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Evaluation of the Non-Specific Effects of Catecholamine and Serotonin Neurotoxins by Injection into the Medial Forebrain Bundle of the Rat

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LORDEN, J. F., G. A. OLTMANS, R. DAWSON, JR. AND M. CALLAHAN. *Evaluation of the non-specific effects of catecholamine and serotonin neurotoxins by injection into the medial forebrain bundle of the rat.* PHARMAC. BIOCHEM. BEHAV. **10**(1) 79–86, 1979.—Low doses of 6-hydroxydopamine (6-OHDA), 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT) that have previously been shown to produce behavioral change following intracerebral infusion were injected into the medial forebrain bundle of the rat. This site contains serotonin (5-HT), norepinephrine (NE), and dopamine (DA) fibers whose anatomical locations have been described. Damage to these fiber systems was quantified by measuring depletion of telencephalic 5-HT, NE and DA. The effects of infusions of 6-OHDA, 5,6-DHT and 5,7-DHT were compared to the effects of unequivocally non-specific electrolytic lesions and copper sulfate infusions. Survival time was varied to evaluate the amount of regeneration that could be expected over periods from 8 to 60 days. Amine levels were found to be stable over the time period examined. With the doses used, evidence was found to support the position that non-specific damage caused by general cytotoxic effects of 6-OHDA and 5,7-DHT is minimized sufficiently to permit the acquisition of useful data on the function of central catecholamine and indoleamine systems.

6-Hydroxydopamine 5,6-Dihydroxytryptamine 5,7-Dihydroxytryptamine Medial forebrain bundle

NEUROTOXINS are important tools in the study of neurochemically-defined pathways in the central nervous system. 6-Hydroxydopamine (6-OHDA), 5,6-dihydroxytryptamine (5,6-DHT), and 5,7-dihydroxytryptamine (5,7-DHT) have been used in a wide variety of anatomical, pharmacological and behavioral studies (e.g., [5, 7, 9, 11, 30]). The usefulness of these toxins is directly related to their specificity; and several studies have addressed this issue.

The initial work with 6-OHDA indicated that this toxin destroyed norepinephrine (NE) and dopamine (DA) neurons, while leaving serotonin (5-HT) and γ-aminobutyric acid fibers intact [31]. A specific catecholamine transport system was believed to concentrate the toxin in catecholamine-containing neurons, thus accounting for the specificity of the toxin [16]. However, recent work has challenged the view that 6-OHDA produces specific depletions of catecholamines [6,25]. Actual estimates of the specificity of 6-OHDA vary widely, depending on the dose, concentration, and speed and site of injection used [6,15].

The strongest arguments for neurochemical specificity have been made for 6-OHDA. The toxins 5,6-DHT and 5,7-DHT which have been proposed as serotonin neurotoxins have been shown to damage NE and DA-containing neurons as well [2, 8, 12]. However, evidence suggests that the specificity of 5,7-DHT can be improved by pretreatment with NE uptake blockers, such as desmethylimipramine (DMI) [3].

Despite the criticism of the specificity of 6-OHDA and the acknowledged lack of specificity of 5,6-DHT and 5,7-DHT, these toxins are still in use. They remain the major alternatives to completely nonspecific lesion techniques for long-term depletion of endogenous NE, DA and 5-HT. Interpretation of the results of any experiment making use of these toxins is, however, complicated. In addition to the issue of non-specificity, another problem may be encountered in experiments involving behavioral measures where several weeks may be required to collect data. With long recovery periods, there is a possibility that regeneration may take place [2, 4, 26, 32]. In such instances, the neurochemical state at the end of the study may not accurately reflect the situation when data collection was initiated, and controls must be included to evaluate this possibility. The present study was designed to provide information relevant to these issues.

The effects of 6-OHDA, 5,6- and 5,7-DHT lesions of the medial forebrain bundle (MFB) were examined. The MFB is a complex region containing many different types of chemically defined afferent and efferent neurons. Analysis of behavioral change resulting from MFB lesions is necessarily difficult. However, the complexity of the area offered an advantage in the present study. The MFB was chosen as a lesion site, since it contains 5-HT, NE and DA fibers whose anatomical locations have been described [14, 23, 30]. With small electrolytic lesions, it is possible to vary the extent of damage to these particular systems by varying the distance of the lesion from the midline. Lesions placed at the medial edge of the MFB, immediately lateral to the fornix, primarily damage 5-HT and NE neurons; lesions at the lateral border of the MFB, adjacent to the internal capsule, damage DA neurons. The degree of damage to each of these fiber systems can be assessed by measuring telencephalic amine content. Thus, the purpose of this is to provide quantitative measures of both the specific and non-specific actions of small doses of the toxins by making the infusion at the medial site known to contain 5-HT and NE neurons. Under these conditions non-specific damage due to a 6-OHDA infusion is reflected in decreases in telencephalic 5-HT content. These effects were compared to depletions produced not only by electrolytic lesions but also by a diffusable, non-specific agent, copper sulfate ($CuSO_4$). Diffusion of the toxins was measured by DA depletion. Survival time was also varied to assess possible regeneration effects.

EXPERIMENT 1

The effects of a 6-OHDA infusion into the MFB were examined in animals sacrificed 8 days after infusion in order to minimize possible neuronal regeneration. Both medial and lateral cannula placements were used. The effects of 6-OHDA on telencephalic amine concentrations were compared to the effects of 5,6-DHT and two types of unequivocally non-specific lesions: electrolytic lesions and infusions of copper sulfate ($CuSO_4$). The lesion locus could be identified histologically in the case of the electrolytic lesions, while the $CuSO_4$ infusion could be expected to have the same diffusion characteristics as the 6-OHDA and 5,6-DHT.

Method

Female albino rats (Holtzman, Madison, WI), weighing between 255 and 300 g at the time of surgery, were used as subjects. All animals were housed in a light and temperature controlled colony room. Purina Laboratory Chow and water were available ad lib throughout the experiment.

All lesions were made at the following coordinates [19]: 2 mm posterior to bregma, 1.3 or 1.5 mm lateral to the midline, and 9.5 mm ventral to the surface of the skull. The incisor bar of the stereotaxic instrument was set 3.5 mm above the interaural line. Ether was used as the anesthetic for all surgery.

Electrolytic lesions were produced by passing a 2 mA cathodal current through a stainless steel insect pin (size 000) for 30 sec. The electrode was insulated except for 1 mm at the tip. 6-OHDA lesions were made by the infusion of 20 or 40 nmol (free base) of 6-OHDA·HBr (Regis Chemical Co.). The 6-OHDA was dissolved at a concentration of 10 μg of the salt/μl. 5,6-DHT lesions were produced by injecting 21 nmol (free base) of 5,6-DHTcreatinine sulfate (Sigma Chemical Co.) at a concentration of 4 μg/μl. A copper sulfate group received a 21 nmol dose of $CuSO_4$ (Mallinkrodt, Reagent Grade Chemical, $CuSO_4 \cdot 5H_2O$). The infusions were delivered through a 26 ga stainless steel cannula over a 2 min period. The infusion vehicle for both the 6-OHDA and 5,6-DHT lesions was 0.9% saline with 0.1% ascorbic acid added to retard oxidation. Vehicle infusion control animals received either 0.5 or 1.0 μl of the vehicle. A sham-operated group received the same surgical treatments as the other groups; however, neither an electrode nor a cannula was lowered into the brain. All infusions and lesions were made bilaterally. The groups and sample sizes are listed in Table 1 for medial placements and Table 2 for lateral placements.

Eight days after surgery, all animals were sacrificed by decapitation. The telencephalic dissection was made by lifting the occipital lobes of the cortex to expose the dorsal diencephalon. When the columns of the fornix were visible, bilateral cuts were made using the corona radiata as guides. A third cut in the coronal plane passing through the anterior commissure severed the telencephalon from the remainder of the brain [22]. Each hemisphere served as a separate sample and was analyzed fluorometrically for 5-HT, NE and DA according to the method of Jacobowitz and Richardson [13].

The brainstems of all animals in the lesion and vehicle infusion groups were placed in neutral buffered formalin and saved for histology. Lesion size and cannula placements were determined in 100 μm frozen sections that were photographed.

Data were analyzed by analysis of variance followed by Newmann-Keuls tests, or by t-tests. Specific hypotheses were tested with one degree of freedom contrasts. The 0.05 level of significance was used throughout, unless otherwise indicated.

Results and Discussion

To minimize differences as a result of variability in the infusion site, all sections included in the neurochemical analysis were verified as having a cannula placed either at a medial site, just lateral to the fornix; or at a lateral site, at the medial border of the internal capsule. The cannula placement sites are presented in Fig. 1a.

The electrolytic lesions (Fig. 1b) were generally very large and frequently covered the area from the internal capsule to the fornix and mamillothalamic tract. The largest electrolytic lesions typically destroyed tissue from immediately below the medial lemniscus to the base of the brain and from the anterior hypothalamic nuclei to the level of the posterior commissure. The considerable lateral extent of the electrolytic lesions indicated possible damage to the nigrostriatal DA fibers.

The average weight ($\pm$ SD) of the telencephalic hemispheres in the experiment was 0.491 g $\pm$ 0.023. No significant differences in section weight occurred between groups. The telencephalic amine content of the sections with histologically verified medial placements is presented in Table 1. The electrolytic lesions, although not so well-localized as the cannula tracks, are also included in Table 1. There were no significant differences in telencephalic amine levels between rats that received 0.5 or 1.0 μl of vehicle; therefore, these groups were combined for data presentation. The vehicle infusion did not produce a significant decrease in the levels of any of the amines measured. All four lesion types, however, produced a significant reduction in 5-HT levels. The smallest amount of damage to 5-HT fibers occurred in

TABLE 1

TELENCEPHALIC AMINE CONTENT 8 DAYS AFTER MEDIAL FOREBRAIN BUNDLE LESIONS (MEDIAL PLACEMENTS)

Group	N	Amine Levels 5-HT	NE	DA
6-OHDA (20 nmol)	4	0.495 ± .068*†	0.118 ± .062*†	0.634 ± .370*
5,6-DHT	7	0.194 ± .077*	0.229 ± .048	0.357 ± .116*
$CuSO_4$	7	0.238 ± .094*	0.173 ± .027*	0.582 ± .182*
Electrolytic	4	0.310 ± .035*	0.233 ± .048	0.300 ± .100*
Vehicle	8	0.658 ± .072	0.246 ± .032	1.026 ± .092
Sham	8	0.708 ± .142	0.269 ± .021	1.165 ± .243

Values expressed as μg of amine/g (M ± SD) fresh weight of brain.
*Differs significantly from both Sham and Vehicle groups.
†Differs significantly from all other lesion groups.

TABLE 2

TELENCEPHALIC AMINE CONTENT 8 DAYS AFTER MEDIAL FOREBRAIN BUNDLE LESIONS (LATERAL PLACEMENTS)

Group	N	Amine Levels 5-HT	NE	DA
6-OHDA (20 nmol)	6	0.625 ± .061	0.150 ± .044*	0.164 ± .138*
6-OHDA (40 nmol)	8	0.558 ± .119	0.117 ± .051*	0.201 ± .140*
5,6-DHT	6	0.307 ± .133*†	0.266 ± .043	0.293 ± .187*
Vehicle	8	0.583 ± .063	0.283 ± .092	1.012 ± .116
Sham	8	0.708 ± .142	0.269 ± .021	1.165 ± .243

Values expressed as μg of amine/g (M ± SD) fresh weight of brain.
*Differs significantly from Sham and Vehicle control group.
†Differs significantly from all groups (Sham, Vehicle, and 6-OHDA).

the 6-OHDA group. This group has significantly more 5-HT remaining than the other lesion groups.

NE depletion was greatest in the 6-OHDA group. Both the 6-OHDA and $CuSO_4$ groups differed significantly from the Sham and Vehicle groups. None of the other lesion groups differed significantly from either control group in NE levels.

DA levels were significantly reduced by all lesions. The 6-OHDA group had the highest DA levels remaining of all the lesion groups. However, the 6-OHDA group differed significantly only from the Electrolytic group. The fact that a significant degree of DA depletion was observed in the 6-OHDA group indicates that the failure of 6-OHDA to produce a reduction in 5-HT levels comparable to that observed in the other lesion groups was not the result of the toxin's failure to diffuse laterally from the injection site.

The data for lateral cannula placements are presented in Table 2. Again, there were no significant differences between vehicle groups; and the vehicle infusion did not cause a significant reduction in amine levels. With cannula placements along the border of the internal capsule, DA depletion was extensive (>75%) in all lesion groups. There were no significant differences between groups in DA levels.

With the lateral placement, a significant reduction of telencephalic 5-HT was observed only in the 5,6-DHT group. This group differed significantly from all other lesion groups in 5-HT levels. Neither the 20 nmol nor 40 nmol dose of 6-OHDA caused a significant reduction in 5-HT, although both doses markedly depleted telencephalic NE. The 5,6-DHT group did not show a significant loss of NE in comparison with either the Sham or Vehicle control groups.

The data from this experiment indicate that low doses of 6-OHDA will cause a moderate decrease in 5-HT levels if infused directly into an area containing 5-HT fibers. Thus, 6-OHDA cannot be considered completely specific for catecholamine neurons. The depletion is not as great, however, as the 5-HT depletion caused by 5,6-DHT lesions or by completely non-specific electrolytic or $CuSO_4$ lesions. Furthermore, if placed adjacent to an area containing 5-HT fibers, even a 40 nmol dose of 6-OHDA in a volume of 1 μl is not sufficient to produce a significant depletion of 5-HT. The failure of 6-OHDA to deplete telencephalic 5-HT in this case is not due to an inaccessibility of the 5-HT neurons. A 21 nmol dose of 5,6-DHT in a 1 μl volume injected at the same far lateral site reduces 5-HT levels by more than 50% in comparison with sham controls.

EXPERIMENT 2

A frequent use for neurotoxins such as 6-OHDA and 5,6-DHT has been in the prolonged behavioral observation of catecholamine- or serotonin-depleted animals. Analysis of behavioral changes that occur after neurochemical lesions would be complicated if amine levels were not stable following the lesions. Thus, Experiment 1 was replicated using a long survival time in order to determine whether significant

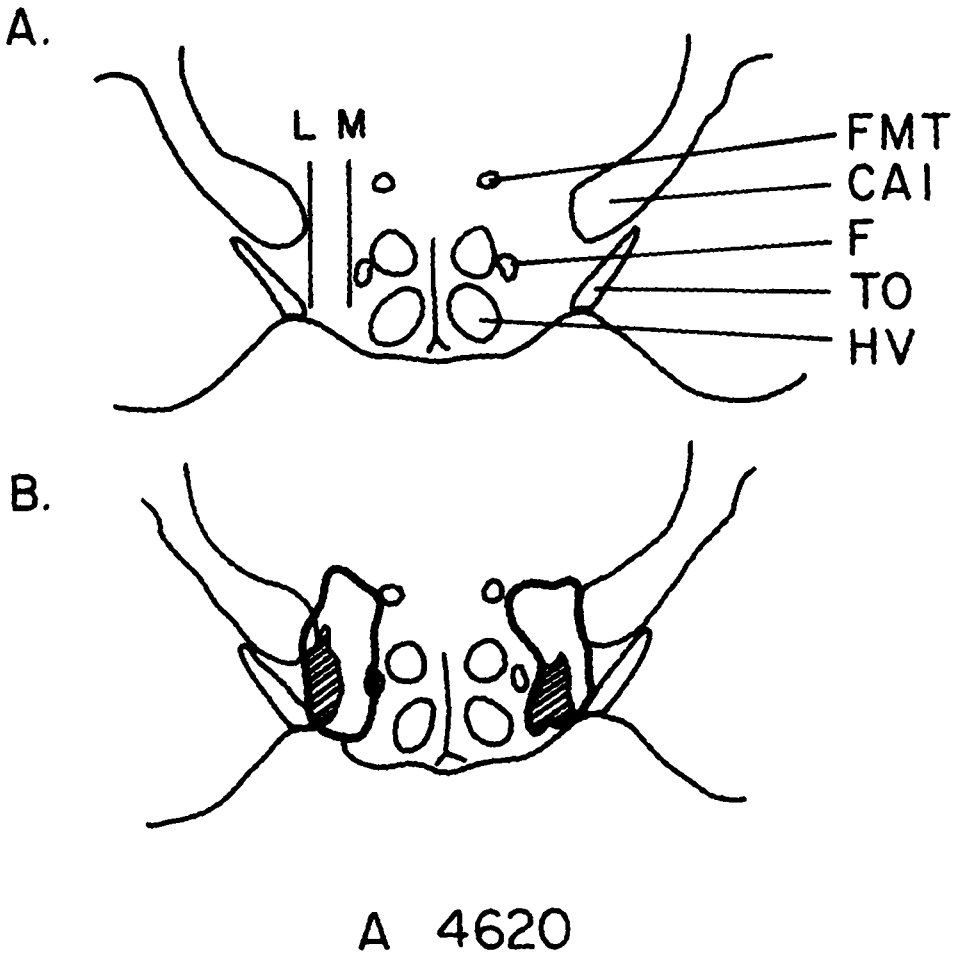

FIG. 1. (A) The position of the cannula tracks for animals included in Experiment 1 is shown. Tracks were located either medially (M) or laterally (L) in the MFB. Abbreviations: CAI, internal capsule; F, Fornix; FMT, mamillothalamic tract; HV, ventromedial nucleus of the hypothalamus; TO, optic tract [18]. (B) The smallest (striped area) and largest (outlined) electrolytic lesions from Experiment 1 are shown.

regeneration should be anticipated with the use of 6-OHDA or 5,6-DHT.

Method

The subjects, surgical prcedures, and assay techniques used in this experiment were identical to those described above. Only medial cannula and electrode placements were examined. Two survival times were used: 30 and 60 days. The groups and sample sizes are listed in Table 3.

Results and Discussion

The range of lesion sizes in the Electrolytic group was similar to that shown in Fig. 1b. As in the 8-day survival group, the electrolytic lesions were large and although aimed medially, caused damage from the fornix to the internal capsule.

The amine levels for all groups are presented in Table 3. Only data for histologically verified medial cannula placements are included. The average section weight (± SD) in this experiment was 0.505 ± 0.025. No significant differences in section weights were noted between groups. In this experiment, vehicle infusions caused significant damage to NE but not DA or 5-HT fibers. A significant 5-HT depletion was observed in the 5,6-DHT and Electrolytic groups only. Despite the 60-day survival time, the depletion of 5-HT in the 5,6-DHT group (68%) was comparable to that obtained with an 8-day survival time in the previous experiment (72%).

5-HT levels were significantly higher in the 6-OHDA groups than in either the 5,6-DHT group or the Electrolytic group. Neither the 20 nor the 40 nmol dose of 6-OHDA produced a significant depletion of 5-HT in comparison with the Sham or Vehicle control groups. Both 6-OHDA groups did, however, sustain a substantial loss (80–86%) of telencephalic NE in comparison with the Sham group. Of the lesion groups in this experiment, only the 6-OHDA groups differed significantly from the Vehicle infusion group in NE levels. The 6-OHDA groups did not differ significantly from each other.

All lesion groups showed a significant loss of telencephalic DA in comparison with both Sham and Vehicle control groups. Depletion ranged from 37% in the 5,6-DHT group to 80% in the 40 nmol 6-OHDA group. The 40 nmol

TABLE 3

TELENCEPHALIC AMINE CONTENT 30 OR 60 DAYS AFTER LESIONS OF THE MEDIAL FOREBRAIN BUNDLE (MEDIAL PLACEMENTS)

Group	N	Survival Time (days)	Amine Levels 5-HT	NE	DA
6-OHDA (20 nmol)	8	30	0.629 ± .149	0.059 ± .026*†	0.507 ± .214*†
6-OHDA (40 nmol)	6	30	0.592 ± .077	0.043 ± .024*†	0.190 ± .158*†
5,6-DHT	7	60	0.233 ± .111*†	0.230 ± .040*	0.592 ± .221*†
Electrolytic	6	30	0.178 ± .043*†	0.203 ± .021*	0.357 ± .402*†
Vehicle (0.5μl)	8	30	0.677 ± .119	0.214 ± .029*	0.852 ± .207
Vehicle (1.0μl)	8	60	0.702 ± .073	0.249 ± .021*	0.841 ± .219
Sham	8	30	0.725 ± .114	0.301 ± .051	0.940 ± .202

Values expressed as μg of amine/g (M ± SD) fresh weight of brain.
*Differs significantly from Sham-operated controls.
†Differs significantly from Vehicle-infused controls.

6-OHDA group differed significantly from all lesion groups except the Electrolytic group. Thus, in equal infusion volumes, 6-OHDA was more effective in depleting NE and DA than was 5,6-DHT; however, 5,6-DHT was significantly more effective in depleting 5-HT. Even a relatively high dose of 6-OHDA was ineffective in producing any significant long-term loss of telencephalic 5-HT.

The results indicate that neurochemical and electrolytic lesions placed in the MFB produce long-lasting depletions in telencephalic levels of the biogenic amines. A comparison of telencephalic amine content in lesion groups 8 days following lesion placement (Table 1) with amine content 30 or 60 days following lesion placement (Table 3) did not provide any quantitative evidence of a recovery of amine content for either 5,6-DHT or 6-OHDA lesions. Thus, the neurochemical depletions produced by these toxins when infused directly into the tissue appears to be fairly stable over the time period examined.

EXPERIMENT 3

5,7-DHT administered intraventricularly to animals pretreated with DMI has been reported to deplete 5-HT and DA [3]. In this experiment, the effectiveness of 5,7-DHT in animals with and without DMI pretreatment was compared to the effects of 6-OHDA or electrolytic lesions in the MFB.

Method

Male albino rats weighing between 300 and 375 g at the time of surgery were used. Stereotaxic and assay procedures were identical to those described in Experiment 1. Infusions of 5,7-DHT creatinine sulfate (Regis Chemical Co.) or 6-OHDA·HBr were made through a 30 ga cannula. A 21 nmol (free base) dose of 5,7-DHT and a 16 nmol (free base) dose of 6-OHDA were used. The infusion vehicle was 0.9% saline and 0.02% ascorbic acid. Concentrations were the same as those in Experiments 1 and 2.

Animals in the 5,7-DHT-DMI group received 25 mg/kg of desmethylimipramine HCl (USV Pharmaceutical Co.) administered IP 45 min prior to the 5,7-DHT infusion. The 5,7-DHT-Sal group received an equivalent volume of physiological saline as a pretreatment. The electrolytic lesions were made with size 000 insect pins with 0.5 mm uninsulated tips. Half of the vehicle group received a 0.5 μl infusion. The groups and sample sizes are listed in Table 4. Only medial cannula placements were examined. All animals were sacrificed 60 days after surgery.

Results

The electrolytic lesions included in this experiment were smaller and more ventrally placed than the average lesions in Experiments 1 and 2. The lesions in the present experiment were comparable to the smallest lesion in Experiment 1, shown in Fig. 1b. Cannula placements were similar to those described above.

The average telencephalic section weights (M ± SD) in Experiment 3 were comparable to those in the first two experiments (0.532 ± 0.047). Again, no significant differences in section size occurred between groups. 5,7-DHT and electrolytic lesions in the MFB, immediately lateral to the fornix, produced 35–54% reductions in 5-HT (Table 4). Both the 5,7-DHT and Electrolytic groups differed significantly from the Vehicle and Sham groups and also from the 6-OHDA group. The 5,7-DHT and Electrolytic groups did not, however, differ significantly from each other. The 6-OHDA lesion caused a 10% reduction in 5-HT. This was not significantly different from the effects of a vehicle infusion.

The greatest reduction in NE was observed in the 6-OHDA group. This was the only group that differed significantly in NE levels from both the Vehicle and Sham groups. The 5,7-DHT-DMI group showed no more NE depletion relative than to the Vehicle group and had significantly more NE remaining than the other lesion groups. Thus, although a smaller dose of 6-OHDA than 5,7-DHT was used, 6-OHDA was more effective at depleting NE than was 5,7-DHT.

DA depletion ranged from 15–33% of sham levels in the lesion groups. Only the electrolytic lesions, however, caused a significant reduction in DA.

GENERAL DISCUSSION

Any type of lesion which requires the entry of an electrode or cannula into the brain cannot be entirely neurochemically specific. Therefore, the question addressed here is not whether any non-specific damage is caused by the

TABLE 4

TELENCEPHALIC AMINE CONTENT 60 DAYS AFTER LESIONS OF THE MEDIAL FOREBRAIN BUNDLE (MEDIAL PLACEMENTS)

Group	N	Amine Levels 5-HT	NE	DA
5,7-DHT + DMI	4	0.306 ± .060*†	0.363 ± .061	1.228 ± .228
5,7-DHT − Sal	4	0.276 ± .029*†	0.301 ± .044*	1.037 ± .347
6-OHDA (16 nmol)	6	0.544 ± .059	0.256 ± .070*†	1.221 ± .239
Electrolytic	4	0.391 ± .023*†	0.279 ± .061*	0.957 ± .329*
Vehicle	7	0.549 ± .078	0.351 ± .061	1.400 ± .251
Sham	8	0.601 ± .069	0.452 ± .080	1.434 ± .213

Values expressed as μg of amine / g (M ± SD) fresh weight of brain.
*Differs significantly from Sham-operated controls.
†Differs significantly from Vehicle-infused controls.

use of 6-OHDA, 5,6-DHT or 5,7-DHT. Rather, the issue is whether an analytical advantage is derived from using one of these toxins in preference to an electrolytic lesion or to an agent such as copper sulfate.

Critics of 6-OHDA's specificity have argued that the destruction of brain tissue which occurs with an infusion of the toxin is marked by a general cytotoxicity which is indistinguishable from that caused by an injection of hydrogen peroxide or $CuSO_4$ [6,25]. The results presented here offer some support for such a position, but in general support the notion that neurotoxins such as 6-OHDA and 5,7-DHT are useful agents for minimizing non-specific neuronal damage as a consequence of the lesion procedure. This was particularly true for the catecholamine neurotoxin 6-OHDA.

In the current study 6-OHDA was introduced directly into an MFB site containing 5-HT fibers. If the neurotoxic effect of 6-OHDA was completely non-specific, one would predict destruction of the 5-HT fibers and a reduction of telencephalic 5-HT content comparable to that found with a non-specific toxin such as $CuSO_4$. This was not the case. With an 8-day post-lesion survival period, the 6-OHDA infusion did produce a significant reduction in telencephalic 5-HT content; but this reduction was significantly less than that produced by an equimolar dose of $CuSO_4$. Thus, the 6-OHDA was significantly less toxic than the $CuSO_4$ when 5-HT depletion was used as the measure of non-specific damage. With longer post-operative survival periods (e.g., 30 or 60 days, Tables 3 and 4) no significant effect on telencephalic 5-HT content was found with the 6-OHDA lesion. This latter finding raises an important issue with respect to studies requiring a prolonged post-operative data collection period. The data indicate that neuronal damage which can be chemically assessed early in the post-operative period is masked later on by neurochemical events which result in a recovery of the original 5-HT levels. This demonstrates the necessity of extreme care in interpreting the results of studies using such toxins when long survival periods are required (as in behavioral studies). Similar conclusions have been reached about behavioral change following intraventricular 6-hydroxydopa lesions [26]

It appears, however, that while 6-OHDA will produce general damage around the infusion site, this destruction is not extreme, and the non-specific effects do not extend for a great distance. Twenty and 40 nmol doses of 6-OHDA, which produced 56–86% depletion of telencephalic NE (Tables 1 and 3), caused at most a 30% depletion of 5-HT in comparison with sham-operated control rats. Thus, as reflected in measures of telencephalic 5-HT, 6-OHDA consistently caused significantly less damage to 5-HT fibers than did equimolar or lower doses of 5,6-DHT and $CuSO_4$. The effects of 5,6-DHT, $CuSO_4$ and electrolytic lesions clearly demonstrated that the infusion site used was an effective one for causing depletion of telencephalic 5-HT. These lesions resulted in a 66–73% loss of telencephalic 5-HT in comparison with Sham control groups. Since the 6-OHDA infusions were histologically verified as being in the same area as the 5,6-DHT and $CuSO_4$ infusions, it appears that the 6-OHDA produced less damage to 5-HT neurons than the other procedures examined. Furthermore, the effects of 6-OHDA on NE were similar, whether it was infused medially or laterally in the MFB, while the significant effect on 5-HT found with medial placements was not present when the infusion site was moved away from the main pathway of the 5-HT fibers. Thus, the slight damage produced by direct infusion of 6-OHDA into tissue agrees with the analyses of other investigators who have applied histological techniques to the nigro-striatal DA system and supports the concept of the existence of a zone of selective neuronal destruction [1, 15, 28].

DA neurons are clearly susceptible to the effects of both 6-OHDA and 5,6-DHT. 5,6-DHT infused into the MFB has been shown to be as effective in lowering DA levels as 6-OHDA, $CuSO_4$ and electrolytic lesions. However, 5,6-DHT is less effective than other methods in depleting NE, even when this toxin is infused at sites where 6-OHDA causes massive depletion of telencephalic NE. The effects of 5,6-DHT on NE and DA in this study are in agreement with those of other investigators who used a wider range of doses and a 10–12 day survival time [27]. Less damage to DA neurons was observed with the use of 5,7-DHT than with 5,6-DHT. Furthermore, infusion of 5,7-DHT in DMI pretreated animals caused less damage to NE neurons than a smaller dose and volume of 6-OHDA. Both 5,7-DHT and 5,6-DHT caused significant depletion of 5-HT at the doses used whether medial or lateral cannula placements were examined; however, somewhat less depletion of 5-HT (54% versus 68–78%) was obtained with 5,7-DHT.

Survival time did not appear to have any large effects on amine levels. 5-HT levels were slightly higher in 6-OHDA-treated rats given 20 nmol and allowed to survive 30

days rather than 8 days. No significant difference in 5-HT levels was observed in animals treated with 5,6-DHT and allowed to survive 60 rather than 8 days. DA levels did not show any recovery following 6-OHDA lesions, if one compares the animals that survived 8 days with those that survived 30. However, DA levels were higher in the 5,6-DHT group that survived 60 days than in the group sacrificed 8 days post-operatively.

The choice of toxin doses in the studies reported here was governed by considerations of behavioral effectiveness. The doses of 6-OHDA, 5,6-DHT and 5,7-DHT used were effective in producing large reductions in telencephalic amine levels. Doses in these ranges have previously been shown to be effective in producing behavioral change [7, 9, 10, 17, 20, 21]. We have argued elsewhere [24] that use of 6-OHDA does not guarantee lesion specificity. With intracerebral injections of 6-OHDA, doses over 16–20 nmol do not increase NE depletion but do increase the number of behavioral effects observed. Stahl and others [29] have presented data that suggest that non-specific damage which occurs with high doses of 6-OHDA results from the extracellular release of H_2O_2. At lower doses, however, 6-OHDA may act via a selective uptake into catecholamine neurons and an intracellular release of H_2O_2. The data presented here support this position.

The problems in data interpretation that occur with the use of large doses of neurotoxins have been well-documented by other investigators [6,25]. The results of the present study suggest that at lower doses, non-specific damage caused by general cytotoxic effects of 6-OHDA and 5,7-DHT is minimized sufficiently to allow useful data on the function of catecholamine and indoleamine systems to be obtained. The non-specific damage is not so great that adequate control procedures such as vehicle infusion or use of more than one cannula site or dose cannot overcome the disadvantages of intracerebral injection of diffusible substances. Improvements in the effective use of these neurotoxins can be expected as more detailed information becomes available about the neurochemical anatomy of the sites at which the toxins are injected.

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