Mechanisms Involved in Electrically-induced Responses of Rat Seminal Vesicles

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Abstract

Contractile responses of rat isolated seminal vesicle were elicited by electrical field stimulation (EFS, 10 Hz, 1 ms, 40 V for 5 s), noradrenaline (1×10^{-5} M) and carbachol (1×10^{-5} M).

Guanethidine (2×10^{-5}–5×10^{-4} M) progressively reduced the contraction induced by EFS and carbachol to 24 ± 2 and $10 \pm 2\%$, respectively, at the highest concentration (n = 6), while potentiating noradrenaline contraction to a maximum of $154 \pm 14\%$ at 2×10^{-5} M (n = 6). Prazosin (1×10^{-6} M) and atropine ($2 \cdot 5 \times 10^{-7}$ M) completely abolished the response to the corresponding agonist and each reduced the response to EFS to 64 ± 8 and $61 \pm 3\%$, respectively (n = 6). In the presence of both atropine and prazosin a small contraction to EFS remained ($14 \pm 4\%$, n = 6), which is unlikely to be due to ATP, since exogenous ATP did not induce a contractile response and had an inhibitory effect on EFS-induced responses. Clonidine ($1 \cdot 25 \times 10^{-5}$ M) completely blocked responses to noradrenaline and reduced the response to EFS to $68 \pm 7\%$ (n = 6). However, when both the adrenergic and cholinergic components of EFS were blocked by prazosin and atropine, clonidine potentiated the remaining response to EFS ($323 \pm 82\%$, n = 4). Yohimbine (1×10^{-5} M) blocked the response to noradrenaline and reduced the response to EFS to $37 \pm 5\%$ (n = 6) while the carbachol response was unaffected. Both cholinergic and noradrenergic components contribute to the response to EFS but there appears to be little involvement of presynaptic α_2-adrenoceptors in regulating neurotransmitter release.

The actions of clonidine and yohimbine are compatible with the suggestion that their effects are due to postsynaptic α_1-adrenoceptor blockade.

The smooth muscle layers of rat seminal vesicle are densely innervated by sympathetic fibres from the hypogastric nerve (Wakade & Kirpekar 1971) and parasympathetic cholinergic fibres. In addition, the presence of other types of neurons has been demonstrated, whose contents include peptides such as vasoactive intestinal polypeptide, neuropeptide Y, gastrin-releasing peptide, substance P and enkephalin (Alm et al 1980; Vaalasti et al 1980; Stjernquist et al 1983, 1987; Moss et al 1987; Yuri 1990; Lange & Unger 1990). However, there is little information about the functional significance of these peptides, although some authors point to the possibility of co-transmission between noradrenaline and neuropeptide Y, or ATP (Nakanishi & Takeda 1973; Meldrum & Burnstock 1985; Stjernquist et al 1987; Wali & Greenidge 1989). Of the post-synaptic receptors in rat vesicular smooth muscle, adrenoceptors are mainly of the α_1-subtype (Gokhale & Sharif 1983; Adenekan 1989; Sharif et al 1990), and the cholinoceptors are muscarinic. The nature of any pre-synaptic receptors is unknown. The present study investigates the effects of some drugs on contractions of rat seminal vesicle induced by electrical field stimulation (EFS), noradrenaline and carbachol.

Materials and Methods

Male Wistar rats, 200–300 g, were killed by cervical dislocation or a blow on the head, followed by exsanguination. The

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seminal vesicles were removed, placed in McEwen's solution (composition (mM): NaCl, 130; KCl, 5·6; NaH_2PO_4, 1·2; $NaHCO_3$, 25; $CaCl_2$, 2·2; glucose, 11; and sucrose, 13) at room temperature (21°C), trimmed and separated before the contents were gently squeezed out.

Tissues were suspended in McEwen's solution at 37°C and bubbled with 5% CO_2 in O_2. From a resting tension of 0·5 g, isometric contractions, elicited by EFS or drugs, were recorded using a UF1 transducer and displayed on a Devices MX2 pen recorder.

EFS was delivered (Stimulator, Pharmacology Department Workshop, Leeds) through parallel platinum wire electrodes (2 cm long, 0·5 cm apart) in trains (5 s) of rectangular pulses (width 1 ms, frequency 10 Hz, 40 V output unless stated otherwise) at 1-min intervals. Drugs were added to the organ bath in volumes usually not exceeding 5% of bath volume. The spasmogens, noradrenaline or carbachol, were added at 4-min intervals with 30 s contact time, each at 1×10^{-5} M. With these stimuli the smooth muscle contractions were 60–80% of the maximum response to the appropriate agonist.

The effects of drugs on induced contractions were studied using a single-dose regime, each concentration remaining in contact for 7–10 min before washing out. The tissue was then exposed to the next drug concentration for 7–10 min, and so on. Some drugs were studied by cumulative addition also on a 7–10-min cycle. In any one tissue, the effects of no more than two drugs were studied on drug- or EFS-induced contractions. When appropriate, experiments were conducted in parallel with time-matched controls using tissue from the same animal adding vehicle in lieu of drug.

Table 1. Frequency-response relationship of rat seminal vesicle to electrical field stimulation (EFS). The stimulating parameters were: pulse width 1 ms, output 40 V, train duration 5 s applied every 1 min. Data are shown as mean ± s.e.m. (n = 6).

Frequency (Hz)	EFS (g tension)
2·5	0·05 ± 0·01
5·0	0·12 ± 0·01
10·0	0·24 ± 0·02
20·0	0·74 ± 0·17

Measurements and statistical analysis

Contractions were measured as maximum changes in tension from pre-drug/stimulation baseline within the stimulating period and expressed in g or as percentages of initial values. Mean and s.e. values were calculated for each group of results and inter-group comparisons were made with one-way analysis of variance and further compared with their time-matched controls using unpaired Student's t-test. Differences were considered statistically significant for $P < 0.05$.

Drugs and solutions

The following drugs were used: (−)-noradrenaline bitartrate, carbachol chloride, clonidine hydrochloride, yohimbine hydrochloride, prazosin hydrochloride, atropine sulphate, dibucaine hydrochloride, guanethidine monosulphate and adenosine 5-triphosphate disodium salt (ATP). Prazosin and yohimbine were initially dissolved in 70% alcohol, dilution being made in McEwen's solution. Other drugs were initially dissolved in distilled water and further diluted in either distilled water or McEwen's solution as appropriate. All drugs were purchased from Sigma.

Results

Rat seminal vesicle contracted rapidly to EFS (10 Hz), reaching a peak within 5 s which was then followed by a second peak of variable magnitude; rapid relaxation followed cessation of EFS. The frequency–response relationship is shown in Table 1. The monophasic responses to noradrenaline and carbachol developed more slowly, reached their peaks after 20–25 s contact, after which the tissue usually began to relax slightly before the drug was washed out.

Guanethidine (2 × 10^{-5}–5 × 10^{-4} M) caused a small reduction in baseline and progressively attenuated the contractions induced by EFS and carbachol to 24 ± 2 and 10 ± 2%, respectively (n = 6) at its highest concentration, while potentiating noradrenaline contractions to 154 ± 14% (n = 6) at 2 × 10^{-5} M. Removal of guanethidine by washing rapidly restored the carbachol response, but EFS remained blocked.

Atropine (2·5 × 10^{-7} M) and prazosin (1 × 10^{-6} M) at

concentrations which totally blocked carbachol and noradrenaline, respectively, without affecting the alternative spasmogen, reduced the response to EFS to 61 ± 3 and 64 ± 8%, respectively (n = 6). Administered together, atropine and prazosin at these concentrations were additive, 14 ± 4% (n = 6) of the original response to EFS remaining in their combined presence.

ATP (up to 5 × 10^{-3} M) had no spasmogenic effects on rat seminal vesicle, indeed a small relaxation of resting basal tension was often seen. EFS-induced contractions were progressively reduced by ATP in concentrations from 1 × 10^{-5} to 1 × 10^{-3} M. At 1 × 10^{-3} M, ATP reduced responses to EFS to 50 ± 5% (n = 6) and responses to noradrenaline to 50 ± 6% (n = 6) but did not affect responses to carbachol. These effects were rapidly reversed by washing. In two experiments, the effects of a higher concentration of ATP (5 × 10^{-3} M) were assessed on EFS, almost total inhibition of the response being achieved (Fig. 1).
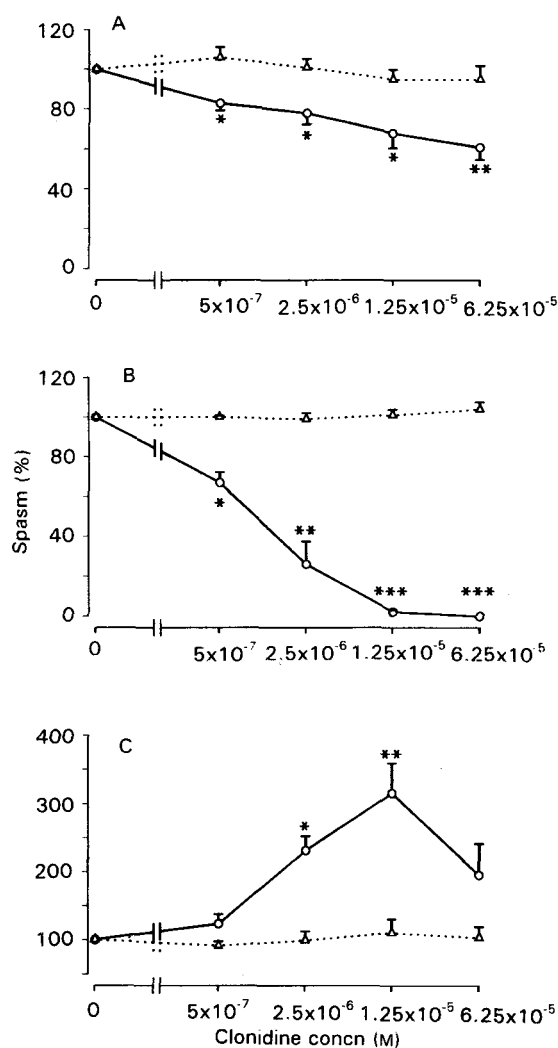
FIG. 2. Effect of clonidine on tension (% of contraction) developed in rat isolated seminal vesicle to: A, electrical field stimulation (EFS, 10 Hz); B, noradrenaline (1 × 10^{-5} M); and C, carbachol (1 × 10^{-5} M). Dotted lines join mean values for time-matched control tissues treated with vehicle in equivalent volume. Results are given as means and vertical bars indicate s.e.m. (n = 6). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (t-test).
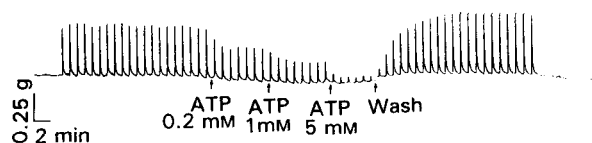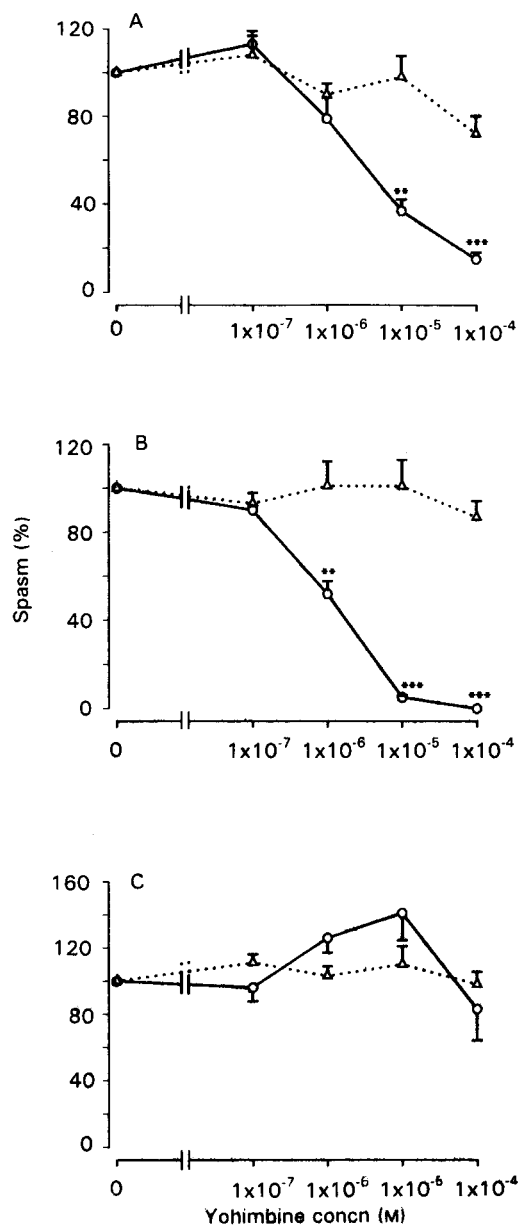
FIG. 1. The effect of cumulative addition of ATP on tension development in rat isolated seminal vesicle to electrical field stimulation (frequency 10 Hz pulse width 1 ms, train duration 5 s, and 40 V output).

prazosin (1×10^{-7} M, which abolished the response to noradrenaline), clonidine no longer blocked the reduced response to EFS; indeed a slight potentiation occurred. In the presence of both atropine and prazosin (5×10^{-8} and 1×10^{-7} M, respectively), clonidine caused a clear potentiation of the remaining responses to EFS ($323 \pm 82\%$ at $6 \cdot 25 \times 10^{-5}$ M, n = 4). In the absence of other drugs, yohimbine reduced the response to noradrenaline in a concentration-dependent manner, complete inhibition occurring with 1×10^{-5} M, at which concentration EFS was reduced to $37 \pm 5\%$ (n = 6), but carbachol was slightly potentiated ($141 \pm 16\%$; n = 6) (Fig. 3).

Discussion

The adrenergic-neuron blocking agent guanethidine augmented responses to noradrenaline (presumably a consequence of blockade of the uptake-1 process (Maxwell et al 1962)) and produced incomplete inhibition of EFS suggesting a contribution to the response from non-noradrenergic neurons. This is supported by the fact that both atropine and prazosin, added separately, caused partial blockade. The small component of contraction remaining in the presence of both atropine and prazosin is most likely due to the release of additional spasmogens from neurons, weight being given to this conclusion by the report from Fedan et al (1977), that atropine and another α-adrenoceptor antagonist, phentolamine, produced similar partial blockade of rat vesicular contractions to EFS, the remaining component of contractions being blocked by tetrodotoxin.

The possibility that ATP may contribute to the contraction induced by neuronal stimulation in rat seminal vesicle was suggested by Wali & Greenidge (1989). However, our findings suggest that this is unlikely. First, exogenous ATP in any concentration used had no spasmogenic action on rat seminal vesicle. Secondly, ATP caused concentration-dependent inhibition of both EFS and noradrenaline, without affecting the carbachol responses. The mechanism causing these effects is unclear.

Unlike the situation in many noradrenergic neurons (Berlan et al 1992), our findings show that presynaptic α_2-adrenoceptors do not appear to play a major part in regulating neurotransmitter release from rat seminal vesicle. Although clonidine reduced EFS by up to 40%, it concurrently caused total blockade of exogenous noradrenaline suggesting a non-specific effect. Furthermore, the α_2-adrenoceptor blocker yohimbine did not potentiate EFS, as would be expected if presynaptic α_2-adrenoceptors are substantially modulating evoked transmitter release. Yohimbine did not affect EFS until 1×10^{-5} M was present, at which stage inhibition occurred. The inhibitory effect of each of these drugs on EFS and on noradrenaline may be due to a common mechanism, namely postsynaptic α_1-adrenoceptor blockade. Such a mechanism was suggested by Sharif et al (1990) who performed a detailed study of clonidine on noradrenaline and phenylephrine responses in rat seminal vesicle, concluding that clonidine acted as a competitive antagonist at α_1-adrenoceptors. Inhibition of noradrenaline by yohimbine is suggested to be due to antagonism of post-junctional α_1-adrenoceptors (Adenekan 1989; Sharif et al 1990), since the selectivity of yohimbine between α_1- and

Fig. 3. Effect of yohimbine on tension (% of contraction) developed in rat isolated seminal vesicle to: A, electrical field stimulation (EFS, 10 Hz); B, noradrenaline (1×10^{-5} M); and C, carbachol (1×10^{-5} M). Dotted lines join mean values for time-matched control tissues treated with vehicle in equivalent volume. Results are given as means and vertical bars indicate s.e.m. (n = 6). $**P < 0 \cdot 01$, $***P < 0 \cdot 001$ (t-test).

Clonidine produced a concentration-dependent block of responses to noradrenaline, which was complete at $1 \cdot 25 \times 10^{-5}$ M, whilst reducing the response to EFS to $61 \pm 6\%$ (n = 6) at $6 \cdot 25 \times 10^{-5}$ M; carbachol contractions were enhanced approximately threefold at $1 \cdot 25 \times 10^{-5}$ M (Fig. 2). When experiments were carried out in the presence of atropine (5×10^{-8} M, which blocked the response to carbachol), the inhibitory effect of clonidine on the noradrenaline response was unaffected, but the reduction of EFS response was less than when atropine was absent ($77 \pm 9\%$ at $6 \cdot 25 \times 10^{-5}$ M, n = 6). In the presence of

α_2-adrenoceptors is in the order of tenfold (Kobinger & Pichler 1980; McGrath 1982). The reported absence of α_2-binding sites in this tissue (Shima 1993) is entirely compatible with the effects of clonidine and yohimbine reported above.

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