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Behavioral Reactivity Following 5-MeODMT Administration in 5,7-DHT-Pretreated Killer Rats

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CUADRA, G. R. AND V. A. MOLINA. *Behavioral reactivity following 5-MeODMT administration in 5,7-DHT-pretreated killer rats*. PHARMACOL BIOCHEM BEHAV **36**(2) 287–290, 1990.—Grouped, nonkiller and killer animals were centrally injected either with vehicle or with 5,7-dihydroxytryptamine. After a period of 7–10 days, forepaw treading and hindlimb abduction were induced by 5-MeODMT administration in all sham and lesioned rats. As expected, behavioral supersensitivity was observed in grouped and nonkiller lesioned rats. A reduced increase in 5-MeODMT-induced behaviors was obtained in killer lesioned animals. 5-HT uptake studies showed a comparable reduction of 5-HT uptake within all the lesioned rats. This evidence suggests an altered capacity to promote adaptive changes on 5-HT sites in killer rats following the destruction of central serotonergic fibers.

Nonkiller Killer 5-HT behavioral syndrome 5-HT behavioral supersensitivity

A reduced serotonergic neurotransmission seems to be critically involved in muricidal display (22,31). Thus, selective reduction of serotonin (5-HT) levels after p-chlorophenylalanine (PCPA), electrolytical or chemical lesions of the dorsal or median raphe nucleus or with a tryptophan-free diet induce mouse-killing behavior (MKB) (1–3, 7, 11). On the contrary, a clear reduction of MKB was observed when 5-HT neurotransmission was stimulated following administration of 5-HT precursors, 5-HT agonists, or neuronal uptake inhibitors (3, 7, 16, 17). In addition, these evidences are supported by biochemical experiments, since a decrease of 5-HT turnover in killer (K) rats has been reported (13,30).

Recently, we have observed similar values of forepaw treading and hindlimb abduction in isolated K and nonkiller (NK) rats after the administration of a 5-HT agonist (5), indicating comparable reactivity of 5-HT sites between these two groups of rats.

Numerous evidence have shown that a decrease in monoaminergic activity (reduced turnover rate, denervation produced by different lesions, etc.) is usually accompanied by an increased reactivity of monoamine sites in order to maintain a normal level of response (8, 24, 26). These phenomena seem to be altered in K animals, since these animals present a reduced turnover rate of 5-HT neurotransmission with a comparable reactivity of 5-HT sites. Therefore, we decided to explore the capacity of K rats in inducing adaptive changes of 5-HT sites. With this purpose we studied the behavioral reactivity of 5-HT sites of K and NK animals following the ICV administration of 5,7-dihydroxytryptamine (5,7-DHT), since several reports have observed behavioral supersensitivity after chemical lesions of central 5-HT projections (19, 20, 29).

METHOD

Animals

Male adult (3-month-old) wistar rats weighing 250–350 g at the start of the experiments were used. They were maintained in a 12-hr light-dark cycle (light off 7 p.m.) with food and water freely available.

Mouse-Killing Behavior

Rats were submitted to one month of social isolation and tested for muricidal activity. To determine MKB, a mouse was placed in the cage and only those rats that killed mice effectively and in less than 5 min were used and classified as K animals. Killed mice were removed from the cages. All rats which killed mice were tested again on each of five consecutive days and any rat who failed to kill rapidly on any day was discarded. Animals which did not kill mice in a 30 min test during five succesive days were considered isolated Nonkiller rats (NK). Another group of rats was

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isolated for only one day and tested for muricidal activity to verify the presence of spontaneous MKB in grouped rats (G).

5,7-DHT Lesions

K, NK and G rats were pretreated with desipramine (25 mg/kg IP) 45 min prior to infusion of neurotoxin in order to prevent its uptake into noradrenergic neurons. 5,7-DHT was dissolved in saline with 0.1% ascorbic acid at a concentration of 15 μg/μl. Bilateral injections (75 μg of base/ventricle) were performed at a flow rate of 1 μl/min. Injections were made with a 10 μl microsyringe (Hamilton) under pentobarbital anesthesia (40 mg/kg IP) using a stereotaxic technique and the microsyringe needle was left in place for 3 min after each injection. Sham-operated animals from K, NK and G groups were injected with the same volume of the vehicle. Coordinates with bregma as reference were as follows: A/P: −2; M/L: ±2 and D/V: +4.

A delay of 7–10 days was used before the first behavioral test, in order to allow degeneration of 5-HT fibers and development of compensatory mechanisms. All 5,7-DHT-lesioned animals were tested for muricidal activity and then submitted to the analysis of the 5-HT behavioral syndrome. After completion of behavioral experiments (day 13 postlesion) rats were sacrificed to assay high-affinity 5-HT uptake.

Behavioral Procedure

For behavioral studies, rats were placed singly in clear plastic cages. The observer of the behavior parameters tested was always blind to drug treatment and the experimental condition of the rat. Throughout all the analysed behaviors, two experimental groups were studied. One composed by sham K, NK and G rats. The other one was formed by 5,7-DHT-lesioned Killers (5,7-DHT-K), 5,7-DHT-lesioned Nonkillers (5,7-DHT-NK) and 5,7-DHT-lesioned Grouped rats (5,7-DHT-G). In any case, animals were used more than once in the different behavioral experiments. Forepaw treading and hindlimb abduction were observed 2 min after 5-methoxy-N,N-dimethyl-tryptamine (5-MeODMT; 2.5 mg/kg IP) administration, the behaviors were evaluated during a 2-min session and repeated at 1-min intervals over a total period of 15 min. The ranked intensity scale was: 0 = absent, 1 = equivocal, 2 = present and 3 = intense. At the end of the 15-min period, scores of each 2-min session were summed and the total behavioral score was obtained (25).

High-Affinity 5-HT Uptake Studies

5-HT uptake was assessed according to Hrdina *et al.* (10) with minor modifications. Following 1–2 days after completion of behavioral experiments, 5,7-DHT-K, 5,7-DHT-NK, 5,7-DHT-G and sham rats were sacrificed and their brain cortex and hippocampus rapidly dissected and homogenized in 20 vol of 0.32 M sucrose in 0.002 M Tris-HCl buffer pH 7.4, while homogenates were centrifuged at 1000 × g for 10 min. The supernatant was centrifuged at 17000 × g for 30 min. The resulting pellet was suspended in the original volume of sucrose and the suspension was used to measure ^{3}H-5-HT uptake. Each tube contained 500 μl of the incubation medium, 50 μl of the tissue suspension preincubated for 7 min at 37°C. ^{3}H-5-HT (specific activity: 29.2 Ci/mmol) was added in a final concentration of 5 nM and the incubation continued for an additional period of 5 min, this incubation was finished by diluting the total volume in 5 ml of ice-cold incubation medium and vacuum filtering. Filters were rinsed twice with 5 ml of ice-cold incubation medium and placed for radioactivity determination in a Beckman scintillation counter.

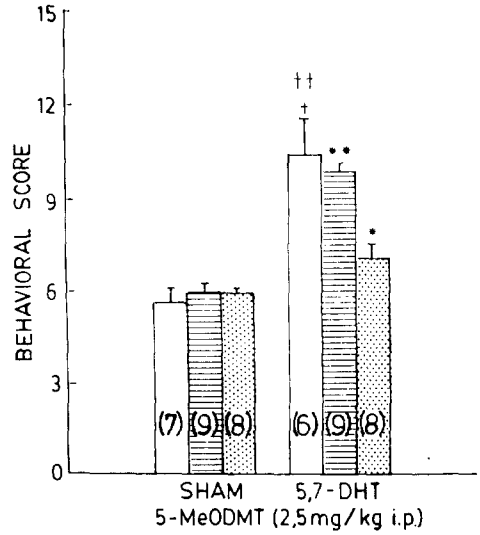

FIG. 1. Effect of the administration of ICV 5,7-DHT (150 μg) on 5-MeODMT (2.5 mg/kg)-induced forepaw treading. Sham controls were injected with 0.1% ascorbic acid instead of 5,7-DHT. The intensity of the behavioral syndrome was determined as described in text. Open bars: G; striped bars: NK; and dotted bars: K animals. *$p<0.05$ compared to sham K; **$p<0.002$ compared to sham NK; †$p<0.002$ compared to sham G; ††$p<0.004$ compared to 5,7-DHT-K rats.

"Blank" samples were incubated in parallel at 0°C. Protein determination was carried out by the method of Lowry *et al.* (12).

Drugs

5-Hydroxy(N)-^{3}H tryptamine creatinine sulphate was purchased from N.E.N., Boston, MA. 5-MeODMT and 5,7-dihydroxytryptamine creatinine sulfate were provided by Sigma, St. Louis, MO. Desipramine HCl (DMI) was obtained from Montpellier Lab., Buenos Aires, Argentina.

Statistics

Forepaw treading and hindlimb abduction were tested using Kruskal-Wallis one-way analysis of variance (ANOVA) followed by the two-tailed Mann-Whitney U-test. Uptake data were analysed by Student's *t*-test.

RESULTS

Analysis of 5-MeODMT-Induced Behaviors on Sham and Lesioned Rats

As previously described (5), comparable values of 5-MeODMT-induced behaviors were observed between G, NK and K sham-lesioned rats (Figs. 1 and 2). A significant increase in forepaw treading and hindlimb abduction was obtained in G and NK rats following the central administration of 5,7-DHT (Figs. 1 and 2). In the same line, lesioned K animals showed a higher forepaw treading after 5-MeODMT as compared to intact K rats (Fig. 1). However, the magnitude of the increase of 5-MeODMT-induced behaviors in K was significantly lower as compared to values obtained for lesioned G and NK animals (Figs. 1 and 2). It is important to point out that under these experimental conditions none of the lesioned G and NK rats exhibited MKB. In addition,

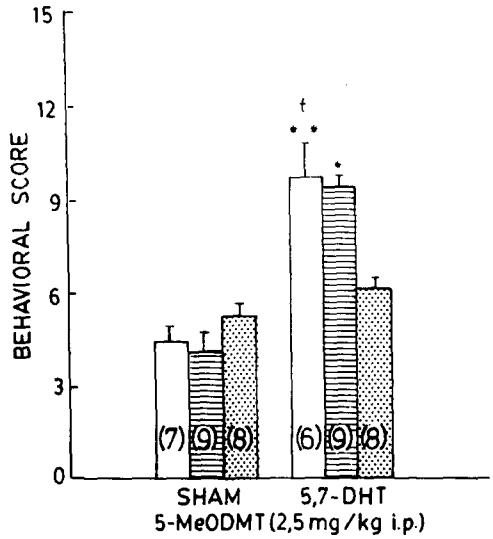

FIG. 2. Effect of the administration of ICV 5,7-DHT (150 μg) on 5-MeODMT (2.5 mg/kg) induced hindlimb abduction. Sham controls were injected with 0.1% ascorbic acid instead of 5,7-DHT. The intensity of the behavioral syndrome was determined as described in text. Open bars: G; striped bars: NK; and dotted bars: K animals. *$p<0.002$ compared to sham NK and to 5,7-DHT-K; **$p<0.002$ compared to sham G; †$p<0.004$ compared to 5,7-DHT-K rats.

the muricidal activity of K animals remain unaltered after the 5,7-DHT injection.

5-HT Uptake in Cortical and Hippocampal Tissue of Sham and Lesioned Rats

The ICV injection of 5,7-DHT produced a clear decrease in the high-affinity 5-HT uptake in cortical and hippocampal tissue (Table 1). A similar reduction of 5-HT uptake on both brain regions was observed among all the lesioned rats (Table 1).

TABLE 1

^{3}H-5-HT UPTAKE IN BRAIN CORTEX AND HIPPOCAMPUS OF KILLERS, NONKILLERS AND GROUPED ANIMALS FOLLOWING ICV ADMINISTRATION OF 5,7-DIHYDROXYTRYPTAMINE (VALUES ARE EXPRESSED AS fmol/mg PROTEIN ± SE)

	Brain Cortex	% of Controls	Hippocampus	% of Controls
SHAM (n = 12)	598.2 ± 24.5		301.0 ± 15.2	
5,7-DHT-K (n = 4)	41.1 ± 7.3*	6.9	41.6 ± 9.5*	13.8
5,7-DHT-NK (n = 4)	47.5 ± 7.4*	7.9	36.5 ± 8.2*	11.8
5,7-DHT-G (n = 4)	45.7 ± 8.3*	7.6	40.1 ± 10.7*	13.3

The crude synaptosomal pellet was prepared as described in the Method section. In parentheses, number of determinations. Sham animals data were pooled, since no significant changes were observed within groups. Values are also given in percentage of controls (100%).

*$p<0.001$ significantly different from mean values of sham-lesioned rats.

DISCUSSION

Confirming previous observations, similar behavioral scores were obtained among G, K and NK sham-lesioned animals. These results suggest that social isolation does not modify the reactivity of those 5-HT sites involved in the 5-HT behavioral syndrome.

A reduced 5-HT turnover in the pons-medulla area, but in no other brain regions, was observed on K animals (13). Similarly, a reduction of 5-HT turnover was reported in K rats as opposed to that observed in "friendly" rats, although "indifferent" animals also show decreased rates of serotonergic activity (30). Taking into account these evidences and the fact that different pharmacological treatments, including agents that act directly on 5-HT sites, clearly block MKB (16,17), a decrease in inhibitory serotonergic neurotransmission in K rats is highly probable as suggested by numerous reports. This impaired performance seems not compensated by a higher reactivity of 5-HT sites as shown in this paper, since a similar behavioral response was obtained between K and NK. However, other possibilities could also account for the reduced activity of 5-HT neurotransmission in K rats. With the present experimental evidence, we cannot definitively conclude that this probable lack of compensation in 5-HT sites of K rats is the critical factor in the decrease serotonergic neurotransmission shown by these rats. Most monoamine neurotransmitter receptors are regulated in response to neuronal activity. Thus, normally receptor supersensitization occurs as an adaptive change of postsynaptic neurons to a functional loss of presynaptic input. However, when K rats were centrally injected with 5,7-DHT, a significantly lower increase of the behavioral response was observed as compared to NK-lesioned rats, suggesting an altered modulation process to promote adaptive changes on 5-HT sites following the functional loss of presynaptic serotonergic projections.

Although definite conclusions on up-regulation of 5-HT receptors cannot be postulated by radioligand binding studies (6, 8, 18, 23), an enhanced responsiveness to microiontophoretically applied 5-HT was observed after denervation of 5-HT projections (32). In addition, behavioral experiments quite convincingly support the phenomena of behavioral supersensitivity of 5-HT sites (8, 9, 29). In fact, one of the behavioral models most frequently used for the analysis of serotonergic supersensitivity is the behavioral syndrome induced by serotonin-mimetic drugs after the central application of 5,7- or 5,6-DHT (19, 20, 29).

A clear reduction in the high-affinity 5-HT uptake in cortical and hippocampal tissue of lesioned rats was found following the ICV administration of 5,7-DHT. In addition, a comparable decrease was observed within all lesioned rats from the three different experimental groups, discarding that differences in the development of behavioral supersensitivity between K and NK may be due to a different denervation of 5-HT terminals following the central injection of the neurotoxin.

During the last years, numerous reports have suggested the presence of multiple 5-HT receptors in the central nervous system. Recognition sites are now divided in two different populations 5-HT$_1$ and 5-HT$_2$ sites (4, 8, 21). Moreover, the 5-HT$_1$ receptor is composed of at least two or probably three different subtypes, 5-HT$_{1a}$, 5-HT$_{1b}$ and 5-HT$_{1c}$ (4). In this line, several papers have proposed that some components of the 5-HT behavioral syndrome, especially forepaw treading, after 5-HT agonists are mediated by the 5-HT$_{1a}$ subtype (25,26). Therefore, and according to our results, K rats shows a relative impairment to induce adaptive change in 5-HT$_{1a}$ sites, as compared to that observed in G and NK rats, in response to a decrease of presynaptic neuronal activity. In accordance with these data, the administration of 8-OH-DPAT, a 5-HT agonist with a great affinity for the 5-HT$_{1a}$ receptor (15), induces a strong antimuricidal effect on several models of MKB (16,17).

The modulation of 5-HT_1 sites in other models of aggression has been not yet studied, however, we have observed a higher inhibitory effect on shock-induced fighting following 5-MeODMT in those rats previously lesioned with 5,7-DHT (unpublished results), indicating that the stimulation of supersensitized 5-HT sites also provokes a functional inhibitory influence in the aggression induced by electric footshock.

Finally, and considering the importance of 5-HT neurotransmission in the maintenance of central homeostasis mechanism, it seems likely to suggest that the dysfunction of 5-HT modulatory mechanism in K rats could result in a decrease ability to display an appropriate behavioral response (14).

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