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THE EFFECTS OF PREGANGLIONIC AND POSTGANGLIONIC DENERVATION ON THE RESPONSES OF THE NICTITATING MEMBRANE TO SYMPATHOMIMETIC SUBSTANCES

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Postganglionic denervation selectively enhances the chronotropic action of noradrenaline but not of adrenaline (Innes & Kosterlitz, 1951). Ephedrine has no effect on the responses of the acutely denervated heart to adrenaline, noradrenaline and cobefrine while cocaine potentiates the action of noradrenaline and cobefrine (Innes & Kosterlitz, 1954). These facts do not seem to support the theory proposed by Burn & Robinson (1952) that the effects of postganglionic denervation and of cocaine can be explained by loss or inhibition of amine oxidase. Since this theory originated in observations on the nictitating membrane, it seemed worth while to adapt for this sympathetic effector the procedure employed for the analysis of the chronotropic responses. Use is also made of the finding of Lockett (1950) that preganglionically and postganglionically denervated membranes differ greatly in their responses to adrenaline, noradrenaline and tyramine.

METHODS

Experimental. The adrenal medullae were inactivated as described in the preceding paper (Innes & Kosterlitz, 1954) and in some animals the nictitating membrane was denervated at the same time. In the others, when denervation of shorter duration (16–72 hr) was to be examined, the membrane was denervated at a second operation performed under chloroform-ether anaesthesia. Preganglionic denervation ('decentralization') was obtained by excising about 2 cm of the cervical sympathetic, and postganglionic denervation ('denervation') by removing the superior cervical ganglion.

For the final stage the cats were anaesthetized with pentobarbitone sodium (45 mg/kg). Raising the total dose of pentobarbitone to 75 mg/kg did not alter the responses of the nictitating membrane. A few experiments were performed in spinal preparations with the brain destroyed (Bülbring & Burn, 1938); the

results did not differ in any way from those obtained with pentobarbitone anaesthesia.

The contractions of the nictitating membrane were recorded by means of isotonic gimbal levers, the tension exerted on the membranes being 5 g. The magnification of the movements of the writing point was × 29.

TABLE 1. List of sympathomimetic substances used

Name	Chemical formula of base: (R_1)	(R_2)	(R_3)	(R_4)	(R_5)
β-Phenylethylamine HCl	H	H	H	H	H
Tyramine acid phosphate	OH	H	H	H	H
DL-'Nor-sympatol' HCl (Win 5512)	OH	H	OH	H	H
DL-'Nor-neosynephrine' HCl (Win 5501)	H	OH	OH	H	H
Hydroxytyramine HCl	OH	OH	H	H	H
L-Noradrenaline bitartrate	OH	OH	OH	H	H
DL-Cobefrine HCl	OH	OH	OH	CH_3	H
DL-Sympatol bitartrate	OH	H	OH	H	CH_3
L-Neosynephrine HCl	H	OH	OH	H	CH_3
Epinine HCl	OH	OH	H	H	CH_3
L-Adrenaline	OH	OH	OH	H	CH_3
L-Ephedrine HCl	H	H	OH	CH_3	CH_3

Drugs. The names and chemical formulae of the sympathomimetic amines are given in Table 1. The solutions of adrenaline and noradrenaline were prepared as described previously (Innes & Kosterlitz, 1954) while the solutions of the other amines were made up freshly for each experiment by weighing out a small amount and dissolving it in Ringer-Locke solution containing ascorbic acid (0·2 mg/ml.). The injections were made into the femoral vein; the volume varied between 0·2 and 1 ml. and the time taken for the injection was always 15 sec. The quantities injected refer to the free bases except in the case of ephedrine HCl and cocaine HCl.

RESULTS

The final effects of decentralization and denervation

While denervation and decentralization caused similar changes in the responses of the nictitating membrane to sympathomimetic amines during the first 20–30 hr after operation, the responses were very different from about 48 hr onwards. Although adrenaline, noradrenaline, cobefrine and tyramine were selected to illustrate in some detail the changes occurring at different stages of decentralization and denervation, reference will be made later to other sympathomimetic substances.

The response of the decentralized membrane to adrenaline was either equal to, or smaller than, that of the denervated membrane (Figs. 1, 2). The decentralized membrane was always less sensitive to noradrenaline and cobefrine

than the denervated one, the difference being particularly large when the adrenaline responses of the two membranes were not equal. The tyramine response on the denervated was always smaller than on the decentralized side,

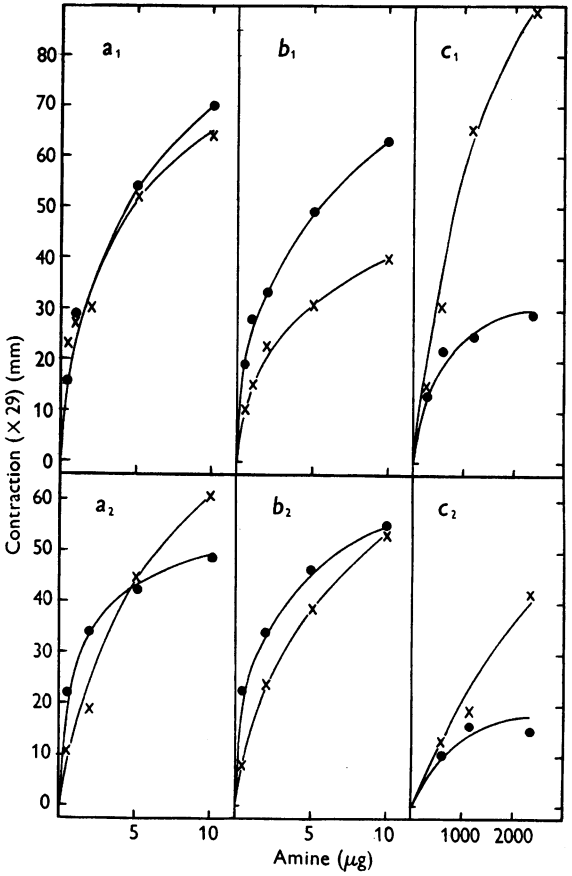

Fig. 1. Expt. 101, 3·8 kg. Dose-response curves of the denervated (●) and decentralized (×) nictitating membranes, 49 days after operation. *a*, L-adrenaline; *b*, L-noradrenaline; *c*, tyramine. a_1–c_1 before, a_2–c_2 after, cocaine HCl (4 mg/kg).

the discrepancy becoming more marked with increasing doses of tyramine. These relationships were not altered when the acute experiment was performed as late as 3 months after denervation and decentralization. Injection of cocaine slightly reduced the adrenaline contraction of the decentralized membrane, markedly enhanced the noradrenaline response and very considerably diminished the contraction after tyramine; this resulted in making the responses of the decentralized membrane more like those of the denervated membrane (Fig. 1). All responses of the denervated membrane were somewhat depressed by cocaine.

The membrane decentralized for 2–3 weeks was much more sensitive to adrenaline, noradrenaline and tyramine than the acutely decentralized membrane (Fig. 3). Ephedrine hydrochloride in doses between 100 and 300 μg/kg potentiated the responses of the acutely decentralized membrane so that they imitated those of a chronically decentralized but never of a chronically denervated membrane. Whatever dose of ephedrine was used, the contraction after noradrenaline always remained smaller than after adrenaline.

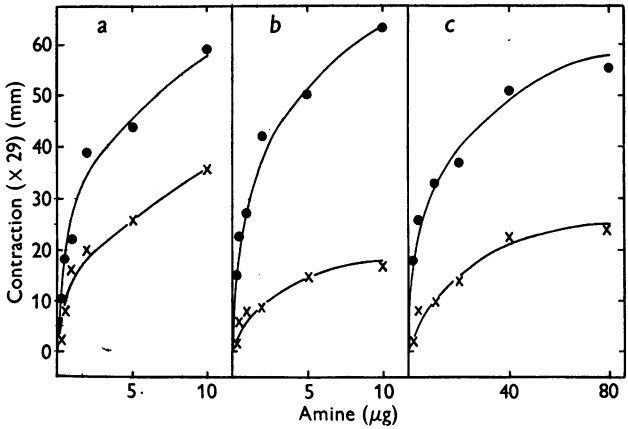

Fig. 2. Expt. 111, 3·2 kg. Dose-response curve of the denervated (●) and decentralized (×) nictitating membranes, 19 days after operation. *a*, L-adrenaline; *b*, L-noradrenaline *c*, DL-cobefrine.

Comparison of the action of ephedrine and cocaine

It would appear that, on the whole, injection of ephedrine simulated the effect of chronic decentralization and injection of cocaine that of chronic denervation. This view was supported by other observations. When cocaine was given after ephedrine had converted the responses of an acutely decentralized membrane to those of a chronically decentralized membrane, then the responses became those of a chronically denervated membrane (Innes & Kosterlitz, 1954; fig. 3). Cocaine, but never ephedrine, changed the responses of a chronically decentralized membrane to those of a denervated membrane (Fig. 4). When ephedrine had been given first and had caused a prolonged contraction, cocaine often led to a relaxation of the membrane, a fact already observed on the intact membrane by Bacq (1936). There was no effect of cocaine on the chronically denervated membranes apart from a slight prolongation of the contractions.

The responses of the acutely decentralized membrane to adrenaline and noradrenaline were affected by very small doses of cocaine (0·1–0·2 mg/kg) in a way which in some respects was similar to the potentiation by ephedrine

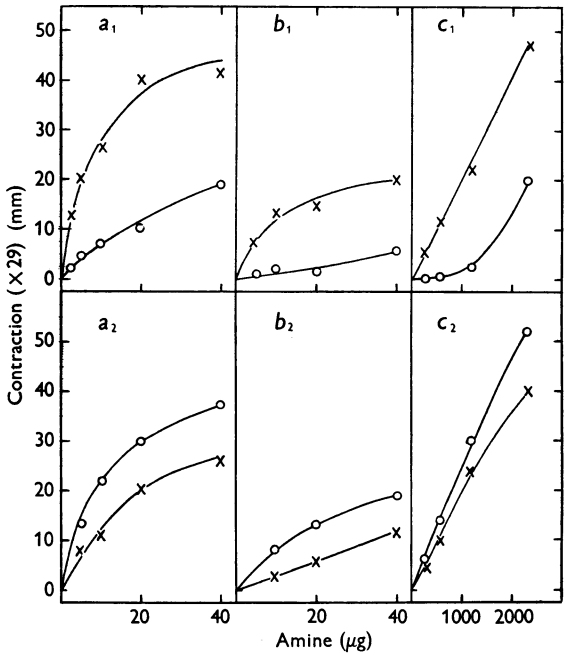

Fig. 3. Expt. 104, 3 kg. Dose-response curves of the acutely decentralized (○) and chronically decentralized (×) nictitating membranes, 18 days after operation. *a*, L-adrenaline; *b*, L-noradrenaline; *c*, tyramine. a_1–c_1 before, a_2–c_2 after L-ephedrine, given in two doses of 400 μg each with an interval of 80 min between them.

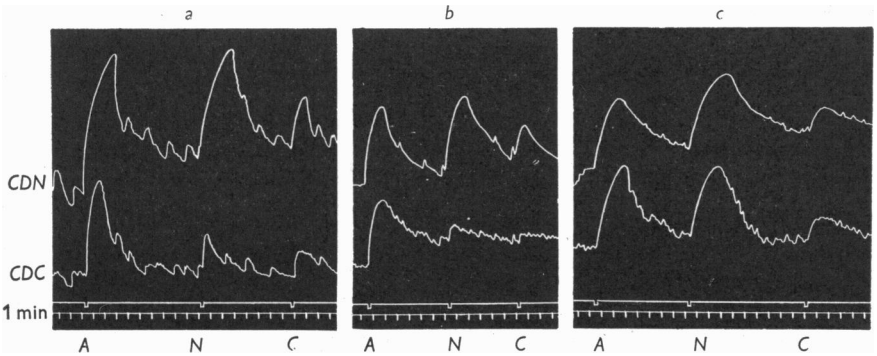

Fig. 4. Expt. 37, 2·5 kg. The effects of ephedrine and cocaine on the responses of chronically denervated (*CDN*) and chronically decentralized (*CDC*) membranes, 15 days after operation to 5 μg L-adrenaline (*A*), 5 μg L-noradrenaline (*N*) and 10 μg DL-cobefrine (*C*). 200 μg L-ephedrine HCl were given 20 min before *b* and 2 mg/kg cocaine 10 min before *c*.

(Fig. 5). There was, however, no increase in the tyramine responses. Larger doses of cocaine, about 2 mg/kg usually being sufficient, equalized the responses to adrenaline and noradrenaline.

Amylocaine, in doses of 20–80 mg/kg, had effects very similar to those of cocaine (Innes & Kosterlitz, 1952). This confirms the observations of Bacq &

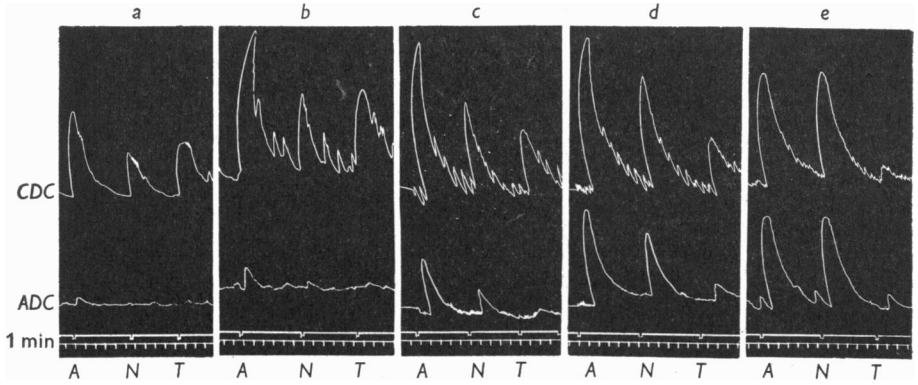

Fig. 5. Expt. 58, 4·3 kg. The effect of repeated small doses of cocaine on the responses of the acutely (*ADC*) and chronically (*CDC*) decentralized nictitating membranes, 24 days after operation, to 10 μg L-adrenaline (*A*), 10 μg L-noradrenaline (*N*) and 1·2 mg tyramine (*T*). Cocaine HCl was injected intravenously: 0·1 mg/kg 10 min before *b*, 0·1 mg/kg 10 min before *c*, 0·2 mg/kg 10 min before *d*, 0·4 mg/kg 40 min and 0·7 mg/kg 10 min before *e*.

Lefebvre (1934) and also agrees with the findings that amylocaine can replace cocaine in potentiating the chronotropic action of noradrenaline (Innes & Kosterlitz, 1954).

The apparently depressing effect of ephedrine on the action of adrenaline and noradrenaline on the chronically decentralized nictitating membrane was probably due to the fact that the chronically, but not the acutely, decentralized membrane responded with marked and prolonged contractions to ephedrine (Fig. 6, Expt. 62). A second injection of the same amount of ephedrine often caused an even larger contraction of the chronically decentralized membrane and then also a considerable shortening of the acutely decentralized membrane. Sometimes it was found that with repeated doses of ephedrine the contractions of the acutely decentralized or denervated membrane eventually became larger than those of the chronically decentralized membrane (Fig. 6, Expt. 45). Ephedrine obviously sensitized the acutely decentralized membrane to further injections of ephedrine. In a similar way, cocaine and amylocaine did not cause a contraction of the acutely decentralized membrane while the chronically decentralized membrane responded to these drugs with prolonged contractions.

The early effects of preganglionic denervation (decentralization)

There was no difference between the responses of intact, acutely decentralized and acutely denervated membranes, and no change was found during the first 16 hr after decentralization. A slight increase in sensitivity was observed 24 hr

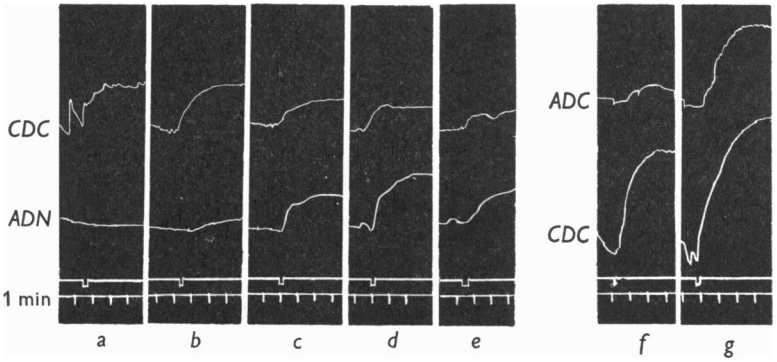

Fig. 6. Expts. 45, 2·4 kg (*a–e*) and 62, 3·1 kg (*f*, *g*). The responses of chronically decentralized (*CDC*) and acutely decentralized (*ADC*) and of denervated (*ADN*) nictitating membranes to repeated doses of L-ephedrine HCl. Before each injection the writing points were readjusted to the original base-line. Doses (μg) and times. Expt. 45: *a*, 100, 2·54; *b*, 200, 3·30; *c*; 200, 4·04; *d*; 300, 4·46; *e*, 300, 5·08. Expt. 62: *f*, 400, 10·15; *g*, 400, 10·58. Operation 15 days (Expt. 45) and 7 days (Expt. 62) prior to experiments.

after operation and became more pronounced after an interval of a further 24 hr (Figs. 7, 8). At this stage, small doses of ephedrine very often had no effect and larger doses a depressant effect on the adrenaline and noradrenaline responses while the tyramine responses were potentiated by small doses and scarcely altered by the larger doses. The responses of the acutely decentralized membrane (Fig. 8) were potentiated by each successive dose of ephedrine until they became, if anything, larger than those of the chronically decentralized membrane.

The early effects of postganglionic denervation

The changes observed during the first 2 days after denervation were much more rapid and complex than after decentralization. At about 16 hr there was often a slightly increased sensitivity to all three amines tested, adrenaline, noradrenaline and tyramine (Fig. 9a_1–c_1), which was made more pronounced by small doses of ephedrine.

The most rapid changes occurred during the period 16–26 hr after operation. They consisted of a very marked sensitization of the membrane to all three amines; the response to noradrenaline was still less than that to adrenaline and the action of tyramine, particularly in small doses, was very considerably enhanced and not depressed as in later stages of denervation (Figs. 9a_2–c_2

and 10). The potentiation of tyramine on the first day of denervation has already been observed by Bülbring & Burn (1938) and Fleckenstein & Burn

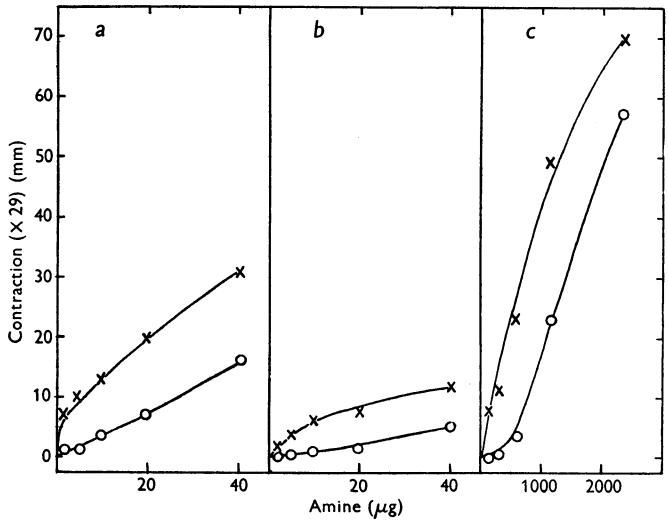

Fig. 7. Expt. 106, 1·9 kg. Dose-response curves of acutely (○) and chronically (×) decentralized nictitating membranes, 48 hr after operation. *a*, L-adrenaline; *b*, L-noradrenaline; *c*, tyramine.

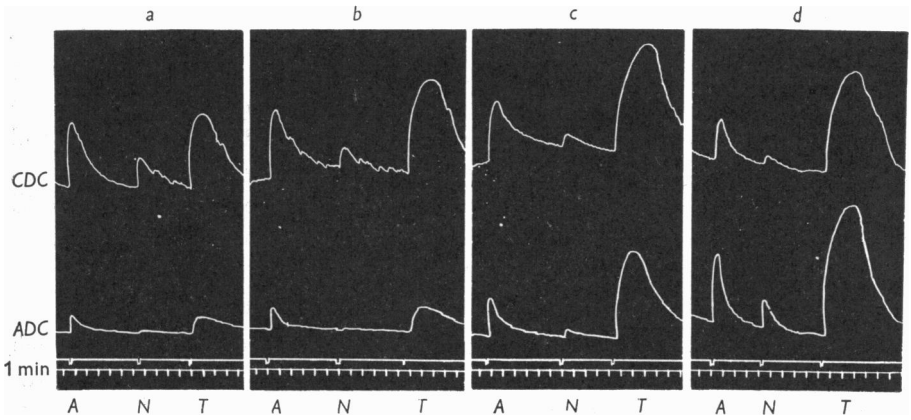

Fig. 8. Expt. 63, 2·5 kg. The effect of ephedrine on the responses of acutely (*ADC*) and chronically (*CDC*) decentralized nictitating membranes, 48 hr after operation, to 10 μg L-adrenaline (*A*), 10 μg L-noradrenaline (*N*) and 1·2 mg tyramine (*T*). L-ephedrine HCl was injected intravenously: 100 μg 20 min before *b*, 200 μg 20 min before *c* and 300 μg 20 min before *d*. The ephedrine contractions were small in *CDC* and absent in *ADC*.

(1953). The membrane was at this stage very sensitive to ephedrine which, even in small doses, caused a powerful contraction with a concomitant de-

pression of the responses to adrenaline, noradrenaline and tyramine (Fig. 10). It is of interest that, when measured at the beginning of the experiment with a tension of 5 g, the denervated membrane was always less extended than the

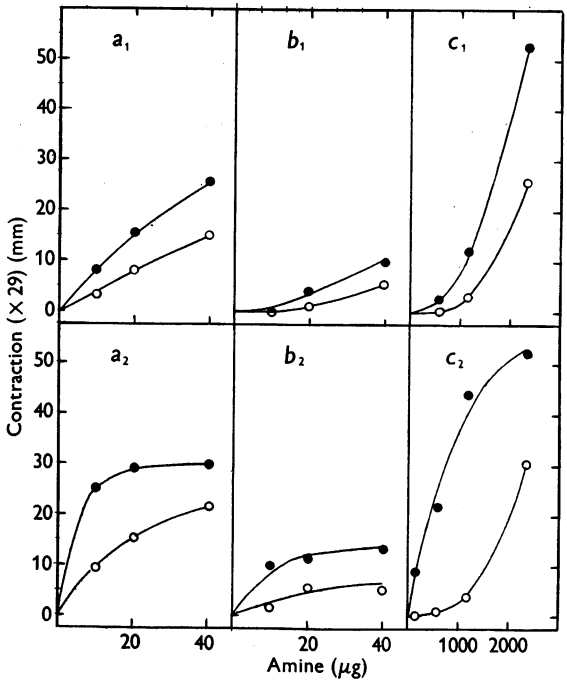

Fig. 9. Expt. 90, 2·8 kg. Dose-response curves of intact (○) and chronically denervated (●) nictitating membranes 1 hr (a_1–c_1) and 6 hr (a_2–c_2) after the beginning of the acute expt. Operation 16 hr prior to beginning of expt. a_1 and a_2, L-adrenaline; b_1 and b_2, L-noradrenaline; c_1 and c_2, tyramine.

decentralized membrane, an observation which agrees well with that obtained on the pupil, viz. during the first 2 days after operation the denervated pupil is larger than the decentralized pupil (Budge, 1855).

During the period of 24–48 hr after denervation, the responses changed in a manner characteristic of postganglionic denervation, the response to noradrenaline increasing and that to tyramine becoming depressed (Fig. 11). This was particularly well seen in an experiment 22 hr after denervation. During the next 11 hr the noradrenaline response increased until it equalled adrenaline while tyramine became less effective (Fig. 12). Although the general sensitivity of the membranes continued to increase after the second day following denervation, there was no further change in the relative magnitudes of the contractions produced by the various amines.

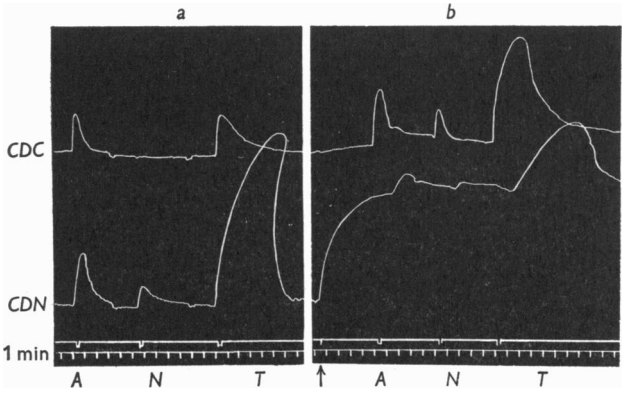

Fig. 10. Expt. 70, 2·4 kg. The responses of chronically decentralized (*CDC*) and denervated (*CDN*) nictitating membranes, 26 hr after operation, to 10 μg L-adrenaline (*A*), 10 μg L-noradrenaline (*N*) and 1·2 mg tyramine (*T*); 200 μg L-ephedrine HCl were injected at the arrow in *b*.

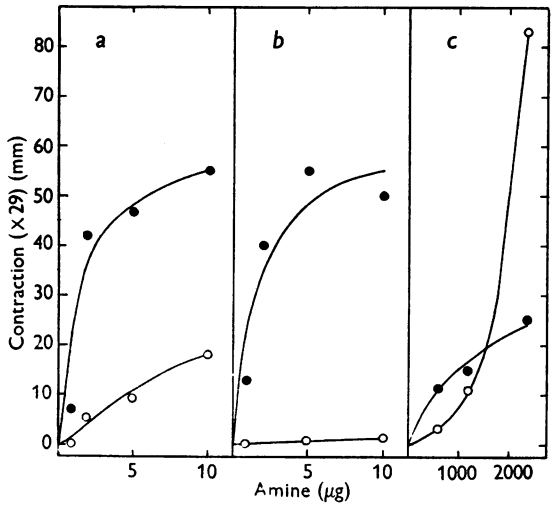

Fig. 11. Expt. S3, 3·4 kg (spinal preparation). Dose-response curves of acutely decentralized (○) and chronically denervated (●) nictitating membranes, 48 hr after operation. *a*, L-adrenaline; *b*, L-noradrenaline; *c*, tyramine.

Spontaneous increase in the sensitivity of the intact nictitating membrane

Although in recovery experiments decentralization had no sensitizing effect until about 16 hr after operation, spontaneous increases in sensitivity of the intact or acutely decentralized membrane were sometimes observed within a few hours after the beginning of an acute experiment. These changes always simulated those of chronic decentralization and never of chronic denervation.

There was nothing obvious in such experiments which could help to explain this phenomenon. In the course of some twenty experiments designed to investigate this problem it was found that the sensitization could be brought

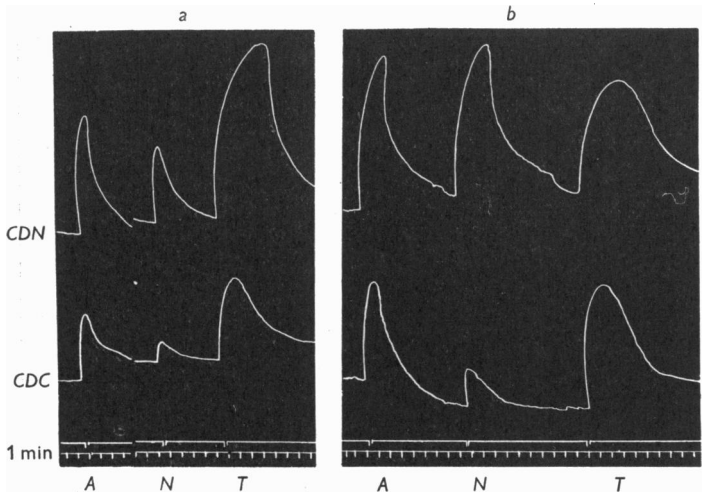

Fig. 12. Expt. 85, 2 kg. The responses of the chronically denervated (*CDN*) and decentralized (*CDC*) nictitating membranes, 22 hr after operation, to 10 μg L-adrenaline (*A*), 10 μg L-noradrenaline (*N*) and 1·2 mg tyramine (*T*). *a*, 20 min; *b*, 11 hr after the beginning of the acute experiment.

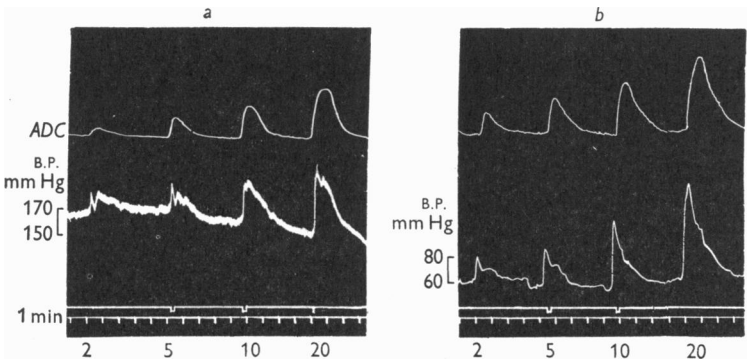

Fig. 13. Expt. 149, 1·8 kg. Effect of haemorrhage on the responses of the acutely decentralized (*ADC*) nictitating membrane to 2, 5, 10 and 20 μg L-adrenaline. The remaining left adrenal was removed at the beginning of the experiment. Immediately before *b*, 10 ml./kg blood were withdrawn. The potentiation was more than 100%.

about most readily by lowering the arterial B.P. to about 60 mm Hg by a haemorrhage of 10–15 ml. blood/kg body weight (Fig. 13). Re-infusion of the blood sometimes but not always reduced the responses. It made no difference whether the adrenals were present or not. Severing the vagus and sinus nerves

did not prevent the increase in sensitivity caused by haemorrhage. A particularly marked and rapid sensitization was found in one experiment in which the B.P. fell spontaneously from 175 to 60 mm Hg in the course of less than 2 hr (Fig. 14).

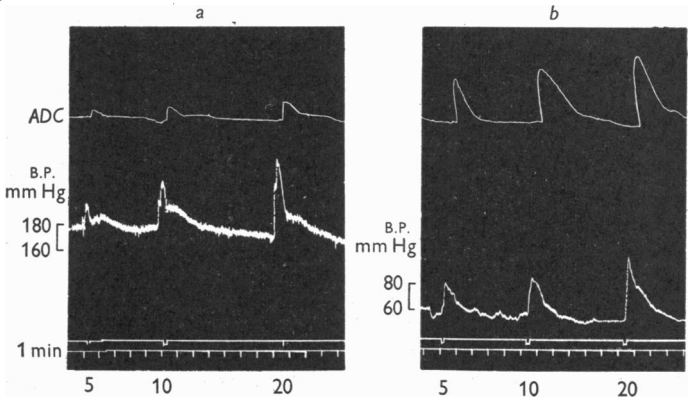

Fig. 14. Expt. 151, 1·8 kg. The 'spontaneous' potentiation of the responses of the acutely decentralized (*ADC*) nictitating membrane to 5, 10 and 20 μg L-adrenaline. Both adrenals were removed at the beginning of the experiment. *a*, at 10·17 a.m. *b*, at 12·04 p.m. Potentiation considerably more than fourfold.

Absence of potentiation of adrenaline and noradrenaline by cobefrine

Burn & Robinson (1952) suggested that the potentiation of the action of cobefrine by denervation or cocaine was due to the fact that less amine oxidase was present and less of the cobefrine would combine with the enzyme, leaving more cobefrine free to act on the membrane. If this were so, cobefrine should, in the acutely decentralized membrane, saturate the enzyme and thus potentiate the response to adrenaline. Such an effect was never observed when adrenaline or noradrenaline was injected 5 min after cobefrine. Further, when 5 μg of adrenaline were given together with 50 or 100 μg of cobefrine, there was no potentiation of the adrenaline contraction of the acutely or chronically decentralized membranes. The action of noradrenaline also remained unaffected.

The effect of decentralization and denervation on the action of some other sympathomimetic substances

Decentralization caused an apparently unspecific increase in the responses of all substances tested. The shapes of the dose-response curves of epinine, neosynephrine and 5-hydroxytryptamine were similar to those of adrenaline and noradrenaline (Fig. 3), while the curves of sympatol and β-phenylethylamine were on the whole more like that of tyramine.

The responses of the denervated membrane were compared with those of the decentralized membrane as a standard of reference in order to allow for

unspecific increases in sensitivity. When amines were tested which differed from adrenaline by the loss of the CH_3 group, noradrenaline (Fig. 15*b*), or the loss of the phenolic OH group in the para- position, neo-synephrine (Fig. 15*c*), or the loss of both of these groups, 'nor-neosynephrine' (Fig. 15*d*), then the responses of the denervated membrane showed only minor differences while

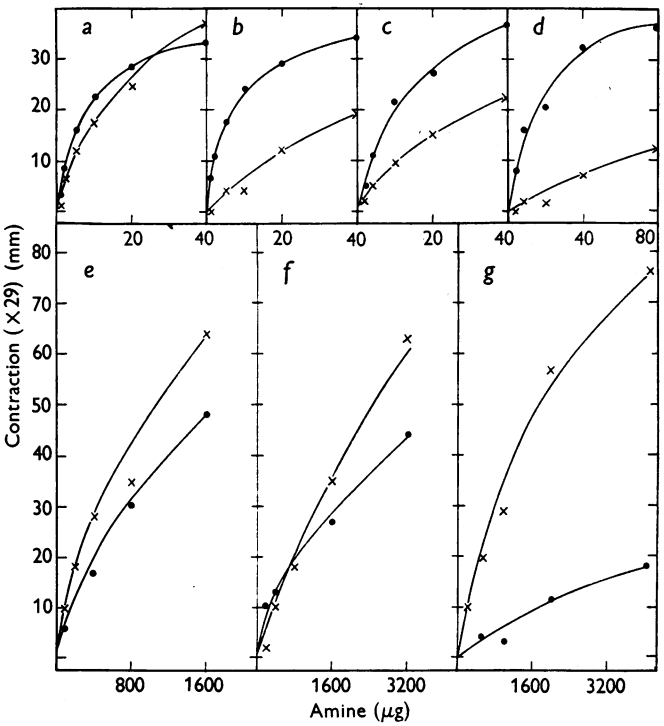

Fig. 15. Expt. 159, 3·1 kg. Dose-response curves of chronically denervated (●) and decentralized (×) nictitating membranes, 48 hr after operation. *a*, L-adrenaline; *b*, L-noradrenaline; *c*, L-neosynephrine; *d*, DL-'nor-neosynephrine'; *e*, DL-sympatol; *f*, DL-'nor-sympatol'; *g*, tyramine.

those of the decentralized membrane were considerably reduced. This was so whether the response to adrenaline of the decentralized membrane was equal to, or less than, that of the denervated membrane. The means of the relative activities of these substances, tested on decentralized and denervated membranes, are summarized in Table 2. As far as the responses of the decentralized membrane were concerned, the loss of the CH_3 (noradrenaline) and of the phenolic OH in the para- position (neosynephrine) led to similar losses in activity. When both these groups were absent, there was a very marked decrease in activity ('nor-neosynephrine).

The responses to the amines without the OH group in the meta- position of

TABLE 2. Comparison of the action of adrenaline, noradrenaline, neosynephrine, 'nor-neosynephrine' and epinine on the chronically decentralized and denervated nictitating membranes (2–18 days)

Amine	Activity expressed in multiples of the dose of L-adrenaline giving the same contraction of the nictitating membranes (range in brackets)	
	Decentralized	Denervated
L-Noradrenaline (11 expts.)	5·3 (4–10)	1·2 (1–2)
L-Neosynephrine (9 expts.)	5·4 (3–10)	1·9 (1–7)
DL-'Nor-neosynephrine' (8 expts.)	40 (20–180)	3·8 (2–8)
Epinine (4 expts.)	42 (33–53)	9 (4–18)

It is probable that L-'nor-neosynephrine' is almost twice as active as the racemic compound which was used. As the number of experiments in which the membrane was denervated 13–18 days prior to the experiment was small, it is not certain whether the duration of denervation has any effect on the responses.

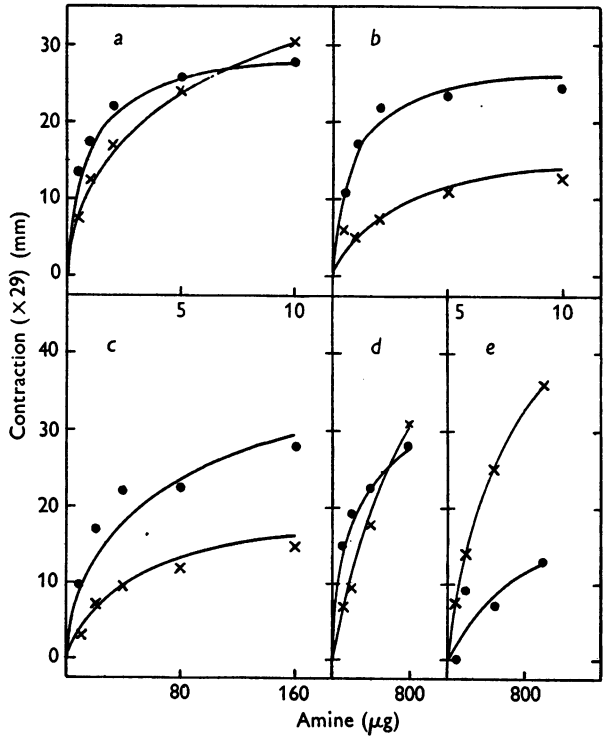

Fig. 16. Expt. 175, 2·2 kg. Dose-response curves of chronically denervated (●) and decentralized (×) nictitating membranes, 48 hr after operation. *a*, L-adrenaline; *b*, L-noradrenaline; *c*, epinine; *d*, hydroxytyramine; *e*, tyramine.

the benzene ring were very different from those described above. Sympatol (Fig. 15*e*) and 'nor-sympatol' (Fig. 15*f*) were both much less active than the compounds having an OH group in the meta- position and the responses of the denervated membrane were slightly less than those of the decentralized membrane. 'Nor-sympatol' differs from tyramine in having an alcoholic OH group in the side chain; thus, introduction into the tyramine molecule of this group scarcely altered the response of the decentralized membrane but improved that of the denervated membrane very considerably.

The loss of the alcoholic OH group from adrenaline, leading to epinine (Fig. 16*c*), or from noradrenaline, leading to hydroxytyramine (Fig. 16*d*), caused a loss of activity on the decentralized membrane similar to that found when the phenolic OH in the meta- position was absent. Compared with tyramine, the response of the denervated membrane to hydroxytyramine was improved while there was little difference between the responses of the decentralized membrane. The responses to hydroxytyramine were rather like those after 'nor-sympatol' in that there was relatively little difference between the decentralized and denervated sides. On the other hand, epinine always caused a much better response on the denervated than on the decentralized side (Table 2).

Cocaine (3 mg/kg) potentiated the responses of the chronically decentralized membrane to noradrenaline, 'nor-neosynephrine', epinine and slightly to neosynephrine. The responses to sympatol, nor-sympatol and hydroxytyramine were scarcely affected by cocaine.

DISCUSSION

The differences between the responses of decentralized and denervated membranes to adrenaline, noradrenaline and tyramine were first pointed out by Lockett (1950). Our observations fully confirm and extend her findings, viz. that, while decentralization unspecifically enhances the responses of the nictitating membrane of the cat to sympathomimetic amines, denervation specifically potentiates the response to noradrenaline and depresses that to tyramine.

It would appear that the changes brought about in the effector system are much more complex after denervation than after decentralization. During the first 24 hr after operation the differences between a denervated and a decentralized membrane are mainly of a quantitative nature: the increase in sensitivity affects all the amines in an unspecific manner but the rate of this increase is greater after denervation than after decentralization. During the second day the sensitivity of the decentralized membrane continues to increase without significant changes in the relative activity of the amines while the denervated membrane becomes more sensitive to noradrenaline and cobefrine but responds less to tyramine, particularly in large doses. This qualitative

change occurs at the time when it becomes impossible to produce a contraction of the nictitating membrane by stimulating the postganglionic fibres subserving it. This loss of conduction is not likely to be the direct cause of the specific alterations as a membrane whose responses have been potentiated by cocaine still contracts after electrical stimulation of the postganglionic fibres.

The changes produced by decentralization and denervation can also be imitated by ephedrine and cocaine. Thus, after injection of ephedrine, an acutely decentralized membrane responds like a chronically decentralized membrane and, after cocaine, like a chronically denervated membrane. As is to be expected, ephedrine has no potentiating but rather a depressing effect on the responses of a chronically decentralized or denervated membrane and cocaine does not significantly affect a denervated membrane.

Burn & Robinson (1952) found that the amine oxidase content of the nictitating membrane falls after denervation. On the strength of this finding and the fact that cocaine inhibits amine oxidase (Philpot, 1940), they formulated their theory that the changes caused by denervation or cocaine are due to a loss or inhibition of amine oxidase. In view of the general rise in sensitivity to sympathomimetic amines which is found after decentralization of the nictitating membrane, it would be of interest to know what happens to the amine oxidase content of such membranes. So far no data are available in support of a possible hypothesis that the increase in sensitivity is due to a loss of enzyme. Such a view, however, would be in agreement with the fact that ephedrine, a competitive inhibitor of amine oxidase (Blaschko, Richter & Schlossmann, 1937), changes the responses of the acutely decentralized membrane so that they become indistinguishable from those of a chronically decentralized membrane. There is only one observation which does not seem to fit, namely the potentiating effect of ephedrine on the action of cobefrine which is immune to amine oxidase from liver and does not compete for the enzyme with adrenaline or noradrenaline. However, the enzyme of the nictitating membrane may be different from that of the liver.

In our opinion, chronic denervation produces changes which are more complex than those after decentralization. It is unlikely that the loss of amine oxidase found in denervated membranes (Burn & Robinson, 1952) can explain the difference between decentralized and denervated membranes. It seems to be difficult to explain on this basis why the denervated membrane responds more to noradrenaline and cobefrine and less to tyramine than the decentralized membrane. Fleckenstein & Burn (1953) also arrived at the conclusion that the potentiating effect of denervation on the action of cobefrine could not readily be explained by a loss of amine oxidase. As far as the depression of the tyramine response was concerned, they assumed that tyramine had no direct action on the normal membrane but competed for the enzyme with the transmitter, noradrenaline; in this way a contraction was

caused by the transmitter itself which had been liberated from the resting nerve fibres. So far, such a competition between the transmitter and tyramine has not been shown by direct experiment. Whatever the explanation of the action of tyramine may be, the fact that ephedrine potentiates the response to tyramine quite readily and to a considerable extent while small or large doses of cocaine do not, indicates that the cocaine effect is different from that of ephedrine and not solely due to an inhibition of amine oxidase. In this connexion it appears to be of importance that cocaine does not prevent the contraction of the nictitating membrane when the postganglionic fibres are stimulated; obviously the transmitter is still liberated when cocaine has been given.

It is not yet possible to formulate a theory which would explain the changes observed after decentralization and denervation. There seem to be, however, a number of facts which may be of significance. Since denervation in its early stages results in changes which qualitatively are indistinguishable from decentralization, it may be justified to assume that the late changes, seen after about 40 hr, are of a secondary nature, superimposed upon the primary changes brought about in the first phase of denervation, i.e. about 24 hr after operation. While these secondary changes occur simultaneously with the degeneration of the postganglionic fibres, they are not necessarily a direct result of the loss of conduction in these fibres. It is more likely that both cocaine and the degeneration of the postganglionic fibres lead to the same, so far unknown, alterations in the effector system. It is tempting to assume that the changes during the first phase of denervation are the same as those occurring in an acutely decentralized membrane after injection of ephedrine and after chronic decentralization of any duration. It is not impossible that a decrease or complete absence of impulses from the cells in the superior cervical ganglion plays a role in producing these changes in the effector system. The fact that after decentralization, but not after denervation, a certain number of impulses will still reach the effector system may serve to explain the particularly rapid, unspecific sensitization which is observed in the first phase of denervation.

The findings obtained with derivatives of adrenaline and noradrenaline lacking one or both phenolic OH groups and the alcoholic OH group also indicate that the changes in the effector after denervation are fundamentally different from those found after decentralization. The actions of some of these amines have already been investigated (Bacq & Lefebvre, 1934; Bacq, 1936, 1938; Bülbring & Burn, 1938; Fleckenstein & Burn, 1953). Our observations are in fair, but not complete, agreement with the results of these authors. The reason for any discrepancy probably lies in the fact that they used for comparison the intact or the acutely decentralized membrane, while in our experiments the chronically decentralized membrane served as a standard of

reference in order to allow for unspecific increases in sensitivity. The results obtained seem to suggest that denervation leads to an inability of the effector cells to detect the absence from the adrenaline molecule of the methyl group and the phenolic OH group in the para- position. Further, after denervation the membrane is no longer able to respond with powerful contractions to large doses of amines not possessing a phenolic OH group in the meta-position, particularly when the alcoholic OH group in the side chain is also absent. These inabilities are not found after decentralization and it is tempting to speculate that they are associated with degenerative changes presumed to occur in the denervated effector cells.

The mechanism of the spontaneous increase in sensitivity which is sometimes observed in intact or acutely decentralized membranes is not clear. The sensitization is of the same type as that found after decentralization or ephedrine and may be caused by similar changes in the effector system. It is observed when the arterial B.P. is lowered spontaneously or by haemorrhage. It was at first thought that this might be an emergency reaction for which cortical steroids would be responsible, but the adrenals were found to be unnecessary for this response. The increased sensitivity is not caused by a reflex from the baroreceptors since the vagi and sinus nerves are also unnecessary.

SUMMARY

1. Chronic preganglionic denervation (decentralization) sensitizes the cat's nictitating membrane in an unspecific manner to adrenaline, noradrenaline, cobefrine, tyramine and all other sympathomimetic substances tested so far. When compared with these responses, chronic postganglionic denervation (denervation) causes either no or only a slight increase in the adrenaline response, a marked potentiation of the noradrenaline and cobefrine responses and a depression of the tyramine effect.

2. The effect of decentralization but not that of denervation can be brought about by injection of ephedrine while cocaine imitates the effect of denervation. Sometimes changes similar to chronic decentralization occur spontaneously in the intact or acutely decentralized membrane in the course of an acute experiment.

3. During the first 24 hr after operation there is no qualitative difference between the responses of denervated and decentralized membranes although the sensitization proceeds much more rapidly after denervation. Between 24 and 48 hr, secondary changes supervene in the denervated membrane which convert its responses to those of a chronically denervated membrane.

4. After denervation, but not after decentralization, the membrane loses its ability to detect the absence from the adrenaline molecule of the methyl group or the phenolic OH group in the para- position. Further, the denervated

membrane is no longer able to contract powerfully after moderate and large doses of derivatives not possessing the phenolic OH group in the meta-position.

5. The evidence presented makes it unlikely that a loss or inhibition of amine oxidase is the only cause for the changes in the responses of membranes which have been chronically denervated or treated with cocaine. Nothing is known as to the nature of the changes in the effector system of such membranes. The general increase in sensitivity of the membrane after decentralization or ephedrine may be due to diminished activity of amine oxidase.

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