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EFFECTS OF COCAINE AND ANTIDEPRESSANT DRUGS ON THE NICTITATING MEMBRANE OF THE CAT

BY

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Cocaine, imipramine and pipradol potentiated the contractions to adrenaline and noradrenaline, but not to tyramine, on the nictitating membrane of the spinal cat. Pheniprazine and dexamphetamine potentiated the responses to adrenaline, noradrenaline and tyramine, whereas nialamide only potentiated the response to tyramine. Potentiation of the response to stimulation of either the preganglionic or the postganglionic sympathetic nerve trunks was observed with imipramine, pipradol, pheniprazine and dexamphetamine. Only dexamphetamine and pheniprazine caused substantial contractions of the membrane when the preganglionic nerve was cut (acutely decentralized), or when the superior cervical ganglion was removed (acutely denervated). Cocaine produced contractions of the innervated but not of the acutely decentralized membrane. The significance of the peripheral effects of these antidepressant drugs in relation to their central actions is discussed.

Several workers, for example, Rothballer (1957), Costa & Zetler (1959), Sigg (1959), and Maxwell, Sylwestrowicz, Plummer, Povalski & Schneider (1960), have speculated on the possible relationship between the central actions of many stimulant drugs and their effects on the peripheral actions of adrenaline. In the present investigation five antidepressant drugs (imipramine, pipradol, pheniprazine, nialamide and dexamphetamine), which have all been used with varying degrees of success in the treatment of depressive illness (Rees, 1960), and cocaine, have been compared for their ability to modify the actions of adrenaline, noradrenaline, tyramine and sympathetic nerve stimulation on the nictitating membrane of the spinal cat. Tyramine was included since it is thought to be metabolized *in vivo* by monoamine oxidase (Corne & Graham, 1957), and the effect of tyramine on the membrane is known to be potentiated by iproniazid, which is an inhibitor of the enzyme (Griesemer, Barsky, Dragstedt, Wells & Zeller, 1953).

It was hoped that the accumulation of the results of this investigation might show certain similarities in some of the peripheral actions of these antidepressant compounds, and thus contribute to an understanding of their mechanism of action.

METHODS

Preparation of spinal cat. The spinal cord was cut at the level of the second cervical vertebra and the brain was destroyed under ether anaesthesia (Burn, 1952). Seventy-five such preparations were used.

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Nictitating membrane. The contractions of the nictitating membrane were recorded by a frontal writing lever, writing on a smoked drum. The contractions were magnified 10 times. Records of the size of the contractions were those measured on the drum. Usually the membrane was acutely decentralized by cutting the preganglionic nerve, but in some experiments the innervation was left intact; when the postganglionic nerve was stimulated the superior cervical ganglion was removed at the beginning of the experiment (acute denervation). An interval of about 1 hr was then allowed to elapse before continuing. Injections were made into the femoral vein. Blood pressure was recorded with a mercury manometer from either a carotid or a femoral artery.

Effect of drugs. The test doses of adrenaline and noradrenaline ranged from 1 to 40 μg, and produced contractions of 2 to 15 mm of the decentralized membrane. Tyramine was given in a dose of 0.25 to 2 mg and gave contractions of 1 to 40 mm. Adrenaline and noradrenaline were injected alternately at intervals of about 5 to 10 min, and tyramine was injected at intervals of 15 to 60 min. However, in a few experiments it was possible to inject tyramine at 10 min intervals without a reduction in sensitivity occurring.

One estimate of the degree of potentiation was made by determining the ratio of the doses producing the same size of contraction before and after the test drug. In most instances the effects of single doses of the antidepressant drugs were observed until a maximum potentiation had been obtained. Another estimate of potentiation was determined from the duration of the contractions, which was expressed as the half-life, that is, the time to 50% recovery. Then the potentiation of the duration was expressed as the ratio of the half-life after the test drug to the half-life of the control response.

Sympathetic nerve stimulation. In order to investigate the potentiating effect of drugs on sympathetic nerve stimulation, the cut preganglionic cervical sympathetic nerve was stimulated submaximally (Trendelenburg, 1959), so as to give contractions between 20% and 60% of the maximum. In some experiments the contractions of the contralateral membrane to stimulation of the stump of the postganglionic nerve were recorded as well. In these experiments the superior cervical ganglion on one side was removed. Square-wave stimuli, of 0.5 msec duration, were delivered to the nerve at a frequency of 10/sec for 15 sec every 2 min. Since it was difficult to maintain constant responses to submaximal stimulation over long periods, the test substances were injected when the contractions remained steady, or were declining slightly for about 10 min, and only those effects which were observed soon after the injection were considered to be significant.

Drugs. The drugs used were cocaine, imipramine, pheniprazine, pipradol, adrenaline, and tyramine as the hydrochlorides, dexamphetamine sulphate, noradrenaline bitartrate, 5-hydroxytryptamine creatinine sulphate, hexamethonium bromide, pyrogallol and nialamide. The doses are expressed in terms of the salt, except for nialamide and pyrogallol, which were injected as the pure compounds dissolved in water.

RESULTS

The main results on all the compounds are summarized in Fig. 1, and in Table 1 these results have been expressed quantitatively where this was possible. Whereas it was often feasible to determine the degree of potentiation of the height of contraction for adrenaline, as described in the methods, it was not often possible for noradrenaline since the nictitating membrane was very insensitive to this substance, even though adrenaline and noradrenaline were roughly equipotent in causing pressor responses: in 17 experiments the average control contraction to 20 μg of noradrenaline was only 4 ± 1.7 mm (standard error), in contrast to adrenaline (10 μg) which produced an average contraction of 24 ± 5.4 mm.

The half-life of the contractions to adrenaline and noradrenaline ranged from 0.5 to 1.5 min in different experiments, and did not vary by more than a factor of

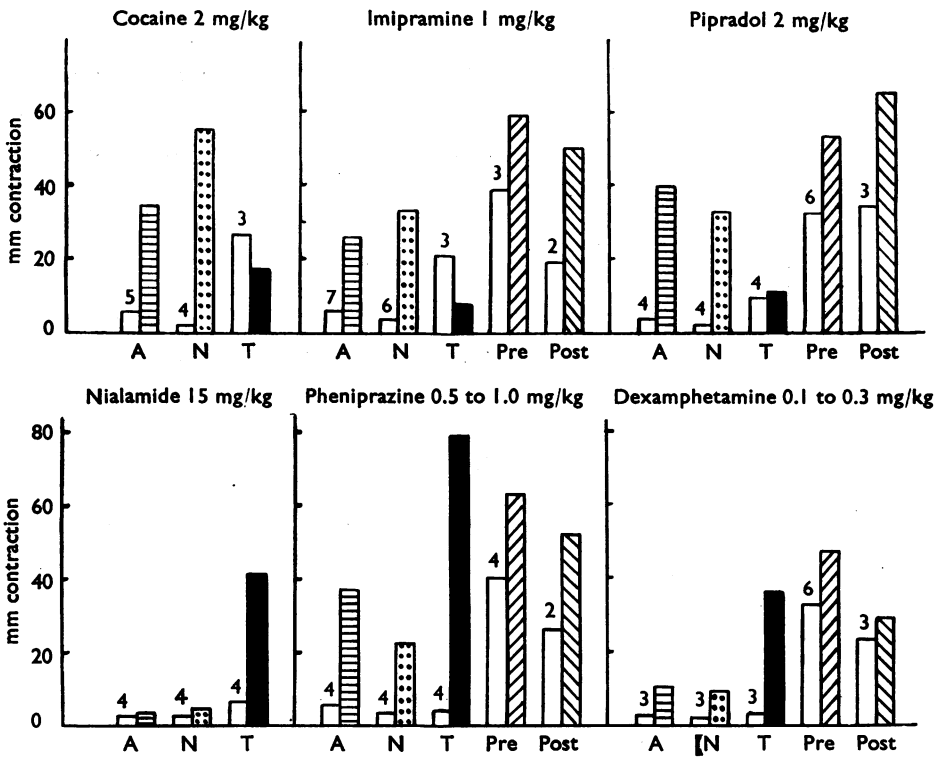


Fig. 1. Spinal cats. Effects of cocaine and antidepressant drugs on contractions of the acutely decentralized nictitating membrane to adrenaline (A), noradrenaline (N), tyramine (T) and submaximal stimulation of the preganglionic nerve (Pre), and on contractions of the acutely denervated membrane to submaximal stimulation of the postganglionic nerve (Post). Control observations are indicated by the blank columns, and the number of experiments from which the average heights of contraction were calculated are shown above the control columns. A dose of 1 mg/kg of pheniprazine was used in experiments on A, N and T, and 0·5 mg/kg was used in experiments on Pre and Post. The dose of dexamphetamine varied from 0.1 to 0.3 mg/kg in different experiments.

two over an 8-fold increase in the dose in any given experiment. The half-life of the contractions to tyramine ranged from 1 to 5 min and also did not vary by more than a factor or two on increasing the dose. Thus in Table 1 a potentiation of the duration of the contractions of less than two cannot be considered to be significant.

Cocaine. The intravenous injection of 0.25 to 2 mg/kg of cocaine produced only a small contraction (2 ± 0.7 mm) of the acutely decentralized membrane (11 experiments). This result is in agreement with the observation made by Trendelenburg (1959). He found that cocaine (0.2 to 6.2 mg/kg) produced a negligible contraction of the acutely decentralized membrane in the spinal cat. In contrast to this small effect, there was often a much larger contraction of the normally innervated membrane, although the response was extremely variable (Table 2). The size of this contraction ranged from 2 to 110 mm, and the duration varied from 3 min to more than 45 min.

TABLE 1

EFFECT OF COCAINE AND ANTIDEPRESSANT DRUGS ON CONTRACTIONS TO SYMPATHOMIMETIC AMINES ON ACUTELY DECENTRALIZED NICTITATING MEMBRANE OF THE SPINAL CAT

The numbers of experiments are shown in parentheses. Potentiation was calculated as the ratio of the doses producing the same size of contraction before and after the test drug

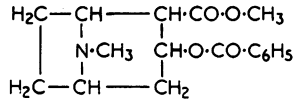
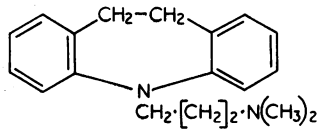
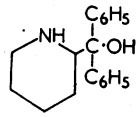
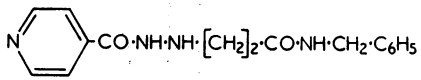
	Formula	Total no. of cats	Dose mg/kg injected intravenously	Adrenaline: Average potentiation of height	Adrenaline: Average potentiation of duration	Noradrenaline: Average potentiation of height	Noradrenaline: Average potentiation of duration	Tyramine: Average potentiation of height	Tyramine: Average potentiation of duration	Direct effect on membrane (average contraction in mm)
Cocaine		3	0·5	1·4 (1)	1·0 (3)	—	1·4 (3)	0·8 (1)	1·0 (1)	1·0 (1)
		2	1·0	3·5 (1)	1·0 (1)	—	1·0 (1)	0·5 (1)	1·0 (1)	0·0 (2)
		6	2·0	2·8 (2)	1·6 (5)	—	2·0 (4)	0·6 (3)	1·0 (3)	0·0 (6)
Imipramine		11	1·0	2·4 (5)	4·0 (7)	>2 (1)	1·3 (4)	0·5 (4)	1·0 (4)	0 (11)
Pipradol		4	2·0	2·0 (2)	1·6 (4)	2·5 (2)	3·3 (4)	1·1 (3)	1·0 (4)	0 (4)
Nialamide		4	15·0	1·0 (4)	1·0 (4)	1·0 (4)	1·0 (4)	2·0 (1)	>15·0 (4)	0 (4)
Pheniprazine	$C_6H_5.CH_2.CH(CH_3).NH.NH_2$	7	1·0	3·5 (3)	1·9 (4)	3 (4)	1·9 (4)	>5 (4)	7·5 (4)	23 (7)
Dexamphetamine	$C_6H_5.CH_2.CH(CH_3).NH_2$	4	0·05	1·8 (3)	1·5 (3)	1·7 (3)	1·3 (3)	>2 (1)	1·4 (3)	9 (4)
		4	0·1 to 0·3	2·0 (1)	1·5 (3)	—	1·3 (3)	>2 (1)	1·4 (3)	16 (4)

TABLE 2

COMPARATIVE EFFECTS OF COCAINE ON THE NORMALLY INNERVATED AND ON THE ACUTELY DECENTRALIZED NICTITATING MEMBRANES OF THE SPINAL CAT

Duration of contraction is expressed as the half-life, that is, time to 50% recovery

Intravenous dose of cocaine mg/kg	Normally innervated membrane			Acutely decentralized membrane		
	Average height of contraction (mm)	Range (mm)	No. of experiments	Average height of contraction (mm)	Range (mm)	No. of experiments
0·25	6	0 to 12	2	0	—	1
0·5	30	0 to 95	7	1·8	0 to 7	6
1·0	5	0 to 10	3	0·8	0 to 5	6
2·0	35	5 to 110	6	0·5	0 to 4	8
	Average duration of contraction (min)	Range (min)		Average duration of contraction (min)	Range (min)	
0·25	8	—	1	—	—	—
0·5	>12	3 to >45	6	13	3 to 24	2
1·0	>9	7 to >12	2	5	—	1
2·0	>20	3 to >30	6	3	—	1

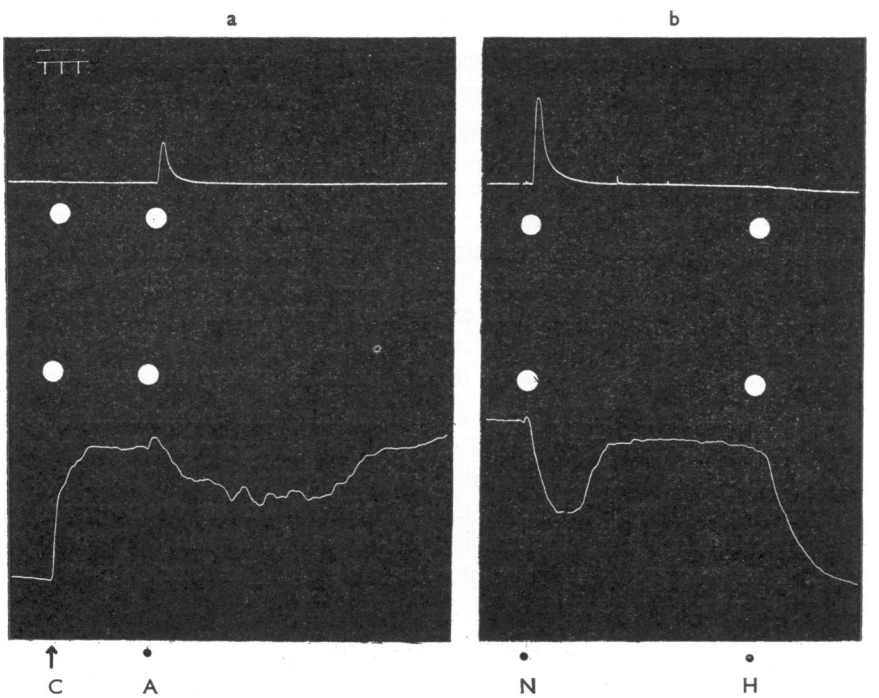


Fig. 2. Spinal cat. Records of contractions of nictitating membrane. Above: acutely decentralized; and below: normally innervated. C=cocaine, 0.5 mg/kg; A=adrenaline, 2 μg; N=noradrenaline, 10 μg; and H=hexamethonium 0.15 mg/kg. Time scale=1 min. Interval of 6 min between *a* and *b*. All injections made intravenously.

The record shown in Fig. 2 is from an experiment in which records were obtained from both the normally innervated and the acutely decentralized membranes. There was a 40 mm contraction of the normally innervated membrane, produced by a second dose of cocaine of 0.5 mg/kg (the first dose produced a larger contraction, 80 mm, but it was of shorter duration). The injection of 2 μg of adrenaline or 10 μg of noradrenaline, which initially produced small contractions (2 to 4 mm) of both membranes, now produced a partial relaxation of the innervated membrane. The membrane also relaxed after hexamethonium. When the effect of hexamethonium had partly worn off, after 12 min, cutting the preganglionic nerve again caused the membrane to relax ; this effect of cutting the preganglionic nerve was seen in two other experiments. The relaxation produced by adrenaline and noradrenaline was probably due to ganglionic blockade, since adrenaline is known to inhibit transmission through sympathetic ganglia (Marrazzi, 1939 ; Bülbring, 1944 ; Matthews, 1956).

In two other experiments adrenaline and noradrenaline (0.5 to 20 μg) produced a partial relaxation of the membrane. The maximum effect observed was an 80% reduction in the height of the contraction.

It was thought that one reason for the failure consistently to reproduce the prolonged contractions of innervated membrane after injections of cocaine may have been due to the time relationship between preparation of the cat and the injection of cocaine. Therefore, two experiments were carried out in which cocaine was injected as soon as possible after cutting the spinal cord and setting up the preparation. In both experiments cocaine (0.5 mg/kg) caused large contractions (30 to 70 mm) of both the acutely decentralized and the normally innervated membranes. However, in one of these experiments the contraction of the innervated membrane was of longer duration than that of the decentralized membrane. Since these large contractions of the decentralized membrane were not observed in the 11 experiments in which the usual 1 hr was allowed to elapse before starting the experiment, it is thought that these contractions may be due to the potentiating effects of cocaine on the circulating catecholamines, released as the result of the ether anaesthesia (Sollmann, 1957) or the trauma from cutting the spinal cord.

Cocaine potentiated the effects of adrenaline and noradrenaline on the decentralized membrane (Fig. 1 and Table 1) ; the maximum potentiation occurred within 10 min. The effect of tyramine, however, was reduced. These results are in agreement with Trendelenburg (1959), who made similar observations on the potentiating effects of cocaine in the spinal cat. He also showed that the contractions produced by submaximal stimulation of either the preganglionic or the postganglionic nerves were increased by cocaine.

Imipramine. Imipramine, in a dose of 1 mg/kg, had no effect on the tone of the acutely decentralized membrane (Table 1). Even higher doses (2 to 4 mg/kg) produced only a small contraction (15 mm) in one out of seven experiments. On the normally innervated membrane doses of 1 to 4 mg/kg had no effect (4 experiments) except in one experiment where a small contraction (10 mm) was obtained.

The effects of imipramine on the responses of the acutely decentralized membrane to adrenaline, noradrenaline and tyramine (Figs. 1 and 3) resembled the effects

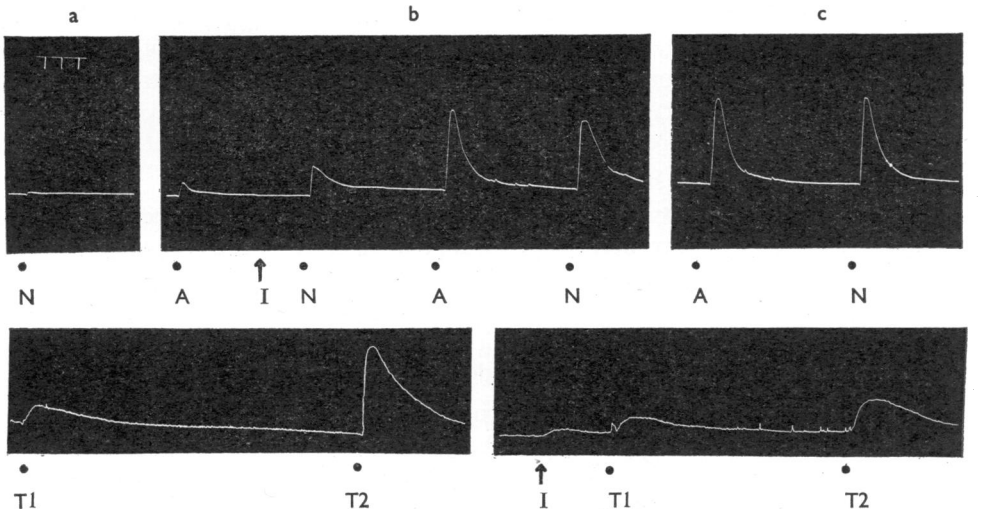


Fig. 3. Spinal cats. Records of contractions of acutely decentralized membranes in two experiments. I=imipramine, 1 mg/kg. Above: N=noradrenaline, 16 μg; A=adrenaline, 4 μg. Interval of 10 min between *a* and *b*, and 60 min between *b* and *c*. Below: T1=tyramine, 1 mg; T2=tyramine, 2 mg; 20 min interval between records. Time scale=1 min. Intravenous injections.

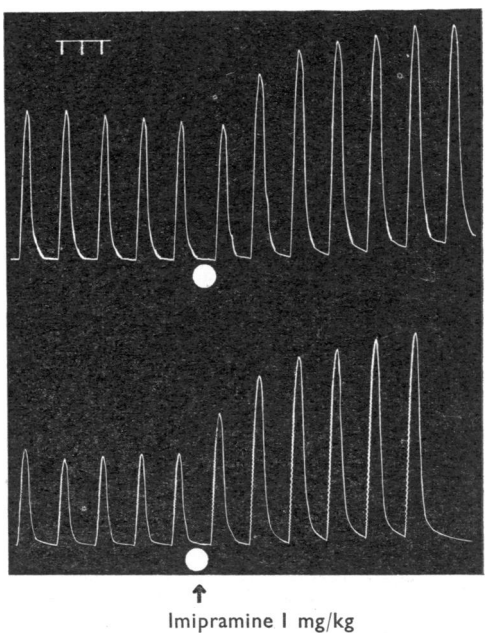


Fig. 4. Spinal cat. Records of contractions of nictitating membrane to submaximal sympathetic nerve stimulation. Above: acutely decentralized membrane (preganglionic stimulation). Below: acutely denervated membrane (postganglionic stimulation). At O imipramine 1 mg/kg was injected intravenously.

obtained with cocaine. As with cocaine, the effects of imipramine were maximal within 10 min of the injection. Fig. 4 illustrates the potentiating effect of imipramine on submaximal stimulation of the preganglionic and postganglionic trunks of the cervical sympathetic nerve. Trendelenburg (1959) showed that cocaine also potentiated the effect of preganglionic and postganglionic stimulation of the cervical sympathetic nerve in the spinal cat.

In view of the similarity between the effects of imipramine and cocaine, it was of interest to see whether imipramine would antagonize the contraction due to amphetamine, since cocaine is known to have this property (Fleckenstein & Stöckle, 1955). It was found (2 experiments) that imipramine (1 mg/kg) caused an immediate relaxation of both the decentralized and the denervated membranes, which were made to contract by an injection of dexamphetamine (0.4 mg/kg).

Furchgott (1955) has suggested that iproniazid, which is an inhibitor of monoamine oxidase, can be used as a tool to study the mechanism of the potentiating effect of cocaine on responses to catecholamines. He showed that cocaine potentiated the contractions of the isolated aortic strip to adrenaline and noradrenaline, equally well before and after treatment with iproniazid. From this he concluded that the potentiating effect of cocaine was not due to inhibition of monoamine oxidase. It was decided to use a similar technique to determne whether the potentiation by imipramine could be due to inhibition of o-methyl transferase. Pyrogallol is known to interfere with the activity of o-methyl transferase (Axelrod & Laroche, 1959). Therefore, experiments were carried out to see whether imipramine potentiated the response of the decentralized nictitating membrane to adrenaline and noradrenaline equally well after pretreatment with pyrogallol as in the absence of pyrogallol.

Three experiments were carried out in which pyrogallol was given shortly before imipramine. Pyrogallol alone had a slight potentiating effect on the contractions to adrenaline but not to noradrenaline ; the height of the contractions to adrenaline was increased from an average of 4 mm before to 14 mm after 3 to 10 mg/kg of pyrogallol, and higher doses (20 to 30 mg/kg) produced no further potentiation. When imipramine (1 mg/kg) was injected after pyrogallol (20 to 30 mg/kg), a much greater potentiation of the responses to adrenaline and noradrenaline was obtained: the contraction to adrenaline was increased from an average of 8 mm before to 62 mm after imipramine, and the contraction to noradrenaline was increased from 3 mm before to 52 mm after imipramine. This effect was certainly not less than that obtained with imipramine alone (Fig. 1). Thus it is concluded that the potentiating effect of imipramine is not due to inhibition of o-methyl transferase.

Pipradol. Pipradol (2 mg/kg) had virtually no effect on the tone of the acutely decentralized membrane (out of 10 experiments a small contraction of 10 mm was observed in only one experiment). Like cocaine and imipramine, pipradol potentiated the contractions to adrenaline and noradrenaline, but not to tyramine (Table 1, Figs. 1 and 5), and the potentiation was maximal in 10 min. Pipradol also potentiated the effects of submaximal stimulation of the preganglionic and postganglionic nerve trunks (Figs. 1 and 5). There was a further similarity to cocaine, since pipradol (2 mg/kg) caused an immediate relaxation of the decentralized membrane made to contract by dexamphetamine (2 experiments).

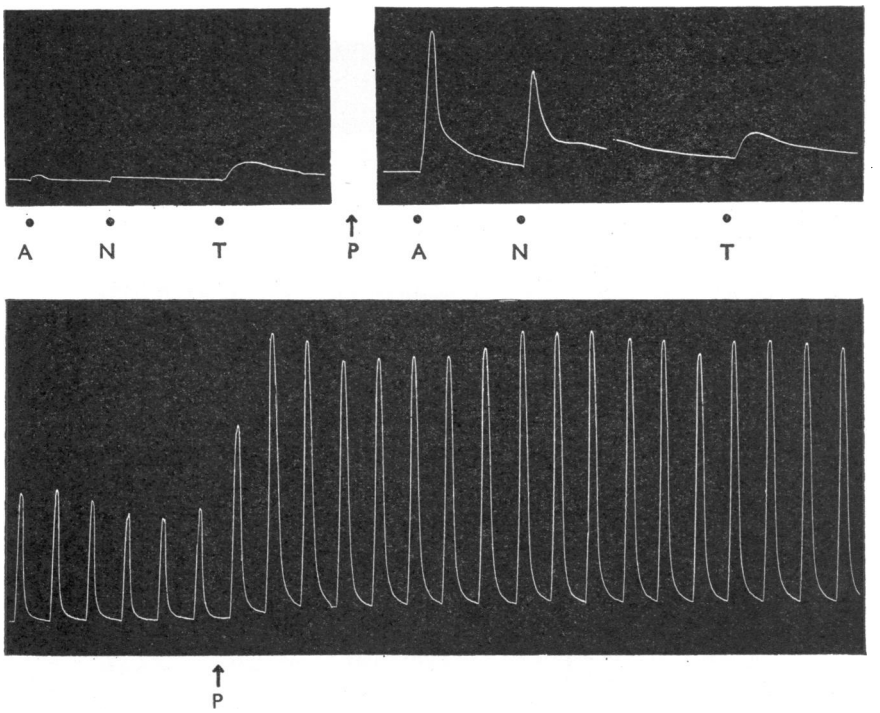


Fig. 5. Spinal cats. Records of contractions of acutely decentralized nictitating membranes in two experiments. Above: A=adrenaline, 4 μg; N=noradrenaline, 20 μg; and T=tyramine, 1 mg. Below: submaximal stimulation of preganglionic cervical sympathetic nerve. P=pipradol, 2 mg/kg. Time scale=1 min. All drugs were injected intravenously.

Nialamide. Nialamide, in a dose of 15 mg/kg, had no effect on the tone of the acutely decentralized membrane (4 experiments) when observations were continued for 2 to 4 hr after the injection. There was no obvious effect on the contractions produced by adrenaline or noradrenaline, but the size and duration of the contractions to tyramine were potentiated (Table 1, Figs. 1 and 6). The potentiation of the response to tyramine was first observed 1.5 hr to 4 hr after the injection of nialamide. Whereas control injections of up to 4 mg of tyramine produced a maximum duration of contraction of 5 min in different experiments, after nialamide the contraction due to tyramine persisted for periods exceeding 30 to 60 min: observations were not made over longer periods. Because of this greatly prolonged action of tyramine it was not possible to determine when nialamide had produced a maximum effect. Since the action of nialamide was slow in onset it was not practicable to test its effects on submaximal nerve stimulation.

Pheniprazine. Unlike the four compounds described previously, pheniprazine produced a marked contraction of the acutely decentralized membrane (Table 1). A dose of 2 mg/kg (two experiments) produced contractions of 45 mm and 120 mm respectively, with durations of more than 20 min. A dose of 1 mg/kg (10 experiments) produced contractions of 6 to 75 mm (average 29 ± 10 mm) and the duration

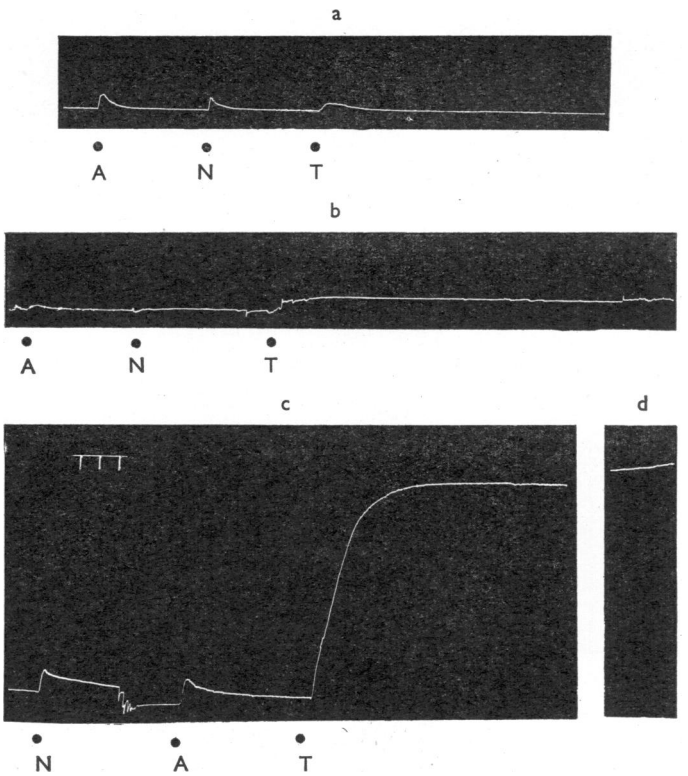


Fig. 6. Spinal cat. Records of contractions of acutely decentralized nictitating membrane. A= adrenaline, 4 μg; N=noradrenaline, 20 μg; T=tyramine, 1 mg. (*a*) Control; (*b*) 1 hr after 15 mg/kg of nialamide; (*c*) 2 hr later; (*d*) 20 min after (*c*). Time scale=1 min. All drugs were injected intravenously.

varied from 4 to 8 min. However, in 3 experiments, after 0.5 to 1 hr, there was a second more gradual increase in the tone of the membrane. An injection of 0.5 mg/kg caused only a small contraction (average 1 ± 0.8 mm in 5 experiments). Therefore, doses of 0.5 to 1 mg/kg, which usually produced relatively brief effects, were used in most of the experiments. These contractions could not have been due to nicotine-like ganglion stimulant effects such as Reinert (1959) has described for amphetamine, since in two experiments contractions were still observed with pheniprazine after removal of the superior cervical ganglion (Fig. 8).

Pheniprazine potentiated the effect of adrenaline and noradrenaline about 3 times, and the response to tyramine was potentiated more than 5 times (Table 1, Figs. 1 and 7). Although there was only a slight increase in the duration of the contractions to adrenaline and noradrenaline, the response to tyramine was considerably prolonged (Table 1 and Fig. 7).

Potentiation of the contractions to the three sympathomimetic amines was observed within 15 min of the injection of pheniprazine, but the maximum potentiation of the contractions to adrenaline and noradrenaline was not observed

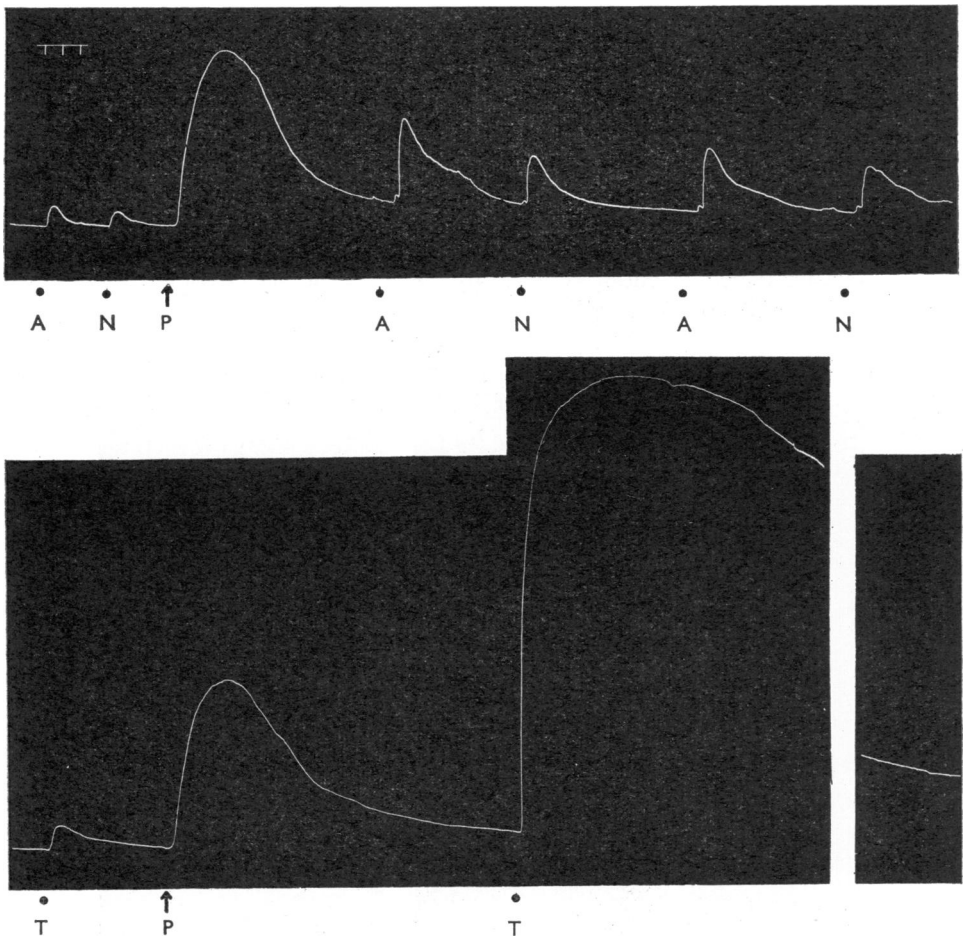


Fig. 7. Spinal cats. Records of contractions of acutely decentralized nictitating membrane in two experiments. P=pheniprazine, 1 mg/kg. Above: A=adrenaline, 5 μg; N=noradrenaline, 20 μg. Below: T=tyramine, 1 mg; interval of 20 min between records. Time scale=1 min. All drugs were injected intravenously.

until 0.5 to 3 hr after pheniprazine. No attempt was made to determine when a maximum effect on the tyramine response had been achieved. Since 5-hydroxytryptamine is a good substrate for monoamine oxidase (Blaschko, 1957), a few experiments were carried out to investigate the potentiating effect of pheniprazine on 5-hydroxytryptamine. The average contraction to 20 to 40 μg of 5-hydroxytryptamine was 5 mm. After 1 mg/kg of pheniprazine the average contraction to 5-hydroxytryptamine was 20 mm and the maximum potentiation was observed in 30 to 150 min (3 experiments). This effect was slight in comparison with the potentiation of the contractions to tyramine (Figs. 1 and 7). This may be due to the fact that there may be other metabolic pathways for 5-hydroxytryptamine, other than monoamine oxidase, which are not important to tyramine. For example,

Weissbach, Redfield & Udenfriend (1958) have shown that conversion to the O-glucuronide may be an important metabolic pathway for 5-hydroxytryptamine.

Fig. 8 illustrates one of the experiments summarized in Fig. 1, in which the effect of pheniprazine on submaximal sympathetic nerve stimulation was investigated. An injection of 0.5 mg/kg potentiated the contractions produced by stimulation of the preganglionic and postganglionic sympathetic nerves. A higher dose of 1 mg/kg caused both membranes to contract. Since the superior cervical ganglion on one side had been removed, neither the potentiation of the contractions to nerve stimulation nor the contraction produced by pheniprazine *per se* could be explained by a stimulant effect on the ganglion.

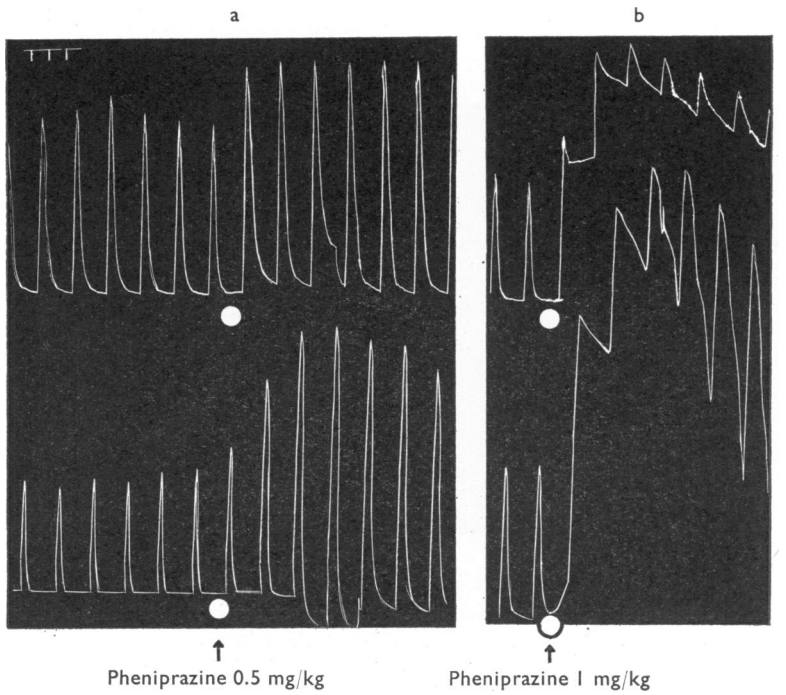


Fig. 8. Spinal cat. Records of contractions of nictitating membranes to submaximal sympathetic nerve stimulation. Above: acutely decentralized membrane (preganglionic stimulation). Below: acutely denervated membrane (postganglionic stimulation). Time scale=1 min. At the white dot pheniprazine was injected intravenously.

Dexamphetamine. Like pheniprazine, dexamphetamine caused a contraction of both the decentralized membrane (Table 1) and the acutely denervated membrane. The duration of these contractions was prolonged (greater than 30 min) at all of the doses used.

Since Reinert (1959) showed that amphetamine had a nicotine-like stimulant effect on the superior cervical ganglion when the drug was injected into the fluid perfusing the ganglion, it was of interest to determine whether the contraction of the decentralized membrane produced by an intravenous injection of amphetamine was

partly due to an effect on the ganglion. Thus in 3 experiments hexamethonium (5 to 10 mg/kg) was injected during the contraction produced by an intravenous injection of dexamphetamine (0.2 to 1 mg/kg). Since there was no effect whatever on the contraction, it is concluded that on intravenous injection the contraction produced by dexamphetamine was not due to a stimulant effect on the ganglion.

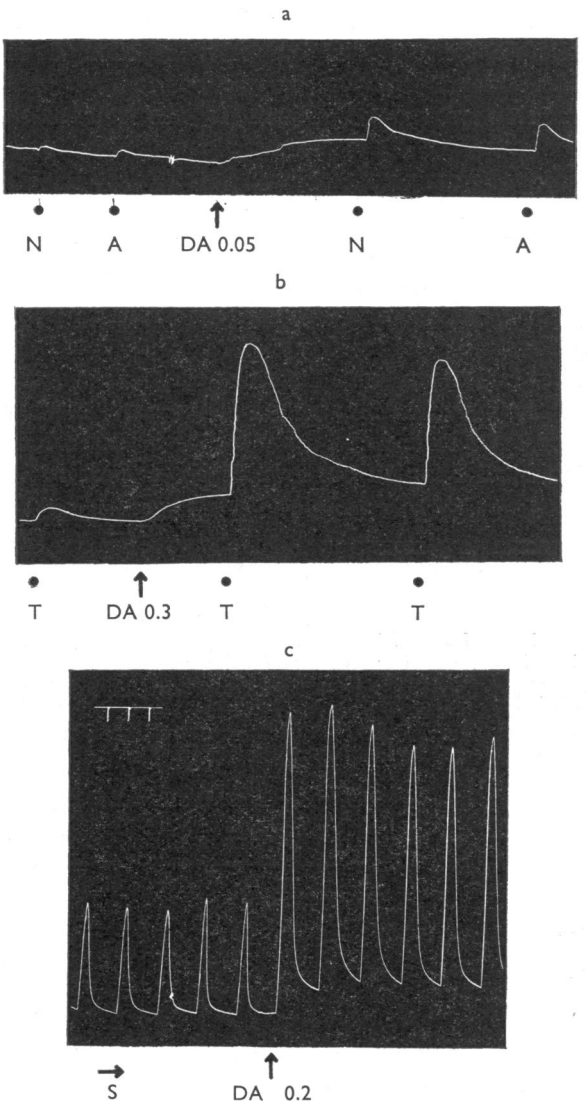


Fig. 9. Spinal cats. Records of contractions of acutely decentralized nictitating membrane in three experiments (*a*, *b* and *c*). DA 0.05, DA 0.2 and DA 0.3 = dexamphetamine 0.05, 0.2 and 0.3 mg/kg respectively. N = noradrenaline, 5 μg; A = adrenaline, 1 μg; T = tyramine, 1 mg; and S = submaximal stimulation of preganglionic cervical sympathetic nerve. Time scale = 1 min. All drugs were injected intravenously.

This is in agreement with observations made by Bejrablaya, Burn & Walker (1958), who found that hexamethonium did not reduce the stimulant effect of amphetamine on the heart.

Dexamphetamine 0.05 to 0.3 mg/kg slightly increased the size of the contractions produced by adrenaline and noradrenaline, but had a more marked effect on the response to tyramine (Table 1, Figs. 1 and 9). A maximum potentiation was observed 30 to 60 min after the injection of dexamphetamine. However, this apparent potentiation may be partly due to an additive action (Gaddum, 1948), since dexamphetamine itself caused prolonged contractions at these doses. Although the size of the contractions produced by tyramine was potentiated, there was no potentiation of the duration of the contractions (Table 1). In this respect dexamphetamine differed from pheniprazine.

Dexamphetamine produced extremely variable effects on the contractions produced by submaximal stimulation of the preganglionic and the postganglionic nerve. The effect on preganglionic stimulation varied from no effect in one experiment to the large effect shown in Fig. 9, where dexamphetamine increased the size of the contractions from 28 mm control to 76 mm. A similar effect was observed in one other experiment, but in 3 experiments there was only a 25% increase in the height of the contraction. In these last 3 experiments the effect of dexamphetamine on stimulation of the postganglionic nerve trunk was also determined: there was only a small potentiation, similar to that observed on preganglionic stimulation (Fig. 1).

Effects on the blood pressure. The major part of the investigation was concerned with effects on the nictitating membrane. However, since the blood pressure was

TABLE 3

EFFECT OF COCAINE AND ANTIDEPRESSANTS ON PRESSOR RESPONSES TO ADRENALINE, NORADRENALINE AND TYRAMINE IN THE SPINAL CAT

Doses of adrenaline and noradrenaline ranged from 1 to 40 μg, those of tyramine from 0·25 to 2 mg. * No significant result could be obtained since the effect of nialamide was very slow in onset, and increases in pressor responses to adrenaline and noradrenaline may have been due to spontaneous changes in sensitivity

	Intravenous dose mg/kg	No. of expts. showing at least 20 mm mercury increase (+) or decrease (−) in pressor response/total no. of expts.		
		Adrenaline	Noradrenaline	Tyramine
Cocaine	0·5–3·0	+5/5	+4/5	−5/5
Imipramine	1·0–4·0	+0/9	+5/8	−5/5
Pipradol	2·0	+3/4	+4/4	−3/4
Nialamide	15·0	No result*	No result*	+4/4
Pheniprazine	1·0	+3/4	+3/4	+3/4
Dexamphetamine	0·05–0·3	+3/5	+3/5	−1/3

recorded simultaneously, it is of interest to describe briefly the effects of cocaine and the antidepressant drugs on the pressor responses to adrenaline, noradrenaline and tyramine, and Table 3 summarizes the results obtained.

On the whole the results were similar to those obtained on the nictitating membrane, but there were a few differences. Firstly, imipramine potentiated the pressor response

to noradrenaline, but not the response to adrenaline, whereas on the nictitating membrane the contractions to both catecholamines were potentiated. Courvoisier, Leau, Ducrot, Fournel, Julou & Bardone (1959) and Sigg (1959) also found that imipramine potentiated the pressor effect of noradrenaline but not that of adrenaline. Secondly, pipradol reduced the pressor effect of tyramine, whereas it had no effect on the contraction of the nictitating membrane to tyramine, and, thirdly, dexamphetamine did not potentiate the pressor effect of tyramine, in contrast to the result obtained on the nictitating membrane, when the height (but not the duration) of the contractions to tyramine was potentiated.

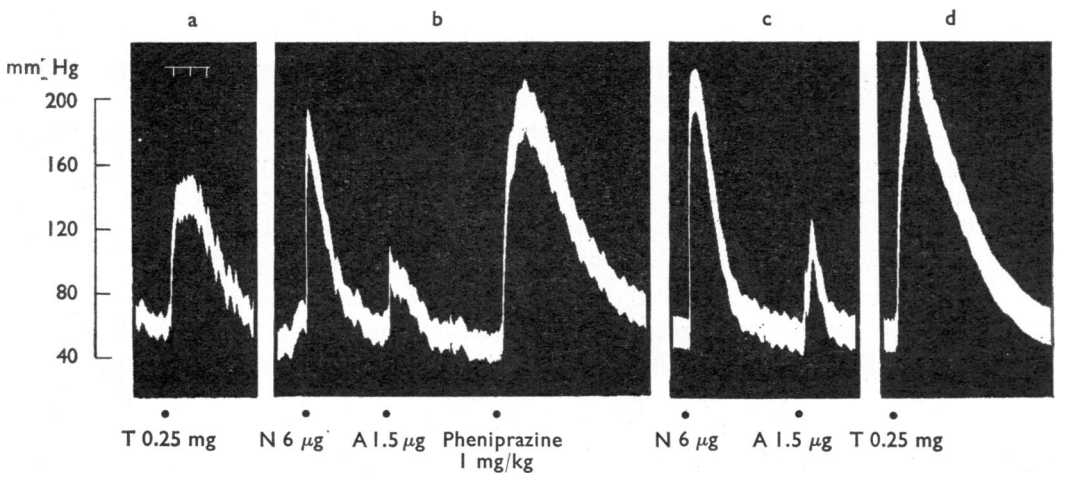


Fig. 10. Spinal cat. Effect of pheniprazine on pressor responses to tyramine (T), noradrenaline (N), and adrenaline (A). Interval of 20 min between *a* and *b*, 8 min between *b* and *c*, and 8 min between *c* and *d*. Time scale=1 min. All drugs were injected intravenously.

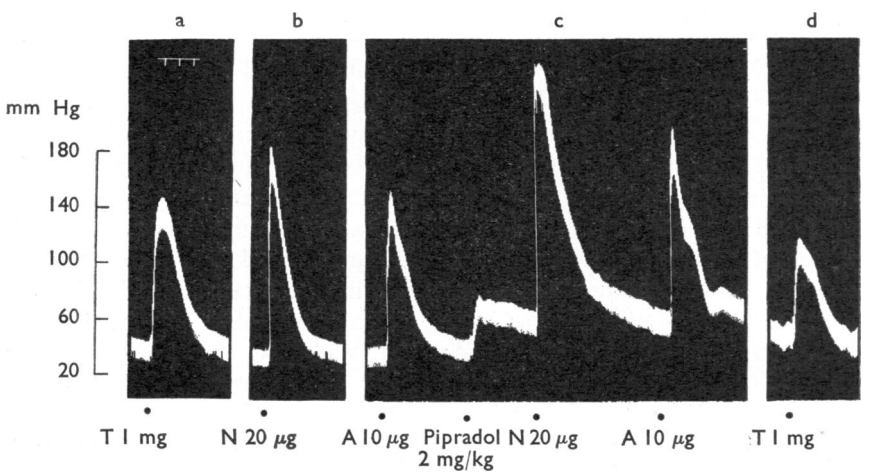


Fig. 11. Spinal cat. Effect of pipradol on pressor responses to tyramine (T), noradrenaline (N), and adrenaline (A). Interval of 10 min between *a* and *b*, 10 min between *b* and *c*, and 15 min between *c* and *d*. Time scale=1 min. All drugs were injected intravenously.

All the compounds, with the exception of nialamide, produced pressor effects. The average pressor effect obtained with cocaine, imipramine and pipradol was of the order of 25 mm of mercury, whereas after pheniprazine or dexamphetamine the average pressor responses were 85 and 50 mm of mercury respectively.

Figs. 10 and 11 illustrate experiments with pheniprazine and pipradol. Pheniprazine (Fig. 10) potentiated the pressor effects of adrenaline, noradrenaline and tyramine, whereas pipradol (Fig. 11) potentiated the action of adrenaline and noradrenaline, but reduced the effect of tyramine.

DISCUSSION

The effects of cocaine were very interesting. It contracted the innervated nictitating membrane, which is in agreement with the earler observation of Burn & Robinson (1952), and, although the size and duration of the contractions varied considerably, in those experiments in which they were sufficiently prolonged the membrane relaxed immediately when either the preganglionic nerve was cut or when hexamethonium was injected. Adrenaline or noradrenaline also caused a relaxation of the membrane, and this effect is thought to be due to the ganglion blocking action of these amines (Marrazzi, 1939 ; Bülbring, 1944 ; and Matthews, 1956).

One tentative explanation of the above action of cocaine is that it might increase the nervous activity in the preganglionic nerve, but this has not been investigated. Macmillan (1959) suggested that cocaine potentiated the action of catecholamines by reducing their uptake by storage sites and thus allowing more to occupy receptor sites. This hypothesis is in agreement with the results of Whitby, Hertting & Axelrod (1960), who found that cocaine reduced the uptake of injected noradrenaline by the tissues and raised the plasma concentrations. Therefore, an alternative explanation of the contraction to cocaine is that cocaine reduces the uptake by storage sites of the noradrenaline released by spontaneous activity in the sympathetic nerve. It is unlikely that cocaine actually increases the amount of noradrenaline released by the spontaneous activity in the sympathetic nerve fibres, since Trendelenburg (1959) could not find any increase in the output of noradrenaline from the spleen on stimulation of the splenic nerve after cocaine.

The effects of cocaine and the antidepressant drugs on the nictitating membrane could be divided into three main types. The first type of effect was a potentiation of the contractions to adrenaline or noradrenaline, accompanied by no effect on, or a reduction of the contractions to, tyramine. Cocaine, imipramine and pipradol had this type of effect. Furthermore, Fleckenstein & Stöckle (1955) found that cocaine antagonized the contractions to amphetamine, and it has now been shown that imipramine and pipradol have a similar action to cocaine in antagonizing the contractions to amphetamine. Recently Axelrod, Whitby & Hertting (1961) have demonstrated a further similarity between cocaine and imipramine: both reduced the uptake of noradrenaline by the tissues. Therefore, it is possible that cocaine, imipramine and pipradol potentiate the effects of adrenaline and noradrenaline by the same mechanism.

The second type of effect observed was a potentiation of the height and duration of the contractions to tyramine, as shown by pheniprazine and nialamide. Since

tyramine is a good substrate for monoamine oxidase (Wiener, 1960), and both pheniprazine and nialamide are potent inhibitors of this enzyme (Zbinden, Randall & Moe, 1960), it is reasonable to conclude that the potentiation of the response to tyramine by pheniprazine and nialamide is due to inhibition of monoamine oxidase. Dexamphetamine also increased the size of the contractions to tyramine. However, dexamphetamine did not prolong the contractions to tyramine or potentiate its pressor effect, and dexamphetamine is only a weak inhibitor of monamine oxidase (Zbinden *et al.*, 1960). Therefore, it is unlikely that dexamphetamine potentiated the size of the contraction to tyramine by an inhibitory effect on monoamine oxidase.

The significance of monoamine oxidase in limiting the action of catecholamines is not clear. However, Spector, Kuntzman, Shore & Brodie (1960) have suggested that, whereas catechol-O-methyltransferase may be important in the inactivation of circulating catecholamines, it is monoamine oxidase which is important at the site of formation and release of these amines in tissues such as the brain and the heart.

The third type of effect was a direct stimulant action on the membrane. This effect was shown by dexamphetamine and pheniprazine. Since Reinert (1959) found that amphetamine had a nicotine-like action on the superior cervical ganglion when the drug was injected into the fluid perfusing the ganglion, it was noteworthy that intravenous injections of dexamphetamine or pheniprazine caused contractions of the membrane after removal of the ganglion. Therefore, the contractions obtained on intravenous injection were clearly not due to nicotine-like ganglion stimulant effects.

Trendelenburg (1959) found that cocaine potentiated the contractions of the membrane to submaximal stimulation of the preganglionic and postganglionic sympathetic nerves. Thus, it was extremely interesting to find that imipramine, pipradol, pheniprazine and dexamphetamine, all of which have central stimulant or antidepressant properties, had similar effects to cocaine. Furthermore, Kamijo, Koelle & Wagner (1956) found that another antidepressant drug, iproniazid, also potentiated the contractions to preganglionic nerve stimulation.

It is possible that cocaine, imipramine and dexamphetamine potentiate sympathetic nerve stimulation by the same mechanism since they reduce the uptake of noradrenaline by the tissues (Whitby *et al.*, 1960 ; Axelrod *et al.*, 1961), but it is not known whether pipradol has the same effect. Axelrod *et al.* (1961) also showed that pheniprazine did not affect the tissue levels of noradrenaline and therefore there must be an alternative explanation (possibly inhibition of monoamine oxidase) for pheniprazine.

Since it seems likely that a central adrenergic mechanism may be important in the maintenance of alert behaviour (Dell, Bonvallet & Hugelin, 1954 ; Rothballer, 1959), it is tempting to speculate that there may be a similarity between the effects of these central stimulant and antidepressant drugs on electrical stimulation of peripheral adrenergic nerves and their effects on catecholamines released within the brain. For two of these drugs, cocaine and amphetamine, there is some suggestive evidence (Rothballer, 1957) for potentiation of the central effects of adrenaline ; they were shown to increase the alerting effect of injected adrenaline as shown on the electroencephalogram.

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