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Evidence that Respiratory Depression by Serotonin Agonists may be Exerted in the Central Nervous System

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MUELLER, R. A., D. LUNDBERG AND G. R. BREESE. *Evidence that respiratory depression by serotonin agonists may be exerted in the central nervous system.* PHARMAC. BIOCHEM. BEHAV. 13(2) 247–255, 1980.—Resting with CO_2 stimulated respiration were measured by means of a whole body plethysmograph in rats lightly anesthetized with halothane. The respiratory effects of different doses of the serotonin precursor 5-HTP, and the serotonin agonist 5-methoxy-N,N-dimethyltryptamine were studied as well as the effects of a serotonin antagonist methysergide and p-chlorophenylalanine, an inhibitor of serotonin synthesis. The serotonergic agonists decreased tidal volume and minute volume in a dose dependent manner and produced a respiratory acidosis. The respiratory depressant effect was antagonized by methysergide, and the serotonergic antagonist and synthesis inhibitor alone stimulated respiration. Rats given intraventricular 5-methoxy-N,N-dimethyltryptamine also evidenced a decrease in tidal volume, and this response was greater in animals given 5,7-dihydroxytryptamine. It seems likely that CNS serotonin receptors are involved in the control of both basal and CO_2 stimulated respiration.

5-Hydroxytryptophan 5-Methoxy-N,N-dimethyltryptamine 5,7-Dihydroxytryptamine Serotonin
Respiratory control Halothane Methysergide

RECENTLY it has been proposed that monoaminergic neuronal systems may be involved in the control of respiration. Thus, both serotonin and dopamine containing cells may function in the peripheral chemo-receptive mechanisms of the carotid body [16, 25, 27]. In addition, however, central monoaminergic neurons may also be important to the control of respiration. For example, both histochemical fluorescence [6,17] and biochemical [28] studies have demonstrated that serotonergic neurons are located near or within the brainstem areas associated with respiratory functions.

Recently our laboratory found that systemic apomorphine causes a dose dependent increase in respiratory frequency and minute volume in rats anesthetized with halothane [21]. Since dopaminergic and serotonergic activity often demonstrate reciprocal interaction, the purpose of the present study was to explore the possible role of serotonergic systems in respiratory regulation in the rat. Olson *et al.* have recently reported in awake rats that interference with normal serotonin availability, such as inhibitors of serotonin synthesis or neuronal integrity, produced a significant decrease in arterial CO_2 tension, whereas 5-hydroxytryptophan antagonized this effect [26]. However, the central nervous system location of these responses in this species was not addressed. We pursued our studies in halothane anesthetized rats in order to assume a constant anesthetic depth, to measure the changes in mechanical properties of respiration directly, and to be able to conveniently administer drugs into the brain. In the present study, the effects of different doses of the serotonin precursor, 5-hydroxytryptophan (5-HTP), and serotonin agonist, 5-methoxy-N,N-dimethyltryptamine (5-MDMT) on the resting and CO_2 stimulated respiratory activity in rats lightly anesthetized with halothane were examined, as well as the effect of a serotonin antagonist, methysergide and of p-chlorophenylalanine (PCPA), an inhibitor of serotonin synthesis.

The present results show that the serotonergic agonists depress tidal volume and minute volume in a dose dependent manner and induce a respiratory acidosis, whereas PCPA or methysergide alone cause an increase in respiration and methysergide antagonizes the effects of the serotonergic agonists. Finally, intraventricular administration of 5-MDMT to rats treated neonatally with 5,7-dihydroxytryptamine (5,7-DHT) produced a greater decrease in minute respiration than when given to normal rats. These

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findings suggest that 5-MDMT depresses respiration by activation of serotonin receptors within the central nervous system.

METHOD

Measurement of Respiration

Sprague-Dawley rats weighing 200–300 g were used. To prepare the rats for measurement of respiration, the animals were given ether and the tail artery was cannulated using tapered PE_{50} tubing (Clay-Adams). This cannula was kept patent with an infusion (1 ml/hr) of heparinized (10 μ/ml) saline (0.9%). A 22 ga polyethylene cannula (Quick-Cath®) was then inserted and sutured into the peritoneal cavity. At this point an incision was made through the strap muscles of the neck to expose the trachea and permit verification of tracheal intubation with an orally placed 14 ga catheter (Angiocath®). The animal was then allowed to breathe halothane (0.7% in O_2) by means of a Draeger vaporizer (periodically calibrated with a Carle-Gas Chromatograph) and was placed in a closed body plethysmograph. The cylindrical three piece Plexiglas plethysmograph (30×7.5 cm) was rendered airtight after connecting 3-way stopcocks to the arterial, intraperitoneal cannulae and the endotracheal tube. Changes in pressure inside the chamber generated by the respiratory movements of the rats were recorded from a fourth stopcock, using a low pressure Validyne® (Model MP45-2) transducer connected to a Hewlett-Packard (Model 7788A) polygraph, which permitted quantification of tidal volume and respiratory frequency. Arterial blood pressure was measured by means of a pressure transducer (Micron Inst.) and the heart rate was calculated from the blood pressure signal. The body temperature was controlled and if necessary adjusted with a heating blanket. A volatile anesthetic, halothane, was chosen for these experiments since at equilibrium the partial pressure in the brain, and thus anesthetic depression, is not altered by changes in alveolar ventilation, nor do metabolism and re-distribution produce a variable anesthetic depth as is the case with other non-volatile anesthetic drugs [18]. Animals which received intraventricular saline, artificial CSF or drugs had cannulae placed into the right lateral ventricle 48 hr previously. All placements were verified by dye injection at the conclusion of the respiratory studies, and only animals with staining of the floor of the fourth ventricle are included.

Experimental Design

All experiments started with a stabilization period of 20 min during which the rat was breathing O_2 (however, halothane (0.7%) was always included in all inhalation gas mixtures). This was followed by a test of CO_2 responsiveness using mixtures of 5% and 10% CO_2 in O_2. Each gas was given for 5 min at which point changes in tidal volume and respiratory frequency had stabilized for at least 2–3 min. Separate studies in other rats also revealed that the arterial CO_2 tension after 10 min of inhalation of either CO_2 concentration is not significantly greater than that observed at 5 min. After a further 10 min on O_2, by which time the respiratory parameters had returned to the pre-CO_2 values, the drug under study was given via the intraperitoneal or intraventricular catheter. At various intervals after drug administration (see Results) the two CO_2 tests were repeated. At the end of the experiment the rat was paralyzed by an intraarterial injection of pancuronium bromide (Pavulon®; 1 mg/kg) and the plethysmograph was calibrated by recording the changes in pressure induced by injections of graded volumes (1–3 ml) of air into the tracheal cannula.

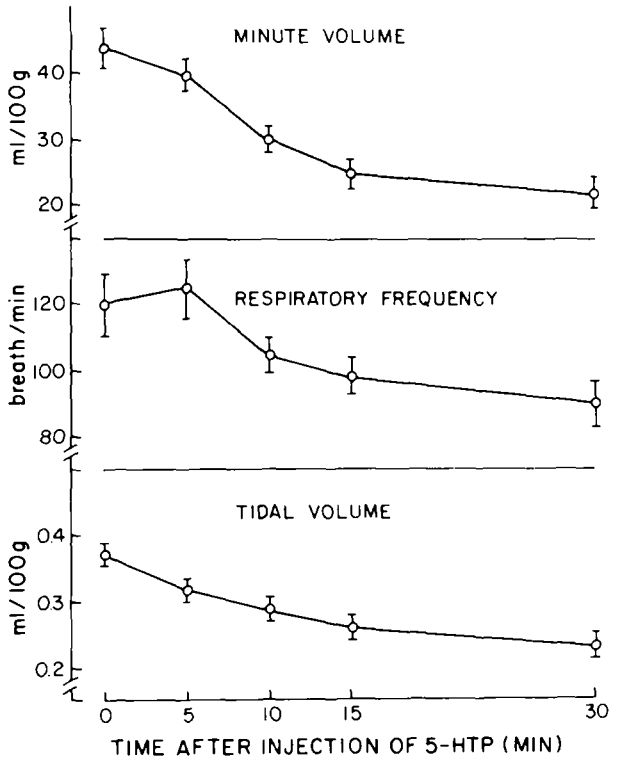

FIG. 1. Time course of respiratory effects of 5-HTP (9 mg/kg, IP) injected 40 min after pargyline (50 mg/kg, IP). Each point represents the mean of 4 rats; vertical brackets indicate ±S.E.M. The lack of representative values at 20 and 25 min after 5-HTP is due to the CO_2 exposure of the rats (see Method) during that time interval.

Blood Gas Measurements

In a separate set of experiments (otherwise designed as above) 0.4 ml of arterial blood was withdrawn at certain intervals (maximum two samples per rat) to permit determination of arterial pH, CO_2 ($PaCO_2$) and O_2 (PaO_2) tensions with a blood gas analyzer (Instrumentation Laboratory, Inc. 113). Immediately after each withdrawal of blood an equivalent volume of saline (0.9%) was slowly injected intraarterially.

Analyses of Brain Monoamine Levels

Some rats used for the respiratory experiments as adults were treated with 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT; Regis Chemicals, Chicago, Ill.; 50 μg in 10 μl) intra-cisternally at 3 days of age [13]. One group of rats was treated with p-chlorophenylalanine methyl ester (PCPA; 400 mg/kg IP). In order to verify the biochemical effects of PCPA the brains were dissected out immediately after the end of the experiments and stored at −50°C. Later the levels of serotonin were estimated by means of a fluorometric procedure [7].

Drugs

Pargyline hydrochloride, 5-HTP or p-chlorophenylalanine methyl ester (PCPA) was dissolved in saline (0.9%).

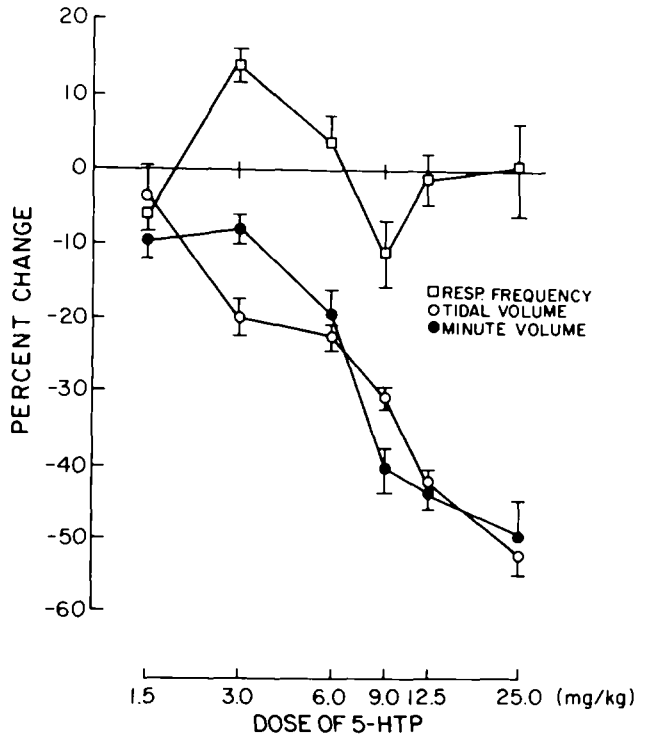

FIG. 2. Respiratory effects 15 min after various IP doses of 5-HTP. All rats were pretreated with pargyline (50 mg/kg) 40 min before 5-HTP. Values 40 min after pargyline and immediately before 5-HTP administration were (n=26): respiratory frequency (□) 100±2.8 breath/min, tidal volume (○) 0.46±0.01 ml/100 g, and minute volume (●) 45.6±1.7 ml/100 g. Each point represents the mean of 4–5 rats and vertical brackets indicate ±S.E.M., and each rat received only one dose of 5-HTP.

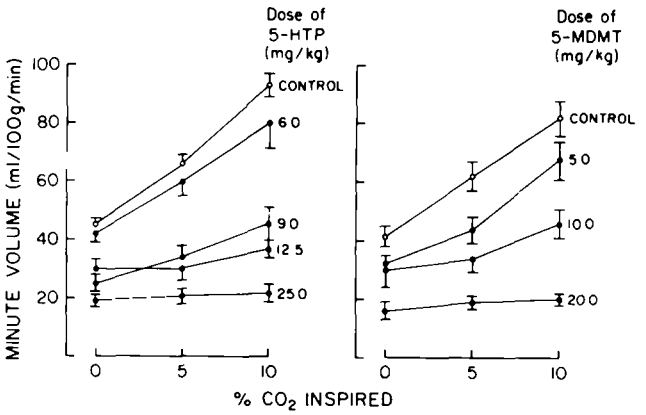

FIG. 3. Effects of various IP doses of 5-HTP injected 40 min after pargyline (50 mg/kg, IP) and of 5-MDMT on CO_2 stimulated minute volume. Two respiratory test periods were run; one before 5-HTP or 5-MDMT, and one 15 min (5-HTP) or 10 min (5-MDMT) later. Each test period consisted of recordings of respiration at rest (0% CO_2) and after inhalation for 5 min each of 5% CO_2 and 10% CO_2 in O_2. The control CO_2 response curve is constructed by pooling of values obtained during the control periods of all 5-HTP treated rats (n=26) and of all 5-MDMT treated rats (n=13) respectively. Otherwise each point represents the mean of 4–5 rats. Vertical brackets indicate ±SEM. Doses of 1.5 mg/kg and 3 mg/kg of 5-HTP did not alter the CO_2 response. All CO_2 stimulated values in the left panel, and all but the 5.0 mg/kg of 5-MDMT (10% CO_2) are significantly different from control ($p<0.001$). The differences between mean CO_2-induced increase in minute volume (change at 10% CO_2—change at 5% CO_2) obtained before and after 5-HTP (or 5-MDMT) were significantly different ($p<0.05$) for all doses except 6 mg/kg 5-HTP and 5 mg/kg 5-MDMT.

methysergide tartrate in H_2O after gentle heating, whereas 5-MDMT was dissolved in H_2O after addition of 3–5 drops of 1 N HCl per 10 ml (to a pH of 4–5). Doses of all drugs are expressed as the salts. For studies of this drug when administered into the cerebral ventricles the pH of the 5-MDMT solution was adjusted to 7.35 with HCl with tonicity maintained using an artificial CSF solution, as necessary.

Statistical analyses employed Student's *t*-test (paired or unpaired) [30].

RESULTS

Effect of 5-HTP and 5-MDMT on Resting Respiration

Since no data is available on the changes serotonergic receptor stimulation produce in halothane anesthetized rats, we first sought to determine the appropriate doses of 5-HTP and 5-MDMT. In order to increase the efficacy of 5-HTP, pargyline (50 mg/kg) was injected 40 min before 5-HTP. In separate experiments, pargyline itself did not affect the respiratory pattern at rest or during CO_2 stimulation when tested 20 to 40 min after injection. 5-HTP was found to depress the minute ventilation mainly by reducing the tidal volume (Fig. 1 and Fig. 2). As is shown in Fig. 1, the respiratory depressing effect of 9 mg/kg of 5-HTP was pronounced and stable 15 min after administration with little further change 30 min after the 5-HTP injection. The respiratory effects of 5-HTP were, therefore, studied during this interval. Increasing doses of 5-HTP from 1.5 to 25 mg/kg progressively decreased the tidal volume and the minute ventilation whereas the respiratory frequency was not consistently altered (Fig. 2).

Although it seemed likely that the effects observed with 5-HTP were due to its conversion to serotonin, it seemed desirable to also examine a directly acting serotonin agonist, like 5-MDMT, to determine if similar effects on respiration were noted. When a dose of 5 mg or more of 5-MDMT was injected, respiratory depression ($p<0.05$) was again produced as after 5-HTP; i.e., a decrease in tidal volume, with no significant change in respiratory rate at doses up to 20 mg/kg. Since the duration of action was not as long for 5-MDMT as for 5-HTP-pargyline, the respiratory effects of 5-MDMT were studied between 10 and 20 min after injection.

Effect of 5-HTP and 5-MDMT on CO_2 Stimulated Respiration

The respiratory minute volume may be directly controlled by regions of the brain stem which are sensitive to CO_2 [12]. Therefore, the change in minute volume with change in inspired CO_2 tension has been used here as a measure of CO_2 stimulated respiration. Fifteen min after 9 mg/kg 5-HTP, before the first CO_2 exposure, the $PaCO_2$ had increased from 42.4±2.0 (pH=7.35±0.01) to 63.2±6.5 (pH=7.20±0.01) ($p<0.001$). Doses of 6.0–25 mg/kg of 5-HTP not only depressed the resting ventilation, but also the ventilatory response to CO_2 exposure (see Fig. 3, left panel), while doses below 6 mg/kg were without effect. Since the end tidal alveo-

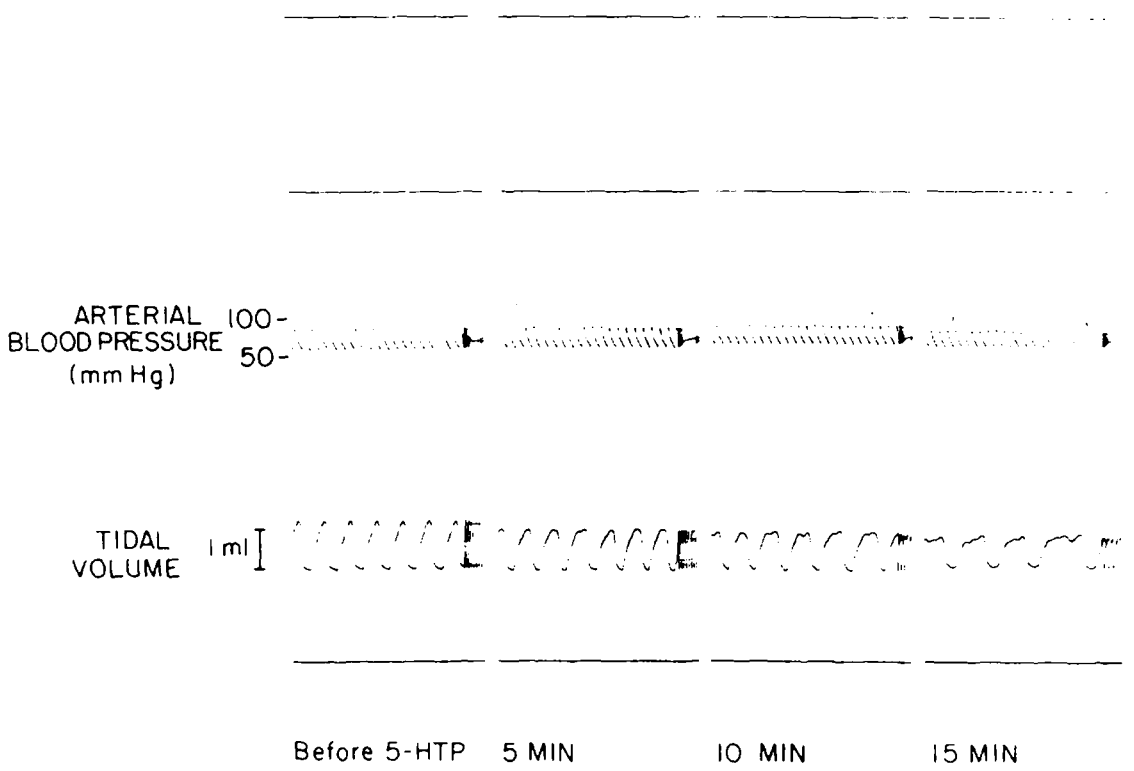

FIG. 4. Respiratory and arterial blood pressure recordings from a typical experiment. The rat was pretreated with pargyline (50 mg/kg, IP) 40 min before the injection of 5-HTP (9 mg/kg, IP). Inspiration is upwards on the respiratory recording. Note the prolonged inspiratory pause which gradually develops after 5-HTP.

lar concentration of CO_2 at rest (0% CO_2) is increased with increasing doses of 5-HTP, the actual $PaCO_2$ produced by breathing one concentration of CO_2 is different at each 5-HTP dose. However, the difference in $PaCO_2$ while breathing 5% and 10% CO_2 is less variable at equilibrium, and thus was chosen as a less biased CO_2 challenge by which to compare the minute volume response.

In the groups treated with 12.5 or 25.0 mg/kg of 5-HTP the relative increase in tidal volume induced by this 5% increment in CO_2 (10–5%) was significantly reduced. At low doses of 5-HTP which still depressed the resting respiration, the slope of the CO_2 response curve seemed to be, if anything, increased, indicating a biphasic pattern of the respiratory effects of 5-HTP. Regardless, higher doses of 5-HTP seem to be needed to depress the sensitivity to CO_2 than those required to depress the resting respiration.

Experiments similar to those described for 5-HTP were also performed using 5-MDMT. As was seen in the above experiments with 5-HTP, administration of 5-MDMT decreased the minute volume response to CO_2 exposure in a dose dependent manner (Fig. 3, right panel).

Effect of 5-HTP on the Shape of the Respiratory Curve

At dose levels higher than 3 mg/kg, 5-HTP (after pargyline pretreatment, see above) quite regularly changed the shape of each inspiratory phase. This was also true for 5-MDMT at 5 or 10 mg/kg. Figure 4 illustrates a typical experiment in which the rat was treated with 9 mg/kg of 5-HTP 40 min after pargyline. Immediately before the injection of 5-HTP the respiration was regular with the expiratory phase longer than the inspiratory time. Five and 10 min later the respiration was still regular, but there was a clearcut tendency of a prolonged inspiratory phase or an apneustic type of inspiration.

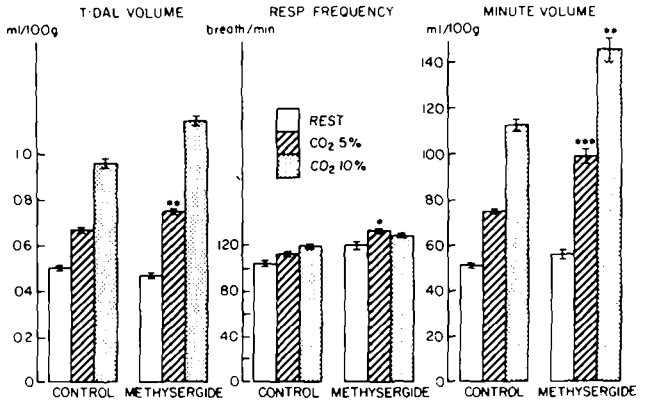

FIG. 5. Effects of methysergide on resting and CO_2 stimulated respiration. The rats were given pargyline (50 mg/kg IP) 40 min before methysergide. A test period consisting of recordings of respiration at rest and after inhalation for 5 min of 5% CO_2 and 10% CO_2 in O_2, respectively, was performed before the injection of methysergide (10 mg/kg, IP) and again 5 min later. Each column represents the mean of 6 rats and the vertical brackets indicate ± S.E.M. Statistically significant differences between values of pre- and post-drug periods are depicted as *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$).

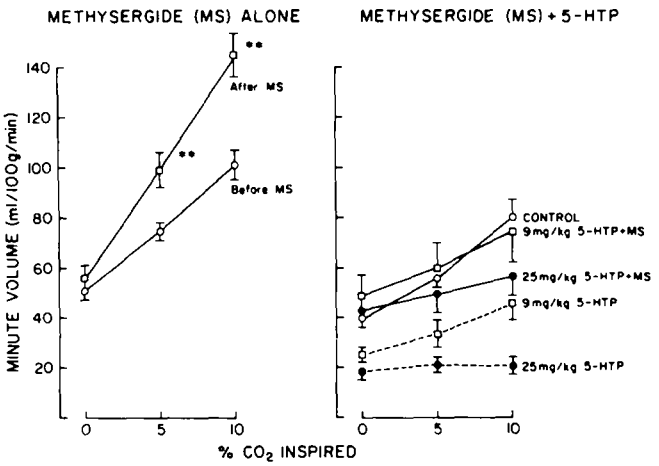

FIG. 6. Effects of methysergide (MS) and 5-HTP plus methysergide on CO_2 stimulated minute volume. All rats were pretreated with pargyline (50 mg/kg, IP) 40 min before methysergide (left panel) or 5-HTP (right panel). Two respiratory test periods were run in connection with methysergide; one before the injection of the drug and one 5 min later. Each test period consisted of recordings of respiration at rest (0% CO_2) and after inhalation for 5 min each of 5% CO_2 and 10% CO_2 in O_2. When methysergide was given 30 min after 5-HTP (right panel) the respiratory depression induced by 5-HTP was equivalent to that found 15 min after 5-HTP (see Fig. 1). The results are shown as mean changes in the resting minute volume and vertical brackets indicate ± S.E.M. The control CO_2 dose-response curve (right panel) is constructed by pooling of V_I values obtained during the control periods of all rats treated with 5-HTP (n=8). Otherwise each point represents the mean of 4–6 rats. Statistically significant differences exist between mean values obtained before and after methysergide for both 5-HTP treated groups ($p<0.05$). Statistically significant differences were found between mean minute ventilation before and after methysergide at both CO_2 concentrations. **=($p<0.01$).

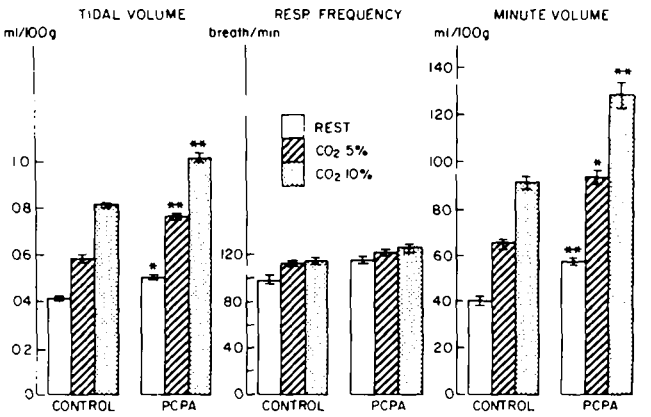

FIG. 7. Effects of p-chlorophenylalanine (PCPA) on resting and CO_2 stimulated respiration. PCPA (400 mg/kg IP) was injected 16–20 hr before the experiment. The controls were untreated. The respiratory test period consisted of recordings of the respiration at rest and after inhalation for 5 min of 5% CO_2 and 10% CO_2 in O_2, respectively. Each column represents the mean of 5 rats and the vertical brackets indicate ± S.E.M. The statistically significant differences between means of the two groups are depicted as *($p<0.05$) and **($p<0.01$).

This tendency was even more evident at 15 min after the administration of 5-HTP, at which time respiration also appeared to be irregular.

Effect of Methysergide on Respiratory Changes Induced by 5-HTP and 5-MDMT

The respiratory effects of methysergide (10 mg/kg) were studied between 5 and 15 min after the injection. As is shown in Fig. 5 methysergide alone did not change the respiration, but increased the ventilatory response to CO_2 significantly by affecting both the tidal volume and the respiratory frequency. The ability of methysergide to augment the CO_2 response is also shown in Fig. 6 (left panel).

Rats treated with pargyline (50 mg/kg) and 5-HTP (9.0 or 25.0 mg/kg), see above, were subsequently given methysergide. Methysergide (10 mg/kg) was injected 30 min after the 5-HTP, at which time the 5-HTP induced respiratory depression was equivalent to that found at 15 min after 5-HTP (see Fig. 1). The resting respiration was measured before the methysergide injection and 5 min later. Two sets of CO_2 tests (5 min 5% CO_2 and 5 min 10% CO_2 in O_2) were conducted in connection with the administration of methysergide—one from 5 to 15 min before the injection and another one from 5 to 15 min after the drug. Within 5 min after methysergide the resting tidal volume had almost returned to normal values in both 5-HTP groups (Fig. 6, right panel). The 5-HTP-induced depression of CO_2-dependent tidal volume was also significantly antagonized by methysergide. It is, however, noteworthy that although 10 mg/kg of methysergide effectively restored the resting tidal volume, the 5-HTP-induced depression of the slope of the CO_2 response curve was not completely reversed, although higher doses of methysergide were not examined. Twenty-five min after 10 or 20 mg/kg 5-MDMT rats given 10 mg/kg of methysergide also demonstrated a reversal of the respiratory depression caused by this serotonin agonist.

Effect of p-chlorophenylalanine (PCPA) on Resting and CO_2 Stimulated Respiration

Olson *et al.* observed in awake rats that PCPA induced a significant decrease in arterial CO_2 tension, suggesting a significant hyperventilation [26]. In order to verify this result in halothane anesthetized animals, rats were injected with 400 mg/kg of the tryptophan hydroxylase inhibitor p-chlorophenylalanine (PCPA) IP 16–20 hr before the respiratory experiment. This treatment decreased the levels of serotonin in the brains at the end of the experiments to 25–35% of the controls (Table 1). The PCPA treated rats displayed increasing resting and CO_2 stimulated and basal minute volume and tidal volume, whereas the respiratory frequency was not significantly augmented. However, the relative increase in minute volume induced by CO_2 (10–5%) was not changed by PCPA (Fig. 7). It was also found (Table 1) that the PCPA induced respiratory stimulation had physiological significance since it resulted in a respiratory alkalosis with significantly decreased $PaCO_2$ and increased arterial pH.

Effects of Serotonergic Agonists or Antagonists on Mean Arterial Blood Pressure and Heart Rate

Forty minutes after pargyline (50 mg/kg IP) the mean arterial blood pressure in the pooled group of all the 5-HTP treated animals (n=26; BP=80 ± 1.2 SEM) was slightly lower ($p<0.01$) than that of the 5-MDMT treated rats (not

TABLE 1

EFFECTS OF p-CHLOROPHENYLALANINE (PCPA) ON BRAIN SEROTONIN LEVELS AND ARTERIAL BLOOD GASES

	Serotonin (mg/g)		Blood gases		
	Brain stem	Rest of brain	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	pH
Controls* (n=5)	419 ± 20	369 ± 28	40.8 ± 3.0	198.2 ± 24.0	7.36 ± 0.03
PCPA treated† (n=5)	212§ ± 26	126§ ± 13	25.6‡ ± 1.75	256.0 ± 36.0	7.46‡ ± 0.02

Rats were anesthetized with 0.7% halothane in O_2. Twenty min later, just before the initial measurement of resting respiration and exposure to increased concentrations of CO_2 (5% and 10%) for two periods of 5 min, arterial blood (0.4 ml) was withdrawn. Fifteen min after the CO_2 test, while still on 0.7% halothane in O_2, the rats were sacrificed by decapitation. "Brain stem" includes only the pons-medulla region, with "rest of brain" comprising all remaining areas.

*Controls were untreated except for halothane and CO_2.

†400 mg/kg of PCPA was injected IP 16–20 hr before the experiment.

Means ± SEM are shown. Statistically significant differences between means of the two groups are depicted as ‡($p<0.01$) and §($p<0.001$).

TABLE 2

EFFECTS OF 5-HTP OR 5-METHOXYDIMETHYLTRYPTAMINE (5-MDMT) ON MEAN ARTERIAL BLOOD PRESSURE AND HEART RATE

	Before injection		After injection	
Drug dose	BP (mmHg)	HR (beats/min)	BP (mmHg)	HR (beats/min)
5-HTP (12.5 mg/kg)* n=5	79 ± 1.9	352 ± 8.0	76 ± 3.7	388 ± 13.6
(25.0 mg/kg)* n=4	81 ± 2.4	376 ± 14.4	69† ± 3.8	373 ± 13.8
5-MDMT (5.0 mg/kg) n=4	93 ± 4.0	355 ± 20.6	65§ ± 3.5	285§ ± 3.5
(10.0 mg/kg) n=5	84 ± 3.3	340 ± 12.7	62§ ± 3.7	316§ ± 9.8
(20.0 mg/kg) n=4	93 ± 3.2	380 ± 28.3	63‡ ± 5.8	280§ ± 16.4

Rats were anesthetized with 0.7% halothane in O_2 and exposed to increased concentrations of CO_2 (5% and 10%) for two periods of 5 min. Ten min after the CO_2 test, while still on 0.7% halothane in O_2, 5-HTP or 5-MDMT was injected IP. Arterial BP and HR were recorded just before the injection of the drug and 15 min (5-HTP) or 10 min (5-MDMT) later.

*The rats were pretreated with pargyline (50 mg/kg IP) 40 min before 5-HTP.

Means ± SEM are shown. Statistically significant differences between means of pre-drug and post-drug values are depicted as †($p<0.05$), ‡($p<0.01$) and §($p<0.001$).

given pargyline) measured at the same time interval after the start of the experiment (n=13; BP=89±2.3). The heart rate, however, was not changed by the pargyline treatment. Except at the highest dose level tested, (25 mg/kg), subsequent 5-HTP did not significantly alter the mean arterial blood pressure (Table 2). The heart rate was not affected in any of the pargyline plus 5-HTP treated groups. In contrast, 5-MDMT at doses which induced an equivalent degree of respiratory depression (see Fig. 2) clearly decreased both the mean arterial blood pressure and the heart rate (Table 2). The reason for this disparity of hemodynamic effects of the two serotonin agonists is unclear.

Neither methysergide (10 mg/kg IP) or PCPA (400 mg/kg IP, 16–20 hr in advance) significantly changed the mean arterial blood pressure or the heart rate.

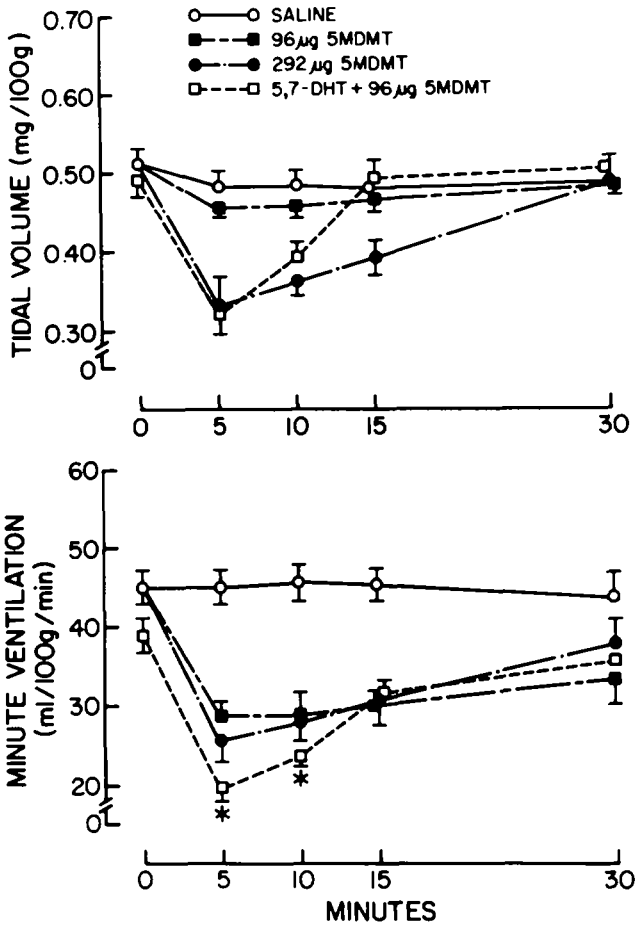

FIG. 8. Effect of intraventricular 5-MDMT on respiration in naive and 5,7-DHT pretreated rats. Cannulae were placed in the right lateral ventricle and cemented in place under ether anesthesia 48 hr before respiratory responses were examined. Animals received saline (or artificial CSF) or the indicated drug dosages in 5 λ volumes at 0 time, 40 min after the start of halothane administration (10 min after the last pre-drug CO_2 test was ended—see Method). The solution of 292 μg 5-MDMT in water was isosmotic with the artificial CSF solution and had an identical pH. The 96 μg 5-MDMT solution was prepared by mixing 2 parts of artificial CSF with 1 part of isotonic 5-MDMT solution. Each point represents the mean±S.E.M. of 3–5 animals. All drug treatments significantly ($p<0.001$) depressed the minute ventilation 5 min after administration. The change in minute ventilation and tidal volume produced by 5-MDMT (96 μg) was significantly greater ($p<0.05$) in 5,7-DHT treated rats then in naive controls.

Effect of Intraventricular 5-MDMT on Respiration in Rats Given 5,7-DHT Neonatally

At the end of the experiments on respiration, those adult rats given 5,7-DHT neonatally (see Method) evidenced a whole brain serotonin depletion to only 12% of control ($p<0.001$). Basal respiratory parameters of this group were: Tidal volume 0.55±0.02 ml/100 g, frequency 71±2/min, and minute ventilation 38.6±0.8 ml/100 g/min (control values were 0.49±0.02, 92±5 and 45±2.8 respectively), revealing a significant reduction in minute volume ($p<0.05$) largely as a consequence of reduced respiratory frequency ($p<0.01$). Subsequent administration of 5λ of saline or artificial CSF to control rats did not significantly alter respiratory mechanical parameters, and a high dose of 5-MDMT (292 μg) produced a greater maximum reduction in minute ventilation and tidal volume than did 96 μg of 5-MDMT (Fig. 11). When 5,7-DHT treated animals were given the lower dose of 5-MDMT, the absolute (Fig. 8) and relative decrease (−49%) was significantly greater than the comparable changes (Fig. 8, −36%) produced in control rats. Although the maximum decrease in tidal volume with the low dose of 5-MDMT in rats given 5,7-DHT was as great as that produced by the high dose of this drug in naive rats, the duration of effect was shorter. In all rats given either dose of 5-MDMT ICV, the respiratory depressant effect was preceeded by 10–15 seconds of increase in tidal volume, an effect not noted after saline or artificial CSF administration.

The administration of the high dose of 5-MDMT ICV resulted in a marked decrease in blood pressure to 68±8 mm Hg (control=98±4) and heart rate 346±20 min^{-1} (control=404±10). The smaller doses of 5-MDMT produced very similar changes in both naive (blood pressure 74±6 mm Hg and heart rate of 323±22 min^{-1}), and in 5,7-DHT treated rats (68±2 and 336±28, respectively).

DISCUSSION

The possibility that monoaminergic neurons participate in the regulation of respiration has recently been pointed out since the anatomical and biochemical basis for a possible role of monoaminergic mechanisms in respiratory control systems is documented both in the carotid body [16, 23, 25, 27] and in pontomedullary areas of the brain [6, 17, 28]. More specifically, several reports suggest a functional role of central catecholamine neurons [9, 10, 11, 24] and serotonergic neurons [4, 5, 20] in the modulation or control of respiration. Most of these studies have administered receptor agonists or antagonists by a peripheral route, and thus the CNS or peripheral site of action is not clear. We wished to examine the respiratory sensitivity of the halothane anesthetized rat to drugs which alter serotonergic function and determine if similar responses followed the central administration of a serotonin agonist.

In the present study, the serotonin precursor, 5-HTP, depressed both the resting and the CO_2 stimulated ventilation in lightly anesthetized and pargyline pre-treated rats in a dose-dependent manner by selectively decreasing the tidal volume. These results are in accordance with the findings of Armijo and Flórez that serotonin precursors injected peripherally reduced ventilation in cats [4]. However, in contrast to the present findings, administration of 5-HTP to cats affected respiratory rate rather than tidal volume. This disparity in effect may well be explained by species differences, different experimental preparations, or different anesthetic techniques. The physiological importance of the 5-HTP induced depression in our experimental model was illustrated by the finding that 9 mg/kg of 5-HTP after pargyline produced a significant respiratory acidosis which would usually increase respiration, yet in fact respiration was decreased even at these increased arterial CO_2 tensions.

One difficulty in the interpretation of the results of the 5-HTP experiments is the possibility that the drug may have exerted a relatively nonspecific effect by altering dopamine release, since it is known that 5-HTP under certain circumstances may be converted to serotonin within non-serotonergic neurons [14]. This seems unlikely for two reasons. First, activation of dopaminergic receptors in our ex-

perimental model results in an increase in respiratory rate [21]. Second, 5-MDMT, which is a directly acting serotonin agonist, shared all aspects of the 5-HTP induced respiratory depression, favoring the view that the 5-HTP was activating serotonergic receptors to affect the respiratory control system in the rat. This idea is further strengthened by the finding that methysergide blunted the respiratory depressing effect of both 5-HTP and 5-MDMT.

Both serotonergic agonists caused a reduction in the slope of the CO_2 response curve (10–5% CO_2) in a dose-dependent way. Such a drug-induced finding suggests change in the setpoint as well as in the gain, i.e., the sensitivity to CO_2, in the CO_2 regulating system [12]. It was quite clear, however, that the resting respiration was more sensitive to the depressing activity of the serotonin agonists than CO_2 stimulated respiration. Furthermore, methysergide, which almost fully abolished the depression of the resting tidal volume induced by high doses of 5-HTP, failed to reverse fully the depressed CO_2 sensitivity. These findings may indicate a pharmacological distinction between the mechanisms maintaining resting respiration and those involved in CO_2 stimulated respiration. The distinction might be based, among other things, on differences in blood perfusion or anatomical differences such as density of serotonergic neurons and/or receptors. The finding that the direct acting serotonergic agonist 5-MDMT behaved like 5-HTP seems to rule out a receptor-related mechanism for the disparity in effect. This view is also favored by the fact that PCPA, which acts intraneuronally by inhibiting the synthesis of serotonin, stimulated both the resting and the CO_2 stimulated respiration, whereas methysergide given alone only increased the CO_2 stimulated ventilation. In addition, although the matter is obviously quite complex, the stimulatory effects of PCPA and methysergide suggest serotonergic tone may be exerted on the respiratory control system both during rest and during conditions with increased partial pressure of CO_2.

We have recently found that in the rat using identical experimental conditions activation of dopaminergic receptors with apomorphine results both in augmented resting and CO_2 stimulated respiration. Also haloperidol, which inhibited the apomorphine induced respiratory stimulation, given alone clearly depressed the CO_2 stimulated respiration [21]. Thus, as in other paradigms such as spontaneous motor activity in rats, there seems to be a reciprocal interaction between dopaminergic and serotonergic neuronal systems in respiratory control [31]. In fact such a similar input to respiratory and motor control might explain the rapid increase in respiration which occurs with exercise.

It might be argued that the serotonergic agonists or antagonists changed the respiratory pattern simply by affecting the level of the anesthesia which was maintained throughout the experiment by a constant concentration of halothane. However, it has recently been shown that in the rat acute depletion of central serotonergic neurons by specific neurotoxins (to 34% of control) [18] or by selective lesion of nucleus raphe dorsalis [29], in fact decreased the concentration of halothane needed for maintaining a certain level of analgesia. Thus, a decrease in central serotonin availability potentiated the analgesic effect of halothane. Therefore, it seems unlikely that stimulation of serotonin receptors, as in the present experiments, would have potentiated the respiratory depressing activity of halothane. Furthermore, the serotonergic agonists quite regularly changed the shape of the respiratory curve into an apneustic form with an increased inspiratory pause. A similar change in shape of the respiratory curve is not seen during an equivalent respiratory depression induced by high concentrations of halothane (Mueller *et al.*, unpublished data), suggesting that the respiratory depression induced by serotonergic stimulation and halothane anesthesia are not simply produced by a common mechanism.

At all dose levels tested, except at the very highest (25 mg/kg), systemic 5-HTP induced a respiratory depression without significantly changing mean arterial blood pressure or heart rate. In contrast, 5-MDMT in doses which cause a similar degree of respiratory depression did decrease significantly both the arterial blood pressure and the heart rate, whether given systemically or intraventricularly. These hemodynamic findings confirm other previous reports in which serotonergic agonists were given systemically and it has been claimed to be centrally mediated [2,3]. The reason why pargyline plus 5-HTP acted differently from 5-MDMT is not clear. At any rate, the respiratory changes observed are not merely a response to changes in circulatory dynamics.

Since 5-MDMT given intraventricularly can produce a marked decrease in tidal volume at doses which are ineffective when given systemically, the effect is presumably generated within the CNS. This conclusion is supported by the greater sensitivity of animals given intracisternal 5,7-DHT after pargyline as neonates to intraventricular 5-MDMT. Such animals demonstrate an apparent supersensitivity in some motor behavioral responses to serotonin agonists [13]. It also seems likely that since intracisternal 5,7-DHT is not known to exert effects outside the CNS, the enhanced 5-MDMT respiratory response in such rats is exerted within the CNS. Recently our laboratory has also found that rats treated neonatally with intracisternal 5,7-DHT are supersensitive as adults to the respiratory depressant activity of intraperitoneally administered 5-HTP or 5-MDMT and that this effect is not significantly blunted by either vagotomy or denervation of the carotid bodies. Furthermore, inhibition of peripheral aromatic amino acid decarboxylase by RO-4-4602 did not reduce the respiratory depressant effect of 5-HTP [22]. These findings indicate that the systemically administered serotonergic agonists are acting at sites which impact on the central respiratory control system.

It seems unlikely, however, that whatever central serotonergic receptors are responsible are a critical component of the respiratory neurons which control inspiration, expiration and respiratory timing. Most central serotonin containing neurons depolarize at a slow constant rate [1]. Moreover, iontophoretic administration of serotonin to neurones, which fire phasically with different portions of the respiratory cycle, has revealed a paucity of consistent responses to serotonin [8, 15, 19]. Thus, although serotonergic receptors can alter respiratory patterns and the response to CO_2, their location is probably not on the same cells which control respiratory timing.

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