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MECHANICAL RESPONSES OF RAT ISOLATED UTERINE HORNS TO TRANSMURAL STIMULATION

M. HOLLINGSWORTH

Departments of Pharmacology, Materia Medica and Therapeutics and Child Health, Medical School, University of Manchester, Manchester, M13 9PT

1 Transmural stimulation of rat isolated uterine horns at low pulse width produced contractions. These were antagonized by hyoscine or tetrodotoxin and potentiated by physostigmine.

2 In the presence of hyoscine, and during bradykinin-induced contractions, transmural stimulation produced inhibition. This inhibition was antagonized by guanethidine, propranolol or tetrodotoxin.

3 Hexamethonium or pempidine did not affect responses to transmural stimulation.

4 It is suggested that transmural stimulation is a method of exciting cholinergic motor and noradrenergic inhibitory postganglionic neurones to the rat myometrium.

Introduction

Mechanical responses of uteri from several species to sympathomimetic amines and muscarinic agonists are well characterized. Methods have been described for electrical stimulation of the extrinsic hypogastric nerve supply in the rabbit *in vivo* (Langley & Anderson, 1895; Schofield, 1952) and *in vitro* (Varagić, 1956) and the guinea-pig *in vivo* (Rüsse & Marshall, 1970) with observable mechanical responses. Negligible effects on uterine motility have been seen in the rabbit after electrical stimulation of the pelvic nerve supply *in vivo* (Langley & Anderson, 1895; Schofield, 1952; Bower, 1966). The pelvic and hypogastric nerves are considered to be cholinergic and noradrenergic respectively (Sjoberg, 1967; Marshall, 1970).

In most species both innervations to the myometrium involve 'short' postganglionic neurones arising from ganglia adjacent to the cervix, as section of the extrinsic nerves does not significantly alter myometrial innervation demonstrated histochemically (Sjoberg, 1967; Kanerva, Mustonen & Teräväinen, 1972). Therefore mainly preganglionic neurones would be stimulated by the above methods. Transmural stimulation has been widely used as a method of exciting postganglionic neurones in other tissues. A method of transmural stimulation of isolated uterine horns of the rat producing cholinergic-mediated contractions and noradrenergic-mediated inhibitions is described.

Methods

Virgin Sprague-Dawley rats, 175 to 250 g, were ovariectomized and, 2 to 4 weeks later, injected subcutaneously with 17β-oestradiol (1.8×10^{-8} mol/kg) in arachis oil daily for 7 days and killed on day 8.

The isolated uterine horns were mounted in a tissue bath containing Krebs solution (mM: Na^+ 143.5, K^+ 5.94, Ca^{++} 2.55, Mg^{++} 1.19, Cl^- 128.4, HCO_3^- 25.0, $SO_4^=$ 1.19, $H_2PO_4^-$ 1.19, glucose 11.1) at 32° C under a resting tension of 0.5 gram. Tension changes were recorded isometrically. Isolated uterine horns under these conditions show minimal spontaneous mechanical activity (Hollingsworth, 1974).

A Grass S8 stimulator was used to stimulate the tissue transmurally via a pair of parallel stainless steel wire electrodes, 0.5 cm apart. The intraluminal electrode was positive. Pulses lasting 0.5 ms and of supra-maximal voltage (40 V) were applied.

Contractile responses to transmural stimulation

After obtaining constant contractions to acetylcholine (1×10^{-6} mol/l), an ascending frequency-effect curve was determined by stimulating transmurally, using 10 s trains applied every 100 seconds. Responses were obtained to acetylcholine and other drugs, applied for a

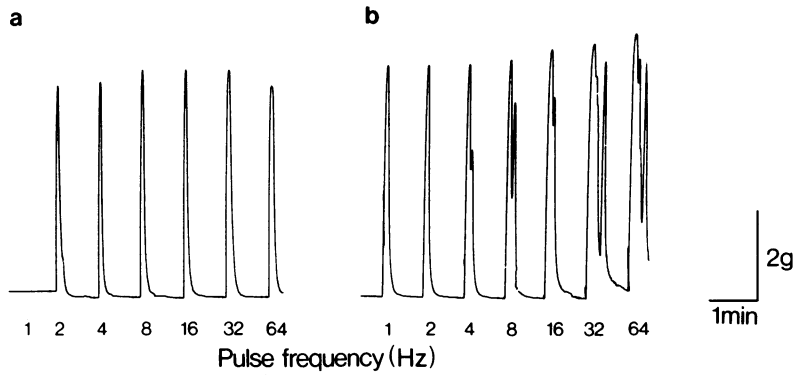

Figure 1 (a) Contractions of an isolated uterine horn of the rat produced by transmural stimulation in Krebs solution and (b) in the presence of physostigmine (1×10^{-5} mol/l). Stimulation parameters: 0.5 ms pulses; 40 V, supra-maximal voltage; 10 s trains applied every 100 seconds.

contact time of 30 s (or 45 s for bradykinin) every 3 minutes.

Inhibitory responses to transmural stimulation

For these experiments the Krebs solution contained hyoscine (1×10^{-8} mol/l). Repeated sustained contractions of the rat uterine horns were achieved by adding a standard concentration of bradykinin (either 9.4×10^{-9} or 4.7×10^{-8} mol/l) for 2 min every 5 minutes. This concentration of bradykinin produced a contraction which was 90% of the maximal contraction to acetylcholine. One min after the addition of the bradykinin, the tissue was stimulated transmurally at a pulse frequency of 2 Hz for 10 seconds. A frequency-effect curve was obtained by repeating the bradykinin standard and using higher frequencies. Concentration-effect curves to noradrenaline and aminophylline were determined in a similar manner by adding them 40 s after the addition of bradykinin.

Modification of responses to transmural stimulation and drugs

Control responses were obtained to transmural stimulation and drugs. Responses were then re-examined, after 30 min incubation, either in Krebs solution (controls) or in Krebs solution containing the modifying drug (test).

Contractile responses to transmural stimulation and drugs were measured as pen deflections (mm). Inhibitory responses were measured as percentage inhibition of bradykinin contractions. These were converted to percentages of the maximal control responses either to acetylcholine (contraction experiments) or to noradrenaline (inhibition experiments). Potencies of drugs are expressed as the mean negative log EC_{50} ± s.e. mean. Horizontal shifts of the EC_{50}'s were used to determine the extent of antagonism or potentiation.

The Mann-Whitney U-test or the Wilcoxon matched pair signed rank test (Siegel, 1956) were used to test the significance of differences.

Drugs

The following drugs were used: acetylcholine chloride (Lematte et Boinot); aminophylline (United Chemists Association); bradykinin (Sandoz); guanethidine sulphate (CIBA); hexamethonium bromide (May & Baker); 5-hydroxytryptamine creatine sulphate (Koch-Light); hyoscine hydrobromide (Koch-Light); nicotine hydrogen tartrate (BDH); 17β-oestradiol (BDH); pempidine tartrate (May & Baker); physostigmine salicylate (BDH) ç propranolol hydrochloride (I.C.I.) and tetrodotoxin (Sankyo).

Results

Contractile responses to transmural stimulation

Transmural stimulation of the isolated uterine horns produced single phasic contractions with a latency and duration of approximately 5 s and 30 s respectively (Figure 1). Contractions were more consistently obtained with a pulse duration of 0.5 ms than with one of 0.2 ms. The lowest frequency at which the virtually all-or-none contractions were obtained varied from 1 to 4 Hz in different preparations, accounting for the

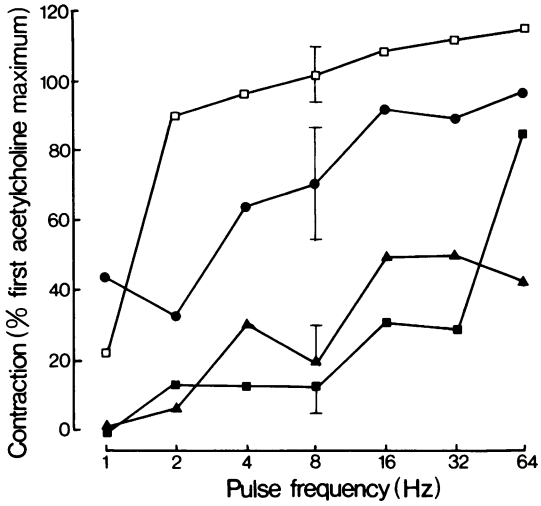

Figure 2 Contractile responses of isolated uterine horns of rat to transmural stimulation in controls (●; $n = 10$) or in the presence of physostigmine (□; 1×10^{-5} mol/l; $n = 8$), hyoscine (▲; 1×10^{-8} mol/l; $n = 9$) or tetrodotoxin (■; 3.1×10^{-7} mol/l; $n = 7$). Stimulation parameters: 0.5 ms pulses, 40 V, 10 s trains every 100 seconds. Means are shown and representative s.e.'s indicated. The means for physostigmine at 2 Hz, for hyoscine at 16 and 64 Hz and for tetrodotoxin at 4, 8, 16 and 32 Hz differed ($P < 0.05$) from the corresponding control means.

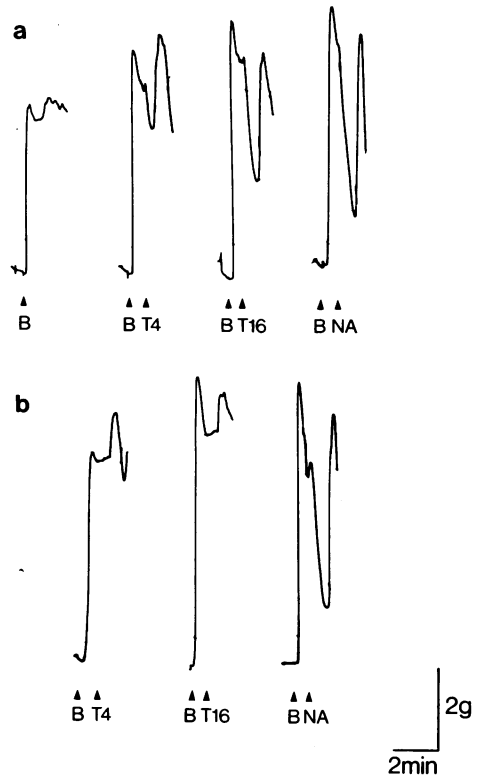

Figure 3 Inhibitory responses of an isolated uterine horn of rat to transmural stimulation (T4 = 4 Hz; T16 = 16 Hz) and to noradrenaline (NA; 5×10^{-7} mol/l), (a) in Krebs solution and (b) in the presence of guanethidine (5×10^{-6} mol/l). Bradykinin (B, 9.4×10^{-9} mol/l) was added for 2 min every 5 min. Stimulation parameters: 0.5 ms pulses; 40 V, supra-maximal voltage; 10 s trains. The Krebs solution contained hyoscine (1×10^{-8} mol/l).

frequency-effect curves in Figure 2. The maximal tension to transmural stimulation was 88% of the maximum response to acetylcholine. At 37°C, when spontaneous contractions were seen, drugs and transmural stimulation induced multi-phasic contractions.

Hyoscine (1×10^{-8} mol/l) and tetrodotoxin (3.1×10^{-7} mol/l) greatly reduced the responses to transmural stimulation ($P < 0.05$) at most frequencies (Figure 2). Physostigmine (1×10^{-5} mol/l) increased both the duration (Figure 1) and the maximal response to transmural stimulation at most frequencies (Figure 2). Repeat control responses to transmural stimulation did not differ from the initial control responses.

The concentration-effect curves with acetylcholine were shifted to the right 31.6-fold by hyoscine ($P < 0.01$) and shifted to the left 2.1-fold by physostigmine ($P < 0.05$). Repeat control concentration-effect curves to acetylcholine, 5-hydroxytryptamine and bradykinin and responses to 5-hydroxytryptamine and bradykinin in the presence of hyoscine did not differ from the initial control responses.

Hexamethonium (1×10^{-5} mol/l) had no effect on contractions to transmural stimulation or acetylcholine ($n = 3$). Hexamethonium (1×10^{-4} mol/l) produced a 50% reduction in maximal reponses to transmural stimulation and to acetylcholine.

Inhibitory responses to transmural stimulation

Before inhibitory responses of the rat isolated uterine horns could be observed tone had to be induced. Among the contractile agents tested (acetylcholine, 5-hydroxytryptamine, oxytocin, angiotensin and bradykinin), only bradykinin induced a sustained tone for more than 1 min (Figure 3). Even with bradykinin, there were small

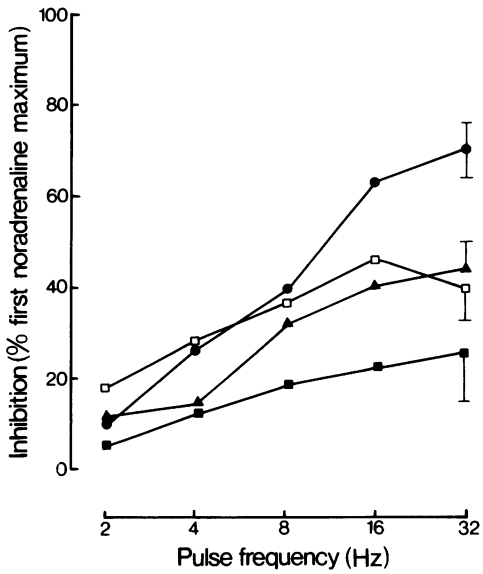

Figure 4 Inhibitory responses of rat isolated uterine horns to transmural stimulation in controls (●; $n = 10$) or in the presence of propranolol (□; 1×10^{-8} mol/l; $n = 8$), guanethidine (▲; 5×10^{-6} mol/l; $n = 10$) or tetrodotoxin (■; 3.1×10^{-7} mol/l; $n = 9$). Transmural stimulation (0.5 ms pulses, 40 V, 10 s trains) was performed during a bradykinin-induced contraction. Results are means and representative s.e.'s are indicated. The means for all test curves differed ($P < 0.05$) from the controls at 16 and 32 Hz.

fluctuations in the sustained tone ($17 \pm 2\%$ in controls, $n = 43$; $8 \pm 3\%$ in repeated controls, $n = 27$; measured as a percentage of the first noradrenaline maximum) which have been called spontaneous inhibitions. There was no marked tachyphylaxis in the contractile response to bradykinin when added to the tissue for 2 min every 5 min over a 3 h period.

Transmural stimulation, during a contraction induced by bradykinin, and in the presence of hyoscine (1×10^{-8} mol/l), induced inhibitions after a latency of 5 to 10 s (Figure 3). The inhibitions tended to be multiphasic. The magnitude of the inhibitions were proportional to pulse frequency up to 32 Hz (Figure 4). The response at this frequency was 71% of the maximum inhibition produced by noradrenaline and a 51% inhibition of the bradykinin contraction. At higher pulse frequencies an increase in tone was often seen preceding the inhibition. Repeat control responses to transmural stimulation did not differ from the initial control responses. Both noradrenaline (2×10^{-8} to 3.1×10^{-4} mol/l; Figure 3) and aminophylline (2.5×10^{-4} to 4×10^{-3} mol/l) produced concentration-related inhibitions of bradykinin induced contractions.

Inhibitory responses to transmural stimulation were greatly reduced by tetrodotoxin (3.1×10^{-7} mol/l, $P < 0.05$), and to a lesser extent by propranolol (1×10^{-8} mol/l, $P < 0.05$) or guanethidine (5×10^{-6} mol/l, $P < 0.05$) (Figures 3 and 4). In two experiments nicotine (2×10^{-8} to 6.25×10^{-5} mol/l) produced small and inconsistent inhibitions of bradykinin-induced contractions. In two further experiments pempidine (1×10^{-5} mol/l) did not modify these responses or the responses to transmural stimulation.

The specificity of the modifying agents was determined by any shifts they produced in the concentration-effect curves to noradrenaline and aminophylline (Table 1). Propranolol

Table 1 Potencies, as mean negative log molar EC_{50}'s, of noradrenaline and aminophylline in inhibiting bradykinin-induced contractions of isolated uterine horns of rat in controls, or after 30 min incubation with a modifying agent

Modifying agent		*Noradrenaline*	*Aminophylline*
None	Control	6.72 ± 0.13 (11)	3.09 ± 0.07 (7)
	Repeat control	6.70 ± 0.19 (11)	3.04 ± 0.12 (6)
Propranolol (1×10^{-8} mol/l)	Control	6.88 ± 0.04 (6)	3.26 ± 0.07 (5)
	Test	6.18 ± 0.16 (8)*	3.40 ± 0.07 (4)
Guanethidine (5×10^{-6} mol/l)	Control	6.79 ± 0.22 (10)	3.30 ± 0.06 (9)
	Test	6.96 ± 0.23 (7)	3.39 ± 0.07 (6)
Tetrodotoxin (3.1×10^{-7} mol/l)	Control	7.00 ± 0.10 (9)	–
	Test	6.80 ± 0.20 (7)	

Results are means with s.e. mean. Number of values in parentheses.
* Significant change of $-\log EC_{50}$ ($P < 0.05$, Wilcoxon matched pair signed rank test).

(1×10^{-8} mol/l) produced a 6-fold shift to the right of the concentration-effect curve to noradrenaline without affecting responses to aminophylline. This concentration of propranolol abolished the spontaneous inhibitions; higher concentrations also reduced bradykinin contractions. Guanethidine and tetrodotoxin did not alter the mean $-\log EC_{50}$'s to noradrenaline or aminophylline.

Discussion

Electrical stimulation at low pulse width has been widely used as a method of eliciting neurotransmitter release in a variety of tissues. The method has been little used with uterine tissues and then only for producing contractile responses. Paterson (1965) has described transmural stimulation of isolated uterine horns of the rat without analysis of the responses. Nakanishi, McLean, Wood & Burnstock (1969) and Nakanishi & Wood (1971) have suggested that contractile responses of isolated strips of human uterus to transmural stimulation had both noradrenergic and cholinergic components, although contractions were little affected by atropine. The contractile and inhibitory responses obtained here to transmural stimulation of rat uterine horns were antagonized by tetrodotoxin, suggesting that they were nerve-mediated.

The contractile responses to transmural stimulation at low pulse width were antagonized by hyoscine and potentiated by physostigmine. This, plus similar results using a perfused uterine horn preparation (Hollingsworth, 1974), where contractions of circular smooth muscle were probably being measured, suggests that both circular and longitudinal smooth muscle receive a cholinergic motor innervation. Histochemical findings support this (Adham & Schenk, 1969; Hervonen, Kanerva & Lietzén, 1973; Hollingsworth, 1974). Electrical stimulation of the pelvic parasympathetic nerves in the rabbit *in vivo* only occasionally produced uterine horn contractions (Schofield, 1952) and did not induce action potentials in uterine nerves (Bower, 1966). The effects seen here in the rat but not in the rabbit might be a species difference or might be related to the oestrogen pre-treatment used in this work which increased acetylcholinesterase staining, typical of cholinergic nerves, suggesting an increased density of innervation (Hervonen *et al.*, 1973; Hollingsworth, unpublished observations).

Physostigmine produced potentiation of contractile responses to both transmural stimulation and exogenous acetylcholine. It is therefore likely that cholinesterases of the rat uterine horns that have been demonstrated biochemically (Foley & McPhillips, 1973) and histochemically, limit the effect of released neurotransmitter.

The method developed here allows the quantitative study of inhibitory responses to transmural stimulation, or exogenous drugs, in uterine horns which do not exhibit an inherent sustained tone. Guanethidine and propranolol antagonize the inhibitory responses to transmural stimulation and this suggests a noradrenergic innervation. Both inhibitory and contractile uterine horn responses to hypogastric nerve stimulation have been described in the rat, depending on the animal's hormonal state (Labate, 1941; Butterworth & Randall, 1970). It is possible that the inhibitory responses to transmural stimulation were due to adrenaline release as rat uteri have a high adrenaline content (Wurtman, Chu & Axelrod, 1963). Collection and assay methods are necessary to prove whether the released transmitter is noradrenaline or adrenaline.

Only a sparse noradrenergic innervation of the rat myometrium has been described histochemically (Hervonen *et al.*, 1973; Hollingsworth, 1974). This is supported by the absence of potentiation of noradrenaline responses by guanethidine here compared to tissues containing a dense noradrenergic innervation, where potentiation is seen. However, the mechanical activity of the rat uterus is readily affected by hypogastric nerve stimulation (Labate, 1941; Butterworth & Randall, 1970) and transmural stimulation. Mechanical responses to transmural stimulation were seen at the same pulse frequencies as the frequencies of action potential discharge recorded by Bower (1966) in rabbit uterine nerves. This suggests a rôle for the autonomic innervation in regulating uterine motility. Rüsse & Marshall (1970) have suggested from *in vivo* studies that one function of the uterine noradrenergic innervation of the guinea-pig is to modulate responses to contractile agents. Use of the present *in vitro* method will allow the study of the mechanism of any modulating action.

The failure of hexamethonium and pempidine to modify responses to transmural stimulation suggests that predominantly postganglionic neurones are being stimulated by this technique. This agrees with histochemical evidence that the majority of noradrenergic and cholinergic postganglionic neurones to the myometrium have their synapses in the paracervical ganglion adjacent to the cervix (Sjoberg, 1967; Mustonen & Teräväinen, 1971; Kanerva *et al.*, 1972). It has been suggested that these 'short' noradrenergic neurones, which are confined to the urogenital tract, display functional differences from 'long' noradrenergic neurones. They are largely un-

affected by postnatal administration of nerve growth factor antiserum, they are resistant to the effects of reserpine and 6-hydroxydopamine, they are difficult to deplete by nerve stimulation and the sex steroids can modify the transmitter content of the tissue (Owman & Sjoberg, 1973). This *in vitro* technique could be used to see if there are other functional differences.

Failure of the blocking drugs to abolish completely the contractile and inhibitory responses to transmural stimulation could be interpreted in two ways. Firstly, the concentrations used were inadequate to abolish the actions of released acetylcholine and noradrenaline, or secondly, that transmural stimulation elicited the release of some other transmitter.

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