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EFFECT OF ANTISYMPATHOMIMETIC DRUGS ON THE PLASMA CONCENTRATIONS OF CATECHOL AMINES

BY

G. L. WILLEY

From the Department of Pharmacology, University of Leeds, and the Smith Kline & French Research Institute, Welwyn Garden City, Herts

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Intravenous injection of phenoxybenzamine, choline 2,6-xylyl ether bromide (xylocholine, TM 10), piperoxane or dihydroergotamine increases the vasopressor activity of blood plasma, collected from cats under chloralose anaesthesia. The increased vasopressor activity that follows the administration of piperoxane is due to an increase of adrenaline and noradrenaline in the plasma. Cross-circulation experiments show that catechol amines are liberated from the spleen by piperoxane.

Brown & Gillespie (1957) showed that when the splenic nerves are stimulated, the noradrenaline concentration in the venous effluent is increased in the presence of phenoxybenzamine. They suggested that this is due to inactivation of the noradrenaline receptors which are "also probably responsible for the destruction and removal of liberated noradrenaline." Other authors have also found that antisympathomimetic drugs increase the plasma concentration of catechol amines. Thus, Burger, Giger, Kägi & Langemann (1957) showed that phentolamine increased the plasma concentration of noradrenaline. Millar, Keener & Benfey (1959) obtained similar results with phenoxybenzamine. An increased urinary excretion of noradrenaline has also been demonstrated after giving phenoxybenzamine (Schapiro, 1958 ; Benfey, Ledoux & Melville, 1959).

The present experiments extend these observations to two other established antisympathomimetic drugs, piperoxane and dihydroergotamine, and to choline 2,6-xylyl ether bromide, a compound showing transient antisympathomimetic effects.

However, some of the drugs produce central sympathetic stimulation (Handovsky, 1935 ; Gross, Tripod & Meier, 1951) which may account, at least in part, for the results of Burger *et al.* (1957), Millar *et al.* (1959), Schapiro (1958), and Benfey *et al.* (1959).

Therefore, in the later experiments described here, this complicating factor of central stimulation was eliminated, so that any observed increase in catechol amines was the result of effects of piperoxane on peripheral mechanisms.

METHODS

Cats of either sex, treated with atropine sulphate, 1 mg/kg intraperitoneally, either spinal or anaesthetized with chloralose (70 mg/kg, intravenously), were used. Injections were made

into the right femoral vein through a metal cannula. Heparin (500 i.u./kg) was injected and blood samples collected from the left femoral artery into ice-cooled, silicone-coated, calibrated centrifuge tubes containing 200 i.u. heparin. The cooled blood was centrifuged for 15 min at 3,500 rev/min. A sample of plasma was removed, avoiding the region of the buffy coat, and kept on ice until assayed.

In some preliminary experiments, venous blood was collected from the spleen of eviscerated and adrenalectomized cats under chloralose anaesthesia by the method of Brown & Gillespie (1957).

Assays. The vasopressor activity of cat plasma was compared with noradrenaline on the arterial blood pressure of pithed rats treated with atropine sulphate 1 mg subcutaneously (Brown & Gillespie, 1957). In some experiments the vasopressor substances were extracted and subjected to chromatographic separation, and, after eluting the separated amines from the appropriate portions of the developed chromatogram, the adrenaline and noradrenaline contents of the eluates were assayed on pithed rats. The adrenaline content of the appropriate eluate was sometimes also estimated on rat uterus stimulated by carbachol (Gaddum & Lembeck, 1949).

Chromatography. Plasma extracts, prepared as described by Vogt (1952), were subjected either to ascending or descending chromatography on Whatman no. 4 paper with water-saturated phenol as solvent (Crawford & Outschoorn, 1951), or to descending chromatography on Whatman no. 1 paper with a mixture of phenol and 0.1 N hydrochloric acid as solvent (Vogt, 1952). The amines were eluted from the chromatogram, and the eluates prepared for assay, using the methods described by Crawford & Outschoorn (1951). The reference compounds were adrenaline, noradrenaline and dopamine. As ascending chromatography gives R_F values for noradrenaline, adrenaline and dopamine that are not sufficiently dissimilar (0.32, 0.5 and 0.47 respectively) to permit separation of adrenaline and dopamine, the descending technique, with phenol/hydrochloric acid as the solvent, was used to separate adrenaline and dopamine. With this descending technique the R_F values for noradrenaline, adrenaline and dopamine were 0.24, 0.54 and 0.45 respectively. Orange G, which has an R_F value of 0.52 with phenol/hydrochloric acid as the solvent, was used in these latter experiments to ascertain the relative positions of the solutes.

With these chromatographic techniques, recoveries of adrenaline, noradrenaline and dopamine, added to plasma to give a final concentration of each amine of 2 μg/ml., were between 65 and 85%. With a lower amine concentration (0.05 μg/ml.) the recoveries of adrenaline and noradrenaline were between 40 and 50%. The values in this paper are those determined experimentally and have not been adjusted for incomplete recovery.

Cross-circulation experiments. The splenic artery and splenic vein, of an eviscerated heparin-treated cat under chloralose anaesthesia, were cannulated with polythene tubes of 1 mm and 2 mm internal diameter respectively. Damage to the splenic nerves was carefully avoided during this operation. The spleen was perfused with whole blood from a heparin-treated spinal cat, the femoral artery and femoral vein being used for this purpose. Venous blood from the spleen was collected periodically from a T-piece inserted in the polythene tube linking the splenic vein to the spinal cat.

RESULTS

Anaesthetized cats. In preliminary experiments, either phenoxybenzamine or choline 2,6-xylyl ether bromide (xylocholine) was administered to eviscerated and adrenalectomized cats. The vasopressor activity of splenic venous blood was estimated before, and at various times after, giving the drug. There was a marked increase in the vasopressor activity of the blood after injection of either of these drugs. The results of individual experiments are shown in the first part of Table 1.

Similar experiments, in which blood was simultaneously collected from both the splenic vein and from the left femoral artery, show that after the injection of either

TABLE 1

VASOPRESSOR ACTIVITY OF CAT PLASMA FROM THE FEMORAL ARTERY AND FROM THE SPLENIC VEIN BEFORE AND AFTER INJECTION OF PHENOXYBENZAMINE OR XYLOCHOLINE

		Vasopressor activity (ng/ml. " noradrenaline ")					
			Min after injection of drug				
Drug	Origin of plasma	Initial conc	1	5	10	25	55
Phenoxybenzamine (10 mg/kg)	Splenic vein	40	—	—	175	—	—
	Splenic vein	16	—	—	133	—	—
	Splenic vein	10	—	—	115	63	30
Xylocholine (5 mg/kg)	Splenic vein	29	41	—	—	—	—
	Splenic vein	25	63	—	—	34	—
Phenoxybenzamine (10 mg/kg)	Femoral artery	24	—	—	40	—	27
	Splenic vein	23	—	—	41	—	27
	Femoral artery	30	—	—	55	60	125
	Splenic vein	50	—	—	60	100	155
Xylocholine (10 mg/kg)	Femoral artery	10	12·5	32	—	—	12·5
	Splenic vein	12·5	22·5	30	—	—	17·5

phenoxybenzamine or xylocholine, there is also an increase in the vasopressor activity of blood collected from the femoral artery. These results are shown in Table 1.

Following these latter observations further antisympathomimetic drugs were tested for their ability to increase the vasopressor activity of blood collected from the femoral artery of non-eviscerate cats with intact adrenals. Table 2 shows the results obtained.

A typical assay of the plasma samples, collected before and after the injection of piperoxane, is illustrated by Fig. 1. The results of this assay are illustrated graphically in Fig. 2.

Of the six drugs listed in Table 2 cocaine hydrochloride and 2(2,6-dimethyl-phenoxy)propyl trimethyl ammonium bromide (subsequently referred to as β-methyl

TABLE 2

VASOPRESSOR ACTIVITY OF CAT PLASMA FROM THE FEMORAL ARTERY BEFORE AND AFTER INJECTION OF ANTISYMPATHOMIMETIC DRUGS

			Vasopressor activity (ng/ml. " noradrenaline ") Min after injection of drug						
Drug	Dose (mg/kg)	Initial conc	1	5	10	25	55	120	180
Phenoxybenzamine	15	25	50	25	25	85	93	—	66
	10	50	—	—	60	100	—	—	—
Xylocholine	10	19	24	19	16	—	14	—	—
	10	11	31	27·5	12·5	12·5	12·5	—	—
Piperoxane	5	25	—	160	50	25	13	—	—
	5	31	31	50	58	50	25	—	—
	5	30	30	50	68	50	40	—	—
	5	13	23	42	63	63	52	50	38
	5	10	25	50	40	17	13	—	—
Dihydroergotamine methanesulphonate	0·75	13	50	9	—	—	—	—	—
	1	18	98	15	13	—	—	—	—
Cocaine hydrochloride	5	13	10	9	10	13	15	—	
β-Methyl xylocholine	10	13	13	11	10	—	10	—	—

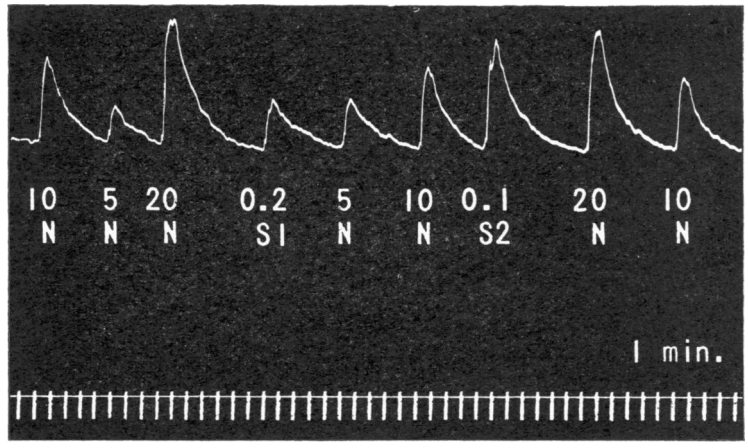

Fig. 1. Pithed rat, treated with atropine sulphate 1 mg. Record of arterial blood pressure showing assay of plasma from femoral arterial blood of cat collected before (S1) and after (S2) injection of 5 mg/kg piperoxane. The volume of each injection of plasma is given in ml. The standard responses are to noradrenaline; the dose is shown in ng.

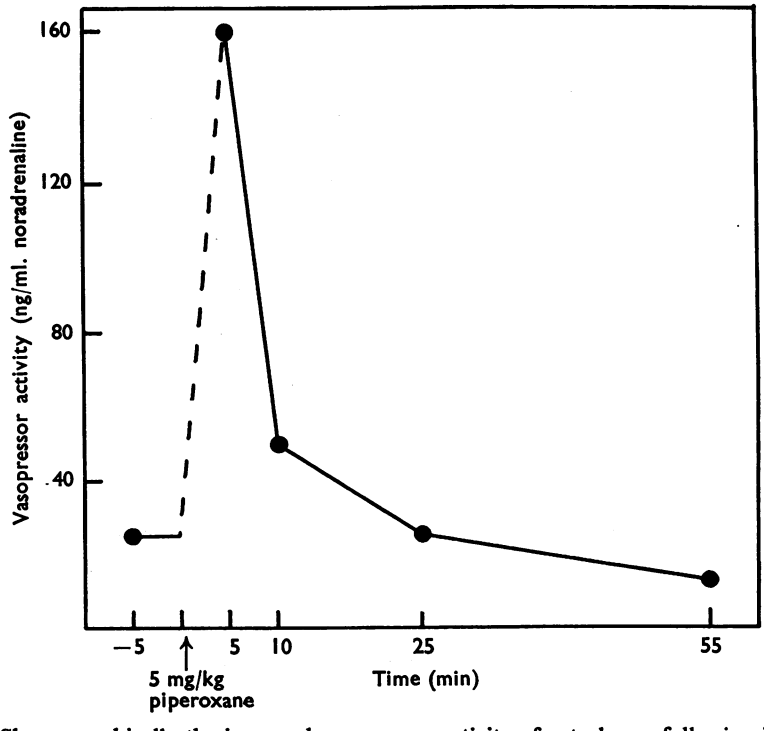

Fig. 2. Shows graphically the increased vasopressor activity of cat plasma following injection of 5 mg/kg piperoxane.

xylocholine) did not increase the vasopressor activity of blood. Xylocholine, in the dose used, caused only a slight increase in the vasopressor activity as compared with the other drugs. But this increase was seen 1 min after the injection, and the vasopressor activity had fallen to the original level 10 min later. Dihydroergotamine methanesulphonate likewise caused an increased concentration 1 min after injection, but, unlike xylocholine, the concentration had fallen to normal 5 min afterwards. The effects of phenoxybenzamine and piperoxane were, however, rather delayed, particularly with the former drug.

A feature of some interest is the time during which the increased vasopressor activity persists, after injection of the antisympathomimetic drug. The antisympathomimetic effects of phenoxybenzamine and piperoxane are prolonged, and Table 2 shows that increased levels of amines are detectable for at least 30 min after injection of either of these drugs.

Similarly, with xylocholine, which exhibits only transient antinoradrenaline effects (Willey, 1957), the increased level of vasopressor activity is only detectable for a relatively short time after injection of the drug. Moreover, injection of β-methyl xylocholine, which shows weaker and even more transient antinoradrenaline effects than xylocholine (Willey, unpublished observations), does not result in a detectable increase in the vasopressor activity.

Nature of vasopressor substance. Of the drugs listed in Table 2, only the substance responsible for the increased vasopressor activity resulting from injection of piperoxane has been examined.

Blood samples were collected 15 min before, and 10 min after, injection of 5 mg/kg piperoxane. The plasma was separated, and the vasopressor activities of aliquots of the two plasma samples were compared with noradrenaline on the blood pressure of a pithed rat. Five ml. aliquots of the plasma samples were extracted and submitted to paper chromatography. Following separation and elution of the appropriate parts

TABLE 3

EFFECT OF PIPEROXANE (5 MG/KG) ON THE ADRENALINE (A) AND NORADRENALINE (NA) CONCENTRATION IN CAT FEMORAL ARTERIAL PLASMA, USING DIFFERENT CHROMATOGRAPHIC METHODS TO SEPARATE THE AMINES

Expt.	Solvent	Technique	Vasopressor activity of plasma before extraction (ng/ml. "noradrenaline")		Catechol amine conc in plasma after extraction and separation (ng/ml.)	
			Before piperoxane	After piperoxane	Before piperoxane	After piperoxane
1	Phenol/water/SO_2	Ascending on Whatman no. 4 at room temperature for 21 hr	18	50	3·6 NA 2·5 A	8·4 NA 3·6 A
2	Phenol/water/SO_2	Ascending on Whatman no. 4 at room temperature for 21 hr	20	70	5 NA 0 A	18 NA <2 A
3	Phenol/water/SO_2	Descending on Whatman no. 4 at 25° C for 15 hr	12·5	40	0 NA 0 A	2·4 NA 9 A
4	Phenol/HCl/N_2	Descending on Whatman no. 1 at 25° C for 40 hr	7·8	30	<1 NA <1·5 A	3·5 NA 3·5 A

of the chromatogram, the eluates were assayed for their adrenaline, noradrenaline or dopamine contents.

Table 3 shows the results of these experiments in which various methods were used to separate the amines. The results indicate that adrenaline and noradrenaline are partly, if not wholly, responsible for the increased vasopressor activity—the proportion of noradrenaline varying from 20 to 90%. There was no evidence that piperoxane increased the amount of dopamine in the blood. But the possibility that the dopamine content is increased is not excluded, for the pithed rat is rather insensitive to this drug and requires from 0.1 to 0.2 μg to produce a measurable blood-pressure response.

TABLE 4

EFFECT OF IPRONIAZID (15 MG/KG) ON THE INCREASE IN THE VASOPRESSOR ACTIVITY OF CAT FEMORAL ARTERIAL PLASMA RESULTING FROM INJECTION OF 5 MG/KG PIPEROXANE

	Vasopressor activity of plasma (ng/ml. " noradrenaline ")			
	Initial conc	Min after injection of piperoxane		
		5	10	55
Before iproniazid	18·5	20	25	12·5
After iproniazid	21	30	—	—
Before iproniazid	25	52·5	31·5	—
After iproniazid	63	100	75	—

Effect of monoamine oxidase inhibitor. The possibility that the destruction of the catechol amines by monoamine oxidase may be affected was investigated by studying the effect of piperoxane before and after treatment with an amine oxidase inhibitor. Iproniazid 15 mg/kg was given intravenously—a dose which was shown by Corne & Graham (1957) to inhibit monoamine oxidase.

Table 4 shows that, after iproniazid, the injection of piperoxane still causes an increase in circulating catechol amines even though the iproniazid itself increases the initial concentrations.

Spinal cats. Intravenous injection of 5 mg/kg piperoxane does not increase the circulating catechol amines in spinal cats (see Table 5). Even after 10 mg/kg

TABLE 5

VASOPRESSOR ACTIVITY OF PLASMA COLLECTED FROM THE PERFUSED SPLEEN OF CHLORALOSED CATS AND FROM THE CAROTID ARTERY OF SPINAL CATS

The femoral blood vessels of the spinal cats were used to perfuse the spleen. Plasma samples were collected before and after injection of piperoxane (5 mg/kg)

		Vasopressor activity of plasma (ng/ml. " noradrenaline ")			
			Min after injection of piperoxane		
Expt.	Origin of plasma	Initial conc	5	25	55
1	Carotid artery	10	10	10	—
	Splenic vein	12·5	37	30	27
2	Carotid artery	9·3	10·5	6·3	—
	Splenic vein	9·8	25	14·5	12·5
3	Carotid artery	2·4	2·8	2·8	—
	Splenic vein	3·5	11·3	7·3	5

piperoxane—that is, after twice the dose which will increase the circulating catechol amines in cats anaesthetized with chloralose—no increase was demonstrable.

Cross-circulation experiments. The experiments with spinal cats suggest that the catechol amine increase, after intravenous piperoxane, is associated in some way with the central nervous system. Cross-circulation experiments were done to test this point. In these the spleen of an anaesthetized cat, with the splenic innervation intact, was perfused with blood from a spinal cat. Drugs injected into the blood

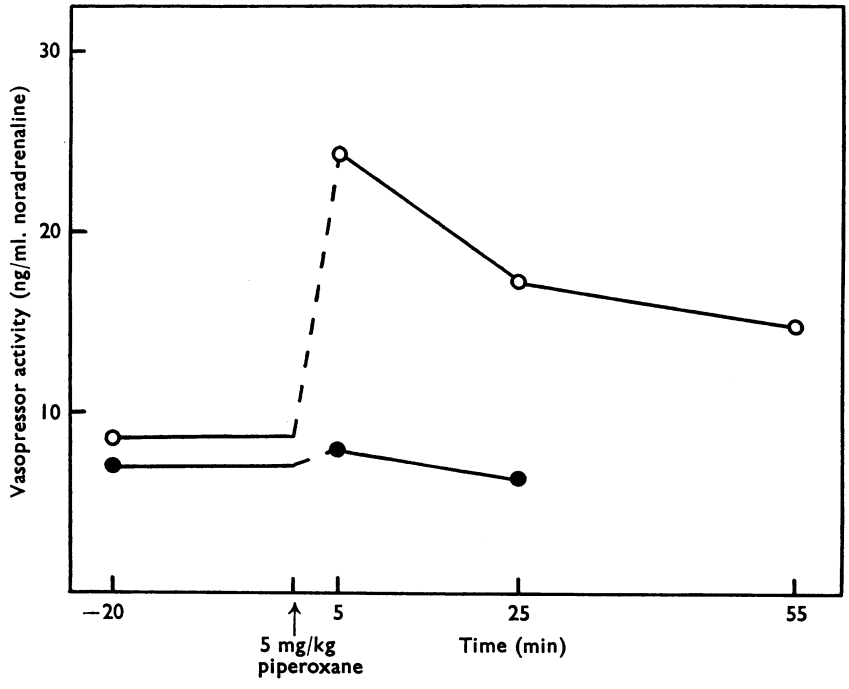

Fig. 3. Vasopressor activity originating from the perfused spleen on administration of 5 mg/kg piperoxane (○——○). (●——●) shows the vasopressor activity of plasma collected from the donor cat at the same times.

stream of the spinal cat cannot reach the central nervous system of the anaesthetized cat, and so any actions seen are direct ones upon the spleen. Blood samples were collected simultaneously from the venous cannula inserted in the splenic vein of the recipient (anaesthetized) animal, and from the carotid artery of the donor (spinal) cat. The samples were collected before and at intervals after the intravenous injection of piperoxane into the spinal cat. These experiments show that, after injection of piperoxane, the catechol amine content of blood from the spleen of anaesthetized cats is substantially increased, while that in the general circulation of spinal cats is only very slightly raised. The detailed results are shown in Table 5 and the mean results illustrated in Fig. 3.

DISCUSSION

An increase in the vasopressor activity of blood collected from cats, anaesthetized with chloralose, follows the intravenous injection of phenoxybenzamine, of piperoxane, of dihydroergotamine methanesulphonate and of xylocholine. This increased vasopressor activity, after giving piperoxane, is partly, if not entirely, accounted for by an increase in the plasma content of adrenaline and noradrenaline.

The most obvious mechanisms whereby the drugs could bring about such an increase are: (i) by releasing amines from the adrenals ; (ii) by inhibiting monoamine oxidase, leading to delayed inactivation of the amine ; (iii) by stimulating ganglia ; (iv) by inducing compensatory reflex mechanisms in response to hypotension ; (v) by stimulating the central nervous system ; (vi) by increasing the amounts of amines released ; or (vii) by diminishing the uptake of amines by tissues.

The adrenals, however, are not entirely responsible, for, in cats with the adrenals removed, the increase is still seen after administration of xylocholine or phenoxybenzamine. Similarly, though complete inactivation of monoamine oxidase by iproniazid raises the resting level of catechol amines, it does not prevent a further rise in response to piperoxane. In any event, Brown & Gillespie (1957) showed that monoamine oxidase plays no apparent part in the destruction of the adrenergic transmitter in the spleen.

That ganglionic stimulation might play a part in raising the plasma adrenaline and noradrenaline was suggested by the fact that some antisympathomimetic drugs, such as xylocholine (Hey & Willey, 1954 ; Edge, Mason & Wyllie, 1957) and piperoxane (Exley, personal communication), are known to stimulate autonomic ganglia. The fact that no increase in plasma catechol amines was detected in spinal cats following injection of piperoxane provides evidence that ganglionic stimulation plays little, or no, part in causing an increase in circulating amines. It would also appear improbable that ganglionic stimulation is responsible for this increase since xylocholine, the most active ganglion stimulant among the drugs studied, raises the catechol amine level only very slightly.

Compensatory reflex mechanisms have been suggested by Benfey, Ledoux & Segal (1959) as the means by which phenoxybenzamine causes an increased amine concentration. They found that phenoxybenzamine raised the urinary excretion of noradrenaline in cats only when there was an accompanying hypotension, and that if the blood-pressure fall was prevented by giving vasopressin there was no increased excretion of noradrenaline in response to phenoxybenzamine. Reflex release due to hypotension, although possibly accounting for the results with phenoxybenzamine, is unlikely to apply to antisympathomimetic drugs in general. Xylocholine and dihydroergotamine methanesulphonate, for example, both raise the blood pressure, and piperoxane lowers it only slightly.

Some of these antisympathomimetic drugs are known to be central nervous stimulants, and this may account in part for the increased amine content of the blood. The absence of any increased amounts of amines in the blood of spinal cats after injection of piperoxane would accord with this fact. But the perfused spleen experiments show that direct central nervous stimulation is not the only

mechanism contributing to the increase. In these experiments the spleen was isolated from the circulation of an anaesthetized cat and included in the circulation of a spinal cat—previous experiments having demonstrated that intravenous injection of piperoxane does not increase the catechol amines circulating in the blood of spinal cats. Increased amounts of catechol amines in the venous blood from the spleen (of the recipient) but not in blood from the carotid artery of the donor suggest that the amines originate from the spleen. It would appear, therefore, that catechol amines are released only from an innervated structure. This observation accords with the suggestion put forward by Brown & Gillespie (1957), namely, that after giving an antisympathomimetic drug, there is a diminished destruction of normally released transmitter. Further evidence supporting this hypothesis is the close parallelism between the time during which an increased level of catechol amines persists, and the duration of the antisympathomimetic effect itself.

Huković (1959) and Boyd, Chang & Rand (1960) have suggested that the amount of amines liberated on sympathetic nerve stimulation is increased in the presence of phenoxybenzamine. Boyd *et al.* showed that some antisympathomimetic drugs possess anticholinesterase activity, and pointed out that increased amounts of transmitter could be released in the presence of an anticholinesterase it there were a cholinergic process involved in the release of catechol amines. Xylocholine exhibits anticholinesterase activity (Willey, 1957) and this observation is further evidence in favour of the suggestion of Boyd *et al.*

Another contribution to the increase may be the displacement of transmitter from the inactivated receptor sites into the general circulation, together with an inhibition of the uptake of transmitter as a consequence of receptor inactivation. If the receptor is responsible for the inactivation of noradrenaline then cocaine, which Trendelenburg (1959) has suggested causes a delayed inactivation of noradrenaline, might be expected to increase the circulating amines; the author has confirmed Trendelenburg's observations that this is not so.

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