Br. J. Pharmac. (1970), **40**, 630–638.

Central effects of 6-hydroxydopamine on the body temperature of the rat

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Summary

1. Rats which had been pretreated with intraventricular injections of 6-hydroxydopamine (6-OHDA) to cause a selective depletion of brain noradrenaline (NA) to 20·7% of control brain NA and brain dopamine (DA) to 34·6% of control brain DA retained an unimpaired ability to regulate their body temperatures on exposure to heat or cold. This is discussed in relation to the possible role of brain NA in the central control of body temperature.

2. Intraventricular injections of 6-OHDA in normal rats at room temperature caused an acute, dose dependent hypothermia of up to 4·5° C which lasted for 4–5 hours. Depletion of brain NA and DA by prior treatment with 6-OHDA markedly reduced the hypothermic response to a subsequent dose of 6-OHDA. Selective depletion of brain NA without affecting brain DA did not reduce the response to 6-OHDA. The acute hypothermic response to 6-OHDA, may therefore, be related to a release of DA in the brain.

Introduction

Injection of 6-hydroxydopamine (6-OHDA) into brain tissue or into the cerebrospinal fluid in rat brain results in a long-lasting depletion of brain noradrenaline (NA) and dopamine (DA) (Ungerstedt, 1968; Bloom, Algeri, Groppetti, Revuelta & Costa, 1969; Uretsky & Iversen, 1969, 1970; Bartholini, Richards & Pletscher, 1970). It has been proposed that these changes may be due to a selective destruction of catecholamine-containing neurones in the brain. Such an action would be consistent with the observed destruction of adrenergic nerve endings in the peripheral sympathetic nervous system following systemic administration of 6-OHDA (Thoenen & Tranzer, 1968). The intraventricular doses of 6-OHDA required to deplete brain NA and DA have no significant effect on the concentrations of 5-hydroxytryptamine or γ-aminobutyric acid in the brain (Uretsky & Iversen, 1970), nor are the doses sufficiently large to have any direct effect on the peripheral sympathetic nervous system (Thoenen & Tranzer, 1968).

In view of the accumulated evidence suggesting a role for NA in the central control of body temperature in the rat (Costa & Neff, 1966; Gordon, Spector, Sjoerdsma & Udenfriend, 1966; Corrodi, Fuxe & Hökfelt, 1967; Feldberg & Lotti, 1967; Duce, Crabai, Vargiu, Piras, Adamo & Gessa, 1968; Myers & Yaksh, 1968; Reid, Volicer, Smookler, Beaven & Brodie, 1968; Simmonds, 1969), it was of interest to see how pretreatment with 6-OHDA by the intraventricular route affected the

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ability of the rat to thermoregulate. During the course of these experiments, an acute effect of 6-OHDA on the body temperature of the rat was observed and this, also, was investigated.

Methods

Female Wistar rats (180–220 g) were kept in the laboratory at an environmental temperature of 22–25° C for at least 18 h before the start of an experiment.

Injection of 6-OHDA. 6-OHDA was dissolved in a vehicle of artificial cerebrospinal fluid containing ascorbic acid (1 mg/ml) and adjusted to pH 5·0. In most experiments, 6-OHDA was injected by the intraventricular route with light ether anaesthesia as described by Uretsky & Iversen (1970). In one experiment, however, an injection guide was mounted on each of four rats' skulls under ether anaesthesia to facilitate intraventricular injection without anaesthetic. The guide was constructed from the boss of a syringe needle and this was mounted vertically over the hole drilled in the skull for injection. The guide was fixed by means of dental cement to three screws tapped into the skull. The dura was punctured while the rats were still anaesthetized and the guide was then plugged with plastic foam. Two days later, 6-OHDA was injected without anaesthesia by removing the plug and inserting a cannula which penetrated to the lateral ventricle.

In an experiment in which 6-OHDA was administered systemically, the compound was injected into a tail vein and was washed in with 1·0 ml 0·9% NaCl solution.

Measurement of rectal temperature. Rats were maintained in individual cages throughout each experiment from at least 60 min before the start of temperature measurements. For each measurement the rat was removed from its cage and held in the hand while a Grant Thermistor probe was inserted 5–6 cm into the rectum. The total time for which the animal was removed from its cage was usually between 20 and 30 seconds.

In the experiments in which the effects of 6-OHDA pretreatment on the temperature responses of rats to heat and cold were investigated, two intraventricular injections of 250 μg 6-OHDA or vehicle were administered 2 days apart. Twelve–17 days later, groups of rats treated with 6-OHDA and control groups were each divided into two halves. One half was exposed to heat (32° C) on one day and then to cold (9° C) 3–5 days later, while for the other half the sequence was reversed. The results from both halves were pooled.

To investigate the acute effects of intraventricularly injected 6-OHDA on rectal temperature, rats were exposed to a room temperature of 22–25° C throughout. Experiments were performed on control rats, rats pretreated with two intraventricular doses of 250 μg 6-OHDA 5 and 3 days earlier, rats pretreated with three intraventricular doses of 25 μg 6-OHDA 11, 9 and 7 days earlier and rats pretreated with protriptyline hydrochloride 15 mg/kg i.p. 120 min before injection of 6-OHDA. As a control, the temperature responses of rats to intravenous injections of 250 μg 6-OHDA were also followed in one experiment.

The temperatures recorded in each rat were expressed as changes from the temperature recorded in the same rat immediately before the start of the experiment.

Assay of NA and DA. At the end of some experiments, the rats were killed and their brains removed and assayed for NA and DA as described previously (Uretsky & Iversen, 1970).

Statistical analysis. The significance of the differences between means was determined by Student's *t*-test. A value of $P<0.05$ was taken to be significant.

Results

Effect of 6-OHDA pretreatment on temperature responses to heat and cold

The changes in rectal temperature of rats at various times after commencement of exposure to heat (32° C) or cold (9° C) are shown in Fig. 1. The rats were maintained at room temperature before the start of the experiment. On exposure to heat, the rectal temperatures of both groups of rats rose significantly during the first 30 min and were maintained at these elevated values without further change for at least 210 minutes. The rise in rectal temperature of 6-OHDA pretreated rats was significantly less than that of control rats when the values at 30, 60 and 240 min were pooled and compared. On exposure to cold, however, the rectal temperatures of 6-OHDA pretreated rats did not differ significantly from those of control rats.

The concentrations of NA and DA in the brains of the 6-OHDA pretreated rats are each expressed as a percentage of the corresponding value in control rats in

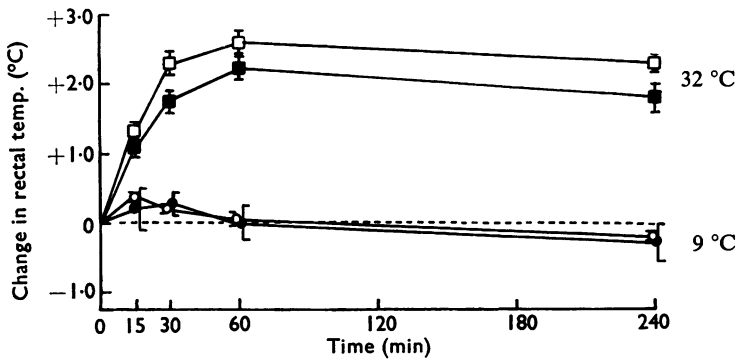

FIG. 1. Changes in the rectal temperatures of rats exposed to 9° C (●, ○) or 32° C (■, □) after maintenance at room temperature (22–25° C). Solid symbols indicate rats pretreated with two intraventricular doses of 250 μg 6-OHDA; open symbols indicate rats pretreated with vehicle. Each point is the mean ± S.E. of values from six rats. The rectal temperature of 6-OHDA pretreated rats at room temperature was 38·4 ± 0·17° C and of control rats at room temperature was 38·5 ± 0·10° C (mean ± S.E. of twelve readings in each case).

TABLE 1. *Effects of various doses of 6-OHDA on the concentrations of NA and DA in rat brain*

	Dose of protriptyline (mg/kg i.p.)	Dose of 6-OHDA (μg i.ventric.)	Brain amine concentrations (% control ± S.E.) NA	DA
(1)		250+250	20·7 ± 6·2 (4)	34·6 ± 6·4 (4)
(2)		25+25+25	25·0 ± 2·9 (4)	89·5 ± 9·1 (4)
(3)	0	150	26·9 ± 2·6 (4)	
	15	150	51·5 ± 3·5 (4)	

The concentrations of NA and DA in control animals were NA 0·35 ± 0·03 μg/g tissue (eight) and DA 0·54 ± 0·07 μg/g tissue (eight). Numbers of animals in parentheses. (1) Doses injected 2 days apart; rats killed 17 days after first injection. (2) Doses injected at intervals of 2 days; rats killed 11 days after first injection. (3) Protriptyline injected 120 min before 6-OHDA; rats killed 240 min after injection of 6-OHDA.

Table 1. Following 6-OHDA pretreatment, both amines showed significant falls which were similar to previously published figures (Uretsky & Iversen, 1970).

Acute effects of 6-OHDA on rectal temperature

Intraventricular 6-OHDA with ether anaesthesia. During the course of pretreating rats with intraventricular doses of 6-OHDA, the animals' rectal temperatures were depressed for several hours after the initial injection. This was further investigated by measuring the rectal temperatures of untreated rats before and after various doses of 6-OHDA. The responses to three different doses and vehicle are shown in Fig. 2. There was a small fall in rectal temperature due to the injection procedure, which included ether anaesthesia, but the temperature returned to normal

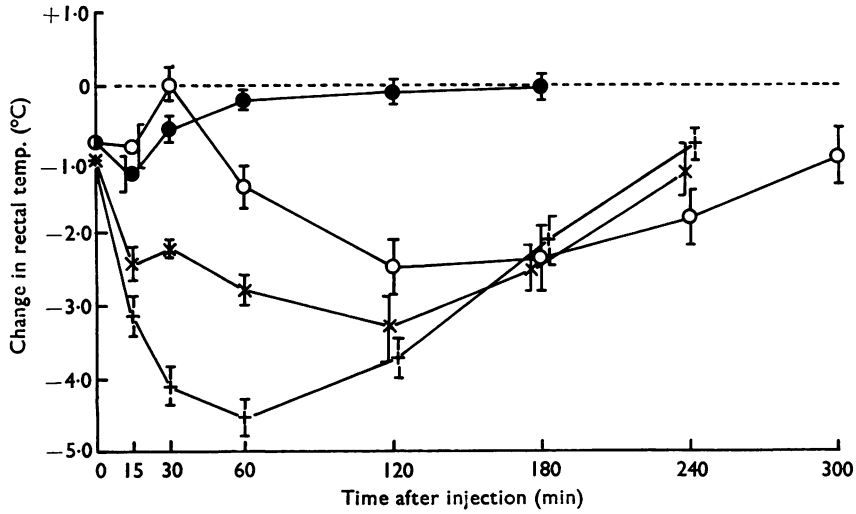

FIG. 2. Changes in the rectal temperatures of rats following intraventricular injection of various doses of 6-OHDA under ether anaesthesia at room temperature. (—●—), Vehicle; (—○—), 12·5 μg 6-OHDA; (—×—), 50 μg 6-OHDA; (—+—), 250 μg 6-OHDA. Each point is the mean ±S.E. of values from five to eight rats.

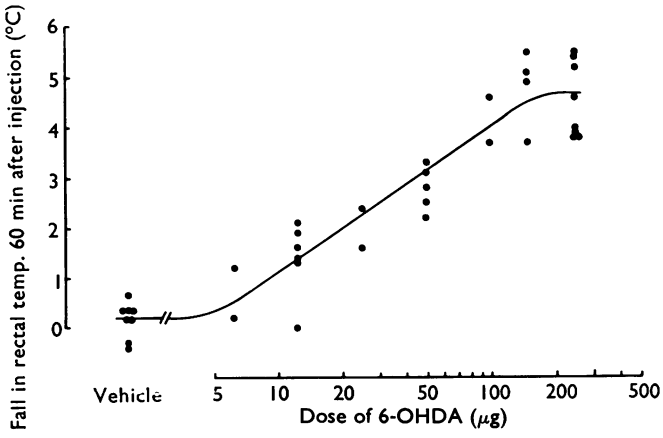

FIG. 3. Relationship between log dose of 6-OHDA and the fall in rectal temperature of rats 60 min after intraventricular injection of 6-OHDA under ether anaesthesia at room temperature. Each point is the result obtained from one rat. The line was drawn through the points by eye.

within 60 minutes. 6-OHDA, however, caused a much more prolonged fall in temperature. With increasing doses of 6-OHDA, the minima in the temperature curves became progressively lower and were reached at progressively earlier times. The falls in temperature 60 min after injection are plotted against log dose of 6-OHDA in Fig. 3.

Intraventricular 6-OHDA without anaesthesia. To confirm that the hypothermic effects of 6-OHDA were independent of the small fall in temperature caused by ether, 250 μg 6-OHDA was injected intraventricularly into two rats without anaesthesia. Two other rats similarly received vehicle. Apart from the absence of the initial ether effect, the responses to 6-OHDA were similar to those shown in Fig. 2 for rats which were injected with 6-OHDA under ether anaesthesia.

Intravenous 6-OHDA with ether anaesthesia. As a further control to demonstrate that 6-OHDA was acting in the C.N.S. and not in the periphery, 250 μg 6-OHDA was injected intravenously into four rats anaesthetized with ether. Four more rats received vehicle. There were no significant differences between the changes in rectal temperature following 6-OHDA and those following vehicle. In both cases, the course of the temperature response was similar to that following intraventricular injection of vehicle under ether anaesthesia (Fig. 2).

Effect of 6-OHDA pretreatment on the temperature response to a subsequent dose of 6-OHDA

To determine whether the hypothermic effects of 6-OHDA were due to a direct action of the compound or to the release of catecholamine, rats were pretreated with two intraventricular doses of 250 μg 6-OHDA. This procedure depleted the concentrations of NA and DA in brain to 20·7% and 34·6% of control, respectively, (Table 1). If 6-OHDA was causing hypothermia by the release of catecholamines, a third dose of 250 μg 6-OHDA would be expected to have less effect than the first dose. That this was the case is shown in Fig. 4. In the rats pretreated with 6-OHDA, the fall in rectal temperature following the third dose was no larger than the fall recorded when vehicle was injected. Between 120 and 240 min after the third dose of 6-OHDA, however, rectal temperatures were significantly elevated above their pre-injection levels.

To distinguish further whether the hypothermic effects of 6-OHDA were due to the release of NA or DA, NA was selectively depleted with low doses of 6-OHDA (Uretsky & Iversen, 1970). Three intraventricular doses of 25 μg 6-OHDA caused a significant depletion of NA to 25·0% of control while the concentration of DA was not significantly reduced at 89·5% of control (Table 1). When rats pretreated in this way were challenged with a dose of 250 μg 6-OHDA, the hypothermic response was no smaller than the response to the same dose of 6-OHDA in control rats (Fig. 5).

Effect of protriptyline on the temperature response to 6-OHDA

Desipramine causes a selective impairment of the uptake of catecholamines into NA-containing neurones with much less effect on the uptake into DA-containing neurones (Haggendal & Hamberger, 1967; Ross & Renyi, 1967; Snyder, Green & Hendley, 1968). This difference was exploited by Evetts & Iversen (1970) who showed that a related compound, protriptyline, administered in a dose of 15 mg/kg

i.p. 120 min before an intraventricular injection of 150 μg 6-OHDA, substantially reduced the depletion of NA due to 6-OHDA without affecting the depletion of DA. Under similar conditions in the present experiments, it was found that protriptyline did not significantly reduce the hypothermic effect of 150 μg 6-OHDA (Fig. 6). At the same time, it was confirmed that the depletion of NA by 6-OHDA was substantially reduced in the presence of protriptyline (Table 1).

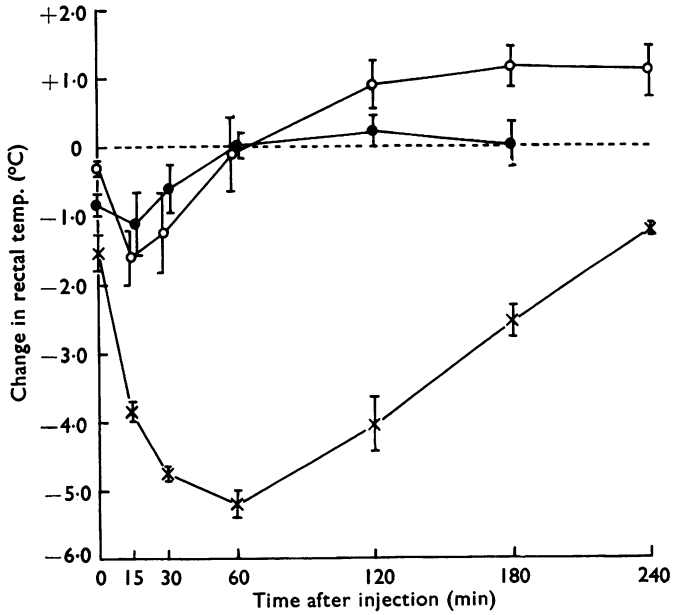

FIG. 4. Effect of pretreatment with large intraventricular doses of 6-OHDA on the hypothermic response of rats to a subsequent intraventricular injection of 6-OHDA under ether anaesthesia at room temperature. (●——●), Response to vehicle following pretreatment with vehicle; (×——×), response to 250 μg 6-OHDA following pretreatment with vehicle; (○——○), response to 250 μg 6-OHDA following pretreatment with two doses of 250 μg 6-OHDA. Each point is the mean ± S.E. of values from four rats.

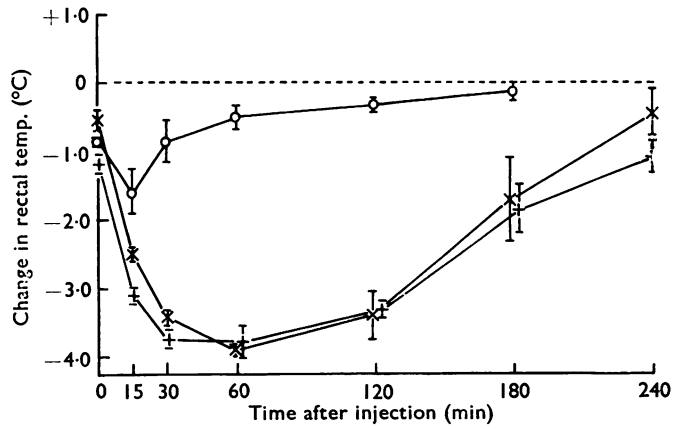

FIG. 5. Effect of pretreatment with small intraventricular doses of 6-OHDA on the hypothermic response of rats to a subsequent intraventricular injection of 6-OHDA under ether anaesthesia at room temperature. (×——×), Response to 250 μg 6-OHDA following pretreatment with vehicle; (+——+), response to 250 μg 6-OHDA following pretreatment with three doses of 25 μg 6-OHDA; (○——○), response to vehicle following pretreatment with three doses of 25 μg 6-OHDA. Each point is the mean ± S.E. of values from three or four rats.

Discussion

It is apparent from the results presented in Fig. 1 that pretreatment with 6-OHDA in no way impaired the ability of rats to regulate their body temperature on exposure to heat or cold. This ability was retained in spite of the loss of 79% of brain NA and 65% of brain DA. Indeed, 6-OHDA pretreated rats showed a significantly smaller hyperthermia than controls. This may be related to the reduced motor activity observed after large doses of 6-OHDA (Evetts, Uretsky, Iversen & Iversen, 1970) since, during heat exposure, normal rats show frequent periods of motor activity which contribute to the hyperthermia (Hainsworth, 1967).

The fact that an extensive loss of brain NA does not impair thermoregulation in the rat is not incompatible with the inhibitory role for NA in the central control of body temperature suggested by Bligh & Cottle (1969) for the sheep, goat and rabbit. Removal of the NA-mediated pathways from the model put forward by these authors would result only in a reduction in the precision of the regulatory mechanism, without interrupting the primary heat loss and heat production pathways. Alternatively, the possibility cannot be excluded that the NA-containing neurones which survived the degenerative effects of 6-OHDA were adequate to maintain the normal function of the central noradrenergic pathways involved in temperature regulation.

The acute hypothermia caused by intraventricular injections of 6-OHDA was very reproducible and showed a clear dose-response relationship. The falls in temperature were substantially larger than those reported to follow similar doses of other catecholamines (Feldberg & Lotti, 1967; Myers & Yaksh, 1968). Also, there was no evidence of a hyperthermic response to 6-OHDA as there is with NA. The

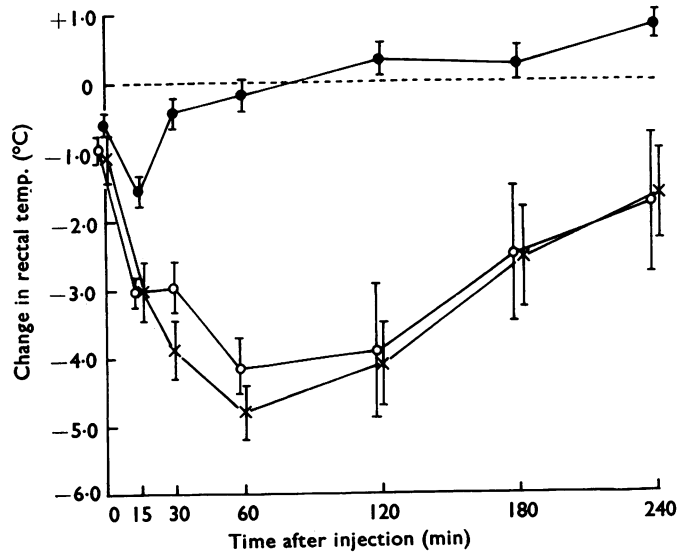

FIG. 6. Effect of protriptyline on the hypothermic response of rats to an intraventricular injection of 6-OHDA under ether anaesthesia at room temperature. Protriptyline was administered in a dose of 15 mg/kg i.p. 120 min before the injection of 6-OHDA. (●——●), Response to vehicle following injection of protriptyline; (○——○), response to 150 μg 6-OHDA following injection of protriptyline; (×——×), response to 150 μg 6-OHDA following injection of saline. Each point is the mean ± S.E. of values from four rats.

temperature responses to injected NA, however, are generally assumed to be due to a direct action of the amines on receptors, while the present experiments suggest that the hypothermic response to 6-OHDA was mediated by the release of endogenous catecholamines. If 6-OHDA had acted directly on catecholamine receptors, the loss of response to subsequent doses of 6-OHDA would be difficult to explain unless a loss of receptors is postulated. In the periphery, 6-OHDA does not cause a loss of catecholamine receptors but rather leads to an increased sensitivity of the receptors (Haeusler, Haefely & Thoenen, 1969). It is also unlikely that the response was due to the direct release of 5-hydroxytryptamine or γ-aminobutyric acid since neither of these substances is chronically depleted by 6-OHDA (Uretsky & Iversen, 1970) and the results in Fig. 4 indicate that the substances being released acutely by 6-OHDA remain chronically depleted.

The difference between the temperature response to injected NA (Feldberg & Lotti, 1967; Myers & Yaksh, 1968) and that to endogenous catecholamines released by 6-OHDA may be due to the apparent involvement of DA rather than NA in the hypothermic response to 6-OHDA. The results presented in Figs. 5 and 6 show that a reduction in the amount of NA available for release or an impairment of the release of NA did not reduce the hypothermic response to 6-OHDA, provided that the release of DA was not affected at the same time (compare Fig. 4). Intraventricular injections of DA, however, have little effect on the body temperature of the rat, the responses consisting of a small rise in temperature (Myers & Yaksh, 1968). It is possible, therefore, that 6-OHDA in the present experiments released DA in sufficiently high local concentrations to stimulate pathways which cause hypothermia. Since it has been reported that the turnover of brain DA is not increased on exposure of rats to heat or cold (Corrodi *et al.*, 1967), the stimulation of these pathways by DA may not be part of the physiological process of temperature regulation.

In view of the evidence that 6-OHDA causes degeneration of catecholamine-containing nerve terminals in rat brain (Ungerstedt, 1968; Bloom *et al.*, 1969; Uretsky & Iversen, 1969, 1970; Bartholini *et al.*, 1970), it is possible that DA is released as a result of the degenerative process. This may not be the only mechanism of DA release following injection of 6-OHDA, however, since doses of 6-OHDA lower than those required to degenerate DA neurones (Uretsky & Iversen, 1970; Table 1) cause hypothermia (Fig. 3). In addition, even high doses of 6-OHDA do not deplete DA from the striatum until 24–48 h after injection but rather cause a rise in striatal DA concentration during this early period (Bell, Uretsky & Iversen, in preparation). It appears, therefore, that 6-OHDA may release DA by a mechanism which is independent of neuronal degeneration.

We wish to thank Dr. L. L. Iversen for valuable advice and criticism. We are also grateful to Dr. H. Thoenen and Dr. J. P. Tranzer of F. Hoffman-La Roche & Co. Ltd., Basle, Switzerland for generous supplies of 6-hydroxydopamine. This work was done during the tenure of a Beit Memorial Research Fellowship by M. A. S. and U.S. Public Health Service, Postdoctoral Fellowship No. 5 FO2 MH35810-02PE by N. J. U. The work was supported by grants to L. L. I. from the Science Research Council and from the Mental Health Research Fund.

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(*Received June* 22, 1970)