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A QUANTITATIVE STUDY OF THE EFFECT OF COCAINE ON THE RESPONSE OF THE CAT NICTITATING MEMBRANE TO NERVE STIMULATION AND TO INJECTED NORADRENALINE

BY

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Results are reported of a quantitative study of the potentiating effect of cocaine on the responses of the cat nictitating membrane to intravenously and intra-arterially injected noradrenaline, as well as to different types of sympathetic nerve stimulation. Responses of the membrane to noradrenaline were potentiated more with intravenous than with close-arterial injections. From studies of the responses of the nictitating membrane to various forms of sympathetic nerve stimulation before and after injection of cocaine, conclusions are drawn as to the extent to which the transmitter amine liberated by nerve activity is normally removed and its effect thereby limited in duration and extent. This uptake was greatest at low stimulus frequencies. The mechanism by which cocaine potentiates sympathetic responses is discussed.

It has been known for over fifty years that cocaine increases the responses of various tissues to some sympathomimetic amines; the voluminous literature has recently been reviewed by Trendelenburg (1963). There is evidence that the noradrenaline-potentiating effect of cocaine is due to its ability to block the uptake of the amine by sympathetic nerve endings (Whitby, Hertting & Axelrod, 1960; Burn & Burn, 1961; Hertting, Axelrod & Patrick, 1961; Muscholl, 1961; Iversen & Whitby, 1962; Kopin, Hertting & Gordon, 1962; Wolfe, Potter, Richardson & Axelrod, 1962).

The quantitative aspect of the potentiation of noradrenaline by cocaine has been little studied. We considered this side of the problem to be of interest for the following reason. Measurements of the uptake of noradrenaline by tissues before and after administration of cocaine and the failure of tyramine to produce sympathomimetic effects on various organs after appropriate doses of cocaine (unpublished experiments on strips of cat isolated spleen) indicate that cocaine can inhibit almost completely the usual uptake of the amine by sympathetically innervated tissues. A quantitative evaluation of the potentiation phenomenon would permit an estimation of the usual uptake of noradrenaline.

In the investigation described here the effects of cocaine on the responsiveness to noradrenaline of a typical sympathetically innervated organ were studied quantitatively. By this indirect approach it was possible to determine approximately to what extent uptake into sympathetic nerve endings usually limits the response

of this organ to exogenous and endogenous noradrenaline. The nictitating membrane of the cat was chosen as test object because it consists of smooth muscle cells innervated exclusively by postganglionic sympathetic nerve endings of the classical noradrenergic type (Gardiner, Hellmann & Thompson, 1962). Also, graded responses to injected noradrenaline and to sympathetic nerve stimulation can easily be obtained.

Contractions of the nictitating membrane to exogenous noradrenaline are usually obtained by intravenous injections of the amine. This mode of application could not be used for our purpose, since the normal uptake of intravenously injected noradrenaline by all sympathetically innervated tissues considerably reduces the amount of the amine reaching the membrane. With intravenous injections of noradrenaline, the effect of cocaine therefore reflects block of noradrenaline uptake not only within but also outside the nictitating membrane. It was thought that injections into the external carotid artery would overcome this difficulty.

The effect of cocaine on the response of the nictitating membrane to endogenous noradrenaline liberated by stimulation of the cervical sympathetic nerve has not been studied intensively (Rosenblueth & McRioch, 1933 ; Bacq & Frédéricq, 1934 ; Fleckenstein & Bass, 1953 ; Trendelenburg, 1959) ; the results were variable, including augmentation, no change and even diminution of the response of the indirectly stimulated membrane after treatment with cocaine. As it is conceivable that the efficiency of the uptake of the liberated noradrenaline by the nerve endings varies with different states of nervous activity, several well-defined patterns of nerve stimulation were used.

METHODS

Fifty-nine cats, of either sex and weighing between 1.5 and 4 kg, were used. The animals were anaesthetized with 35 mg/kg of pentobarbitone sodium, given intraperitoneally. The preferred anaesthetic, ether, could be used only in reserpinized animals since without this treatment ether released catechol amines and caused prolonged contractions of the nictitating membrane, most conspicuously on the denervated side. The trachea was opened just above the sternum and a cannula introduced. The spinal cord was then cut between occiput and atlas, the brain destroyed and artificial ventilation begun. Both adrenal glands were excluded from the circulation by ligatures. The blood pressure in an iliac artery was recorded, using a mercury manometer. One femoral vein was cannulated for intravenous injections. For intra-arterial injections one or both lingual arteries were ligated and a plastic catheter introduced into the proximal segment. The tip of the catheter lay at a distance of 2 to 3 mm from the external carotid artery. The drugs were injected slowly in 0.2 ml. of 0.9% saline. The contractions of both nictitating membranes were recorded isotonically on a smoked drum and magnified seven-times with a frontal writing lever, the tension on the membranes being 4 g. An electromagnetic vibrator, fixed on the rod supporting the levers, reduced frictional distortion of the record.

Denervation of the nictitating membrane was carried out by aseptic removal of the right superior cervical ganglion of cats anaesthetized with ether or pentobarbitone sodium 9 to 20 days before an experiment.

Nerve stimulation. For preganglionic stimulation the sympathetic trunk was cut just above the sternum and laid on a pair of platinum electrodes. For postganglionic stimulation the oesophagus, the larynx, and the deep muscles of the neck were removed, without damaging the vessls running from the carotid artery to the vagosympathetic trunk and the superior cervical ganglion. The ganglion and the postganglionic nerve were carefully separated and

isolated from the other nerves by a rubber membrane. The nerve was laid on a pair of platinum electrodes. Warm liquid paraffin, bubbled with pure oxygen, prevented drying of the nerve.

The stimulator was a Grass model S4, with stimulus isolation unit. Monophasic pulses of 1 msec duration were used throughout, and the other parameters varied in different experiments.

In a few experiments submaximal stimuli were used. Voltage and frequency were chosen so as to give a contraction comparable in height to that following a certain dose of noradrenaline or that obtained with supramaximal stimuli at lower frequency and shorter duration of stimulation. Usually, however, supramaximal stimuli were used. Stimulating at a low frequency the voltage was increased until no greater contraction occurred. A voltage three- to five-times this maximal voltage was then used throughout the experiment. Supramaximal stimuli have the advantage that they excite virtually all intact nerve fibres and give responses of the membrane constant for many hours. With constant supramaximal voltage, the variation of stimulus frequency and duration of stimulation allowed the study of the effect of cocaine on adrenergic transmission under differing conditions.

A single supramaximal shock produced a rapid and brief contraction of the membrane. The height of this contraction in large cats was about 20% of the maximal one when using optimal parameters.

Frequency/response curves were obtained by stimulating at increasing frequencies until the plateau of tetanic contraction was reached at each frequency. The advantage of this method is its "supramaximal" stimulation since, for a given stimulus frequency the effect cannot be augmented by any change of the other parameters, such as duration of stimulation or voltage. The disadvantage is that tetanic stimulation rapidly causes fatigue with frequencies near or higher than the highest reported to occur under physiological conditions, which, in the efferent autonomic nervous system, is about 5 impulses/sec (Folkow, 1952).

True stimulus number/response curves were obtained by stimulating at a constant frequency but at various durations of the stimulation period. The maximal effect obtainable by this method corresponds to that achieved by tetanic stimulation at the given frequency. As a rule a stimulus frequency of 1.6 shocks/sec was used.

A mixed stimulus number-frequency/response curve was obtained by keeping the duration of the stimulation period constant at 5 sec and varying stimulus frequency so as to give 1, 3, 9, 27, 81 and 243 shocks during this period. The heights of the brief contractions obtained lay on an almost linear curve when the number of stimuli in 5 sec was plotted as the abscissa on a log scale. In a good preparation this mode of stimulation gave constant results over many hours. Comparison of stimulus number-frequency/response curves obtained with different animals showed little variation when the results were expressed as percentages of the individual maximal responses.

In several experiments supramaximal preganglionic stimulation was carried out after acute partial section of the postganglionic trunk. This procedure imitates that using submaximal voltage in that it stimulates only a proportion of the fibres innervating the membrane, but has the advantage of keeping constant the number of stimulated fibres and smooth muscle cells. All the patterns of response to supramaximal stimulation could also be studied after partial section of the postganglionic trunk.

Estimations of the potentiation produced by cocaine were made graphically from the plots of response against log dose or against log stimulus number before and after giving cocaine. The statement that cocaine potentiated three-times means that after administration of cocaine one-third of the dose of noradrenaline or of the number of shocks given initially caused a response similar in size to that before treatment.

Cocaine hydrochloride was injected into the femoral vein. The injection was made slowly, while watching the electrocardiogram, for doses of 3 mg/kg or more. Care was taken to avoid an increase exceeding 5 mm in resting tone of the nictitating membrane. A dose of 10 mg/kg was highly toxic for most cats; even with a slow injection the heart was greatly affected. The first sign in the electrocardiogram was a broadening of the QRS-complex. Bundle-branch block, various ventricular arrhythmias and ventricular arrest often occurred.

(−)-Noradrenaline hydrochloride (Arterenol, Hoechst) was used. Doses refer to the base. Reserpine was given intraperitoneally in a dose of 3 mg/kg 1 day before an experiment.

RESULTS

The sympathomimetic effect of cocaine on the nictitating membrane

Rapid intravenous injection of cocaine (0.3 mg/kg or more), besides increasing blood pressure and heart rate, produced dose-dependent contractions of the nictitating membrane. Tachyphylaxis occurred rapidly. The efficacy of cocaine in contracting the membrane varied greatly between different preparations. Its sympathomimetic potency was of the same order of magnitude as that of tyramine. The duration of the contraction produced by cocaine was shorter than that due to amphetamine but generally longer than for tyramine. The effect was typical of that of an indirectly acting amine, inasmuch as the action of low doses was first seen on the chronically denervated membrane, whereas with higher doses the effect was greater on the innervated side (Haefely, Hürlimann & Thoenen, 1963). In cats treated with 3 mg/kg of reserpine 24 to 48 hr before an experiment, the sympathomimetic effect of cocaine was practically abolished, and even doses as high as 10 mg/kg could be injected rapidly without increasing the resting tone of the membrane. With preparations from cats not treated with reserpine it was necessary to inject cocaine very slowly (doses of 3 and 10 mg/kg in 10 to 15 min) to avoid contractions. Resting nictitating membranes after administration of cocaine very often resembled chronically denervated ones in showing small spike-like contractions.

The effect of cocaine on responses of the nictitating membrane to noradrenaline injected intravenously

Normal membranes. Because of the low sensitivity of the nictitating membrane to intravenously injected noradraline, only the lower part of the dose/response curve could be studied. The horizontal shift of the curve produced by cocaine was measured for each membrane and the potentiations (means and standard errors, with numbers of experiments in parentheses) were 3.55 ± 0.26 (4) for 0.1 mg/kg, 14 ± 2 (7) for 0.3 mg/kg, 26 ± 3.8 (7) for 1 mg/kg, 89 ± 24 (6) for 3 mg/kg and 55 ± 17 (4) for 10 mg/kg of cocaine.

Chronically denervated membranes. The chronically denervated nictitating membrane was 173 ± 22 (6) times more sensitive to noradrenaline than the innervated one. The effect of cocaine on chronically denervated membranes was erratic. The responses to low doses of noradrenaline were often slightly augmented though sometimes a decrease was observed. On the other hand the responses to higher doses were usually diminished by cocaine.

The effect of cocaine on the response of the nictitating membrane to noradrenaline injected intra-arterially

With close-arterial injections of noradrenaline to the nictitating membrane one might expect to obtain dose/response curves without difficulty, since threshold responses occurred with doses about one-thirtieth of those necessary by intravenous injection. It was, however, impossible to obtain responses corresponding to more than 30 to 50% of the height of contraction resulting from maximal electrical stimulation. Above a critical dose of intra-arterially injected noradrenaline, the

response of the nictitating membrane either remained stationary or even decreased. The maximal response obtainable was even smaller than with intravenous injections.

After administration of cocaine the effect of small doses of noradrenaline was always increased, whereas responses to larger doses remained unchanged or often decreased. Kukovetz & Lembeck (1962) had observed this phenomenon in chronically denervated membranes.

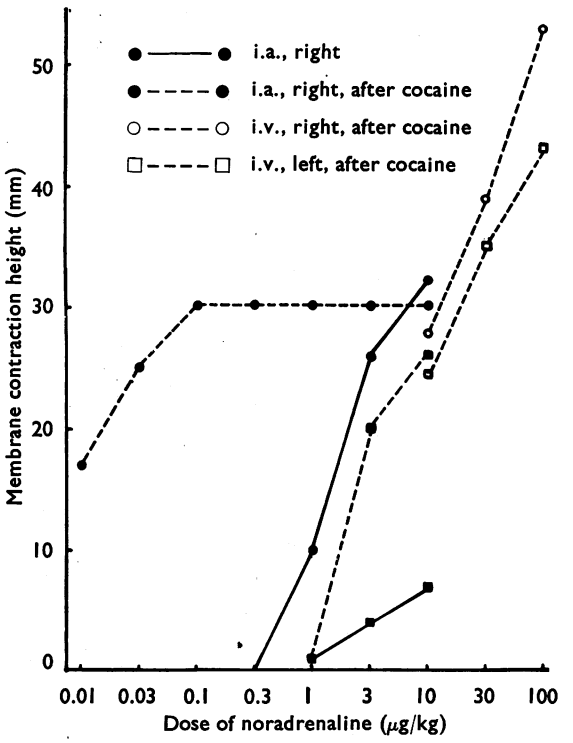

Fig. 1. Response of nictitating membranes to noradrenaline (intra-arterially and intravenously) before and after cocaine (mg/kg). Ordinate: contraction height (mm on the smoked drum); abscissa: dose of noradrenaline (μg/kg). Circles: right-hand membrane; squares: left-hand membrane; solid symbols: intra-arterial injections; open symbols: intravenous injections; solid line: before cocaine; broken lines: after cocaine. After 1 mg/kg of cocaine, the sensitivity of the right-hand nictitating membrane to small doses of noradrenaline injected into the right lingual artery was greatly increased. With 0.1 μg/kg of noradrenaline a point representing approximately half of the maximal response was rapidly attained and could not be surpassed, though a greater response was obtained by intravenous injection. The response of the left-hand membrane after cocaine was of the same magnitude whether the amine was injected intravenously or intra-arterially on the contralateral side.

The altered reaction of the nictitating membrane to noradrenaline after administration of cocaine depended on the site of injection of the amine (Fig. 1). Cocaine (1 mg/kg) greatly augmented the responses of the right-hand membrane to small doses of noradrenaline injected into the right lingual artery. However, a maximal reponse was obtained with 0.1 μg/kg of noradrenaline by this mode of injection.

When 10 μg/kg of noradrenaline were injected intra-arterially, most of the injected amine was directed towards the right-hand membrane, whereas only a small part of the same dose, injected into the femoral vein, could have reached the same membrane, as judged by the height of contraction before injection of cocaine. Despite this difference the responses to both injections after administration of cocaine were practically the same. Higher intravenous doses of noradrenaline could cause larger contractions of the membrane. Both membranes were approximately equally sensitive to noradrenaline. Some of the noradrenaline injected intra-arterially escaped into the general circulation and caused the left-hand membrane to contract. This response was greatly potentiated by cocaine, so that 10 μg/kg of noradrenaline injected intra-arterially had about the same effect on both the left-hand and the right-hand membranes. After higher doses of cocaine it was often possible to obtain larger contractions of the contralateral than of the ipsilateral membrane using intra-arterial injections of noradrenaline.

When noradrenaline was injected into the same animal both intravenously and intra-arterially, cocaine consistently potentiated more the responses to the intra-

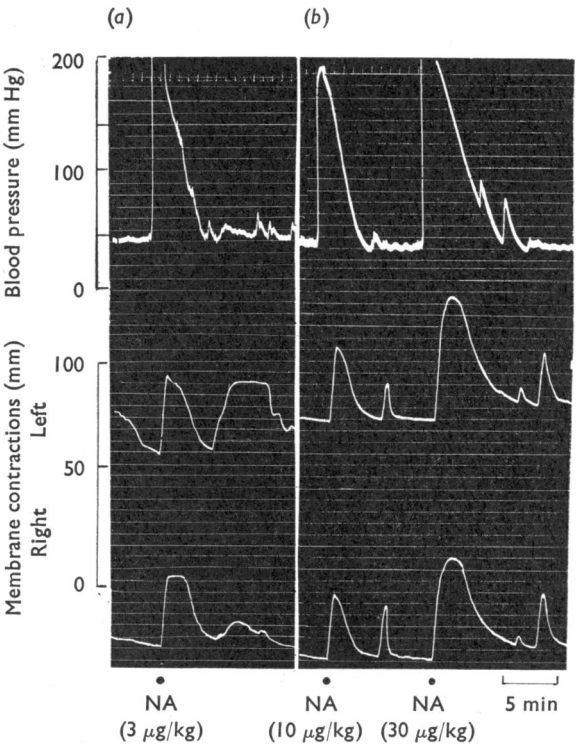

Fig. 2. Modification of the response of nictitating membranes to noradrenaline after cocaine by the vasoconstrictor effect of the amine. Responses of two different cats to intravenous noradrenaline (NA) after 0.3 mg/kg of cocaine (*a*) and 3 mg/kg of cocaine (*b*). From above down: arterial blood pressure, contractions of left- and right-hand nictitating membranes. The immediate response of the membranes to noradrenaline was followed by a second contraction after several minutes.

venously injected amine. Occasionally cocaine caused the early contraction to an intravenous or intra-arterial injection of noradrenaline to be succeeded by a second one, sometimes nearly as large as the first (Fig. 2). The time between these two

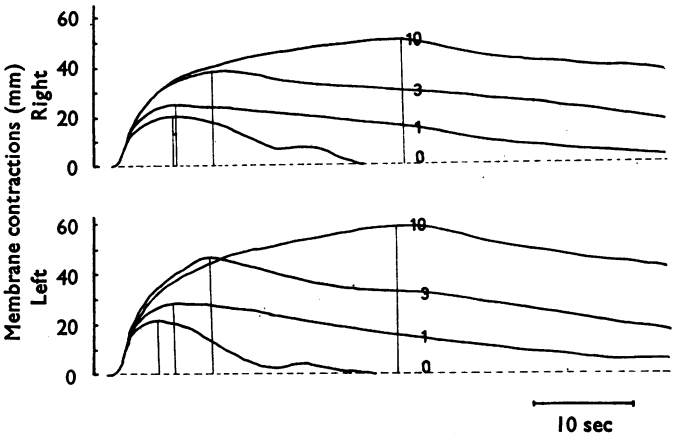

Fig. 3. Effect of increasing doses of cocaine on the responses of nictitating membranes to single supramaximal shocks applied to the preganglionic cervical sympathetic nerve. The responses (ordinate) of both membranes of the same cat are expressed in mm of contraction height on the smoked drum. On each of the curves are shown the doses of cocaine (1, 3, and 10 mg/kg).

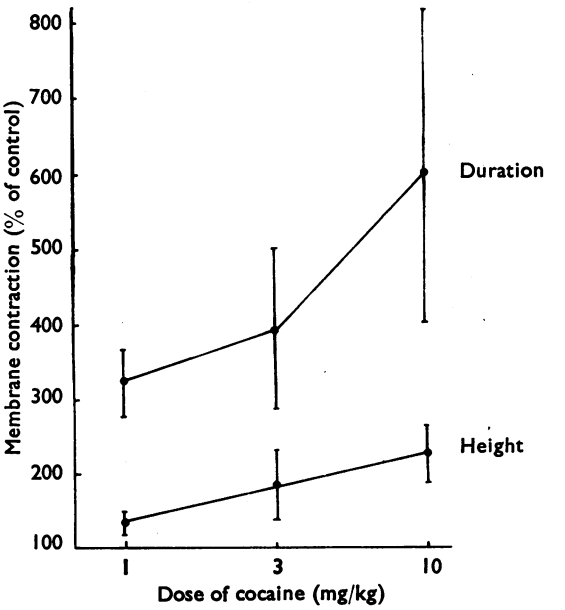

Fig. 4. Effect of increasing doses of cocaine on height (lower curve) and duration (upper curve) of the response (ordinate) of the nictitating membrane to a single supramaximal shock applied to the preganglionic cervical sympathetic nerve. The values obtained before cocaine are taken as 100%. The means from four experiments and their standard errors are plotted. Abscissa: dose of cocaine (mg/kg).

contractions varied from 1 to 3 min. The supersensitivity of the chronically denervated membrane in the absence of cocaine was of the same order of magnitude whether noradrenaline was injected intravenously or intra-arterially.

The effect of cocaine on the response of the nictitating membrane to sympathetic nerve stimulation

Immediately after the injection of cocaine small contractions of the membrane (for example those produced by a single shock to the nerve) were transiently augmented more than larger ones (see also Fig. 1 in Trendelenburg, 1959). A constant degree of potentiation was not obtained until 10 to 15 min after the injection, and then remained so for at least 1 hr.

The effect of cocaine on the response to a single supramaximal shock. Fig. 3 illustrates an experiment with three consecutive doses of 1, 3 and 10 mg/kg of cocaine. After injection of cocaine both height and duration of the contractions

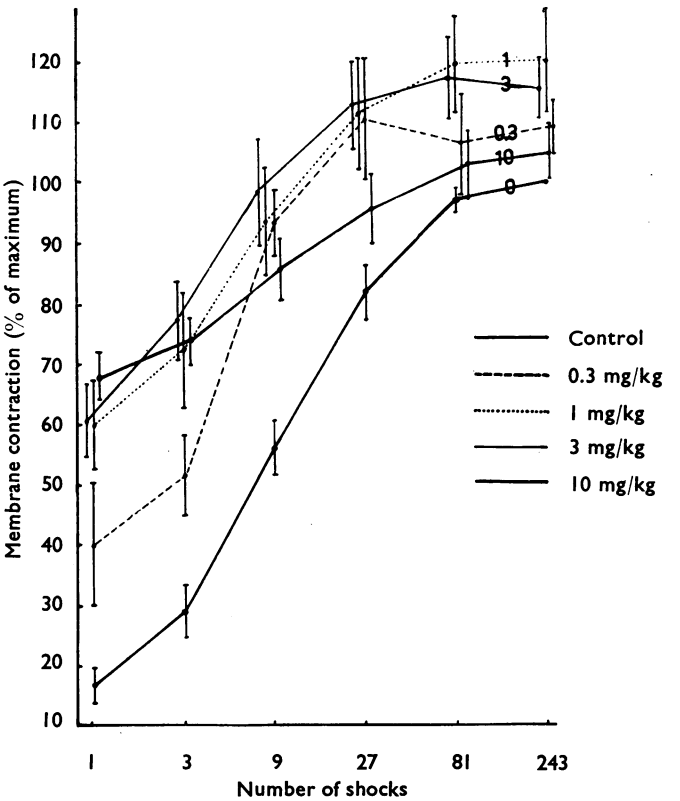

Fig. 5. Effect of dose of cocaine on stimulus number/response curves of a nictitating membrane. Abscissa: number of shocks applied to the preganglionic cervical sympathetic nerve at a constant stimulus frequency (1.6 shocks/sec). Ordinate: the maximal response of the nictitating membrane in the control series is taken as 100% and all values are expressed as percentages of this value. The mean values obtained in at least five experiments and their standard errors are plotted.

were increased, the latter much more than the former. In Fig. 4 the results of several experiments under identical conditions are summarized. The effect on duration of contraction was about three-times greater than that on height. It follows that a more complete evaluation of the potentiating action of cocaine would be possible by taking into account both height and duration of contraction, for example by measuring the area of contraction recorded on the smoked paper.

The effect of cocaine on stimulus number/response curves. For these curves the stimulus frequency was kept constant at 1.6 shocks/sec and the number of shocks applied to the nerve was increased stepwise by factors of three. The contraction of each individual membrane obtained with 243 shocks (the maximal obtainable for this frequency and about 70% of the maximal obtainable with the optimal frequency) was taken as 100%. The responses obtained by varying the number of shocks were expressed as percentages of this maximum. The resulting curves show a sigmoid shape with an approximately linear course in the middle sections (Fig. 5). The standard errors of the plotted means were less than 10% of the maximal response.

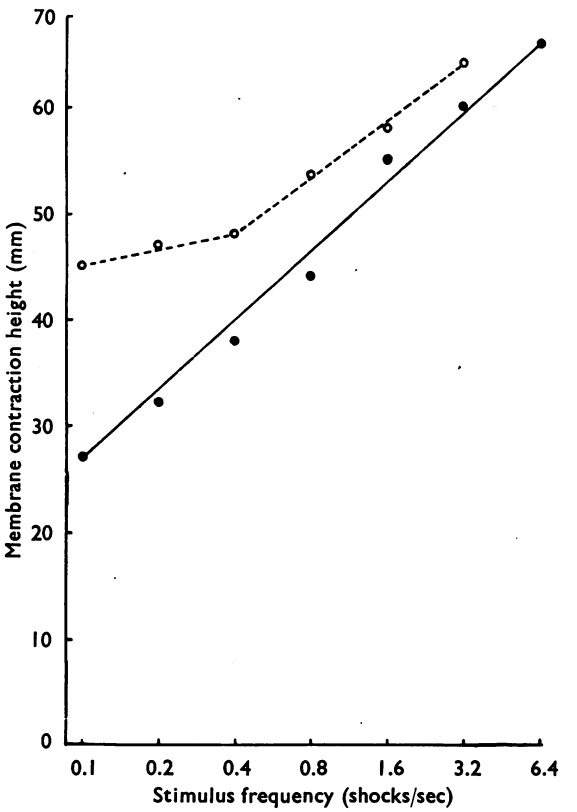

Fig. 6. Effect of cocaine on a stimulus frequency/response curve of a nictitating membrane. Ordinate: contraction (height in mm on the smoked drum). Abscissa: stimulus frequency (shocks/sec). ●——● Control; o- - -o after 3 mg/kg of cocaine. The potentiation by cocaine is greatest at lower frequencies.

The effect of increasing doses of cocaine on the stimulus number/response curve is also shown in Fig. 5. With 0.1 mg/kg or less of cocaine, there was usually only a transient augmentation of the response to a single shock, whereas the response to greater numbers of stimuli remained unaltered. In higher doses, cocaine shifted the curve considerably to the left. After injection of 0.3 mg/kg it was necessary to give only about one-third the number of shocks to obtain the same size of contraction as before. An almost maximal shift was obtained with 1 mg/kg of cocaine. After 10 mg/kg, the effects of higher stimulus numbers—though greater than before injection of cocaine—became smaller than after 0.3 to 3 mg/kg of cocaine. The response to a single shock after injection of 10 mg/kg of cocaine corresponded to one obtained with 15 to 20 shocks before cocaine; the greatest augmentation observed in individual experiments was about thirtyfold.

The effect of cocaine on frequency/response curves. These curves, approximately linear, were obtained by stimulating the preganglionic cervical sympathetic nerve until the height of contraction of the membrane reached a plateau. The stimulus frequency was increased by factors of two beginning with 0.1 shocks/sec. A fused tetanic contraction was obtained when the frequency exceeded 0.8 shocks/sec. The

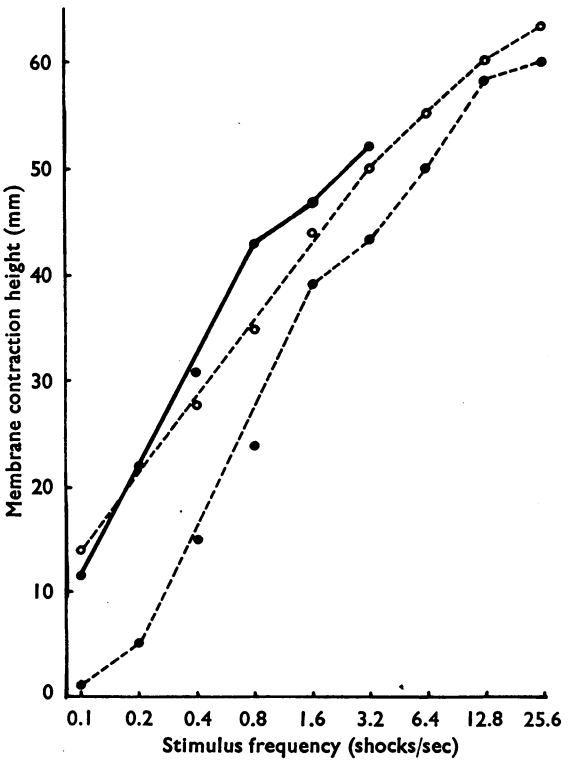

Fig. 7. Effect of cocaine on a stimulus frequency/response curve of a nictitating membrane after partial section of the postganglionic trunk. Ordinate: contraction (height in mm on the smoked drum). Abscissa: stimulus frequency (shocks/sec). ●——● Control; ●- - -● after partial nerve section; ○- - -○ after 1 mg/kg of cocaine and partial nerve section.

maximal contraction was usually obtained at frequencies between 6 and 12 shocks/sec and corresponded to the maximal response of the nictitating membrane obtained by electrical stimulation of the cervical sympathetic nerve.

The effect of cocaine on the frequency/response curve differed from that on the stimulus number/response curves. An experiment showing the usual frequency/response curves obtained before and after giving 3 mg/kg of cocaine is illustrated in Fig. 6. The augmentation of response due to cocaine was greatest at lower stimulus frequencies. Control experiments had shown that it was extremely difficult to obtain two identical frequency/response curves with the same membrane. Repeated tetanic stimulation at frequencies above 3 shocks/sec almost inevitably led to progressively diminishing responses over sequential stimulation periods. In most experiments, therefore, only the range between 0.1 and 3.2 shocks/sec was studied.

The effect of cocaine on mixed stimulus number-frequency/response curves. These curves resembled very closely the true stimulus number/response curves. The effect of cocaine was also very similar. The resulting curve was shifted to the left but remained parallel to the control curve. The dose-dependence of the shift corresponded to that found with stimulus number/response curves.

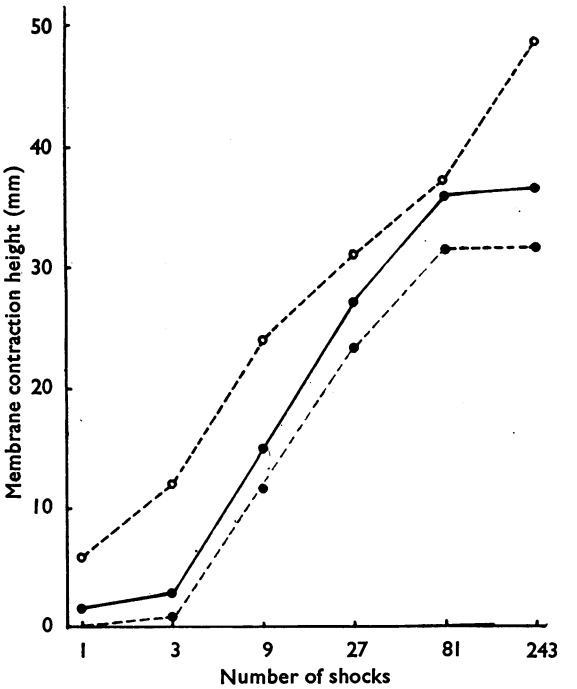

Fig. 8. Effect of cocaine on a stimulus number/response curve of a nictitating membrane after partial section of the postganglionic trunk. Ordinate: contraction (height in mm on the smoked drum). Abscissa: number of shocks applied to the preganglionic cervical sympathetic nerve at a stimulus frequency of 1.6 shocks/sec. ● —— ● Control; ● - - - ● after partial nerve section; ○ - - - ○ after 1 mg/kg of cocaine and partial nerve section.

The effect of cocaine on the response to submaximal stimulation and to supramaximal preganglionic stimulation after partial section of the postganglionic trunk. It was not possible to obtain reproducible graded responses to nerve stimulation with submaximal voltage. It was, however, easy to demonstrate a dose-dependent augmented response of the membrane to a given submaximal stimulation after injection of cocaine. This procedure was used by Trendelenburg (1959) but does not permit quantitative study of the effect of cocaine.

Partial section of the postganglionic trunk caused a shift of all three curves to the right. The shift depended on the number of fibres cut. Cocaine caused a dose-dependent shift to the left. Figs. 7, 8 and 9 illustrate the effects of cocaine after

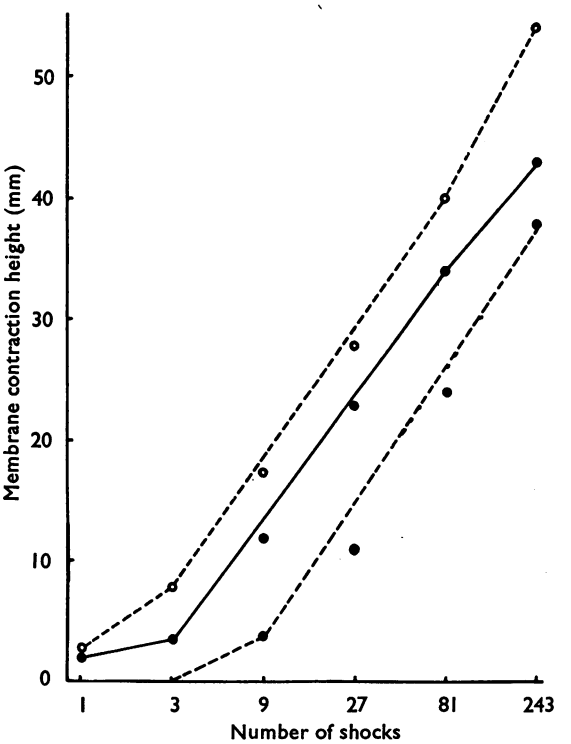

Fig. 9. Effect of cocaine on a mixed stimulus number-frequency/response curve of a nictitating membrane after partial section of the postganglionic trunk. Ordinate: contraction (height in mm on the s moked drum). Abscissa: number of shocks in 5 sec applied to the preganglionic cervical sympathetic nerve. ● —— ● Control; ● - - - ● after partial nerve section; O - - - O after 1 mg/kg of cocaine and partial nerve section.

partial section of the postganglionic trunk for a frequency/response, a stimulus number response and a mixed stimulus number-frequency/response curve.

The question whether cocaine potentiates a submaximal stimulus more than a supramaximal one was studied on stimulus number/response curves with intact and partially cut postganglionic trunks. In both types of experiment, the ratios of potentiation at a stimulation rate of 1.6 shocks/sec were the same.

The influence of reserpine on the effect of cocaine. Previous treatment with reserpine diminishes greatly the sympathomimetic action of cocaine. The effects of 10 mg/kg of cocaine on mixed stimulus number-frequency/response curves of normal cats and of cats treated with 3 mg/kg of reserpine given intraperitoneally 24 hr before an experiment were compared. As already shown by Trendelenburg, Muskus, Fleming & Gomez Alonso de la Sierra (1962), this treatment results in a shift of the curve to the right and to a depression of the maximum. As shown in Fig. 10, the potentiating effect of cocaine was also present in reserpinized animals,

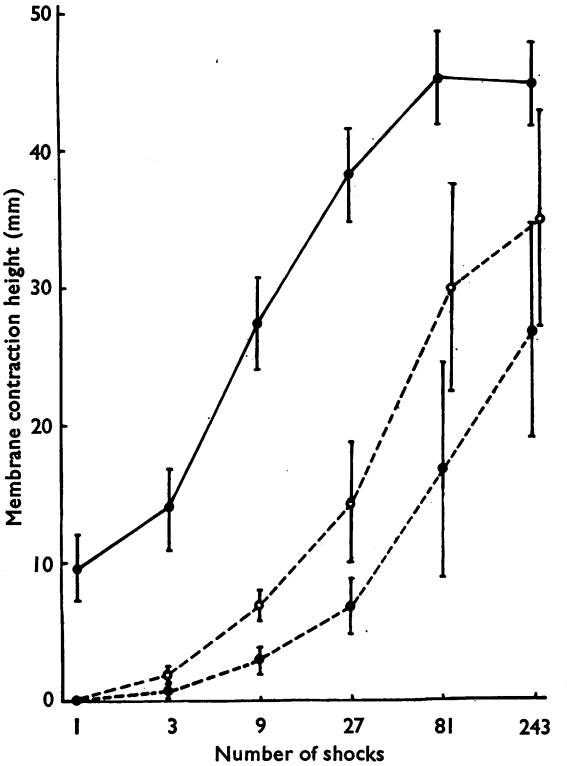

Fig. 10. Effect of cocaine (10 mg/kg) on stimulus number/response curves of a nictitating membrane after treatment of cats with reserpine (3 mg/kg) 24 hr before the experiment. Ordinate: contraction (height in mm on the smoked drum). Abscissa: number of shocks applied to the preganglionic cervical sympathetic nerve at a stimulus frequency of 1.6 shocks/sec. ●——● Control; ●- - -● after reserpine; ○- - -○ after reserpine and cocaine. The mean values in six experiments and their standard errors are given. The control curve for nontreated animals is taken from Fig. 5.

although greatly reduced, the horizontal shift of the curve in nontreated animals corresponding approximately to a twenty-five-fold augmentation, but to only a three-fold augmentation in reserpinized animals.

The effect of cocaine on postganglionic nerve stimulation. Control experiments with stimulation of either one cervical sympathetic nerve alternatively pre- and post-

ganglionically, or the sympathetic nerve on one side pre- and on the other post-ganglionically, showed no difference in the effects of cocaine.

Effect of cocaine on injection of subthreshold doses of noradrenaline immediately before sympathetic stimulation. Fig. 11 shows the result of an experiment in which, 15 sec before sympathetic stimulation, a subthreshold dose of noradrenaline was

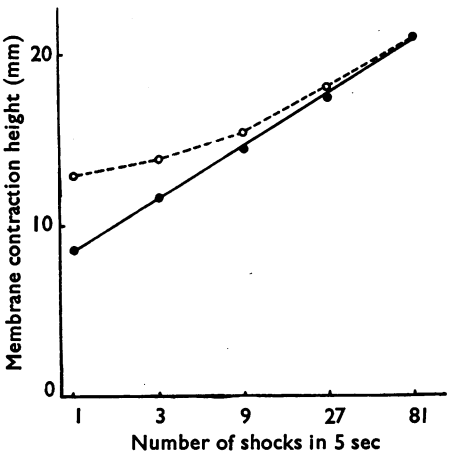

Fig. 11. Synergism of preganglionic cervical sympathetic nerve stimulation applied 15 sec after intravenous injection of a subthreshold dose (0.3 μg/kg) of noradrenaline. Ordinate: contraction of nictitating membrane (height in mm on the smoked drum). Abscissa: number of shocks in 5 sec applied to the nerve. ● —— ● Sympathetic stimulation alone; O - - - O sympathetic stimulation after injection of noradrenaline.

injected intravenously. This injection resulted in an increased response of the membrane to stimulation with a small number of shocks in 5 sec, but the increase declined and disappeared as the number of shocks in 5 sec increased.

DISCUSSION

Our results show that cocaine regularly, and in a dose-dependent manner, potentiates the responses of the nictitating membrane to exogenous noradrenaline as well as to sympathetic nerve stimulation, provided submaximal parameters are used.

Mode of action of cocaine. In addition to its well-documented blocking effect on the uptake of noradrenaline by sympathetically innervated tissues (Iversen & Whitby, 1962; Kopin *et al.*, 1962; Wolfe *et al.*, 1962), the sympathomimetic action of cocaine has to be considered as one of the possible causes of its ability to potentiate noradrenaline. Such an action of cocaine has been demonstrated in isolated atria by Furchgott (personal communication). In our work, evidence that the sympathomimetic effect of cocaine is due to the release of noradrenaline came with the demonstration that depletion of the stores of noradrenaline by reserpine greatly reduced this action of cocaine. By releasing noradrenaline from nerve endings in innervated preparations—even if only in subthreshold amounts

—cocaine can synergistically augment the response to nerve activity. Experiments with sympathetic nerve stimulation immediately after injection of a constant subthreshold dose of noradrenaline (Fig. 11) showed that an increased "background level" of noradrenaline augmented only the contractions produced by lower frequencies of stimulation. Medium doses of cocaine, immediately after their injection, caused an augmentation most pronounced in the lower part of the stimulus number/response curve, whereas, about 15 min after the injection when release of noradrenaline by cocaine may be assumed to have virtually ceased, there was a parallel shift of the curve. On the other hand, the injection of very small doses of cocaine (0.03 to 0.1 mg/kg) usually augmented only transiently the effects of a single shock and did not alter the rest of the resultant curve. In this instance the block of noradrenaline uptake seemed to be too small to shift the curve, whereas some release of noradrenaline by cocaine had occurred. Although the potentiation is probably caused initially by the release of noradrenaline, this phase is followed by the main component of noradrenaline potentiation which is probably due to diminished amine uptake. An increase in concentration of noradrenaline in the vicinity of its receptors can explain all aspects of the noradrenaline potentiation by cocaine observed up to now.

The potentiating effect of cocaine is diminished when the normal uptake mechanisms are greatly impaired, as after treatment with reserpine, and abolished when these mechanisms are destroyed, as after chronic denervation. As one would expect of a drug which slows inactivation of a released transmitter, the effect of cocaine is a dual one; it increases the height as well as the duration of contraction. This result is consistently found for noradrenaline-induced contractions as well as for responses to nerve stimulation. For the latter, the effect on duration is much more marked than on height of contraction.

The influence of cocaine on noradrenaline-induced contractions. Since cocaine inhibits the uptake of noradrenaline by sympathetic nerve endings throughout the animal an augmented effect of intravenously injected noradrenaline on the nictitating membrane would be expected even if cocaine itself had no effect on the membrane. Unfortunately, the intra-arterial injection of noradrenaline, which would permit a quantitative estimation of the effect of cocaine itself on the membrane, proved unsatisfactory because of highly variable responses. We believe that these erratic results are due to the vasoconstrictor effect of noradrenaline, which effect is also potentiated by cocaine and interferes with the access of the amine to the smooth muscle cells of the nictitating membrane. This interference is greater for injections into the carotid artery than for intravenous injections, possibly because the rapid increase of the blood pressure after intravenous injection overcomes the vasoconstrictor effect of noradrenaline in the vessels of the membrane. In spinal cats some areas of the effector organ are likely to be temporarily excluded from the circulation. When the vasoconstrictor effect ceases, some of the retained amine may reach the effector cells by reopened vessels and give rise to a delayed second response (Fig. 2). The same phenomenon has also been observed by Thoenen, Hürlimann & Haefely (unpublished) in the cat isolated perfused spleen and by Ohlin & Strömblad (1958) in the cat parotid gland.

If it is assumed that equal concentrations of noradrenaline are required in the region of its receptors to produce equal responses of the nictitating membrane before and after injection of high doses of cocaine, it is possible to estimate the relative amount of the amine which is usually taken up at other sites and thus excluded from the receptors. As the maximal potentiation of the effect of injected noradrenaline by cocaine was 50- to 100-fold, one arrives at the unexpected result that 98 to 99% of the amine is usually inactivated by uptake. This value agrees well with those obtained by measurements of the recovery of noradrenaline in the venous effluent of the cat isolated spleen perfused with small amounts of the amine before and after addition of cocaine (Thoenen, Hürlimann & Haefely, 1964).

The potentiation by cocaine of the response to sympathetic nerve stimulation. Only the response to a single shock is suitable for evaluating quantitatively the effect of cocaine on the responses to sympathetic nerve stimulation, since with 10 mg/kg of cocaine the upper part of the stimulus number/response curve is depressed. With the same assumption as made for exogenous noradrenaline, it is possible to estimate the proportion of the amine available to its receptors. The potentiation of the contraction (measured as height) by the highest dose of cocaine is about twenty- to thirty-times, which suggests that less than 5% of the total sympathetic transmitter liberated by a single shock becomes effective at the receptors. A more accurate estimate of the ratio of inactivated to released amine would probably be obtained by comparing contraction areas (height and duration) before and after injection of cocaine. The potentiating effect of cocaine diminishes as the stimulus frequency is increased, from which result it is concluded that the ratio of inactivated to liberated amine also becomes progressively smaller.

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