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Evidence against VIP or substance P being the transmitter in non-cholinergic excitatory nerves supplying the guinea-pig bladder

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In the guinea-pig bladder, contractile responses to substance P (0·3 μM) and VIP (3 μM) were unaffected by P_2-purinoceptor desensitization with α,β-methylene ATP (3×10^{-6} M), while the responses to stimulation of the non-cholinergic excitatory nerves (4–16 Hz) were abolished. The evidence presented suggests that ATP or a related purine nucleotide, and not VIP or substance P, is responsible for the non-cholinergic excitatory component of the nerve-mediated response.

The response of the mammalian urinary bladder to parasympathetic nerve stimulation is only partially inhibited by atropine (Langley & Anderson 1895). It has been proposed that the residual response is caused by ATP or a related nucleotide released from non-cholinergic excitatory nerves supplying the bladder (Burnstock et al 1972, 1978). ATP closely mimics the nerve-mediated response of the isolated detrusor muscle from the guinea-pig (Burnstock et al 1972, 1978). The presence of vasoactive intestinal polypeptide (VIP)- and of substance P-containing neurons has been demonstrated by immunohistochemical techniques in the guinea-pig urinary bladder (Hökfelt et al 1978). Both these peptides cause contraction of the guinea-pig urinary bladder (Johns 1979, 1981) and have been proposed as peripheral neurotransmitters in other tissues (Franco et al 1979; Fahrenkrug 1979).

Kasakov & Burnstock (1983) showed that the slowly-degradable ATP analogue α.β-methylene ATP desensitized P_2-purinoceptors and abolished the responses of the guinea-pig urinary bladder to non-cholinergic excitatory nerve stimulation. In this study, the effect of α.β-methylene ATP desensitization on the nerve-mediated response has again been examined, and a comparison made with contractions elicited by substance P and VIP.

Materials and methods

Guinea-pigs of either sex (250–400 g) were stunned and exsanguinated and the abdomen opened. Mucosa-free strips of the detrusor of the bladder were prepared by the method of Ambache & Zar (1970). The preparations were suspended in 2 ml and 10 ml organ baths in modified Krebs solution of the following ionic composition (mM); NaCl 133, KCl 4·7, NaH_2PO_4 1·3, $NaHCO_3$ 16·3, $MgSO_4$ 0·6, $CaCl_2$ 2·5 and glucose 7·7 containing guanethidine (3·4 μM). The Krebs solution was maintained at 36·5 ± 0·5 °C and bubbled with a 95% O_2, 5% CO_2 gas mixture. The preparations were placed initially under 0·5 g resting tension and allowed to equilibrate for 40–60 min.

The mechanical activity was recorded isometrically with a Dynamometer UF1 force transducer and displayed on a Grass polygraph. Electrical field stimulation was achieved by passing square-wave pulses (4 Hz, 0·3 ms duration and supramaximal voltage) to platinum ring electrodes (separated by 10 mm) from a Grass SD9 stimulator. The duration of a period of electrical stimulation was sufficient to allow the neurogenic excitatory responses to decay to one third of the maximal amplitude attained (usually 7–10 s).

Drugs. Atropine sulphate, α,β-methylene-adenosine 5'-triphosphate (α,β-MeATP), substance P, vasoactive intestinal polypeptide (VIP) were all obtained from Sigma. Guanethidine monosulphate was obtained from CIBA.

Statistics. The mean and standard error of the mean (s.e.m.) for the groups of experiments were calculated. The means were compared using the paired *t*-test. A probability of $P < 0.05$ was considered to be significant.

Results

Detrusor strips of guinea-pig urinary bladder responded to electrical field stimulation with a rapid twitch-like response (Fig. 1a). P_2-purinoceptor desensitization was produced by successive additions of α,β-MeATP (3×10^{-6} M) at 2 min intervals. Desensitization was usually complete after 3 applications of the drug (Fig. 1a, b). After desensitization with α,β-MeATP, the nerve response was reduced (Fig. 1a, c). Lower frequencies were inhibited more than higher frequencies by α,β-MeATP desensitization (Fig. 1c). The addition of atropine (1 μM) to the Krebs solution reduced the magnitude of the phasic contractile component of the nerve response (Fig. 1b, c). Higher frequencies of field stimulation were antagonized to a greater extent than lower frequencies (Fig. 1c). Desensitization with α,β-MeATP in the presence of atropine caused abolition of the nerve response.

After desensitization of the P_2-purinoceptor with α,β-MeATP, the responses to substance P (0·3 μM) (n = 8) and VIP (3 μM) (n = 6) were not significantly changed in appearance or magnitude (Fig. 2a, b).

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Discussion

These results demonstrate that in the presence of atropine, nerve-mediated contractions were abolished after P_2-purinoceptor desensitization with α,β-MeATP, whereas the excitatory responses to substance P and VIP were unaffected. Therefore, it seems unlikely that VIP or substance P mediate the non-cholinergic excitation of the guinea-pig bladder. This is consistent with the results of Leander et al (1981) who found that the non-cholinergic excitatory response was unaffected by substance P antagonists. Recently, MacKenzie & Burnstock (1984) in a study of the timecourse and sensitivity of peptides on the detrusor muscle, also argued against the involvement of both VIP and substance P in the non-cholinergic contractile response. In contrast, Hourani (1984) showed that following desensitization of the P_2-purinoceptor with enantiomers of adenylyl 5'-(β,γ-methylene)-diphosphonate (AMP-PCP), the responses to substance P were inhibited. However, it may be that AMP-PCP is less specific than α,β-MeATP in causing desensitization of the P_2-purinoceptor.

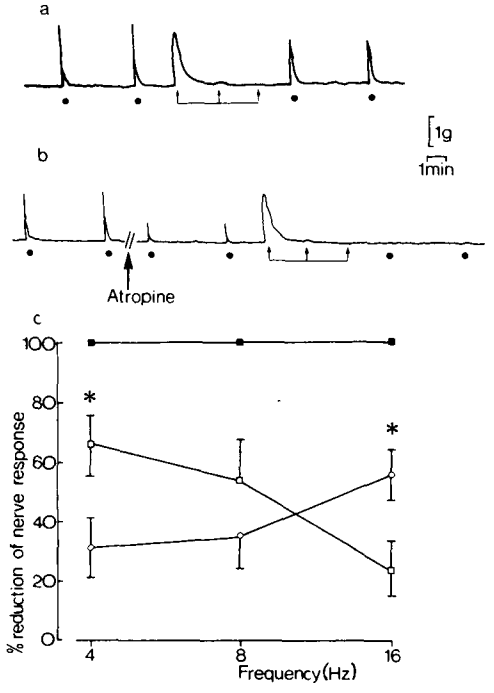

FIG. 1. (a) The effect of α,β-methylene ATP desensitization on responses to nerve stimulation (●) (8 Hz, 0·3 ms, supramaximal voltage for 10 s). α,β-Methylene ATP was added at the points indicated by the arrows. (b) The effect of atropine (1 μM) on the response to nerve stimulation (●) and the subsequent effect of α,β-methylene ATP desensitisation on the non-cholinergic nerve response (●) in the presence of atropine. The break in the trace (//) represents a 30 min incubation period with atropine. (c) The effect of atropine (1 μM) (○, n = 8), α,β-methylene ATP desensitization (□, n = 8) and atropine + α,β-methylene ATP desensitization (■, n = 8) on the responses to nerve stimulation at the given frequencies (0·3 ms, supramaximal voltage for 10 s). Guanethidine (3·4 μM) was present throughout. *$P < 0·05$.

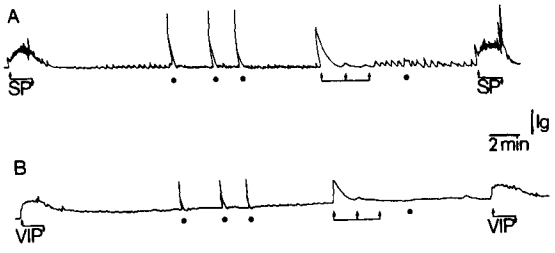

FIG. 2. (a) The effect of α,β-methylene ATP desensitization on the response to substance P (SP) (0·3 μM) and nerve stimulation (●) (8 Hz, 0·3 ms pulse width supramaximal voltage). α,β-Methylene ATP was added at the points indicated by the arrows. (b) The effect of α,β-methylene ATP desensitization on the response to VIP (3 μM) and nerve stimulation (●) (8 Hz, 0·3 ms pulse width, supramaximal voltage).

The results of the present study also show that the nerve-mediated response is more sensitive to α,β-MeATP desensitization at low frequencies and more sensitive to antagonism by atropine at higher frequencies. MacKenzie & Burnstock (1984) also found that the atropine-sensitive portion of the neurogenic response contributes mostly to the initial rapid excitation at frequencies of 8 Hz and over. The results of the present study support the view that ATP, but not substance P or VIP, is responsible for the non-cholinergic excitatory response. It is not yet known whether ATP is released together with acetylcholine as a co-transmitter from postganglionic neurons supplying the guinea-pig bladder as suggested from previous ultrastructural (Hoyes et al 1975) and pharmacological studies (MacKenzie et al 1982), or from separate 'purinergic' nerves as the principal transmitter.

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The putative 5-HT_1 receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor

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The putative central 5-HT receptor agonist, 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole succinate (RU 24969), was found to be a potent inhibitor of the continuous K^+ evoked efflux of [^{3}H]5-HT from superfused rat frontal cortex slices (pD_2 7·45). The effects of RU 24969 were attenuated by the putative 5-HT autoreceptor antagonists, methiothepin, quipazine and (−)-propranolol but not by the α_2-adrenoceptor antagonist, idazoxan. It is concluded that RU 24969 inhibits K^+ evoked efflux of [^{3}H]5-HT from rat frontal cortex slices by stimulation of the 5-HT autoreceptor. Moreover, since RU 24969 potently displaced ligand binding to the 5-HT_1 and 5-HT_{1B} recognition sites but was only weakly active at the 5-HT_2 receptor, the results lend support to the claim for a pharmacological resemblance between the 5-HT autoreceptor and the 5-HT_1 recognition site and in particular the low affinity 5-HT_{1B} subtype.

5-Methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole succinate (RU 24969) is a novel piperidinyl indole which decreases 5-hydroxyindoleacetic acid (5-HIAA) levels in rat brain after peripheral administration (Euvrard & Boissier 1980). The effect is consistent with agonist activity at central 5-hydroxytryptamine (5-HT) receptors, an interpretation strengthened by the observation that RU 24969 is a potent and selective displacer of [^{3}H]5-HT receptor binding (Hunt & Oberlander 1981).

The reduction in brain 5-HIAA concentrations could reflect either an inhibition of 5-HT release following post-synaptic receptor stimulation and a neuronal feedback loop, or a direct interaction of RU 24969 with the 5-HT autoreceptor. This latter possibility has been investigated using inhibition of K^+ evoked efflux of [^{3}H]5-HT from rat brain slices as an index of autoreceptor activity. In addition, the potency of RU 24969 to displace ligand binding to the various central 5-HT receptor subtypes has been measured by receptor binding techniques. The results indicate that RU 24969 is a potent 5-HT autoreceptor agonist with marked activity at the 5-HT_{1B} subtype of the 5-HT_1 recognition site.

Materials and methods

Superfusion studies. The effect of drugs on K^+ evoked efflux of [^{3}H]5-HT was studied using the continuous stimulation technique of Frankhuyzen & Mulder (1982) adapted for rat frontal cortex slices as described by Middlemiss (1984a, b). Briefly, the frontal cortices from two male Sprague-Dawley rats (200–250 g) were chopped in two directions at 250 μm intervals and incubated with 0·1 μM [^{3}H]5-HT in the presence of 10 μM pargyline in Krebs-Henseleit buffer oxygenated with 95% O_2–5% CO_2 at 37 °C. After 15 min the slices were washed and 50 μl aliquots (about 20 mg tissue) were transferred to each chamber of a superfusion apparatus and superfused at 0·38 ml min^{-1} with Krebs buffer. The superfusion solution contained 3·2 μM paroxetine to prevent the neuronal uptake of [^{3}H]5-HT. After 30 min of superfusion, some of the slices were exposed to Krebs buffer containing elevated K^+ ions (25 mM) for 16 min (i.e. t = 30–46 min) and then 18 successive 4 min fractions of the superfusate (continuously superfused with either Krebs or 25 mM K^+ Krebs) were collected (i.e. t = 46–118 min). At the end of the experiment the radioactivity in the slices and in each fraction was determined by liquid scintillation counting.

Cumulative dose-responses to RU 24969 were constructed using four concentrations of agonist (30 nM to 1 μM) with a 16 min interval between each increase in agonist concentration. Experiments with antagonists were carried out by adding drug to the superfusion media (basal and elevated K^+) at t = 30 min, drug was then present throughout the superfusion. Since under these conditions RU 24969, at concentrations up to 1 μM, did not affect the baseline efflux of tritium, basal efflux was taken as the release in the absence of added agonist with or without added antagonist as appropriate. The results were calculated as described by Frankhuyzen & Mulder (1982). Determination of apparent pA_2 values was performed as described by