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Behavioral and Neurochemical Correlates of Morphine and Hypoxia Interactions

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FREEMAN, G. B., P. NIELSEN AND G. E. GIBSON. *Behavioral and neurochemical correlates of morphine and hypoxia interactions*. PHARMACOL BIOCHEM BEHAV 24(6) 1687–1693, 1986.—Decreased oxygen availability (hypoxia) impairs the synthesis of dopamine and serotonin in parallel with a decline in open-field behavior. If hypoxic-induced deficits in dopamine and serotonin metabolism are physiologically important, then stimulation of their synthesis may help reverse hypoxic-induced neurochemical and behavioral deficits. Acute morphine sulfate (50 mg/kg) increased dihydroxyphenylacetic acid/dopamine ratios (DOPAC/DA) (+20%), the conversion of [^{3}H]tyrosine to [^{3}H]dopamine (+73%) and open-field activity (+130%) in CD-1 male mice. However, morphine failed to significantly alter the incorporation of [^{3}H]tryptophan to [^{3}H]serotonin. Morphine antagonized the hypoxic-induced impairment of dopamine metabolism and locomotor activity. DOPAC/DA ratios of hypoxic animals that were treated with morphine were identical to controls, and conversion rates of [^{3}H]tyrosine to [^{3}H]dopamine were increased. Total distance in an automated activity monitor following the combination of morphine and hypoxia increased 79% compared to a 48% decrease with hypoxia alone. These results suggest that both hypoxia and morphine alter the dopaminergic system, but in opposite directions. These interactions may help to explain why morphine is able to ameliorate hypoxic-induced changes in behavior.

Morphine Hypoxia Dopamine Serotonin Locomotor activity Neurotransmitter metabolism

IF the hypoxic-induced impairment in the synthesis of dopamine (DA) and serotonin (5-HT) [3, 4, 7, 8, 12] is physiologically important, then stimulation of their metabolism may help reverse hypoxic-induced neurochemical deficits. Acute morphine enhances dopamine metabolism in rats and mice [1, 21, 24, 36, 40]. Morphine (10 mg/kg) elevates striatal homovanillic acid (HVA) and this increase is blocked by dosages of apomorphine (0.05 mg/kg) that activate inhibitory presynaptic dopamine receptors [37]. Morphine's effects on 5-HT metabolism are controversial. Although the administration of a single large dose (100 mg/kg) of morphine failed to alter the turnover rate of 5-HT [25,34], others report moderate dosages of morphine stimulate 5-HT turnover as assessed by either isotopic or non-isotopic methods in mice [42] and rats [1, 41, 43]. Thus, the effects of morphine on DA and 5-HT metabolism were examined during control and hypoxic conditions. Radioisotopic measures were used to estimate monoamine turnover rates [11]. DOPAC/DA ratios were also utilized to estimate DA turnover; an increase in this ratio suggests increased DA metabolism within the nerve terminal.

If decreased neurotransmitter formation underlies hypoxic-induced decreases in open field behavior [12,14], then stimulation of their synthesis may ameliorate behavioral deficits. Hypoxia reduces acetylcholine synthesis, but cholinergic drug treatment only partially ameliorates hypoxic-induced behavioral deficits [17]. These findings suggest that other neurotransmitter systems may also play a critical role. Acute morphine treatment increases the locomotor activity of mice [28, 31, 33, 40]. The increased locomotor behavior may be primarily mediated through striatal dopaminergic mechanisms [5, 13, 22, 27, 39] or an interaction of DA with other systems [32,35] and may be species-specific as well [40]. Thus, the interaction of morphine and hypoxia on open field behavior was examined.

These interactions were examined in a sodium nitrite model of hypoxia. Sodium nitrite converts hemoglobin to methemoglobin and this diminishes the oxygen carrying capacity of the blood without reducing the blood pO_2. The effects in brain cannot be accounted for by the actions of sodium nitrite, but appear to be due to the tissue hypoxia [15]. In previous studies, the level of $NaNO_2$ (75 mg/kg) used in the present experiments produced brain hypoxia, as assessed with lactate concentrations, equivalent to 10% oxygen [18].

Investigating the effects of morphine on hypoxic-induced deficits is of potential clinical interest because of the possible role of the endogenous opioids in the pathophysiology of ischemic-induced neurological deficits [19, 20, 23]. Ischemia, however, is a complex metabolic insult with numerous si-

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ABBREVIATIONS

DA	dopamine
DOPAC	dihydroxyphenylacetic acid
HVA	homovanillic acid
HIAA	5-hydroxyindoleacetic acid
5-HT	serotonin
TRP	tryptophan
TYR	tyrosine
$NaNO_2$	sodium nitrite
ECR	estimated conversion rate

multaneously changing variables. In the present study, a single component of ischemia, hypoxia, and its interaction with acute opiate administration were examined.

METHOD

Materials

Male CD-1 mice (30–35 days old; 23–25 g) were from Charles River Breeding Laboratory (Stone Ridge, NY). L-[5-^{3}H]tryptophan (28.7 Ci/mmol), L-[ring-2,6-^{3}H]tyrosine (38.1 Ci/mmol) and the liquid scintillation fluid, Aquasol 2, were from New England Nuclear (Boston, MA). [^{14}C]tyrosine (56 mCi/mmol), [^{14}C]-dopamine (56 mCi/mmol), [^{14}C]tryptophan (54 mCi/mmol) and [^{14}C]serotonin (55 mCi/mmol) were from Amersham Corp. (Chicago, IL). The electrochemical detector (LC-4B) and flow cell (LC-17) were from Bioanalytical Systems, Inc. (West Lafayette, IN). The chromatographic column (μBondapak, C-18, reverse phase), sample delivery system (model 710B WISP and 6000A pump) and data reduction system (model 730 data module and model 721 system controller) were obtained from Millipore, Waters Chromatography Division (Milford, MA). The fraction collector (model 201) was from Gilson. Sodium nitrite ($NaNO_2$) was from Baker Chemical Co. (Phillipsburg, NJ) and morphine sulfate was from Wyeth Laboratories (Philadelphia, PA). The digiscan animal activity monitor (model RXY ZCM-16) was from Omnitech Electronics, Inc. (Columbus, OH). Data was printed automatically on an Epson dot matrix printer (model MX-80IIIF/T) that was connected to the digiscan. The polytron homogenizer was from Brinkman Instruments Co. (Westbury, NY).

Procedure

Male CD-1 mice (30–35 days old; 23–25 g) that had been acclimated to our animal facility for one week were fasted the night before the experiment with free access to water and were separated into four treatment groups: saline, $NaNO_2$ (75 mg/kg; 0.0075 ml/g), morphine (50 mg/kg; 0.005 ml/g), and morphine + $NaNO_2$. The morphine dosage was selected on the basis of behavioral changes (see the Results section). Each drug was dissolved in 0.9% saline on the day of the experiment. On the morning of the experiment, animals in each group received two successive but nearly simultaneous intraperitoneal injections 30 minutes prior to sacrifice: control (saline, saline), $NaNO_2$-treated ($NaNO_2$, saline), morphine-treated (morphine, saline), morphine + $NaNO_2$-treated (morphine, $NaNO_2$). Twenty minutes after the intraperitoneal injection, animals were momentarily placed in perforated Plexiglas cylinders and received an intravenous injection of [^{3}H]TRP (100 μCi/mouse) and [^{3}H]TYR (100 μCi/mouse) in 150 μl saline. Animals were sacrificed by head focussed microwave irradiation 10 minutes later. Incorporation of both precursor amino acids into their products is linear at this time [11]. Tissue was prepared and compounds were separated by automated high pressure liquid chromatography and their contents were determined by electrochemical detection. Programmed collection of the TYR, DA, 5-HT and TRP peaks allowed determinations of radioactivity by liquid scintillation [11]. Dihydroxybenzylamine (DHBA) was used as an internal standard for concentrations and [^{14}C]TYR, [^{14}C]TRP, [^{14}C]DA and [^{14}C]5-HT assessed recovery of radioactivity (69%, 58%, 45% and 52%, respectively). The estimated conversion rate (ECR) of the precursor amino acids, TYR and TRP, into DA and 5-HT, respectively, was calculated as follows:

$$\text{ECR} = \frac{\begin{array}{c}\text{dpm in product}\\(\text{DA or 5-HT})\\\text{per mg of tissue}\end{array}}{\begin{array}{c}\text{precursor specific}\\\text{activity}\\(\text{TYR or TRP})\end{array}} \div \text{time in min}$$

Specific activity was dpm per picomole. Statistical analysis was done by analysis of variance with the least significant difference test [38].

Separate, but similarly-treated animals, were used for behavioral testing. Mice were fasted the night before the experiment and allowed water ad lib. On the day of the experiment, mice were divided into the same four treatment groups and received two successive intraperitoneal injections as described above. Thirty minutes later, animals were placed into a digiscan animal activity monitor and locomotor activity was recorded for 10 minutes as previously described [12]. The parameters of interest included: total distance traveled (inches), rest time (difference in seconds between total sample time and time spent moving) and number of vertical movements.

RESULTS

Morphine sulfate increased locomotor activity. There was a dose-dependent increase in total distance traveled with 25 to 75 mg/kg of morphine sulfate [$F(3,20)=3.91$, $p<0.025$, $n=6$ per group; control, 571±76; morphine (25 mg/kg), 1171±221; morphine (50 mg/kg), 1580±239; morphine (75 mg/kg), 1616±361]. Since the 50 mg/kg dosage produced the least variable response in this and other locomotor scores, it was used in subsequent biochemical and behavioral experiments.

Measurements of the concentrations and specific activities of TYR, DA, DOPAC and HVA suggested that DA metabolism was impaired by hypoxia and stimulated by morphine. Hypoxia reduced DOPAC concentrations [−18%; $F(3,33)=7.60$, $p<0.001$] and the DOPAC/DA ratios [−19%; $F(3,32)=8.24$, $p<0.001$] (Table 1). However, hypoxia did not alter the steady state levels of TYR, DA (Table 2) or HVA (Table 1). In contrast, morphine increased the DOPAC concentrations (+19%) and the DOPAC/DA ratio (+20%). As with hypoxia, morphine did not alter the concentration of TYR, DA or HVA. DOPAC and the DOPAC/DA ratio in hypoxic animals that were also treated with morphine differed from hypoxic-mice but not from controls.

Specific activities and dpm/mg tissue also suggest that hypoxia diminishes and morphine stimulates DA formation (Table 2). Morphine increased DA specific activity (+110%) and dpm/mg tissue (+104%), whereas hypoxia decreased DA

TABLE 1

DOPAMINE METABOLITE CONCENTRATIONS IN MOUSE STRIATUM DURING CHEMICAL HYPOXIA WITH OR WITHOUT MORPHINE

	Treatment			
	Control	Morphine	$NaNO_2$	Morphine + $NaNO_2$
DOPAC	5.02 ± 0.29	5.99 ± 0.36*‡	4.12 ± 0.13*†	4.90 ± 0.26†
DOPAC/DA	0.113 ± 0.007	0.136 ± 0.008*‡	0.091 ± 0.003*†	0.118 ± 0.006‡
HVA	6.17 ± 0.35	5.93 ± 0.30	5.46 ± 0.14	6.02 ± 0.20

Values (pmol/mg of tissue) are means ± SEM of 9–10 animals.

Animals were injected with morphine (50 mg/kg) and/or $NaNO_2$ (75 mg/kg) at 0 time, [^{3}H]TYR and [^{3}H]TRP at 20 min and were sacrificed at 30 min microwave irradiation. Symbols denote value differs significantly ($p<0.05$) from *control, †morphine and ‡$NaNO_2$ by analysis of variance with the lsd test.

TABLE 2

CONCENTRATION, SPECIFIC ACTIVITY AND dpm/mg TISSUE OF DOPAMINE AND TYROSINE DURING CHEMICAL HYPOXIA WITH OR WITHOUT MORPHINE

	Treatment			
	Control	Morphine	$NaNO_2$	Morphine + $NaNO_2$
pmol/mg				
TYR	43.95 ± 4.50	41.15 ± 3.78	48.94 ± 3.57	52.63 ± 6.18
DA	44.84 ± 1.45	44.15 ± 1.08	44.40 ± 1.26	44.86 ± 1.48
dpm/pmol				
TYR	41.67 ± 3.45	51.56 ± 1.96*‡	37.44 ± 2.18	42.11 ± 3.58
DA	7.76 ± 0.67	16.27 ± 1.27*‡	5.45 ± 0.44†	9.43 ± 0.88†‡
dpm/mg				
TYR	1748.2 ± 141.3	2124.8 ± 175.7	1802.4 ± 125.7	2182.2 ± 146.6
DA	349.9 ± 29.0	714.3 ± 54.7*‡	255.5 ± 11.6†	447.2 ± 41.3†‡

Animals were injected with morphine (50 mg/kg) and/or $NaNO_2$ (75 mg/kg) at 0 time, [^{3}H]TYR and [^{3}H]TRP at 20 min and were sacrificed at 30 min by microwave irradiation. Values are means ± SEM of 8–10 animals. Symbols denote value differs significantly ($p<0.05$) from *control, †morphine and ‡$NaNO_2$ by analysis of variance with the lsd test.

specific activity and the dpm/mg tissue in DA [−27%; $F(3,34)=26.55$, $p<0.001$]. The combination of morphine and hypoxia produced a nonsignificant stimulation of DA specific activity and dpm/mg tissue compared to controls. These changes occur with minimal alterations in the precursor TYR specific activity. Therefore, the estimated rates of synthesis demonstrated the same pattern (Table 3). Morphine stimulated synthesis by 73% and hypoxia impaired synthesis by 23%, $F(3,32)=30.33$, $p<0.001$, whereas the combination was nearly equal to control.

Serotonin metabolism was impaired by hypoxia and these effects were ameliorated by morphine. Although morphine alone had no effect on TRP or 5-hydroxyindoleacetic acid (5-HIAA) concentrations, it increased 5-HT concentrations by 46%, $F(3,35)=22.50$, $p<0.001$ (Table 4). $NaNO_2$-treatment did not significantly alter the concentration of the measured serotonin-related compounds. However, the combination of morphine and $NaNO_2$ elevated TRP (+27%) and 5-HT (+36%) levels compared to controls. The specific activity of 5-HT revealed an entirely different effect. Both hypoxia alone and in combination with morphine depressed 5-HT specific activity similarly [−24% and −25%, respectively; $F(3,34)=7.31$, $p<0.001$], whereas morphine alone had no effect (Table 5). Although the specific activity was unchanged by morphine, the dpm/mg of 5-HT increased 53% compared to control, $F(3,35)=21.50$, $p<0.001$. On the other hand, hypoxia diminished the specific activity and the dpm/mg tissue (−27%). Accounting for the specific activity of the precursor TRP to estimate rates of conversion (Table 3) suggested that morphine had no effect on 5-HT synthesis, whereas hypoxia reduced synthesis [−31%; $F(3,35)=5.02$, $p<0.01$] and the combination did not vary from the control or the hypoxic group.

Open-field behavior was sensitive to hypoxia and morphine. Replicable demonstration of these effects required careful attention to detail. For example, the increased activity after acute morphine treatment is altered by whether or not food is withheld for the night prior to the experiment. Fasting overnight moderately elevated the total distance traveled (in inches) by control animals, 624±29 (n=32) to 725±23 (n=31), but considerably increased the activity of mice acutely treated with morphine, 1242±147 (n=31) to 1834±143 (n=32). The narcotic-induced hyperactivity (changes in total distance) is also naloxone sensitive, control, 528±54; morphine (50 mg/kg), 1808±145; naloxone (2 mg/kg), 541±37; naloxone/morphine, 402±61. The studies

TABLE 3

THE EFFECTS OF CHEMICAL HYPOXIA AND/OR MORPHINE ON THE ESTIMATED CONVERSION RATES IN MOUSE STRIATUM

Treatment	Estimated Conversion Rate × 100 TYR→DA	TRP→5-HT
Control	86.01 ± 6.81	3.65 ± 0.31
Morphine	149.15 ± 8.22*‡	3.87 ± 0.38‡
$NaNO_2$	66.23 ± 4.65*†	2.50 ± 0.21*†
Morphine + $NaNO_2$	93.78 ± 7.62†‡	3.00 ± 0.19†

Values (pmol/mg per min × 100) are means ± SEM of 8–10 animals.

Animals were injected with morphine (50 mg/kg) and/or $NaNO_2$ (75 mg/kg) at 0 time, [^{3}H]TYR and [^{3}H]TRP at 20 min and were sacrificed at 30 min by microwave irradiation.

$$ECR = \frac{\text{dpm in product (DA or 5-HT) per mg of tissue}}{\text{precursor specific activity (TYR or TRP)}} \div \text{time in min}$$

Symbols denote value differs significantly ($p<0.05$) from *control, †morphine and ‡$NaNO_2$ by analysis of variance with the lsd test.

that examined morphine, hypoxia and their interactions demonstrated significant treatment effects for total distance, $F(3,43)=14.5$, $p<0.001$, and rest time, $F(3,43)=24.4$, $p<0.001$. Sodium nitrite reduced (−48%) total distance traveled (Table 6), whereas morphine markedly increased locomotor activity compared to control (+130%). The hypoxic-induced locomotor activity deficit was no longer evident in $NaNO_2$-treated mice that had also received morphine. Changes in rest time mirrored the drug-induced changes in total distance. Sodium nitrite increased (+42%), whereas morphine (−79%) and the simultaneous administration of morphine and $NaNO_2$ (−36%) decreased rest time. Although the number of vertical movements was a sensitive indicator of the behavioral effects of chemical hypoxia [−55%; $t(21)=3.33$, $p<0.01$], the sensitivity of the vertical sensors to the "straub tail" effect of acute morphine administration made it meaningless in morphine-treated mice.

DISCUSSION

Hypoxia is a typical metabolic encephalopathy and provides a convenient model in which to study the response and interaction of various neurotransmitters and behavior to mild metabolic insults. Although chemical hypoxia reduces the synthesis of acetylcholine, DA, 5-HT and the amino acid neurotransmitters [3, 4, 7, 12, 18], determining which of these is most sensitive to hypoxia is difficult because percent changes from control when comparing different systems do not necessarily denote physiological importance. In addition, variable decreases between studies complicate such comparisons further. For example, the magnitude of the sodium nitrite effect on TYR to DA formation (−23% compared to −41%) and TRP to 5-HT formation (−32% versus −39%) was smaller in the present studies than in previous ones [12].

The results in this study suggest that the behavioral effects of morphine, sodium nitrite and their combination closely reflect neurochemical changes in DA in the striatum. Different changes may occur in other regions or in other neurotransmitters. The results with DA are consistent with the suggestion that morphine stimulates DA formation and hypoxia impairs it. They do not distinguish between the reversal of hypoxic-induced deficits by morphine and an antagonism of morphine's action by hypoxia. Rather, hypoxia and morphine appear to counteract each other's effects. Hypoxia impairs synthesis in the presence or absence of morphine, whereas, morphine stimulates DA under normoxic and hypoxic conditions. These interactive effects on DA metabolism are closely reflected in altered open-field behavior. Although the present results suggest a correlation between neurochemical changes in DA and locomotor activity, they do not prove that the changes in DA metabolism underlie the behavioral effects. Such consistent correlations do not occur between 5-HT synthesis and behavior. Several previous studies suggest that morphine-induced increases in locomotor behavior are related to elevated DA metabolism [5, 13, 22, 27, 39], whereas other investigators have reported that the motor activation responses of mice to morphine is not exclusively dopaminergic [32,35]. Thus, the alteration of hypoxic-induced deficits in DA metabolism may be a good indicator of the beneficial effects of various therapies. Compounds such as tabernanthine which increase DA formation in hypoxic animals [6] may also reverse hypoxic-induced behavioral deficits.

Similar conclusions appear to be true of memory tasks. Acute hypoxic- and hypobaric-hypoxia decrease the conditioned avoidance response in rats and memory is restored by apomorphine (1–1.5 mg/kg) [2, 3, 29, 30]. The protective effect of apomorphine is suppressed by pimozide, a centrally acting DA antagonist [2], but not by domperidone, a DA antagonist, which does not cross the blood-brain barrier [30].

The interaction of morphine and hypoxia may reflect alterations in carbohydrate metabolism. Mild hypoxia (15% O_2, 85% N_2) stimulates cerebral glycolysis as reflected by a 45% increase in glucose utilization and a rise in brain lactate [16]. On the other hand, morphine decreases cerebral glycolysis as demonstrated by increased brain glucose concentrations (+42%) and decreased lactate concentrations (−24%) [10,26]. Acute morphine treatment causes respiratory depression with a concomitant increase in blood CO_2 [26]. In the present experiment, changes in blood CO_2 were not monitored. However, since the effects of morphine and hypoxia were counteractive, it would appear that both the behavioral and neurochemical effects of morphine were not related to a respiratory depressant effect of the drug. An exact relationship remains to be determined.

The effects on 5-HT metabolism in the current study are consistent with the idea tha hypoxia impairs the synthesis of 5-HT, whereas morphine primarily decreased its release. During hypoxia, synthesis and release appear to be reduced in parallel, so that specific activity, dpm/mg tissue and rates of synthesis decline in parallel while levels remain constant. Serotonin concentrations and the dpm/mg tissue increase with morphine treatment, wheras the specific activity and estimated conversion rates remain normal. The relationship of these changes in 5-HT metabolism to the effects of hypoxia on brain function are unclear, since they do not correlate with behavior in these experiments.

TABLE 4

CONCENTRATIONS OF INDOLEAMINE RELATED COMPOUNDS IN MOUSE STRIATUM DURING CHEMICAL HYPOXIA WITH OR WITHOUT MORPHINE

	Treatment			
	Control	Morphine	$NaNO_2$	Morphine + $NaNO_2$
Tryptophan	12.22 ± 1.09	10.18 ± 1.13‡	13.66 ± 1.07†	15.55 ± 1.07*†
Serotonin	2.75 ± 0.11	4.03 ± 0.21*‡	2.54 ± 0.09†	3.73 ± 0.18*‡
5-HIAA	2.41 ± 0.12	2.44 ± 0.24	2.14 ± 0.14	2.34 ± 0.10

Values (pmol/mg of tissue) are means ± SEM of 9–10 animals.

Animals were injected with morphine (50 mg/kg) and/or $NaNO_2$ (75 mg/kg) at 0 time, [^{3}H]TYR and [^{3}H]TRP at 20 min and were sacrificed at 30 min by microwave irradiation. Symbols denote value differs significantly ($p<0.05$) from *control, †morphine and ‡$NaNO_2$ by analysis of variance with the lsd test.

TABLE 5

SPECIFIC ACTIVITY AND dpm/mg TISSUE OF SEROTONIN AND TRYPTOPHAN DURING CHEMICAL HYPOXIA WITH OR WITHOUT MORPHINE

	Treatment			
	Control	Morphine	$NaNO_2$	Morphine + $NaNO_2$
dpm/pmol				
TRP	142.6 ± 6.9	220.9 ± 18.4*‡	148.3 ± 8.0†	178.9 ± 8.8*†
5-HT	19.1 ± 1.0	19.2 ± 1.0‡	14.4 ± 1.1*†	14.4 ± 0.9*†
dpm/mg				
TRP	1789.8 ± 170.6	2131.1 ± 176.6	2002.1 ± 151.6	2738.1 ± 173.6*†‡
5-HT	53.0 ± 4.1	81.0 ± 5.0*‡	36.6 ± 3.0*	53.1 ± 3.5†‡

Animals were injected with morphine (50 mg/kg) and/or $NaNO_2$ (75 mg/kg) at 0 time, [^{3}H]TYR and [^{3}H]TRP at 20 min and were sacrificed at 30 min by microwave irradiation. Values are means ± SEM of 8–10 animals. Symbols denote value differs significantly ($p<0.05$) from *control, †morphine and ‡$NaNO_2$ by analysis of variance with the lsd test.

TABLE 6

EFFECTS OF CHEMICAL HYPOXIA AND MORPHINE ON OPEN-FIELD ACTIVITY

	Treatment			
	Control	Morphine	$NaNO_2$	Morphine + $NaNO_2$
Total distance (inches)	677 ± 44	1556 ± 200*‡	354 ± 54†	1214 ± 175*‡
Rest time (seconds)	285 ± 16	59 ± 21*‡	404 ± 28*†	182 ± 44*†‡
Number of vertical movements	69 ± 10	§	31 ± 5*	§

Animals were injected with morphine (50 mg/kg) and/or $NaNO_2$ (75 mg/kg) at 0 time. At 30 min, mice were placed into the digiscan analyzer and were monitored for 10 min. Values are means ± SEM of 11–12 animals. Symbols denote value differs significantly ($p<0.05$) from *control, †morphine and ‡$NaNO_2$ by analysis of variance with the lsd test. §The number of vertical movements could not be accurately assessed in morphine- and morphine/$NaNO_2$-treated mice due to the sensitivity of the vertical sensors to the "straub-tail" effect of acute morphine administration.

Cyproheptadine and mianserin, two 5-HT receptor antagonists, prevent the stimulatory effect of acute morphine (10 mg/kg) in mice [31]. Similarly, selective destruction of central 5-HT neurons with intracerebroventricular injection of 5,7-dihydroxytryptamine or the administration of metergoline, a 5-HT blocker, caused an antagonism of the stimulatory action of morphine on DA turnover in the rat striatum [9]. These results of other investigators and the significant decline in the TRP to 5-HT conversion rate with hypoxia in the present experiment suggest that a diminution of 5-HT activity may play a role in the hypoxic-induced antagonism of the stimulatory effects of morphine on DA turnover and locomotor activity.

The interaction of morphine and hypoxia suggest opiate receptors may be involved in hypoxic-induced deficits. Although the present experiments provide no empirical evidence of hypoxic-induced changes in opiate receptors, the present studies clearly show that the narcotic-induced hyperactivity (changes in total distance) is naloxone sensitive. The effects of hypoxia on endogenous opiate activity and receptor concentration remain to be determined.

In conclusion, mild acute hypoxia produces many biochemical and behavioral changes that include multiple neurotransmitter deficits. The results in this study suggest that the behavioral effects of morphine, sodium nitrite and their combination more closely reflect neurochemical changes in DA rather than 5-HT metabolism. However, the present experiments cannot provide conclusive evidence that enables a distinction to be made between morphine's protective and hypoxia's antagonistic effects. The opioids may provide a useful tool to further characterize the interaction of DA and 5-HT in the striatum. With an understanding of the above neurotransmitter interactions, it may become possible to develop better treatments of multiple neurotransmitter deficiencies that are a part of many metabolic and chronic degenerative disorders.

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