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# Effects of ovarian steroids upon responses mediated by adrenoceptors in separated layers of the myometrium and in the costo-uterine muscle of the guinea-pig

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**1** This study describes the effects of ovarian steroid hormones upon the responses to adrenoceptor agonists of isolated myometrium, separated into its longitudinal and circular layers, and of costo-uterine muscle from guinea-pigs. The preparations were field-stimulated at 100 s intervals, and the adrenoceptor agonists phenylephrine and isoprenaline produced enhancement or inhibition of the evoked contractions.

**2** Isoprenaline produced propranolol-sensitive inhibitory effects in longitudinal and circular myometrium and costo-uterine muscle preparations from animals from all experimental groups: i.e. from nonsteroid-treated animals (ovariectomized and intact); intact animals treated with either oestrogen or progesterone alone; ovariectomized animals treated with oestrogen; ovariectomized and intact animals treated with progesterone following oestrogen priming; and from animals 1–4 days post-partum. Longitudinal myometrial preparations from progesterone-treated oestrogen-primed and from post-partum animals were most sensitive to this agonist.

**3** Phenylephrine produced phentolamine-sensitive excitatory effects in circular myometrial and costo-uterine muscle preparations from animals from all the experimental groups. In contrast, propranolol-sensitive inhibitory responses to phenylephrine occurred in longitudinal myometrial preparations taken from animals treated with progesterone following oestrogen priming, and from post-partum animals. Longitudinal myometrium from animals from the remaining experimental groups exhibited phentolamine-sensitive excitatory responses to phenylephrine.

**4** The basis for the selective effect upon the longitudinal myometrium of exposure to progesterone following a period of oestrogen priming, is discussed. The results described are consistent with the possibility that in the longitudinal layer of guinea-pig uterus exposed to progesterone following oestrogen priming there is an increase in the proportion of β-adrenoceptors in this layer. This increase may reduce the likelihood of contractions arising via direct stimulation of α-adrenoceptors in this layer in response to sympathetic activation during pregnancy.

## Introduction

It has been widely reported that pregnancy or changes in levels of ovarian steroids can modify the mechanical response of uterine smooth muscle to catecholamines and to sympathetic stimulation (for reviews, see Miller, 1967; Marshall, 1972). These effects have been demonstrated using uterine horns from the guinea-pig (Hermansen, 1960; Pennefather & Isaac, 1967; Isaac, Pennefather & Silva, 1969; Rüsse & Marshall, 1970), the cat (Dale, 1906; Cushny, 1906; Gustavson & van Dyke, 1931; Tsai & Fleming, 1964), the rabbit (Miller & Marshall, 1965), and the rat (Diamond & Brody, 1966; Abdel-Aziz & Bakry, 1973). Electrophysiological studies with guinea-pig myometrium by Szurszewski & Bülbring (1973), have done much to establish the ionic basis of the excitatory and inhibitory actions of catecholamines mediated via actions at uterine α- and β-adrenoceptors. Nevertheless, the underlying mechanisms by which the steroids effect changes in uterine responses to these amines, and the functional significance of these changes, have not been established. Moreover, it is not clear from earlier studies whether the effects of pregnancy and hormones occur in both circular and longitudinal myometrial layers of guinea-pig uterine horns, as is the case with the rat (Kawarabayashi & Osa, 1976; Chow & Marshall, 1981; Kishikawa, 1981).

In the present paper we describe the effects of

administration of ovarian steroids upon the mechanical responses to adrenoceptor agonists of smooth muscle within the longitudinal and circular layers of the guinea-pig uterus. Phenylephrine and isoprenaline, which are relatively selective for α- and β-adrenoceptors respectively, were used. We have also studied the effects of steroid administration upon the response of the costo-uterine muscle to these agents. This tissue, which lies in the ovarian suspensory ligament, contains longitudinally-arranged smooth muscle which is continuous with the longitudinal layer of the uterus (Gabella, 1976). In this species the costo-uterine muscle contains relatively high levels of noradrenaline (Furness & Malmfors, 1971; Thorbert, Alm, Owman & Sjöberg, 1977; Farrar, Handberg, Hartley & Pennefather, 1980). Isoprenaline can cause inhibition and noradrenaline can cause excitation of the smooth muscle of this tissue (Mohsin, Harris & Pennefather, 1975; Mohsin & Pennefather, 1979). Experiments in this laboratory (Farrar *et al.*, 1980; Hartley, Pennefather & Story, 1983) have established that although the fluctuations in ovarian hormone levels occurring during the oestrous cycle of the guinea-pig can modify the levels of catecholamines within the longitudinal myometrium, those within the costo-uterine muscle are unaffected. This difference may be due, in part, to the differences in the innervation of the two tissues, since, unlike the costo-uterine muscle, the longitudinal myometrial layer receives a proportion of its sympathetic innervation via short adrenergic nerves which are susceptible to the influence of ovarian steroids (see review by Marshall, 1981). We therefore considered it of interest to establish whether or not responses mediated via adrenoceptors in the costo-uterine muscle are subject to regulation by ovarian steroids.

Preliminary accounts of the findings of this study have been communicated to the Australian Physiological and Pharmacological Society (Story, Hartley & Pennefather, 1980) and to the 8th International Congress of Pharmacology (Hartley, Story & Pennefather, 1981).

## Methods

### *Animals*

Adult virgin female guinea-pigs of the Dunkin Hartley strain, weighing 400 to 800 g were housed in runs at 22°C with a photoperiod of 12 h light:12 h dark. Oestrous cycles were monitored by daily examination of the vaginal closure membrane and the use of vaginal smears as described previously (Farrar *et al.*, 1980). Mean cycle length of these animals was $15.9 \pm 0.3$ days (number of cycles = 62, number of animals = 51).

Animals were allocated to nine treatment groups as described below. All oestradiol treatments started between days 7 and 11 of the oestrous cycle.

(1) (Day 10) guinea-pigs untreated, and used on their 10th cycle day.
(2) (Sham Day 10) sham ovariectomized, no steroid treatment, used on their 10th cycle day.
(3) (Ovx) ovariectomized at least 14 days before use, no steroid treatment.
(4) (Intact + P) treated with progesterone, 3 mg/animal s.c. daily for 4–5 days, starting day 1 of the cycle.
(5) (Ovx + O) ovariectomized, treated with 20 μg/kg oestradiol cypionate in peanut oil, s.c. every 2–3 days for at least 14 days.
(6) (Intact + O) non-ovariectomized, treated with oestradiol cypionate as in (5).
(7) (Ovx + O + P) ovariectomized, treated with oestradiol cypionate as described above, and in addition given progesterone 3 mg/animal s.c. daily for 4–5 days before use.
(8) (Intact + O + P) non-ovariectomized, treated with oestradiol cypionate and progesterone as in (7).
(9) (Post-partum) guinea-pig 1–4 days post-partum, no steroid treatment.

The operative procedures employed in performing the ovariectomies and sham-ovariectomies have been described previously (Farrar *et al.*, 1980).

### *Preparations*

Animals were killed by cervical dislocation, and the costo-uterine muscles and both uterine horns were removed. Each uterine horn was slit open by a longitudinal incision and carefully separated into the outer longitudinal and inner circular muscle layers. The endometrium was removed from the circular layer. Except in the case of animals under treatment regime (3), (Ovx; no steroid treatment), a longitudinal strip, 5 mm in width was cut from the layer of longitudinal myometrium from each horn, and a strip about 1 cm square was taken from the central region of each circular myometrial layer. Animals from treatment group (3) exhibited considerable atrophy of the uterine horns, and thus the entire layer of the longitudinal myometrium was used rather than a strip. The myometrial and costo-uterine muscle preparations were suspended in 30 ml organ baths, so that the muscle fibres were oriented vertically, under an initial tension of 1 g (500 mg for preparations from treatment group (3)). The bathing solution was maintained at 37°C, and had the following composition ($mmol\,l^{-1}$): NaCl 120, KCl 5, $CaCl_2$ 2.5, $NaHCO_3$ 25, $NaH_2PO_4$ 1, $MgSO_4$ 1, glucose 11, sucrose 10, disodium edetate 0.03 and sodium ascorbate 0.09. The solution was bubbled with 5% $CO_2$ in $O_2$.

Contractions were recorded isometrically using Grass FTO3 transducers and recorded on Grass 7B or Grass 79C polygraphs.

The smooth muscle within the preparations was field-stimulated using bipolar platinum or stainless steel electrodes. Stimulation using Grass S88, or S44 stimulators was applied as 5 s trains of pulses every 100 s throughout the experiment (30 Hz, 2 ms, voltage just submaximal). Each preparation was allowed to equilibrate for at least 1 h, and no drugs were added until the preparation responded with reproducible contractile responses to the stimulation. At that time, any spontaneous contractile activity had either ceased or occurred only in the form of bursts of contraction at intervals of 10 min or greater. In the majority of preparations the spontaneous bursts could be clearly distinguished from drug-induced responses. In a small number of preparations it was not possible to distinguish phenylephrine-induced from spontaneous contractions, and these preparations were abandoned.

Each preparation was exposed to either isoprenaline or phenylephrine by the sequential addition of increasing concentrations of agonist, to establish a concentration-response relationship. Agonists remained in contact with the tissue for 4–6 min. The mean magnitude of three stimulation-induced contractions immediately before drug addition was taken as the control stimulation-induced contraction. This was compared with the magnitude of the smallest stimulation-induced contraction in the presence of an inhibitory agonist, and the response expressed as percentage inhibition of contraction. The maximal tension developed in the presence of an excitatory agonist was compared with the control stimulation-induced contraction, and the response expressed as increase in tension (g).

When a concentration-response relation had been established for either isoprenaline or phenylephrine, the preparation was exposed to propranolol ($1\ \mu mol\ l^{-1}$) or phentolamine ($1\ \mu mol\ l^{-1}$) respectively, for at least 30 min, and then further responses to the agonist were obtained in the presence of the antagonist. Reduction or reversal of the responses confirmed in a qualitative way that they were mediated by β- or α-adrenoceptors respectively.

*Drugs*

(−)-Phenylephrine hydrochloride (ICN Pharmaceuticals Inc.) and (−)-isoprenaline bitartrate (Sigma) were made up daily as stock solutions containing NaCl 154 mmol $l^{-1}$, $NaH_2PO_4$ 1 mmol $l^{-1}$ and ascorbic acid 0.2 mmol $l^{-1}$.

Propranolol hydrochloride (ICI Aust. Ltd.) and phentolamine hydrochloride (Ciba-Geigy) were made up in distilled water. 17-β-Oestradiol-cyclopentyl-proprionate (Hoechst) and progesterone (Calbiochem) were made up weekly in peanut oil and kept in the dark.

*Analysis of concentration-response data*

Individual log concentration-response curves were plotted, and each graph was used to determine the concentrations of agonists producing 'threshold' (≃10% change in the height of electrically evoked contractions), maximal response observed (x-max) and 50% of maximal response ($EC_{50}$); together with the magnitude of the maximal effect (y-max). All measures of agonist potency are expressed as negative logarithms of the respective concentration throughout the results.

*Statistical analysis*

Statistical analysis of the measures for each agonist in each treatment group was by one-way analysis of variance. Where a significant heterogeneity was demonstrated ($P<0.05$), further analysis was undertaken to compare the effects of pairs of treatments using paired or non-paired Student's $t$ tests as appropriate and adopting $P<0.05$ as the criterion of statistical significance.

## Results

*Spontaneous activity and response to electrical stimulation*

Most preparations initially exhibited spontaneous activity; this was observed least frequently with circular myometrial preparations. The longitudinal myometrial and costo-uterine muscle preparations responded with regular contractions to field stimulation for periods of 4–6 h. Stimulation-induced contractions of the circular muscle preparations diminished with time, and experiments were therefore terminated after 2 h.

Neither propranolol ($1\ \mu mol\ l^{-1}$) nor phentolamine ($1\ \mu mol\ l^{-1}$) inhibited the stimulation-induced contractions; previous experiments in this laboratory showed that guanethidine ($10\ \mu mol\ l^{-1}$) was also without effect upon the contractions.

Table 1 shows the mean magnitudes of tension of the three electrically-evoked contractions recorded immediately before the first addition of agonist, for preparations from each of the treatment groups. Since the magnitude of contractions in preparations taken from intact and sham-operated animals on day 10 of the oestrous cycle (groups 1 and 2) were similar, these data from these groups were pooled to serve as a control group for comparison with the remaining experimental groups.

**Table 1** Mean tension developed in response to electrical stimulation (30 Hz, 2 ms, voltage just submaximal), and mean tissue weights* for preparations from different treatment groups

| Treatment group | *Longitudinal myometrium* | | | | *Circular myometrium* | | | | *Costo-uterine muscle* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Mean tension* | | *Mean wt* | | *Mean tension* | | *Meat wt* | | *Mean tension* | | *Mean wt* | |
| | (g) | (*n*) | (mg) | (*n*) | (g) | (*n*) | (mg) | (*n*) | (g) | (*n*) | (mg) | (*n*) |
| 1. Day 10 | 1.00±0.14 | (14) | 39±7 | (4) | 0.45±0.08 | (14) | 62±6 | (3) | 1.69±0.38 | (9) | 20±2 | (4) |
| 2. Sham day 10 | 0.79±0.25 | (8) | — | | 0.24±0.07 | (7) | — | | 1.44±0.44 | (8) | — | |
| 3. Ovx | 0.53±0.11 | (15) | — | | 0.04±0.01 | (6)† | — | | 1.88±0.18 | (18) | 32±2 | (18)‡ |
| 4. Intact + P | 1.13±0.27 | (10) | — | | 0.35±0.07 | (10) | — | | 1.66±0.24 | (10) | — | |
| 5. Ovx + O | 2.25±0.56 | (14)† | 121±7 | (12)‡ | 0.25±0.82 | (11) | 168±13 | (12)‡ | 1.49±0.17 | (12) | 34±9 | (4) |
| 6. Intact + O | 4.45±0.62 | (10)† | 89±6 | (11)‡ | ND | | | | ND | | | |
| 7. Ovx + O + P | 3.75±0.72 | (14)† | 91±3 | (10)‡ | 0.15±0.04 | (16)† | 131±12 | (10)‡ | 1.69±0.17 | (17) | 33±5 | (6) |
| 8. Intact + O + P | 3.68±0.75 | (9)† | 104±7 | (12)‡ | 0.08±0.02 | (9)† | 118±7 | (12)‡ | 1.75±0.24 | (10) | 39±5 | (4)‡ |
| 9. Post-partum | 0.99±0.35 | (13) | 457±75 | (4)‡ | 0.36±0.08 | (13) | 353±81 | (4)‡ | 1.74±0.18 | (16) | 47±6 | (4)‡ |

*Whole costo-uterine muscles were used; longitudinal and circular myometrial preparations taken as described in Methods. Approximately half the longitudinal layer was used, except for preparations from ovariectomized animals, where the whole layer was used, and for the post-partum group, where a smaller proportion of the longitudinal and circular layers was taken so the dimensions were similar to those of preparations from the other groups.
ND = not determined. *n* = number of experiments. Values given are mean ± s.e.mean.
† and ‡ significantly different from control (groups 1 and 2) and day 10 (group 1) respectively, *t* test, $P < 0.05$.

There was no significant effect of treatment upon the mean magnitude of stimulation-induced contraction, or in mean tissue weight of costo-uterine muscles. This was not the case for preparations of longitudinal myometrium in which electrical stimulation evoked particularly large contractions in oestrogen-primed preparations (Groups 5–8). This may be due to myometrial hypertrophy (Table 1), which was clearly apparent from inspection of the whole uterine horns of these animals. The contractions induced by stimulation of preparations from post-partum animals (which were the largest preparations used, see Table 1) did not significantly exceed those of tissues from control animals (Groups 1 and 2).

Circular myometrial preparations from the different treatment groups also showed differences in mean magnitude of evoked contractions, however, these were relatively small and no consistent pattern related to the type of treatment could be discerned.

### *The effects of treatments on responses to agonists*

The results of the analyses of variance to determine the influence of the various treatments upon the concentration-response data are summarized in Table 2.

#### *Longitudinal myometrial layer*

*Phenylephrine* There were clearcut qualitative differences, associated with the hormonal status of the animal, in the response of longitudinal muscle preparations to phenylephine.

This agonist enhanced electrically evoked contractions in preparations from animals which were not exposed to exogenous steroids (Groups 1–3), and from non-ovariectomized guinea-pigs treated with oestrogen alone (Group 6). Excitatory responses to phenylephrine also predominated in tissues from ovariectomized animals treated with oestrogen alone (Group 5), although in two of these there were weak inhibitory responses to a single concentration of phenylephrine ($10\,\mu mol\,l^{-1}$). In preparations from intact animals treated with progesterone alone (Group 4), phenylephrine was excitatory and more potent than in preparations taken from animals on their 10th cycle day (Table 3). Phentolamine consistently antagonized the excitatory responses to phenylephrine. In 13 of 18 preparations this occurred with a concentration of $1\,\mu mol\,l^{-1}$, and in the remainder with a concentration of $10\,\mu mol\,l^{-1}$.

Phenylephrine consistently inhibited electrically-evoked contractions in all preparations from the experiment groups (7, 8 and 9) in which exposure to moderate levels of progesterone followed a period of priming with oestrogen of either exogenous or endogenous origin. Three of the seven post-partum

**Table 2** Results of one-way analysis of variance of concentration-response data (to determine whether there is any significant heterogeneity of variance) for treatment groups

| | *Longitudinal myometrium* | | *Circular myometrium* | | *Costo-uterine muscle* | |
|---|---|---|---|---|---|---|
| | Iso | PE | Iso | PE | Iso | PE |
| Threshold | $P<0.01$ | *$P<0.01$ | NS | NS | $P<0.01$ | $P<0.01$ |
| $EC_{50}$ | $P<0.01$ | $P<0.01$ | NS | NS | $P<0.01$ | $P=0.052$ |
| x-max | $P<0.01$ | $P<0.01$ | NS | NS | $P<0.05$ | $P<0.01$ |
| y-max | $P<0.01$ | ** | $P<0.01$ | $P<0.01$ | $P<0.01$ | $P<0.01$ |

*ANOVA on all thresholds including groups where PE was inhibitory. ANOVA excluding groups where PE was inhibitory showed no significant difference due to treatment.

** Since responses of different groups were different qualitatively it was deemed inappropriate to compare these (for groups where PE was excitatory, ANOVA showed no significant difference).

The concentration-response data for day 10 and sham ovariectomized day 10 animals were not significantly different (*t* test, $P > 0.05$) and thus results for these two groups were pooled and served as a control for comparison with the other experimental groups, with two exceptions, see footnotes to Tables 3 and 4.

guinea-pigs (Group 9) used in this study were used following their first pregnancy. The remainder of the animals were multiparous. Inhibitory responses to phenylephrine were observed in preparations from both groups of animals. Phenylephrine produced inhibition of contractions in longitudinal myometrial preparations from both parturient and non-parturient horns of two guinea-pigs from the multiparous group in which the pregnancy had been unilateral.

Concentrations of phenylephrine producing inhibition in oestrogen-primed, progesterone-treated animals were lower than those required to produce excitatory effects in preparations in control groups (Groups 1 and 2; Table 3). Propranolol ($1\ \mu mol\ l^{-1}$) reversed the inhibitory effects of phenylephrine.

*Isoprenaline* Isoprenaline was consistently inhibitory on preparations from all experimental groups (Table 3). It was least potent in producing threshold inhibition in the four groups in which oestrogen levels were lowest (i.e. in Groups 1 and 2; following ovariectomy (Group 3); and after treatment with progesterone alone (Group 4)). Exposure of oestrogen-primed animals to progesterone (Groups 7–9) led to an approximately 100 fold increase in the threshold potency of isoprenaline compared to Groups 1 and 2. Similar trends are discernible in the $EC_{50}$ (Table 3) and in the x-max values. Furthermore, the magnitude of the mean maximum inhibition produced by isoprenaline was greatest in tissues following combined hormonal exposure (Groups 7, 8 and 9; Table 4).

Propranolol ($1\ \mu mol\ l^{-1}$) usually reduced and occasionally reversed the effects of isoprenaline. High concentrations of isoprenaline ($50\ \mu mol\ l^{-1}$ or greater) in the absence of propranolol produced excitatory responses, which were antagonized by phentolamine ($1\ \mu mol\ l^{-1}$).

In preparations from experimental groups in which both agonists were inhibitory, isoprenaline was significantly more potent, by a factor of approximately 1000 fold, than phenylephrine in producing a threshold inhibition of contraction ($P<0.001$, paired *t* test, d.f. = 14). (Table 3).

*Circular myometrial layer*

Phenylephrine consistently enhanced and isoprenaline consistently inhibited evoked contractions of preparations from all treatment groups. There was no reversal of the excitatory action of phenylephrine, in preparations from the oestrogen-primed groups exposed to progesterone, as there was in the corresponding preparations of longitudinal myometrium (Table 3).

Only 4 of 16 circular myometrial preparations taken from ovariectomized animals (Group 3), responsed consistently to field stimulation. Three were exposed to isoprenaline, which inhibited the evoked contractions, while the remaining preparation was exposed to phenylephrine, which caused excitation.

Analysis of variance of the concentration-response data (Table 2) revealed that the mean threshold concentrations, $EC_{50}$ values, and maximal concentrations were not affected by treatment; however, there were significant influences due to treatment in the maximal responses to both agonists. The difference in the mean magnitude of the maximal response of tissues from ovariectomized, oestrogen-treated animals (Group 5), to phenylephrine significantly exceeded that of tissues taken from the control group (Group 2) (Table 4). Isoprenaline produced a significantly greater maximal inhibition in tissues from

**Table 3** Effect of treatments upon the concentration of agonist producing [a]threshold and [b]50% of maximal response ($EC_{50}$)

| *Treatment Group* | | *Longitudinal myometrium* Iso | PE | *Circular myometrium* Iso | PE | *Costo-uterine muscle* Iso | PE |
|---|---|---|---|---|---|---|---|
| 1 Day 10 | (a) | **8.4±0.5 (5)** | 5.3±0.6 (6) | **7.2±0.4 (6)** | 6.1±0.3 (7) | **7.9±0.2 (8)** | 6.4±0.2 (8) |
| | (b) | **7.5±0.5 (5)** | 4.5±0.5 (4) | **6.5±0.5 (6)** | 5.2±0.2 (7) | **6.9±0.2 (8)** | 5.4±0.3 (7) |
| 2 Sham day 10 | (a) | **7.3±0.4 (4)** | 4.6±0.4 (4) | **7.0 (2)** | 5.9±0.2 (4) | **7.9±0.5 (4)** | 6.3±0.2 (4) |
| | (b) | **6.7±0.2 (4)** | 4.2±0.4 (4) | **5.9 (1)** | 5.1±0.2 (4) | **7.0±0.4 (4)** | 5.6±0.6 (4) |
| 3 Ovx | (a) | **7.2±0.4 (7)** | 5.3±0.5 (5) | **7.8±0.8 (3)** | 6.0 (1) | **8.7±0.5 (6)** | 6.2±0.1 (9) |
| | (b) | **6.9±0.4 (7)** | 4.7±0.5 (5) | **6.5 (2)** | 4.4 (1) | **8.2±0.6 (5)*** | 5.6±0.1 (8) |
| 4 Intact + P | (a) | **7.5±0.1 (5)** | 6.5±0.2 (5) | **7.7±0.3 (3)** | 5.9±0.2 (5) | **8.4±0.4 (5)** | 6.2±0.4 (5) |
| | (b) | **7.3±0.1 (5)** | 6.0±0.2 (5)* | **7.0±0.1 (3)** | 5.5±0.1 (5) | **7.3±0.3 (5)** | 5.6±0.4 (5) |
| 5 Ovx + O | (a) | **8.4±0.2 (8)** | 4.4±0.5 (7) | **7.4±0.2 (4)** | 5.8±0.6 (5) | **7.0±0.5 (6)** | 5.5±0.3 (6)* |
| | (b) | **7.6±0.3 (8)** | 4.9±0.5 (3) | **7.6±0.2 (3)** | 5.1±0.3 (6) | **5.9±0.4 (5)*** | 4.9 (1) |
| 6 Intact + O | (a) | **8.9±0.2 (4)** | 5.6±0.4 (5) | ND | ND | ND | ND |
| | (b) | **8.0±0.2 (4)** | 4.5±0.4 (4) | ND | ND | ND | ND |
| 7 Ovx + O + P | (a) | **9.3±0.4 (8)*** | **6.2±0.4 (6)** | **8.9±0.6 (6)** | 5.2±0.2 (9) | **7.5±0.3 (9)** | 5.4±0.2 (9)* |
| | (b) | **8.2±0.4 (5)** | **6.0±0.5 (5)*** | **8.3±0.7 (5)** | 4.8±0.2 (7) | **7.2±0.3 (9)** | 5.1±0.1 (8) |
| 8 Intact + O + P | (a) | **9.7±0.3 (5)*** | **7.2±0.4 (5)*** | **7.5±0.3 (4)** | 5.9±0.4 (4) | **6.4±0.4 (5)*** | 5.1±0.3 (5)* |
| | (b) | **9.4±0.4 (5)*** | **6.7±0.4 (5)*** | **7.3 (2)** | 5.6±0.5 (3) | **6.0±0.4 (5)*** | 4.7±0.3 (5)* |
| 9 Post-partum | (a) | **9.5±0.2 (5)*** | **6.5±0.2 (5)*** | **8.0±0.7 (4)** | 5.7±0.6 (4) | **8.4±0.4 (7)** | 5.8±0.2 (8) |
| | (b) | **9.2±0.4 (4)*** | **6.4±0.4 (4)*** | **7.0±0.6 (3)** | 5.1±0.3 (4) | **7.4±0.4 (7)** | 5.0±0.2 (8) |

*significantly different from control (Groups 1 and 2) *t* test, $P<0.05$. ND = not determined. **Inhibitory response in bolt type.**
All values are expressed as mean negative log molar concentrations ± s.e.mean (*n*).

**Table 4** Effects of treatments upon the maximal response to agonists

| *Treatment group* | *Longitudinal myometrium* Iso | PE | *Circular myometrium* Iso | PE | *Costo-uterine muscle* Iso | PE |
|---|---|---|---|---|---|---|
| | Mean maximum response to Iso (*% inhibition*) and to PE (increase in force, g and *% inhibition*) | | | | | |
| 1 Day 10 | **47.9± 8.6 (5)** | 1.6± 0.2 (5) | **35.8± 9.7 (6)** | 0.6 ±0.2 (7) | **76.9± 7.0 (8)** | 2.7±0.5 (8)‡ |
| 2 Sham day 10 | **65.0±11.3 (4)** | 1.4± 0.2 (4) | **14.1 (2)** | 0.2 ±0.1 (4) | **56.8± 6.4 (4)** | 5.9±0.6 (4)† |
| 3 Ovx | **77.0± 3.8 (7)*** | 0.8± 0.1 (5) | **68.6±22.5 (3)** | 0.01 (1) | **55.6± 9.2 (5)** | 1.9±0.3 (9)‡ |
| 4 Intact + P | **49.0± 7.7 (5)** | 2.3± 0.4 (5) | **31.6±12.2 (3)** | 0.6 ±0.1 (5) | **75.0± 6.6 (5)** | 3.5±0.5 (5) |
| 5 Ovx + O | **82.0± 6.0 (8)*** | 3.3± 2.1 (4) | **40.2± 4.6 (4)** | 1.4 ±0.7 (5)* | **71.6±11.7 (5)** | 2.4±0.4 (3)‡ |
| 6 Intact + O | **77.1± 9.1 (4)** | 2.1± 0.3 (5) | ND | ND | ND | ND |
| 7 Ovx + O + P | **85.1± 4.5 (8)*** | **66.7±11.3 (6)** | **45.2± 5.6 (5)** | 0.1 ±0.03 (8) | **42.9± 5.8 (9)*** | 1.5±0.4 (8)‡ |
| 8 Intact + O + P | **90.4± 4.1 (5)*** | **71.0± 7.3 (5)** | **41.0±15.5 (3)** | 0.03±0.01 (4) | **43.4± 6.7 (5)*** | 0.5±0.1 (5)† |
| 9 Post-partum | **87.7± 4.7 (4)*** | **90.8± 2.2 (4)** | **83.4± 3.2 (4)*** | 0.3 ±0.1 (4) | **58.3± 7.4 (7)** | 1.8±0.4 (8) |

*significantly different from control (Groups 1 and 2), *t* test, $P<0.05$.
†significantly different from day 10 (Group 1) *t* test, $P<0.05$ (day 10 and sham day 10 not pooled, see footnote Table 2).
‡significantly different from sham day 10 (Group 2), *t* test, $P<0.05$.
All values mean ± s.e.mean (*n*).
**Inhibitory response in bold type.**

the post-partum group than in those from control animals.

Phenylephrine was significantly more potent in causing enhancement of threshold values in the circular than in the longitudinal myometrium from both sham ovariectomized animals, and from untreated animals on their 10th cycle day (Groups 1 and 2) ($P<0.05$, unpaired $t$test). A similar trend was observed with tissues from ovariectomized, oestrogen-treated animals (Group 5) but the differences did not achieve statistical significance ($0.05<P<0.10$). Phenylephrine was approximately equipotent in circular and longitudinal muscle preparations from animals treated with progesterone alone (Group 4).

In oestrogen-primed animals (Groups 5–8), the threshold potency of isoprenaline was greater in the longitudinal than in the circular myometrium ($P<0.05$, unpaired $t$test; Table 3). A similar trend was observed in preparations from post-partum animals (Group 9) but did not achieve statistical significance ($0.05<P<0.10$).

*Costo-uterine muscle*

Isoprenaline was inhibitory and phenylephrine was excitatory in all preparations of costo-uterine muscle. The nature of the excitatory effect of phenylephrine was qualitatively different in preparations taken from animals that were ovariectomized and then treated with oestrogen (Group 5); this difference was not observed in the other ovariectomized groups, and is therefore not a consequence of the operative procedure *per se*. Although there were changes in the potency of phenylephrine in preparations from all groups exposed to oestrogen (Table 3), these were small. In preparations from the intact oestrogen- and progesterone-treated animals (Group 7), the threshold sensitivity to isoprenaline was significantly less than in tissues taken from control animals (Table 3).

Phenylephrine was generally more potent on the costo-uterine muscle than on the uterine layers. Lower concentrations were required to produce threshold excitatory effects in the costo-uterine muscle preparations than in the longitudinal myometrial layer taken from the same animals ($P<0.05$, paired $t$test; see Table 3).

## Discussion

The present study of the effects of ovarian steroids upon the response of the guinea-pig uterus to adrenergic influences differs from earlier studies (Hermansen, 1960; Pennefather & Isaac, 1967; Isaac *et al.*, 1969; Rüsse & Marshall, 1970; Szurszewski & Bülbring, 1973; Elmer, Alm & Thorbert, 1980) in that the two myometrial layers have been studied separately. This major methodological modification has allowed us to establish that the administration of progesterone to oestrogen-primed animals leads, in both ovariectomized and intact animals, to a clearcut, reproducible reversal of the effects of phenylephrine, in the longitudinal, but not in the circular myometrial layer. This particular treatment regime is also associated with a marked increase in the sensitivity of longitudinal layer to the inhibitory β-adrenoceptor agonist, isoprenaline. Similarly, it appeared that the sensitivity to this agonist of the longitudinal myometrium from animals treated with oestrogen alone was also enhanced, though the effect was less marked. The reasons for the apparent tissue selectivity of the effects of steroids upon adrenoceptor-mediated responses are not clear from the present experiments. While doses of steroids higher than those used by us might produce changes in the responses to adrenoceptor agonists of the circular myometrium, it is of interest that in preliminary experiments in this laboratory (Adam, Hartley, Pennefather & Story, unpublished observations) we have established that the circulating levels of progesterone following the combined oestrogen-progesterone treatment regime used in the present study are well in excess of those occurring during the oestrous cycle (Croix & Franchimont, 1975) and indeed are within the range occurring during mid pregnancy (Challis, Heap & Illingworth, 1971). Furthermore, since the placenta is the major source of both oestrogen and progesterone during preganancy in this species, it follows that the circular myometrial layer taken from animals in the post-partum period, when the levels of circulating progesterone remain high (Challis *et al.*, 1971), will have been exposed to both steroids in concentrations at least as high as those reaching the longitudinal layer. Nevertheless, even in preparations from post-partum animals the changes seen were confined to the longitudinal layer.

It has been reported that the two muscle layers of bicornuate and duplex uteri have differing embryological origins (Sobotta, 1891); the longitudinal myometrial layer may develop from the serosa, while the circular layer is derived earlier from the Müllerian duct. This difference may underlie the selective effect of steroid hormones upon the longitudinal layer of the duplex uterus of the guinea-pig. Similarly, in myometrial preparations taken from the rat and the rabbit, the actions of adrenoceptor agonists, and the influence of pregnancy and steroids upon these responses, are different in the two myometrial layers (Nesheim, 1972; 1974; Kawarabayashi & Osa, 1976; Chow & Marshall, 1981).

We have recently found that the oestradiol/progesterone regime used in the present study also

leads, in the longitudinal myometrium, to a selective reversal of the effects of noradrenaline and adrenaline such that their actions at β-adrenoceptors are dominant (Adam, Story, Hartley & Pennefather, 1982). Moreover, these two amines, like phenylephrine, also inhibit electrically-evoked contractions of longitudinal myometrium from post-partum guinea-pigs (Story, Hartley & Pennefather, 1983). These observations, together with those described here, lead us to suggest that exposure of the longitudinal myometrium to progesterone after priming with endogenous or exogenous oestrogen may affect the numbers or affinity of adrenoceptors and/or stimulus effect coupling following agonist-receptor combination. Precedent for such an effect of combined administration of oestradiol and progesterone upon uterine adrenoceptors has been provided by radioligand binding studies using myometrial membranes from the rabbit (Roberts, Insel, Goldfien & Goldfien, 1977; Williams & Leftkowitz, 1977; Hoffmann, Lavin, Lefkowitz & Ruffolo, 1981; Roberts, Insel & Goldfien, 1981). These indicate that oestrogen increases, but that co-administration of progesterone decreases, the numbers of α-adrenoceptor binding sites in the myometrium. However, the myometrial layers were not studied separately in these experiments.

The possibility that steroids increase the affinity or numbers or alter the subtype of β-adrenoceptors in the longitudinal myometrium has not yet been established, but may explain the marked increase in the potency of isoprenaline which we have observed with longitudinal myometrium from doubly treated and post-partum animals. It has, however been established that high concentrations of oestradiol inhibit the extraneuronal uptake of noradrenaline (Iversen & Salt, 1970). This action may have contributed to the increase seen in the potency of isoprenaline in longitudinal myometrium from all groups of animals treated with this steroid (Table 3). However, such an effect would also be expected to produce corresponding increases in the potency of isoprenaline in circular myometrium and in costo-uterine muscle. This was not observed (Table 3).

In the guinea-pig, pregnancy induces degeneration of uterine short adrenergic neurones (Gårdmark, Owman & Sjöberg, 1971; Owman & Sjöberg, 1973; Bell & Malcolm, 1978; Thorbert, Alm, Owman, Sjöberg & Sporrong, 1978; Owman, 1981). This effect, together with an effect of high circulating levels of oestrogen and progesterone causing a functional loss of α-adrenoceptors, such as that reported here, would protect the longitudinal myometrium from excitation arising from sympathetic activation during pregnancy. In contrast to the effects of steroid treatment upon the longitudinal myometrium of the guinea-pig uterus there were no marked steroid-induced or post-partum changes in the response of the costo-uterine muscle to adrenoceptor agonists, although this tissue is anatomically continuous with the longitudinal myometrium. This may reflect the fact that the costo-uterine muscle, is developmentally older than the uterine layer (Mossman, 1977).

The costo-uterine muscle provides the entrance for that portion of the uterine innervation which is via long rather than short neurones, and which is restricted to the tubo-uterine region (Thorbert, 1978; Owman, 1981). It has been suggested that smooth cells in the tubo-uterine junction may provide a uterine pacemaker (Alvarez & Caldeyro-Barcia, 1953; Owman, 1981). Noradrenaline levels within this region, unlike those in the body of the uterus do not change during the oestrous cycle (Farrar *et al.*, 1980), following the administration of oestrogen and progesterone (Handberg, unpublished observations), during pregnancy (Thorbert, 1978) or in the post-partum period (Thorbert, 1978; Hartley, Story, Farrar & Pennefather, 1983). Thus it is possible that neuronal and hormonal sympathetic activation may initiate contractions via effects upon α-adrenoceptors located on these pacemaker cells, irrespective of reproductive status.

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