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Adrenal Demedullation and Peripheral 6-OHDA Administration in the Rabbit: Effects on Body Weight, General Activity and Cardiovascular Responsivity

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JOSEPH, J., W. BOGGAN AND D. A. POWELL. *Adrenal demedullation and peripheral 6-OHDA administration in the rabbit: Effects on body weight, general activity and cardiovascular responsivity* PHARMAC. BIOCHEM BEHAV. 10(6): 875–881, 1979.—Two experiments were undertaken to determine the effects of systemic 6-hydroxydopamine (6-OHDA) administration and adrenal enucleation on body weight, brain and heart norepinephrine, general activity, and cardiovascular responsivity in the rabbit. 6-Hydroxydopamine produced long lasting, dose related decreases in baseline blood pressure (BP) and in pressor responses following electric shock. Partial reversal of the BP changes was observed in nonenucleated animals tested 20 days after 6-OHDA administration but not in enucleated animals tested 5, 10 or 20 days post-injection or in control animals tested 5, 10 or 15 days after vehicle injection. Although 6-OHDA administration produced transient weight loss and activity decrements, no long term weight or activity changes were observed.

6-OHDA Blood pressure Heart rate Activity Rabbits

THE EFFECTS of peripheral catecholamine (CA) depletion have been studied in several different behavioral paradigms [2, 3, 4, 5, 14, 17, 18, 27, 28]. However, most of these experiments have focused upon operant behaviors in rodents, even though a major hypothesis regarding the action of the peripheral CAs on behavior is related to possible Pavlovian conditioning of situational cues in operant situations [4]. A Pavlovian conditioning preparation which has been useful in studying basic learning processes is the nictitating membrane or eyeblink response of the restrained rabbit [9, 19, 23]. Cardiovascular conditioning has also been studied in this preparation, and it has lent itself well to a variety of physiological manipulations (e.g. [13, 20, 21]). However, there is little information regarding peripheral CA depletion or its effects on behavior in the rabbit [8,16].

Prior to studying associative processes as a function of catecholamine depletion in the rabbit, however, it would seem imperative to assess the systemic and unconditioned effects of this manipulation. Thus, in the present experiments the relatively specific neurotoxic agent 6-hydroxydopamine (6-OHDA) [12] was utilized to deplete peripheral CAs and several behavioral and physiological

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measures were assessed. However, 6-OHDA does not penetrate adrenal tissues well [25,26]: in order to prevent the action of adrenal CAs on behavior, the adrenal medulla must be removed. Thus, a procedure for adrenal enucleation in the rabbit is also described. Body weight, heart and brain norepinephrine concentration, open field activity and the response of the cardiovascular system to these manipulations were assessed. Experiment 1 studied dose-response relationships and Experiment 2 studied time-response relationships.

METHOD

Animals

Seventy-two, 40-day old New Zealand rabbits (males and females) obtained locally were used. Thirty-two rabbits were employed in Experiment 1 and 40 in Experiment 2. The animals were maintained on ad lib food and water in an animal ward with a 7 a.m.–7 p.m. light/dark cycle. All experiments were conducted during the daylight portion of this cycle.

Apparatus

During cardiovascular testing (described below) all animals were held in a Plexiglas restrainer with an adjustable head stock. Measures of heart rate (HR) and blood pressure (BP) were recorded on a four channel Model-5 Grass polygraph. During the experimental session, the animals were tested in a deactivated, sound attenuated refrigerator shell through which 75 db white noise was constantly presented The control and recording apparatus was located in an adjacent room. The experiments were controlled by *BRS Digibit* solid-state logic devices programmable via an Allied Systems patchboard receiver. Blood pressure catheters were prepared from 30 ga, Teflon tubing with a 26 ga needle inserted into one end and capped with the plunger and top of a 1 ml disposable syringe. Statham pressure transducers (P23-DC) and a mercury manometer coupled to a Grass DC preamp (SP-1) were used to record blood pressure. The pre-amplifier was calibrated with a mercury manometer so that a 1 mm pen deflection corresponded to a 1 mm change in blood pressure. Heart rate was recorded using Grass 5P6 EKG pre-amplifiers. Activity was monitored in a 90×90 cm open field painted flat black. Archer photobeams mounted in the walls and a Digital counter were used to record the behavior. The photobeams were placed so that four 45×45 cm squares were formed in the field.

Surgical Procedures

Two surgical procedures were employed. The first involved either sham or adrenal demedullation and the second involved cannulation of the medial ear artery in order to measure blood pressure [19]. Approximately 5 days after delivery the rabbits were anesthetized with 60 mg/kg Ketamine Hydrochloride (Vetolar) and 2.5 mg/kg chlorpormazine. A three-inch incision was made diagonally 0.5 in. below the rib cage and the adrenals exposed. After dissecting away the adrenal membranes the adrenal cortex was punctured and the medulla was aspirated through the opening. Sham demedullations were carried out in the same manner except that the adrenal glands remained intact and the medullae were not withdrawn. Following surgery, the animals were given 100,000 units of procaine penicillin as a prophylactic measure. A 30–40 day post-operative recovery period was allowed for the adrenal cortices to heal. During this period, the animals were weighed every other day and any animals which showed continued weight loss were given intraperitoneal injections of 50% Dextrose plus electrolyte solution (Myzon Laboratories, Inc., Kansas City) until weight gain was observed.

Following the recovery period and immediately prior to testing, the restrained animals were cannulated under local anesthesia (Xylocaine) as follows. The right medial ear artery was exposed, and a Teflon cannula, prefilled with sodium heparin in saline (10 mg/ml), was inserted into the artery and sutured to the surrounding tissue. This technique is simple and nontraumatizing to the animal [19]. All animals were tested immediately after cannulation to avoid the loss of experimental animals due to arterial clotting around the cannula.

Drug Injections

Thirty-two rabbits participated in Experiment 1 in which dose-response relationships were examined. Of the 32 rabbits, half (N=16) were sham demedullated and half demedullated. Each of these groups was further divided into subgroups of 4 and received intramuscularly either 0, 5, 30 or 75 mg/kg of 6-OHDA hydrobromide as a free base in ascorbic acid (1 mg/ml) and saline. All doses were based on the salt. The 5 mg/kg injection was given in a single dose: the 30 mg/kg and 75 mg/kg injections were given in two and three multiple daily doses of 15 mg/kg and 25 mg/kg, respectively, since preliminary data indicated that 6-OHDA is highly toxic when given in single doses above 30 mg/kg [10]. Animals in the 0 mg/kg group received only a single vehicle injection. Forty additional animals participated in a second experiment in which the temporal effects of 6-OHDA were examined. Each of these animals was administered either 75 mg/kg 6-OHDA intramuscularly in three multiple daily doses of 25 mg/kg or vehicle, etc., and the period between the last injection and behavioral measurement varied.

Cardiovascular Testing

In Experiment 1 all animals were tested 48 hours after the administration of the vehicle or last 6-OHDA injection. This time period was chosen (a) to allow for maximum destruction of adrenergic terminals, (b) to prevent possible interference with physiological measurements from short-term sympathomimetic effects of 6-OHDA, (c) to allow sufficient time for maximum peripheral catecholamine depletion to occur, and (d) to allow recovery from any possible anorexic or adipsic effects of the injection. During each testing session EKG electrodes (stainless steel safety pins) were implanted in the skin above the right shoulder and left haunch. A series of 16 presentations of electric shock was administered through shock electrodes inserted above and below the orbit of the eye. Four different shock intensities (0, 2, 5 and 10 mA) were administered in random order at intertrial intervals of approximately 3 minutes.

Heart rate and blood pressure were recorded on each trial and consisted of a baseline response measure and postshock measures, as follows. The baseline HR measure consisted of the mean duration of the two blocks of 5 heart beats which occurred immediately prior to shock onset. The postshock measure was based on 12 successive blocks of 5-beat intervals occurring immediately after shock offset. These 5-beat durations were converted to beats per minute in all in-

stances. Blood pressure was measured in mm Hg during each of the blocks corresponding to pre- and post-shock intervals. The BP response consisted of the largest BP change occurring during a particular 5-beat interval [19]. The small lumen and distensibility of the BP cannula precluded measurements of the systolic and diastolic components of the BP wave. Thus, BP changes recorded were an average of these two components: however, it should be noted that this average does not correspond to "mean blood pressure" as it is typically defined.

As noted above, a second experiment examined the temporal effects of 6-OHDA injection on cardiovascular responsivity. In this experiment four additional groups of 8 animals each were tested at either 5, 10, 15 or 20 days following the last 6-OHDA injection. Four animals in each group received adrenal demedullations and four received sham operations. The shock intensities and their order of presentation together with the subsequent cardiovascular measurements were carried out as in Experiment 1.

Activity Measurements

In order to determine any general debilitating effects that might result from 6-OHDA administration, daily activity measurements were taken during both experiments. During Experiment 1 five daily activity measurements were made beginning on the day of cardiovascular testing. During Experiment 2 the activity sessions began 5 days prior to 6-OHDA injection and continued up to and including each animal's designated day of cardiovascular testing. All the animals that received cardiovascular testing in Experiment 2 received 75 mg/kg 6-OHDA. Thus, in this experiment an additional control group (4 sham and 4 demedullated) received 3 daily vehicle injections and was tested on open field activity. These control animals were injected on Days 6, 7 and 8 of the experiment corresponding to the 6-OHDA injections of the experimental group.

Weight Measurements

During Experiment 1 each animal was weighed daily for 5 days prior to the drug injection, during the injection period, and for 5 days after the last injection. During Experiment 2 weight measurements were again taken 5 days prior to and during the drug injection period and throughout the waiting period following the injections until cardiovascular testing took place.

Histology and Biochemical Assay

In order to determine the efficacy of the demedullation procedure and 6-OHDA injections, at the end of behavioral testing the animals were sacrificed by decapitation in a cold room and the hearts, brains and adrenal glands removed. The adrenal glands were placed in 10% buffered Formalin, coded, blocked in paraffin, and sectioned at 5 microns. They were stained with hematoxylin, counterstained in eosin and microscopically examined by an independent investigator for the presence or absence of medullary cells. The hearts and brains were quick frozen on dry ice following removal and analyzed for NE content according to established fluormetric procedures [15].

RESULTS

Histology Results

Examination of the adrenals revealed that medullary enucleation was virtually complete in the demedullated animals, with the medullary layer and a small portion of the *Zona Reticularis* completely absent in all demedullated animals: all other layers (e.g., *Zona Compacta*) were intact, indicating that a regeneration of adrenal cortical tissue, but not medullary tissue, had taken place during the 30-day recovery period.

Tissue Norepinephrine Determination

The biochemical analysis revealed that heart NE was substantially depleted in the 6-OHDA injected animals as compared to the vehicle controls. The data from Experiment 1 are shown in Fig. 1. A one-way analysis of variance showed the differences associated with 6-OHDA dosage to be significant ($F(3,24)=387.18$, $p<0.001$). Duncan post-tests showed (a) that the vehicle group differed significantly from all other groups ($p<0.01$) and (b) that the 5 mg/kg group differed significantly from the 30 mg/kg and 75 mg/kg groups ($p<0.05$). There was, however, no difference between the 30 and 75 mg/kg groups ($p>0.05$). Brain NE levels were unchanged across the four drug conditions, ranging from 1.3 ± 0.04 μg/g in the 30 mg/kg group to 1.7 ± 0.1 μg/g in the 0 mg/kg group.

FIG. 1. Norepinephrine (NE) levels in whole brain and heart tissue of rabbits 48 hr after the administration of graded doses of 6-OHDA.

Assays of the brain and heart tissue of the animals which participated in Experiment 2 revealed that 6-OHDA produced an average cardiac NE depletion of 75% in all animals with no significant depletion produced in brain tissue. An analysis of variance of these data showed that there were no differences between sham and demedullated animals ($F(1,24)=0.08$, $p>0.77$), nor was any differential recovery seen over the course of the experiment (days: $F(3,24)=0.76$, $p>0.05$: days×sham-demedullate: $F(3,24)=0.60$, $p>0.50$). Brain NE levels were essentially the same for all groups of 6-OHDA injected animals regardless of surgery ($F(1,24)=0.87$, $p>0.05$) or time since drug injection ($F(3,24)=0.87$, $p>0.05$).

TABLE 1

MEAN WHOLE BODY WEIGHTS (± 1 SEM) IN KG OF RABBITS IN EXPERIMENT 2 INJECTED WITH 6-HYDROXYDOPAMINE OR VEHICLE FOR (a) 5 DAYS PRIOR TO DRUG INJECTION, (b) DURING THE THREE-DAY INJECTION REGIMEN AND (c–f) FOR FOUR 5-DAY BLOCKS FOLLOWING DRUG INJECTION

	(a) 5 days prior to injection	(b) 3 days during injection	(c) days 1–5 post injection	(d) days 6–10 post injection	(e) days 11–15 post injection	(f) days 16–20 post injection
Vehicle	2.6 ± 0.2	2.7 ± 0.2	2.8 ± 0.3	2.8 ± 0.3	2.9 ± 0.3	2.9 ± 0.3
6-OHDA	2.8 ± 0.3	2.2 ± 0.4	2.3 ± 0.3	2.7 ± 0.5	2.8 ± 0.3	2.8 ± 0.4

Body Weight

During Experiment 1 animals in the 30 and 75 mg/kg groups showed a transient decrease in body weight during the injection regimen, compared to the 5 mg/kg and vehicle groups. A repeated measures analysis of variance of these data using as variables (a) days (repeated, 13 levels), (b) demedullation conditions (2 levels), and (c) 6-OHDA dosage (4 levels) revealed only a significant drug×day interaction ($F(36,288)=4.15$, $p<0.001$). During Experiment 2 all 6-OHDA injected animals revealed a transient weight loss during the drug injection period that endured for 5 to 10 days after the last drug injection. The vehicle injected animals, on the other hand, revealed a steady increase in body weight over the 28-day testing period. An analysis of variance comparing the latter vehicle injected group to the 6-OHDA injected group, which was tested 20 days post-injection, revealed a significant days effect ($F(27,324)=14.61$, $p<0.001$) and a significant days×drug interaction ($F(27,324)=2.79$, $p<0.01$). However, the demedullation effects were not significant. These data, pooled for the demedullation conditions, are shown in Table 1. This table shows the mean body weights of the 6-OHDA and vehicle injected groups which were tested 20 days post-injection, (a) during the 5 days prior to drug injection, (b) during the injections, and (c) for four 5-day blocks after drug injection. This table shows that during drug injection and for the 5-day period following drug injection there were substantial differences between the two groups (during: $t(14)=3.61$, $p<0.01$: 5 days post: $t(14)=2.48$, $p<0.05$). However, no other comparisons were significant. Thus, these differences had essentially disappeared by 10 to 15 days post-injection.

Activity

During Experiment 1, free field activity was unaffected by adrenal demedullation ($F(1,24)<1$, $p>0.10$) or 6-OHDA administration ($F(3,24)<1$, $p>0.10$). There was a general decline in activity over sessions in all groups during Experiment 2. These data are shown in Fig. 2 for the 75 mg/kg 6-OHDA group and for the vehicle control group tested before, during and after injection. The data are pooled for the demedullation conditions, which were not significantly different. The 6-OHDA injected animals were less active than the vehicle animals during the three days on which the drug was administered. However, these differences had essentially disappeared by the second post-injection day. Although an overall analysis of variance revealed no differences between the two groups, separate analysis of (a) pre-injection, (b) during injection and (c) post-injection scores showed that the activity of the 6-OHDA animals during the injection period was significantly lower ($F(1,12)=29.8$, $p<0.003$). However, between-group differences were not significant either prior to this time ($F(1,12)=0.90$, $p>0.6$) or during post-injection sessions ($F(1,12)=0.97$, $p>0.7$).

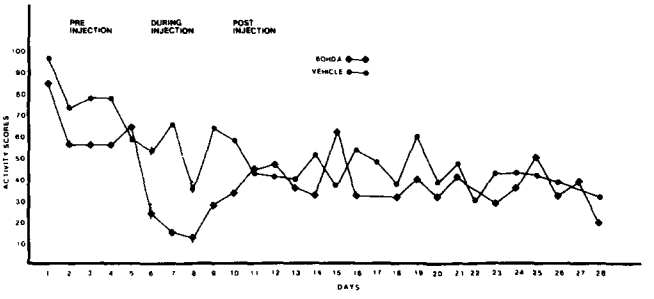

FIG 2. Open field activity scores of rabbits injected with 75 mg/kg 6-hydroxydopamine or vehicle Data are presented for 5 days prior to injection, 3 days during injection and 20 days following injection

Heart Rate and Blood Pressure Changes

During Experiment 1, adrenal demedullation had no effect on either BP or HR measures. However, baseline BP levels decreased as a function of 6-OHDA dosage: while all doses lowered resting BP levels, the higher doses (viz., 30 mg/kg and 75 mg/kg) produced the greatest decreases (see Fig. 3). Analysis of variance showed these differences to be significant ($F(3,24)=8.51$, $p<0.0007$). Duncan post-tests showed that the 30 mg/kg and 75 mg/kg groups differed from the vehicle ($p<0.01$) and 5 mg/kg groups ($p<0.05$).

Figure 4 shows mean changes in blood pressure (top panel) and mean heart rate (bottom panel) in response to the 10 mA shock for each of the four groups of animals in Experiment 1. These data are pooled for the adrenal demedullation conditions since these differences were not significant. BP decreased over shock trials in all animals administered 6-OHDA while the vehicle group showed BP increases. A repeated measures analysis of variance showed that these BP changes were significant ($F(11,204)=11.3$, $p<0.001$) and that the response of the 6-OHDA animals differed significantly from their vehicle controls ($F(33,264)=4.3$, $p<0.001$). Moreover, Duncan post-tests showed that the group which received 5 mg/kg showed less overall change than those animals that had received higher drug doses ($p<0.05$): however, the 5 mg/kg group differed from controls ($p<0.05$).

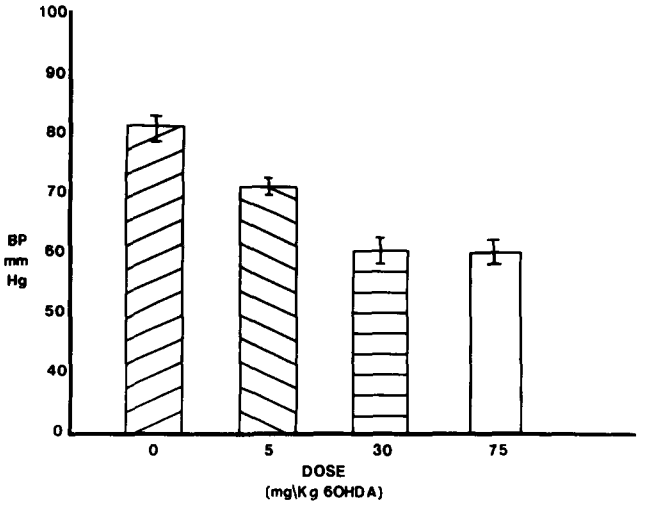

FIG. 3. Mean baseline blood pressure (± 1 SEM) of four groups of rabbits administered different doses of 6-OHDA as shown.

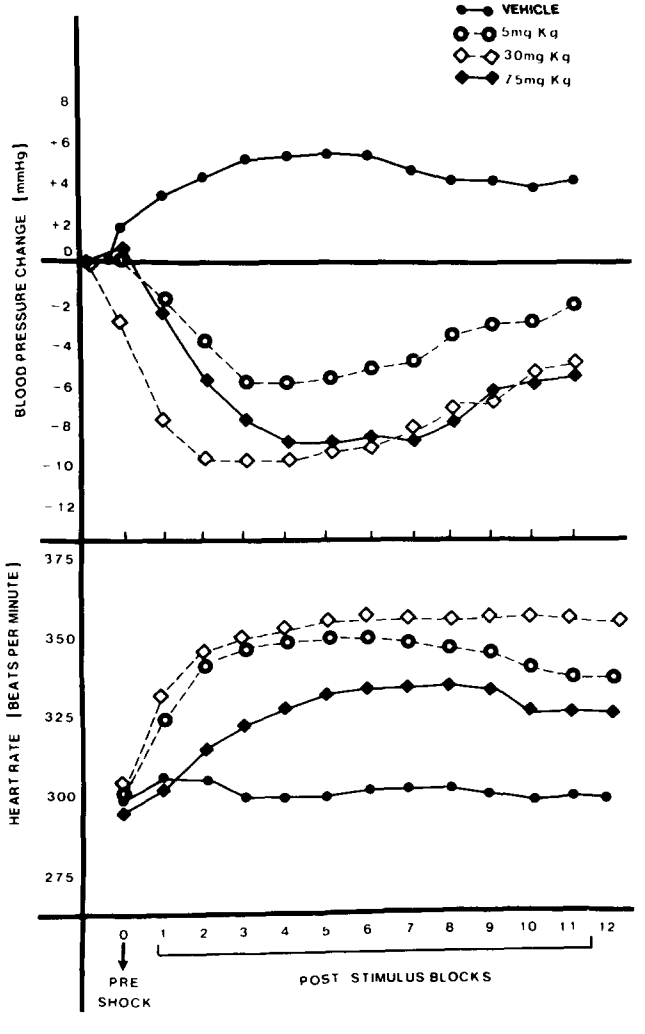

FIG. 4. Mean post-stimulus blood pressure changes and heart rate in response to para-orbital electric shock of rabbits given graded doses of 6-OHDA.

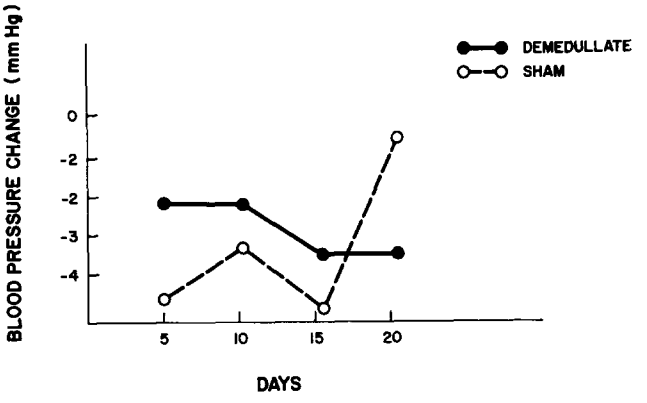

FIG. 5 Mean post-stimulus BP changes in response to paraorbital electric shock in sham and adrenal demedullated groups of rabbits at 5, 10, 15 or 20 days following the last 6-OHDA injection.

Heart rate is shown in the bottom of Fig. 4. An analysis of baseline HR measures (see pre-shock in Fig. 4) showed that the 6-OHDA injected animals did not differ significantly from the vehicle controls ($F(3,24)=2.14$, $p>0.1$). However, as might be expected, changes indicative of cardiac activity compensatory to BP responses were observed when shock was administered ($F(11,204)=3.27$, $p<0.002$). Moreover, 6-OHDA injected animals showed greater heart rate increases over blocks ($F(33,264)=4.79$, $p<0.0001$) than the vehicle-injected animals. Post-tests revealed that the vehicle group showed significantly less tachycardia following shock presentation that any of the drug groups ($p<0.05$).

In Experiment 2 no differences were again obtained in either baseline BP or HR as a result of adrenal demedullation (HR, $F(1,24)=0.088$, $p>0.76$: BP, $F(1,24)=1$, $p>0.98$), nor were significant baseline differences associated with time after 6-OHDA injection (HR, $F(3,24)=0.73$, $p>0.54$. BP, $F(3,24)=1.4$, $p>0.35$). However, baseline BP of all groups of animals was significantly lower than that of the vehicle animals of Experiment 1 as assessed by post hoc t-tests (smallest, $t(14)=2.61$, $p<0.05$). The BP and HR topographies (viz., pattern of change in response to shock) of the rabbits in the second experiment were essentially the same as was found in the animals of Experiment 1 injected with 6-OHDA (see Fig. 4). However, Fig. 5 shows that the BP response was affected by both day of testing ($F(3,24)=3.57$: $p<0.03$) and adrenal demedullation. This figure depicts the mean BP response averaged over shock intensity and the 12 post stimulus blocks of 5-heart beats for the sham and demedullate animals separately. It can also be observed in this figure that the sham 20-day group showed virtually no BP change in response to shock, resulting in a significant sham-demedullate×day interaction ($F(3,24)=5.24$, $p<0.01$). Thus, there appeared to be some recovery of the BP pressor response, but it was confined primarily to the sham operated animals.

DISCUSSION

The results of the present two experiments may be summarized as follows. (a) The histological analysis showed that the demedullation procedure was successful in eliminating adrenal medullary cells. (b) The peripheral specificity of 6-OHDA was confirmed in the rabbit, as it has been in other

species [12]. Brain NE levels remained similar to those of vehicle controls, whereas cardiac NE levels were depleted to 25% of those of controls: interestingly the rabbit seems to be especially sensitive to this compound, since no regular dose dependent changes in norepinephrine were observed with the doses utilized. As little as 5 mg/kg of 6-OHDA produced NE depletion and there was no difference between 30 and 75 mg/kg. (c) 6-Hydroxydopamine did not have a differential effect on cardiovascular responsivity in sham-operated and demedullate animals. (d) The major action of 6-OHDA appeared to be on BP baseline and the topography of the BP response. Heart rate changes appeared to be homeostatic adjustments to these effects. (e) Neither 6-OHDA administration nor adrenal demedullation produced long-term differences in weight or activity compared to controls. (f) Some recovery of cardiovascular function occurred 3 to 4 weeks after injection of 6-OHDA, especially in non-demedullated animals, but cardiac NE levels remained depleted at this time.

The latter finding is especially interesting. This difference was probably due to recovery of function in sympathetic vascular beds. It has been shown in rats, for example, that by 7 days after 6-OHDA injection some regrowth of the adrenergic nerves innervating the mesenteric arteries can be observed [6]. Moreover, it has been found that no prolonged reduction of tyrosine hydroxylase is produced in blood vessels following treatment with 68 mg/kg 6-OHDA IV [1]. It is unlikely, however, that this recovery was due solely to rapid sympathetic vascular reinnervation, since the resumption of sympathetic tone in this case was shown only by the sham group This finding implicates adrenal medullary catecholamines, possibly acting on supersensitive postsynaptic receptor sites, in such recovery.

Chronotropic homeostatic adjustments in HR took place as BP decreased in both experiments, but 6-OHDA effects on HR were not as consistent as its effects on BP. For example, although dose-related decreases in the BP pressor response occurred, this finding was not obtained for the HR response. It was also found that the mean HR response of the vehicle animals was considerably smaller than that of the 6-OHDA injected animals. However, the means shown in Fig. 4 are not indicative of the changes shown by individual animals, since some vehicle-injected animals (N=3) showed decelerative HR responses, although most (N=5) showed accelerative changes. For this reason, the HR means reflect smaller accelerations following shock in the vehicle group. While the variables which determine these differences in HR directionality remain obscure, similar results have been obtained in other experiments [11,24]. More importantly 6-OHDA tends to cancel out these differences and converts, as it were, potential HR "decreasers" to "increasers." These changes are probably the result of a reduction in peripheral vascular tone produced by chemical sympathectomy. Interestingly, decreasing cardiac NE by 6-OHDA did not block the HR response, suggesting that sympathetic innervation of the heart acts mainly to induce positive or negative changes in contractile force [22] allowing other mechanisms, such as vagal excitation or inhibition, to induce chronotropic changes [7].

The results of the activity and body weight analyses indicate that peripheral 6-OHDA injection produced a short-term drop in activity and body weight during the period of drug administration, but they quickly returned to normal. These findings support the results of earlier studies, which have shown temporary decrements in activity as a function of peripheral 6-OHDA administration [18]. These data and those concerning weight changes suggest that 6-OHDA produces no long-term general debilitation. It therefore seems to be a useful tool for investigating the possible contribution of the peripheral sympathetic nervous system to the acquisition of classically conditioned behaviors in rabbits. Moreover, since the sham-operated animals showed some recovery of function over time, the contribution of the adrenal medulla to this conditioning process might also be assessed.

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