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Serotonin Turnover and Supersensitivity After Neonatal 5,7-Dihydroxytryptamine

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MUELLER, R. A., A. TOWLE AND G. R. BREESE. *Serotonin turnover and supersensitivity after neonatal 5,7-dihydroxytryptamine*. PHARMACOL BIOCHEM BEHAV 22(2) 221–225, 1985.— Adult awake rats which received neonatal pargyline and 5,7-dihydroxytryptamine to severely reduce CNS serotonin terminals and perikarya have a reduced rate of accumulation of brain stem 5-hydroxytryptophan after Ro-44602. The rate of accumulation in the cerebral cortex and spinal cord were near normal when adult, even though serotonin and 5-hydroxyindoleacetic acid were sharply reduced in these regions. The respiratory response to 5-methoxy N,N-dimethyl-tryptamine was much more pronounced in pargyline-5,7- dihydroxytryptamine treated rats than in controls. If supersensitivity in serotonin receptors only develops in areas with decreased transmitter turnover, the site of action of serotonin agonists to depress respiration would seem to reside in the brain stem region. The results also suggest that compensatory changes in turnover do not develop to a similar degree in all CNS areas with altered serotonin content.

Serotonin Serotonin turnover Serotonin agonists 5-hydroxyindoleacetic acid 5-hydroxytrptophan Ro-44602 5-methoxy N,N-dimethyltryptamine Respiratory depression 5,7-dihydroxytryptophan

THE use of 5,7-dihydroxytryptamine (5,7-DHT) as a tool to remove central nervous system serotonin containing neurons is now widespread. Central administration of the drug to adult animals appears to produce a pruning of axonal arborizations, with sparing of the cell bodies and ultimately regrowth of a portion of the destroyed axonal structure [1, 2, 32]. However, neonatal administration of 5,7-DHT produces a more pronounced neuronal pruning which appears to include loss of cell bodies and only limited diffuse fiber regrowth into denervated areas [28]. Administration of 5,7-DHT at either age produces supersensitivity to drugs believed to act at serotonergic post-junctional receptors [6, 7, 9, 15, 19, 20]. However, little is known regarding the adjustments in transmitter dynamics which may develop in remaining or regenerated serotonergic nerve terminals after 5,7-DHT treatment. The present study examines alterations in serotonin turnover in several areas of rat brain after neonatal 5,7-DHT. The dosage protocol used is one for which the anatomical and neuropharmacological consequences have been described [7, 22, 28]. In addition, the respiratory depressant effects of 5-methoxy-N,N-dimethyltryptamine in awake rats was examined in unanesthetized control and neonatal 5,7-DHT-treated rats to illustrate the problems in interpretation of receptor supersensitivity and site of action of exogenous agonists in 5,7-DHT-treated rats.

Our results demonstrate that neonatal 5,7-DHT produces remarkable differential changes in 5HT turnover in various brain regions. The apparent supersensitivity which develops to serotonin receptor agonists should be interpreted in the context of regionally specific changes in transmitter turnover.

METHOD

Selective Lesions of Brain Serotonin-Containing Neurons

In order to lesion serotonergic cells and nerve terminals, 3-day-old rats of either sex were given 50 μg (Base) of 5,7-DHT creatinine sulfate 30 minutes after pargyline hydrochloride (50 mg/kg IP) [7]. Control animals received pargyline alone, as above.

Measurement of 5-HTP, 5HT, and 5HIAA

Tissues were homogenized in 5% TCA, centrifuged at 12,000 ×g for 10 minutes and the supernatant analyzed for serotonin, 5-hydroxytryptophan (5-HTP), and 5-hydroxyindoleacetic acid (5HIAA) by HPLC techniques according to the method of Kilts *et al.* [14].

Respiratory Studies in Awake Rats

Animals were lightly anesthetized with ether to permit placement of a PE-50 cannula in the ventral tail artery [11], which was brought out through the skin between the scapulae. The rat was then placed in a circular (20 cm) 20 cm high container, which permitted freedom of movement. Blood pressure and pulse were recorded after reawakening and 0.3 ml blood samples were removed for measurement of pH, $PaCO_2$ and PaO_2 15 and 30 minutes later with a

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Radiometer BMS3 MK2 Blood Micro System blood analyzer. After each sample, blood was replaced with an equal volume of 0.9% saline. Immediately after the thirty-minute period, 5-methoxy-N,N-dimethyltryptamine (5MDMT) was given IP, and all above measurements repeated 5, 10 and 15 minutes later.

Drugs

Ro-44602 N-(DL Seryl)-N^2-(2,3,4-Trihydroxybenzyl) hydrazine (Hoffman-LaRoche, Nutley, NJ) was dissolved in water to provide a solution which was isotonic and given IP, whereas 5-methoxy-N,N-dimethyltryptamine was dissolved in saline after the addition of 3–5 drops of IN HCl per 10 ml (final pH 4–5). Pargyline hydrochloride was purchased from Saber Laboratories, Inc., Morton Grove, IL. The 5,7-DHT creatinine sulfate was purchased from Regis Chemical Company. Doses of all drugs are expressed as the salts.

Statistics

The synthetic rate constants were calculated by dividing the rate of 5-HTP synthesis by the content of serotonin. Statistical analyses employed Student's *t* test (paired and unpaired) or an analysis of variance using Tukey's w procedure to assess significance between groups [26].

RESULTS

Effect of Neonatal 5,7-DHT on Brain Stem Serotonin, 5HIAA, and 5HTP Accumulation

In order to assess the effect of neonatal 5,7-DHT on serotonin synthesis when adult, awake rats (150–300 g, 4 months of age) were given the decarboxylase inhibitor, RO-4-4602 (800 mg/kg, IP) [10] and5HTP was measured 15 and 45 minutes later. The rate of change in 5HTP between

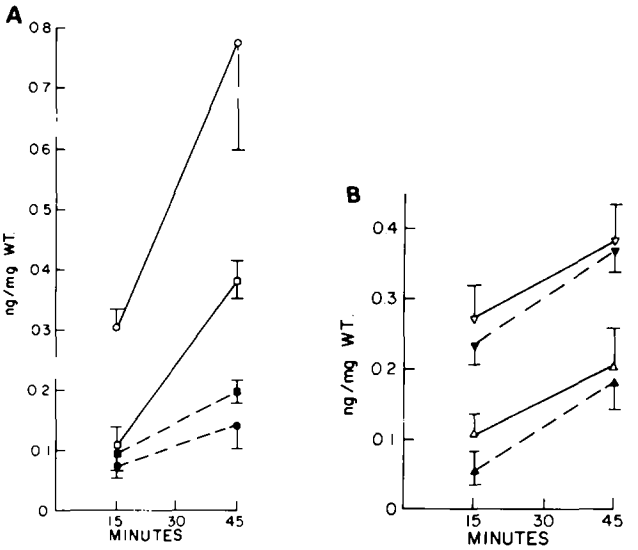

FIG. 1A. Effect of neonatal pargyline +5,7-DHT on 5HTP accumulation in pons medulla and midbrain. Control (pargyline only, solid lines, open symbols) and pargyline plus 5,7-DHT (dashed lines, closed symbols) were prepared neonatally as described in methods. Midbrain=circles; Pons-Medulla=squares. When 250–300 g, 800 mg/kg Ro-44602 was given IP at zero time. Groups of six-seven rats were killed 15 and 45 min later. The rate of accumulation of 5HTP in both tissues in control rats was significantly greater ($p<0.05$) than that observed in 5,7-DHT rats. All serotonin and 5HIAA values were lower in 5,7-DHT rats relative to control (see Table 1).

FIG. 1B. Effect of neonatal pargyline + 5,7-DHT on 5HTP accumulation in parietal cortex and spinal cord. Control (pargyline only, solid lines, open symbols) and pargyline plus 5,7-DHT (dashed lines, closed symbols) were pretreated neonatally as described in methods. Cortex=△; Spinal cord=▽.

TABLE 1

CHANGES IN SEROTONIN SYNTHESIS, CONTENT AND 5HIAA AFTER 5,7-DHT

CNS Area	Treatment	Average 5HTP Synthesis Rate ng/mg wt†	5HT ng/mg wt*	5HIAA ng/mg wt*	Synthetic Rate Constant (hr^{-1})
Brain Stem	Control	0.548	0.504 ± 0.030	0.419 ± 0.026	1.09
	5,7-DHT	0.198	0.082 ± 0.009	0.077 ± 0.002	2.41
Mesencephalon	Control	0.774	1.307 ± 0.059	0.577 ± 0.034	0.59
	5,7-DHT	0.096	0.121 ± 0.031	0.056 ± 0.014	1.09
Caudate	Control	0.206	0.230 ± 0.059	0.413 ± 0.107	0.89
	5,7-DHT	0	ND	ND	0
Spinal Cord	Control	0.220	0.454 ± 0.016	0.187 ± 0.022	0.48
	5,7-DHT	0.288	0.117 ± 0.013	0.014 ± 0.013	2.46
Parietal Cortex	Control	0.212	0.109 ± 0.006	0.164 ± 0.022	1.94
	5,7-DHT	0.260	ND	ND	∞

*Values of 6–7 rats given RO-4-4602 15 minutes previously.
ND=not detectable.
†Average accumulation from 15 to 45 minutes after RO-4-4602 800 mg/kg IP.

these two time points was then used as an estimate of serotonin synthesis rate. Whereas the synthesis rate of 5HTP in control pons-medulla was 548 ng/g/hr, that in 5,7-DHT rats was 198 ng/g/hr, approximately 1/3 control (Fig. 1 ($p<0.01$)). Basal serotonin and 5HIAA were 1/6 and 1/5 of the corresponding values in the control pons-medulla ($p<0.001$) (Table 1). If the flux of serotonin release is approximated by the rate of synthesis at steady state, serotonin release in the brain stem is dramatically reduced in the 5,7-DHT treated rats. The low value of 5HIAA in 5,7-DHT rats would support the absence of a normal or increased rate of release of 5HT in 5,7-DHT treated rats. However, use of 5HIAA alone would seem to underestimate the turnover rate of 5HT in this area.

Serotonin Turnover in Other Areas

In the mesencephalon, serotonin and 5HIAA concentrations in 5,7-DHT treated rats were less than 10% of normal. Even 5-HTP at 15 minutes was only 25% of normal and the relative rate of rise 30 minutes later was less than 12% of normal. In the head of the caudate (anterior to the optic chiasm) no 5HTP, serotonin or 5HIAA was detectable in any animal which received neonatal 5,7-DHT. In the parietal cortex the 5HTP rate of accumulation was 23% greater than normal (when compared to control; Fig. 1) though serotonin content was below the sensitivity of the assay (Table 1). In the spinal cord serotonin in the 15-minute group was 23% of control and 5HIAA 16% of control. However, the rate of accumulation of 5HTP in the spinal cord from 15 to 45 minutes after Ro-44602 administration was 30% greater than control ($p>0.1$).

Essentially identical regional results in serotonin, 5HIAA, and serotonin turnover were obtained in another group of adult rats which received desmethylimipramine (20 mg/kg, IP) instead of pargyline 30 minutes before 5,7-DHT as neonates [4,6].

Functional Assessment of 5HT Receptors: Effects of Neonatal 5,7-DHT on Awake Adult Rat Respiration Response to a Serotonin Agonist

Arterial pH, $PaCO_2$, PaO_2, and heart rate were the same for both control and 5,7-DHT-treated rats 15 and 30 minutes after reawakening from ether anesthesia. The resting blood pressure of rats treated with 5,7-DHT was higher than vehicle treated control rats (Fig. 2). Administration of 5 mg/kg 5MDMT IP produced a significant hypercarbia and hypoxia in 5,7-DHT rats ($p<0.05$), while control rats evidenced no change in $PaCO_2$ or PaO_2 from initial values. The relative hypertension and bradycardia after 5MDMT were similar in both treatment groups.

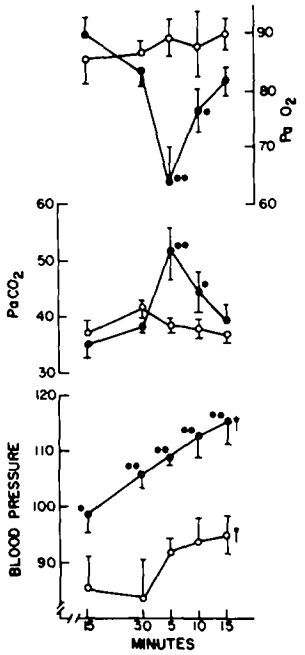

FIG. 2. Changes in PaO_2, $PaCO_2$ and blood pressure in control and 5,7-DHT treated rats. At 3 days of age, animals were given 50 mg/kg pargyline only (controls ○) or combined 30 min later with 50 μg/kg IC 5,7-DHT (●). When 250-300 g body weight, they were lightly anesthetized with ether while a PE 50 cannula was implanted in the ventral tail artery to permit arterial blood sampling and recording of blood pressure and pulse. After discontinuing ether administration, (zero time), the rats were transferred to a cage which permitted free movement (diameter—20 cm, height 20 cm). At thirty minutes, control and 5,7-DHT pretreated rats were given 5 mg/kg 5 Methoxy N, N-dimethyltryptamine IP (2 cc saline/kg), and recordings and samplings repeated 5, 10 and 15 minutes later. At all 5 sample intervals, 0.3 ml blood was removed for arterial pH, $PaCO_2$, and PaO_2 and was replaced slowly with 0.3 ml 0.9% saline. Each point represents the mean ± SEM (brackets) of 6–8 animals. *$p<0.05$; **$p<0.01$ relative to control group. All blood pressure values in 5,7-DHT rats were significantly higher than control. ‡$p<0.05$ compared to 30-minute pre-drug blood pressure value of that group.

DISCUSSION

It is now generally acknowledged that the deficiency of a neurotransmitter at a synapse, as produced after a severe reduction in nerve terminals, could be compensated for by increasing the efficiency of transmission at remaining synapses in at least two ways: increasing the number of receptors (or subsequent receptor dependent processes) or increasing the release or turnover of transmitter from remaining partially injured or regenerating terminals.

The findings of increased sensitivity of various behavioral, endocrine, and respiratory responses to serotonin agonists after 5,7-DHT would seem to imply increased receptor sensitivity [8, 9, 12, 15, 19, 22, 32]. These observations of behavioral supersensitivity have been noted in DMI-5,7-DHT treated rats after administration of a variety of serotonergic agonists such as serotonin [31], 5-methoxy-N,N-dimethyltryptamine [29] and 5HTP [27]. Supersensitivity to iontophoretic application of serotonin after destruction of serotonin nerve terminals with 5,7-DHT has been noted in neurons of the ventral lateral geniculate and amydaloid nucleus [30] but not in the hippocampal pyramidal cells [13]. Trulson *et al.* [29] noted that supersensitivity in 5,7-DHT treated rats (ratio of ED_{50} to control ED_{50}) was 4.5 fold for 5HTP for the behavioral syndrome whereas for 5MDMT the ratio was only 1.8 fold.

The administration of drugs which selectively destroy CNS serotonin containing neurons or electrolytic lesions of areas rich in serotonergic cell bodies has also been associated with increased binding of serotonin to membrane preparations by an increase in number of high affinity binding sites or decrease in K_D for ^{3}H-serotonin [3,24]. Thus, it is clear that receptor changes are one component which may decrease the effectiveness of the transmitter impairment produced after 5,7-DHT lesioning, as well as explain the

enhanced responsiveness to exogenous serotonergic agonists.

The importance of increased synthesis of serotonin in areas which have residual nerve terminals after lesioning of serotonergic cells has received less attention. Bourgoin *et al.* [5] noted that electrolytic lesioning of the B_7 and B_8 serotonergic nuclei, which reduced forebrain 5HT levels by over 75%, showed parallel reductions in 5HIAA and serotonin which suggested that no compensatory hyperactivity develops in the remaining 5HT neurons. We are aware of no other studies of regional serotonin turnover in 5,7-DHT lesioned rats. The results of the present study also fail to document elevated 5HIAA levels relative to 5HT in areas with even greater loss of transmitter, such as the cerebral cortex and spinal cord. Notably 5HIAA content is not normal in these two regions even though turnover as indexed by 5-HTP accumulation appears normal. Therefore, either 5HIAA is even less reliable a reflection of serotonin utilization in 5,7-DHT rats than in controls, or in fact no increased biosynthetic activity is present. In this latter case, the turnover measurements with RO-4-4602 would be in error. Since perfusion of carcases to remove blood before dissection does not alter the apparent synthesis rate (Mueller *et al.*, unpublished observations), blood 5HTP would seem an unlikely source of apparent tryptophan hydroxylase activity.

Our results suggest that if the ratio of serotonin/neuron remains constant after 5,7-DHT, the turnover of individual serotonergic neurons is increased in all areas, since the relative decrease in turnover is always less than the change in 5HT content (Table 1). On the other hand, expressed as regional tryptophan hydroxylating activity, only in the parietal cortex and spinal cord does the turnover return at least to normal. Others have shown that in normal rats serotonin turnover is faster in cell bodies than in terminal areas [16,23]. Curiously, in areas rich in cell bodies such as the brain stem and midbrain the serotonin turnover rate remains significantly decreased in 5,7-DHT-treated rats below values in control rats. On the basis of 5HT or 5HIAA concentrations it would have appeared that nerve terminal areas such as the spinal cord and cortex were the most impaired, not the least.

The observed normal baseline respiratory blood gases in our awake 5,7-DHT rats is at variance with the data of Olson *et al.* [25]. The cause of this discrepancy may be due to the fact that Olson *et al.* [25] treated adult rats with 5,7-DHT after desmethylimipramine, and observed significant depletion of dopamine, a possible respiratory stimulant [17, 18, 21]. We have previously shown that treatment of neonatal rats with 5,7-DHT after either pargyline or desmethylimipramine produces both a more profound and specific depletion of serotonin with no depletion of dopamine or norepinephrine [7]. The responses to 5MDMT in the awake rats of this study resemble the differential responses noted in anesthetized rats given 5MDMT IP [19] or centrally [22] with 5,7-DHT treated rats in both cases appearing more sensitive than controls. Thus, these effects of a serotonergic agonist support the suggestion of Olson *et al.* [25] that supersensitivity to the respiratory depressant effects of serotonin is present in 5,7-DHT treated rats, whether awake or anesthetized. Previous results in 5,7-DHT-treated anesthetized rats demonstrated a 3–4 fold increase in sensitivity when 5HTP plus pargyline was given peripherally [19] or 5MDMT was given intraventricularly [22]. In the present study, the data on brain stem synthesis rate of serotonin at steady state in awake rats using the 5HTP accumulation technique reveals a decrease in rate of synthesis to almost 1/3 of normal. Thus, it is possible that the three-fold increase in serotonin receptor sensitivity represents an appropriate compensation to return receptor activation of post-synaptic events to normal in rats whose synthesis of serotonin is only about 1/3 normal.

With parenteral adminstration of 5MDMT, as with ICV administration, the site(s) in brain most responsible for the depression of respiration by 5MDMT cannot be localized. Based on the observed impaired turnover in the brain stem, it would seem likely that supersensitive receptors should be localized in this general area of brain.

Our data suggest that when the neuroaxis as a whole is considered, serotonergic receptors are supersensitive in the 5,7-DHT treated rats. If basal serotonergic neuronal activity is reflected by turnover, efficiency may well be severely decreased in some areas (e.g., brain stem) but near normal in terminal regions (cortex, spinal cord). Opposite conclusions would have followed assessment of 5HT levels alone.

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