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Effects of intraventricular 2,4,5-trihydroxyphenylethylamine (6-hydroxydopamine) on rat behaviour and brain catecholamine metabolism

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Summary

1. 6-Hydroxydopamine (200 μg injected intraventricularly) caused depletion of noradrenaline from all regions of rat brain within 2 h after injection but depletion of dopamine in the brain was observed only from 2 days after injection. Both catecholamines remained depleted for more than 32 days.

2. Rats treated with intraventricular 6-hydroxydopamine were sedated and lethargic, with reduced spontaneous and exploratory activity, for periods of up to 8 days after injection. Conditioned avoidance responding was abolished or reduced for a similar period.

3. Intraventricular 6-hydroxydopamine caused a prolonged reduction in the amount of labelled catecholamines in store 4 h following an intraventricular injection of ^{3}H-dopamine. During the first 6 h after 6-hydroxydopamine injection, there was a marked increase in neutral and acid metabolites from the labelled catecholamines.

4. A comparison of the behavioural and biochemical effects of intraventricular 6-hydroxydopamine and reserpine suggests that both drugs affect catecholamine storage mechanisms but by different mechanisms. It was not possible from these experiments to correlate behavioural changes with either catecholamine storage or metabolism.

Introduction

6-Hydroxydopamine (2,4,5-trihydroxyphenylethylamine hydrobromide; 6HD) has been shown to deplete peripheral tissue stores of noradrenaline (NA) in mice, dogs, guinea-pigs and kittens (Porter, Totaro & Stone, 1963; Laverty, Sharman & Vogt, 1965), and also to cause morphological degeneration of adrenergic nerve endings in rats (Tranzer & Thoenen, 1968). The associated loss of adrenergic function suggested that at the periphery 6HD produces a 'chemical sympathectomy' (Malmfors & Sachs, 1968).

The administration of 6HD into the cerebrospinal fluid, to avoid the blood-brain barrier, was found to cause a substantial and long-lasting depletion of NA from the rat brain (Uretsky & Iversen, 1969). This result was consistent with the hypo-

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thesis that 6HD produces a selective acute degeneration of NA-containing nerve terminals in the brain similar to that produced in the peripheral sympathetic system (Tranzer & Thoenen, 1968 ; Malmfors & Sachs, 1968). However, despite the large doses of 6HD administered, behavioural changes which might be expected after the acute destruction of central noradrenergic neurones (Schildkraut & Kety, 1967) were not reported.

Marked motor disturbances were observed in rats, after the injection of 6HD directly into the substantia nigra region (Ungerstedt, 1968). This behavioural effect was associated with degeneration of the whole nigro-neostriatal dopamine neurone system.

Both of the above studies involving the intracerebral injection of 6HD suggest that this compound is a valuable tool for the investigation of the physiological role of catecholamines in the brain. Therefore the effects of intraventricular 6HD on rat behaviour and catecholamine uptake, storage and metabolism in the rat brain have been studied.

A report of some of these results was made to the Fourth International Pharmacology Congress, Basel, 1969 ; some preliminary results have been published (Taylor, 1969).

Methods

Male rats (180–230 g) from albino and black strains were injected with 6HD or vehicle into the left lateral ventricle of the brain by a stereotaxic technique (unless otherwise stated). Light ether anaesthesia was used. Solutions of 6HD for intraventricular injection were prepared in sterile 0·9% NaCl solution containing ascorbic acid (3 mmol/l.). Doses are expressed as weight of the salt. The injection volume was always 20 microlitres. All control animals received intraventricular injections of the vehicle.

The exploratory behaviour of naive albino and black rats after receiving intraventricular 6HD was compared with control rats using a Y-shaped runway (Steinberg, Rushton & Tinson, 1961) ; each rat was observed for rearing activity, number of entries, number of faecal pellets and grooming behaviour for a period of 3 min. Nocturnal activity was measured in a few individual animals ; each animal had access from a small home cage to a treadwheel to which a counter was attached. Activity was assessed each night by the number of revolutions of the treadwheel, using a cross-over design to eliminate differences between cages and wheels. Black rats were trained to avoid a 7 s electric shock by escaping to a pole during a 7 s buzzer warning (Cook & Weidley, 1957). The effect of each drug treatment was assessed as the score from ten trials, compared with its pre-drug score.

In order to assay brain catecholamines and their metabolites, rats were decapitated and their brains removed. Tissue samples, either whole brain or dissected brain regions, were assayed for NA and DA using the trihydroxyindole fluorimetric method (Laverty & Taylor, 1968). 6HD did not interfere to any appreciable extent with the assay of either NA or dopamine (DA). Tritiated catecholamines and metabolites were determined 4 h after the intraventricular injection of 10 μCi ^{3}H-dopamine (ring-^{3}H(G) ; specific activity 280 mCi/mmol) by the method of Taylor & Laverty (1969a).

The effect of intraventricular 6HD on the blood pressure of genetically hypertensive male albino rats (200–250 g) was determined (Phelan, 1968).

The behavioural and biochemical effects of reserpine (Ciba or Koch-Light) (2·5 mg subcutaneously) were studied using the same techniques as those used for the 6HD studies.

Results

The effect of intraventricular 6HD (10, 60 or 200 μg) on rat brain regional catecholamine levels and Y-runway activity 24 h after injection is shown in Table 1. NA was significantly reduced in all regions after 60 μg 6HD but Y-runway activity was unaffected. After 200 μg 6HD NA levels were reduced even further and Y-runway activity was completely inhibited. Endogenous DA did not seem to be affected. The general appearance of rats after 10 and 60 μg 6HD was no different from controls but after 200 μg 6HD treated rats were lethargic with a hunched-up posture and they exhibited piloerection. As 200 μg 6HD affected both behaviour and catecholamine levels, this dose was used in all subsequent experiments.

The duration of action of 6HD was studied in white rats over a period of up to 32 days after single injections of 200 μg intraventricularly (Table 2). Whole brain noradrenaline content was diminished within 1 h of injection and levels remained low for the remainder of the period studied. Whole brain dopamine content however, did not fall appreciably until 24 h after the injection, and then remained low for the remainder of the period. Exploratory behaviour in the Y-runway was markedly reduced from 1 h after injection but was largely recovered by 8 days after injection.

Conditioned avoidance response behaviour was measured after intraventricular 6HD in black rats, as this strain was found to be easier to train and more consistent in their CAR behaviour than white rats. Four hours after 6HD (200 μg intraventricularly) CAR behaviour was reduced and remained low for at least 2 days after the injection (Fig. 1). By 8 days after injection most animals had recovered their ability to respond though even at 32 days after injection two of the seven animals still failed to respond consistently to the conditioned stimulus.

Analysis of the brain catecholamines in these black rats after 6HD (Table 3) showed that there was a reduction in noradrenaline content in all regions at 2 days after injection, with only a slight effect on striatal dopamine levels. At 32 days after injection both amines were depleted. Thus black rats seemed to show the same pattern of rapid depletion of noradrenaline but more delayed depletion of dopamine, with depletion of both amines lasting more than 32 days, seen in white rats.

Nocturnal activity was inhibited by 6HD in black rats, as measured by the number of turns of a treadwheel to which each rat had access. In three experiments the activity of 6HD treated rats was reduced to less than 30% of that of controls during the first 8 days after injection.

During these behavioural experiments, particularly during studies on exploration of the Y-runway, it was noticed that 6HD treated rats during the first 2 days after injection had a marked tendency to move in a circle towards the left. Since the 6HD had been injected into the left lateral ventricle, an investigation was made of brain catecholamine levels in each side of the brain, and of rat behaviour, following

TABLE 1. *Effect of 6HD (10, 60 and 200 μg) on regional catecholamine content and Y-maze activity 24 h after injection into the left lateral ventricle of white rats*

	No. of animals	NA content (μg/g)					DA content (μg/g)	Y-runway activity No./3 min	
		Thalamus-midbrain	Cortex	Cerebellum	Pons-medulla	Striate	Striate	Rearings	Entries
Control	9	0·53±0·01	0·26±0·01	0·26±0·01	0·58±0·04	0·33±0·04	3·9±0·2	13±1	8±2
6HD 10 μg	5	0·39±0·02	0·27±0·01	0·23±0·01	0·56±0·01	0·36±0·04	3·9±0·4	12±2	8±1
6HD 60 μg	5	0·25±0·02	0·16±0·01	0·15±0·02	0·29±0·02	0·20±0·01	4·0±0·4	10±3	6±3
6HD 200 μg	5	0·12±0·01	0·11±0·01	0·01±0·00	0·18±0·01	0·13±0·01	3·7±0·1	0±0	0±0

Results are expressed as means ±S.E.M.

TABLE 2. *Effects of 6-hydroxydopamine (200 μg intraventricularly) on brain catecholamines and behaviour in white rats at intervals after injection*

Control	1 h	2 h	4 h	6 h	1 day	2 day	8 day	16 day	32 day
Whole brain noradrenaline content (μg/g)									
0·30 (0·00; 30)	0·14 (0·00; 4)	0·10 (0·00; 4)	0·09 (0·01; 5)	0·12 (0·00; 4)	0·13 (0·01; 9)	0·14 (0·01; 10)	0·11 (0·00; 10)	0·10 (0·01; 10)	0·11 (0·01; 6)
Whole brain dopamine content (μg/g)									
0·69 (0·03; 30)	0·73 (0·06; 4)	0·77 (0·04; 4)	0·64 (0·03; 5)	0·78 (0·04; 4)	0·59 (0·06; 9)	0·42 (0·05; 10)	0·36 (0·03; 10)	0·35 (0·05; 10)	0·33 (0·02; 6)
Y-runway rearings (mean number/3 min session)									
8·6 (0·5; 39)	0 (0·0; 4)	1·0 (1·0; 4)	0·6 (0·3; 5)	1·3 (0·7; 5)	1·8 (0·9; 9)	3·8 (0·8; 10)	5·6 (0·5; 15)	5·7 (0·9; 6)	4·5 (0·9; 6)
Y-runway entries (mean number/3 min session)									
6·4 (0·3; 39)	1·3 (0·7; 4)	0·5 (0·3; 4)	2·0 (0·7; 5)	1·0 (0; 4)	3·2 (0·6; 9)	4·5 (0·6; 10)	6·6 (0·4; 15)	6·5 (0·7; 6)	6·0 (0·4; 6)

Results are expressed as the mean. The S.E.M. and number of rats are given in brackets below each value.

injections of 6HD into the left or right lateral ventricle (Table 4). No difference was found in the brain catecholamine levels on the two sides of the brain 5 h after injection of 6HD into either lateral ventricle, nor was there any difference in the reduction of exploratory activity in the Y-runway. There were, however, significant differences in behaviour of the two groups when in their cages following injection. The animals injected with 6HD on the left side showed the usual pattern of behaviour—that is, slight excitation on awakening after a longer duration of ether anaesthesia, a hunched posture with little activity, similar to that produced by reserpine, and when stimulated to activity a definite tendency to circle to the left side. Animals injected on the right side appeared less tranquillized, and tended to circle to the right during the first 30 min after injection. During the next 2·5 h after injection these rats spontaneously and individually had short periods of violent activity to the extent of convulsions. All animals appeared to be affected at least once, but before and after the convulsive episodes the animals appeared quiet and inactive. By 8 days after 6HD injection it was not possible to distinguish between treated and control rats by simple observation.

Rats treated with intraventricular 6HD maintained a normal growth rate for the 32 days studied. No blood pressure reduction was seen in genetically hypertensive rats (Phelan, 1968) treated with intraventricular 6HD. There was no effect on heart noradrenaline storage 2 days after intraventricular injection of 6HD, nor was any effect of 6HD on body temperature observed during 14 days after 6HD injection.

Uptake and metabolism of ^{3}H-dopamine

Four hours after the intraventricular administration of ^{3}H-dopamine (10 μCi in 20 μl), the principal labelled amine present in the brain was ^{3}H-NA and the principal labelled metabolite occurred in the neutral fraction and was probably largely 4-hydroxy-3-methoxy-phenylglycol (Taylor & Laverty, 1969b ; Fig. 2). In all these experiments, rats were killed 4 hours after injection of ^{3}H-DA so that the effects

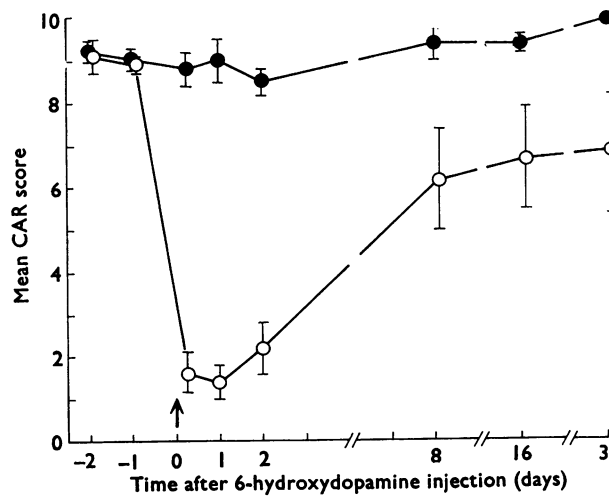

FIG. 1. Effect of 6-hydroxydopamine (200 μg intraventricularly) on a pole-climbing conditioned avoidance response (CAR) in black rats. Results are the mean (±S.E.M.) of each animal's score from ten trials using seven drug treated animals (○——○) and six saline treated controls (●——●).

TABLE 3. *Effect of 6HD (200 μg intraventricularly) on regional brain catecholamines in black rats (means ± S.E.M.)*

	No. of animals	NA content (μg/g)					DA content (μg/g)
		Thalamus-midbrain	Cortex	Cerebellum	Pons-medulla	Striate	Striate
2 days after injection							
Controls	4	0·61±0·03	0·28±0·01	0·28±0·03	0·47±0·05	0·29±0·02	5·2±0·2
6HD	4	0·24±0·02	0·18±0·01	0·07±0·01	0·22±0·03	0·18±0·02	4·3±0·5
32 days after injection							
Controls	7	0·57±0·04	0·32±0·01	0·28±0·01	0·49±0·03	0·21±0·02	3·0±0·4
6HD	7	0·23±0·02	0·10±0·02	0·10±0·02	0·26±0·02	0·11±0·02	0·9±0·2

TABLE 4. *Effect of* 6HD (200 μg) *injected into the left or right lateral ventricle on white rat behaviour, Y-runway activity at 4 h after injection and the concentration of catecholamines in the left and right half of the brain 5 h after injection*

		Catecholamine content (μg/g)				Y-runway activity No./3 min		
		NA		DA				
Injection	Ventricle	Left	Right	Left	Right	Rearings	Entries	Observed behaviour
Saline	Left	0·35±0·01	0·36±0·01	0·63±0·05	0·60±0·04	6±2	7±2	Normal
6HD	Left	0·08±0·00	0·12±0·00	0·78±0·06	0·67±0·02	0±0	0±0	Sedation; left-hand circling
Saline	Right	0·34±0·02	0·35±0·01	0·63±0·02	0·59±0·01	6±2	6±1	Normal
6HD	Right	0·09±0·00	0·07±0·00	0·80±0·03	0·82±0·04	0±0	0±0	Sedation; intermittent convulsions

Each value is the mean ± S.E.M. of five determinations.

of drugs on the metabolite pattern could be measured under standard conditions. Drug treatment of the animals was carried out at various times before killing; hence in experiments in which drug treatment was for less than 4 h, the ^{3}H-DA was given before the drug, whereas in experiments with drug treatments of more than 4 h, the ^{3}H-DA was given after the drug.

In the brains of animals killed 1 and 2 h after the intraventricular injection of 6HD, there was a significant reduction in the amount of stored ^{3}H-NA, a less pronounced fall in stored ^{3}H-DA and significant increases in the amount of acid and neutral metabolites of ^{3}H-NA and ^{3}H-DA (Fig. 2). This metabolite pattern was also present 6 h after the injection of 6HD, except that labelled 3,4-dihydroxyphenylacetic acid was significantly less than in control animals. The fall in this acid metabolite was found for up to 16 days after 6HD treatment. By 24 h after 6HD injection, the levels of other acid and neutral metabolites were normal, though the storage of amines was still reduced; this pattern remained up to 16 days after 6HD treatment.

In one experiment ^{3}H-NA (20 μCi 7-^{3}H-NA in 20 μl) was injected into the ventricles of four rats treated 2 days before with 6HD. There was a significant reduction of uptake of ^{3}H-NA, but little effect on the pattern of metabolites.

Biochemical and behavioural effects of reserpine

Following reserpine (2·5 mg/kg subcutaneously) there was a significant fall in the endogenous brain levels of NA and DA (Fig. 3A). Reserpine caused a smaller

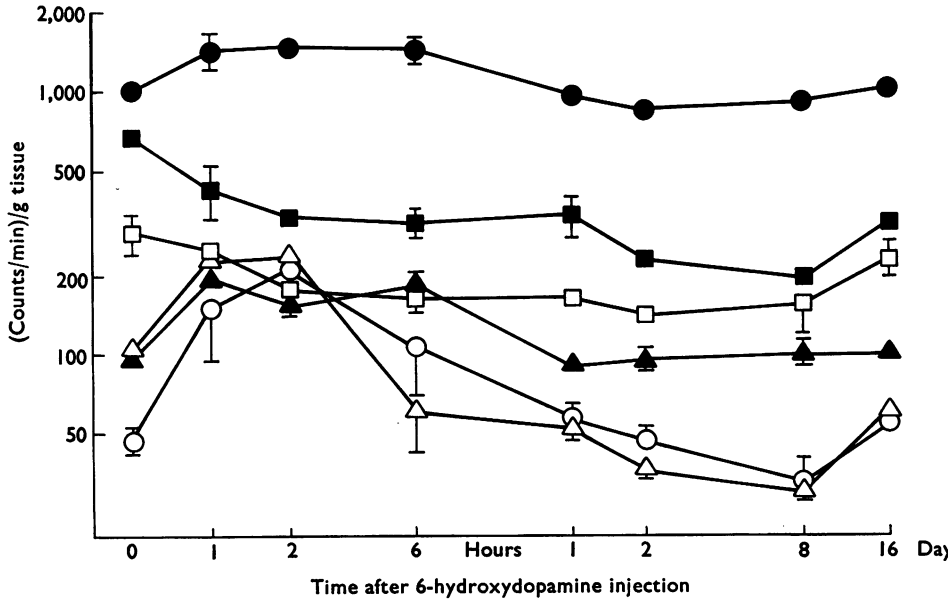

FIG. 2. The content of labelled catecholamines and metabolites in whole rat brain at varying intervals after the intraventricular injection of 6-hydroxydopamine (200 μg). In all cases ^{3}H-dopamine (10 μCi) was given intraventricularly into the brain 4 h before the animal was killed. Each point is the mean (±S.E.M.) of four determinations. ●——●, Alcohol metabolites, for example, 4-hydroxy-3-methoxy-phenylglycol; ■——■, noradrenaline; □——□, dopamine; ▲——▲, 4-hydroxy-3-methoxy and 3,4-dihydroxy—mandelic acids; △——△, 3,4-dihydroxy-phenylacetic acid; ○——○, 4-hydroxy-3-methoxy phenylacetic acid. Note that a log scale is used for both axes.

fall in the concentration of ^{3}H-NA and ^{3}H-DA present in the brain 4 h after the intraventricular injection of ^{3}H-DA (Fig. 3B). There was a significant increase in labelled homovanillic acid 2 h after reserpine, whereas the labelled dihydroxyphenylacetic acid fraction was reduced during the period from 6 h to 2 days, after the reserpine injection.

Reserpine (2·5 mg/kg subcutaneously) inhibited exploratory activity in the Y-runway almost completely within 1 hour of injection ; activity remained reduced even 16 days after injection (Fig. 4). Conditioned avoidance response behaviour was also inhibited completely by reserpine but this had recovered by 9 days after injection (Fig. 4).

Discussion

These results, as well as confirming the effects of intraventricular 6HD on brain catecholamine levels reported by Uretsky & Iversen (1969), show that 6HD can

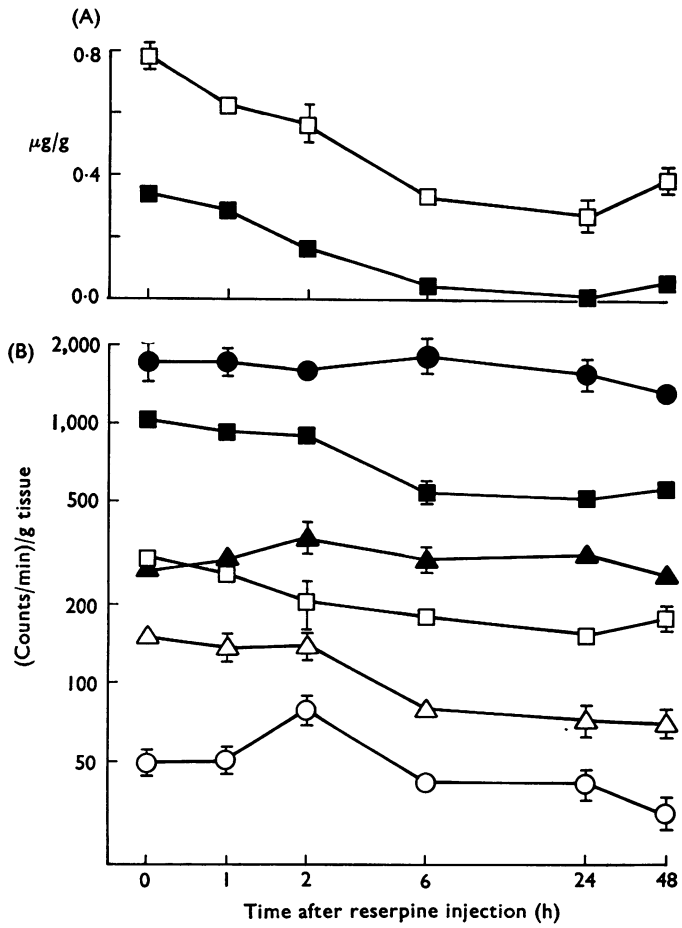

FIG. 3. Effects of reserpine (2·5 mg/kg subcutaneously) on whole brain catecholamine metabolism. Each point is the mean (±S.E.M.) of four determinations. (A), Content of endogenous noradrenaline (■——■) and dopamine (□——□). (B), Content of labelled catecholamines and metabolites, when the animal was killed in each case 4 h after the intraventricular injection of ^{3}H-DA (10 μCi) into the brain. Symbols as for Fig. 2.

affect animal behaviour considerably. The observed sedation, lethargy, and piloerection after intraventricular 6HD were similar to the characteristic behavioural effects of reserpine, so that a comparison has been made between the two drugs. Both drugs inhibited exploratory behaviour and also CAR behaviour in rats for periods of 8 days or longer. Both drugs depleted brain catecholamines for prolonged periods also. However, there are a number of points in which the two drugs differ.

One interesting difference is the delayed effect of intraventricular 6HD on the endogenous DA levels. 6HD depleted brain NA maximally within 2 h, but the DA levels did not fall until 2 days after injection. Reserpine reduced the levels of both amines simultaneously, and by 2 days they were beginning to recover. No recovery was observed after 6DA, both amines remaining depleted for at least 32 days after injection. This prolonged effect of 6HD injected into the brain is similar to that observed in the periphery after systemic administration (Porter *et al.*, 1963 ; Laverty *et al.*, 1965) which is thought to be due to destruction of the adrenergic nerves (Tranzer & Thoenen, 1968).

There were differences between the effects of intraventricular 6HD and reserpine on the metabolism of ^{3}H-HD injected intraventricularly. During the first 6 h after 6HD administration there were significant increases in the acid and neutral metabolites of NA and DA, accompanying a fall in storage of the amines. After reserpine, although there was a more profound fall in the levels of endogenous amines, the effects on ^{3}H-DA metabolism were much less marked. Hence, although the two drugs both affect amine storage, they do not appear to act by exactly the same mechanism. Both drugs, while affecting amine storage, do not appear to affect amine metabolism, apart from the initial few hours after injection, as judged by the pattern of metabolites from ^{3}H-DA injected intraventricularly, yet the behavioural effects of the drugs last for at least 8 days. Conversely, the effect of 6HD on amine storage lasts for at least 32 days, whereas the behaviour is largely normal in most animals by the sixteenth day after injection. Thus from these

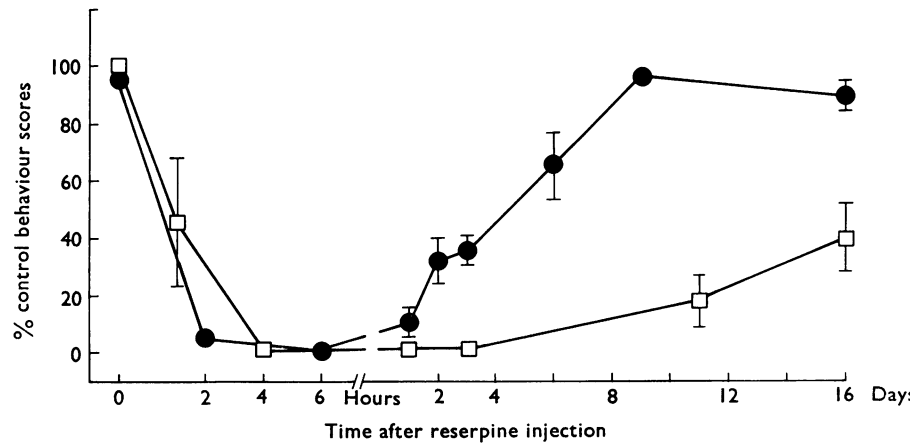

FIG. 4. Effects of reserpine (2·5 mg/kg subcutaneously) on Y-runway exploratory behaviour (□——□) and pole-climbing CAR behaviour (●——●) in rats. Results are expressed as the mean (±S.E.M.) response of treated animals compared with that of controls (groups of at least six animals in each case). Exploratory behaviour was measured as the sum of the observed rearings and entries ; CAR behaviour was the score from ten trials.

experiments it is not possible to correlate the effects of these drugs on behaviour with either amine storage or amine metabolism.

Although 6HD injected intravenously lowered blood pressure effectively in rats (Laverty & Phelan, 1968), intraventricular 6HD had no effect on blood pressure. No effect on body weight was observed, suggesting normal feeding behaviour. Hence it appears that 6HD does not affect the central control of these factors, nor of temperature regulation.

It is difficult to explain satisfactorily the behavioural difference observed when 6HD was injected into the right lateral ventricle rather than the left. The left-hand circling behaviour observed with left-sided injections was common to most of the animals studied, but right-hand circling in animals injected on the right side was more difficult to observe, while the violent convulsive behaviour was not observed in animals injected on the left side. The studies of brain amine levels failed to reveal any differences due to the site of injection, so that further investigations are required to establish the reproducibility and perhaps a mechanism for these observations.

While reserpine and intraventricular 6HD both act on catecholamine storage mechanisms, it is probable that their site of action is different. Reserpine is thought to affect predominantly the intraneuronal storage mechanism. This could explain the considerable fall in endogenous tissue levels of catecholamines while the metabolite pattern remains more or less normal. 6HD, on the other hand, appears to increase the metabolism of the newly acquired or newly synthesized catecholamines to a greater extent than reserpine, since the metabolites are increased markedly during the initial exposure to the drug, whereas the endogenous noradrenaline level is not so markedly depleted. Thus 6HD may be causing a complete breakdown of a proportion of the adrenergic neurones, radically affecting uptake, storage and metabolism of noradrenaline, but leaving other neurones more or less intact, whereas reserpine may have a more limited action affecting all amine-containing neurones.

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