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Cocaine potentiates the responses to methacholine and noradrenaline in the rat vas deferens

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The ability of cocaine (10 μM) to potentiate the contractile responses of the epididymal half of the rat vas deferens to methacholine was reversed by prazosin. Prazosin also partially reversed the ability of cocaine to increase the spontaneous overflow of ^3H following loading of the tissue with [^3H]noradrenaline. We suggest that cocaine potentiated the responses to methacholine by stimulating, directly or indirectly, α_1-adrenoceptors.

In many studies assessing the potency of agonists or antagonists at adrenoceptors, cocaine has been routinely added to inhibit neuronal uptake. Cocaine will only be useful in such studies if it has no additional actions (discussed by Furchgott 1972). However cocaine (i) is a general depressant of cardiac tissue (Trendelenburg 1968; Doggrell & Vincent 1982; Lew & Angus 1983), (ii) has a postjunctional action whereby some blood vessels become supersensitive to a variety of stimuli (e.g. histamine, methoxamine in the rabbit aorta; Kalsner & Nickerson 1969) and (iii) acts as an agonist at α-adrenoceptors and also potentiates responses to acetylcholine by a postjunctional mechanism in the rat anococcygeus muscle (Doggrell & Waldron 1982).

Cocaine has been used to inhibit neuronal uptake in contractility studies with the rat vas deferens (e.g. Demichel et al 1981; Leedham & Pennefather 1982; Doxey et al 1984). We have examined whether cocaine has additional actions on this tissue. Thus we report the effects of cocaine on the contractile responses of the epididymal half of rat vas to methacholine and noradrenaline (NA). The effects of cocaine on the uptake and the spontaneous overflow of ^3H, following preloading of the tissue with [^3H]noradrenaline, were also investigated.

Methods and materials

Mature male Wistar rats (300–450 g) were stunned and exsanguinated. Vasa deferentia were removed, dissected free of surrounding tissue and the epididymal half retained. All experiments were performed in the presence of a modified Krebs solution [composition (mM): NaCl 116; KCl 5·5; $CaCl_2$ 2·5; $MgCl_2$ 1·2; NaH_2PO_4 1·2; $NaHCO_3$ 22·0; D-glucose 11·2; Na_2EDTA 0·04] equilibrated with 5% CO_2 in oxygen at 37 °C. In each series of experiments the individual values obtained were compared by Student's paired *t*-test and were considered significant when $P < 0.05$. Mean values ± s.e.m. were then determined.

For the contractility studies, each epididymal tissue was mounted under 0·5 g tension in a 5 ml organ bath containing Krebs solution, and allowed to equilibrate for 1 h. Concentration-response curves to methacholine and then noradrenaline were obtained non-cumulatively. Exposure to agonist was continued for 30 s or until the response was maximal. The tissues were then washed by over-flow and allowed to recover, for a minimum of 7 min, before further addition of agonist. Contractile responses were recorded isometrically with force displacement transducers (Grass model FT03.C) and displayed on a polygraph (Grass model 79B). When two response curves were obtained 30 min was allowed to elapse between them.

When the effect of cocaine or prazosin on the responses to agonists was studied, the drug was added to one of a pair of mounted vasa, while the other remained untreated. For experiments in the presence of cocaine, the drug was in the Krebs solution in the bath. After all experiments the tissues were blotted and weighed.

Where maximum responses (mg), with or without drug, were not significantly different, responses were calculated as a percentage of the maximum response of the individual response curve (i.e. normalized) and the slope (difference in percentage maximum of the response/logarithm molar concentration of agonist) for each concentration response curve was computed by regression analysis (over the range 20–80% of the

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maximum response). When the maximum responses were significantly different for each pair of tissues, i.e. treated and untreated, all responses were calculated as a percentage of the maximum response of the untreated tissue.

For the accumulation of ^3H, each epididymal tissue was mounted under 0·2–0·5 g tension on a wire frame and equilibrated for 75 min in 5 ml Krebs solution. After exposure to 50 nM [^3H]NA for 15 min, the tissues were blotted, and washed for 10 min in Krebs solution. They were then blotted, weighed, and digested in 1 ml of 'Protosolve' (120 g NaOH in 1 litre methanol). When the tissue had dissolved, 10 ml of a toluene-based scintillation fluid and 0·5 ml of glacial acetic acid were added. The ^3H in the tissue and medium was determined by liquid scintillation spectrometry. To study the effects of cocaine or prazosin on the accumulation of ^3H, the drugs were added to the Krebs 60 min before incubation with [^3H]NA. The accumulation of ^3H was expressed as the tissue/medium ratio.

Before the measurement of ^3H overflow, individual epididymal tissues were mounted under 0·2–0·5 g tension in 3 ml Krebs solution and equilibrated for 15 min. (−)-[^3H]NA final concentration 0·5 μM, was added for 1 h, after which each tissue was placed in 30 ml of prewarmed Krebs for 30 min and a further 30 ml for 30 min. The tissues were transferred to 5 ml of fresh Krebs and this solution was replaced at 5 min intervals for 55 min. During this 55 min period, the effect of cocaine *or* prazosin on the spontaneous overflow of ^3H was studied for the last 25 min, the drugs being added to the bathing solution of one of the epididymal preparations while the other of the pair received no drug. When the effect of cocaine in the presence of prazosin was being examined, prazosin was added to both tissues for the whole of the 55 min.

At the end of the overflow period (i.e. 115 min after exposure to (−)-[^3H]NA) the tissues were digested and the ^3H in the tissue and medium was determined by liquid scintillation spectrometry. Overflow was expressed as % overflow as follows: % overflow = A/A′ × 100, where A = amount of ^3H that overflowed in a 5 min period and A′ = amount of ^3H in the tissue at the beginning of the overflow period (determined by addition of tissue content of ^3H to the ^3H in the media collected after the start of the overflow period). For individual tissues, the spontaneous overflow of ^3H for each 5 min after 90 min was then calculated as a percentage of that between 85 and 90 min. Overflow from treated and untreated tissues was then compared. (−)-[^3H]NA with a specific activity of 24·8 Ci mmol^{-1} was obtained from the New England Nuclear Corpora-

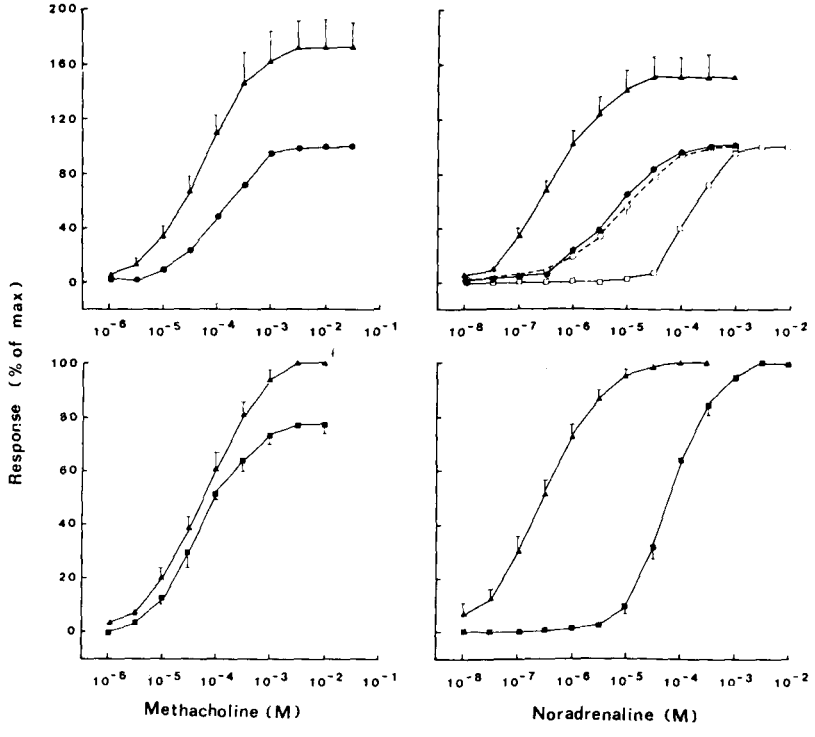

FIG. 1. Contractile responses of the epididymal tissue of the vas deferens to methacholine and NA. Top: responses in the absence (●) and presence (▲) of 10 μM cocaine [n = 10] and in the absence (○) and presence (□) of 0·1 μM prazosin [n = 8]. Bottom: responses in the presence of 10 μM cocaine (▲) and the presence of cocaine and 0·1 μM prazosin (■) [n = 9]. All responses are expressed as a percentage of the maximum response (see Methods for details). Each value is the mean ± s.e.m.

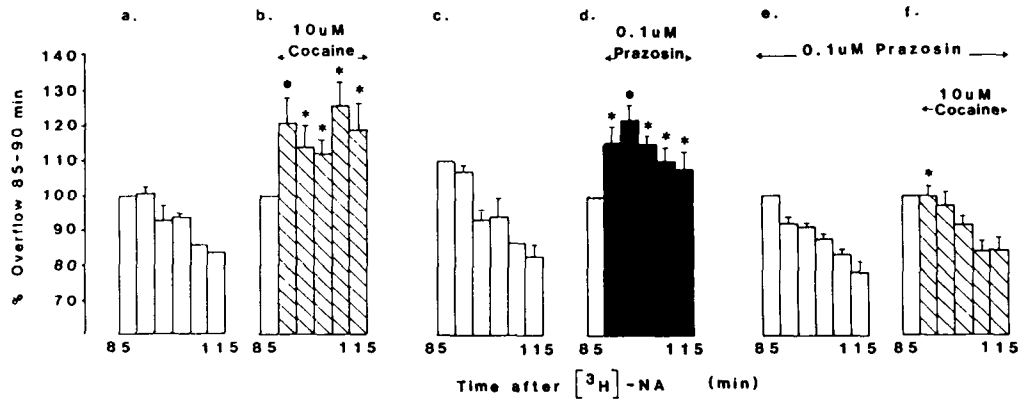

FIG. 2. The effect of cocaine and prazosin on the overflow of ^3H from the epididymal tissue of the rat vas deferens, following incubation with [^3H]NA. Overflow in the absence (a) and presence of 10 μM cocaine (b) [n = 6], in the absence (c) and presence of 0·1 μM prazosin (d) [n = 5], and in the presence of 0·1 μM prazosin throughout, without (e) and with the addition of 10 μM cocaine (f) [n = 8]. Each value is the mean; vertical lines show s.e.m.

tion. The other drugs used were cocaine hydrochloride (May & Baker), methacholine chloride, (−)-noradrenaline bitartrate (Sigma Chemicals Co.) and prazosin hydrochloride (donated by Pfizer Ltd).

Results

The mean wet weights of the epididymal tissues that were untreated and treated with cocaine (10 μM) were 27 ± 2 mg (n = 10) [mean ± s.e.m., n = 10] and 28 ± 2 mg (n = 10), respectively. Under all other conditions the control and test preparations were not significantly different.

Cocaine (10 μM) and prazosin (0·1 μM) did not induce tone in the preparation. Cocaine increased the maximal and submaximal contractile responses to methacholine and NA (Fig. 1). Maximal responses to methacholine were increased from 662 ± 64 mg (n = 10) to 1127 ± 161 mg (n = 10) and to NA from 1716 ± 178 mg to 2316 ± 147 mg (n = 10). Prazosin had no effect on the responses to methacholine or the maximal responses to NA, but inhibited submaximal responses to NA (Fig. 1). In the presence of cocaine (10 μM), prazosin (0·1 μM) reduced the maximal responses to methacholine and the submaximal responses to NA (Fig. 1). The inhibitory effect of prazosin on submaximal responses included an increase in the slope of the concentration-response curve to NA from 50 ± 6 (n = 8) to 72 ± 2 (n = 8) in the absence of and from 44 ± 4 (n = 10) to 65 ± 5 (n = 10) in the presence of cocaine.

The preparations accumulated ^3H from a bathing medium containing 5×10^{-8} M [^3H]NA. Accumulation was reduced from a mean tissue/medium ratio of 3·6 ± 0·5 (n = 6) to 1·7 ± 0·3 (n = 6) by cocaine (10 μM) but not altered by prazosin (0·1 μM).

85 min after the incubation with [^3H]NA, the overflow of ^3H from the preparation was decreasing (Fig. 2a, c). Cocaine (10 μM) or prazosin (0·1 μM) alone increased the overflow of ^3H (Fig. 2b, d). In the presence of prazosin (0·1 μM), the overflow of ^3H also decreased with time (Fig. 2e) and the ability of cocaine (10 μM) to release ^3H was reduced (Fig. 2f).

Discussion

Acetylcholine and methacholine act at an atropine-sensitive site to contract the epididymal but not the prostatic tissue of the rat vas (Doggrell 1981). Although several mechanisms may underlie the observed ability of cocaine to potentiate the responses to methacholine, the effect is probably due to the stimulation of α_1-adrenoceptors either directly or indirectly. This stimulation is subthreshold for contractility. Cocaine releases ^3H which may represent [^3H]noradrenaline or its [^3H]metabolites. Prazosin reverses the ability of cocaine to potentiate responses to methacholine probably because it reduces the ability of cocaine to release ^3H and also antagonizes the stimulatory effects of cocaine, or of the noradrenaline that has been released by cocaine, at α_1-adrenoceptors.

Although the ability of cocaine to potentiate contractile responses to noradrenaline is often attributed to the inhibition of neuronal uptake, the stimulation of α_1-adrenoceptors may also contribute to this effect in the tissue studied. Moreoever this stimulatory effect of cocaine at α_1-adrenoceptors must distort the assessment of the inhibitory effect of prazosin on responses to noradrenaline in the presence of cocaine.

It is necessary to inhibit the neuronal uptake process completely, or as near to completely as possible, before the potency of antagonists at adrenoceptors is assessed when noradrenaline is the agonist (Kenakin 1982). However, the additional effects of uptake inhibitors should be avoided (Furchgott 1972). At 10^{-5} M, cocaine only partially inhibits the neuronal uptake of the noradrenaline in the epididymal tissue. Enough evidence has accumulated from this and other studies (see Introduction) to suggest that it is inadvisable to use

cocaine in any study to assess the potency of an agonist or antagonist.

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The interaction of cimetidine with various antihypertensive agents in the spontaneously hypertensive rat

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The H_2-receptor antagonist cimetidine (250 μg) administered intracerebroventricularly (i.c.v.) 15 and 30 min before clonidine (25 μg kg^{-1} i.v.), significantly antagonized clonidine-induced hypotension in anaesthetized spontaneously hypertensive rats. The hypertensive response of cimetidine was correlated with the inhibition of clonidine-induced hypotension. In addition, cimetidine (250 μg i.c.v.) counteracted the hypotensive effects of pentolinium (5·0 mg kg^{-1} i.v.), guanethidine (5·0 mg kg^{-1} i.v.) and minoxidil (1·0 mg kg^{-1} i.v.) These data do not support previous suggestions that the hypotensive action of clonidine is caused by stimulation of the H_2-receptor, but suggest that central administration of cimetidine causes peripheral vasoconstriction and this may offer resistance to the hypotensive action of different antihypertensive agents.

The interaction between clonidine and histamine H_2-receptor antagonists has indicated that histamine H_2-receptors are involved in the central hypotensive action of clonidine (Karppanen et al 1976, 1977; Finch et al 1978). However, there have been other findings which raise some doubt about this hypothesis, namely the induction of sleep in chicks, and the increase in locomotor activity in rats, are not inhibited by H_2-receptor antagonists (Vogt 1977; Nomura & Segawa 1979). Furthermore, Pilc et al (1979) and Timmermans et al (1980) have demonstrated that, in rat cerebral cortex, clonidine is bound specifically to sites different from the histamine binding sites. We have previously shown that in anaesthetized spontaneously hypertensive (SH) rats, central administration of cimetidine causes a sustained rise in perfusion pressure of the auto-perfused hindquarter (Dohadwalla & Dadkar 1981). In view of these observations, we have investigated the interaction between various antihypertensive agents and the H_2-receptor antagonist cimetidine, in anaesthetized SH rats.

* Correspondence.

Materials and methods

Male SH rats (230–250 g) the strain developed by Okamoto & Aoki (1963) were used. Permanent cannulation of lateral cerebroventricles was performed stereotaxically in pentobarbitone sodium anaesthetized animals as described by Dadkar et al (1984). These rats were allowed to rest for two to three days. On the day of experiment, they were anaesthetized with pentobarbitone sodium (50 mg kg^{-1} i.p.) and blood pressure was recorded on a Hellige physiological recorder using Statham P 23 Db pressure transducer. Mean blood pressure was calculated as diastolic pressure plus one third of pulse pressure. Intravenous administration of drug was carried out by cannulation of right jugular vein. Results were expressed as change in blood pressure over initial value in mmHg for each rat. The site of intracerebroventricular (i.c.v.) injection was confirmed at the end of the experiment by injection of 10 μl of Evans blue, and subsequent microscopic examination.

The drugs used were: clonidine hydrochloride (Boehringer Ingelheim), minoxidil (Upjohn), pentolinium tartrate (May & Baker) and guanethidine hydrochloride (Ciba-Geigy), dissolved in 0·9% sodium chloride and administered intravenously. Cimetidine