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EFFECT OF DRUGS ON THE NORADRENALINE CONTENT OF BRAIN AND PERIPHERAL TISSUES AND ITS SIGNIFICANCE

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Large single doses of methoserpidine (12 mg/kg) given to rabbits lowered the noradrenaline content of sympathetic ganglia but not that of brain; no sedation was observed. Cats responded to doses ranging from 12 to 0.5 mg/kg with loss of noradrenaline from ganglia as well as from brain, and were sedated by the drug. The effect in man resembles that in the rabbit. Only within the group of reserpine-like drugs do sedation and loss in hypothalamic noradrenaline run parallel. These effects are therefore not causally related. Guanethidine lowers the noradrenaline content of sympathetic ganglia (cats and rabbits), but this effect does not explain the blocking action of the drug on the adrenergic nerve. Effects on the noradrenaline of the brain are variable and may be caused reflexly rather than by direct central action of guanethidine. Repeated intravenous injections of dimethylphenylpiperazinium iodide for a period of 4 hr did not produce any significant change in the noradrenaline content of ganglia or brain of rabbits. In contrast, dexamphetamine (20 mg/kg) produced a small but significant mean fall in noradrenaline content of the superior cervical ganglia and in that of the brain, but the effects were not seen in every rabbit. Prolonged administration of the mono-amine oxidase inhibitors pheniprazine and phenylhydrazinobutane raised the noradrenaline content of the brain of rabbits but not that of cats, whereas it raised the noradrenaline of the ganglia of cats but not (or rarely) that of rabbits. The question of correlation between a rise in the noradrenaline content of the brain and certain clinical signs is discussed. Finally, a comparison is made in rabbits between the changes produced by drugs in the noradrenaline content of the heart and of the superior cervical ganglion. The changes run parallel and are only occasionally more pronounced in the heart.

A study was made of the changes in the noradrenaline content of sympathetic ganglia and brain produced by a series of drugs. The first section deals with drugs which have pharmacological actions on different parts of the postsynaptic sympathetic neurones. Two of these drugs, methoserpidine (10-methoxydeserpidine) and guanethidine, cause hypotension, and the question arose whether this effect can be explained by changes in the noradrenaline content of the peripheral sympathetic neurones. A second point was to ascertain whether these drugs have any action on the brain. The actions of the two other drugs, the nicotine-like compound dimethylphenylpiperazinium iodide, which stimulates sympathetic ganglia, and

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amphetamine, which stimulates nerve endings, were examined. If this stimulation was accompanied by a loss in noradrenaline from the sympathetic neurone this would support the theory (Burn, Leach, Rand & Thompson, 1959) that these substances act by releasing noradrenaline.

The second part of this work is concerned with two amine oxidase inhibitors known or reputed to raise the noradrenaline content of tissues, particularly of brain; the object was to test whether there is a correlation of such action with effects on behaviour. Throughout the work profound species differences were encountered and required special attention.

METHODS

The experiments were carried out on rabbits and cats. At different time intervals after the parenteral administration of drugs, the animals were rapidly anaesthetized with chloroform and bled to death. The superior cervical ganglia, the hypothalamus (from which corpora mammillaria and optic tracts had been dissected off) and, in some rabbits, the right atrium (including the auricular appendage and the atrial septum) were excised, quickly weighed, and immersed in acidified ethanol cooled in dry ice for extraction of noradrenaline. The extraction procedure for nervous tissue, followed by paper-chromatographic separation of the noradrenaline from other active substances, and its elution from the paper have been described (Vogt, 1952, 1953, 1954). The heart tissue (125 to 370 mg) was more difficult to extract than nervous tissue (Muscholl, 1959). It was cut up finely with scissors and homogenized in two portions, each in 1.5 ml. acid ethanol, and another 5 ml. acid ethanol used for washing the homogenizer. The residue obtained after evaporation of the acid ethanolic extract was not taken up in dilute hydrochloric acid, but treated like a plasma sample (Vogt, 1952) by extracting it with a total of 2.4 ml. slightly acidified ethanol saturated with sodium chloride, concentrating the extract by evaporation and applying the concentrate directly to paper. The deposit formed during concentration was re-extracted and this washing also applied to the paper.

Because of the small quantities of noradrenaline to be estimated, bioassay on the pithed rat blood pressure (Muscholl & Vogt, 1957) was used in preference to chemical estimation. Frequent checks were made on the recovery of 0.1 to 0.2 μg noradrenaline added to cerebellar tissue which does not contain appreciable amounts of the amine. Recoveries ranged from 65 to 75%. Precautions taken throughout the extraction included avoidance of direct sunlight and cooling of the samples either in ice-water or in dry ice whenever they were stored between different steps of the procedure.

In most experiments, the left superior cervical ganglion was extirpated before any drug was administered. Estimation of its noradrenaline content supplied the control figure for the right ganglion extirpated after treatment with drugs. The extirpation of the left ganglion was done under ether with aseptic precautions, and recovery was uneventful. At this preliminary operation, decentralization of the right superior cervical ganglion was sometimes carried out by removing about 1 cm of the preganglionic sympathetic trunk. Treatment with the drug was begun 13 to 23 days after the preliminary operation in cats; in rabbits the interval was 3 to 28 days. The short intervals were only used when drug treatment was prolonged.

Drugs. Methoserpidine (10-methoxydeserpidine; Decaserpyl, Roussel) was injected intravenously into rabbits and intraperitoneally into cats. The solution was prepared by dissolving 10 to 20 mg in 1 ml. warm polyethylene glycol 400, adding 5 mg tartaric acid and 4.5 mg benzyl alcohol dissolved in a little water, and making up to 5 ml. solution with distilled water. The solution was sterilized by brief boiling before administration to cats.

Guanethidine sulphate [2-(octahydroazocin-1-yl)ethylguanidine sulphate] was injected as 2% solution in 0.9% sodium chloride solution very slowly into the ear vein of rabbits. For intra-

peritoneal injection in cats the solution was sterilized by boiling for 1 min. 1,1-Dimethyl-4-phenylpiperazinium iodide was injected intravenously into rabbits as a 0.2% solution in 0.9% sodium chloride solution. The injections were repeated at frequent intervals. Dexamphetamine sulphate was injected subcutaneously into rabbits as a 2% solution in 0.9% sodium chloride solution. The pressor effects in the pithed rat were studied after intravenous injection of solutions of dexamphetamine sulphate or of racemic amphetamine sulphate. Pheniprazine (α-methylphenethylhydrazine; 1-phenyl-2-hydrazinopropane; amphetamine hydrazide) hydrochloride and phenylhydrazinobutane hemisulphate (1-methyl-3-phenylpropylhydrazine hemisulphate) were injected subcutaneously in cats and rabbits as 2% solutions in 0.9% sodium chloride solution. Immediately before use the solutions, which are slightly acid, were neutralized with solid sodium bicarbonate. Doses of all these substances are expressed in terms of the weight of the salt.

RESULTS

Control experiments

When drugs were used which might have an action on the rat blood pressure, it was important to find out whether the eluates containing the noradrenaline might be contaminated with the drugs. Experiments were therefore performed in which the R_F value of amphetamine salts was estimated in the solvent system used for separating the noradrenaline from other bases (15 ml. 0.1 N hydrochloric acid in 85 g phenol). Ninhydrin, dissolved in a mixture of acetone and glacial acetic acid in the proportions 9:1, was used to detect the location of the amine. It was found that amphetamine hydrochloride travelled to a place near the front of the chromatogram, and therefore far away from the region of noradrenaline. When, however, amphetamine sulphate was chromatographed, an additional elongated spot formed which started in the noradrenaline region. The same result was obtained with the hydrochlorides and sulphates respectively of pheniprazine and of ephedrine.

It would therefore be possible for any of these bases to contaminate the noradrenaline region of the chromatogram if they were present as sulphates in the final tissue extract. This, however, is ruled out by the predominance of chloride in tissues and by the extraction procedures in which hydrochloric acid is the only acid used.

It was, nevertheless, desirable to know how much amphetamine would be required to produce a rise in blood pressure in the pithed rat. Small rises in blood pressure of equal height were obtained by 1 μg dexamphetamine sulphate and 3 ng noradrenaline; when the doses were increased the ratio became increasingly unfavourable to the dexamphetamine, 20 μg for example being equivalent to 10 ng or, in another rat, to 5 ng noradrenaline. There was no danger, therefore, that amounts of dexamphetamine capable of producing a pressor effect were present in the eluates of the noradrenaline region of the chromatograms. Pheniprazine is also pressor in the pithed rat, but less potent than dexamphetamine, and phenylhydrazinobutane is even less effective.

One incidental finding which was contrary to expectation is worth mentioning: in the pithed rat, in which pressure effects must be entirely due to peripheral effects of the drug, there was no difference in the rise obtained by injecting equal weights of dexamphetamine or of racemic amphetamine. The assumption, therefore, that dexamphetamine is less active on the circulation than the laevo-isomer does not hold for the rat.

Control values for the concentration of noradrenaline in the hypothalamus

In most of the experiments on rabbits, the control values were obtained from 31 untreated rabbits of mixed breeds taken at different periods during the course of the work ; the mean amounted to 1.07 ± 0.05 (s.e.) with a range of 0.54 to 1.73 μg/g fresh tissue. One series of experiments was done on a single breed ; in this instance the mean used was that obtained on 6 untreated animals of that breed. The control values for cats were collected over the course of several years on 20 animals ; the mean was 1.40 ± 0.07 and the range 0.9 to 2.1 μg/g. None of these values is corrected for the recovery of about 70%.

Methoserpidine

This isomeride of reserpine carries a methoxyl group on C_{10} instead of on C_{11} of ring A, and has been reported to have a hypotensive effect in man while lacking the central depressant effect of reserpine (Gros, Peterfalvi & Jequier, 1959). This dissociation of central and peripheral actions has also been reported in mice, rats and dogs. The present work was carried out in order to see whether the loss of noradrenaline which accompanies the pharmacological actions of reserpine in both the brain and the peripheral sympathetic neurones would be restricted to the periphery after treatment with methoserpidine. Such a finding would strengthen the view that the hypotensive effect of reserpine and its analogues is essentially due to a peripheral action on the sympathetic system.

Experiments were carried out on rabbits ; intravenous injection of 2 and 6 mg/kg methoserpidine produced very small falls in the noradrenaline content of ganglia, and 12 mg/kg was required in order to obtain an average fall of 59% (Table 1) ; even at this dose the drug caused no sedation, and there was no effect on the catecholamines in the hypothalamus. Only during the first few minutes were there clinical signs: slowing, followed by acceleration, of respiratory rate, closure of the eyelids and muscular weakness and tremors ; no sedation, miosis or hypersensitivity to light occurred as after an injection of reserpine. Injection of the vehicle produced no signs at all.

These experiments show clearly that, in the rabbit, methoserpidine reduces the noradrenaline of ganglia by nearly 60% without exerting a similar effect on the brain ; however, this substance is much less potent than reserpine, which, in a single dose of 2 mg/kg, reduces the ganglionic noradrenaline by about 90% ; this is the degree of depletion required to produce failure of impulse transmission from the nerve to the tissue (Muscholl & Vogt, 1958). There is little doubt that repeated administration of methoserpidine, as with reserpine, would exert a cumulative effect so that lack of transmitter would account for the lowering of the vasomotor tone.

The experiments were then extended to cats, which are much more sensitive to reserpine than rabbits and in this respect resemble the human patient. The results were very surprising. Doses of methoserpidine 12 mg/kg which produced no changes of behaviour nor any persistent autonomic signs in the rabbit had a reserpine-like action on cats ; relaxation of the nictitating membrane, miosis and a decrease in spontaneous activity were prominent, and chemical analysis revealed loss of noradrenaline from sympathetic ganglia and brain alike (Table 1). The

TABLE 1

EFFECT OF METHOSERPIDINE ON THE NORADRENALINE CONCENTRATION IN HYPOTHALAMUS AND SUPERIOR CERVICAL GANGLIA

Noradrenaline in μg/g fresh tissue (means, with range in brackets). [1] Standard error of the mean. Significant falls marked * ($P<0.05$) or ** ($P<0.001$). † In these cats both superior cervical ganglia were excised after giving the drug and the noradrenaline concentrations compared with that of untreated cats

Species	No. of animals	Dose, mg/kg	Interval between injection and removal of tissues (hr)	Ganglia			Hypothalamus	
				Control (noradrenaline, μg/g)	After treatment (noradrenaline, μg/g)	% change	After treatment (noradrenaline, μg/g)	% change from normal mean of 1.07 ± 0.05^{1} μg/g
Rabbits	1	2	19	4·9	4·3	−12	0·88	−18
	1	6	20	3·7	2·9	−21	1·03	−4
	7	12	17–26	5·9 (3·9–8·1)	2·4 (1·7–3·1)	−59**	1·01 (0·83–1·34)	−5
								% change from normal mean of 1.40 ± 0.07^{1} μg/g
Cats	2	12	18–19	4·7 (4·4, 5·0)	1·8 (1·8, 1·8)	−61*	0·41 (0·39, 0·44)	−71*
	2	4	18–19	3·2 (2·5, 3·8)	0·5 (0·5, 0·6)	−84*	0·64 (0·61, 0·67)	−54*
	5†	0·5	18	4·2 (2·5–4·6) (normal mean)	2·4† (1·8–3·5)	−43**	1·06 (0·82–1·40)	−24*
	3	0·25	18–19	4·0 (3·1–4·6)	3·5 (2·3–5·3)	−12	1·47 (1·43–1·54)	+5

same happened with the dose of 4 mg/kg ; the percentage of noradrenaline lost from brain was smaller than that lost from sympathetic ganglia, but was still over 50%. When the dose was reduced to 0.5 mg/kg, loss of noradrenaline from ganglia and from hypothalamus was less, but still significant for both tissues. Even at this low dose level, the peripheral sympathetic tissue was only slightly more affected than the brain. In four of the five cats, sedation was quite obvious, though not severe. By reducing the dose to 0.25 mg/kg a further attempt was made to obtain changes in the peripheral sympathetic neurones and not in the hypothalamus: the last row of Table 1 shows that this aim was not achieved: neither ganglionic nor hypothalamic noradrenaline were significantly altered, and no autonomic signs or changes in behaviour were observed. We must therefore conclude that in the cat methoserpidine, like reserpine itself, acts indiscriminately on brain and peripheral sympathetic neurones, and that there is no preferential effect on the sympathetic system as was found in the rabbit. This is the more surprising because the dog, which is at least as sensitive to reserpine as is the cat, tolerates daily parenteral injections of 1 mg/kg methoserpidine for 3 months without showing any central effects (Peterfalvi & Jequier, 1960). Whether the substance does not penetrate into the brain of rabbits and dogs whereas it enters the cat brain, or whether, what is more likely, brain tissue of rabbit and dog is not susceptible to the action of the drug, cannot be stated.

Guanethidine

Guanethidine is not related chemically to reserpine. However, it has a prolonged hypotensive effect (Page & Dustan, 1959), the biochemical background of which is not clear. Physiological experiments (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960) have shown that its hypotensive effect is not explained by a block of autonomic ganglia or failure of conduction in adrenergic nerves.

In experiments done on rabbits, 15 to 20 mg/kg guanethidine invariably reduced the noradrenaline content of sympathetic ganglia (Table 2), the change being little different whether the drug was allowed to act for 2, 4 or 24 hr. Depletion was never greater than 65% and is therefore unlikely to be the cause of the functional failure of these neurones which occurs very soon after injection of the drug (see Discussion). The results agree with observations by other authors (Cass, Kuntzman & Brodie, 1960 ; Butterfield & Richardson, 1961) on the catechol amine concentration of the heart of rabbits and dogs. They differ from the results of Cass *et al.* (1960) in that, in the majority of the experiments, a diminution was also found in the noradrenaline content of the hypothalamus. However, it is obvious from the ranges (see Table 2) that the effect on the brain tissue was variable from animal to animal, so that a special experiment was conducted on 6 rabbits (Table 2, row 4) in which the hypothalami only were analysed. The rabbits were injected with guanethidine and their brain noradrenaline compared with that of 6 untreated rabbits from the same strain: there was a small, but significant, mean fall in the treated group, but again it was obvious that the ranges were overlapping (Table 2). All rabbits showed transient cardiac arrhythmia, gasping and muscular weakness for a few minutes after the injection, but appeared normal for the next 2 hr ; later, however, diarrhoea and dilatation of the denervated pupil indicated autonomic disturbances.

TABLE 2

EFFECT OF GUANETHIDINE ON THE NORADRENALINE CONCENTRATION IN HYPOTHALAMUS AND SUPERIOR CERVICAL GANGLIA

Noradrenaline in μg/g fresh tissue (means, with range in brackets). * Falls significant ($P<0.05$). [1] This experiment was carried out on a different breed of rabbits in which the mean concentration of hypothalamic noradrenaline of 6 controls was 1·74±0·067[3] (range 1·58–2·00). [2] The percentages are reduced to 25 and 23% respectively if evaluated against the mean of 2 litter mate controls (1·1±0·18[3] μg/g). [3] Standard error of the mean

Species	No. of animals	Dose, mg/kg	Interval between injection and removal of tissues (hr)	Ganglia			Hypothalamus	
				Control (noradrenaline, μg/g)	After treatment (noradrenaline, μg/g)	% change	After treatment (noradrenaline, μg/g)	% change from normal mean of 1·07±0·05[3] μg/g
Rabbits	3	15	2	4·3 (3·0–5·1)	2·1 (1·3–2·7)	−51*	1·05 (0·92–1·27)	−2
	4	15	4	5·8 (5·4–6·3)	2·2 (1·1–4·0)	−62*	0·83 (0·37–1·49)	−23
	3	20	4	4·7 (4·0–5·6)	2·7 (1·8–3·8)	−41*	0·59 (0·51–0·65)	−45*
	6	20	4	—	—	—	1·38 (1·04–1·60)	−21*[1]
	3	20	24	5·3 (4·6–6·4)	1·8 (1·3–2·2)	−66*	0·90 (0·82–0·96)	−16
								% change from normal mean of 1·4±0·07[3] μg/g
Cats	1	15	2	4·0	2·5	−37	0·67	−52
	5	15	4–5	4·2 (3·1–5·7)	2·5 (1·5–3·4)	−40*	0·74 (0·53–1·0)	−47*[2]
	4	15	24	4·4 (3·0–6·1)	2·3 (1·2–3·8)	−47	0·77 (0·54–1·08)	−45*[2]

In the cat, intraperitoneal injections of 15 mg/kg guanethidine caused a reduction of noradrenaline in the superior cervical ganglion averaging about 40%, or a little less than in the rabbit. The noradrenaline concentration in the hypothalamus was usually reduced, but the effect was variable and the mean reduction very small if calculated as percentage of the noradrenaline content of untreated litter-mates (explanations of Table 2). The signs, too, were variable and differed in cats from different colonies. Whereas one breed frequently showed nothing but a gradual relaxation of the nictitating membranes which started as early as 15 min after the injection, and later a general decrease in motor activity and a tendency to lie still in a corner, another breed went usually through an early phase of some excitement, widening of the pupils (denervated and innervated) and contraction of both nictitating membranes before the decline in activity and the signs of peripheral sympathetic inhibition set in. The analyses of catechol amines represented in Table 2 are all from the second breed, and it is interesting that the concentrations of noradrenaline in the hypothalamus obtained on the first breed (Dagirmanjian & Vogt, to be published) were less frequently below normal. An interpretation of this difference will follow in the discussion.

Dimethylphenylpiperazinium iodide

This nicotine-like substance causes a stimulation of autonomic ganglia which is short-lived and not followed by paralysis (Chen & Portman, 1954); consequently, the same effect can be produced repeatedly, and the compound appeared ideally suited to test the hypothesis (Burn *et al.*, 1959) that the action of nicotine on the vessels of the rabbit ear, the cat nictitating membrane and piloerectors, and other organs supplied with sympathetic fibres was produced indirectly by a release of noradrenaline and not due to a direct action of the drug on the ganglia. The site of the stores from which the noradrenaline would be released could be either the sympathetic neurones or the tissue itself. As there is no proof for the location of noradrenaline outside the sympathetic neurones within these tissues (Coupland & Heath, 1961) and as the catechol amines disappear after degeneration of the sympathetic nerves, it seemed reasonable to assume that any depletion would affect or at least include the sympathetic ganglia.

Since large single doses of dimethylphenylpiperazinium are lethal, repeated small doses were given intravenously to rabbits. The single dose was 0.17 to 0.3 mg/kg, and the total dose ranged from 3.5 to 11.1 mg/kg. The injections were repeated every 7 to 10 min for a period of 4 hr. During and for a brief period after each injection there were signs of parasympathetic stimulation, and these were followed by a more prolonged period of signs of sympathetic discharge. Rise in temperature and tremor were seen when a series of injections had been given. The intensity of the signs was not steady throughout the 4 hr, some responses becoming less and others more intense as injections were repeated.

Preliminary experiments, in which the noradrenaline content of the two superior cervical ganglia was compared with that of normal controls, showed inconsistent results after 4.5 mg/kg dimethylphenylpiperazinium, but a significant fall occurred when the total dose injected was 7.5 mg/kg. The experiments were then repeated with this dose employing the more precise method of using the noradrenaline con-

centration of the left ganglion as control for that of the right. The results (Table 3) indicated a small fall (22%), but the difference of the means was not significant. Further increase in the dose did not appear to change the results. Observations on the hypothalamus were also not consistent. There were some low figures after 4.5 mg/kg and one after 9 mg/kg (Table 3), but the fact that these were only found occasionally suggested that they were not caused by a direct action of the drug.

TABLE 3

EFFECT OF DIMETHYLPHENYLPIPERAZINIUM ON THE NORADRENALINE CONCENTRATION IN HYPOTHALAMUS AND SUPERIOR CERVICAL GANGLIA

Noradrenaline in μg/g fresh tissue (means, with range in brackets). [1] Standard error of the mean. * Change non-significant

		Ganglia			Hypothalamus	
No. of rabbits	Total dose, mg/kg	Control (noradrenaline, μg/g)	After treatment (noradrenaline, μg/g)	% change	After treatment (noradrenaline, μg/g)	% change from normal mean of 1·07±0·05[1] μg/g
6	7·5	5·4 (3·5–5·6)	4·2 (2·8–5·1)	−22*	1·0 (0·8–1·3)	−5*
1	9·0	4·2	3·3	−22	0·5	−53
1	11·0	4·1	4·2	+2	1·0	−5

Amphetamine

Burn & Rand (1958) have suggested that amphetamine, which loses its action after denervation of the sympathetic supply to an organ, is sympathomimetic because it releases noradrenaline at its site of action. Experiments on rabbits were carried out in order to see whether experimental evidence for this theory could be obtained either at a peripheral or a central site. From previous experiments (Vogt, 1954) it was known that the related compound ephedrine did not reduce the noradrenaline content of the hypothalamus of cats. Amphetamine, however, is much more potent than ephedrine as a central stimulant, and, for example, acts as an antagonist of the depression produced by reserpine, or by 5-hydroxytryptamine injected into the cerebral ventricles.

Rabbits were injected twice at 2-hourly intervals with dexamphetamine sulphate 10 mg/kg, and the tissues were examined 4 hr after the first injection. Usually, but not invariably, there was a fall in the noradrenaline content of the superior cervical ganglia and of the hypothalamus (Table 4). The mean difference from the controls was below 30% but significant. A few additional experiments, not listed in Table 4, confirmed the general trend, but it was obvious that individual rabbits responded differently, although the signs presented by the rabbits were very similar. There was first agitation and apparent apprehensiveness, followed by very characteristic automatisms consisting of licking and chewing movements: the rabbits chewed the bars of their cage but refused food. At this stage they paid little attention to external stimuli. There was dilatation of the pupil, exophthalmos and retraction of the nictitating membrane only on the innervated side, thus confirming the findings of Marley (1961) and the similar observations on ephedrine made by Burn & Tainter (1931). Two experiments (Table 4, row 2) were done after decentralization of the superior cervical ganglion by section of the preganglionic fibres. This procedure reduced the effects of the drug on the eye, and, as far as one can

TABLE 4

EFFECT OF DEXAMPHETAMINE SULPHATE (20 MG/KG SUBCUTANEOUSLY) ON THE NORADRENALINE CONCENTRATION IN HYPOTHALAMUS AND SUPERIOR CERVICAL GANGLIA OF RABBITS

Noradrenaline in μg/g fresh tissue (means, with range in brackets). The drug was given in two equal doses at 0 and 2 hr, and the rabbits were killed at 4 hr. [1] Standard error of the mean. * Fall significant ($P<0.02$)

	Ganglia			Hypothalamus	
No. of rabbits	Control (noradrenaline, μg/g)	After treatment (noradrenaline, μg/g)	% change	After treatment (noradrenaline, μg/g)	% change from normal mean of 1.07 ± 0.05^{1} μg/g
11	5·5 (2·4–7·8)	3·9 (2·9–5·2)	−29%*	0·78 (0·53–1·18)	−27%*
2 (right ganglion decentralized)	4·3 (3·4, 5·2)	4·1 (2·8, 5·5)	−4%	0·94 (0·56, 1·33)	−12%

tell from the limited number of observations, also prevented the fall in ganglionic noradrenaline. Owing to the smallness and variability of the effect in the intact ganglion, it did not appear justifiable to repeat this experiment on the large number of animals which would have been required to obtain a statistically valid difference. The moderate pupillary dilatation seen in these rabbits demonstrates a peripheral component of the action of amphetamine, a fact which is also in accordance with the findings of Marley (1961) and with the effect on the blood pressure of the pithed rat described under the section headed "Control Experiments."

Pheniprazine and phenylhydrazinobutane

Noradrenaline concentrations. Pheniprazine and its homologue phenylhydrazinobutane are potent inhibitors of mono-amine oxidase and therefore might cause the accumulation of a number of amines in the tissues. However, from previous work with another inhibitor of mono-amine oxidase, iproniazid, it was known that, at least in the cat, brain noradrenaline is lowered, not raised, after a single dose of 100 mg/kg (Vogt, 1959). The two drugs used in the present experiments are more potent inhibitors than iproniazid, and were usually given repeatedly. A rise of brain noradrenaline after a 5-day treatment has been reported for the rabbit (Biel, Drukker, Shore, Spector & Brodie, 1958).

As a precaution, it was first necessary to ascertain that any rise in the noradrenaline content of tissues which might be found was produced by an accumulation of the amine *in vivo* and not by a protection from enzymatic breakdown during the dissection and homogenization of the tissue. This was done by injecting 6 rabbits intraperitoneally with 15 mg pheniprazine/kg and analysing the tissue 1 hr later. This is ample time for the substance to enter the brain, since 1 hr after a subcutaneous injection the brain 5-hydroxytryptamine is already maximally increased (Spector, Shore & Brodie, 1960). There was a rise of 5% over the normal mean for the noradrenaline in ganglia, and of 8% for that in brain, differences which are not significant.

In the first experiments, pheniprazine 20 or 30 mg/kg was injected, the latter quantity in divided doses, and the rabbits were killed about 17 hr after the first dose. The noradrenaline rose in the brain but not in the ganglia. Then 20 mg/kg was administered on two consecutive days: this killed all rabbits except one which also showed a high noradrenaline content of the hypothalamus (row 3, Table 5). Death

TABLE 5

EFFECT OF INHIBITORS OF MONOAMINE OXIDASE ON THE NORADRENALINE CONCENTRATION IN HYPOTHALAMUS AND SUPERIOR CERVICAL GANGLIA

Noradrenaline in μg/g fresh tissue (means, with range in brackets). * Rise significant ($P<0.05$). [1] Standard error of the mean. [2] Only 5 pairs of ganglia analysed. [3] Only 6 pairs of ganglia analysed. The drugs were injected subcutaneously in single doses except for the 30 mg/kg, which was given in two doses

					Ganglia			Hypothalamus	
Species	No. of animals	Drug	Daily dose mg/kg	No. of days of treatment	Control (noradrenaline, μg/g)	After treatment (noradrenaline, μg/g	% change	After treatment (noradrenaline, μg/g	% rise above normal mean of 1·07±0·05[1] μg/g
Rabbits	3	Pheniprazine	20	1	6·2 (5·3–7·6)	6·7 (6·4–7·1)	+8	1·9 (1·8–2·0)	80*
	3	hydro-	30	1	6·0 (5·1–6·8)	5·9 (5·5–6·1)	−2	1·8 (1·7–2·0)	71*
	1	chloride	20	2	4·7	3·8	−19	2·3	115*
	2		5	3	6·0 (5·3, 6·7)	8·2 (8·1, 8·3)	+37·5	2·5 (2·1, 2·9)	131*
	4		5	5	4·0 (3·3–4·2)	5·0 (4·2–7·2)	+28	3·9 (2·7–5·5)	266*
	4		5	10	5·6 (4·7–6·4)	8·4 (8·0–8·9)	+49*	2·2 (1·9–2·7)	107*
	1		5	19	—	—	—	2·3	115*
	4	Phenyl-	20	1	3·9 (2·9–5·9)	4·4 (2·9–6·1)	+12	1·9 (1·4–2·2)	73*
	4	hydrazino-	30	1	3·5 (2·7–4·3)	4·8 (3·6–6·7)	+39	1·6 (0·8–2·2)	47
	6	butane	5·3	4	4·7 (3·7–6·2)	4·9 (1·0–7·8)	+5[2]	2·7 (2·1–3·3)	150*
	5	sulphate	5·3	5	4·0 (2·2–6·7)	5·6 (3·1–7·7)	+40	2·6 (1·7–3·6)	146*
									% rise above normal mean of 1·4±0·07[1] μg/g
Cats	7	Pheniprazine hydrochloride	3	5	4·2 (3·0–5·1)	7·0 (5·3–9·1)	+67*[3]	1·9 (1·1–3·7)	32

occurred from respiratory embarrassment. The dose was then reduced to 5 mg/kg/day, which appeared to be tolerated indefinitely. In experiments lasting from 3 to 19 days the brain noradrenaline was always high, with a maximum at 5 days; the mean ganglionic noradrenaline rose slowly, the rise reaching about 50% and statistical significance after 10 injections.

Essentially the same results were obtained with phenylhydrazinobutane, only that the accumulation of noradrenaline in the hypothalamus was slightly less than with the lower homologue.

Experiments were then conducted on cats, since their response to another mono-amine oxidase inhibitor, iproniazid, was shown to be a fall, not a rise, in hypothalamic noradrenaline. Pheniprazine was chosen and the dose reduced to 3 mg/kg/day since preliminary tests showed larger doses not to be tolerated. The results brought out another species difference: after 5 days' treatment the noradrenaline of the ganglia was considerably raised whereas the mean value for the brain was not significantly affected (Table 5, bottom row). Five of the values were within $\pm 22\%$ of normal, but two of them, 2.3 and 3.7 μg/g, were above the range of 0.9 to 2.1 determined on 20 normal cats, and point to the possibility that a rise in brain noradrenaline can occur in the cat after treatment with a mono-amine oxidase inhibitor.

Clinical observations and changes in behaviour. After single large doses of pheniprazine rabbits showed dilatation of the innervated pupil, some exophthalmos, and a rise in temperature and in rate of respiration. The rabbits appeared more alert than normal. Repeated smaller doses gave rise to some pupillary dilatation, most obvious shortly after each injection, and, after 2 or 3 injections, to an increase in alertness which was maintained to a variable degree till the end of the experiment; a few rabbits became fierce. The same signs occurred after injection of the butane homologue, but often in an attenuated form. With this compound, too, some rabbits became fierce, and a special experiment (see below) was therefore designed in order to determine whether there were any real differences in the response to the two compounds.

It may be noted that the automatisms described in a preceding section and so characteristic for amphetamine were completely absent with either of the hydrazino-derivates.

Cats given pheniprazine in small daily doses showed pupillary dilatation, exophthalmos and retraction of the nictitating membrane from the third day onwards, very much like the rabbits. Five of the seven animals, however, developed rigidity of the limbs and ataxia, signs not seen in the rabbit, and only the remaining two cats were alert and excited on the last two days of treatment. The two cats with a high brain noradrenaline belonged to the group showing ataxia. Large single doses given to cats (25 mg/kg) were reported to produce the same signs (including ataxia) as those seen in the present work with repeated small doses (Eltherington & Horita, 1960).

Ataxia has also been observed in dogs after very much more prolonged treatment with this compound (Maling, Highman & Spector, 1961). The dogs were injected for 3 to 52 weeks, and at the end of the experiments there was no change in the noradrenaline content of the brain stem. This shows that, in this respect too, cats and dogs respond in the same way.

The peculiar observation of fierce behaviour of single rabbits after treatment with the amine oxidase inhibitors prompted an experiment designed to decide whether this was a genuine effect of the drugs and whether there was a difference between the effect of pheniprazine and phenylhydrazinobutane. Equimolar solutions of the two drugs were prepared and the experimenter injected those, and a control solution of buffer (*p*H 3), for 5 days into two rabbits each, without knowing the composition of the solutions. The task of the experimenter was to try to identify the drug injected by observing the behaviour elicited in the rabbits, on the assumption that the pheniprazine was the more potent drug. The rabbits injected with the buffer were so easily identified by their docility that the contrast with the other animals left no doubt about the efficacy of the two drugs; of the two pairs of rabbits given drugs, however, one animal became wild and aggressive, rushing toward the observer and trying to bite, whereas the other three became simply agitated. The aggressive rabbit had been injected with pheniprazine. Another two pairs of rabbits were then injected in the same way, and again only one animal became aggressive, this time after treatment with phenylhydrazinobutane. The responses of the other three rabbits were alertness and some unfriendliness. It became obvious that the individuality of the rabbit rather than the drug determined the response. Four docile rabbits were therefore chosen and two each injected with pheniprazine and phenylhydrazinobutane as before. This time two rabbits became aggressive (after pheniprazine) and two excited only (after phenylhydrazinobutane). A cross-over test was then made after an interval of eight weeks, normal behaviour having returned as early as five days after cessation of the injections. The same two rabbits which had become aggressive before did so again, but this time after phenylhydrazinobutane. Nineteen weeks later the experiment was repeated; this time, however, the rabbits which had become aggressive were given a different inhibitor each, and so were the remaining two rabbits. Injections were continued for seven days. As before, the two rabbits which had responded with aggressive behaviour to either drug were the only ones to do so now. All four rabbits were killed at the end of this experiment and the hypothalamic noradrenaline determined: the "aggressive" rabbits showed an increase of 202 and 301%, the less affected rabbits of 223 and 311%. The smaller increases were after phenylhydrazinobutane, the larger ones after pheniprazine. The different degree of accumulation of catechol amines was therefore not related to the difference in behaviour but to the choice of drug.

These experiments do not substantiate the view that there is much difference in the effect on behaviour between pheniprazine and its butane homologue, though they confirm the greater effect of the propane derivate on the accumulation of noradrenaline in the brain. They further demonstrate that the constitution of the individual rabbit plays a large role in determining the effect of amine oxidase inhibitors on behaviour.

Comparison of the changes in noradrenaline content produced by drugs in heart and superior cervical ganglion

The object of this last group of experiments was to see to what extent changes obtained by drugs in the noradrenaline content of sympathetic ganglia reflected changes occurring in an organ like the heart which contains few, if any, sympathetic

ganglion cells but a large number of postganglionic fibres. The work of Schaepdryver & Preziosi (1959) on the heart of mice suggested that changes in the noradrenaline content are more readily produced in the heart than in ganglia. From the functional point of view, the availability of transmitter at the nerve endings should be more important than the stores in the nerve cells.

Experiments with methoserpidine (12 mg/kg, rabbits killed 18 to 26 hr after the injection) showed greater depletion of noradrenaline from the heart than from the superior cervical ganglion only in one of three animals (no. 1, Table 6). When guanethidine was injected (15 or 20 mg/kg, excision of tissue 2, 4 or 24 hr after

TABLE 6

EFFECT OF DRUGS ON THE NORADRENALINE CONCENTRATION (μG/G FRESH TISSUE) OF THE RIGHT ATRIUM AND SEPTUM OF THE RABBIT HEART
(Comparison with effects on superior cervical ganglion)

* Mean±s.e. of the mean of 10 normal rabbits. † Four daily doses, rabbits killed 18 to 21 hr after the last injection

				Noradrenaline concentration				
				Atrium				
Rabbit no.	Drug and mode of injection	Dose mg/kg	Duration of experiment	μg/g	% change from normal mean	Control ganglion μg/g	Ganglion after treatment μg/g	% change from control side
	None	None		1·73±0·12*	0			
1	Methoserpidine intravenously	12	18 hr	0·39	−77	5·46	3·09	−45
2		12	21 hr	0·40	−77	8·10	2·77	−66
3		12	22 hr	0·54	−69	6·15	1·74	−72
4	Guanethidine intravenously	15	2 hr	0·79	−54	4·80	2·72	−44
5		15		0·89	−49	3·03	1·25	−59
6		15	4 hr	0·64	−64	5·83	1·13	−81
7		15		0·35	−80	5·76	1·87	−68
8		20	4 hr	0·63	−64	5·62	3·84	−32
9		20		0·30	−83	4·02	2·65	−34
10		20		0·70	−60	4·31	1·77	−58
11		20	24 hr	0·74	−57	4·63	1·95	−58
12		20		0·34	−80	4·83	1·33	−72
13		20		0·15	−91	6·42	2·17	−66
14	Phenylhydrazinobutane sulphate subcutaneously	5·3	4 days	2·34	+35	4·34	4·00	−8
15		5·3		2·52	+46	5·26	5·00	−5
16		5·3		3·06	+77	6·25	7·81	+25

the injection), 3 of the 10 rabbits (nos. 8, 9, 13) showed greater depletion in the heart than in the ganglia, but the reverse was true of rabbit 6. When a drug was given which tended to raise the noradrenaline content of tissues (phenylhydrazinobutane, 5.3 mg/kg for 4 days), there was a significant rise in the heart of each rabbit, whereas the changes in the ganglia were not significant.

From these experiments it follows that the noradrenaline content of the heart is, in some circumstances, more readily changed by drugs than the content of the superior cervical ganglion, but only rarely does the change in the ganglion not reflect the events in the peripheral tissue.

DISCUSSION

Methoserpidine. Several aspects of the results obtained with this drug are of interest. The lack of sedation observed in the rabbit even with high doses was correlated with a failure to find a loss of noradrenaline from the hypothalamus. Yet this drug caused a disappearance of noradrenaline from the peripheral sympathetic neurones. Since methoserpidine is known to lower the blood pressure in hypertensive man and rat, it appears justified to attribute the hypotensive effect of reserpine-like drugs to loss of transmitter from the peripheral adrenergic neurones and not to changes in the brain ; only some members of the group, such as reserpine itself, cause functional and biochemical changes in both the brain and the peripheral adrenergic neurones. A dissociation of central and peripheral biochemical effects similar to, but not as drastic as, that seen after methoserpidine has been reported in the rabbit for syrosingopine, an ethoxycarbonyl ester of reserpine (Orlans, Finger & Brodie, 1960). Syrosingopine, too, causes hypotension in man or dog, in doses which produce little sedation, and Orlans *et al.* (1960) also concluded that hypotension after reserpine and its analogues was therefore due to a peripheral action of these drugs. In mice, Leroy & Schaepdryver (1961) found syrosingopine to lower the noradrenaline of the heart but not of the brain ; however, no effect on either heart or brain was produced with 25 mg/kg methoserpidine. Since mice are very insensitive to reserpine derivatives, and rabbits required at least 12 mg/kg, these negative findings are probably explained by the dosage.

Even more striking than the dissociation of central and peripheral actions of this drug is the fact that this dissociation occurs in some species and not in others. In the cat, all doses which produced any effect on catechol amines in sympathetic ganglia also affected the noradrenaline in the brain, and these same doses also produced sedation. For syrosingopine, too, dissociation was found to be less complete in the cat than in the rabbit (Vogt, unpublished). There are two practical conclusions to be drawn from these observations: within the reserpine group of drugs, any screening tests intended for compounds having hypotensive but lacking depressant action in man should be carried out in rabbits or possibly in dogs, but not in cats ; within this same group of drugs it seems safe to assume that sedative action is absent if there are no changes in brain amines. Though from other evidence (Vogt, 1962) it is known that the fall in hypothalamic noradrenaline, which is only one of several biochemical effects produced, is not causally connected with sedation, the occurrence of such a fall can serve as an index for the presence of more relevant biochemical changes in the brain.

Guanethidine was shown to lower the noradrenaline content of the peripheral sympathetic neurones, but, in contrast to the findings with reserpine-like drugs, the loss of transmitter was too small and too slow to explain the failure of sympathetically innervated tissue to respond to electrical stimulation of its nerves. It may be recalled that intravenous guanethidine exerts its blocking effect within minutes of the injection (McCubbin, Kanako & Page, 1961), and that its addition to an isolated piece of intestine, the mesenteric nerves of which are being stimulated, abolishes the response after one minute (Achari & Dutta, 1961). Yet only about half of the ganglionic noradrenaline was lost in the course of 2 to 4 hr (Table 2), a loss which is not sufficient to cause functional failure (Muscholl & Vogt, 1958).

The occurrence of a fall in the noradrenaline concentration of the hypothalamus in a number of cats need not be a direct depleting effect of the drug but may be secondary to a reflex stimulation of the sympathetic centres. Drugs which cause a prolonged central sympathetic stimulation produce such a fall (Vogt, 1954), and the inconsistency of the response to guanethidine would favour the view that we are dealing with a secondary effect. There would then be no need to assume that such a strongly ionized drug as guanethidine penetrates the blood-brain barrier.

Dimethylphenylpiperazinium. In spite of repeated injections of this drug during the course of 4 hr no significant change in the noradrenaline content of the superior cervical ganglion was obtained. This result agrees well with the failure to decrease the noradrenaline content of the superior cervical ganglion of the dog by prolonged electrical stimulation (Vogt, 1954). It does not answer the question whether nicotine-like substances act by liberating noradrenaline since resynthesis might be fast enough to compensate for loss by utilization. Lindmar & Muscholl (1961) succeeded in showing that the perfused rabbit heart loses more noradrenaline into the perfusion fluid when stimulated by dimethylphenylpiperazinium than when beating normally, but these experiments do not give information on the speed of resynthesis in the tissues during the stimulation. In other experiments on cats' atria, however, treatment with dimethylphenylpiperazinium did not reduce the noradrenaline content of the isolated tissue (Muscholl, 1961).

The occasional large falls in hypothalamic noradrenaline after frequent injections of dimethylphenylpiperazinium may be explained by assuming that some rabbits respond to the whole procedure of repeated injections and the accompanying autonomic effects with an increase in the activity of the sympathetic centres, such an increase in activity being known to be associated with loss of noradrenaline from the brain.

Amphetamine. The interpretation of results which are " statistically significant " and yet inconsistent is always very difficult. The most likely reason for this inconsistency is that several factors are at play, and that the sum of the effects is variable. There are several known or supposed properties of amphetamine which might lower the ganglionic noradrenaline: according to Burn & Rand (1958) it acts by releasing noradrenaline. This view has recently been made more precise by the demonstration (Schümann & Philippu, 1961) that tyramine " liberates " catechol amines from adrenal medullary granules and from perfused adrenals by a process of replacement, the amount of drug taken up by the granules being equimolar to the quantity of catecholamine lost. (Similar experiments with amphetamine were reported verbally at the 1st International Pharmacological Meeting.) Assuming that the same process takes place in the ganglia, this would be one way in which a fall in noradrenaline could occur. Secondly, amphetamine accelerates the destruction of intravenously administered radioactive catechol amines in the mouse (Axelrod & Tomchick, 1960). This action might be secondary to, or independent of, the action described by Schümann & Philippu (1961). Furthermore, amphetamine inhibits *in vitro* the side-chain oxidation of dopamine to noradrenaline (Goldstein & Contrera, 1961). If this effect also took place *in vivo,* it might lead to a fall in the noradrenaline content of tissues. Opposing a fall would be the inhibiting action of amphetamine

on amine oxidase. This action, however, has not been demonstrated *in vivo*, so that its role is uncertain.

The observation that the decentralized pupil shows less mydriasis than the normal pupil indicates that amphetamine causes some central sympathetic discharge ; this may lead to a lower noradrenaline content of the brain. The complicating factors mentioned in connexion with the sympathetic ganglia may also modify the effect produced in the brain and thus account for the variability of the results.

Pheniprazine and phenylhydrazinobutane. Striking species differences were encountered in the response to these substances: in the rabbit, it was easy to increase the noradrenaline content of the hypothalamus, and nearly impossible to change that of the sympathetic ganglia. In the cat, the opposite held: a significant increase in the noradrenaline content of the ganglia was achieved, while the brain of only two out of seven cats showed values which were above the normal range. It would therefore appear that the role played by amine oxidase in the metabolism of noradrenaline is not only subject to species differences but also varies in different parts of the nervous system.

Another explanation of the low noradrenaline content of the sympathetic ganglia is possible. There is evidence that the inotropic effect of those monoamine-oxidase inhibitors which, like pheniprazine, are ephedrine-like in structure, is produced by the release of catechol amines from sympathetic nerve endings in the cat heart (Lee, Shin & Shideman, 1961). If this phenomenon also holds for the ganglia it would explain the failure of the catecholamines to accumulate in spite of a decrease in the activity of mono-amine oxidase.

The ease with which the noradrenaline can be raised in rabbit brain and not in cat brain has previously been pointed out by Spector *et al.* (1960), and correlated with the excitement produced with amine oxidase inhibitors in the rabbit but not in the cat. For the individual animal, the results of the present work do not confirm such a correlation: neither did those rabbits show the greatest degree of excitement which had the highest noradrenaline concentrations in the brain, nor did the noradrenaline content of the cat brains bear any relation to whether or not the animal had responded with excitement. In work with other drugs (morphine, β-tetrahydronaphthylamine) excitement was indeed accompanied by a fall and not by a rise in hypothalamic noradrenaline (Vogt, 1954). Brodie, Spector & Shore (1959) themselves point out that the general scheme which tries to relate central stimulation by inhibitors of mono-amine oxidase with accumulation of noradrenaline in the brain breaks down when 1-isopropyl-2-α-methylphenethylhydrazine (1-phenyl-isopropyl-2-isopropylhydrazine) is tested after repeated administration to rabbits. The authors observed a rise in the noradrenaline content of the brain stem of the same magnitude as that obtained after pheniprazine, but changes in behaviour or signs of sympathetic stimulation were lacking. It seems impossible, at the present state of our knowledge, to decide whether the mono-amine oxidase inhibitors exert their stimulant effect on the brain by raising the noradrenaline of the brain or by an action on other amines or by totally different biochemical processes.

The toxicity of the inhibitors of mono-amine oxidase was much greater in cats than in rabbits. Whereas rabbits became alert or, at most, aggressive, cats often developed

rigidity and ataxia ; as demonstrated in dogs (Maling *et al.*, 1961) these signs are a result of brain lesions.

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