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Hypothermia Induced by Hyperbaric Oxygen is Not Blocked by Serotonin Antagonists

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FENTON, L. H., B. BECK, S. DJALI AND M. B. ROBINSON. *Hypothermia induced by hyperbaric oxygen is not blocked by serotonin antagonists.* PHARMACOL BIOCHEM BEHAV **44**(2) 357-364, 1993.—Exposure to HBO causes hypothermia, bradycardia, head weaving, resting tremor, piloerection, and straub tail in rats. These physiological and behavioral responses can also be evoked by selective activation of serotonin$_{1A}$ (5-HT$_{1A}$) receptors. The purpose of the current study was to determine if hypothermia caused by HBO is due to increased activation of 5-HT$_{1A}$ receptors. The levels of brain biogenic amines were measured in brain regions of Sprague-Dawley (SD) rats exposed to HBO. Exposure to HBO caused an increase in the levels of 5-hydroxyindoleacetic acid (5-HIAA) in the striatum (92%, $p < 0.05$) and occipital-temporal cortex (116%, $p < 0.05$), but not in other brain regions. Exposure to HBO did not change the levels of tryptophan, serotonin (5-HT), other biogenic amines, or their metabolites. It is hypothesized that the Fawn Hood (FH) rat, which is reported to be resistant to hypothermia induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), has an abnormality of 5-HT$_{1A}$ receptor activity. Although the FH rat was more resistant to hypothermia induced by HBO than the SD rat, we were not able to confirm that this rat was resistant to hypothermia induced by 8-OH-DPAT. The 5-HT receptor antagonists, 1-(1H-Indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol (Pindolol), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN-190), and methysergide, did not block hypothermia induced by HBO in SD rats. A series of control experiments were used to confirm that the antagonists blocked hypothermia induced by serotonin agonists. These data suggest that although HBO alters levels of the serotonin metabolite 5-HIAA in the striatum and occipital-temporal cortex of the rat brain, coincident hypothermia is not due to increased activation of 5-HT$_{1A}$ receptors.

Hypothermia Hyperbaric oxygen 8-OH-DPAT Pindolol 5-Hydroxytryptophan NAN-190 5-HT$_{1A}$ receptor

INCREASED partial pressure of oxygen is used in the management of respiratory distress syndrome and other medical conditions that require intensive care (22,39). The maximum concentration of oxygen that can be achieved under normal conditions at sea level is a partial pressure of oxygen (pO_2) of 760 mm Hg or 1 atmosphere absolute (ATA) of oxygen. Hyperbaric oxygen (HBO) ($pO_2 > 760$ mmHg) is used as a more aggressive form of oxygen therapy in the treatment of arterial gas embolism, decompression sickness, carbon monoxide poisoning, osteomyelitis, radiation necrosis, and diabetic ulcers (16). In addition, exposure to HBO routinely occurs in diving, aviation, and aerospace environments (24). Exposure to increased partial pressure of oxygen can cause significant physiological, behavioral, and toxic side effects (5,16,34). A better understanding of the basic mechanisms that underlie these effects will contribute to the optimal medical and occupational use of oxygen.

Although some of the effects of oxygen are thought to be due to increases in free radicals (13), evidence suggests that alterations in neurotransmitter function may also be involved (6,9,32,36,38). HBO causes several physiological/behavioral effects that are also seen with activation of 5-HT$_{1A}$ receptors. Exposure to increased partial pressure of oxygen causes bradycardia (5), hypothermia (33), and behaviors similar to those reported as "serotonin syndrome" (straub tail, piloerection, tremor, and rigidity) (34). Activation of 5-HT$_{1A}$ receptors causes similar side effects—bradycardia (27), hypothermia (15,21), and behaviors of the 5-HT syndrome (23,26). A relationship between oxygen exposure and 5-HT activity has been proposed (8,19,31) and is supported by observations of changes in 5-HT and 5-HIAA levels from rats exposed to increased partial pressure of oxygen (6,8,9,19,31). Interpretation of the data, however, is complicated by differences in methods (time and dose of oxygen exposure) and variability of results.

The purpose of this study was to test the hypothesis that

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oxygen-induced hypothermia is the result of increased activation of 5-HT_{1A} receptors. The levels of biogenic amines and their metabolites were measured in specific brain regions of rats that had been exposed to 4 ATA oxygen. Two strategies were then used to evaluate the relationship between HBO-induced hypothermia and 5-HT_{1A} receptor activation. First, the Fawn Hood (FH) rat, an animal model reported to be deficient in 5-HT_{1A} receptors (17), was exposed to HBO. It was predicted that if oxygen caused hypothermia through increased activation of 5-HT_{1A} receptors, then the hypothermic effect of oxygen would be less in FH rats than in Sprague-Dawley (SD) rats. The hypothermic effect of oxygen was less in the FH rat than in the SD rat. Second, the effect of 5-HT receptor antagonists on body temperature of rats exposed to HBO was measured in SD rats. To confirm that the 5-HT_{1A} antagonists block 5-HT related hypothermia, 5-HT_{1A} antagonists were used to reduce the hypothermia caused by the 5-HT agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), and the 5-HT precursor, 5-hydroxytryptophan (5-HTP). Although 5-HT receptor antagonists blocked 5-HT-related hypothermia, these compounds did not block hypothermia induced by HBO. A preliminary report of these findings has been presented (11).

METHOD

Materials

Male SD rats were obtained from Charles River (Wilmington, MA). Male FH rats were obtained from the New York State Department of Health (Albany, NY). Rats were housed in a climate-controlled room (22–24°C), kept on a 12 L : 12 D cycle, and fed Purina high-fiber rat chow. Rats weighed between 250 and 400 g. 8-OH-DPAT [Research Biochemicals Inc. (RBI) Natick, MA] was dissolved in sterile normal saline to an injection volume of 1 ml/kg. 5-Hydroxytryptophan (RBI) was dissolved in normal saline to an injection vol. of 10 ml/kg. Methysergide (RBI) and NAN-190 (RBI) were dissolved in DMSO to an injection vol. of 1 ml/kg. (±) Pindolol (Sigma Chemical Co., St. Louis, MO) was dissolved in 100 μl 1N HCl (G. Gudelsky, personal communication), and diluted with sterile normal saline to an injection vol. of 1.0 ml/kg (pH = 6.8). 8-OH-DPAT was given by subcutaneous injection and all other drugs were given by intraperitoneal injection.

Chamber for Regulation of Temperature, Pressure, and Oxygen Content

To minimize the effects of diurnal variation of temperature, experiments were started between 11 a.m. and 1 p.m. (7,34). Experiments were done in a NATL-BD model #197 animal hyperbaric chamber (Piersol-Pine, Oaks, PA). For oxygen exposures, the chamber was flushed with 100% oxygen, and chamber pressure was increased to 4 atmospheres absolute (ATA) at a rate of 1 ATA/min. Chamber temperature and humidity were measured continuously with a Thermo Hygrometer (model no. 3309-60; Cole Parmer Instrument Co., Chicago, IL). A Servoxmax oxygen analyzer (model no. 580-A; Sybron/Tayor, Rochester, NY) was used for continuous measurement of oxygen content throughout the experiment. Carbon dioxide content was continuously measured by infrared gas analyzer model IR-702 (Infrared Industries Inc. Santa Barbara, CA). Oxygen content was maintained ≥98% and carbon dioxide accumulation was limited to ≤0.03% by continuous chamber ventilation at 60–80 ml/min. A Lyton heat exchanger (model no. 2006; Forma Scientific, Division of Mallinckrodt, Inc. Marietta, OH) was used to maintain chamber temperature between 25°C and 27°C. Decompression to 1 ATA was made at a rate of 1 ATA/min. Air controls were exposed to atmospheric conditions (21% O_2 at 1 ATA total pressure, which provides a pO_2 equal to 150 mmHg). Pressure controls were exposed to 5% O_2 /95% N_2 at 4 ATA total pressure to maintain a pO_2 equal to 150 mmHg.

Measurement of Biogenic Amines

Groups of four SD rats were housed in a 10 × 16 × 8 in. Plexiglas box and exposed to 100% oxygen at 4 ATA pressure for 2.5 h in a recompression chamber. The exposure schedule was chosen to maximize possible changes in brain biogenic amine levels while limiting the incidence of oxygen-induced seizure activity (33). Immediately following decompression, the rats were sacrificed and brain tissue was dissected. Occipital-temporal cortex, frontal-parietal cortex, hippocampus, thalamus, striatum, hypothalamus, and brain stem were dissected and frozen on dry ice. The mean time between sacrifice and freezing was 7 min. Specimens were stored (−70°C) until assay. Tissue specimens were sonicated in a sodium acetate buffer (100 mM, pH = 5.0), and the supernatant was collected following centrifugation (29,000 g × 15 min). 5-Hydroxyindoleacetic acid (5-HIAA), norepinephrine (NA), dopamine (DA), and homovanillic acid (HVA) were measured by HPLC using an ESA (Bedford, MA) electrochemical detector (Coulochem, 5100A). The mobile phase [0.05 M sodium acetate, 1 mM sodium octyl sulfate, 0.1 mM disodium ethylenediamine-tetraacetate (EDTA), and 3% acetonitrile (pH adjusted to 3.0 with phosphoric acid)] was filtered and degassed. The flow rate was 1 ml/min (40). Serotonin and tryptophan were measured by HPLC using a McPherson (Acton, MA) fluorometric detector (model no. FL 750) with 295 nm excitation and a 320 nm broad-band emission filter. The mobile phase consisted of 0.05 M sodium acetate, 0.1 mM disodium ethylenediamine-tetraacetate (EDTA), and 12% methanol. The pH was adjusted to 4.5 with phosphoric acid, and the flow rate was 1.2 ml/min (2). External standards of biogenic amines were injected after every fifth specimen. A reverse phase C18 column was used for both HPLC analyses.

Measurement of Temperature

Rats were brought to the laboratory and kept without food or water for 3 h. The rats were mildly restrained in wire mesh cages and placed into the recompression chamber. Body temperature was measured with YSI model 402 thermistor rectal probes inserted 6 cm and a YSI Tele Thermometer model no. 43TF (Yellow Springs Instrument Co., Yellow Springs, OH). The rats were acclimated to these conditions for 45 min; a length of time determined necessary for temperature stabilization (<0.1°C variation over the last 15 min prior to injections). After stabilization, animals received injections (vehicle or drug). After an additional 15 min, rats were exposed to 100% oxygen at 4 ATA pressure. Time of exposure was between 60 and 150 min. Exposure duration was chosen to maximize the drug (15,17,18,20,21) and oxygen (11,33,34) effects. Temperatures were measured continuously and recorded at 5-min intervals. Baseline temperature was the average of the last three temperatures taken. An identical procedure was followed for experiments that required the use of 5-HTP, 8-OH-DPAT, or 5-HT antagonists.

The effect of 8-OH-DPAT on body temperature of unrestrained FH and SD rats was measured for comparison with

previous studies (17). Briefly, FH and SD rats were given free access to food and water and allowed one hour for stabilization following transfer from the vivarium to the laboratory. Rectal temperatures were taken in unrestrained rats immediately before and 30 min after 8-OH-DPAT (0.1 mg/kg) administration. Each experiment included both FH and SD rats.

Statistical Analysis

All data presented are the mean ± SEM and are the results of at least two experiments done on separate days. Statistical analysis of biogenic amine neurotransmitter levels was done by one-way analysis of variance (ANOVA). Tests of individual comparison following a significant F value were performed using Fisher PLSD. Statistical significance of changes in temperature over time was assessed by two-factor repeated measures ANOVA (Stat View 512 TM, Brain Power Inc., Calabasa, CA). The changes in temperature from baseline over time (from time = 0 to the end of the experiment) were used for statistical comparisons, except for the comparisons in Fig. 1, which compared the curves from t = 15 min. $p < 0.05$ was considered significant.

RESULTS

Effects of Oxygen on Levels of Brain Biogenic Amines

Rats were exposed to HBO, and the levels of biogenic amines in seven brain regions were compared with the levels obtained in air and pressure controls. With the exception of 5-HIAA, there was no significant difference between the levels of biogenic amines in rats exposed to HBO, air, and pressure (data for striatum shown in Table 1A). In the striatum, the levels of 5-HIAA were 52% greater in the HBO-exposed rats than in the air controls, and 92% greater in the HBO-exposed rats than in the pressure controls. In the occipital-temporal cortex (OT), the levels of 5-HIAA were 64% greater in the HBO-exposed rats than in the air controls, and 116% greater in the HBO-exposed rats than in the pressure controls. No changes in the levels of 5-HIAA were seen in the other brain regions tested (Table 1B).

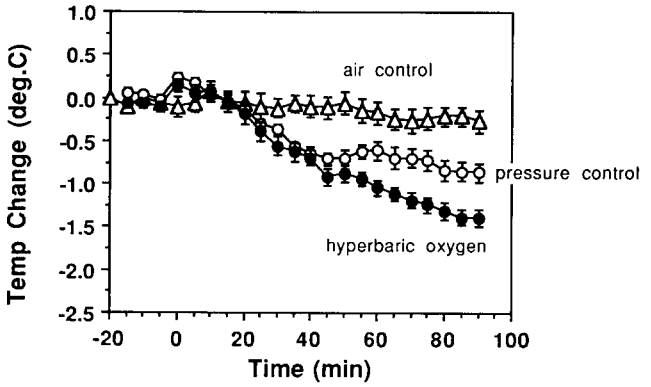

FIG. 1. Effect of HBO on body temperature in SD rats. Rectal temperature was measured in rats exposed to 4 ATA oxygen and compared to air and pressure controls. The decrease in body temperature was greater in the group treated with oxygen compared to pressure controls [$F(1, 19) = 4.7$; $p = 0.04$] and compared to air controls [$F(1, 20) = 28.5$; $p = 0.0001$]. Data are the mean ± SEM of 14 rats for the oxygen group, 8 rats for the air controls, and 7 rats for the pressure controls from 4 independent experiments.

Tryptophan levels were also measured to determine if the increase in 5-HIAA could be explained by an oxygen-induced increase in tryptophan. HBO did not significantly affect the levels of tryptophan in occipital-temporal cortex or striatum. The levels of tryptophan in the occipital-temporal cortex were 23.7 ± 2.1 with 4 ATA O_2, 25.1 ± 1.7 with air controls, and 24.4 ± 2.9 with pressure controls (mean pmol/mg tissue ± SEM, $p > 0.8$). Tryptophan levels in the striatum were 47.9 ± 2.8 with 4 ATA O_2, 41.5 ± 6.3 with air controls, and 50.6 ± 5.2 with pressure controls (mean pmol/mg tissue ± SEM, $p > 0.8$).

The level of oxygen exposure used in these studies was chosen to maximize possible changes in neurotransmitter levels during oxygen-induced hypothermia yet limit the confounding effects of seizure activity on neurotransmitter levels. Tonic/clonic seizure activity occurred in 18% (7 of 39) of the oxygen-treated rats. For purposes of analysis, we divided the oxygen-treated animals into those with and without clinical evidence of seizures. Data represent biogenic amine levels from oxygen-treated rats that did not have clinical evidence of seizure activity. In striatum, the level of 5-HIAA of oxygen-treated rats with seizures (5.8 ± 1.0) did not significantly differ from the level of 5-HIAA of oxygen-treated rats without seizures (6.1 ± 0.9) (mean pmol/mg tissue ± SEM, $p > 0.9$). In occipital-temporal cortex, the level of 5-HIAA of oxygen-treated rats with seizures (3.2 ± 0.21) did not significantly differ from the level of 5-HIAA of oxygen-treated rats without seizures (3.3 ± 0.4) (mean pmol/mg tissue ± SEM, $p > 0.8$).

Effects of Oxygen on Body Temperature

The effect of HBO on rectal temperatures of SD rats was measured and compared with air and pressure controls (Fig. 1). A pressure control group of rats was exposed to increased pressure but "normal" oxygen concentration. HBO-exposed rats developed significantly greater hypothermia than pressure controls (ANOVA, $p = 0.04$) and air control (ANOVA, $p = 0.001$) (Fig. 1). Pressure controls had a maximum decrease (0.9 ± 0.1°C) of body temperature at 90 min. HBO-exposed rats had a maximum decrease (1.4 ± 0.1°C) of body temperature at 90 min. HBO-induced hypothermia persisted for at least 2.5 h or until the onset of seizure activity (data not shown).

The FH rat has decreased 5-HT uptake (3) and may also have a decreased hypothermic response to the 5-HT_{1A} agonist 8-OH-DPAT (17). The effect of oxygen exposure on body temperature was compared in FH rats and SD rats (Fig. 2A). HBO caused less hypothermia in FH rats than in SD rats (ANOVA, $p = 0.02$). Exposure to 100% oxygen at 4 ATA pressure for 90 min caused a 0.2 ± 0.2°C decrease in the body temperature of FH rats, and a 1.2 ± 0.2°C decrease in the body temperature of SD rats.

Serotonin antagonists were used to test the hypothesis that hypothermia induced by HBO is caused by increased activation of 5-HT_{1A}receptors. Pindolol (1 mg/kg) has been shown to cause a 75% decrease in the hypothermia induced by 8-OH-DPAT (0.1 mg/kg) (18). In our studies, pindolol (10 mg/kg) did not block the hypothermia induced by oxygen (ANOVA, $p = 0.2$). In fact, there appeared to be a greater decrease in body temperature in pindolol-treated rats than in controls (Fig. 3A). The effect of pindolol on rat body temperature of air controls was minimal (Fig. 3A).

Experiments were done to confirm that pindolol blocks hypothermia due to activation of 5-HT_{1A} receptors. A dose of

TABLE 1A

THE LEVELS (pmol/mg tissue) OF BIOGENIC AMINES IN THE STRIATUM FROM RATS EXPOSED TO 4 ATA OF 100% OXYGEN

Biogenic Amine	Air Control	Pressure Control	HBO-Treated
5-HT	1.0 ± 0.1	1.3 ± 0.3	1.1 ± 0.2
5-HIAA	4.4 ± 0.5	3.6 ± 0.6	6.1 ± 0.9*
DA	7.2 ± 0.8	5.9 ± 0.8	7.0 ± 1.3
HVA	8.7 ± 0.5	6.6 ± 1.2	7.3 ± 1.4
NA	15.0 ± 2.5	11.8 ± 2.4	13.1 ± 3.0

The levels of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), homovanilic acid (HVA), and norepinephrine (NA) in the striatum of rats exposed to HBO were measured and compared to air and pressure controls. HBO caused a significant increase in the levels of 5-HIAA. *$p < 0.05$.

the experimental 5-HT_{1A} agonist 8-OH-DPAT (0.1 mg/kg) induced a degree of hypothermia comparable to that produced by HBO (100% O_2 at 4ATA). Pindolol (10 mg/kg) reduced the hypothermia caused by 8-OH-DPAT (ANOVA, $p = 0.03$). At 50 min, the decrease in temperature for rats given 8-OH-DPAT and pindolol (0.5 ± 0.1°C) was 58% less than the decrease in temperature in rats given 8-OH-DPAT and vehicle (1.2 ± 0.2°C) (Fig. 3B).

Because 8-OH-DPAT is not an endogenous agonist, the effects of the serotonin precursor, 5-HTP, on temperature was examined at 25, 50, 100, or 200 mg/kg. 5-HTP caused a dose-dependent decrease in the body temperature of SD rats (ANOVA, $p = 0.001$) (Fig. 4A). 5-HTP (50 mg/kg) induced a degree of hypothermia comparable to that produced by HBO (100% O_2 at 4ATA). Pindolol (10 mg/kg) reduced the hypothermia caused by 5-HTP (ANOVA, $p = 0.001$). At 90 min, the decrease in temperature of rats given 5-HTP and pindolol (0.4 ± 0.3°C) was 75% less than the decrease in temperature of rats given 5-HTP and vehicle (1.6 ± 0.13°C) (Fig. 4B). Methysergide, a nonspecific serotonin antagonist, partially blocked hypothermia induced by 5-HTP (Fig. 4C, ANOVA, $p = 0.02$). The effects of the 5-HT_{1A}-selective antagonist NAN-190 and the nonselective 5HT antagonist methysergide on hypothermia induced by oxygen were measured. NAN-190 did not block hypothermia induced by oxygen (Fig. 5A) at a dose (10 mg/kg) that blocks hypothermia induced by 8-OH-DPAT (30). In fact, the hypothermia induced by oxygen was greater in the animals treated with NAN-190 than in the vehicle controls (ANOVA, $p = 0.02$). The decrease in body temperature caused by NAN-190 before and during oxygen exposure is consistent with the small decrease in body temperature previously reported (30). Methysergide (1 mg/kg) did not block hypothermia induced by oxygen (Fig. 5B, ANOVA, $p = 0.3$).

The decreased response of the FH rat, compared to SD rats, to the hypothermic effect of HBO (Fig. 2A), and the previously reported decreased response of the FH rat to 8-OH-DPAT (17), is consistent with the hypothesis that HBO causes hypothermia by increased activation of 5-HT_{1A} receptors. Additional experiments were done to verify the resistance of the FH rat to 8-OH-DPAT-induced hypothermia. First, using the methods employed for all of the previous experiments, the effects of 8-OH-DPAT on rectal temperature were compared in FH and SD rats. The decrease in body temperature caused by 8-OH-DPAT (0.1 mg/kg) was the same in SD and FH rats (Fig. 2B). Since this observation does not agree with the

TABLE 1B

LEVELS (pmol/mg tissue) OF 5-HIAA IN SPECIFIC BRAIN REGIONS OF RATS EXPOSED TO 4 ATA OF 100% OXYGEN

Brain Region	Air Control	Pressure Control	HBO-Treated
OT	2.0 ± 0.3	1.5 ± 0.1	3.3 ± 0.4*
FP	2.2 ± 0.4	2.7 ± 0.3	2.5 ± 0.6
HC	2.0 ± 0.2	1.8 ± 0.2	1.8 ± 0.3
THAL	5.2 ± 1.0	5.8 ± 0.6	6.2 ± 0.5
BS	1.3 ± 0.1	1.5 ± 0.2	1.6 ± 0.3
STR	4.4 ± 0.5	3.6 ± 0.6	6.1 ± 0.9*
HT	2.5 ± 0.8	3.7 ± 0.8	3.5 ± 1.0

The effect of HBO on the levels of 5-HIAA in the occipital-temporal cortex (OT), frontal-parietal cortex (FC), hippocampus (HC), thalamus (THAL), brainstem (BS), striatum (STR), and hypothalamus (HT) were examined. In addition to the increase in 5-HIAA levels seen in the striatum, HBO also caused an increase in 5-HIAA levels in the occipital-temporal cortex. There was no significant effect of HBO on the levels of the other biogenic amines in these brain regions (data not shown). Data are the mean ± SEM of 9 rats from three independent experiments. *$p < 0.05$.

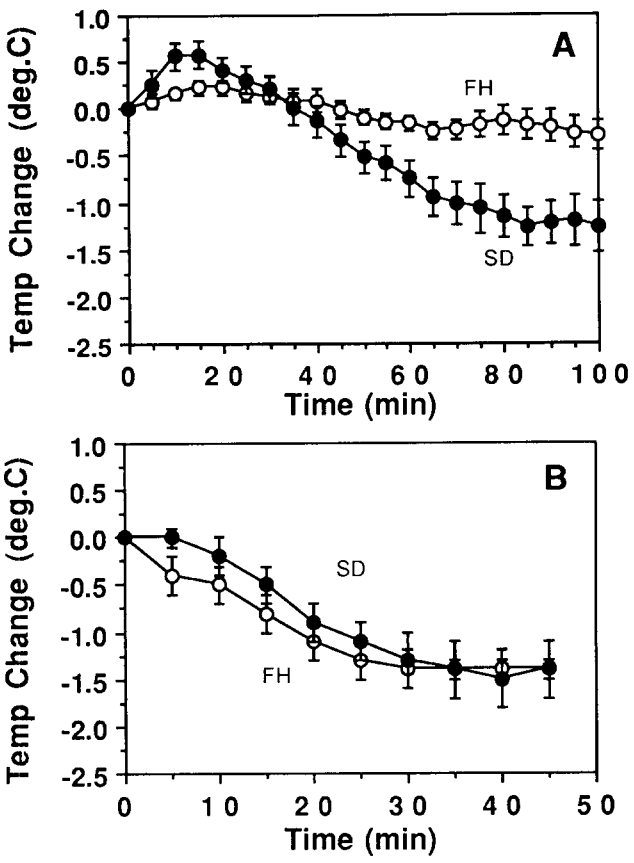

FIG. 2. Effect of HBO (A) and 8-OH-DPAT (B) on body temperature in FH and SD rats. (A) FH rats were resistant to oxygen-induced hypothermia compared with SD rats, [$F(1, 16) = 7.2$; $p = 0.02$]. At 90 min, the decrease was 1.2 ± 0.2°C in SD and 0.2 ± 0.2°C in FH rats. Data are the mean ± SEM of eight rats from three independent experiments. (B) The effect of 8-OH-DPAT on temperature changes in FH and SD rats was examined. FH rats and SD rats were mildly restrained. After a 45 min adaptation period, rats were injected with 8-OH-DPAT (0.1 mg/kg) at time = 0 min. Rectal temperatures were monitored continuously and recorded at five min intervals. When FH and SD rats were compared, no differences in the effects of 8-OH-DPAT on body temperature were observed [$F(1, 14) = 0.73$; $p = 0.4$]. Data are the mean ± SEM of 7 rats from three independent experiments.

previously reported observation (17), the effects of 8-OH-DPAT on rectal temperature were compared in FH and SD rats using the methods previously described (17). The temperature decrease in FH rats (1.1 ± 0.3°C) was the same as the temperature decrease in SD rats (1.3 ± 0.14°C) (mean ± SEM of eight rats from four separate experiments, $p > 0.5$ by two-tailed t-statistic).

DISCUSSION

A number of similarities between the effects of activation of 5-HT_{1A} receptors and the side effects seen with exposure to HBO (see Introduction) prompted the current study to test the hypothesis that hypothermia induced by oxygen may be caused by activation of 5-HT_{1A} receptors.

The results from several early studies of the effects of increased partial pressure of oxygen on the levels of biogenic amines are inconsistent. Increases (19), decreases (9), and no change (6,8,31) in serotonin have all been reported. This variability may be due to the use of a flourometric assay that lacks adequate specificity (4). In addition, since previous studies were done using whole brain homogenates, alterations in specific brain regions would not have been identified. In the current studies, the levels of biogenic amines were measured in specific brain regions. 5-HIAA was increased in the striatum and occipital-temporal cortex of rats exposed to HBO (Table 1). This observation is consistent with a selective increase in serotonin turnover related to oxygen exposure. The levels of tryptophan were not changed by HBO, suggesting that the elevations of 5-HIAA are not due to alterations in the availability of precursor. It has been postulated that elevation in 5-HIAA, following exposure to increased partial pressure of oxygen, is due to increased activity of the rate limiting step for synthesis of 5-HT by tryptophan hydroxylase (8). The anatomic specificity of the increase in 5-HIAA that is suggested by this study may be related to time of exposure, changes in cerebral blood flow, or other factors.

Increased partial pressure of oxygen causes hypothermia in rats (33,34). Since convective respiratory heat loss is a function of gas density that increases as barometric pressure increases (37), the hypothermia associated with HBO might be due to convective heat loss due to pressure alone. In the pres-

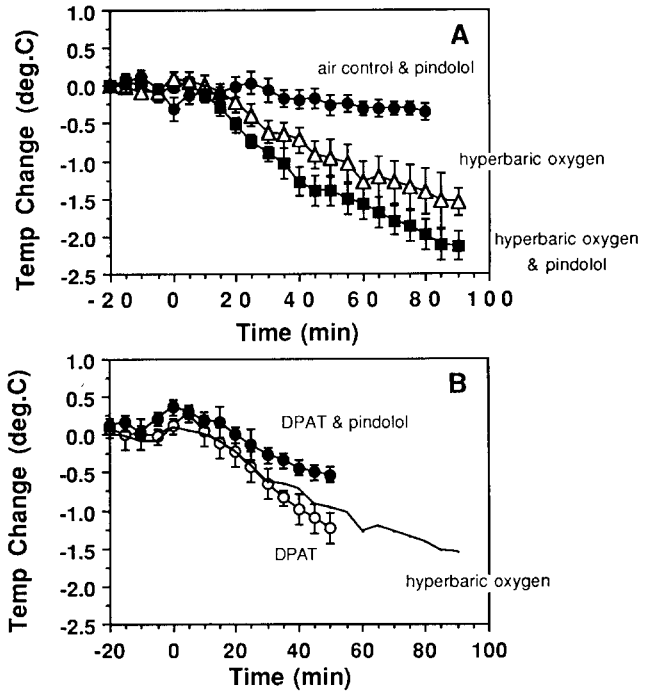

FIG. 3. The effect of pindolol on hypothermia induced by 4 ATA oxygen (A) or 8-OH-DPAT (B). (A) Fifteen minutes before exposure to oxygen (time = −15 min), animals were injected with 10 mg/kg pindolol or vehicle. Pindolol did not reduce the hypothermia caused by 4 ATA oxygen [$F(1, 17) = 1.8$; $p = 0.2$]. Air controls were also injected with Pindolol. (B) Fifteeen minutes before injection with 8-OH-DPAT or saline (time = −15 min), animals were injected with pindolol (10 mg/kg) or vehicle. Pindolol reduced the hypothermia induced by 8-OH-DPAT [$F(1, 10) = 6.8$; $p = 0.03$]. The solid line represents the data for 4 ATA O_2 presented in Fig. 4A. Data are the mean ± SEM of six rats from two independent experiments.

ent study, oxygen-treated rats developed a greater decrease in temperature than pressure controls (Fig. 1). This indicates that the increased partial pressure of oxygen causes a loss in body temperature that cannot be attributed to conductive heat loss due to pressure alone.

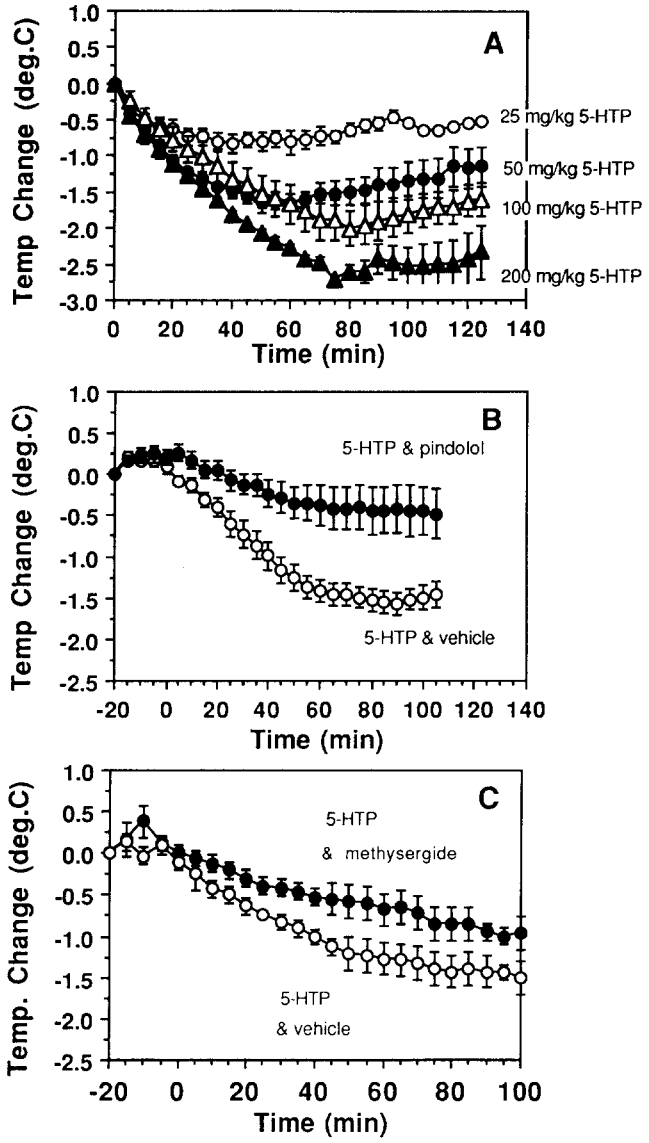

FIG. 4. The effect of 5-HTP on body temperature (A), inhibition of 5-HTP-induced hypothermia by pindolol (B), and the inhibition of 5-HTP induced hypothermia by methysergide (C). (A) Rats were injected (t = 0) with 25 mg/kg, 50 mg/kg, 100 mg/kg, or 200 mg/kg 5-HTP. 5-HTP caused a dose-related decrease in body temperature of SD rats [$F(3, 17) = 10.6$; $p = 0.001$]. Data are the mean ± the SEM of 6 observations for 25, 50, and 100 mg/kg and 3 observations for 200 mg/kg. These averages represent three experiments done on separate days. All doses were included in each experiment. (B) Pindolol (10 mg/kg) or vehicle was given 15 min before ($t = -15$) injection of 5-HTP (50 mg/kg) ($t = 0$). Pindolol reduced the hypothermia caused by 5-HTP [$F(1, 19) = 13.9$; $p = 0.001$]. Data are the mean ± SEM of 10 rats. (C) Methysergide (1 mg/kg) reduced the hypothermia induced by 5-HTP (50 mg/kg) [$F(1, 12) = 6.9$; $p = 0.02$]. Data are the mean ± SEM of 7 rats from two independent experiments.

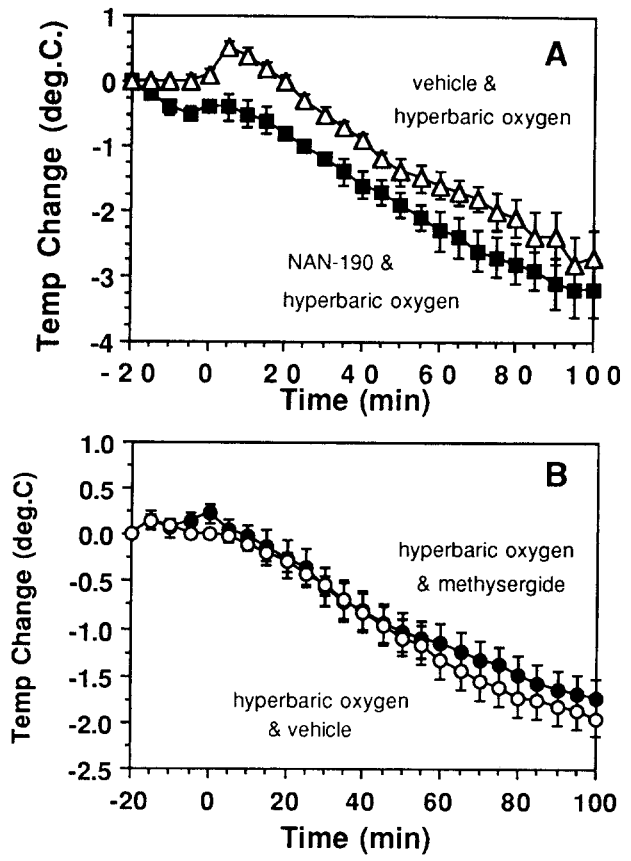

FIG. 5. The effect of the 5-HT_{1A} antagonist, NAN-190 (A) and methysergide (B), on hypothermia induced by 4 ATA oxygen. (A) NAN-190 (10 mg/kg) did not protect rats from oxygen-induced hypothermia. In fact, NAN-190 produced a significantly greater loss in temperature compared with vehicle controls [$F(1, 11) = 7.6$; $p = 0.02$]. The data are the mean ± SEM of 6 rats from two independent experiments. (B) Methysergide (1 mg/kg) had no significant effect on the change in body temperature induced HBO [$F(1, 18) = 1.0$; $p = 0.3$]. The data represent the mean ± SEM of at least nine observations from three separate experiments.

The general anesthetic properties predicted for nitrogen under these experimental conditions (5) might explain why the pressure controls had decreased body temperature compared to the air controls, but experiments to establish such a relationship were not done. Pressure controls also had an increased risk for developing decompression sickness upon depressurization. Autopsy of pressure controls following exposure confirmed the presence of gas bubbles in the mesenteric vasculature in approximately 30% of the animals, indicating decompression injury.

The relevant finding for the purposes of this study was that the oxygen treated group had greater hypothermia than both control groups. Based on our observation that rats exposed to oxygen have increased 5-HIAA levels (Table 1), and the observation that activation of 5-HT_{1A} receptors results in hypothermia (15,21,28), we hypothesized that HBO might cause hypothermia by activation of 5-HT_{1A} receptors. In previous studies, the effects of serotonin depletion using para-chlorophenylalanine on hypothermia induced by HBO have been examined (34). Although para-chlorophenylalanine po-

tentiated the hypothermia, the possibility that activation of 5-HT_{1A} and 5-HT_2 receptors may have opposing effects on temperature (18) makes interpretation of this result difficult. To determine if 5-HT_{1A} receptor activation is involved in hypothermia induced by HBO, we compared the effect of HBO on FH rat and SD rats, and we measured the effect of 5-HT_{1A} receptor antagonists on SD rats exposed to HBO.

The FH rat is reported to be more resistant to 8-OH-DPAT-induced hypothermia than the SD rat (17). If hypothermia due to HBO involves increased activation to 5-HT_{1A} receptors, then the FH rat would be resistant to the hypothermic effect of oxygen. The FH rat was less responsive to HBO than the SD rat (Fig. 2A). We were unable, however, to confirm the resistance of the FH rat to hypothermia induced by 8-OH-DPAT. We did not determine if there were differences between the FH and SD rat at other doses of 8-OH-DPAT. The difference between our results and those previously reported (17) cannot be currently resolved, but it is possible that the resistance of the FH rat to 8-OH-DPAT has changed during the 5 years of breeding between the studies.

The effects of 5-HT_{1A} receptor antagonists were examined. Pindolol is somewhat selective for 5-HT_{1A} receptors, but also blocks beta adrenergic receptors (1). At a dose shown to reduce hypothermia induced by 8-OH-DPAT (Fig. 3B), pindolol did not reduce hypothermia induced by HBO (Fig. 3A). The interaction of pindolol with beta adrenergic receptors could mask the effect of pindolol at 5-HT_{1A} receptors. Therefore, the effect of NAN-190, a 5-HT_{1A} antagonist with $alpha_1$ adrenergic, but not beta adrenergic antagonist activity (14), was examined. At a dose previously shown to block hypothermia induced by 8-OH-DPAT (30), NAN-190 did not block hypothermia induced by HBO (Fig. 5A). Methysergide is a nonselective 5-HT receptor antagonist (25) without known direct effects on adrenergic receptors. At a dose previously shown to block hypothermia induced by 8-OH-DPAT (35), methysergide did not block hypothermia induced by HBO (Fig.5B).

A series of studies were done to confirm that these receptor antagonists block hypothermia caused by serotonergic agents. The 5-HT_{1A} agonist 8-OH-DPAT was given to induce a degree of hypothermia comparable to that caused by HBO. At a dose that did not block oxygen-induced hypothermia, pindolol did block 8-OH-DPAT-induced hypothermia (Fig. 3B). Because 8-OH-DPAT is not the endogenous agonist, the effects of 5-HTP were examined. In rats, IP injection of 5-HTP causes an increase in extracellular concentration of serotonin in the hypothalamus (12), the behaviors observed in the serotonin behavioral syndrome (23,29), and hypothermia (20). In this study, 5-HTP caused a dose-dependent decrease in body temperature (Fig. 4A) that was essentially blocked by pindolol (Fig. 4B). Methysergide partially blocked the hypothermic effect of 5-HTP (Fig. 4C). The dosage of pindolol and methysergide given should have decreased the hypothermia induced by HBO if 5-HT_{1A} activation were the underlying mechanism of the oxygen effect.

We conclude the following: (a) HBO causes an increase in 5-HIAA levels in the striatum and occipital-temporal cortex in rats. (b) Hypothermia in rats exposed to increased partial pressure of oxygen is due to oxygen. (c) HBO-induced hypothermia is not due to activation of 5-HT_{1A} receptors. (d) 5-HTP causes a dose-related hypothermia, which is due to activation of 5-HT_{1A} receptors. (5) The FH rat is resistant to HBO-induced but not 8-OH-DPAT-induced hypothermia. This suggests that the difference between thermoregulatory function of the FH and SD rat is not defined by an abnormality of 5-HT_{1A} receptor function.

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