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Iprindole potentiates responses to acetylcholine in the rat anococcygeus muscle

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Those tricyclic antidepressants commonly used therapeutically e.g. amitriptyline, imipramine, either have no effect or, at high concentrations, inhibit the contractile responses to acetylcholine (ACh) in the rat anococcygeus muscle (Doggrell & Woodruff 1977). In contrast, iprindole, also a tricyclic antidepressant, potentiates the responses to ACh by an unknown, mechanism (Doggrell & Woodruff 1977). As several drugs (e.g. guanethidine) potentiate contractile responses to ACh in the rat anococcygeus by releasing (−)-noradrenaline [(−)-NA] (Doggrell & Paton 1978), we have set out to determine whether this occurs with iprindole. Thus we report the effects of iprindole on the overflow of ^3H (following preloading of the tissue with (−)-[^3H]NA) and on the contractile responses of the rat anococcygeus muscle to ACh under a variety of conditions.

Materials and Methods

Mature male Wistar rats were stunned and exsanguinated, and anococcygeus muscles were dissected free. All tissues were used in a modified Krebs solution of the following composition (mM): NaCl 166, 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. The values obtained under different conditions were compared by Student's paired or unpaired *t*-test, as appropriate, and differences were considered to be significant when $P \leq 0{\cdot}05$. Mean values ± s.e.m. were also determined.

Before measuring ^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]NA, final concentration 0·5 μM, was added for 1 h, after which each muscle was placed in 24 ml of pre-warmed Krebs for 30 min and then in 36 ml for 45 min. The tissues were transferred to 4 ml of fresh Krebs and this solution was replaced at 5 min intervals for 75 min. The effect of iprindole or yohimbine on the spontaneous overflow of ^3H was studied, after 90 min of overflow (i.e. 90 min after the incubation with (−)-[^3H]NA), for 45 min by adding iprindole or yohimbine to the bathing solution of one of the anococcygeus muscles while the other muscle of the pair received no iprindole or yohimbine. When cocaine (10 μM) or yohimbine (1 μM) was used they were added to the Krebs solution of one of the muscles from 75 min of overflow, onwards, and both muscles of the pair were exposed to 10 μM iprindole after 90 min of overflow for 45 min.

At the end of the overflow period (i.e. 150 min after exposure to (−)-[^3H]NA) 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 = $A/A' \times 100$, 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 the contractility studies, each anococcygeus muscle was mounted under 0·5 g tension in a 5 ml organ bath containing Krebs solution, and allowed to equilibrate for 3 h unless otherwise stated. Dose response curves to ACh were determined non-cumulatively. Exposure to ACh was for 30 s or until a maximum response was obtained. Contractile responses were recorded isometrically with force displacement transducers (Grass model FTO3.C) and displayed on a polygraph (Grass model 79B). Iprindole was present in the solution bathing one of the anococcygeus muscles from the beginning of the equilibration period while the other muscle of the pair received no iprindole. When experiments were carried out in the presence of desipramine, and α-adrenoceptor antagonist, neostigmine, atropine, ouabain, cocaine or iprindole, these drugs were present in the Krebs solution of both muscles from the beginning of the equilibration period. Only one drug was used for each pair of muscles.

To desensitize muscles to ACh, each was incubated in the presence of 1mM ACh for 3 h and then washed in Krebs solution for 30 min before the addition of iprindole to one tissue of the pair. The tissues were then equilibrated for 30 min before a dose-response curve to ACh was initiated.

Where maximum responses (g), with or without iprindole, were not significantly different, they were calculated as a percentage of the maximum response of the particular response curve (i.e. normalized).

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The slope (difference in percentage maximum of the response/unit of logarithm molar concentration of ACh and a pD_2 value (negative logarithm of molar concentration of producing 50% of the maximum response) for each dose response curve was computed by regression analysis (over the range 20–80% of the maximum response).

(−)-[^{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* (Ciba-Geigy), iprindole hydrochloride* (John Wyeth & Brother Ltd.), cocaine hydrochloride (May & Baker), prazosin hydrochloride* (Pfizer Ltd.) and acetylcholine chloride, atropine sulphate, neostigmine bromide, ouabain octahydrate, and yohimbine hydrochloride (Sigma Chemicals Co.). Compounds with an asterisk were donated.

Results and discussion

After incubation of the anococcygeus muscle for 1 h with 0·5 μM (−)-[^{3}H]NA, there is a rapidly declining overflow of ^{3}H for 15 min followed by overflow declining slowly over 15–45 min (Doggrell & Waldron 1982), after which no further significant reduction in overflow is observed (Doggrell & Waldron 1982). After 90 min of overflow neither cocaine at 10 μM (Doggrell & Waldron 1982) nor yohimbine at 1 μM (this study) had any effect. Iprindole at 1μM had no effect but at 10 μM it increased the overflow of ^{3}H. This effect was maintained in the presence of 10 μM cocaine (Fig. 1) or of 1 μM yohimbine.

Table 1. The effect of iprindole on the contractile responses to ACh in the rat anococcygeus muscle.

	Acetylcholine pD_2 (mean ± s.e.m.)
Control	4·37 ± 0·09 (5)
1 μM iprindole	4·44 ± 0·08 (5)
Control	4·20 ± 0·10 (6)
10 μM iprindole	4·50 ± 0·08 (6)*
1 μM desipramine	3·86 ± 0·19 (6)
1 μM desipramine, 10μM iprindole	4·21 ± 0·16 (6)*
1 μM phentolamine	4·23 ± 0·07 (5)
1 μM phentolamine, 10 μM iprindole	4·92 ± 0·26 (5)*
0.1 μM prazosin	4·32 ± 0·09 (8)
0.1 μM prazosin, 10 μM iprindole	4·92 ± 0·11 (8)*
1 μM yohimbine	4·33 ± 0·06 (8)
1 μM yohimbine, 10 μM iprindole	5·01 ± 0·09 (8)*
1 μM neostigmine	6·12 ± 0·09 (6)
1 μM neostigmine, 10 μM iprindole	6·47 ± 0·10 (6)*
50 μM ouabain	4·27 ± 0·10 (6)
50 μM ouabain, 10 μM iprindole	4·70 ± 0·06 (6)*
Following ACh incubation (1 mM for 3 h):	
Control	3·73 ± 0·09 (6)
10 μM iprindole	4·13 ± 0·11 (6)*
10 μM atropine	3.21 ± 0·06 (6)
10 μM atropine, 10 μM iprindole	4·24 ± 0·15 (6)*
10 μM cocaine	4·33 ± 0·19 (6)
10 μM cocaine, 10 μM iprindole	4·47 ± 0·15 (6)
10 μM iprindole	4·84 ± 0·13 (6)
10 μM iprindole, 10 μM cocaine	5·02 ± 0·28 (6)

*$P \leq 0.05$, Student's paired t-test of individual values. (n) = number of observations.

The accumulation of NA from low concentrations of the amine by the rat anococcygeus is predominantly neuronal (Nash et al 1974). Furthermore, after incubation with (−)-[^{3}H]NA all of the ^{3}H in the rat anococcygeus behaves as authentic (−)-NA (Doggrell & Woodruff 1977). In the present study we used a low concentration of (−)-[^{3}H]NA to promote neuronal accumulation, and washed the tissue for 90 min to remove the loosely bound ^{3}H. Following neuronal accumulation of (−)-[^{3}H]NA the spontaneous overflow of ^{3}H will represent (−)-[^{3}H]NA and the [^{3}H]metabolites of (−)NA. Iprindole (10 μM) inhibits the neuronal accumulation of (−)-[^{3}H]NA in the rat anococcygeus (Doggrell 1981a). In the present study iprindole increased the overflow of ^{3}H. Cocaine, a potent inhibitor of the neuronal uptake process, has no effect on the spontaneous overflow of ^{3}H in the rat anococcygeus (Doggrell & Waldron 1982). Thus it is unlikely that iprindole increases overflow by inhibiting uptake. However, as accumulation is equal to the uptake minus the overflow of ^{3}H it is possible that the ability of iprindole to increase overflow contributed to the inhibition of accumulation.

The iprindole-induced overflow of ^{3}H is not dependent on iprindole being transported by the neuronal (−)NA uptake carrier as the overflow was maintained in the presence of cocaine, nor is it due to antagonism at prejunctional α_2-adrenoceptors as yohimbine, a selective α_2-adrenoceptor antagonist, had no effect alone

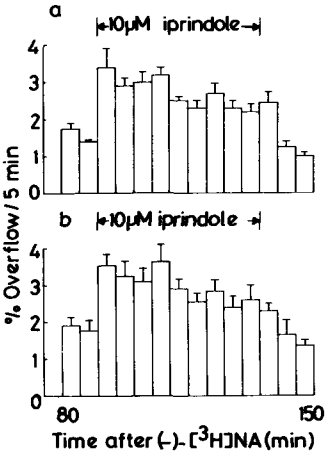

FIG 1. the effect of 10 μM iprindole on the overflow of ^{3}H from the rat anococcygeus muscle, following incubation with [^{3}H]NA, in the absence (a) and presence (b) of 10 μM cocaine. Each value is the mean from 5 preparations; vertical lines show s.e.m.

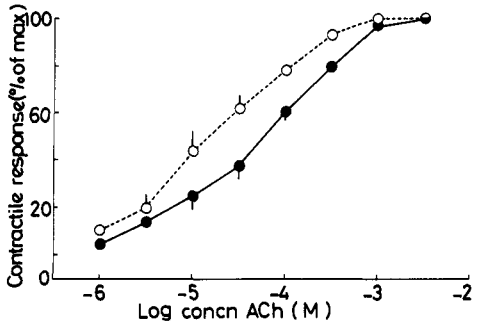

FIG. 2. The effect of iprindole on contractile responses to ACh in the rat anococcygeus muscle, in the absence (●) and presence (○) of 10 μM iprindole. All responses are expressed as a % of the maximum response. Each value is the mean ± s.e.m.

and did not modify the iprindole's action. From the contractility studies it seems unlikely that iprindole releases endogenous (−)-NA (see below) and thus it is probable that the iprindole-induced overflow of ^3H predominantly represents [^3H]metabolites of (−)-NA rather than (−)-[^3H]NA.

Iprindole, desipramine, phentolamine, yohimbine neostigmine (all at 1 μM), prazosin, 0·1 μM, ouabain, 50 μM and atropine, 1 nM or 1 μM, did not induce tone in the resting anococcygeus muscle. 10 μM iprindole alone induced tone (< 1½g) in 5 of 6 preparations tested which lasted up 2 h. Once established the contractions to 10 μM iprindole were not altered by the addition of 1 μM phentolamine or 1 μM atropine. The ability of iprindole to induce tone was not abolished in the presence of desipramine (4 of 6; 4 of 6 tissues challenged with 10 μM iprindole contracted), phentolamine (5 of 5), prazosin (2 of 8), yohimbine (2 of 8), neostigmine (3 of 6), ouabain (4 of 6), or 10 μM atropine (6 of 6). Following a 3 h incubation in the presence of 1 μM ACh and a 30 min wash, during which the tone of the preparation returned to its resting level, the addition of 10 μM iprindole induced tone in 2 of 8 preparations. These two tissues were discarded.

To ensure that no effects on tone were involved, tissues were incubated in the presence of iprindole for 3 h (except after incubation with ACh) before a dose-response curve to ACh was initiated. Iprindole (1 and 10 μM) had no effect on the maximal response to ACh (expressed as g) or on the slopes of the dose-response curves. Submaximal responses were unaltered and potentiated by iprindole at 1 and 10 μM, respectively (Table 1, Fig. 2). This ability of 10 μM iprindole to potentiate responses to ACh was also observed in the presence of desipramine, phentolamine, prazosin, yohimbine, neostigmine, ouabain or 10 μM atropine (Table 1). The pD_2 values for ACh were decreased following incubation of the muscles with 1 mM ACh for 3 h, suggesting that the tissue had been partially desensitized to ACh. Under this condition the ability of iprindole to potentiate responses to ACh was retained (Table 1).

Cocaine (10 μM) induces tone in some anococcygeus muscles which lasts up to 1 h. Subsequently, cocaine potentiates responses to ACh (Doggrell & Waldron 1982). Following a 3 h equilibration, in the presence of 10 μM cocaine, iprindole at 10 μM had no effect on responses to ACh (Table 1). Conversely, in the presence of iprindole, cocaine had no effect on responses to ACh (Table 1).

The rat anococcygeus muscle has postjunctional α-adrenoceptors and cholinergic muscarinic receptors (Gillespie 1972, 1980). The release of (−)NA which acts at α-adrenoceptors, underlies the induction of tone and the potentiation of contractile responses to ACh in the rat anococcygeus by guanethidine and labetalol, as both of these effects were abolished by preincubating the tissue with 6-hydroxydopamine or by the addition of phentolamine (Doggrell & Paton 1978). However it seems unlikely that a release of (−)-NA contributes to either the contractions or the potentiation of responses to ACh with iprindole as these effects were maintained in the presence of antagonists for α_1- and/or α_2-adrenoceptors. The iprindole-induced contractions are also not mediated by muscarinic receptors as they were atropine-insensitive. Thus the iprindole contractions are either mediated by a receptor other than an α-adrenoceptor or muscarinic receptor or are non-receptor (directly) mediated.

The second action of iprindole (10 μM) observed in this study was to increase sensitivity to ACh. As the sensitizing action occurred after the iprindole-induced contractions had stopped, it seems unlikely that the contractions had any role in the action. Several other mechanisms have also been eliminated as underlying the action. The contractile responses to ACh in the rat anococcygeus are potentiated by anticholinesterases, e.g. neostigmine (Doggrell 1981b). Iprindole is not an anticholinesterase as the potentiating effect on responses to ACh was maintained in the presence of neostigmine. Desipramine is a potent inhibitor of the neuronal accumulation of (−)-[^3H]NA in the rat anococcygeus (Doggrell & Woodruff 1977). Iprindole (10 μM) may inhibit the neuronal uptake of NA but it seems unlikely that this action causes potentiation of responses to ACh as the effect of iprindole persisted in the presence of desipramine. Effects on Na+/K+ ATP-ase are also not implicated as iprindole potentiated responses to ACh in the presence of ouabain. It was also observed that the ability of iprindole to potentiate responses to ACh was retained after the tissue had been desensitized to ACh or in the presence of an antimuscarinic agent i.e. atropine.

Recently we have demonstrated that cocaine increases the sensitivity to ACh by a postjunctional mechanism (Doggrell & Waldron 1982). It seems likely that iprindole has a similar postjunctional action to cocaine in this tissue as the potentiating effect of

iprindole on responses to ACh was abolished in the presence of cocaine and vice versa.

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REFERENCES

Doggrell, S. A. (1981a) J. Pharm. Pharmacol.: 33, 796–797
Doggrell, S. A. (1981b) Br. J. Pharmacol. 73: 829–835
Doggrell, S. A., Paton, D. M. (1978) J. Pharm. Pharmacol. 30: 453–454
Doggrell, S. A., Waldron, J. B. (1982) Ibid 34: 408–410
Doggrell, S. A., Woodruff, G. N. (1977) Br. J. Pharmacol. 59: 403–409
Gillespie, J. S. (1972) Ibid. 45· 404–416
Gillespie, J. S. (1980) TIPS 1: 453–457
Nash, C. W., Gillespie, J. S., Robertson, E. N. (1974) Can. J. Physiol. Pharmacol. 52: 430–440

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Increased ^{45}Ca-efflux from smooth muscle microsomes by a rise in an extramicrosomal Ca ion concentration, and the effect of thymol

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It is well accepted that myoplasmic Ca ion concentration regulates muscle contractility. In skeletal muscle, in the contraction process, Ca ion is supplied exclusively from an intracellular Ca source in the sarcoplasmic reticulum, whereas, in smooth muscle, both intracellular and extracellular Ca sources have been proposed to contribute to contraction (Bolton 1979). At present, it is unclear where the intracellular Ca source (Ca store) is located in smooth muscle cells and how Ca ions are released from this store. Using guinea-pig mesentric artery chemically skinned with saponin, which makes the cell membrane, but not the sarcoplasmic reticulum, selectively permeable to solute molecules (Endo 1977), Itoh et al (1981) recently demonstrated the presence of Ca-induced release of Ca preloaded by ATP-dependent processes, and that its nature appears to resemble that observed in skeletal muscle. We now report that increased efflux of ^{45}Ca preloaded in microsomes from guinea-pig taenia caecum is caused by a rise in extramicrosomal Ca ion concentration. Moreover, to assess this phenomenon pharmacologically, the effect of thymol on Ca-induced stimulation of ^{45}Ca-efflux was also examined.

Methods and results

Taeniae caeci from guinea-pigs of either sex (300–500 g) were minced and disrupted in a buffered sucrose solution (0·3 M sucrose, 1 mM dithiothreitol, 0·1 mM phenylmethylsulphonyl fluoride, 10 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethane-sulphonic acid, pH 7·5) in a Potter-type homogenizer by 6-strokes, followed by 3-bursts of 5 s with a Polytron PT-10 at setting no. 7. Large particles and mitochondria were removed by centrifugation, first at 2500 *g* for 10 min and then 15 000*g* for 20 min. The supernatant was centrifuged at 100 000*g* for 1 h. The resulting pellet was subjected to treatment with 0·6 M KCl for 2·5 h and resedimented. Microsomal concentration was determined by the method of Lowry et al (1951), using bovine serum albumin as a standard. ^{45}Ca-efflux was carried out as follows. At first, microsomes were preloaded with ^{45}Ca in 0·5 ml of a solution which contained: 30 mM Tris/maleate (pH 7·0), 100 mM KCl, 5 mM ATP, 5 mM $MgCl_2$, 5 mM NaN_3, 10 mM creatine phosphate, 100 μg ml^{-1} creatine kinase, 4 μCi ml^{-1} ^{45}Ca(16·8 Ci mg^{-1}), 0·1 mM $CaCl_2$, 0·045 mM ethyleneglycol-bis-(β-aminoethylether)-*NN'*-tetra-acetic acid (EGTA), 0·5 mg ml^{-1} microsomes. The concentration of free Ca ion in the reaction medium was calculated to be 10 μM from the apparent affinity constant ($1·26 \times 10^6$ M^{-1}) between EGTA and Ca at pH 7·0 (Ogawa 1968). After preloading microsomes with ^{45}Ca by incubation at 32 °C for 10 min, ^{45}Ca-efflux was carried out by diluting the uptake medium 10-fold with a buffer to give final concentrations of 30 mM Tris/maleate (pH 7·0), 100 mM KCl, 5 mM ATP, 5 mM $MgCl_2$, 5 mM NaN_3, 10 mM creatine phosphate, 100 μg ml^{-1} creatine kinase, 2 mM $CaCl_2$, and 2·13 (or 7·29) mM EGTA (pCa = 5 (or 6·5)). Since, by this method, $^{45}Ca/^{40}Ca$ was decreased to 1/200 after dilution, reuptake of ^{45}Ca during efflux was almost negligible.

^{45}Ca content in microsomes was trapped on a Millipore filter (HAWP) by a vacuum filtration, and counted as described by Takayanagi et al (1979). When necessary, thymol in ethanol (1:1 w/v) was added in a diluting buffer, where the final concentrations of thymol and ethanol were 0·5 mM and 0·0075%, respectively. ^{45}Ca-efflux was not influenced by this concentration of ethanol as vehicle (data not shown).

Microsomes obtained as described above took up Ca

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