Br. J. Pharmac. (1974), 52, 539-547

THE INNERVATION OF THE RAT CERVIX AND ITS PHARMACOLOGY *In vitro* AND *In vivo*

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1 Contractions of the rat cervix and uterine horns were measured both *in vitro* and *in vivo* as changes in luminal perfusion pressure during perfusion at constant flow.

2 Spontaneous cervical and uterine horn contractions *in vitro* were reduced from rats pre-treated with 17β-oestradiol compared to those ovariectomized only or those pre-treated with 17β-oestradiol plus progesterone.

3 The rat cervix exhibited similar *in vitro* sensitivities to methacholine, oxytocin, isoprenaline and phenylephrine as did the uterine horns.

4 Transmural stimulation produced cervical and uterine horn contractions, which were blocked by hyoscine or tetrodotoxin, suggesting a cholinergic motor innervation. This was supported by histological findings.

5 *In vivo,* the injections of propranolol (1.9×10^{-7} mol/kg) or pempidine (3.2 or 6.4×10^{-6} mol/kg) were followed by large cervical and smaller uterine horn contractions, suggesting an adrenergic inhibitory tone mediated by β-adrenoceptors.

6 The evidence for a cervical sphincter is discussed.

Introduction

There have been many pharmacological studies on uterine horns of various species but few on the adjacent cervical region. It has been suggested on the basis of histological studies in the human (Danforth, 1947) and stress-strain studies on isolated rat cervix (Harkness, 1964) that the mechanical properties of the cervix are determined mainly by the connective tissue. However, both *in vitro* and *in vivo,* in a variety of species, cervical tissue can exhibit spontaneous mechanical activity and will respond to drugs with contractions or relaxations (Adler, Bell & Knox, 1944; Schild, Fitzpatrick & Nixon, 1951; Schofield, 1952; Fitzpatrick, 1957, 1958; Najak, Hillier & Karim, 1970).

The purpose of the present experiments was to investigate drug responses and motor innervation of the rat cervix *in vitro* and *in vivo* and to compare them with the responses of the uterine horns. Some of these results have been communicated to the British Pharmacological Society (Hollingsworth, 1974).

Methods

Histology

Longitudinal and transverse frozen sections of rat cervix and uterine horns from 17β-oestradiol pre-treated rats (see later) were studied. Sections were treated according to the Gomori (1950) modification of Masson's Trichrome method to show smooth muscle distribution. Adjacent sections were treated according to the Spriggs, Lever, Rees & Graham (1966) modification of the Falck (1962) fluorescence histochemical technique to demonstrate catecholamines. The Koelle thiocholine method, as described by Pearse (1972), was used to show the presence of acetylcholinesterase and cholinesterase, for which inhibitors were BW284C51 (1:5-bis-(4-allyl dimethylammoniumphenyl pentan-3-one di-iodide, 10^{-5} M) and *iso*-OMPA (tetra-*iso*propyl-pyrophosphoramide, 10^{-5} M) respectively. Substrates used were acetylthiocholine iodide and butyrylthiocholine iodide. Tissues were incubated for 4 hours.

In vitro *perfusion*

One cervical canal was cannulated at the uterine horn end and the lumen perfused with Krebs solution at 37°C at a constant flow rate of 1.5 ml/minute. Changes in perfusion pressure were detected by a pressure transducer attached to a side-arm of the perfusion apparatus. Uterine horns were perfused and responses measured in a similar manner. Spontaneous contractions were measured as maximum pressure in mmHg

(1 mmHg ≈ 133 Pa) or contraction frequency (number/min) or integrated pressure (in mmHg min; measured planimetrically as the area under the pressure curve above atmospheric pressure over 5 min periods). Tissues were perfused for 1 h before drugs were either injected into the perfusing fluid (in a constant dose volume of 0.05 ml at 15 min intervals) or added to the Krebs reservoir. Drug responses were measured as either maximum pressure or integrated pressure.

The perfused tissues were transmurally stimulated through a pair of parallel stainless steel wire electrodes, 0.5 cm apart, one of which passed through the total lumen of each tissue. A Grass S6 stimulator was used to apply 0.2 ms pulses of supra-maximal voltage (40 V) in 5 s trains every 2 minutes.

In vivo *perfusion*

Rats were anaesthetized with urethane (1.4×10^{-2} mol/kg, i.p.) and the tracheae cannulated. The right external jugular vein was cannulated for the injection of drugs. Arterial blood pressure was recorded from the right carotid artery by means of a pressure transducer. The right cervical canal was cannulated at the uterine horn end and perfused with Krebs solution at a flow rate of 1.5 ml/min as *in vitro.* A tube was placed in the vagina to carry Krebs to waste. The lumen of a 2 cm length of the left uterine horn was perfused with Krebs from the ovarian end. The temperature of the Krebs perfusing the tissues was 33 ± 1°C. This was the body temperature as maintained by an overhead lamp. Heparin, 2,000 units/kg, was injected intravenously.

Fifteen min after completion of cannulation, drugs were injected intravenously in ascending dose order at 10 min intervals, before and after injection of a modifying agent (or 0.9% w/v NaCl solution (saline)). Intra-cervical and intra-uterine horn perfusion pressures were recorded as *in vitro* and responses measured as maximum perfusion pressure or inhibition of integrated pressure measured over 5 min periods. Falls in diastolic blood pressure were expressed as percentages of the pre-drug diastolic blood pressure.

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

Animals

Virgin Wistar rats, 175 to 250 g, were ovariectomized and 2 to 4 weeks later injected subcutaneously daily for 7 days with the solvent, arachis oil (0.5 ml/kg; ovariectomized group) or 17β-oestradiol (1.8×10^{-8} mol/kg; oestradiol group) or 17β-oestradiol plus progesterone (1.8×10^{-8} mol/kg and 1.6×10^{-5} mol/kg respectively; progesterone group) and killed 22 ± 2 h after the last dose.

Drugs

The following drugs were used: hyoscine hydrobromide (Koch-Light); (±)-isoprenaline hydrochloride (Sigma); methacholine chloride (acetyl β-methylcholine chloride; Koch-Light); nicotine hydrogen tartrate (BDH); 17β-oestradiol (BDH); oxytocin (synthetic; Sigma); pempidine tartrate (May & Baker); (−)-phenylephrine hydrochloride (Sigma); progesterone (BDH); (±)-propranolol hydrochloride (ICI); tetrodotoxin (Sankyo); urethane (BDH).

The Krebs solution composition (mM) was 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.

Results

Histology

The rat cervix contains two cervical canals, each of which connect the uterine body lumen to the vagina via an external os. The trichrome-staining method showed that the cervix and uterine horns contained outer, predominantly longitudinally arranged, and inner, predominantly circularly arranged, smooth muscle layers. The cervix had a higher connective tissue content than the uterine horns. There was a virtual absence of smooth muscle near the external ossa. This is as described by Harkness & Harkness (1959). However, a thickening of the circularly arranged smooth muscle was noted at the junction between the hard, opaque cervical tissue containing parallel, narrow lumina, with the wider lumina of the uterine body.

With the Falck technique, a virtual absence of fluorescent fibre-like structures was noted in both the cervix and the uterine horns, except around small blood vessels ($n = 14$). Occasional fluorescent fibre-like structures were seen in a small inner longitudinal smooth muscle layer running towards the epithelium. Intensely fluorescent cell bodies were seen in the periphery of the cervix near the cervical-vaginal junction which probably correspond to ganglion cells and 'small-intensely fluorescent' cells described by Hervonen, Kanerva & Lietzen (1973). Fluorescence was absent from control sections heated without paraformaldehyde.

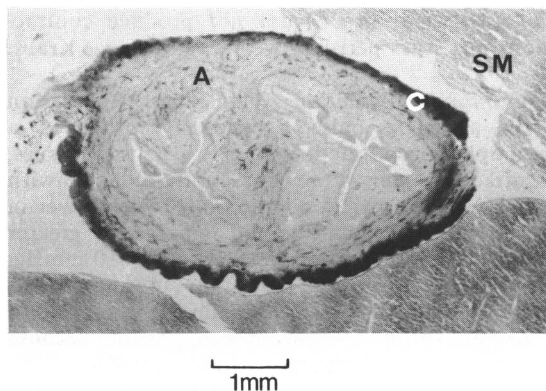

Fig. 1 Transverse section of the uterine body end of a rat cervix, stained by the Koelle method. A fairly dense network of acetylcholinesterase-staining fibres (A), typical of cholinergic nerves, can be seen running in the circular cervical smooth muscle. The outer longitudinal smooth muscle stained for cholinesterase (C). The tissue was mounted in skeletal muscle (SM).

A dense network of acetylcholinesterase-staining fibre-like structures was seen running in the longitudinal and circular smooth muscle layers of the cervix and uterine horns ($n = 8$, Figure 1). This staining appeared denser in the cervix than in the uterine horns and greatest at the uterine body-cervical junction. Acetylcholinesterase-staining fibres were also seen running in the connective tissue towards the external ossa. Cholinesterase-staining followed a similar distribution, although the outer longitudinal smooth muscle stained relatively much more intensely (Figure 1). Specific staining was absent from sections incubated without substrates.

In vitro *perfusion*

Spontaneous contractions On commencing luminal perfusion there was a large temporary pressure rise (52 ± 14 mmHg; $n = 9$) in the cervix only. Thereafter, both tissues exhibited low resting perfusion pressures (< 10 mmHg; Figure 2). Spontaneous cervical and uterine horn contractions, after 1 h perfusion, from the 17β-oestradiol pretreated rats were significantly ($P < 0.01$) less than from either the ovariectomized or the progesterone pre-treated groups (Table 1). Therefore all subsequent experiments were performed on tissues from 17β-oestradiol pre-treated rats.

Drug responses Injected methacholine and oxytocin induced phasic cervical and uterine horn contractions (Figure 2). Although methacholine and oxytocin produced greater increases in intra-cervical than intra-uterine horn integrated pressures, the drugs were approximately equipotent on the two tissues (Figure 3).

Oxytocin (1×10^{-2} iu/ml) added to the Krebs reservoir produced regular contractions of the two tissues which could be inhibited by isoprenaline ($ED_{50} = 6.3 \times 10^{-11}$ mol, cervix; $= 5.6 \times 10^{-11}$ mol, uterine horn) and phenylephrine ($ED_{50} = 1.1 \times 10^{-7}$ mol, cervix; $= 4.0 \times 10^{-8}$ mol, uterine horn). The two tissues were equally sensitive to the sympathomimetic amines. Isoprenaline was approximately 1700 and 700 times more potent than phenylephrine on the cervix and uterine horns respectively. Phenylephrine (up to

Table 1 Comparison of spontaneous contractions of the rat cervix and uterine horns *in vitro*

	Pre-treatment		
	Ovariectomized (n = 12)	*17β-oestradiol* (n = 7)	*17β-oestradiol + progesterone* (n = 6)
Frequency of pressure increases (n/min)			
Cervix	0.92 ± 0.08	0.37 ± 0.13	0.80 ± 0.10
Uterine horn	1.40 ± 0.14	0.43 ± 0.12	1.00 ± 0.07
Maximum pressure (mmHg)			
Cervix	63.5 ± 4.2	20.7 ± 9.6	64.7 ± 7.5
Uterine horn	47.0 ± 7.0	18.7 ± 5.6	37.2 ± 2.7
Integrated pressure (mmHg/min)			
Cervix	98.7 ± 12.2	7.4 ± 5.0	58.4 ± 10.3
Uterine horn	59.6 ± 4.3	2.0 ± 2.0	42.0 ± 7.5

Spontaneous contractions of luminally perfused rat cervix and uterine horns *in vitro* measured over a 5 min period 1 h after starting perfusion. Results are means with s.e. mean. All parameters of spontaneous contractions from the 17β-oestradiol pretreated group were significantly ($P < 0.01$) less than the corresponding parameter from either the ovariectomized or the progesterone pre-treated group (Mann Whitney U-test).

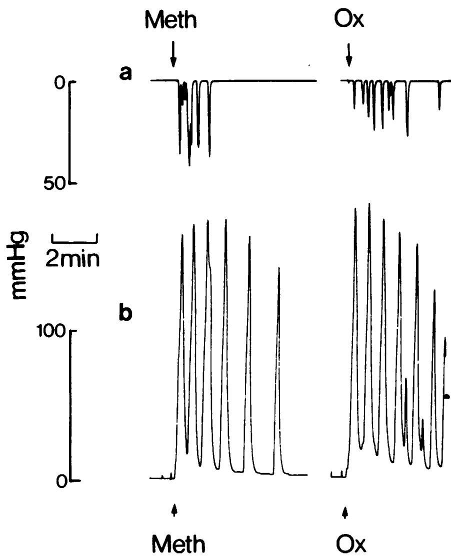

Fig. 2 Isolated perfused uterine horn (a) and cervix (b) of the rat. Single doses of methacholine (Meth; 4.5×10^{-8} mol) and oxytocin (Ox; 1.4×10^{-2} iu) induced phasic increases in perfusion pressure.

2.7×10^{-7} mol; $n = 6$) did not produce contractions in tissues perfused with oxytocin-free Krebs.

Transmural stimulation Transmural stimulation with a single pulse occasionally produced a monophasic contraction of the perfused tissues, contractions were more consistently obtained with pulse trains. Cervical contractions were slower in onset, longer in duration and significantly greater ($P < 0.01$) in maximum pressure (97 ± 10 mmHg; $n = 10$) than those of the uterine horns (52 ± 6 mmHg; $n = 10$). No inhibitory responses to transmural stimulation were seen, possibly because of the absence of resting perfusion pressure. Both tissues exhibited a similar relationship between frequency and response (Figure 4).

After obtaining a frequency-response curve to transmural stimulation, responses were obtained to single doses of methacholine (4.5×10^{-8} mol) and oxytocin (4×10^{-2} iu) which produced maximum pressure rises of a similar order to those produced by transmural stimulation. Drug responses and the maximum response to transmural stimulation were defined as 100%. The three were repeated 15 min after starting perfusion with Krebs containing hyoscine (2.5×10^{-8} M). Hyoscine abolished methacholine responses but had no significant effect ($P > 0.05$) on responses to oxytocin.

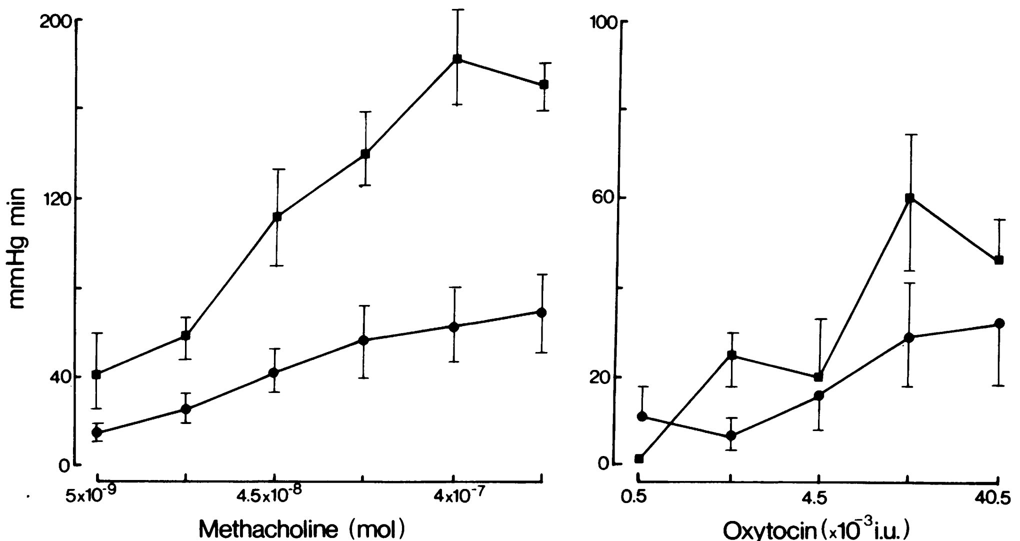

Fig. 3 Isolated perfused cervix (■) and uterine horns (●) of the rat. Log dose-effect curves to methacholine ($n = 8$) and oxytocin ($n = 5$). Results are means; vertical lines indicate s.e. mean.

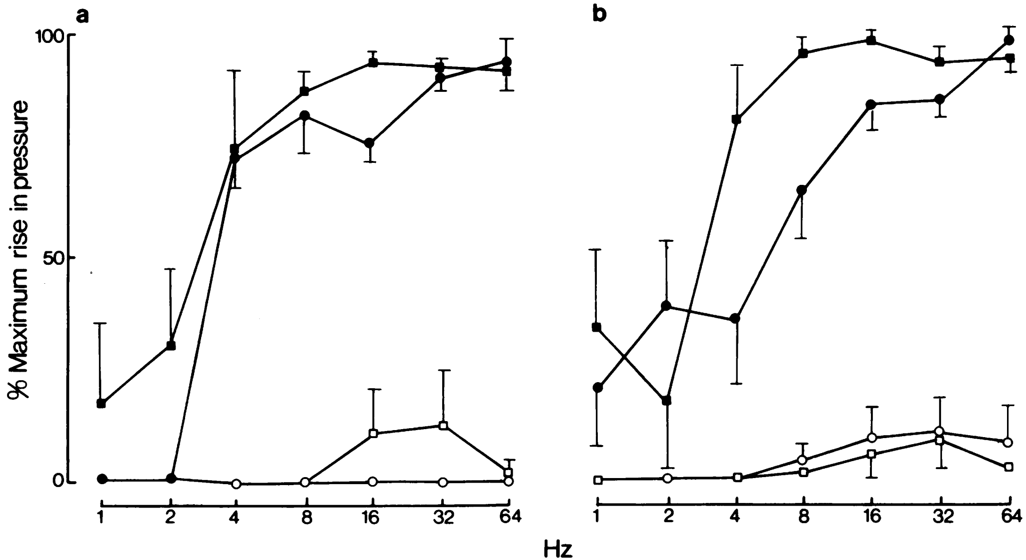

Fig. 4 Isolated perfused cervix (■) and uterine horns (●) of rat. Effects of (a) hyoscine (2.5×10^{-8} M) and (b) tetrodotoxin (3.1×10^{-7} M) on responses to transmural stimulation. Closed symbols, control responses; Open symbols, responses obtained in the presence of the modifying agent. Values are means; vertical lines indicate s.e. mean, $n = 6$.

Cervical responses to transmural stimulation were markedly reduced by hyoscine and those of the uterine horn were abolished (Figure 4).

The same experimental design was adopted substituting tetrodotoxin (3.1×10^{-7} M) for hyoscine. Tetrodotoxin had no significant effect ($P > 0.05$) on responses to methacholine or oxytocin but markedly reduced cervical and uterine horn responses to transmural stimulation (Figure 4).

Control experiments showed that repeated cervical ($n = 6$) and uterine horn ($n = 12$) responses to transmural stimulation, methacholine and oxytocin were not significantly different ($P > 0.05$) from initial responses.

In vivo *perfusion*

As *in vitro*, on starting cervical perfusion there was a large pressure rise, which *in vivo* usually exceeded 100 mmHg. After 1 to 2 min perfusion, resting perfusion pressures were generally less than 10 mmHg and spontaneous contractions were infrequent.

Methacholine (1.6×10^{-8} to 1.3×10^{-7} mol/kg) produced a fall in blood pressure, single or biphasic cervical contractions but only small uterine horn contractions ($n = 10$, Figures 5 and 6). Control experiments ($n = 3$) showed that these methacholine responses were reproducible. Hyoscine (3.3×10^{-8} mol/kg) had no effect on resting perfusion pressures. Hyoscine produced a 6.4-fold shift to the right of the vasodepressor dose-effect curve for methacholine but about a 20-fold shift of the cervical constrictor dose-effect curve for methacholine ($n = 4$; Figure 6). Oxytocin (0.5 to 2×10^{-2} iu/kg) produced cervical and small uterine horn contractions with no change in blood pressure ($n = 2$). These responses were unaffected by hyoscine (3.3×10^{-8} mol/kg).

For investigation of the effects of isoprenaline (1.2×10^{-9} to 1.9×10^{-8} mol/kg) *in vivo*, the cervix and uterine horns were perfused with Krebs solution containing oxytocin (1×10^{-2} iu/ml) (Figure 5). The potencies of isoprenaline in reducing oxytocin-induced contractions of the two tissues appeared similar but were difficult to measure as oxytocin did not produce the same regularity of contractions as *in vitro*. Isoprenaline did not reduce resting perfusion pressures. Propranolol (1.9×10^{-7} mol/kg) produced a 7.9-fold shift to the right of the vasodepressor dose-effect curve for isoprenaline and antagonized the inhibitory action of isoprenaline on the cervix and uterine horns ($n = 8$).

Nicotine (6.1×10^{-8} to 2.5×10^{-7} mol/kg) produced dose-related increases in blood pressure without inducing cervical and uterine horn

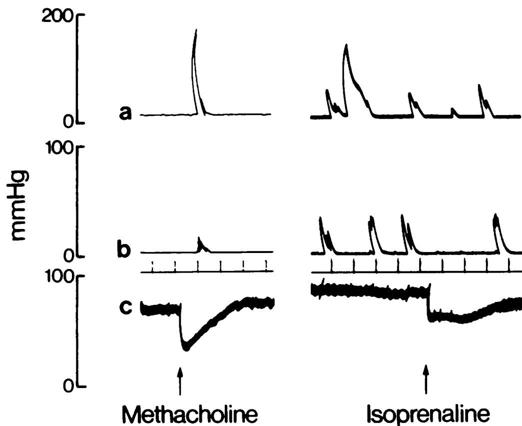

Fig. 5 *In vivo* rat cervical (a) and uterine horn (b) perfusion pressures and arterial blood pressure (c). Effect of methacholine (3.2×10^{-8} mol/kg, i.v.) and isoprenaline (4.8×10^{-9} mol/kg, i.v.). Tissues were luminally perfused with Krebs (plus oxytocin, 1×10^{-2} iu/ml, in the experiment with isoprenaline). Results from 2 rats. Time marks at 1 min intervals.

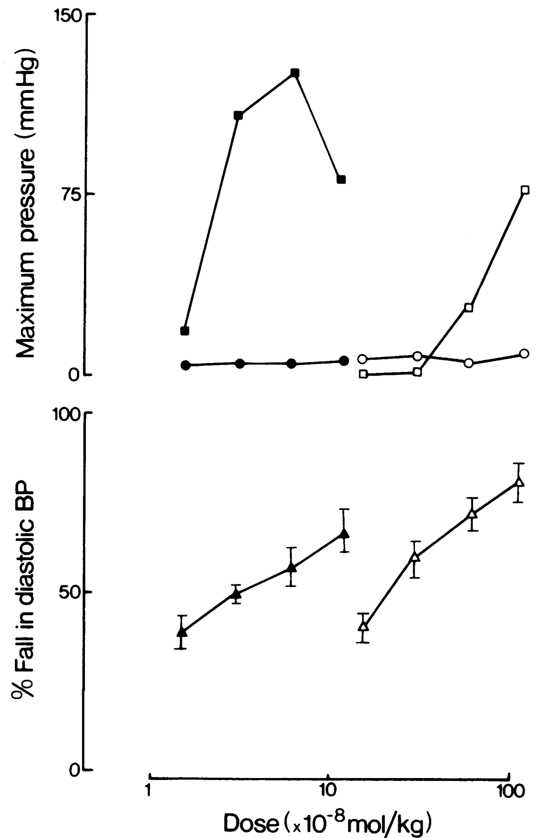

Fig. 6 *In vivo* rat cervical (■) and uterine horn (●) perfusion pressures and % fall in diastolic blood pressure (▲). Log dose-effect curves for methacholine alone (closed symbols; $n = 6$) and repeated after hyoscine (3.3×10^{-8} mol/kg; open symbols; $n = 4$). Values are means; vertical lines indicate s.e. for % fall in diastolic blood pressure.

contractions ($n = 6$). Pempidine (3.2 or 6.4×10^{-6} mol/kg) produced an immediate fall in blood pressure (44 ± 6%) and abolished the pressor effect of nicotine.

It was observed that the injection of propranolol or pempidine was followed, within 2 to 3 min, by the onset of large cervical and smaller uterine horn contractions which were maintained for at least 20 min (Fig. 7); thereafter the tissues remained quiescent. These effects of propranolol and pempidine were not related to previous administrations of isoprenaline or nicotine respectively as they were seen in rats given saline in place of the agonists ($n = 3$).

Discussion

The present results clearly show that a portion of the rat cervix exhibits spontaneous mechanical activity and can respond to drugs *in vitro* and *in vivo*. This suggests that the smooth muscle of the rat cervix may possess functional roles in the non-pregnant amimal and possibly during pregnancy and needs to be recognized in interpreting drug effects on total uterine motility. The histological findings suggest that the pressure changes were a function of smooth muscle contractions of the region at the uterine body-cervical junction rather than near the external ossa where the smooth muscle content was much less. This may also apply to cervical contractions recorded by others (see introductory section) as cervical smooth muscle distribution is similar between species although gross anatomy varies (Hafez, 1973).

The ability of tetrodotoxin and hyoscine to antagonize contractions induced by transmural stimulation at low pulse width suggests that the rat cervix and uterine horns receive a cholinergic motor innervation. This suggestion is supported by the histochemical demonstration of dense acetylcholinesterase-staining in the present study and by other workers (Adham & Schenk, 1969; Hervonen *et al.*, 1973). Nakanishi & Wood (1971) have described a human uterine motor innervation as cholinergic, although responses were little affected

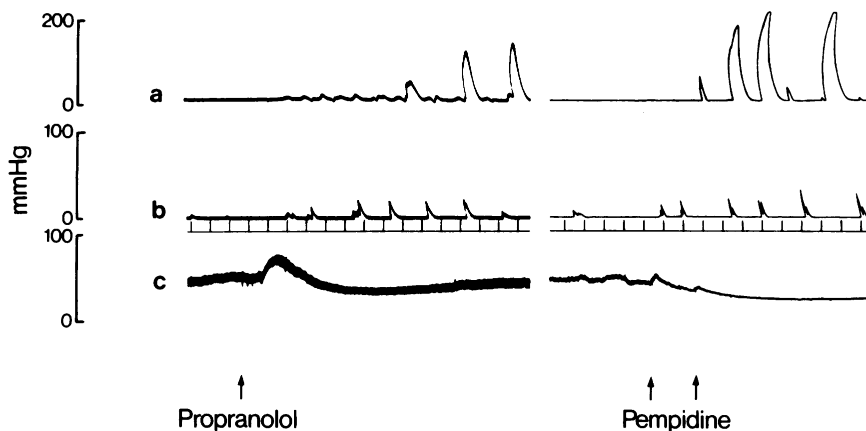

Fig. 7 Onset of large cervical (a) and smaller uterine horn (b) contractions after intravenous injections of propranolol hydrochloride (1.9×10^{-7} mol/kg) or pempidine tartrate ($2 \times 3.2 \times 10^{-6}$ mol/kg) in 2 rats. Arterial blood pressure (c) was also measured. Time marks at 1 min intervals.

by atropine. Also, electrical stimulation of the pre-ganglionic sacral parasympathetic nerves in the rabbit *in vivo* only occasionally produced cervical or uterine horn contractions (Schofield, 1952) and did not induce action potentials in uterine nerves (Bower, 1966). Therefore, to date, there has been little evidence for a cholinergic motor uterine innervation. However, it is interesting that division of the sacral parasympathetic nerves prolongs parturition in the rat (Carlson & De Feo, 1965) and dog (Shabanah, Toth & Maughan, 1964), although this may result from factors other than removal of a motor innervation.

There have been many studies of uterine noradrenergic innervation (Marshall, 1970). Hypogastric nerve stimulation can produce inhibition or contraction of rat uterine horns depending on the hormonal state of the animal (Butterworth & Randall, 1970). The effect of any released noradrenaline from electrical stimulation of noradrenergic nerves would produce inhibition in the present experiments, as did isoprenaline and phenylephrine. The large increase in cervical (and smaller uterine horn) contractions after propranolol or pempidine *in vivo* suggests the presence of an adrenergic inhibitory tonic influence on these organs. The doses of propranolol and pempidine used produced significant antagonism at vascular β-adrenoceptors and nicotinic receptors respectively. This tone could derive from local noradrenergic nerves or alternatively, might involve the adrenal medulla. Spriggs (1965) has described a high adrenaline output from the adrenal medullae of rats anaesthetized with urethane.

A sphincter function has been ascribed to the rat cervix in regulating uterine fluid discharge into the vagina (Blandau, 1945; Armstrong, 1968) and to the human isthmus in regulating the isthmal canal diameter (Asplund, 1952; Mann, McLaren & Hayt, 1961). Three further criteria can be applied to define a sphincter region.

The first criterion is a thicker layer of circular smooth muscle in the sphincter region compared to the adjoining non-sphincter region. This was seen here at the uterine body-cervical junction and with an associated dense cholinergic innervation.

The second criterion is the presence of a nervous or hormonal tone to the smooth muscle *in vivo* producing contraction. There was no marked resting perfusion pressure of the rat cervix *in vivo* although this is seen in the rabbit isthmus (Levy & Lindner, 1972) which is described as a sphincter (Brundin, 1969). Sphincter tone may not have been observed in the present *in vivo* studies due to the use of an anaesthetic. Also, the extent of cervical constriction in non-anaesthetized rats, as measured by the uterine retention of dye solutions, is proportionately related to the quantity of uterine luminal fluid present (Blandau, 1945), the latter being small in the animals used here. There was no evidence for any cholinergic constrictor tone *in vivo* as hyoscine, in doses which antagonized methacholine responses, did not reduce cervical perfusion pressure or alter contractions.

The third criterion has been the presence of α-adrenoceptors mediating sphincter contraction but inhibition of the adjacent non-sphincter smooth muscle, such as has been described for the

choledochoduodenal junction (Persson, 1971). However, α-adrenoceptors mediate uterine horn contractions (Marshall, 1970). There have been suggestions as to differences in cervical drug sensitivities compared to the uterine horns (Adler *et al.*, 1944; Newton, 1937; Schild *et al.*, 1951), although only Fitzpatrick (1957) attempted to compare dose-effect curves. In the rat, *in vitro*, there was no evidence here for marked differences in drug potencies between the two tissues. The greater intra-cervical compared to intra-uterine horn pressures, whether spontaneous, drug-induced or electrically-induced, were probably a reflection of the initial difference in their respective internal diameters. Under other hormonal states, or in pregnancy, differences in cervical and uterine horn drug sensitivities may be apparent.

There is therefore some functional and histological evidence but little pharmacological evidence for a cervical sphincter.

References

ADHAM, N. & SCHENK, E.A. (1969). Autonomic innervation of the rat vagina, cervix and uterus and its cyclic variation. *Am. J. Obstet. Gynec.*, **104**, 508-516.

ADLER, J., BELL, G.H. & KNOX, J.A.C. (1944). The behaviour of the cervix uteri in vivo. *J. Physiol. Lond.*, **103**, 142-154.

ARMSTRONG, D.T. (1968). Hormonal control of uterine lumen fluid retention in the rat. *Am. J. Physiol.*, **214**, 764-771.

ASPLUND, J. (1952). The uterine cervix and isthmus under normal and pathological conditions. *Acta radiol. suppl.*, **91**, 3-76.

BLANDAU, R.J. (1945). On the factors involved in sperm transport through the cervix uteri of the albino rat. *Am. J. Anat.*, **77**, 253-272.

BOWER, E.A. (1966). The characteristics of spontaneous and evoked action potentials recorded from the rabbit's uterine nerves. *J. Physiol. Lond.*, **183**, 730-747.

BRUNDIN, J. (1969). Pharmacology of the oviduct. In: *The Mammalian Oviduct.* ed. Hafez, E.S.E. & Blandau, R.J., Ch. 10, pp. 251-269, Chicago: Chicago University Press.

BUTTERWORTH, K.R. & RANDALL, M.J. (1970). Effects of α- and β-adrenoceptor blocking agents on the responses of the rat uterus to catecholamines throughout the oestrous cycle. *Br. J. Pharmac.*, **40**, 160P.

CARLSON, R.R. & DE FEO, V.J. (1965). Role of the pelvic nerves versus the abdominal sympathetic nerves in the reproductive function of the female rat. *Endocrinology*, **77**, 1014-1022.

DANFORTH, D.N. (1947). The fibrous nature of the human cervix and its relation to the isthmic segment in gravid and non-gravid uteri. *Am. J. Obstet. Gynec.*, **53**, 541-557.

FALCK, B. (1962). Observations on the possibilities of the cellular localisation of monoamines by a fluorescence method. *Acta physiol. scand.*, **56**, suppl. 197.

FITZPATRICK, R.J. (1957). The activity of the uterine cervix in ruminants. *Vet. Rec.*, **69**, 713-717.

FITZPATRICK, R.J. (1958). The response of the cervix uteri of ruminants to adrenaline. *J. Comp. Path.*, **68**, 219-231.

GOMORI, G. (1950). Rapid one-step trichrome strain *Am. J. Clin. Path.*, **20**, 661-664.

HAFEZ, E.S.E. (1973). The comparative anatomy of the mammalian cervix. In: *The Biology of the Cervix.* ed. Blandau, R.J. & Moghissi, K. pp. 23-56. Chicago: University of Chicago Press.

HARKNESS, M.L.R. & HARKNESS, R.D. (1959). Changes in the physical properties of the uterine cervix of the rat during pregnancy. *J. Physiol. Lond.*, **148**, 524-547.

HARKNESS, R.D. (1964). The physiology of the connective tissue of the reproductive tract. *Int. Rev. of Connect. Tissue Res.*, **2**, 155-211.

HERVONEN, A., KANERVA, L. & LIETZEN, R. (1973). Histochemically demonstrable catecholamines and cholinesterases of the rat uterus during estrous cycle, pregnancy and after oestrogen treatment. *Acta physiol. scand.*, **87**, 283-288.

HOLLINGSWORTH, M. (1974). The *in vitro* rat cervix. *Br. J. Pharmac.*, **50**, 470P.

LEVY, B. & LINDNER, H.R. (1972). The effects of adrenergic drugs on the rabbit oviduct. *Europ. J. Pharmac.*, **18**, 15-21.

MANN, E.C., McLAREN, W.D. & HAYT, D.B. (1961). The physiology and clinical significance of the uterine isthmus. *Am. J. Obstet. Gynec.*, **81**, 209-222.

MARSHALL, J.M. (1970). Adrenergic innervation of the female reproductive tract; anatomy, physiology and pharmacology. *Ergebn. Physiol.*, **62**, 6-67.

NAJAK, Z., HILLIER, K. & KARIM, S.M.M. (1970). The action of prostaglandins on the human isolated, non-pregnant cervix. *J. Obstet. Gynec. Br. Commonw.*, **77**, 701-709.

NAKANISHI, H. & WOOD, C. (1971). Cholinergic mechanisms in the human uterus. *J. Obstet. Gynaec. Br. Commonw.*, **78**, 716-723.

NEWTON, W.H. (1937). The insensitivity of the cervix uteri to oxytocin. *J. Physiol. Lond.*, **89**, 309-315.

PEARSE, A.G.E. (1972). Cholinesterases. In: *Histochemistry, Theoretical and Applied.* 3rd edition. vol. 2, pp. 766-807. Edinburgh and London: Churchill.

PERSSON, C.G.A. (1971). Adrenoceptor functions in the cat choledochoduodenal junction *in vitro*. *Br. J. Pharmac.*, **42**, 447-461.

SCHILD, H.O., FITZPATRICK, R.J. & NIXON, W.C.W. (1951). Activity of the human cervix and corpus uteri. Their response to drugs in early pregnancy. *Lancet*, **i**, 250-253.

SCHOFIELD, B.M. (1952). The innervation of the cervix and cornu uteri in the rabbit. *J. Physiol. Lond.*, **117**, 317-328.

SHABANAH, E.H., TOTH, A. & MAUGHAN, G.B. (1964). The role of the autonomic nervous system in uterine contractility and blood flow. II: The role of the parasympathetic neurohormone acetylcholine in uterine motility and blood flow. *Am. J. Obstet. Gynec.*, **89**, 860-880.

SIEGEL, S. (1956). *Non-parametric statistics for the behavioural sciences.* New York: McGraw-Hill.

SPRIGGS, T.L.B. (1965). The effects of anaesthesia induced by urethane or phenobarbitone upon the distribution of peripheral catecholamines in the rat. *Br. J. Pharmac. Chemother.*, **24**, 752-758.

SPRIGGS, T.L.B., LEVER, J.D., REES, P.M. & GRAHAM, J.D.P. (1966). Controlled formaldehyde-catecholamine condensation in cryostat sections to show adrenergic nerves by fluorescence. *Stain technol.*, **41**, 323-327.

(Received May 22, 1974)