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MECHANICAL RESPONSES OF THE ISOLATED CERVIX AND UTERINE HORN OF PREGNANT RATS NEAR TERM TO DRUGS

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1 The responses of circularly cut strips of cervix and uterine horn from rats on days 20 and 22 of pregnancy to drugs were compared *in vitro*.

2 The cervix exhibited similar responses and sensitivities to acetylcholine, bradykinin, prostaglandin (PG) $F_{2\alpha}$ (day 20) and isoprenaline (day 20) as did the uterine horn but was less sensitive or responded less consistently to isoprenaline (day 22), oxytocin and PGE_1. PGE_2 was more potent on the cervix (day 20).

3 Before term the relatively inextensible connective tissue of the cervix plus contractions of the smooth muscle would help to prevent foetal expulsion. At term the cervical smooth muscle is sufficiently unresponsive to allow cervical dilatation.

Introduction

During the second half of gestation the rat cervix becomes progressively more extensible (Harkness & Harkness, 1959; Hollingsworth & Isherwood, 1977). This is associated with a fall in collagen concentration. Similar changes take place in the human cervix where a marked increase in glycosaminoglycan content has also been measured (Danforth, Veis, Breen, Weinstein, Buckingham & Manalo, 1974).

While the main component of the cervix is connective tissue, in all species there appears to be some smooth muscle (El-Banna & Hafez, 1972). In the non-pregnant rat, some thickening of the circularly arranged smooth muscle at the uterine horn end of the cervix has been noted (Hollingsworth, 1974). Cervical tissue *in vitro* and *in vivo* of several species can exhibit spontaneous mechanical activity and will respond to drugs with both contractions and relaxations (Newton, 1934; Bonnycastle & Ferguson, 1941; Adler, Bell & Knox, 1944; Schild, Fitzpatrick & Nixon, 1951; Schofield, 1952; Fitzpatrick, 1957; 1958; Najak, Hillier & Karim, 1970; Hollingsworth, 1974; Coutinho & Darzé, 1976; Mackenzie, 1976).

The relative importance of these two tissue components in controlling the function of the cervix during gestation and at parturition is undecided. The objective of the present investigation was to compare the responses of the smooth muscle of the isolated cervix and uterine horn from 20 and 22 day pregnant rats to a variety of drugs and hormones. Some of the hormones tested (prostaglandins, oxytocin and bradykinin) have been implicated in the control of parturition. Muscarinic and β-adrenoceptor responses of the tissues were tested with acetylcholine and isoprenaline.

Methods

Tissues were obtained from Sprague-Dawley rats on days 20 and 22 of gestation (day of finding copulation plug = day 1). The daily light period was between 07 h 00 min and 20 h 00 min. The majority of the rats of this strain deliver between 12 h 00 min on day 22 and 12 h 00 min on day 23.

Longitudinal and transverse frozen sections of cervix and uterine horn from rats on day 20 of gestation were treated according to the Gomori (1950) modification of Masson's trichrome method to show smooth muscle distribution.

The cervix was defined as the less vascular tissue with parallel lumina between the uterine horns and the vagina. The cervical septum was cut to produce a cylinder of tissue from which a circularly cut preparation could be prepared. A circularly cut strip of uterine horn of similar dimensions was also prepared. These isolated tissues were mounted in tissue baths at 37°C under a resting tension of 0.5 g in modified Krebs solution bubbled with 95% O_2 and 5% CO_2. Mechanical activity was measured isometrically and displayed on a Grass 89C polygraph.

For those drugs, like acetylcholine, which produced an immediate and relatively sustained contraction, the

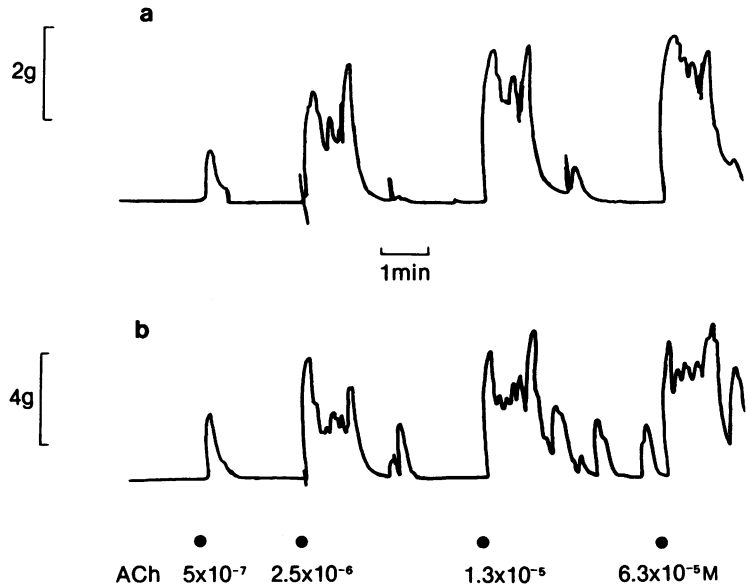

Figure 1 Effect of acetylcholine (ACh) on isolated, circularly cut strips of cervix (a) and uterine horn (b) from a rat on day 20 of gestation. Contact time 1 min, time cycle 5 minutes.

measure of drug effect was the peak amplitude of response during the time of the tissue drug contact (1 min with a time cycle of 5 minutes). Where drugs, such as the prostaglandins, induced increased frequency of contractions, the measured response was the integral under the contraction curve produced by feeding the tension signal above resting tension through a Grass 7P10B preamplifier. The integrated response was measured in gram minutes. The time of tissue drug contact here was 10 min with a time cycle of 15 minutes. To measure the inhibitory effects of isoprenaline or prostaglandin E_2 (PGE_2), an EC_{80} concentration of acetylcholine was added to the tissues for 1 min every 5 minutes. Isoprenaline or PGE_2 were added 2 min before the acetylcholine and the inhibition of the contraction to acetylcholine used as a measure of their effect. Where possible, concentration-effect curves were constructed to drugs and molar EC_{50}s determined so that the sensitivities of the two tissues could be compared. Tissues were incubated in solutions containing antagonists for 30 min before agonist drugs were repeated.

The composition of the modified Krebs solution (mM) was: Na^+ 143.5, K^+ 5.94, Ca^{2+} 0.64, Mg^{2+} 1.19, Cl^- 124.6, HCO_3^- 25.0, SO_4^{2-} 1.19, $H_2PO_4^-$ 1.19 and glucose 5.65.

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

Results

Histology

The rat cervix contains two cervical canals, each of which connect the lumen of one uterine horn to the vagina via an external os. The trichrome-staining method showed that the inner, mainly circular, smooth muscle layer of the uterine horn extended into the uterine horn end of the cervix. As with the cervix of the non-pregnant rat (Hollingsworth, 1974) there was some thickening of the circularly-arranged smooth muscle in this region. There was less smooth muscle in the vaginal end of the cervix.

In vitro *studies*

The spontaneous contractions of the uterine horn were greater in amplitude, frequency and integral but of shorter duration than those of the cervix. The spontaneous motility of the cervix declined to zero more rapidly than that of the uterine horn.

The cervix and uterine horn responded in a qualitatively similar manner to acetylcholine (Figure 1). Both tissues exhibited similar sensitivities to the sustained contractile actions of acetylcholine and bradykinin (Table 1). Hyoscine (1×10^{-8} mol/l) produced similar rightward shifts of the log-concentration effect curves to acetylcholine on the two tissues but had

Table 1 Potencies of drugs, as negative log molar EC_{50}s, on isolated tissues in modified Krebs solution from rats in late gestation

	Cervix	*Uterine horn*
Day 20		
Acetylcholine	5.87 ± 0.08 (12)	5.94 ± 0.19 (10)
Bradykinin	8.34 ± 0.13 (11)	8.22 ± 0.13 (9)
Isoprenaline	6.34 ± 0.11 (14)	6.21 ± 0.13 (14)
Day 22		
Acetylcholine	5.56 ± 0.23 (13)	6.03 ± 0.17 (14)
Isoprenaline	6.37 ± 0.11* (8)	7.67 ± 0.11 (8)

Values are means ± s.e. mean. No. of tissues in parentheses.
* Potency on the cervix significantly less than on the uterine horn ($2P < 0.02$, Mann Whitney U-test).

no effect on responses to bradykinin. Isoprenaline, which inhibited acetylcholine-induced contrations, was equipotent on the two tissues from day 20 rats but 32 fold more potent on the uterine horn from day 22 rats.

The tensions developed by the two tissues to concentrations of acetylcholine (3×10^{-4} mol/l) and bradykinin (1.25×10^{-7} mol/l) which produced maximal contractions were determined in g (Table 2). The cervical and uterine horn tissues used were of similar dimensions and wet weight. The uterine horn developed about twice the tension compared to the cervix to both drugs.

Apart from these three agonist drugs, uterine horn and cervical responses were dissimilar. Oxytocin (0.1 to 100 mu/ml; Figure 2) and prostaglandin $F_{2\alpha}$, 1×10^{-6} to 1×10^{-4} mol/l), particularly at lower concentrations, produced contractions of the uterine horn which were regular in frequency and amplitude but produced irregular contractions of the cervix.

In twelve experiments where EC_{50}s could be determined, oxytocin was of similar potency on the cervix ($EC_{50} = 1.2$ mu/ml) as on the uterine horn ($EC_{50} = 0.52$ mu/ml) (day 20). In the remaining four experiments, the cervix (day 20) did not respond to oxytocin in concentrations up to 100 mu/ml. $PGF_{2\alpha}$ was approximately equipotent on the two tissues (EC_{50}s about 1×10^{-6} mol/l).

The uterine horn generally responded consistently to PGE_1 (EC_{50} about 1×10^{-6} mol/l) with the magnitude of contraction being related to concentration (day 20). In contrast, only a small proportion of the cervical preparations responded and in an irregular manner.

PGE_2 was more potent in inducing an increased frequency of contractions of the cervix (EC_{50} approximately 1×10^{-8} mol/l) than the uterine horn (EC_{50} about 1×10^{-6} mol/l) from day 20 rats. Acetylcholine-induced contractions of both tissues were inhibited by higher concentrations of PGE_2 ($>10^{-5}$ mol/l). PGE_2 had a prolonged inhibitory action on the cervix after wash-out of the drug. This inhibitory effect of PGE_2 was unaffected by propranolol (1×10^{-8} mol/l) which significantly inhibited responses to isoprenaline.

In one experimental group ($n = 7$) rats were given indomethacin (5 mg/kg s.c.) in arachis oil on day 19 and killed on day 20. The integral of the spontaneous contractility was measured for the first hour after placing the tissues in modified Krebs solution. Indomethacin pretreatment virtually abolished uterine horn contractions but had no effect on those of the cervix (Table 3). The maximal spasmogenic responses of both tissues to acetylcholine as well as the integrated responses to $PGF_{2\alpha}$ (10^{-7} to 10^{-5} mol/l) and the cervical contractions to oxytocin (0.1 to 10 mu/ml)

Table 2 Maximum tensions (g) developed by isolated cervix and uterine horn from pregnant rats to acetylcholine and bradykinin

	Day 20		*Day 22*	
	Cervix	*Uterine horn*	*Cervix*	*Uterine horn*
Acetylcholine	4.1 ± 0.3*	8.4 ± 0.3	3.9 ± 0.3*	8.7 ± 0.5
Bradykinin	3.5 ± 0.3*	6.1 ± 0.6	—	—

Each value is the mean ± s.e. mean of 12 observations.
* Significantly less ($2P < 0.01$) than corresponding uterine horn value (Mann-Whitney U-test).

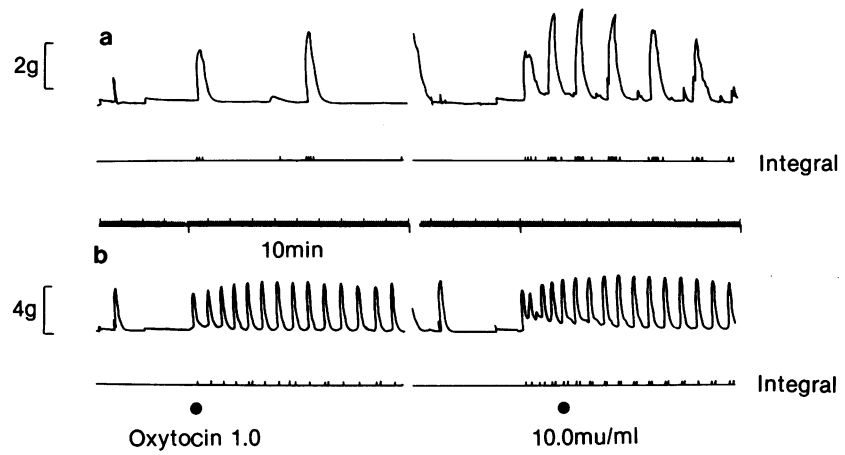

Figure 2 Effect of oxytocin in isolated, circularly cut strips of cervix (a) and uterine horn (b) from a rat on day 20 of gestation. Contact time 10 min, time cycle 15 minutes.

were unaffected by indomethacin. The uterine horn response to the highest concentration of oxytocin (10 mu/ml) was significantly increased.

On day 22, PGE_1, PGE_2, $PGF_{2\alpha}$, and oxytocin induced increased frequency of contractions of the cervix in the same range of concentrations as those effective on the uterine horn (EC_{50}s for the prostaglandins about 1×10^{-6} mol/l and 5 mu/ml for oxytocin). However, the maximum integrated responses of the cervix were only 34.5, 19.0, 17.6 and 47.6% respectively of those of the uterine horn in contrast to the more pronounced cervical responses on day 20 (Table 4).

Discussion

The results of the present investigation indicate that the cervix of the rat on days 20 and 22 of gestation contains smooth muscle which can exhibit spontaneous contractility and can respond mechanically to a variety of drugs. Similar observations have been made with the cervix of other species (see Introduction).

The cervix of the late pregnant rat exhibited similar sensitivities to acetylcholine, bradykinin, $PGF_{2\alpha}$ and isoprenaline (day 20); was less sensitive or less consistently responsive to oxytocin, isoprenaline (day 22) and PGE_1, and more sensitive to PGE_2 (day 20) than the uterine horn. This is in contrast to these tissues from the non-pregnant rat which exhibited similar sensitivities to methacholine, oxytocin, isoprenaline and phenylephrine (Hollingsworth, 1974). Oxytocin has been shown to be less potent on the cervix of the pregnant cow *in vivo* than the uterus (Fitzpatrick, 1957) while ergometrine is more potent on the human cervix than the uterus (Schild *et al.*, 1951; Mackenzie, 1976). The reasons for these differences in drug potencies between the cervix and uterine horn are not as yet explained.

Contractile responses to PGE_2 at low concentrations and inhibitory actions at high concentrations suggest two receptor systems which display differing sensitivities to PGE_2. PGE_2 has been shown to in-

Table 3 Effect of indomethacin (5 mg/kg s.c.) given on day 19 on the integrated spontaneous contractility (g min) of tissues from day 20 rats for 1 h after placing in modified Krebs solution.

	Cervix	*Uterine horn*	*2P*
Control	4.9 ± 1.0 (8)	20.7 ± 6.7 (8)	<0.05
Indomethacin	3.8 ± 0.7 (8)	0.8 ± 0.7 (8)	<0.05
2P	>0.05	<0.01	

Each value is the mean ± s.e. mean. No. of tissues in parentheses.
2P is the significance of difference by the Mann-Whitney U-test.

Table 4 Maximal integrated responses (g min) developed by isolated cervix and uterine horns from pregnant rats to drugs

	Day 20		Day 22	
	Cervix	*Uterine horn*	*Cervix*	*Uterine horn*
Oxytocin	4.5 ± 1.3 (7)	4.8 ± 0.9 (7)	3.8 ± 1.0 (14)	11.0 ± 1.2 (14)
Prostaglandin E_1	2.1 ± 0.8 (8)	6.7 ± 0.7 (8)	1.2 ± 0.4 (6)	6.3 ± 1.3 (6)
Prostaglandin E_2	2.8 ± 0.8 (8)	4.4 ± 0.3 (8)	0.9 ± 0.4 (6)	5.1 ± 0.4 (6)
Prostaglandin $F_{2\alpha}$	5.4 ± 0.5 (4)	6.3 ± 1.6 (4)	2.0 ± 0.8 (6)	4.2 ± 0.6 (6)

Tissues were incubated with drugs for 10 minutes. Values are means ± s.e. mean. No. of tissues in parentheses.

duce relaxations of the cervix of the non-pregnant human *in vitro* (Najak *et al.*, 1970) and *in vivo* (Coutinho & Darzé, 1976) but generally contracts the uterus of the non-pregnant human and both tissues during pregnancy (Mackenzie, 1976). The inhibitory action of PGE_2 was not blocked by propranolol demonstrating it was not a β-adrenoceptor-mediated effect. The cervix of the pregnant rat (day 20) was more sensitive to both actions of PGE_2 than the uterine horn but showed little response to PGE_2 at term.

It has been suggested that the contractile actions of oxytocin (Vane & Williams, 1973) and bradykinin (Chivers & Whalley, 1977) on the uterine horn of the non-pregnant rat and its spontaneous motility *in vitro* (Vane & Williams, 1973) are partially mediated by the release of a prostaglandin, perhaps $PGF_{2\alpha}$. Williams, Sneddon & Harney (1974) have shown that the uterine prostaglandins derive mainly from the endometrium rather than from the myometrium. Only the uterine horn end of the cervix possesses an epithelial lining like the uterine endometrium (Hamilton, 1947). Perhaps prostaglandin production per g of cervix is less than that of the uterine horn and this could explain some of the differences between cervical and uterine horn drug responses. To test this hypothesis indirectly, rats were pretreated with indomethacin, a moderately selective prostaglandin synthesis inhibitor (Flower, 1974). While the uterine horn contractions were almost abolished, consistent with the observations of others (Aiken, 1972; Vane & Williams, 1973), cervical contractions were unaffected. This could be explained by the cervix having a major and the uterine horn a minor non-prostaglandin mediation of spontaneous contractility. *In vitro* responses to oxytocin were not reduced by indomethacin pretreatment and those of the uterine horn were enhanced. This is in agreement with the *in vivo* observations of Fuchs, Smitasiri & Chantharaksri (1976) and could be interpreted as indicating that the actions of oxytocin on the cervix and uterine horn of the late pregnant rat do not involve prostaglandins.

The uterine horn developed about twice the maximal tension compared to the cervix to acetylcholine and bradykinin. This is similar to the relative proportions of smooth muscle in the two tissues as determined histologically (Harkness & Harkness, 1959).

It would seem valid to separate the physiological changes of the cervix during gestation into those occurring before and at parturition. There is a progressive increase in cervical extensibility in the second half of gestation in the rat (Harkness & Harkness, 1959; Hollingsworth & Isherwood, 1977) perhaps as a result of increasing plasma concentrations of relaxin (O'Byrne & Steinetz, 1976). Before parturition the circularly arranged cervical smooth muscle is able to respond to local and circulating hormones and to neurotransmitters. This plus the relatively inextensible connective tissue would help to prevent any foetal expulsion.

Cervical tissues from rats on day 22 exhibited small integrated responses to the prostaglandins (E_1, E_2, and $F_{2\alpha}$) and oxytocin which have putative roles in parturition. During parturition the cervical smooth muscle will not therefore contract sufficiently to prevent cervical dilatation and foetal expulsion caused by coordinated uterine contractions.

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