ACh) metabolism during hypoxia is behaviorally important, the effects of cholinergic drugs and 4-aminopyridine, an enhancer of ACh release, were examined in hypoxic mice. Chemical hypoxia (150 mg/kg $NaNO_2$) reduced tight rope test scores from 13.2±0.2 to 2.8±0.3. 4-Aminopyridine partially reversed the scores of hypoxic mice (7.3±0.7). Physostigmine improved performance by hypoxic mice (7.9±0.4) when it was given before $NaNO_2$ and this effect was blocked by pretreatment with atropine (3.5±0.4) or mecamylamine (2.4±0.6). Neostigmine (0.002–0.2 mg/kg) was ineffective. Performance also improved if atropine (7.0±0.5) or mecamylamine (7.2±0.5) was given before $NaNO_2$. Nicotine (5.5±0.7) or the muscarinic agonist arecoline (5.4±0.5) improved performance when given during the hypoxic episode, and when the drugs were combined, the score was even higher (8.2±1.0). Neither epinephrine (0.002–2.0 mg/kg) nor norepinephrine (0.00002–2.0 mg/kg) improved performance by hypoxic mice. These results suggest that hypoxia produces a behaviorally important impairment of the cholinergic system perhaps through a primary alteration of acetylcholine release.

Hypoxia Oxygen Acetylcholine Aminopyridine Behavior Nicotine Physostigmine Atropine Mecamylamine Release Muscarine

THE metabolic encephalopathies are a group of disorders in which brain function is altered secondarily to systemic changes [18]. Despite diverse etiologies (i.e., hypoxia, hypoglycemia, hyperammonemia, heavy metal poisoning or nutritional deficits), these diseases lead to a decline in short term memory, reduced critical judgement, progressive loss of performance on intellectual and motor tasks, mental confusion and eventually unconsciousness [16,20]. Since these disorders share similar symptoms, elucidation of the molecular mechanism of one may provide insight into all of them.

Hypoxia, a reduction in oxygen availability, is a well standardized animal model of these metabolic encephalopathies. The brain depends upon a continuous supply of oxygen, but the molecular basis for this requirement has not been determined. ATP or the adenylate energy charge are unaltered in even severe hypoxia [15,21]. The brain's need for oxygen may be due to oxygen's role in neurotransmitter metabolism. Although hypoxia decreases catecholamine and serotonin synthesis [5, 6, 7], the physiological importance of these changes remains unclear since they can be reversed by stress [3,4]. The synthesis of the glucose derived neurotransmitters acetylcholine (ACh), glutamate, aspartate, 4-aminobutyrate, serine, glycine, glutamine, and alanine decline in parallel during moderate reductions in oxygen tensions and during chemical hypoxia [12].

Although changes other than those in ACh metabolism accompany hypoxia, the deficits in the cholinergic system appear to play a role in the behavioral deficits. Pretreatment of hypoxic-hypoxic rats [19] or anemic hypoxic mice [9] with the acetylcholinesterase inhibitor physostigmine prolongs the time until seizures and death. These studies used severe hypoxia, crude indicators of behavioral deficits and a single dosage of one cholinergic drug. The present studies used a sensitive indicator of behavioral deficits [1, 2, 10, 12], and a complete dose response of numerous cholinergic drugs to determine which components of the cholinergic system are altered by chemical hypoxia. Previous studies [12] showed that this model of chemical hypoxia with $NaNO_2$-injections and hypoxic-hypoxia produce similar neurochemical changes.

METHOD

Male CD-1 mice (18–30 g) from Charles River Breeding Laboratory (Wilmington, MA) were acclimated to our animal facility for a minimum of two days. Drugs are listed with their molecular weights in parentheses. Sodium nitrite ($NaNO_2$; 69.0) was from J. T. Baker Chemical Co. (Phillipsburg, NJ), mecamylamine hydrochloride (203.8) from Merck, Sharpe and Dohme Research Labs (West Point, PA); adrenaline chloride (183.2) from Parke-Davis Pharmaceutical (Morris Plains, NJ) and levophed bitartrate (norepinephrine; 169.2) was from Breon Laboratories (New York, NY). Neostigmine methyl sulfate (334.4), physostigmine salicylate (413.5), atropine sulfate (676.8), atropine methyl bromide (methatropine; 384.3), arecoline hydrobromide (236.2), nicotine (162.2), hexamethonium bromide (362.2), decamethonium bromide (418.4) and 4-aminopyridine (94.1) were from Sigma Chemical Co. (St. Louis, MO).

The quantitative scoring system [2] for the tight rope test

TABLE 1
SCORING PROTOCOL FOR THE TIGHT ROPE TEST

Behavior	Time Interval	Points	Maximum Points
each paw on string	5 sec	1	4
tail on string	5 sec	1	1
change in body position from vertical horizontal	none	1	1
each paw used to traverse	5 sec	1	4
travel in one direction	5 sec	3	3
freeze on string	4 sec	−1	−3
fall during time interval	0–15 sec	−3	
fall during time interval	16–30 sec	−2	
fall during time interval	31–60 sec	−1	
reaches vertical pole within	0–15 sec	3	
reaches vertical pole within	16–30 sec	2	
reaches vertical pole within	31–60 sec	1	

A mouse is placed in the middle of an elevated taut string and scored on his ability to maneuver along it until he reaches one of the two vertical poles or falls. The testing time is one minute. Maximum score is 16. Minimum score is −3. Animals must freeze for 4 sec to lose 1 point.

of Miquel and Blasco [17] has high day to day reproducibility and small within day variation. The sensitivity of this test was increased by expansion of the scoring criteria (Table 1). The tight rope appartus consisted of a string (length 50 cm; diameter 2 mm) that was tied tightly between two vertical poles and suspended 40 cm over a foam pad. The animal was held by the tail over the midpoint of the string; timing and scoring began when the mouse grasped the string with its forepaws and ended if the animal fell, reached one of the vertical poles or after one min. The scoring of the test was done by the same person who gave the injections. Since subjective judgements are not required and each experiment contains 4–6 treatments with 5 animals in each, this approach probably did not introduce a bias. One animal from each group was treated and/or tested before two animals in any treatment and that sequence was repeated. Furthermore, the short time interval (0.5 to 1 min) between treatment and testing of mice from different groups did not permit the observer time to decode the treatment of the mouse that was being tested.

Mice were fasted the night before the experiment but allowed water ad lib. To determine control scores, mice were pretested and those with scores that were 11 or less (about 20%) were eliminated. The control test scores of the remaining mice were ranked to evenly distribute them among the treatment groups. Multiple testing of the same animal does not alter tight rope performance ([1, 2, 12], unpublished results).

On the day of the experiment, the drugs were dissolved in 0.9% saline and the pH was adjusted to 7.4 with either 1 N HCl or 1 N NaOH. The drugs were injected intraperitoneally (0.0075 ml/g body weight). Mild hypoxia was induced by injection of $NaNO_2$ (150 mg/kg), which converts hemoglobin to methemoglobin and thus reduces the oxygen carrying capacity of the blood. Preliminary experiments determined that mean scores at 10 and 20 min after injection of $NaNO_2$ injection had larger standard errors than those at 30 min (unpublished results). Thus, all hypoxic mice were tested 30 min after the injection of $NaNO_2$.

The percent methemoglobin was measured to determine if the drugs altered the $NaNO_2$-hemoglobin interaction. For methemoglobin determinations the mice were treated as in the behavioral studies, except that at the testing time they were decapitated and blood was collected and heparinized (30 units per sample). The percent methemoglobin was determined by modification of the method of Evelyn and Malloy [8]. Heparinized blood (0.05 ml) was added to 4.95 ml of 0.017 M phosphate buffer (pH 6.6) and the absorbance was read at 635 nm. Neutralized cyanide (0.04 ml; from equal volumes of 12% acetic acid and 13.3% potassium cyanide) was added and 1 ml of sample was transferred to 4.0 ml of 0.067 M phosphate buffer (pH 6.6) and absorbance was read at 540 nm.

The total number of $NaNO_2$-treated animals was large (>125) compared to any other treatment group. Statistical comparison between drug treatment groups were only made with animals that were treated with $NaNO_2$ on the same experimental day to avoid the Type 1 errors [22]. All comparisons were done by analysis of variance with the least significant difference test (lsd; [22]).

RESULTS

The tight rope test was a sensitive behavioral indicator of mild hypoxia in mice. Performance declined linearly with increasing dosages of $NaNO_2$ (Fig. 1). No other behavioral deficits in rodents have been reported for the milder degrees of hypoxia. The 150 mg/kg dosage of $NaNO_2$ was chosen for the drug trials because it had smaller standard errors than the other treatments and allowed considerable room for improvement. Although performance was measured at 10, 20 and 30 min after injection of $NaNO_2$, testing at 30 min gave the most highly reproducible scores. The percent methemoglobin at 30 min was 4.0 ± 0.4 ($n=5$) in controls and 54 ± 3 in the $NaNO_2$-injected animals. None of the drugs at their most efficacious dosage altered the $NaNO_2$-hemoglobin interaction since no treatment changed the percent methemoglobin ($n \geq 3$). Nor were the drug-induced changes in tight-rope per-

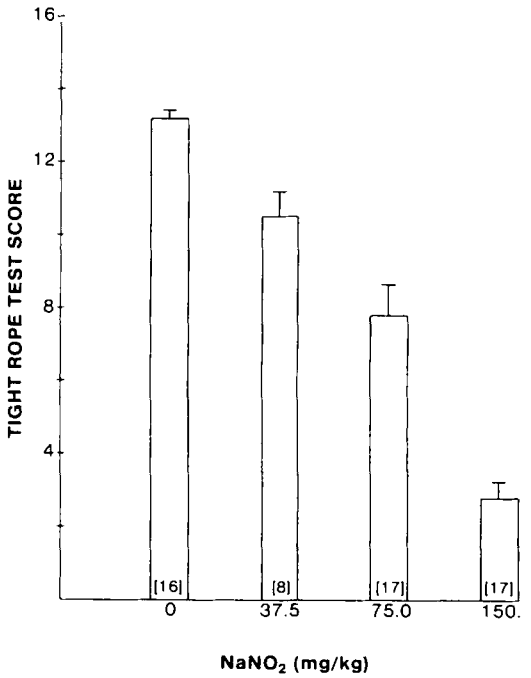

FIG. 1. $NaNO_2$ dose-response. $NaNO_2$ was injected 30 min prior to the tight rope test. The scores at each dosage level differ from one another by the lsd test ($p<0.05$). Values are means±S.E.M. of the number of mice in brackets.

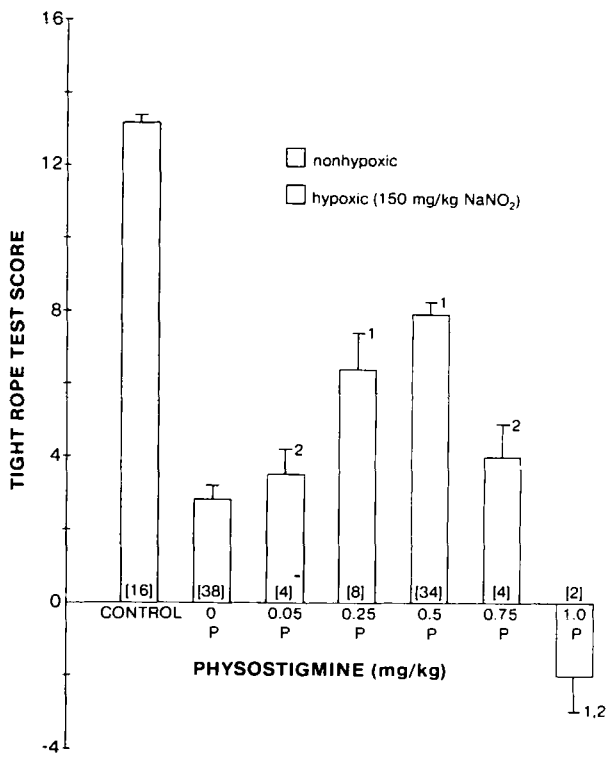

FIG. 2. Physostigmine dose-response. Physostigmine salicylate (P) was injected 10 min prior to the $NaNO_2$ (40 min before testing). If physostigmine (0.5 mg/kg) was given 10 min after $NaNO_2$ (20 min before testing), scores did not improve (n=15). [1]Denotes score differs significantly ($p<0.05$) from $NaNO_2$ value. [2]Denotes score less than the optimal physostigmine dosage (0.5 mg/kg) by the lsd test ($p<0.05$). All treatment scores were significantly lower ($p<0.05$) than the control value. Values are means±S.E.M. of the number of mice in brackets. When nonhypoxic mice were given physostigmine (0.5 mg/kg) 40 min prior to testing, scores declined ($p<0.05$) from the control level of 13.2±0.1 (n=40) to 6.0±0.7 (n=8).

formance due to a general stimulation that might be produced by the injection, since saline did not alter scores in either hypoxic or non-hypoxic mice (unpublished observations).

Physostigmine salicylate (0.5 mg/kg), a centrally and peripherally acting acetylcholinesterase inhibitor, improved tight rope test scores in hypoxic mice (Fig. 2), when injected 10 min before $NaNO_2$ (40 min before testing). At dosages greater than 0.25 mg/kg, salivation increased and tremors appeared. A post-treatment with physostigmine (0.5 mg/kg; 10 min after $NaNO_2$ and 20 min before testing) did not improve hypoxic scores (legend Fig. 2). A seven point decline in the scores was noted when physostigmine (0.5 mg/kg) was given to non-hypoxic mice 40 min before the tight rope test (legend Fig. 2). Neostigmine methyl sulfate, which primarily inhibits peripheral acetylcholinesterase, either had no effect (0.02 mg/kg) or reduced scores (0.002 or 0.2 mg/kg) when it was given before or after $NaNO_2$. In non-hypoxic mice, 0.2 mg/kg reduced scores from 13.2±0.1 (n=40) to 9.0±1.5 (n=8).

To determine if the lack of effect of physostigmine as a treatment during hypoxia was a pre- or a post-synaptic effect agonists were tested during the hypoxic insult. Centrally acting muscarinic drugs improved tight rope test performance in hypoxic mice. Arecoline hydrobromide (1.0 or 2.0 mg/kg), which is primarily a muscarinic agonist with central and peripheral actions, improved the performance of hypoxic mice (Fig. 3), when given 15 min after $NaNO_2$ (15 min before testing). Some of this effect may have been due to its nicotinic action [14]. The same dosages decreased test scores in non-hypoxic mice (legend Fig. 3). The muscarinic antagonist atropine sulfate (2.0 mg/kg), that acts both centrally and peripherally, significantly improved test scores of hypoxic mice (Fig. 4), if given 25 min before $NaNO_2$ (55 min prior to testing). Dosages one order of magnitude higher or lower than the optimal dosage (2.0 mg/kg) did not improve performance in hypoxic mice. Atropine (2.0 mg/kg) was not effective in hypoxic mice when it was given after or simultaneously with $NaNO_2$ and it decreased the scores in non-hypoxic mice (legend Fig. 4). Methatropine (0.1, 1.0, or 10 mg/kg), which is a muscarinic blocker that acts predominantly in the periphery, did not improve tight rope test performance. The highest dosage (10 mg/kg) lowered the scores of $NaNO_2$ injected mice. Performance declined from 13.2±0.1 (n=40) to 7.9±1.7 (n=8) in methatropine-treated non-hypoxic mice.

Nicotinic drugs also improved tight rope test performance in hypoxic mice. Several low dosages ($<5\times10^{-2}$ mg/kg) of nicotine, a cholinergic agonist that acts both centrally and peripherally, reversed the hypoxic decrease in behavior (Fig. 5) when injected 15 min after $NaNO_2$ (15 min before testing). Higher dosages produced tremors, but had no effect (5×10^{-1} mg/kg) or decreased (5 mg/kg) tight rope performance. When non-hypoxic mice were given nicotine (5×10^{-2} mg/kg) their scores declined (legend Fig. 5). Since treatment of hypoxic animals was successful, prehypoxic treatment was not examined. Mecamylamine hydrochloride (2.0 and 0.5 mg/kg), a

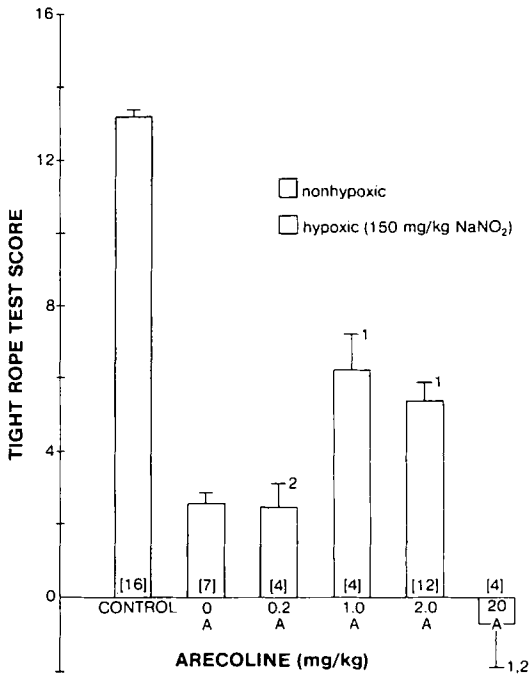

FIG. 3. Arecoline dose-response. Arecoline hydrobromide (A) was injected 15 min after $NaNO_2$ (15 min prior to testing). [1]Denotes values higher than the $NaNO_2$ level. [2]Denotes score lower than both the 1.0 and 2.0 mg/kg dosage levels by the lsd test ($p<0.05$). Values are means±S.E.M. of the number of mice in brackets. When nonhypoxic mice were given arecoline (2.0 mg/kg), scores declined ($p<0.05$) from 13.2±0.1 (n=40) to 7.0±1.1 (n=8).

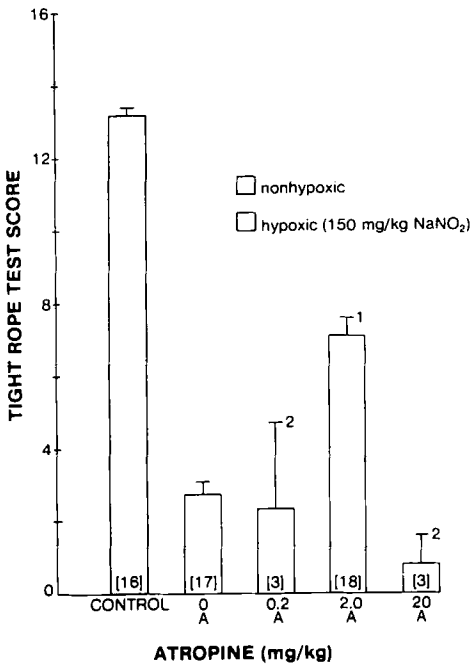

FIG. 4. Atropine dose-response. Atropine (A) was injected 25 min before $NaNO_2$ (55 min prior to testing). When atropine (2 0 mg/kg) was injected 15 min after (4.2±0.6, n=4) or at the same time (3.2±0.5; n=4) as the $NaNO_2$ injection, mice scored significantly lower than the atropine pretreated group. [1]Denotes score is lower than the optimal (2.0 mg/kg) dosage level by the lsd test ($p<0.05$). Values are means±S.E.M. of the number of mice in brackets. When nonhypoxic mice were given atropine (2.0 mg/kg) 55 min prior to testing, scores decreased ($p<0.05$) from 13.2±0.1 (n=40) to 9.8±1.0 (n=8).

central and peripheral nicotinic antagonist, improved scores (Fig. 6) when it was given 25 min before $NaNO_2$ (55 min before testing) at the time when it effectively blocked the actions of physostigmine. The highest dosage (50 mg/kg) decreased performance of hypoxic mice. Mecamylamine (2.0 mg/kg) reduced test scores in non-hypoxic mice when given 55 min before testing (legend Fig. 6). Hexamethonium bromide (0.5 and 0.2 mg/kg), a nicotinic blocker in autonomic ganglia, improved scores (Fig. 7) when given 25 min before $NaNO_2$ (55 min prior to the tight rope test), but decreased scores (0.5 mg/kg) in non-hypoxic mice (legend Fig. 7). Decamethonium bromide (0.2 mg/kg), a depolarizing blocker at the neuromuscular junction, improved test performance (Fig. 8) when given 25 min (55 min before testing) before $NaNO_2$. However, in non-hypoxic mice it decreased scores (legend Fig. 8).

4-Aminopyridine (0.1 mg/kg) improved scores in hypoxic mice (Fig. 9) when given 15 min after $NaNO_2$ (15 min before testing), but was ineffective when given before $NaNo_2$. The dosage of 4-aminopyridine that optimally reversed the hypoxic-induced decline in performance did not reduce scores in non-hypoxic mice (legend Fig. 9).

Drugs that alter blood pressure [epinephrine (0.002, 0.02, 0.2 or 2 mg/kg) or norepinephrine (2×10^{-5}, 2×10^{-4}, 2×10^{-3}, 2×10^{-2}, 2×10^{-1} or 2 mg/kg)] did not improve tight rope test performance of hypoxic mice.

Drug combinations were used to help elucidate the interaction of hypoxia with the cholinergic system. To fully characterize these interactions, each agonist and antagonist combination would have to be tested at several dosages and times of administration. In the present studies, the drugs were administered at the concentration and time that optimally improved tight rope test scores in hypoxic mice when they were given individually. The combination of arecoline (Fig. 3) and nicotine (Fig. 5) produced higher scores (Table 2) than either drug alone, but that of atropine (Fig. 4) and mecamylamine (Fig. 6) did not improve scores (Table 2) more than either drug alone.

Combinations of the acetylcholinesterase inhibitors and either muscarinic or nicotinic antagonists were also evaluated for their effectiveness at the dosages and times that improved performance in hypoxic mice when given alone. Prior administration of either atropine (Fig. 4) or methatropine diminished the beneficial effect of physostigmine (Table 2). If neostigmine was given after atropine, the improvement with atropine in hypoxic mice was partially blocked. Nicotinic antagonists also altered the effects of the acetylcholinesterase inhibitors during hypoxia. When mecamylamine was given before physostigmine, the beneficial effect of physostigmine (Fig. 2) in hypoxic mice was blocked (Table 2). If mecamylamine was administered after physostigmine it did not alter the action of physostigmine. If neostigmine was given after mecamylamine, the improvement with mecamylamine in hypoxic mice was partially blocked (Table 2).

DISCUSSION

The tight rope test is a sensitive behavioral indicator of the effects of hypoxia ([12] and present study), thiamin defi-

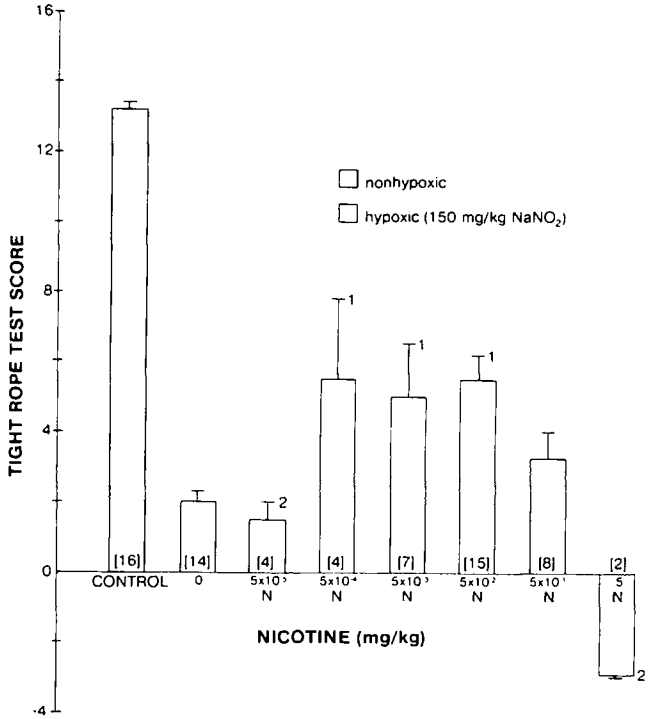

FIG. 5. Nicotine dose-response. Nicotine (N) was injected 15 min after $NaNO_2$ (15 min prior to testing). [1]Denotes score is higher than the hypoxic level. [2]Denotes scores significantly lower than the dosages from 5×10^{-2} to 5×10^{-4} mg/kg by the lsd test ($p<0.05$). Values are means±S.E.M. of the number of mice in brackets. When nicotine (5×10^{-2} mg/kg) was given to nonhypoxic mice 15 min before testing, scores decreased ($p<0.05$) from 12.0±0.1 (n=40) to 7.0±1.3 (n=8).

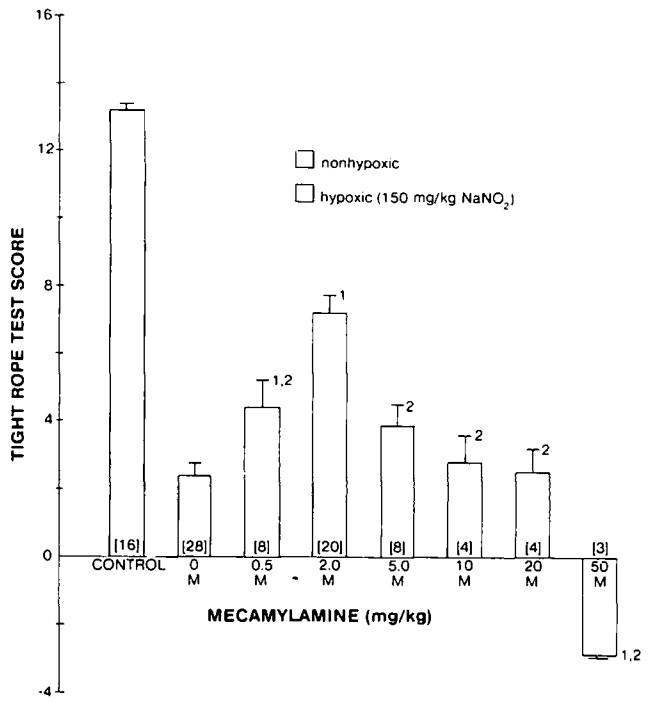

FIG. 6. Mecamylamine dose-response. Mecamylamine hydrochloride (M) was injected 25 min prior to $NaNO_2$ (55 min before testing). Mecamylamine (2.0 mg/kg) improved test scores in hypoxic mice when given 15 min after $NaNO_2$ (15 min prior to testing; 8.1±1.3; n=7). When the 2.0 mg/kg dosage was given at the same time as the $NaNO_2$ (i.e., 30 min before testing) scores did not improve (3.8±0.6; n=4). [1]Denotes score is lower than the 2.0 mg/kg dosage level by the lsd test ($p<0.05$). Values are means±S.E.M. of the number of mice in brackets. Mecamylamine (2.0 mg/kg) 55 min before testing decreased scores ($p<0.05$) in nonhypoxic mice from 13.2±0.1 (n=40) to 7.1±1.6 (n=8).

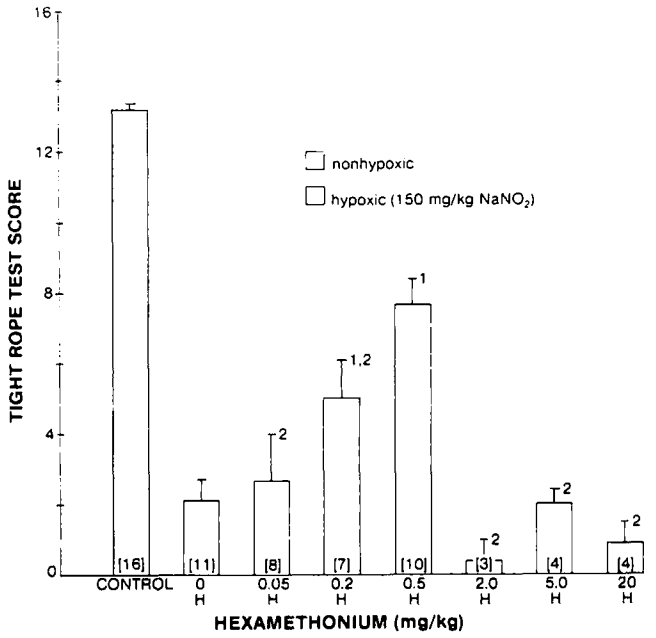

FIG. 7. Hexamethonium dose-response. Hexamethonium bromide (H) was given 25 min before $NaNO_2$ (55 min before testing). [1]Denotes score is higher than $NaNO_2$. [2]Denotes score is lower than the 0.5 mg/kg dosage group by the lsd test ($p<0.05$). Values are means±S.E.M. of the number of mice in brackets. When hexamethonium (0.5 mg/kg) was given to nonhypoxic mice, scores dropped ($p<0.05$) from 13.2±0.1 (n=40) to 7.5±1.3 (n=8).

ciency [1,2] and aging [10] that can be manipulated with various drug treatments. Performance in non-hypoxic mice declined or was unchanged when they received drug regimens that were beneficial to hypoxic mice. Different dosages may have improved tight rope test scores in controls, but our purpose was to determine whether the dosages that reversed hypoxic deficits altered scores in non-hypoxic mice. The drug-induced decreases of scores in non-hypoxic mice may have been due to perturbations of the cholinergic system that was already in balance.

The present studies demonstrate that the cholinergic deficit in hypoxia is behaviorally important. Cholinergic agonists (arecoline and nicotine) partially improved performance on the tight rope test during hypoxia, but only if they were administered at the appropriate dosage during the hypoxic insult. On the other hand, cholinergic antagonists (hexamethonium, decamethonium, atropine, and mecamylamine) were only beneficial if they were injected before the hypoxic insult. Perhaps, the prior exposure to these drugs stimulated the cholinergic system so that it could better withstand subsequent hypoxic insults. However, the incomplete reversal of behavior with cholinergic drugs implies that hypoxia also alters other aspects of metabolism. Since none of the drugs altered the methemoglobin levels during chemical hypoxia, they probably had no direct interaction with $NaNO_2$ or with methemoglobin.

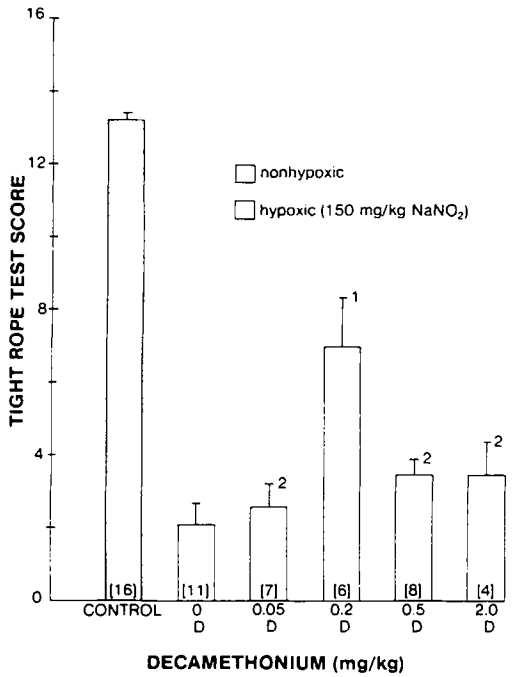

FIG. 8. Decamethonium dose-response. Decamethonium bromide (D) was injected 25 min before $NaNO_2$ (55 min before testing). [1]Denotes score is higher than the hypoxic level. [2]Denotes score is lower than the 0.2 mg/kg dosage group by the lsd test ($p<0.05$). Values are means±S.E.M. of the number of mice in brackets. When nonhypoxic mice were given decamethonium (0.2 mg/kg) 55 min prior to testing, scores decreased ($p<0.05$) from 13.2±0.1 (n=40) to 6.0±1.5 (n=8).

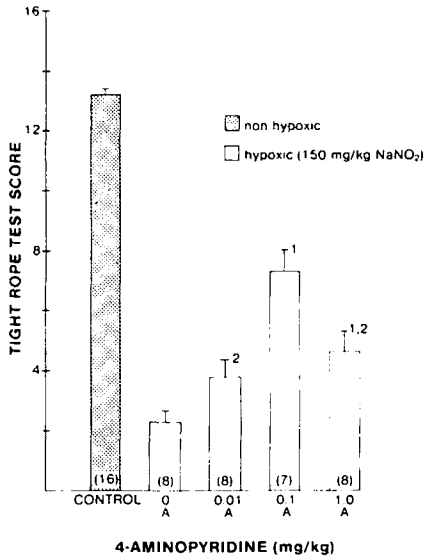

FIG. 9. 4-Aminopyridine dose-response. 4-Aminopyridine (A) was injected 15 min after $NaNO_2$ (15 min prior to testing). When mice were treated with 4-aminopyridine (0.1 mg/kg) 15 min before $NaNO_2$, scores did not improve over the hypoxic level (3.5±0.9; n=4). [1]Denotes scores higher than $NaNO_2$. [2]Denotes scores lower than the 0.1 mg/kg dosage level by the lsd test ($p<0.05$). Values are means±S.E.M. of the number of mice in brackets. When nonhypoxic mice were given 4-aminopyridine (0.1 mg/kg) 15 min before testing, scores were unchanged.

These *in vivo* data are consistent with the hypothesis that hypoxia may impair the cholinergic system by inhibition of the calcium dependent release of ACh. *In vitro*, this decrease can be overcome by 4-aminopyridine [11], which stimulates calcium influx into the nerve terminal (see [13]). The improvement in tight rope test performance with 4-aminopyridine suggests that impaired release may underlie the hypoxic induced behavioral deficits. This would explain why physostigmine is only beneficial as a pretreatment; since release is impaired during hypoxia there would be little ACh in the synaptic cleft to protect from hydrolysis. This hypothesis would also predict that agonists would be effective during the hypoxia, but that antagonists would not.

The cholinergic deficit in hypoxia appears to have a muscarinic and nicotinic component. Thus, both nicotine and arecoline can reverse hypoxic symptoms if they are given during the hypoxic insult and are more effective together than either is individually. Injections of mecamylamine or atropine before the hypoxic insult partially protect against hypoxia. Furthermore, physostigmine's beneficial effects are blocked by prior administration of mecamylamine, a nicotonic blocker or atropine, a muscarinic antagonist. This lack of distinction between different cholinergic receptors further supports the concept of a presynaptic blockade of ACh release by hypoxia.

Hypoxia also alters the peripheral cholinergic system. The improvement in tight rope test performance with decamethonium and hexamethonium indicate alterations in the peripheral nicotinic component of the ganglion. In addition, methatropine partially blocks the beneficial peripheral effects of physostigmine. The ineffectiveness of neostigmine or methatropine alone suggest that a central cholinergic component is also important.

Catecholamine administration demonstrated that peripheral sympathetic effects could not account for the alteration in behavior. Norepinephrine and epinephrine at concentrations (0.002–2 mg/kg) that are known to increase blood pressure, failed to improve tight rope test performance in hypoxic mice. Both elevate blood pressure, but through two different mechanisms [14]. Since neither drug reversed the hypoxic score deficit, the impairment of tight rope test performance with hypoxia and its improvement with cholinergic drugs is probably independent of the cholinergic drugs' effects on the sympathetic nervous system.

In conclusion, pharmacological manipulations of the behavioral deficits due to chemical hypoxia directly implicate the cholinergic system as a behaviorally important component in the hypoxic syndrome. The results are consistent with the hypothesis that hypoxia impairs the release of ACh. Hypoxia altered both muscarinic and nicotinic components of the central cholinergic system. The pharmacological manipulations suggested that peripheral aspects of the cholinergic system were also altered, although the studies with norepinephrine and epinephrine demonstrate that this is not strictly due to changes in blood pressure. Since cholinergic drug treatments did not produce a complete recovery, the deficits in behavior are not only due to cholinergic dysfunction.

TABLE 2

CHOLINERGIC DRUG COMBINATIONS IN CHEMICAL HYPOXIA

	Dosages (mg/kg)	Min Before Tight Rope Test	Tight Rope Test Score Mean ± S.E.M.	(n)	Significance
Control	—	—	13.4 ± 0.2	(16)	1
$NaNO_2$	150	30	2.6 ± 0.2	(125)	—
DRUG INTERACTIONS					
Agonists					
Nicotine + Arecoline	5×10^{-2}, 2.0	15, 15	7.7 ± 0.7	(11)	1, 3, 5
	5×10^{-2}, 1.0	15, 15	6.3 ± 1.2	(3)	1
	5×10^{-3}, 2.0	15, 15	8.2 ± 1.0	(4)	1, 3, 5
	5×10^{-3}, 1.0	15, 15	8.0 ± 1.5	(4)	1, 3, 5
	5×10^{-4}, 1.0	15, 15	7.4 ± 1.1	(4)	1
	5×10^{-4}, 1.0	15, 15	4.2 ± 0.9	(4)	—
Antagonists					
Atropine + Mecamylamine	2.0, 2.0	55, 55	6.7 ± 1.8	(7)	1
Acetylcholinesterase Inhibitors + Muscarinic Antagonists					
Atropine + Physostigmine	2.0, 0.5	55, 40	3.5 ± 0.4	(8)	4, 6
Physostigmine + Atropine	0.5, 2.0	40, 15	1.5 ± 0.6	(4)	4, 6
Methatropine + Physostigmine	2.0, 0.2	55, 40	5.7 ± 0.3	(6)	1, 3, 6
Atropine + Neostigmine	2.0, 0.2	55, 40	4.8 ± 0.8	(8)	1, 4, 5
Acetylcholinesterase Inhibitors + Nicotine Antagonists					
Physostigmine + Mecamylamine	0.5, 2.0	40, 15	8.7 ± 1.1	(6)	1
Mecamylamine + Physostigmine	2.0, 0.5	55, 40	2.4 ± 0.6	(7)	4, 6
Mecamylamine + Neostigmine	2.0, 0.2	55, 40	5.2 ± 1.6	(8)	1, 4, 5

Mice were given $NaNO_2$ (150 mg/kg) 30 min before the tight rope test. Both dosages and timings are in the same order as the drugs listed under Drug Interactions. Each drug was given at the concentration at which it alone optimally reversed hypoxic-deficits. Differences ($p<0.05$) were determined by the lsd test: 1. higher than $NaNO_2$; 2. lower than $NaNO_2$; 3. higher than first drug alone; 4. lower than first drug alone; 5. higher than second drug alone; 6. lower than second drug alone.

ACKNOWLEDGEMENTS

This work was supported in part by NIH grants NS 16997, NS 03346 and NS 15125, the Brown and Williamson Company, the Burke Relief Foundation and the Will Rogers Institute. The authors thank Chantel Stern for her assistance in early stages of these experiments.

REFERENCES

1. Barclay, L. L., G. E. Gibson and J. P. Blass. Impairment of behavior and acetylcholine metabolism in thiamine deficiency. *J Pharmacol Exp Ther* **217:** 537–543, 1981.
2. Barclay, L. L., G. E. Gibson and J. P. Blass. The string test: An early behavioral change in thiamine deficiency. *Pharmacol Bicohem Behav* **14:** 153–157, 1981.
3. Brown, R. M., S. R. Snider and A. Carlsson. Changes in biogenic amine synthesis and turnover induced by hypoxia and/or foot shock stress. II. The central nervous system. *J Neural Trans* **35:** 293–305, 1974.
4. Carlsson, C., M. Hagerdal, A. E. Kaasik and B. K. Siesjö. A catecholamine-mediated increase in cerebral oxygen uptake during immobilization stress in rats. *Brain Res* **119:** 223–231, 1977.
5. Davis, J. N. and A. Carlsson. Effect of hypoxia on tyrosine and tryptophan hydroxylation in unanesthetized rat brain. *J Neurochem* **20:** 913–915, 1973.
6. Davis, J. N., A. Carlsson, V. MacMillian and B. K. Siesjo. Brain tryptophan hydroxylation: Dependence on arterial oxygen tension. *Science* **182:** 72–74, 1973.
7. Davis, J. N., L. T. Gibson, E. Stanton and W. Maury. The effect of hypoxia on brain neurotransmitter systems. *Adv Neurol* **26:** 219–223, 1979.
8. Evelyn, K. A. and H. T. Malloy. Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. *J Biol Chem* **126:** 655–662, 1938.
9. Gibson, G. E. and J. P. Blass. Impaired synthesis of acetylcholine in brain accompanying mild hypoxia and hypoglycemia. *J Neurochem* **27:** 37–42, 1976.
10. Gibson, G. E., C. Peterson and D. J. Jenden. Acetylcholine synthesis decreases during senescence. *Science* **213:** 674–676, 1981.
11. Gibson, G. E. and C. Peterson. Decreases in the release of acetylcholine *in vitro*. *Biochem Pharmacol* **32:** 111–114, 1982.
12. Gibson, G. E., C. Peterson and J. Sansone. Decreases in amino acid and acetylcholine metabolism during hypoxia. *J Neurochem* **37:** 192–201, 1981.

13. Glover, W. E. The aminopyridines. *Gen Pharmacol* **13:** 259–285, 1982.
14. Goodman, L. S. and A. Gilman. *The Pharmacological Basis of Therapeutics*, sixth edition. New York: MacMillan Company, 1980.
15. Gurdjian, E. S., W. E. Stone and J. E. Webster. Cerebral metabolism in hypoxia. *Arch Neurol Psychiatr* **54:** 474–477, 1944.
16. Luft, U. C. Aviation physiology—the effects of altitude. In: *Handbook of Physiology, vol 2, Section 3: Respiration*, edited by W. O. Fenn and H. Rahn. Washington, D.C.: American Physiological Society, 1965, pp. 1099–1145.
17. Miquel, J. and M. Blasco. A simple technique for evaluation for vitality loss in aging mice, by testing muscular coordination and vigor. *Exp Gerontol* **13:** 389–396, 1978.
18. Plum, F. *The Nervous System vol. 2. Clinical Neurosciences*, edited by D. Tower. New York: Raven Press, 1975, pp. 193–201.
19. Scremin, A. M. E. and O. U. Scremin. Physostigmine-induced cerebral protection against hypoxia. *Stroke* **10:** 142–143, 1979.
20. Siesjö, B. K. *Brain Energy Metabolism*. New York: Wiley Press, 1978.
21. Siesjö, B. K. and L. Nilsson. The influence of arterial hypoxemia upon labile phosphates and upon extracellular and intracellular lactate and pyruvate concentrations in the rat brain. *Scand J Clin Lab Invest* **27:** 83–96, 1971.
22. Steel, R. G. D. and J. H. Torrie. *Principles and Procedures of Statistics*. New York: McGraw Hill, 1960.