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Effects of Three Dihydroxylated Derivatives of Tryptamine on the Behavior and on Brain Amine Content in Mice[1]

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MASSOTTI, M., A. SCOTTI DE CAROLIS AND V. G. LONGO. *Effects of three dihydroxylated derivatives of tryptamine on the behavior and on brain amine content in mice.* PHARMAC. BIOCHEM. BEHAV. 2(6) 769–775, 1974. – This investigation deals with the effects on behavior and on cerebral bioamine content in 3 dihydroxylated tryptamine derivatives (5,6-DHT, 5,7-DHT and 6,7-DHT) administered intracerebrally to mice. Both 5,6-DHT and 5,7-DHT caused a 50–70% lowering of 5-hydroxytryptamine (5-HT) content in whole brain which lasted for the entire experimental period (20 days). 5,6-DHT and 5,7-DHT differed in their effects on norepinephrine (NE) and dopamine (DA). While the former induced a DA diminution to an extent comparable to that observed for 5-HT and a rise in NE, the latter induced a lowering of NE and affected DA only slightly. 5,6-DHT decreased spontaneous activity of treated mice at 21 and 43 μg; the effect was dose-related. 5,7-DHT diminished spontaneous activity only at 43 μg. Following either 5,6-DHT or 5,7-DHT, 5-HTP induced an exaggerated tremorigenic response; this potentiation may be related to an imparied uptake of 5-HT by the terminals. 6,7-DHT, while more toxic than the other two drugs, showed a much weaker effect both on brain amines content and on behavior.

5,6-Dihydroxytryptamine 5,7-Dihydroxytryptamine 6,7-Dihydroxytryptamine L-DOPA
5-Hydroxytryptophan Brain catecholamine content Brain indoleamine content

BAUMGARTEN *et al.* [2,5] presented evidence that 5,6-dihydroxytryptamine (5,6-DHT), injected into the cerebral ventricles of rats was selectively concentrated in serotonergic central terminals, where it induced degenerative changes similar to those caused by 6-hydroxydopamine (6-OHDA) with respect to catecholaminergic neurons. These alterations were accompanied by a selective long-lasting depletion of brain and spinal cord 5-hydroxytryptamine (5-HT). These findings were subsequently confirmed by others [10, 11, 12]. More recently, Baumgarten and Lachenmayer [4] studied the central effect of 5,7-dihydroxytryptamine (5,7-DHT). They found that 5,7-DHT was less toxic than 5,6-DHT while resembling the latter as to its effect on serotonergic neurons.

Baumgarten and his associates reported a hyperexcitability and fighting behavior during the first few days after 5,6-DHT and 5,7-DHT treatment, respectively, [3, 4, 6]. Da Prada *et al.* [12] observed an increase in copulatory activity in rats after administration of 5,6-DHT; this phenomenon was also present, but to a lesser degree, after 5,7-DHT treatment [4]. Ho *et al.* [16] reported that, in mice, 5,6-DHT prevented the development of tolerance and dependence induced by morphine pellet implantation.

This investigation is concerned with the effects in mice of intracerebral administration of three dihydroxylated derivatives of tryptamine, 5,6-dihydroxytryptamine (5,6-DHT), 5,7-dihydroxytryptamine (5,7-DHT), and 6,7-dihydroxytryptamine (6,7-DHT) on behavior and on brain amine content.

We tested, in particular, the behavioral response of mice to 5-hydroxytryptophan (5-HTP) administered at various intervals after intracerebral injection of 5,6-DHT, 5,7-DHT, and 6,7-DHT. A hypersensitivity to 5-HTP could be expected in these animals since potentiation of the effects of L-DOPA was found in animals treated with 6-OHDA, which destroys catecholaminergic terminals [1].

[1] Some of the results described were obtained by one of the authors (V.G.L.) at the Department of Pharmacology, Loyola Medical School, Maywood, Illinois, USA; the help and advice of Professor A. G. Karczmar is gratefully acknowledged. Dr. A. Manian, Psychopharmacology Research Branch NIMH, USA has kindly furnished us with the various tryptamine derivatives.

METHOD

Animals

A total of 650 adult mice of both sexes, weighing 25–35 g were injected intracerebrally [14]. Creatinine sulphate dihydrate salts of 5,6-DHT and of 5,7-DHT, and creatinine sulphate monohydrate salt of 6,7-DHT were dissolved in 0.1% ascorbic acid solution; only freshly prepared solutions were used. The volume injected was always 10 μl; the same volume of ascorbic acid solution was injected intracerebrally to the mice which served as controls. L-DOPA was dissolved in distilled water with sufficient HCl (0.1 N) to produce a 1% solution at pH 3–4; pargyline and D, L-5-HTP were dissolved in water. All drug doses refer to the weight of the base. Unless otherwise stated, all these latter drugs were administered intraperitoneally.

Apparatus and Procedure

Spontaneous activity was measured by means of Animex activity meters (Farad Inc., Hagersten, Sweden). The activity of groups of 5 animals was recorded for 3 hr from 10 a.m. to 1 p.m. The tremors and the incoordinated motor activity induced by administration of 5-HTP were recorded by placing small plastic cages containing one animal per cage over a Grass FT-10C force displacement transducer connected to a polygraph. Evaluation of the response of mice to L-DOPA was carried out as described Everett [13], without pretreating the animals with pargyline. In brief, groups of 4 animals treated with L-DOPA (100 mg/kg) were placed in large containers; during the first hour after L-DOPA injection the mice were evaluated every 10 min for the presence of piloerection, salivation and Straub tail phenomenon, as well as for reactivity to external stimuli (evidenced by jumping, squeaking, running) and aggressive and stereotypic behavior. The behavioral responses were evaluated by two independent observers, one of whom unaware of the treatment, and a global score of +1, +2 or +3 was assigned to each group of animals.

Extraction and Assay of Brain Amines

Measurements of the whole brain levels of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) were carried out. The amine content was assessed in treated and control mice at 3, 10 and 20 days after intracerebral administration of 5,6-DHT, 5,7-DHT or 6,7-DHT.

Mice were deprived of food for 12 hr and killed by decapitation; the entire brain (including cerebellum) was weighed, homogenized in *n*-butanol acidified to pH 2 with concentrated HCl (0.85 ml/1000 ml) and centrifuged at 4°C for 15 min at 8000 rpm. The supernatant was pipetted into a test tube and diluted with a double volume of *n*-heptane-HCl 0.1 N (10:1 v/v). This mixture was shaken vigorously for 30 sec and recentrifuged at 3000 rpm for 10 min. One third of the resultant aqueous phase was used for the determination of serotonin as described by Maickel *et al.* [18]. The remaining aqueous phase was washed with chloroform and divided into two equal aliquots for the estimation of DA [9] and NE [18]. Fluorescence of the three amines was read in an Aminco-Bowman spectrophotofluorometer following calibration. Recovery of exogenous amines added to the tubes prior to the first shaking step was as follows: DA, 82%; NE, 87%; 5-HT, 93%. In animals treated with solvent alone the values for DA, NE and 5-HT were 1.18 ± 0.05, 0.42 ± 0.02 and 0.89 ± 0.02 μg/g wet brain, respectively (uncorrected for recovery).

RESULTS

Effects of 5,6-DHT on Mice

5,6-DHT was administered at dose levels at 10.5, 21, 43 and 86 μg per animal. As the highest dose caused death of 70% of the animals within 24 hr, the present results refer to experiments carried out with the three lower doses. For the first 5–6 hr after the injection there was no discernible difference in gross behavior between 5,6-DHT and vehicle-treated animals. Twenty-four hours following treatment with 21 μg spontaneous activity of the animals was reduced; the decrease in spontaneous activity lasted for a week. This effect was more marked in mice treated with 43 μg (Fig. 1); in these animals recovery was observed two weeks after treatment.

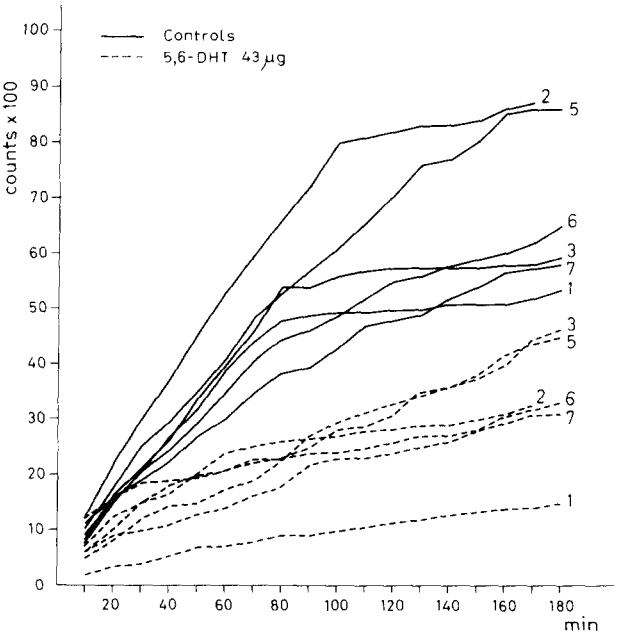

FIG. 1. Effects of 5,6-DHT on spontaneous activity of mice. Cumulative records for two groups of 5 mice each, obtained in the week following 5,6-DHT (43 μg) treatment (– – – –), or control injection (———). The numbers indicate the days of testing. Note that on all days tested 5,6-DHT treated animals displayed less spontaneous activity than controls. Ordinate: Print-out of Animex counter. Abscissa: Time in minutes.

Effects of Drugs in Mice Pretreated with 5,6-DHT

5-Hydroxytryptophan. As in rodents the central effects of 5-HTP are minimal unless the animals are pretreated with a MAO inhibitor [8], all animals received 20 mg/kg of pargyline 4 hr prior to 5-HTP challenge. 5-HTP was administered 2, 4, 10 and 20 days after treatment with 43 μg of 5, 6-DHT (two groups of 4 mice each) or with the vehicle (same number of mice). An enhancement of the 5-HTP tremorigenic effects was evident 2, 4 and 10 days after intracerebral injection of 5,6-DHT. Within 2 min after 5-HTP injection, the 5,6-DHT-treated animals began to shake their heads and subsequently exhibited violent and sustained whole body tremors, lasting about one hour. In vehicle-treated mice, 5-HTP induced a milder reaction 7–8 min after administration. These animals showed only

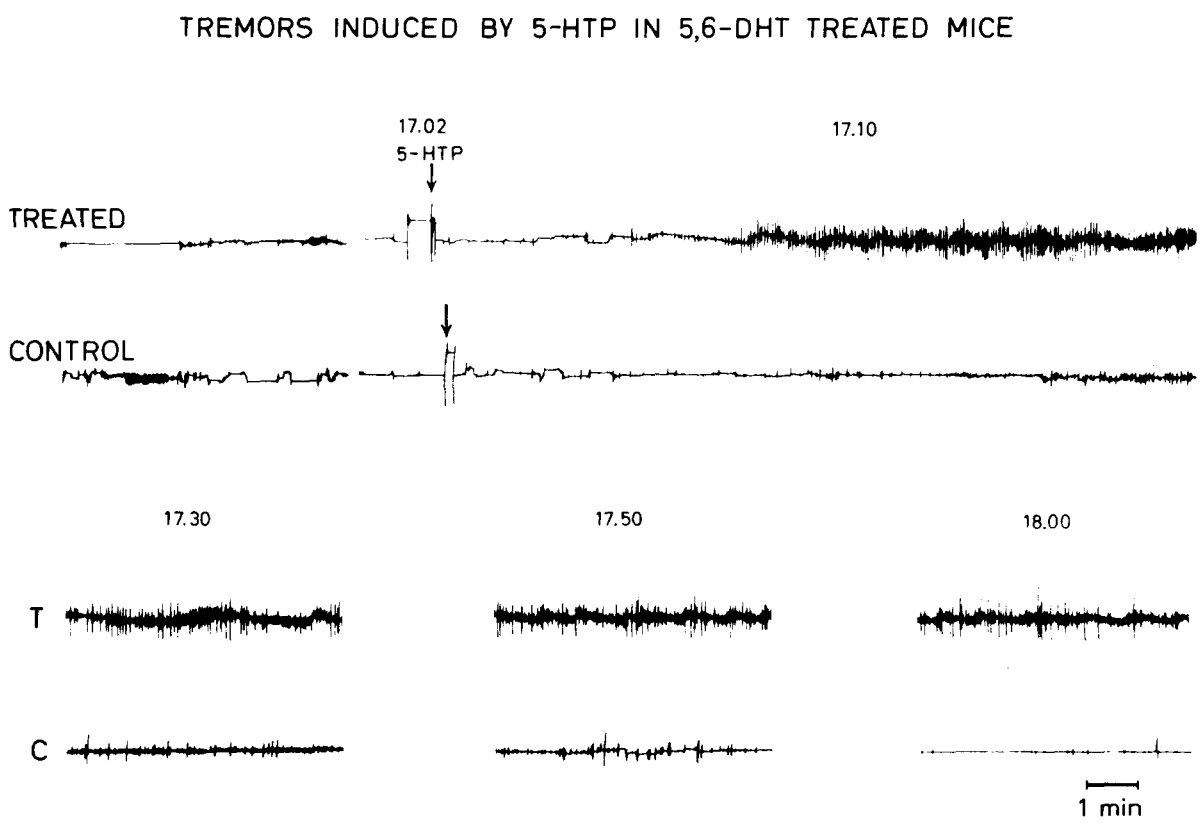

FIG. 2. Representative record of the tremors induced by 5-HTP in mice. The treated animal received intracerebrally 43 μg of 5,6-DHT two days in advance. Arrows indicate i.p. injection of 5-HTP, 75 mg/kg.

head twitches; the whole body tremors, if present, occurred only for a brief period (Fig. 2). By the 20th day this potentiation of the 5-HTP action in 5,6-DHT treated animals, while diminished, was still evident.

In mice pretreated with 21 μg of 5,6-DHT the potentiation of the response to 5-HTP was inconsistent and, when present, was less intense than that observed in mice treated with 43 μg of 5,6-DHT.

Since 6-OHDA potentiates L-DOPA behavioral effects even in mice not treated with MAO inhibitors [1], two groups of 4 animals treated with 43 μg of 5,6-DHT were challenged 2 and 4 days later with 75 mg/kg of 5-HTP in the absence of pargyline; in these animals the action of 5-HTP was not enhanced.

L-DOPA. The specificity of the potentiation of the 5-HTP response was tested in two experiments. First, L-DOPA was given to 2 groups of 4 mice each, 2, 4 and 10 days following the intracerebral injection of 43 μg of 5,6-DHT; L-DOPA was also given to the same number of mice treated with vehicle alone. Two and four days after 5,6-DHT treatment L-DOPA effects were potentiated; all mice showed hyperreactivity to external stimuli, squeaking, aggressiveness and stereotypic behavior, reaching a score of +3. Vehicle-injected animals, treated with L-DOPA scored +1. The L-DOPA potentiation was absent 10 days following 5,6-DHT treatment. In mice treated with 21 μg of 5,6-DHT some potentiation of the L-DOPA response was noticed 2 and 4 days after treatment (score of +2, as compared to the +1 of the controls); the potentiation disappeared by the tenth day (Fig. 3).

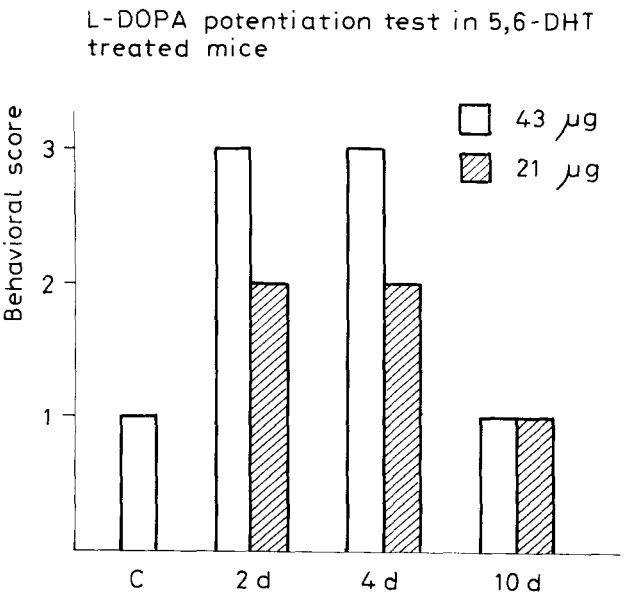

FIG. 3. Potentiation of the behavioral effects of L-DOPA in 5,6-DHT treated mice. L-DOPA, 100 mg/kg, was administered i.p. to groups of 4 mice each. Behavior was evaluated for one hour after injection. Ordinate: Behavioral score (cf. Methods); abscissa: time in days after intracerebral injection of the drugs. c = vehicle-treated animals. Note that potentiation of L-DOPA effects was found 2 and 4 days but not 10 days after treatment with both 21 μg and 43 μg.

The second experiment dealt with the effects of 5-HTP in animals pretreated with 6-OHDA. Four groups of 4 animals each were pretreated with 100 μg of 6-OHDA administered intracerebrally; an equal number of mice received the vehicle alone. Two days later, two groups of 6-OHDA pretreated mice were challenged with 75 mg/kg of 5-HTP, 4 hr after treatment with pargyline, 20 mg/kg; the other two groups underwent the same treatment 4 days after 6-OHDA. In both instances the tremors induced by 5-HTP were of greater intensity than in the vehicle-treated animals. The tremors started 7–8 min after 5-HTP injection, i.e. after a delay comparable to tnat observed in the control animals. The tremors observed in the 6-OHDA pretreated mice differed from those observed in 5,6-DHT pretreated mice; they consisted of an increase in the head twitchings and jerks; sustained fine tremors characteristic of the 5,6-DHT treated animals were not observed.

Effects of 5,6-DHT on brain amine content. Estimation of brain amines was carried out concomitantly with behavioral experiments. The mice were treated intracerebrally with 10.5, 21 or 43 μg of 5,6-DHT and assessed for brain amine content on the 3rd, 10th and 20th day. The dose of 10.5 μg of 5,6-DHT did not induce any significant modification in brain amine content. However, a significant decrease in DA and 5-HT was observed on all experimental days after 21 μg and 43 μg of the drug (Fig. 4). The decrease in 5-HT and DA ranged from 50 to 65% of the control values. No changes in NE were found on the 3rd and 20th day, while on the 10th day a slight but significant increase was present.

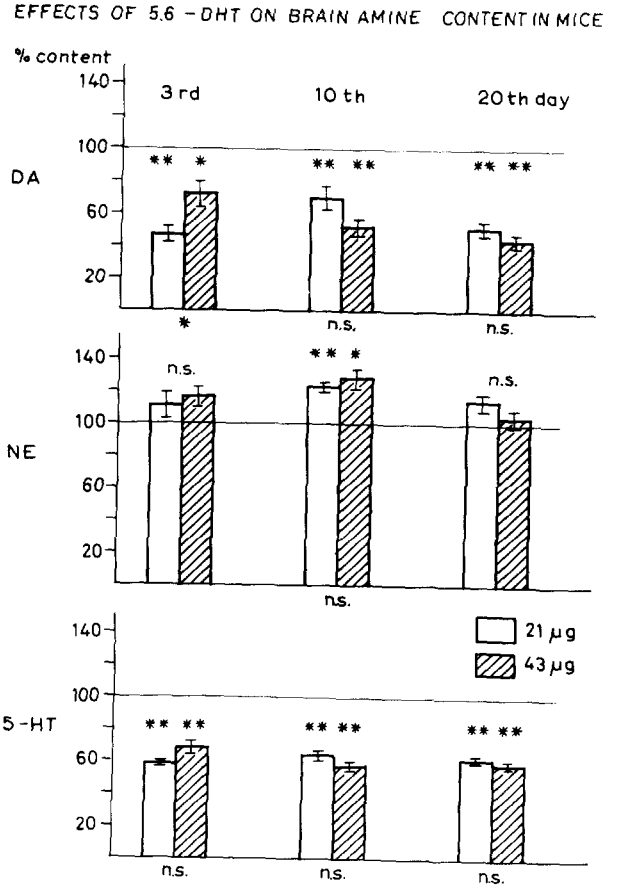

FIG. 4. Effects of 5,6-DHT on brain amine content in mice. The histogram summarizes the data obtained 3, 10 and 20 days after intracerebral administration of 21 and 43 μg of the drug. At least 8 animals were used for each determination. Results are expressed as percent of control values ± S.E. Evaluation of data was carried out by Multiple Comparison applied to the analysis of variance. *P* values for differences from controls are indicated above the columns; *p* values for interdose difference are shown below the columns. * = $p<0.05$; ** = $p<0.001$; n.s. = non significant.

Effects of 5,7-DHT on Mice

5,7-DHT was administered at dose levels of 10.5, 21, 43 and 86 μg. The two highest doses caused death of 90 and 30% of the animals respectively within 24 hr. The present results refer to experiments carried out with the 3 lower doses.

For the first 5–6 hr after the injection there was no discernible difference in gross behavior between 5,7-DHT and vehicle-treated animals; spontaneous motor activity was decreased only in the case of animals treated with 43 μg. Recovery was observed 4 days after treatment.

Effects of Drugs in Mice Pretreated with 5,7-DHT

5-Hydroxytryptophan. 5-HTP (75 mg/kg) was administered to two groups of 4 mice which received two days earlier 21 and 43 μg of 5,7-DHT, respectively. 5-HTP was also administered to an equal number of vehicle-treated mice. All animals received 20 mg/kg of pargyline, 4 hr before the 5-HTP challenge. 5-HTP induced intense and long lasting tremors in both groups of 5,7-DHT treated mice. Potentiation of the 5-HTP response was similar to that obtained in mice treated with 43 μg of 5,6-DHT. Essentially the same results were obtained in mice pretreated 4 and 10 days previously with 21 or 43 μg of 5,7-DHT.

L-DOPA. Two groups of 4 mice each were challenged with L-DOPA (100 mg/kg) 2, 4 and 10 days after intracerebral injection of 5,7-DHT (43 μg); L-DOPA was also given to an equal number of mice treated with vehicle alone. Two and 4 days after 5,7-DHT treatment there was a potentiation of the L-DOPA effects similar in all respects to that observed following the 5,6-DHT treatment, all mice reaching a score of +3. The L-DOPA potentiation was absent 10 days following the 5,7-DHT treatment. The same experiment was carried out in mice treated with 21 μg of 5,7-DHT 2, 4 and 10 days before L-DOPA challenge. The exaggerated response (score +2) to the L-DOPA was present 2 and 4 days after 5,7-DHT treatment; it disappeared by the tenth day.

Effects of 5,7-DHT on brain amine content. The dose of 10.5 μg did not alter significantly brain amine levels. A significant diminution in 5-HT, ranging from 50 to 70% of the controls, was found on all experimental days, following 21 or 43 μg of 5,7-DHT (Fig. 5). In contrast with the results obtained with 5,6-DHT, NE was found diminished; DA levels were also lower than those of the controls, but the diminution was less marked than that observed with 5,6-DHT.

Effects of 6,7-DHT on Mice

In doses higher than 10.5 μg 6,7-DHT caused death of almost all animals treated; the dose of 10.5 μg caused death of 40% of the mice within 24 hr. The surviving animals

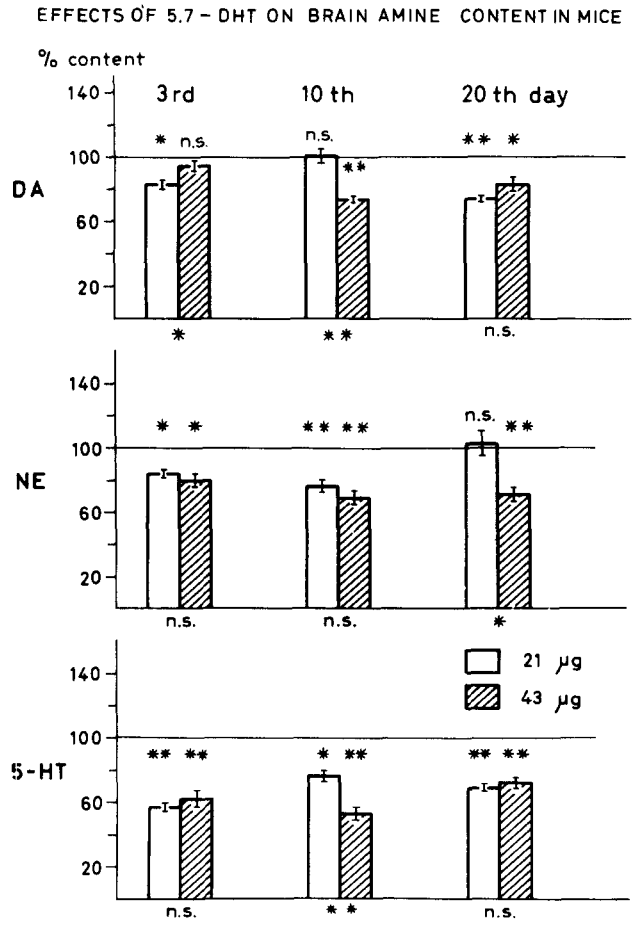

FIG. 5. Effects of 5,7-DHT on brain amine content in mice. The histogram summarizes the data obtained 3,10 and 20 days after intracerebral administration of 21 and 43 µg of the drug. At least 8 animals were used for each determination. Results are expressed as percent of control values ± S.E. Evaluation of data was carried out by Multiple Comparison applied to the analysis of variance. For the identification of p values, cf. Fig. 4.

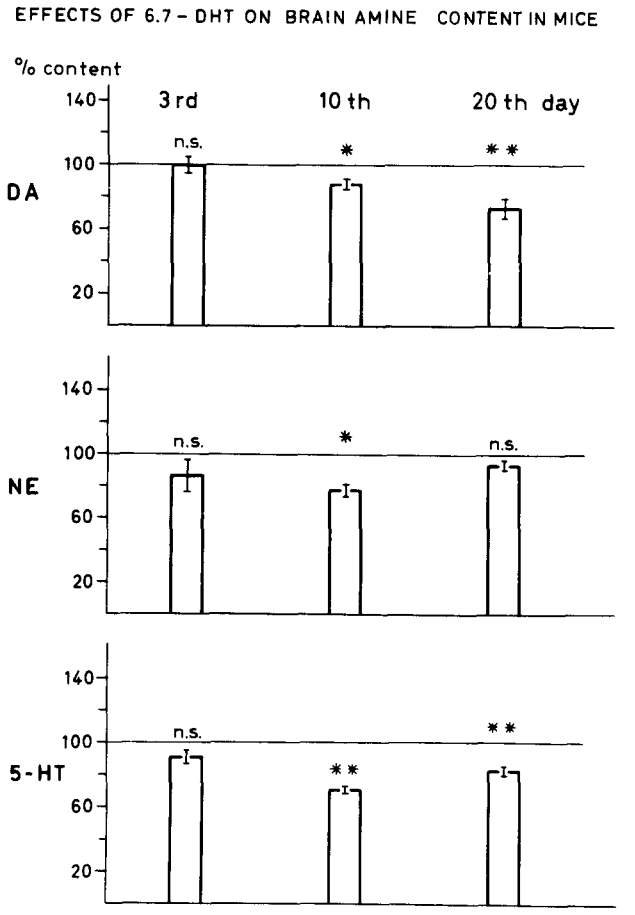

FIG. 6. Effects of 6,7-DHT on brain amine content. The histogram summarizes the data obtained 3,10 and 20 days after intracerebral administration of 10.5 µg of the drug. At least 8 animals were used for each determination. Results are expressed in percent of control values ± S.E. Evaluation of data was carried out by the Student t. For the identification of p values, cf. Fig. 4.

appeared depressed for the first 5–6 hr after injection. Twenty four hours following treatment a slight (statistically not significant) diminution in spontaneous activity was still present; full recovery was reached 2–3 days after treatment.

Effects of Drugs in Mice Pretreated with 6,7-DHT

5-Hydroxytryptophan. Two, 4 and 10 days after treatment with 10.5 µg of 6,7-DHT, 5-HTP (75 mg/kg) was administered to two groups of 4 mice. 5-HTP was also administered to an equal number of mice pretreated intracerebrally with the vehicle alone. All animals received 20 mg/kg of pargyline 4 hr before the 5-HTP challenge. Only on the fourth day after treatment was a mild potentiation of the response to 5-HTP noticed.

L-DOPA. Two groups of mice each were challenged with L-DOPA (100 mg/kg) 2, 4 and 10 days after intracerebral injection of 6,7-DHT; the same number of mice treated with vehicle alone was also injected with L-DOPA. There was no significant difference in the L-DOPA response of the controls and 6,7-DHT treated mice.

Effects of 6,7-DHT on brain amine content. Three days after treatment with 10.5 µg of 6,7-DHT there was no significant modification in brain content of the three amines. On the tenth and twentieth day, 5-HT content fell to 70% and 80%, respectively, of the control values. On the 20th day a fall in DA to 70% of the control values was also noticed (Fig. 6).

DISCUSSION

Both 5,6-DHT and 5,7-DHT caused a lowering of 5-HT content in the whole brain, reaching 50–70% of control values and lasting for the entire experimental period (20 days); doses of 21 and 43 µg proved equally effective. Similarly, Ho *et al.* [16] reported that 40 and 80 µg of 5,6-DHT, injected intracerebrally, depleted 5-HT to 60% of controls. In rats treated with 5,6-DHT the 5-HT content of discrete brain areas was affected differentially [2,10];

marked reduction was noticed in the spinal cord but not in the pons and medulla oblongata. This has been attributed to the technique of administration of the drug, which, being injected into the spinal fluid, may not reach deeply located zones. The investigations of Baumgarten *et al.* [5] and Baumgarten and Lachenmayer [4], carried out with the microscopic fluorescence technique demonstrated that degeneration of the serotonergic system after 5,6-DHT or 5,7-DHT involves not only the nerve endings but also the distal part of the axons. If neurotransmitter depletion depended specifically on the destruction of the terminals, the extensive damage of serotonergic neurons may not result in an equally striking diminution in 5-HT brain content. This may explain why in our experiments as well as in those of others lowering of 5-HT levels was never very marked.

Our results indicate that 5,6-DHT and 5,7-DHT produce different effects with respect to NE and DA. While the former induced a DA diminution to an extent comparable to that observed for 5-HT as well as a rise in NE on the tenth day, the latter induced a lowering of NE and had only a slight effect on DA. These data are in partial agreement with results of Baumgarten *et al.* [2], who reported for the rat treated with 5,6-DHT a late increase in NE, reaching its maximum on the tenth day, and a lowering in DA; this lowering, however, lasted for a much shorter time period (5 days) than that observed by us. That 5,6-DHT destroys DA-containing nerve terminals was indicated by the data of Bjorklund *et al.* [7], who found a reduced fluorescence in the caudate nuclei of rats treated intraventricularly with 5,6-DHT. We are not aware of published data on the effect of 5,7-DHT on cerebral amine content. Baumgarten and Lachenmayer [4] reported that, in the rat, doses of up to 75 μg of 5,7-DHT did not induce any gross alterations in fluorescence intensity of catecholaminergic axons, which appeared damaged only after higher doses.

Contrary to what was reported for the rat [3,12], administration of 5,6-DHT and 5,7-DHT did not elicit in mice any sign of hyperirritability or hypersexuality; mice treated with 5,6-DHT and 5,7-DHT showed a diminution in spontaneous activity which was dose-related. For 5,6-DHT this diminution occurred with the dose of 21 μg and was more marked after 43 μg. In the case of 5,7-DHT, the effect on spontaneous activity could be demonstrated only after 43 μg; 6,7-DHT did not have any noticeable effect on this parameter. There may be no direct relationship between amine depletion and behavioral depression as 21 μg of 5,6-DHT or of 5,7-DHT which only slightly affected the spontaneous activity, caused the same diminution in brain amines as that observed after 43 μg.

The exaggerated response to 5-HTP found in our experiments may be due to a mechanism analogous to that postulated by ourselves and others [1] to explain the enhancement of the behavioral effects of L-DOPA following intracerebral administration of 6-OHDA, i.e., to the impaired uptake of 5-HT by the terminals. Under these circumstances, 5-HT generated by the administration of 5-HTP may act longer on the receptor, giving rise to an enhanced effect. This hypothesis is consistent with data of Daly *et al.* [11] and Bjorklund *et al.* [7] who have described a loss of 5-HT uptake sites in rats treated with 5,6-DHT.

Mice treated with 5,6-DHT or with 5,7-DHT also show a hypersensitivity to L-DOPA. In a previous study [17] of 5,6-DHT, this phenomenon was attributed to the damage of the DA system, as evidenced by the diminution in brain DA content. However, 5,7-DHT diminished only slightly the cerebral DA content (Fig. 5). The impairment of functions of the nerve endings may also be achieved through mechanisms other than destruction. 5,6-DHT is taken up into DA nerve tracts [11]; this may induce a dysfunction of the terminals, and a similar phenomenon may occur with 5,7-DHT.

6,7-DHT while more toxic than the other two drugs, showed a much weaker effect both on brain amines content and on behavior. This compound lacks the hydroxy group in position 5 and the steric conformation may play a role in the uptake of the drug by the 5-HT terminals.

In conclusion, the present results and data of other workers clearly demonstrate that drugs such as 5,6-DHT and 5,7-DHT injected into the cerebrum damage 5-HT neurons. However, this effect is far from being selective since 5,6-DHT and 5,7-DHT seem to affect also the catecholaminergic neurons. In this context, Heikkila and Cohen [15] demonstrated that 5,6-DHT inhibits both DA and 5-HT uptake in brain tissue slices. It is difficult to decide at this time whether the DHT effect on the 5-HT or on catecholaminergic neurons is directly related to the observed modifications of behavior.

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