Brit. J. Pharmacol. (1965), **24**, 549–560.

ACTION OF 2, 4, 5-TRIHYDROXYPHENYLETHYLAMINE ON THE STORAGE AND RELEASE OF NORADRENALINE

BY

R. LAVERTY,* D. F. SHARMAN AND MARTHE VOGT

From the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

(*Received September* 18, 1964)

Porter, Totaro & Stone (1963) have reported that a single injection of 6-hydroxydopamine (2,4,5-trihydroxyphenylethylamine) lowered the noradrenaline content of the mouse heart by more than 50% for longer than 25 days. Porter *et al.* (1963) proposed that this prolonged action was due to destruction of the noradrenaline binding sites by the 6-hydroxydopamine. However, since 6-hydroxydopamine is very similar to noradrenaline in its chemical properties (Senoh & Witkop, 1959) it was possible that the 6-hydroxydopamine or some metabolite replaces the noradrenaline in the heart tissue and is then bound there, preventing the resynthesis or replacement of the lost noradrenaline.

In order to investigate this question a method was devised to isolate and measure the 6-hydroxydopamine content of tissue samples, and so to see whether its persistence in the tissues could explain its action on the storage of noradrenaline. The functional state of the nervi accelerantes was tested at different time intervals after the administration of 6-hydroxydopamine, and the effect of the drug on the release of catechol amines from the adrenal medulla was examined.

METHODS

Adult male and female mice, young female guinea-pigs, and kittens and puppies of either sex were used in these experiments. 6-Hydroxydopamine was injected intraperitoneally or intravenously as the hydrobromide; all doses of 6-hydroxydopamine and other amines are expressed in terms of the bases.

Cardiac nerve stimulation. Kittens were anaesthetized with ether followed by intravenous chloralose (80 mg/kg) and the chest was opened under artificial ventilation. In some kittens the adrenal glands were removed before opening the chest; in many, the blood pressure was measured by a mercury manometer connected to the carotid artery. The right stellate ganglion was exposed and the strand of postganglionic nerves going to the heart was placed on shielded electrodes. Rectangular pulses of 1 msec duration from a Grass SD5 stimulator were used at a frequency of 15 shocks/sec; the voltage applied was usually 20 V. The heart rate was measured from the electrocardiogram recorded on a Grass Polygraph.

Perfusion of adrenal glands. Adrenal glands of puppies were perfused by gravity at 35° C with Locke solution saturated with 95% oxygen and 5% carbon dioxide. The perfusion cannula was tied into the superior mesenteric artery. The dissection was carried out as previously described (Vogt, 1951), but the isolated segments of aorta and vena cava to which the left adrenal gland was attached were excised from the body together with the adrenal gland and placed on a perforated platform resting in a funnel. The

* Beit Memorial Research Fellow. Present address: Wellcome Research Institute, University of Otago Medical School, Dunedin, New Zealand.

6-hydroxydopamine was freshly dissolved in bicarbonate-free Locke solution to make a 0.2% solution. Of this solution, 0.2 ml. was slowly infused by means of a precision syringe fitted to a narrow polyethylene tube which opened into the tip of the arterial cannula. In this way it was possible to give the drug without addition of reducing chemicals to prevent its destruction.

Catechol amine estimations. Mice were stunned and bled to death. Guinea-pigs and kittens were anaesthetized with chloroform or chloralose and bled to death. The hearts and other tissues were removed, blotted dry and stored for less than 3 hr at −16° C. In the kitten experiments only the atria of the heart were used; mouse hearts were handled as groups of four to six. Heart tissues were frozen in liquid nitrogen, crushed while frozen and then homogenized with twice their weight of 0.1 N-hydrochloric acid; brain tissues were homogenized directly in 0.1 N-hydrochloric acid. The proteins were precipitated with perchloric

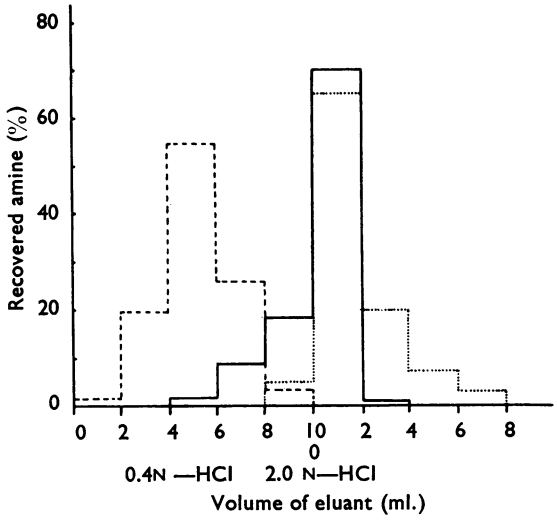

Fig. 1. Elution pattern of noradrenaline (– – – –), 6-hydroxydopamine (——) and dopamine (. . . .) from a Dowex 50 X-8 ion-exchange resin column (2.5 × 0.4 cm) using 0.4 N- and 2 N-hydrochloric acid.

acid, and the amines were extracted by passing the deproteinized extract, after adjusting to *p*H 4 with potassium carbonate, through a column of Dowex 50 X-8 ion-exchange resin (2.5 × 0.4 cm) (Bertler, Carlsson & Rosengren, 1958). The column was washed with 4 to 6 ml. of water. Noradrenaline was eluted with 10 ml. of 0.4 N-hydrochloric acid, and other catechol amines were removed with 8 ml. of 2 N-hydrochloric acid (Fig. 1). When separation of the catechol amines was to be carried out by paper chromatography they were eluted from the column with 8 ml. of 2 N-hydrochloric acid.

Noradrenaline in the first eluate was determined fluorimetrically after oxidation by ferricyanide (Euler & Lishajko, 1961; for details see Sharman, Vanov & Vogt, 1962). The catechol amines in the second eluate were estimated fluorimetrically after acetylation (Laverty & Sharman, 1964) by condensation with ethylenediamine; 4 ml. of column eluate, after treatment with 0.3 ml. of acetic anhydride followed by excess of sodium bicarbonate, were heated for 20 min at 65° C with 0.5 ml. of a mixture of 3 vols of ethylenediamine and 2 vols of 2 N-hydrochloric acid. The reaction mixture was cooled, saturated with sodium chloride and extracted with 3 ml. of isobutanol. The fluorescence of the organic phase was measured.

The quantitative measurements of the catechol amines were carried out on a Locarte filter fluorimeter. For noradrenaline the filters used were primary, Chance OX1, and secondary, Ilford Bright Spectrum 625. For catechol amines after ethylenediamine condensation the primary filter was a combination of Corning 3389 and Corning 5113 (half standard thickness); the secondary filters were Ilford Bright Spectrum 623 and Ilford Bright Spectrum 625 (see later).

For further purification by paper chromatography, the column eluate was treated with acetic anhydride and sodium bicarbonate and the total acetylated amines were extracted from aqueous solution with

dichloromethane; the dichloromethane extract was evaporated almost to dryness and the concentrated extract was applied to alkali-washed Whatman No. 50 paper (Sharman, 1963). The developing solvent was a modification of the system used for steroids (Eberlein & Bongiovanni, 1955) and consisted of the organic phase from a mixture of petroleum ether (boiling point, 80 to 100° C), water and *tert.*-butanol (8 : 8 : 3). The paper was equilibrated in the presence of the aqueous phase for 12 to 18 hr and a descending chromatogram was developed for 24 to 48 hr. Authentic catechol amines were acetylated and run on each chromatogram and visualized by heating the paper after spraying with a five-fold dilution of the ethylenediamine–hydrochloric acid mixture described above. The chromatograms of tissue extracts were cut into 1.5-cm lengths and each piece was eluted with 4 ml. of water. The catechol amines were estimated fluorimetrically after condensation with ethylenediamine as described above.

In the experiments on kittens and guinea-pigs, the small quantities of noradrenaline present in the superior cervical and stellate ganglia were measured biologically on the pithed rat, following separation of the amines by paper chromatography using a phenol–hydrochloric acid solvent (Vogt, 1954). Because 6-hydroxydopamine and noradrenaline are " isographic " (Senoh & Witkop, 1959), any 6-hydroxydopamine present in the extract would be found on the chromatogram with the noradrenaline. However, since the pressor activity of noradrenaline is about 3,000-times that of 6-hydroxydopamine, the amount which might conceivably have been present in the tissue extract was far below threshold dose in the bioassay.

Separation and estimation of 6-hydroxydopamine. 6-Hydroxydopamine gave no fluorescent product when subjected to the ferricyanide oxidation method used for noradrenaline and adrenaline; 1 μg of 6-hydroxydopamine gave less fluorescence than 1 ng of noradrenaline. Using the iodine oxidation technique of Carlsson & Lindqvist (1962), 1 μg of 6-hydroxydopamine gave a fluorescence equivalent to less than that of 30 ng of dopamine but, when condensed with ethylenediamine, it formed a fluorescent product of similar intensity and wavelength to that of dopamine.

After acetylation, condensation of catechol amines with ethylene diamine gave a greater fluorescence intensity than before (Laverty & Sharman, 1964). The same held for 6-hydroxydopamine. In isobutanol the acetylated 6-hydroxydopamine derivative had similar fluorescence characteristics to the acetylated noradrenaline derivative, but different ones from those of the acetylated dopamine derivative (Laverty & Sharman, 1965).

Separation of 6-hydroxydopamine from other catechol amines was attempted using both column and paper chromatography. The elution pattern of 6-hydroxydopamine from Dowex 50 X-8 resin using hydrochloric acid is compared in Fig. 1 with those of noradrenaline and dopamine. It will be seen that the 6-hydroxydopamine is eluted predominantly in the dopamine eluate (the 2 N-hydrochloric acid eluate); any 6-hydroxydopamine eluted with the noradrenaline in the 0.4 N-hydrochloric acid eluate did not interfere with the estimation of noradrenaline by the ferricyanide-oxidation technique. The total recovery of amine was for noradrenaline 89% from 0.2 μg, for 6-hydroxydopamine 90% from 5 μg, and for dopamine 82% from 2 μg.

On paper, 6-hydroxydopamine is " isographic " with noradrenaline (Senoh & Witkop, 1959). In order to separate the two amines, the acetyl derivatives were formed and run in a descending paper chromatogram with the solvent system described above for catechol amines.

In this solvent system, acetylated noradrenaline, adrenaline, 6-hydroxydopamine and dopamine were separated (Fig. 2), though there was, in some experiments, a tendency for the tail of the dopamine spot to overlap the 6-hydroxydopamine spot. The mean recovery of amines after acetylation, extraction with dichloromethane and chromatography was for noradrenaline 71% from 0.2 to 1.0 μg, for adrenaline 68% from 1 μg, for 6-hydroxydopamine 49% from 1 to 5 μg, and for dopamine 63% from 0.2 to 1.0 μg.

Since both column and paper chromatography separated the 6-hydroxydopamine and dopamine from the noradrenaline, a method was required for the measurement of 6-hydroxydopamine in the presence of dopamine. After condensation with ethylenediamine the fluorescence spectra of acetylated dopamine and 6-hydroxydopamine were sufficiently different to make a differential estimation feasible. With an Ilford Bright Spectrum 623 filter, the fluorescence intensity per μg of dopamine was approximately twice that of 6-hydroxydopamine whereas, using an Ilford Bright Spectrum 625 filter, it was approximately six-times that of 6-hydroxydopamine. Using these filter sets it was possible to determine with reasonable precision quite small amounts of one amine in the presence of the other (Table 1).

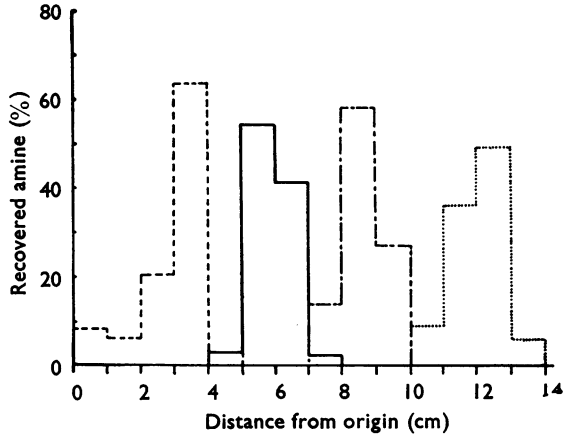

Fig. 2. Separation of the acetylated derivatives of noradrenaline (– – – –), adrenaline (——), 6-hydroxydopamine (– . – . –) and dopamine (. . . .) on Whatman No. 50 paper using a petroleum ether, water and *tert.*-butanol (8 : 8 : 3) solvent system. The chromatogram was developed downwards for 24 hr, and then cut into 1-cm strips.

TABLE 1

THE ESTIMATION OF DOPAMINE AND 6-HYDROXYDOPAMINE IN MIXTURES

The amines were acetylated, condensed with ethylenediamine and the amounts measured fluorimetrically using two different fluorescence wavelengths

	Dopamine (ng)		6-Hydroxydopamine (ng)	
	Added	Measured	Added	Measured
A. Sample in 4 ml. of water				
	200	220	2	−33
	200	213	5	−22
	200	196	10	7
	200	201	50	42
	100	98	100	135
	100	96	100	114
	50	47	200	185
	50	52	500	476
	25	22	500	462
	10	10	500	419
	100	96	500	476
B. Sample added to 4 ml. of eluate				
	100	88	300	324
	100	92	300	290
	100	90	300	302
	100	93	300	302
	100	106	300	323
	100	102	300	316
	100	99	300	332

RESULTS

Effect of 6-hydroxydopamine on catechol amine concentrations in mouse tissues. In a preliminary experiment on two groups of three mice each, it was found that 16 hr after an injection of 6-hydroxydopamine (6.7 mg/kg, intraperitoneally) the noradrenaline content of the hearts was reduced from 0.42 to 0.16 μg/g; there was no observable change in the

noradrenaline content of the brain, spleen or lung, nor in the dopamine content of any of these four tissues. In all further experiments on mice, only the noradrenaline and 6-hydroxydopamine contents of the hearts were measured.

Two strains of mice were used, one for the experiments involving intraperitoneal injections of 6-hydroxydopamine and dopamine, the other for the intravenous experiments. They were found to differ in the initial noradrenaline concentration of their hearts. The results are summarized in Fig. 3,*a*. It will be seen that the noradrenaline content of the

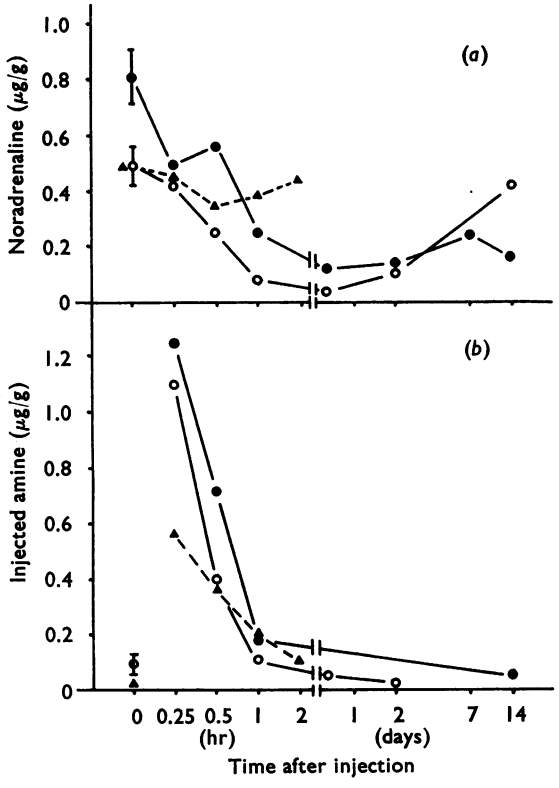

Fig. 3. The concentration in heart tissue of noradrenaline (*a*) and 6-hydroxydopamine or dopamine (*b*) following the injection of 10 mg/kg of 6-hydroxydopamine (○, intraperitoneally; ●, intravenously) or 10 mg/kg of dopamine (▲, intraperitoneally). The means and standard deviations given for control values (zero time) were obtained using six groups of five mice each; other points represent the means from two or more such groups.

mouse heart fell rapidly following the injection of 6-hydroxydopamine (10 mg/kg) by either route, the fall being almost complete at 1 hr after the injection. The level of noradrenaline recovered very slowly, and was still quite low, particularly in the intravenously injected group, as long as 14 days after the injection. However, with a dose of 6.7 mg/kg intraperitoneally, the noradrenaline content of the heart was back to 70% of its initial value at 17 hr. After injection of dopamine (10 mg/kg), used for comparison, there was no significant change in the noradrenaline content of the heart.

Estimation of 6-hydroxydopamine concentrations in mouse heart by differential fluorimetry. After injection of 6-hydroxydopamine its concentration in the heart rose to over 1 μg/g 15 min after the injection, and then fell steeply, being almost back to control values at 1 hr (Fig. 3,*b*). The values of 6-hydroxydopamine given for control hearts probably represent a background fluorescence from undetermined materials rather than an actual tissue content of 6-hydroxydopamine, because no 6-hydroxydopamine could be detected in control hearts following further purification by paper chromatography. Small amounts of materials having fluorescence characteristics similar to those of 6-hydroxydopamine were detected on the paper in places other than those occupied by known catechol amines.

The tissue concentration of injected dopamine was measured in order to compare the time course of its accumulation in, and disappearance from, tissue with that of 6-hydroxydopamine. The uptake of dopamine by the heart was not as complete as the uptake of 6-hydroxydopamine, the tissue containing only 0.6 μg/g 15 min after the injection. The tissue content of dopamine fell at a slightly slower rate than that of 6-hydroxydopamine, and had not returned to control values 2 hr after the injection.

With a lower dose of 6-hydroxydopamine (6.7 mg/kg, intraperitoneally), the amount in the heart 1 and 17 hr after the injection was similar to that following 10 mg/kg.

The recovery of 6-hydroxydopamine, added to a tissue sample and carried through the column extraction procedure, was rather variable; the mean recovery in five experiments in which 1 μg of 6-hydroxydopamine was added to tissue was 60%. The recovery of dopamine (0.5 μg) in similar experiments was 73%. All figures given are uncorrected for recovery.

It was noticed that many animals killed 15 and 30 min after an injection of 6-hydroxydopamine had a pronounced red coloration of their urine. No such colour was seen in control animals or in animals injected with dopamine.

Detection of 6-hydroxydopamine and metabolites in mouse hearts after paper chromatography. In order to confirm the results obtained by differential fluorimetry, treated hearts were extracted for amines by ion-exchange chromatography and the amines in the column eluates were then acetylated, extracted with dichloromethane and separated on a paper chromatogram. After elution, condensation with ethylenediamine was carried out and the fluorescence plotted. Pooled extracts from 13 to 20 mouse hearts were used. An equal number of hearts from uninjected controls was analysed simultaneously: in none of the three experiments was there any 6-hydroxydopamine found. The amount detectable by the present technique was less than 30 ng; allowing for an approximate total recovery of 30%, this would place an upper limit on the tissue concentration of 6-hydroxydopamine in mouse hearts at 0.04 μg/g. It was possible in these experiments to detect the presence of adrenaline and dopamine in some extracts of mouse hearts; the amounts were too small to be measured accurately but were of the order of 0.03 μg/g for adrenaline and 0.01 μg/g for dopamine.

The hearts of mice which had been treated with 6-hydroxydopamine 15 min and 16 hr before being killed were extracted and analysed. At 15 min after injection of 6-hydroxydopamine, at a time when the noradrenaline content was only slightly lower than that of the control hearts, there was a fluorescence peak on the chromatogram (Fig. 4) corresponding to approximately 1.1 μg of 6-hydroxydopamine. This amount represents a tissue

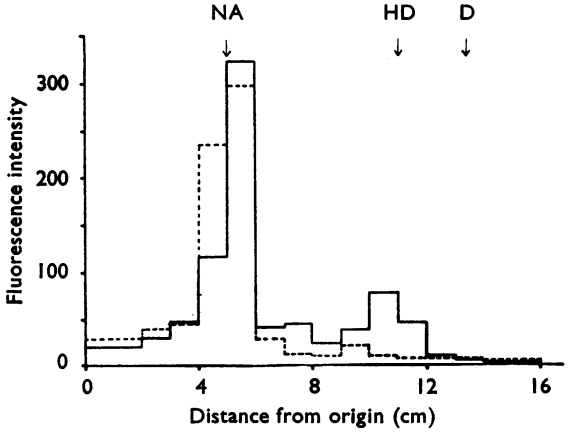

Fig. 4. Fluorescence scan of chromatograms from extracts of hearts. (- - - -) Control mice, (——) mice killed 15 min after intraperitoneal injection of 6-hydroxydopamine (10 mg/kg). The extracts were from approximately 3 g of heart and chromatograms were developed downwards for 48 hr in a petroleum ether, water and *tert.*-butanol (8 : 8 : 3) solvent. Control spots of known catechol amines run in parallel were found at, for noradrenaline (NA) 5 to 6 cm, 6-hydroxydopamine (HD) 11 to 12 cm, and dopamine (D) 14 cm from the origin. The fluorescence is measured in arbitrary units.

concentration similar to that obtained by direct differential fluorimetry. The chromatogram from mouse hearts 16 hr after the injection of 6-hydroxydopamine showed a pronounced fall in the noradrenaline content from the control values, but no detectable 6-hydroxydopamine peak.

Since, therefore, residual 6-hydroxydopamine did not appear to replace the noradrenaline of the heart, two experiments were carried out in which a search was made for any other phenolic or indolic bases which might have been formed as metabolites of 6-hydroxydopamine. Groups of about twenty mice were killed 3 hr after intraperitoneal injection of 6-hydroxydopamine (10 mg/kg), and chromatograms of the heart extracts run as before.

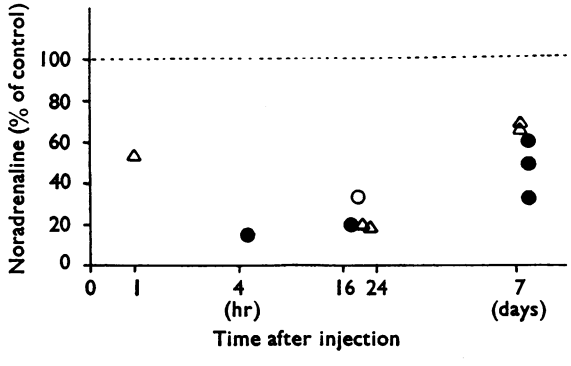

Fig. 5. The depletion of the noradrenaline from kitten hearts by 6-hydroxydopamine. The doses used were 10 mg/kg intraperitoneally (△), 20 mg/kg intravenously (○), and 30 mg/kg intravenously (●). The results are calculated in terms of the percentage of the noradrenaline content of hearts from litter-mate controls; the noradrenaline content (mean and standard deviation) of the hearts of eight kittens was 0.90 ± 0.30 μg/g.

They were scanned with ultraviolet light, but no absorbing spots were seen. A portion of the chromatogram was then sprayed with diazotized *p*-nitroaniline to detect any phenolic compounds, and the other portion with Ehrlich's *p*-dimethylaminobenzaldehyde reagent to look for indoles. No difference from strips prepared from control hearts was seen; these tests are not sensitive to less than microgram quantities.

The effect of 6-hydroxydopamine on the kitten and guinea-pig heart and on other tissues. The injection of 6-hydroxydopamine into kittens caused a profound and prolonged fall in the noradrenaline content of the heart (Fig. 5), of similar degree and time-course as that observed in mice. However, the 6-hydroxydopamine content of the hearts of the treated animals fell more slowly, being 1.6 μg/g 1 hr after intraperitoneal administration of 10 mg/kg, but undetectable 21 hr after injection. When 30 mg/kg were given intravenously, some 6-hydroxydopamine (0.1 μg/g) was found 4.5 hr later.

Relaxation of the nictitating membrane was seen when large doses of 6-hydroxydopamine had been injected 6 hr or more previously; therefore the noradrenaline content of the superior cervical ganglia was estimated in these experiments. No significant changes were seen (Table 2). Neither was there any change in the noradrenaline content of stellate ganglion of the cat examined 18 hr after the highest dose; it was 5.1 μg/g which is even

TABLE 2

THE EFFECT OF 6-HYDROXYDOPAMINE ON THE NORADRENALINE CONTENT OF NERVOUS TISSUES IN KITTENS

Injections were intravenous

Litter No.	Dose (mg/kg)	Duration	Noradrenaline content (μg/g) Superior cervical ganglia	Hypothalamus
1	Control		3·6	2·3
	30	4·5 hr	3·1	0·7
	30	18 hr	3·2	1·1
2	Control		4·4	1·6
	20	20 hr	4·4	0·6
3	Control		—	1·3
	Control		—	1·9
	30	8 days	—	0·7
	30	8 days	—	1·2
	30	8 days	—	1·3

higher than the average normal figure (Muscholl & Vogt, 1958). The noradrenaline content of the hypothalami of the kittens treated with 20 or 30 mg/kg of 6-hydroxydopamine was found to be lowered significantly up to 24 hr after injection (Table 2). The single low figure 8 days after the injection was that of a cat which had developed a severe infection.

The functional state of the sympathetic nerves leading to the heart was tested in a series of kittens. In six control experiments stimulation of the right nervi accelerantes caused an average increase of heart rate of 16% from a mean rate, after vagotomy, of 235 beats/min. When 6-hydroxydopamine was injected while records were being made, acceleration of the heart by stimulation of the cardiac nerves was immediately abolished. However, in one cat, which had not been adrenalectomized, the heart rate rose spontaneously by 25% (to 342) in the course of the next 90 min. A smaller rise of 10% occurred in an adrenalectomized cat following the intravenous injection of 6-hydroxydopamine; since it only developed slowly, it was not responsible for the immediate failure of nerve stimulation to increase heart rate.

In four kittens that had been injected with 10 mg/kg of 6-hydroxydopamine either 1 or 7 days previously, stimulation of the cardiac sympathetic nerves caused a mean increase in heart rate of 11%. However, when the dose was increased to 20 to 30 mg/kg, even 8 days later stimulation of the cardiac nerves did not accelerate the heart or raise the blood pressure. In one experiment in which the heart failed to respond to nerve stimulation after treatment with 6-hydroxydopamine, an intravenous injection of noradrenaline caused a prolonged increase in heart rate of 20%. There was no correlation between the ability of the heart to respond to nerve stimulation and the noradrenaline content of the heart.

Experiments in guinea-pigs gave similar results to those obtained in mice and kittens, in that 6-hydroxydopamine depleted the heart and spleen of noradrenaline without affecting the noradrenaline content of the sympathetic ganglia (Table 3). Yet comparison with

TABLE 3

EFFECTS OF 6-HYDROXYDOPAMINE IN THE GUINEA-PIG

Injections were intraperitoneal in the first, and intravenous in the other experiments

Dose (mg/kg)	Duration (hr)	Noradrenaline content				6-Hydroxydopamine content of heart (μg/g)
		Heart (μg/g)	Stellate ganglia (μg/g)	Superior cervical ganglia (μg/g)	Spleen (μg/spleen)	
Control		1·5	2·8	6·1	0·38	0·01
		1·5	2·5	4·6	0·50	0·00
		1·7	1·2	4·3	0·42	
			2·8	5·0		
			3·9	7·0		
10	16	1·0	3·4	5·5	0·21	0·25
10	2	0·34	3·4	3·8	0·10	1·10
15	2·5	0·35	2·2	3·4	0·12	0·50

Fig. 3 shows certain differences: 16 hr following intraperitoneal injection of 10 mg/kg of 6-hydroxydopamine, only a small fall in the noradrenaline content of the heart had occurred in the guinea-pig, and the 6-hydroxydopamine did not disappear from the heart as rapidly as in the mouse.

The release of catechol amines from the adrenal gland by 6-hydroxydopamine. The effect of 6-hydroxydopamine on the storage mechanism of adrenomedullary amines was investigated in the isolated perfused adrenal gland of the dog. A dose of 0.27 mg infused over a period of 75 to 100 sec caused an immediate and reversible increase in the release of both noradrenaline and adrenaline into the perfusate collected over a 2-min period (Table 4). There was no damage to the gland which was seen to revert to a lower secretion rate after the end of the infusion and to respond subsequently to stimulation by tyramine hydro-

TABLE 4

MEDULLARY AMINES RELEASED BY THE ISOLATED PERFUSED ADRENAL GLAND OF THE DOG IN RESPONSE TO AN INFUSION OF 0·27 mg OF 6-HYDROXYDOPAMINE

Adrenal weight (g)	Noradrenaline released			Adrenaline released		
	Before infusion (ng/min)	During infusion (ng/min)	Increase (%)	Before infusion (ng/min)	During infusion (ng/min)	Increase (%)
0·25	53	90	70	300	500	67
0·28	45	138	206	300	500	67
0·41	50	120	140	450	1,100	144

chloride, prenylamine or dimethylphenylpiperazinium iodide. The flow through the adrenal gland was not affected by the drug. The percentage methylation (adrenaline as percentage of the total amines) was not or very little changed during stimulation by the drug.

DISCUSSION

The present experiments confirm those of Porter *et al.* (1963) concerning the prolonged action of 6-hydroxydopamine on the noradrenaline content of the mouse heart. The action of this drug has a rapid onset, but even after a single injection the effect lasts for more than 1 week in both mice and kittens. We have also shown in guinea-pigs and kittens that 6-hydroxydopamine has no appreciable action on the noradrenaline content of sympathetic ganglia; our findings differ from those in dogs (Stone, Stavorski, Ludden, Wenger, Ross, Totaro & Porter, 1963) with 6-aminodopamine in that a high dose of 6-hydroxydopamine (20 to 30 mg/kg) depleted the hypothalamic noradrenaline in cats. Thus 6-hydroxydopamine appears to have selective effects on different parts of the peripheral and central nervous system.

6-Hydroxydopamine not only lowers the noradrenaline content of heart tissue but also releases catechol amines from the dog adrenal medulla (Table 4). This effect explains the rapid increase in heart rate seen in anaesthetized kittens immediately following an intravenous injection of 6-hydroxydopamine, an effect which was reduced but not abolished by adrenalectomy. In dogs, also, 6-hydroxydopamine caused a prolonged increase in heart rate (Stone *et al.*, 1963).

It has been suggested that 6-hydroxydopamine might be a naturally occurring amine, at least in the rat (Senoh, Creveling, Udenfriend & Witkop, 1959). Our experiments using a paper chromatographic separation failed to show any 6-hydroxydopamine in untreated mouse hearts. This does not, however, eliminate the possibility that quantities not detectable by our methods may occur, particularly in tissues rich in dopamine.

A number of phenylethylamine derivatives are thought to lower noradrenaline concentration in tissues by direct replacement of the noradrenaline; tyramine (Schümann & Philippu, 1962), α-methylnoradrenaline (Maître & Staehelin, 1963) and dopamine (Harrison, Levitt & Udenfriend, 1963) have all been shown to replace noradrenaline stoichiometrically. However, it is obvious from our experiments that, except during the first hour after injection, there is not sufficient 6-hydroxydopamine present in the tissue of mice and kittens to replace the lost noradrenaline. Since 6-hydroxydopamine disappeared so rapidly from the tissue, it was possible that a metabolic product was formed which was responsible for its action. However, no basic metabolite could be detected in extracts from treated mouse hearts. This may have been due to the metabolites being acidic or neutral, chemically unstable and thus lost during extraction, or being present only in small quantities. Such a metabolite would be unlikely to act as a replacement for a considerable quantity of noradrenaline for periods of longer than a week. 6-Hydroxydopamine does not affect uptake of noradrenaline into the rat heart so that it appears not to act in the tissue like a sympathomimetic amine (Iversen, 1964).

It is possible that only small amounts of 6-hydroxydopamine need be present to release, and to maintain the depletion of, stored noradrenaline. Such small amounts may persist in

tissues, as do small amounts of reserpine (Maggiolo & Haley, 1964) and guanethidine (Bisson & Muscholl, 1962). Some other mode of action than replacement must then be postulated.

The fact that the noradrenaline content of heart remains depressed after a single injection of 6-hydroxydopamine for many days has been interpreted (Porter *et al.*, 1963) as permanent damage to the binding sites in the heart. This view is supported by the demonstration of rapid disappearance of the compound from the tissues and our inability to find any persisting metabolite. 6-Hydroxydopamine is a strong reducing agent, very unstable and forming red oxidation products readily. It is, therefore, not unlikely that its metabolic path is totally different from that of sympathomimetic amines and that it causes irreversible damage to receptor sites by virtue of its great chemical reactivity.

In addition to damage to storage mechanisms in the heart, there is functional failure of transmission at sympathetic endings of several organs. In the heart, this occurs immediately after an intravenous injection, when depletion has not yet had time to develop, and is still present a week later, when the noradrenaline content of the heart is reduced by about 50% at most. Furthermore, relaxation of the nictitating membrane was seen at periods ranging from 6 hr to 3 days after an injection of 6-hydroxydopamine (30 mg/kg); in the earlier part of the experiment, it was probably overshadowed by release of amines from the adrenal medulla. Yet the noradrenaline content of the superior cervical ganglia was normal, as was, indeed, that of the stellate ganglion at a time when impulse transmission to the heart was abolished. A similar lack of correlation between block of transmission and depletion of tissue noradrenaline was seen in the dog by Stone *et al.* (1963). It is possible to explain these observations on a multiple-pool concept of storage of tissue noradrenaline, by assuming a complete depletion of the readily available noradrenaline without necessarily a complete depletion of more firmly bound stores. However, they could be explained equally well by assuming that 6-hydroxydopamine has a bretylium-like action on adrenergic nerves. This hypothesis would be more consistent with the observations that the noradrenaline content of the stellate and other ganglia, from which the adrenergic neurones originate, is normal when the peripheral nerve ending is no longer releasing noradrenaline.

SUMMARY

1. A single injection of 6-hydroxydopamine (10 to 30 mg/kg) lowered the noradrenaline content of mouse, kitten and guinea-pig hearts for a period ranging from less than 1 hr to more than 1 week.

2. Methods for the isolation and fluorimetric estimation of 6-hydroxydopamine in tissue were developed. Ion-exchange chromatography, alone or followed by paper chromatography, was used for separation of 6-hydroxydopamine from catechol amines. The 6-hydroxydopamine did not remain in the heart of the mouse for more than 1 hr after the injection.

3. No bases which might have been metabolites of 6-hydroxydopamine were detected in the heart 3 hr after injection of the drug.

4. In kittens and guinea-pigs, 6-hydroxydopamine did not lower the noradrenaline content of the superior cervical or stellate ganglia; large doses (20 to 30 mg/kg) reduced the hypothalamic noradrenaline in kittens.

5. Large doses of 6-hydroxydopamine in kittens abolished the increase in heart rate due to stimulation of the cardiac postganglionic sympathetic nerves; smaller doses (10 mg/kg) reduced this response. No correlation was found between the response to nerve stimulation and the noradrenaline content of the heart.

6. 6-Hydroxydopamine released noradrenaline and adrenaline from the isolated perfused adrenal gland of the dog.

7. The prolonged and specific depletion of noradrenaline from peripheral stores is not due to replacement by 6-hydroxydopamine. Other possible mechanisms of action are discussed.

We are grateful to J. E. McEwen, F.I.S.T., for his skilful help in the noradrenaline estimations. We would like to thank Dr C. S. Miller (Merck Sharp & Dohme Research Laboratories Inc.) for a gift of 6-hydroxydopamine.

REFERENCES

BERTLER, Å., CARLSSON, A. & ROSENGREN, E. (1958). A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta physiol. scand.*, **44**, 273–292.

BISSON, G. M. & MUSCHOLL, E. (1962). Die Bezeihung zwischen der Guanethidin-Konzentration im Rattenherzen und dem Noradrenalingehalt. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **244**, 185–194.

CARLSSON, A. & LINDQVIST, M. (1962). *In vivo* decarboxylation of α-methyl DOPA and α-methyl meta-tyrosine. *Acta physiol. scand.*, **54**, 87–94.

EBERLEIN, W. R. & BONGIOVANNI, A. M. (1955). New solvent systems for the resolution of corticosteroids by paper chromatography. *Arch. Biochem.*, **59**, 90–96.

EULER, U. S. VON & LISHAJKO, F. (1961). Improved technique for the fluorimetric estimation of catecholamines. *Acta. physiol. scand.*, **51**, 348–355.

HARRISON, W. H., LEVITT, M. & UDENFRIEND, S. (1963). Norepinephrine synthesis and release *in vivo* mediated by 3,4-dihydroxyphenethylamine. *J. Pharmacol. exp. Ther.*, **142**, 157–162.

IVERSEN, L. L. (1964). *The Uptake of Noradrenaline by the Isolated Heart.* Ph.D. Thesis, University of Cambridge.

LAVERTY, R. & SHARMAN, D. F. (1964). A sensitive method for the estimation and identification of dopamine. *J. Physiol. (Lond.)*, **175**, 27*P*.

LAVERTY, R. & SHARMAN, D. F. (1965). The estimation of small quantities of 3,4-dihydroxyphenylethylamine in tissues. *Brit. J. Pharmacol.*, **24**, 538–548.

MAGGIOLO, C. & HALEY, T. J. (1964). Brain concentration of reserpine-H^3 and its metabolites in the mouse. *Proc. Soc. exp. Biol. (N.Y.)*, **115**, 149–151.

MAÎTRE, L. & STAEHELIN, M. (1963). Effect of α-methyl-DOPA on myocardial catecholamines. *Experientia (Basel)*, **19**, 573–575.

MUSCHOLL, E. & VOGT, M. (1958). The action of reserpine on the peripheral sympathetic system. *J. Physiol. (Lond.)*, **141**, 132–155.

PORTER, C. C., TOTARO, J. A. & STONE, C. A. (1963). Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. *J. Pharmacol. exp. Ther.*, **140**, 308–316.

SCHÜMANN, H. J. & PHILIPPU, A. (1962). The mechanism of catecholamine release by tyramine. *Int. J. Neuropharmacol.*, **1**, 179–182.

SENOH, S., CREVELING, C. R., UDENFRIEND, S. & WITKOP, B. (1959). Chemical, enzymatic and metabolic studies on the mechanism of oxidation of dopamine. *J. Amer. chem. Soc.*, **81**, 6236–6240.

SENOH, S. & WITKOP, B. (1959). Non-enzymatic conversions of dopamine to norepinephrine and trihydroxyphenethylamine. *J. Amer. chem. Soc.*, **81**, 6222–6231.

SHARMAN, D. F. (1963). A fluorimetric method for the estimation of 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid) and its identification in brain tissue. *Brit. J. Pharmacol.*, **20**, 204–213.

SHARMAN, D. F., VANOV, S. & VOGT, M. (1962). Noradrenaline content in the heart and spleen of the mouse under normal conditions and after administration of some drugs. *Brit. J. Pharmacol.*, **19**, 527–533.

STONE, C. A., STAVORSKI, J. M., LUDDEN, C. T., WENGER, H. C., ROSS, C. A., TOTARO, J. A. & PORTER, C. C. (1963). Comparison of some pharmacologic effects of certain 6-substituted dopamine derivatives with reserpine, guanethidine and metaraminol. *J. Pharmacol. exp. Ther.*, **142**, 147–156.

VOGT, M. (1951). Cortical secretion of the isolated perfused adrenal. *J. Physiol. (Lond.)*, **113**, 129–156.

VOGT, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol. (Lond.)*, **123**, 451–481.