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Effect of cocaine on tone and contractile responses to acetylcholine in the rat anococcygeus muscle

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Cocaine is a potent inhibitor of the neuronal uptake of noradrenaline (e.g. Doggrell 1981), a local anaesthetic (Ritchie & Green 1980), and has a postjunctional action whereby some tissues become supersensitive to contractile responses mediated by a variety of stimuli (e.g. histamine, methoxamine in the rabbit aorta; Kalsner & Nickerson 1969). We have examined the effects of cocaine on contractile responses to acetylcholine using the rat anococcygeus muscle, a tissue which has no cholinergic innervation (Gillespie 1972). The effect of cocaine on the spontaneous overflow of ^3H following preloading of the tissue with (−)-[^3H]noradrenaline is reported.

Materials and methods

Mature male Wistar rats were stunned and exsanguinated. Anococcygeus muscles were dissected free. All experiments were performed in the presence of a modified Krebs solution of the following composition (mM): NaCl 116, KCl 5·4, $CaCl_2$ 2·5, $MgCl_2$ 1·2, NaH_2PO_4 1·2, $NaHCO_3$, 22·0, D-glucose 11·2, and Na_2EDTA 0·04, equilibrated with 5% CO_2 in O_2, at 37 °C.

Before the measurement of ^3H overflow, individual anococcygeus muscles were mounted under 0·2–0·5 g tension in 3 ml Krebs solution and equilibrated for 15 min. (−)-[^3H]Noradrenaline, final concentration 0·5 μM, was added for 1 h. At the end of the 1 h the muscles were treated as follows: (i) Spontaneous overflow. Each muscle was transferred to 4 ml of fresh Krebs solution. The replacement of Krebs solution was repeated at 5 min intervals for 2 h. (ii) Effect of cocaine on overflow. Each tissue was placed in 24 ml of prewarmed Krebs for 30 min and then in 36 ml for 45 min. The tissues were then transferred to 4 ml of fresh Krebs and this solution was replaced at 5 min intervals for 75 min. When the effects on spontaneous overflow were studied, cocaine or tyramine was added after 90 min of overflow for 30 min.

At the end of the overflow period the tissues were 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 fluor and 0·5 ml of glacial acetic acid were added. The ^3H in the tissue and each medium was determined by liquid scintillation spectrometry. Overflow was expressed as % overflow as follows: % overflow = $A/A' \times 100$, 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. The values obtained under different conditions were compared by Student's unpaired *t*-test, and differences were considered to be significant when $P < 0.05$.

For the contractility studies each anococcygeus muscle was mounted under 0·5 g tension in 5 ml organ baths containing Krebs solution. The tissues were allowed to equilibrate for 2 h 30 min. Concentration-response curves for acetylcholine were determined non-cumulatively. Exposure to acetylcholine was continued for 30 s or until a maximum response was obtained. Tissues were then allowed to recover for a minimum of 5 min before further addition of acetylcholine. Contractile responses were recorded isometrically with force displacement transducers (Grass FT03.C) and displayed on a polygraph (Grass 79B).

When the effect on responses was examined, cocaine was present in the Krebs solution of the anococcygeus muscle from the beginning of the equilibration period, while the other muscle of the pair remained in the absence of the drug throughout. When experiments were carried out in the presence of desipramine, phentolamine, neostigmine, atropine, or ouabain, these drugs were in Krebs solution from the beginning of the equilibration period.

When the maximum responses (g) under different conditions, were not significantly different, responses were calculated as a percentage of the maximum response of the individual response curve (i.e. normalized). The slope (difference in percentage maximum of the response/unit of

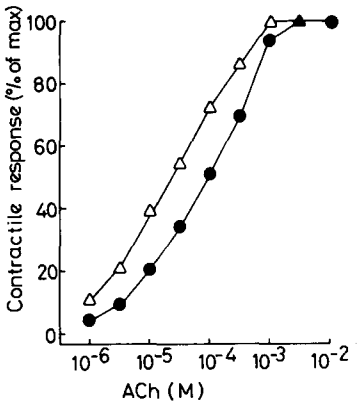

FIG. 1. The effect of cocaine on contractile responses to acetylcholine in the rat anococcygeus muscle. Responses to acetylcholine in the absence (●) and presence (△) of 10 μM cocaine. All responses are expressed as a percentage of the maximum response (ordinate). (Each value is the mean ±) s.e.m. within the symbols).

* Correspondence.

logarithm molar concentration of agonist) and a pD_2 value (negative logarithm of molar concentration of agonist producing 50% of the maximum response) for each concentration-response curve was computed by regression line analysis (over the range 20–80% of the maximum response). For each pair of tissues, the ability of a drug to potentiate or to inhibit responses was expressed as the dose-ratio (the antilogarithm of the difference between the pD_2 values in the presence and, from the other tissue of the pair, in the absence of drug). The individual values (maximum responses, percentage of the maximum response, slopes, pD_2 values, and dose-ratio) under different conditions, were compared using Student's paired *t*-test and were considered significantly different when $P < 0.05$. Mean values ± s.e. mean were also determined.

(−)-[^{3}H]Noradrenaline with a specific activity of 2·2 Ci mmol^{-1} was obtained from the New England Nuclear Corporation. The other drugs used were desipramine hydrochloride, phentolamine mesylate (donated by Ciba-Geigy), cocaine hydrochloride (May & Baker), and acetylcholine chloride, atropine sulphate, neostigmine bromide, ouabain octahydrate and tyramine hydrochloride (Sigma).

Results and discussion

After incubation of the rat anococcygeus muscle for 1 h in the presence of 0·5 μM (−)-[^{3}H]noradrenaline, there was a rapidly declining overflow of ^{3}H for 15 min followed by a period during which overflow declined slowly, 15–45 min. After 45 min no further significant reduction in overflow was observed. When the overflow of ^{3}H was no longer declining 10 μM cocaine had no effect. Tyramine, 10 μM, increased the overflow of ^{3}H, × 5.

Desipramine, phentolamine, neostigmine (all at 1 μM), atropine, 1 nM– 1 μM, and ouabain (50 μM) did not induce tone in the resting anococcygeus muscle. Cocaine 10 μM by itself induced tone (≤500 mg) in 7 of 8 preparations which lasted up to 1 h. The ability of cocaine to induce tone was abolished in the presence of phentolamine, 1 μM, but not by desipramine, neostigmine, atropine (all at 1 μM) or ouabain, 50 μM. To ensure that no effects on tone were involved, tissues were incubated in the presence of cocaine for 2½ h before acetylcholine was added. Cocaine, 10 μM, had no effect on the magnitude of the maximal responses or on the slopes of the concentration-response curves to acetylcholine. Submaximal responses to acetylcholine were potentiated, × 4·3, by cocaine (Fig. 1). This ability of 10 μM cocaine to potentiate responses was also observed in the presence of desipramine, phentolamine, neostigmine, atropine or ouabain (Table 1).

The ability of high concentrations of cocaine (≥10 μM) to induce tone of the rat anococcygeus muscle has been attributed to an indirect sympathomimetic action (Gillespie & McGrath 1974; Gillespie 1980). The results of the present study do not confirm this. Thus cocaine induced tone of the rat anococcygeus in the presence of desipramine. Desipramine is a potent inhibitor of the neuronal accumulation of ^{3}H from (−)-[^{3}H]noradrenaline and of the contractile responses to tyramine in the rat anococcygeus (Doggrell & Woodruff 1977). Also in the present study tyramine, an indirect sympathomimetic agent, increased the overflow of ^{3}H (following preloading of the tissue with (−)-[^{3}H]noradrenaline) whereas cocaine did not. The small cocaine-induced contractions were probably due to a direct action at α-adrenoceptors as they were only abolished by phentolamine (at a concentration which acts as a specific α-adrenoceptor antagonist in the rat anococcygeus (Doggrell & Vincent 1981).

Table 1. The effect of cocaine on contractile response to acetylcholine in the rat anococcygeus muscle.

	Acetylcholine pD_2 (mean ± s.e.m.)
Control	4·08 ± 0·05 (8)
Cocaine 10 μM	4·65 ± 0·08 (8)[1]
Desipramine 1 μM	3·86 ± 0·08 (6)
Desipramine 1 μM, Cocaine 10 μM	4·24 ± 0·08 (6)[1]
Phentolamine 1 μM	3·92 ± 0·07 (6)
Phentolamine 1 μM, Cocaine 10 μM	4·20 ± 0·07 (6)[1]
Neostigmine 1 μM	6·20 ± 0·09 (6)
Neostigmine 1 μM, Cocaine 10 μM	6·43 ± 0·11 (6)[1]
Atropine 1 μM	3·71 ± 0·07 (6)
Atropine 1 μM, Cocaine 10 μM	4·06 ± 0·04 (6)[1]
Ouabain 50 μM	4·31 ± 0·22 (4)
Ouabain 50 μM, Cocaine 10 μM	4·77 ± 0·14 (4)[1]

[1] $P < 0.05$, Student's paired *t*-test of individual values. (n) = number of observations.

The second action of cocaine, 10 μM, observed in the present study was to increase the sensitivity to acetylcholine. The observation conflicts with a previous report that this concentration of cocaine had no effect on responses to acetylcholine in the rat anococcygeus (Gibson & Pollock 1973). The reason for this discrepancy is unknown but as the potentiation we observed was small, it may have been undetectable using an unpaired tissue protocol. As the sensitizing action occurred after the cocaine-induced contractions had stopped and in the presence of phentolamine, it seems unlikely that direct α-adrenoceptor stimulation had any role in this action. Other mechanisms eliminated as underlying this increased sensitivity to acetylcholine with cocaine were (i) inhibition of noradrenaline uptake (as the effect was observed in the presence of desipramine), (ii) anticholinesterase activity (the effect was maintained in the presence of neostigmine) and (iii) effects on Na^+/K^+ ATP-ase (the action of cocaine was not altered by ouabain). It seems likely that this is a similar postjunctional action of cocaine to supersensitize the rat anococcygeus to a variety of stimuli as has been reported in some other tissues (e.g. rabbit aorta, Kalsner & Nickerson 1969). Thus the ability of cocaine by itself to potentiate responses to noradrenaline in the rat anococcygeus may not be solely due to inhibition of neuronal uptake as has been previously suggested (Gibson & Pollock 1973) but may also have a postjunctional component.

In many studies assessing the potency of agonist or antagonists at adrenoceptors, cocaine has been routinely

added to the medium in order to inhibit neuronal uptake only. As our study has shown cocaine to be a direct agonist at α-adrenoceptors and also to potentiate responses to acetylcholine by a postjunctional mechanism in the rat anococcygeus the use of cocaine in such studies with this tissue would seem inadvisable.

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REFERENCES

Doggrell, S. A. (1981) J. Pharm. Pharmacol. 33: 795–796
Doggrell, S. A., Vincent, L. (1981) Ibid. 33: 720–724
Doggrell, S. A., Woodruff, G. N. (1977) Br. J. Pharmacol. 59: 403–409
Gibson, A., Pollock, D. (1973) Ibid. 49: 506–513
Gillespie, J. S. (1972) Ibid. 45: 404–416
Gillespie, J. S. (1980) TIPS 1: 453–457
Gillespie, J. S., McGrath, J. S. (1974) Br. J. Pharmacol. 50: 109–118
Kalsner, S., Nickerson, M. (1969) Ibid. 35: 428–439
Ritchie, J. M., Green, N. M. (1980) in: Gillman, A. G., Goodman, L. S., Gillman, A. (eds) The Pharmacological Basis of Therapeutics, 6th edn, Macmillan, New York, pp 300–320

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Leukotriene B_4 in synovial fluid

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Leukotriene B_4 (LTB_4) is generated enzymatically from arachidonic acid by human leucocytes in vitro (Smith 1981). Recent work suggests that LTB_4 is a putative mediator of inflammation in vivo since it both increases leucocyte infiltration and, in the presence of vasodilatory prostaglandins, causes increased vascular permeability (Smith 1982). A second criterion is whether it is present at sites of inflammatory reactions and here the only quantitative evidence is that of Klickstein et al (1980). The LTB_4 was separated from 2 to 3 ml samples of synovial fluid by solvent extraction, column chromatography and reverse phase high performance liquid chromatography (h.p.l.c.); recognized by a comparison of retention times with standard material and measured by ultraviolet absorption techniques. In synovial fluid from eighteen patients with rheumatoid arthritis the reported values (mean ± s.e.m.) were 141 ± 34 ng ml^{-1} (Klickstein et al 1980).

There are objections to relying on physicochemical methods only for the measurement of LTB_4. There are a number of isomers of the leukotriene which are not only difficult to separate completely from the authentic material but also are either much less active or devoid of the relevant biological activities. Also, more recent work (Hansson et al 1981) has shown that LTB_4 is metabolized by human leucocytes to more polar hydroxy and carboxy compounds which are far less potent as leucotactic agents.

We have therefore repeated the work measuring the LTB_4 in the final h.p.l.c. fraction both by ultraviolet absorption and a sensitive and specific bioassay method (Cunningham et al 1980). In synovial fluid specimens, ranging in volume from 10 to 24 ml, from 12 patients with rheumatoid arthritis we could find no LTB_4 using the ultraviolet absorption method. In our hands the lower limit of detection with this technique is 50 ng per whole specimen i.e. approximately 2·5 ng ml^{-1}. The more sensitive bioassay procedure yielded a mean value of 0·34 ± 0·14 ng ml^{-1} of LTB_4.

It seems likely that the reason only traces of LTB_4 are present in synovial fluid is that the leukotriene is rapidly metabolized by the inflammatory cells present in the exudate. In other experiments human peripheral PMNs, human synovial fluid cells or rat peritoneal PMNs metabolized radiolabelled LTB_4 to more polar compounds and caused a loss of biological activity from unlabelled LTB_4 within minutes.

If LTB_4 is generated by leucocytes which enter and accumulate in inflammatory exudates, such as synovial fluid in patients with rheumatoid arthritis, then it will not persist as such but will be removed by metabolism. In this respect it resembles other inflammatory mediators including histamine, bradykinin and the complement-derived cytotaxin, C5a.

REFERENCES

Cunningham, F. M., Shipley, M. E., Smith, M. J. H. (1980) J. Pharm. Pharmacol. 32: 377–380
Hansson, G., Lindgren, J. A., Dahlen, S. E., Hedqvist, P., Samuelsson, B. (1981) FEBS Lett. 130: 107–112
Klickstein, L. B., Shapleigh, C., Goetzl, E. J. (1980) J. Clin. Invest. 66: 1166–1170
Smith, M. J. H. (1981) Gen. Pharmacol. 12: 211–216
Smith, M. J. H. (1982) in: Samuelsson, B., Paoletti, R. (eds) Leukotrienes and other lipoxygenase products. Raven Press: New York, pp 283–292