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INVESTIGATION OF THE ROLE OF CALCIUM IN THE SUPERSENSITIVITY PRODUCED BY COCAINE IN CAT SPLEEN STRIPS

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1 Cocaine (2×10^{-6} M and 10^{-5} M) produced 2 and 7 fold shifts to the left of the dose-response curve to (−)-noradrenaline recorded isotonically in isolated splenic capsular strips of the cat.

2 The same concentrations of cocaine also produced increases in the maximum response of the tissue to 117% and 126.7% of control.

3 Desmethylimipramine (DMI, 10^{-7} to 10^{-6} M) produced no significant potentiation of the response of cat spleen strips to (−)-noradrenaline. At 10^{-5} M DMI decreased the maximum response.

4 Cocaine (10^{-5} M) produced a 3.3 fold shift to the left of the dose-response curve whereas DMI (10^{-6} M) had no effect on the dose-response curve to oxymetazoline in cat splenic capsular strips.

5 Cocaine (10^{-5} M) in the presence of phentolamine (10^{-6} M) produced a shift to the left and an increase in the maximum response to K^+, an agonist which is believed to produce muscle contraction by increasing the membrane calcium flux.

6 Cocaine (10^{-5} M) had no effect on the dose-response curve to angiotensin which is believed to contract vascular muscle by releasing calcium from intracellular storage sites.

7 The potentiating effect of cocaine (10^{-5} M) on responses of spleen strips to (−)-noradrenaline was blocked by the calcium flux inhibitor SKF 525A (2.65×10^{-5} M).

8 It is concluded that the results are compatible with the view that cocaine enhances the influx of calcium across the cell membrane during responses to agonists that utilize the extracellular pool of calcium and that this effect is responsible for a large part of the potentiation of the response.

Introduction

Cocaine can produce supersensitivity in adrenergically innervated tissues by at least two mechanisms. The first mechanism is inhibition of neuronal uptake which is important in densely innervated tissues such as the cat nictitating membrane and results in a shift to the left of the dose-response curve (Graefe & Trendelenburg, 1970; Trendelenburg, Maxwell & Pluchino, 1970; Trendelenburg, Graefe & Eckert, 1972) with no increase in the maximum tension which the muscle is capable of developing. The second mechanism is of postsynaptic origin and reflects an action of cocaine at the level of the α-adrenoceptor or beyond (Maxwell, Wastila & Eckhardt, 1966; Kasuya & Goto, 1968; Kalsner & Nickerson, 1969; Varma & McCullough, 1969; Greenberg & Long, 1971; Greenberg & Innes, 1976). The postsynaptic action of cocaine produces a change in the smooth muscle such that when exposed to α-agonists it is capable of generating an increased maximum tension. This type of supersensitivity has been called type 2 sensitization (Kalsner, 1974). There are indications that certain types of type 2 sensitization involve a change in the metabolism of Ca^{2+}, for instance, the supersensitivity induced by reserpine in rabbit aortic strips (Carrier & Hester, 1976). Calcium is necessary in smooth muscle for activation of the contractile proteins. It can be supplied from both extracellular and intracellular sites. The extracellular sites include the extracellular fluid and Ca^{2+} bound to elastin and the outside of smooth muscle membrane. The intracellular sites probably include the sarcoplasmic reticulum, mitochondria and the plasma membrane with associated surface vesicles (Bohr, 1973).

Agonists could produce contraction of smooth muscle by increasing the influx or decreasing the efflux of Ca^{2+} or by triggering its release from intracellular storage sites. Depolarization by potassium has been shown to produce a large influx of Ca^{2+} and it is likely that this initiates contraction since the contraction is abolished by removal of extracellular Ca^{2+} or blockade of influx by means of La^{3+} (van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973). Noradrenaline utilizes both extracellular and intracellular stores of Ca^{2+} to produce contraction. In cat spleen strips the initial fast phase of contraction is due to release of the intracellular Ca^{2+}

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and the subsequent slow phase to influx of extracellular Ca^{2+} (Woodward, Bose & Innes, 1970). The response of vascular smooth muscle to angiotension II on the other hand is thought to be dependent almost entirely on release of Ca^{2+} from the intracellular sites (Kalsner, Nickerson & Boyd, 1970; van Breemen, Farinas, Gerba & McNaughton, 1972).

The present experiments investigate whether the postsynaptic supersensitivity induced by cocaine in cat spleen strips is due to an effect on Ca^{2+} metabolism and, if so, which source of Ca^{2+} is involved.

Methods

Cats (of either sex, 2 to 3.5 kg) were anaesthetized with halothane (1.5%) in a N_2O:O_2 mixture (2:1 v/v). The spleen was removed through a midline abdominal incision and washed in Krebs-Henseleit solution. Capsular strips (approx. 1.5 × 0.2 × 0.05 cm; wt. 33 ± 3 mg, $n = 40$) were cut along the long axis of the spleen with a tissue microtome based on the Stadie-Riggs-microtome (6727-010) (Blakeley & Summers, 1977). The strips were stored before use for up to 36 h at 4°C in Krebs-Henseleit solution. Control experiments showed that storage in this way did not affect the responses. The strips were suspended in a 25 ml organ bath containing Krebs-Bicarbonate solution of the following composition (mM): $NaHCO_3$ 25, NaCl 120, KCl 4.5, NaH_2PO_4 1.83, $CaCl_2$ 1.25, $MgSO_4$ 1.00 and glucose 11.1, bubbled with 95% O_2 and 5% CO_2 and maintained at 37°C. The tissue was allowed to equilibrate with several changes of bathing solution for at least 40 min under a tension of 1 g. Isotonic recording of the contractions of the strips was by means of a Sangamo-Weston displacement transducer (type NDI) and SE 3006 u.v. recorder. Isometric recording was by a Grass force displacement transducer and Devices M2 pen recorder. The baseline tension in experiments involving isometric recording was 0.8 g. Responses were recorded as increases in tension (g) above this baseline tension.

Protocol

Doses of agonists were added to the organ bath cumulatively, such that the concentration in the bath increased by a factor of 3 with each addition. Two dose-response curves were determined in each preparation. After the first dose-response curve, the bath was washed out repeatedly and the strips allowed to relax to their original length over 40 min. The test drug or vehicle was added at this point and allowed to equilibrate for 10 min before the next addition of agonist.

Analysis of dose-response curves

Sensitivity changes can be classified into 2 types (Kalsner 1974): type 1 sensitization involves a change in the effective concentration of agonist at the receptors and was interpreted by examination of the shift in the dose-response curve at the ED_{50} level; type 2 sensitization involves a change in the responding tissue beyond the initial combination of agonist and receptor and was interpreted by examination of the change in maximum response of the tissue.

Statistical evaluation of results

Points obtained from individual experiments were plotted as log dose-response curves. Points lying between 20 and 80% of the maximum response (i.e. those on the linear part of the log dose-response curve) were fitted by means of linear regression analysis. This line was used to describe the gradient and the ED_{50} for each dose-response curve. Results are expressed as mean ± s.e. mean. Estimation of the significance of changes in the gradient, ED_{50} and maximum response between groups of experiments was by Student's t test.

Drugs

The following drugs were used (−)-noradrenaline bitartrate (Sigma), SKF 525A (Smith, Kline & French Laboratories, Philadelphia PA), cocaine hydrochloride (E. Coburn, Ltd.), angiotensin II (hypertensin, Ciba), desmethylimipramine hydrochloride (Pertofran, Geigy Pharmaceuticals Ltd.), chlorpheniramine maleate (Allen & Hanbury's Research Ltd.) and oxymetazoline hydrochloride (E. Merck Ltd.). The remaining chemicals were 'Analar' grade.

Results

Reproduceability of the dose-response curve to (−)-noradrenaline

Addition of (−)-noradrenaline to the spleen strips produced contractions which were recorded isotonically. After completion of the dose-response curve the agonist was washed out and a second curve obtained. The responses to the second addition of agonist were expressed as a percentage of the maximum response obtained during the first exposure (see Figure 1).

There was no significant difference in the maximum response or gradient obtained with the second exposure and only a small shift to the right of the dose-response curve at the level of the ED_{50}. These results show that two dose-response curves to (−)-noradrenaline can be obtained in the same preparation

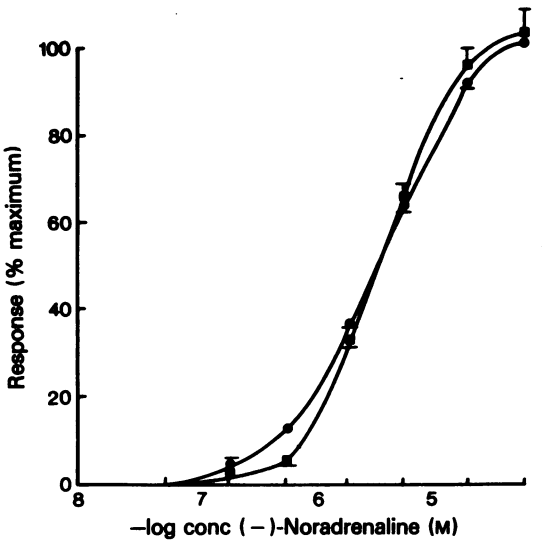

Figure 1 Comparison of two log dose-response curves to (−)-noradrenaline obtained in the same capsular splenic strip. Responses are expressed as a percentage of the maximum response obtained in the first dose-response curve: (●) first dose-response curve; (■) second dose response curve ($n = 8$).

without a major alteration in sensitivity or maximum response.

Effect of cocaine on the dose-response curve to (−)-noradrenaline

Cocaine (2×10^{-6} M and 10^{-5} M) had no effect on the resting length of capsular splenic strips. These concentrations produced respectively 2 and 7 fold shifts to the left of the log dose-response curve to (−)-noradrenaline at the level of the ED_{50} (see Figure 2). These concentrations also increased the maximum response to $117 \pm 4\%$ ($n = 13$) and $126.7 \pm 6.4\%$ ($n = 11$) of control. There was no significant change in the gradient of the curves. These results confirm those of other workers (Granata & Langer, 1973; Guimaraes & Brandao, 1973; Granata, Stefano & Langer, 1974).

Effect of cocaine on isometrically recorded responses to (−)-noradrenaline

It was considered important to determine whether the increased maximum response observed in the previous section was due to an increase in the ability of the muscle to shorten (perhaps caused by changes in elasticity or other physical properties) or whether it reflected an increased ability of the muscle to generate tension.

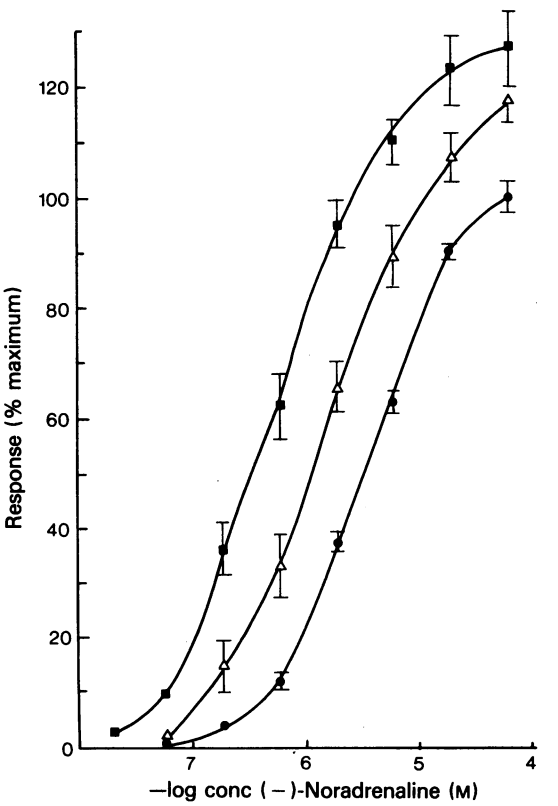

Figure 2 The effect of cocaine on the log dose-response curve to (−)-noradrenaline in capsular splenic strips: (●) control ($n = 8$); (△) cocaine, 2×10^{-6} M ($n = 13$); (■) cocaine, 10^{-5} M ($n = 11$).

Control experiments using the isometric recording technique showed, as with isotonic recording, that the dose-response curves obtained with 2 exposures to (−)-noradrenaline did not differ significantly with regard to the ED_{50} or maximum response (see Table 1). Addition of cocaine (10^{-5} M) to the bathing solution in these experiments again shifted the log dose-response curve to the left and increased the maximum response. It is concluded that the increased maximum response reflects an increase in the ability of the muscle to generate tension in the presence of cocaine.

Effect of desmethylimipramine and chlorpheniramine on the dose-response curve to (−)-noradrenaline

Table 2 shows the effect of DMI, a competitive neuronal uptake inhibitor (Iversen, 1967) on the dose-response curve to (−)-noradrenaline.

DMI (10^{-7}, 10^{-6} and 10^{-5} M) produced no significant potentiation of the response to (−)-noradrena-

line. Only at a concentration of 10^{-5} M did DMI have any effect on the maximum response; at this concentration the response was reduced to 89.5% of the control value. This decrease in maximum response probably reflects the known antagonistic action of DMI at high concentrations (Westfall, 1973).

Certain antihistaminic drugs have been reported to possess 'cocaine-like' activity, potentiating the contractile response of isolated atria to noradrenaline (Isaac & Goth, 1965). As shown in Table 3, chlorpheniramine maleate (3×10^{-6} M) did not significantly alter the ED_{50}, the slope of the dose-response curve or the maximum response to (−)-noradrenaline in cat splenic strips.

Table 1 The effect of cocaine on isometric responses of isolated strips of cat spleen to (−)-noradrenaline

Groups	n	ED_{50} (μM)	*95% confidence interval of the* ED_{50} (μM)	*Shift in the* ED_{50}	*Slope = % max response / log concentration* (M)	*Maximal tension* (*g*)
(a) 1st control	14	2.24	1.53–3.27		56.6 ± 1.7	2.4 ± 0.15
(b) 2nd control	6	1.95	0.89–4.28	+1.15 from (a)	64.4 ± 5.4	2.6 ± 0.1
				***	***	***
(c) Cocaine (10^{-5} M)	8	0.34	0.16–0.73	+6.51 from (a)	87.0 ± 3.2	3.1 ± 0.1

*** $P < 0.001$ when tested against control group (a). Positive shifts of the log dose-response curve are shifts to the left.

Table 2 The effect of desmethylimipramine (DMI) on responses of isolated strips of cat spleen to (−)-noradrenaline

Groups	n	ED_{50} (μM)	*95% confidence interval of the* ED_{50} (μM)	*Shift in the* ED_{50}	*Slope = % max response / log concentration* (M)	*% maximal response*
(a) Control	8	2.59	1.95–3.45		53.1 ± 1.0	100
(b) DMI, 10^{-7} M	7	2.55	1.17–5.56	+1.00 from (a)	53.8 ± 1.3	97.8 ± 3.0
(c) DMI, 10^{-6} M	5	1.98	1.03–3.80	+1.91 from (a)	52.9 ± 8.0	100.7 ± 3.4
(d) DMI, 10^{-5} M	8	3.20	1.36–7.13	−1.20 from (a)	45.4 ± 4.1	89.9 ± 5*

* $P < 0.01$ when tested against control group (a). Positive shifts of the log dose-response curve are shifts to the left.

Table 3 The effect of chlorpheniramine on responses of isolated strips of cat spleen to (−)-noradrenaline

Groups	n	ED_{50} (μM)	*95% confidence interval of the* ED_{50} (μM)	*Shift in the* ED_{50}	*Slope = % max response / log concentration* (M)	*% maximal response*
(a) Control	8	1.50	1.20–1.87		63.2 ± 1.7	100
(b) Chlorpheniramine (3×10^{-6} M)	4	1.26	0.18–7.69	+1.25 from (a)	63.8 ± 0.4	99.4 ± 2.18

Effect of cocaine on the dose-response curve to oxymetazoline

If the potentiating effect of cocaine on the response to noradrenaline is not due entirely to blockade of neuronal uptake then cocaine should also potentiate the response to oxymetazoline, a directly acting α-adrenoceptor agonist which is not a substrate for neuronal uptake (Mujic & Van Rossum, 1965; Birmingham, Paterson & Wojcicki, 1970).

Since oxymetazoline could not be satisfactorily washed out of the strips, nor submaximal concentrations be repeated reproducibly, a change in experimental protocol was necessary. In each spleen strip, only one dose-response curve to oxymetazoline was determined in the presence or absence of cocaine or DMI. All responses were expressed as a percentage of the individual maximum response. As shown in Table 4, 10^{-5} M cocaine significantly shifted the dose-response curve to the left 3.3 fold, the ED_{50} shifting from 0.79 to 0.24 μM. DMI (10^{-6} M) had no effect on the dose-response curve to oxymetazoline.

Effect of cocaine on the response produced by K^+

K^+ produces depolarization of smooth muscle cells by reducing the K^+ concentration gradient across the cell membrane. However, at high concentration it also depolarizes nerve endings causing the release of noradrenaline and dopamine β-hydroxylase (Thoa, Wooten, Axelrod & Kopin, 1975). To prevent the action of noradrenaline released from nerves on the smooth muscle, these experiments were conducted in the presence of the α-adrenoceptor antagonist, phentolamine (10^{-6} M), which has been shown to block the response to 10^{-5} M (−)-noradrenaline in cat spleen strips (Granata, *et al.*, 1974). Under these conditions K^+ produced contraction of the splenic strips (Figure 3).

The effect of K^+ is thought to be caused almost exclusively by an increase in membrane flux of Ca^{2+} derived from the extracellular compartment (Daniel, 1965; Hinke, 1965; Hudgins & Weiss, 1968). In spleen strips it produced a dose-related contraction. In the presence of cocaine (10^{-5} M) there was a shift to the left and an increase in the maximum response obtained with K^+. These results would suggest that the presence of cocaine increases the influx of Ca^{2+} which occurs in the presence of K^+.

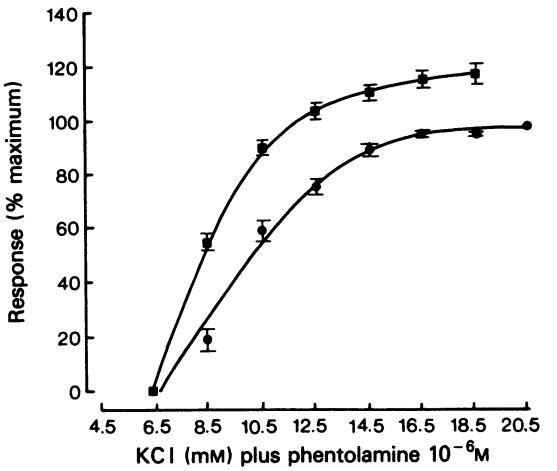

Figure 3 The effect of cocaine on responses of isolated strips of cat splenic capsule to K^+ in the presence of phentolamine (10^{-6} M). Responses are expressed as a percentage of the control maximum response. (●) Responses to KCl; (■) responses to KCl in the presence of cocaine (10^{-5} M) ($n = 4$).

The effect of cocaine on responses to angiotensin

In vascular tissue the response to angiotensin is dependent on the intracellularly bound Ca^{2+} (Kalsner, *et al.*, 1970; van Breemen, *et al.*, 1972). If cocaine were to potentiate the responses to angiotensin it would indicate that it has an effect on the intracellular binding of Ca^{2+}.

Splenic strips were 17 times more sensitive to angiotensin than (−)-noradrenaline. Cocaine had no significant effect on the ED_{50}, slope or maximum response of the dose-response curve to angiotensin (Figure 4). This would indicate that cocaine does not

Table 4 The effect of cocaine on responses of isolated strips of cat spleen to oxymetazoline

Groups	n	ED_{50} (μM)	*Shift in the ED_{50}*	*Slope = % max response / log concentration* (M)	*% maximal response*
(a) Control/oxymetazoline	26	0.79		52.6 ± 2.3	100
(b) Oxymetazoline/cocaine 10^{-5} M	14	0.24	+3.34*** from (a)	39.6 ± 1.8	100

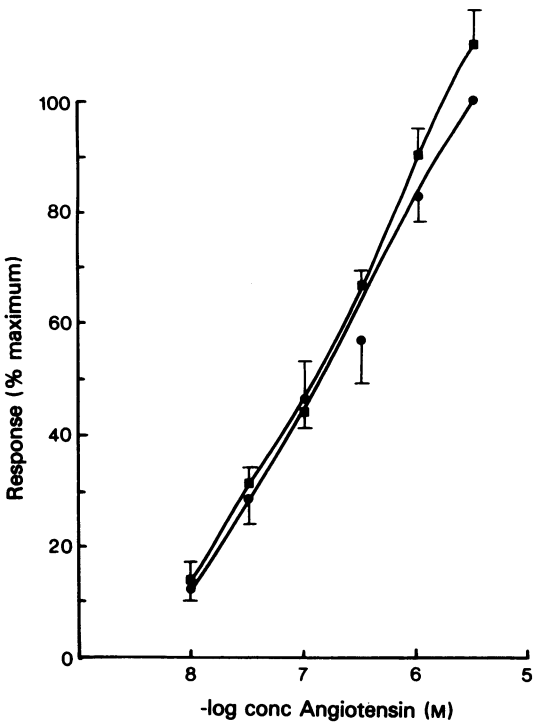

Figure 4 The effect of cocaine on the log dose-response curve to angiotensin II in isolated strips of cat splenic capsule: (●) responses to agiotensin II (n = 8); (■) responses to angiotensin II in the presence of cocaine (10^{-5} M) (n = 6).

produce supersensitivity by altering intracellular binding of Ca^{2+}.

The effect of cocaine on responses to (−)-noradrenaline in the presence of SKF 525A

SKF 525A (β-diethylaminoethyldiphenylpropylacetate) is an inhibitor of Ca^{2+} influx (Kalsner *et al.*, 1970). Given alone at a concentration of 2.65×10^{-5} M it produced a small shift to the right of the dose-response curve to noradrenaline (−1.3) at the level of the ED_{50} and a decrease in the maximum response of 13%. This was to be expected in view of the fact that part of the response to (−)-noradrenaline is believed to be due to the influx of Ca^{2+} (Woodward *et al.*, 1970).

Control experiments were performed in which two dose-response curves to (−)-noradrenaline were constructed in the presence of SKF 525A. There was no significant difference in the ED_{50}, slope or maximum response in these control experiments (Figure 5).

In the presence of SKF 525A, cocaine (10^{-5} M) produced neither a significant shift to the left nor an increased maximum response. These results would indicate that SKF 525A prevents the potentiation normally observed with cocaine (Figure 6).

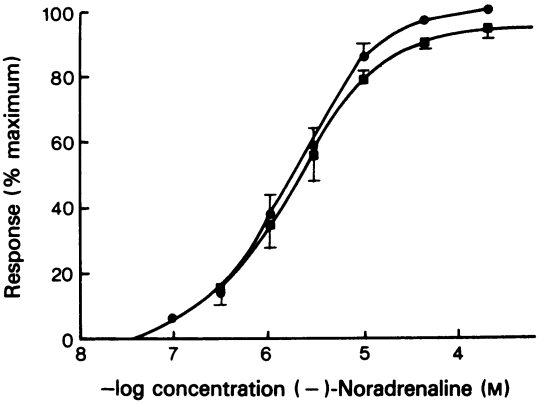

Figure 5 Comparison of two log dose-response curves to (−)-noradrenaline obtained in the same capsular splenic strip in the presence of SKF 525A (2.65×10^{-5} M): (●) first dose-response curve (n = 10); (■) second dose-response curve (n = 5).

Discussion

At least three explanations have been put forward to explain cocaine-induced postjunctional supersensitivity; changes in numbers or properties of receptors; an increase in cell to cell communication; or alterations in calcium metabolism. An early explanation for postjunctional supersensitivity was that cocaine and other procedures acted near the postsynaptic receptors either to increase the number of receptors or to alter, by an allosteric conformational change, either the intrinsic activity of the receptor, the receptor-agonist affinity, or binding (Maxwell, Plummer, Povalski, Schneider & Coombs, 1959; Carrier & Holland, 1965; Maxwell, *et al.*, 1966; Nakatsu & Reiffenstein, 1968; Reiffenstein, 1968; Bito & Dawson, 1970; Innes & Karr, 1971). This hypothesis has been questioned by Green & Fleming (1968) who reasoned that if cocaine altered the sensitivity of receptors then a change in affinity of noradrenaline for the receptor should be detectable by determination of the PA_x values for agonist-antagonist interaction. No such changes in the affinity of adrenoceptors for noradrenaline were observed in the supersensitive cat nictitating membrane (Green & Fleming, 1967), cat spleen (Green & Fleming, 1968; Innes & Mailhot, 1973) or aortic strips (Taylor & Green, 1971). The idea that cocaine induced postjunctional supersensiti-

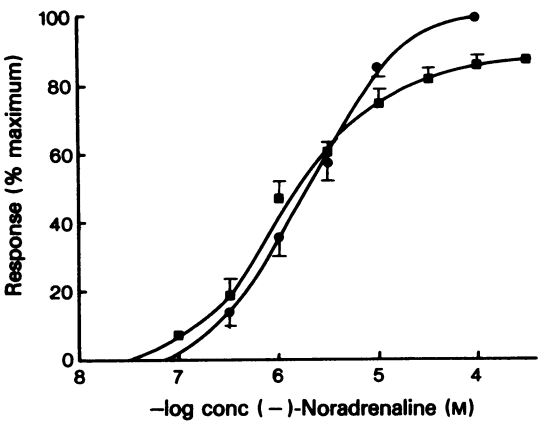

Figure 6 The effect of cocaine on the log dose-response curve to (−)-noradrenaline in the presence of SKF 525A in isolated strips of cat splenic capsule: (●) responses to (−)-noradrenaline in the presence of SKF 525A (2.65×10^{-5} M) ($n = 10$); (■) effect of cocaine (10^{-5} M) on responses to (−)-noradrenaline in the presence of SKF 525A (2.65×10^{-5} M) ($n = 6$).

vity occurs at the level of the receptors is, therefore, unlikely.

Another explanation for increased responses to noradrenaline is that synchronisation of contraction may be improved, i.e. cell to cell communication may be enhanced (Westfall, McClure & Fleming, 1972). Consistent with this hypothesis is the observation that denervation increases the number of cell to cell contacts in rat vas deferens (Westfall, Lee & Stitzel, 1975). The increase in number of cell to cell contacts may develop in parallel with membrane depolarization, since reduction in membrane bound Ca^{2+} has been shown to cause depolarization and initiate membrane fusion (Poste & Allison, 1973). This idea has recently been questioned on histological grounds (Paton, Buckland-Nicks & John, 1976) and instead it has been suggested that spontaneous activity is associated with membrane depolarization arising from a decrease in Ca^{2+} bound to the cell membrane, (Fleming & Westfall, 1975) or to changes in tissue content of ATP since these are increased by procedures which produce postjunctional super-sensitivity (Westfall *et al.*, 1975).

Changes in Ca^{2+} metabolism provide an explanation for postjunctional supersensitivity (Barnett, Greenhouse & Taber, 1968; Greenberg & Innes, 1968; 1976; Kasuya & Goto, 1968; Greenberg & Long, 1971; Shibata, Hattori, Sakurai, Mori & Fujiwara, 1971).

There are two main areas where cocaine could affect calcium metabolism so that more Ca^{2+} was made available for contraction. Cocaine could either affect intracellular storage sites of Ca^{2+} or membrane Ca^{2+} fluxes. Several studies have indicated that different agonists utilize different stores of Ca^{2+} in the production of contractile responses (Waugh, 1962; Bohr, 1964; Hinke, Wilson & Burnham, 1964; Daniel, 1965; Hinke, 1965; van Breemen & Daniel, 1966; Hudgins & Weiss, 1968).

In the present studies the effect of cocaine on responses to 3 agonists was studied. K^+-induced contractions have been shown to be associated with an influx of Ca^{2+} from loosely bound extracellular stores (Daniel, 1965; Hinke, 1965; Hudgins & Weiss, 1968). Since in spleen strips, cocaine produced a shift to the left and an increase in the maximum response to K^+ this would indicate an effect of cocaine on Ca^{2+} influx. The response to noradrenaline is produced by an initial release of Ca^{2+} from the bound intracellular store followed by an influx of Ca^{2+} from the extracellular pool (Waugh, 1962; Hinke, 1965; Hudgins & Weiss, 1968). Cocaine potentiated the response of spleen strips to noradrenaline. This effect cannot be explained by the inhibitory action of cocaine on neuronal uptake for the following reasons. Firstly, cocaine produced an increase in maximum response as well as a shift to the left of the dose-response curve to noradrenaline. An increase in maximum response is not associated with inhibition of uptake and suggests a postjunctional action (Kalsner, 1974). Secondly, cocaine also potentiates responses to oxymetazoline, an α-adrenoceptor agonist which is not a substrate for neuronal uptake (Birmingham *et al.*, 1970). One point which is not evident from Table 4 but which can be observed in Figure 7(a) is that after cocaine not only is the dose-response curve to oxymetazoline shifted to the left but the gradient becomes less. If however, as in Figure 7(b) the dose-response curve in the presence of cocaine is replotted, assuming that the increased maximum response had occurred as was the case with noradrenaline, it can be seen that cocaine then produces a parallel shift of the dose-response curve to the left which is of similar magnitude to that observed with noradrenaline. A third point is that other inhibitors of neuronal uptake such as desmethylimipramine or chlorpheniramine have remarkably little effect on the position of the dose-response curve to noradrenaline, only shifting the curve 1.9 and 1.25 fold to the left respectively (not significantly different from controls) and having no effect on the maximum response. This relative lack of effect of neuronal uptake inhibitors on responses of spleen strips is probably related to the density of innervation of the tissue which is much less than that in tissues such as the rat anococcygeus muscle in which DMI (10^{-6} M) produces a 12.2 fold shift to the left of the dose-response curve to noradrenaline (Summers & Tillman unpublished observations). The postsynaptic effect of cocaine would therefore be

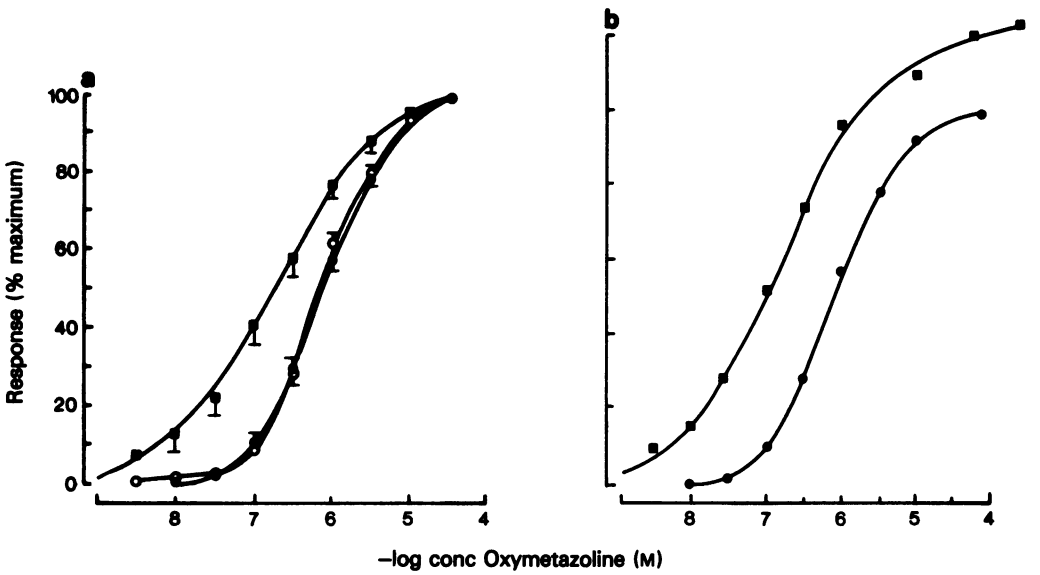

Figure 7 Effect of cocaine and desmethylimipramine (DMI) on the log dose-response curve to oxymetazoline. In (a) responses are expressed as a percentage of the maximum response obtained in each experiment: (●) control dose-response curve to oxymetazoline ($n = 26$); (■) effect of cocaine (10^{-5} M) ($n = 14$); (○) effect of DMI (10^{-6} M) ($n = 6$). In (b) responses, in the presence of cocaine (10^{-5} M), have been recalculated so that the maximum response is assumed to be similar to that obtained with (−)-noradrenaline in the presence of cocaine (126%).

expected to be most obvious in tissues in which the density of noradrenergic innervation is relatively low.

From studies with inhibitors of Ca^{2+} flux such as La^{3+} and SKF 525A, the third agonist angiotensin appears to initiate contraction almost exclusively by mobilizing the calcium firmly bound to the intracellular membrane (Kalsner, *et al.*, 1970; van Breemen, *et al.*, 1972). Cocaine had no effect in the present experiments on responses to angiotensin indicating no effect on intracellular Ca^{2+} metabolism. The experiments with all three agonists support the conclusion that in cat spleen strips the increased response to noradrenaline is the result of an increased Ca^{2+} flux across the cell membrane following treatment with cocaine.

Cocaine has also been shown either to increase (Kasuya & Goto, 1968; Hashiguchi, Ito & Kuriyama, 1974) or have no significant effect (Pennefather, 1976) on the response to K^+ in other tissues such as the rat and guinea-pig vas deferens but not in the rat anococcygeus (Gibson & Pollock, 1973) or the human umbilical artery (Reiffenstein & Triggle, 1974). Therefore the ability of cocaine to enhance the response to K^+ appears to be both tissue and species specific. Other evidence which supports the idea that cocaine acts on Ca^{2+} influx in spleen strips is the finding that SKF 525A prevented the enhancement of the response to noradrenaline by cocaine. Exposure of strips to SKF 525A partly inhibited the response to noradrenaline. This is to be expected as the latter part of the response to noradrenaline is thought to be dependent on influx of Ca^{2+} (Woodward, *et al.*, 1970). In the presence of SKF 525A cocaine neither shifted the dose-response curve nor increased the maximum response. This is supported by the findings of Shibata *et al.*, 1971, who showed in rabbit aortic strips that cocaine does not potentiate the response to noradrenaline in Ca^{2+} free medium or in the presence of Mn^{2+} or Co^{2+} which are thought to inhibit specifically Ca^{2+} influx (Hagiwara & Nakajima, 1966; Geduldig & Junge, 1968; Shibata, 1969). In addition, in the same preparation, cocaine effectively potentiated the contraction produced when Ca^{2+} was added to a Ca^{2+}-free, high K^+ medium.

The effect of cocaine on influx of Ca^{2+} as an explanation for supersensitivity to noradrenaline is by no means generally accepted (Barnett *et al.*, 1968; Kasuya & Goto, 1968; Greenberg & Innes, 1968; 1976; Pennefather, 1976). In particular, in cat spleen strips it has been suggested that cocaine potentiates the response to noradrenaline by making the tightly bound intracellular Ca^{2+} store available for contraction (Greenberg & Innes, 1976). Evidence for this suggestion is that cocaine potentiates the response to

noradrenaline in a Ca^{2+}-free medium and in strips which have been previously depleted of Ca^{2+} by treatment with the chelating agent disodium edetate (EDTA). There is no obvious explanation which would explain the different conclusion arrived at in the present experiments but it is possible that after EDTA treatment there is sufficient redistribution of Ca^{2+} to provide the small amount of extracellular Ca^{2+} required to affect the mechanical response (Lullman, 1970; van Breemen, *et al.*, 1973).

Great caution must be exercised in interpretation of the results of experiments in which the actions of agonists and antagonists on particular pools of calcium have been used to investigate an aspect of the mode of action of another drug. One assumption that has been made is that the drugs act specifically on particular calcium pools in splenic strips and this has not been proved by direct experiment. It would also be desirable to measure the effect of cocaine on the calcium fluxes across the splenic smooth muscle membrane as well as on responses. In spite of these reservations all the evidence we have obtained suggests that cocaine enhances the influx of calcium across the cell membrane during responses to agonists that utilize the extracellular pool of calcium and that this effect is responsible for a large part of the observed potentiation of responses.

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