Brit. J. Pharmacol. (1966), **26**, 678–685.

RESPONSE OF THE MAMMALIAN UTERUS TO PROSTAGLANDINS UNDER DIFFERING HORMONAL CONDITIONS

BY

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(Received November 26, 1965)

The biological actions of prostaglandins of the E and F series on a variety of smooth muscle have been reported on (Horton & Main, 1963, 1965 ; Horton, 1965 ; Pickles, Hall, Clegg & Sullivan, 1966). However, the effect of oestrogens and progesterone on the response of uterine smooth muscle to prostaglandins has not been extensively studied, and such results as have been published have often been conflicting (Änggård & Bergström, 1963 ; Best & Pickles, 1963 ; Bygdeman, 1964). It therefore seemed to be of interest to extend this work further.

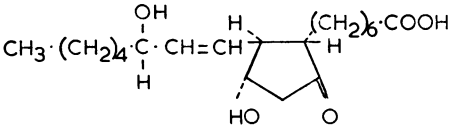
METHODS

Prostaglandins E_1 and $F_{2\alpha}$ were used in these experiments. Their formulae are shown in Fig. 1. Virgin female guinea-pigs of 450–500 g and rats of 170–190 g were used, and the uterine horns were studied in dioestrous or under the influence of oestrogens when the animals were in natural oestrus. The hormonal state of the animals was assessed by vaginal cytology, and those animals which were in natural oestrus were given oestradiol benzoate by subcutaneous injection 24 hr before experiments, 50 μg to guinea-pigs and 20 μg to rats. Each uterine horn was set up in a perspex organ bath of 5 ml. capacity. Two silver wire electrodes were fixed into grooves inside the organ bath and connected to leads from a stimulator. A modified Krebs solution at 30° C of the following composition was used (mM/l.): NaCl 123.1, $NaHCO_3$ 26.2, KCl 4.97, $MgSO_4$ 3.54, KH_2PO_4 12.5, $CaCl_2$ 0.27. Dextrose 1 g/l. was added. Gas (5% CO_2 in oxygen) was bubbled through the organ bath during experiments. The low calcium concentration in the Krebs solution and the comparatively low temperature ensured that spontaneous activity was abolished. In all experiments the myometrium was stimulated at 50 c/s sinusoidal a.c., the optimal stimulus strength being 15V for 5 sec repeated at intervals of 1 min. The apparatus used for measuring uterine tension has been previously described in detail (Styles & Sullivan, 1962 ; Sullivan, 1963). Briefly, isometric contractions were recorded with a differential capacitance transducer, the output voltage from which was coupled to a servo recorder, from which a kymograph tracing was obtained, and to an integrator motor. By integrating uterine tension in g against time in sec, the area under the curve of the kymograph tracing was measured and the results expressed as integrated tension in g sec. In all experiments doses of 0.2 ml. were left in the organ bath for 180 sec and the tension developed recorded on the integrator.

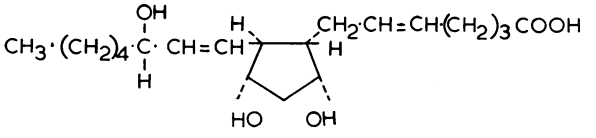
Progesterone in aqueous solvent (Primolut, Schering A.G. Berlin) was added to the organ bath in some experiments.

RESULTS

A possible qualitative difference between the responses of the guinea-pig uterus to prostaglandins E_1 and $F_{2\alpha}$ became apparent in these experiments. It was found that after washing out any dose of E_1, the response of the uterus to electrical stimulation was considerably greater than during the control period. The duration of this enhanced



E_1: 11a, 15-dihydroxy-9-oxo-prost-13-enoic acid



$F_{2\alpha}$: 9a, 11a, 15-trihydroxy-prost-5,13-dienoic acid

Fig. 1. The formulae of prostaglandins E_1 and $F_{2\alpha}$.

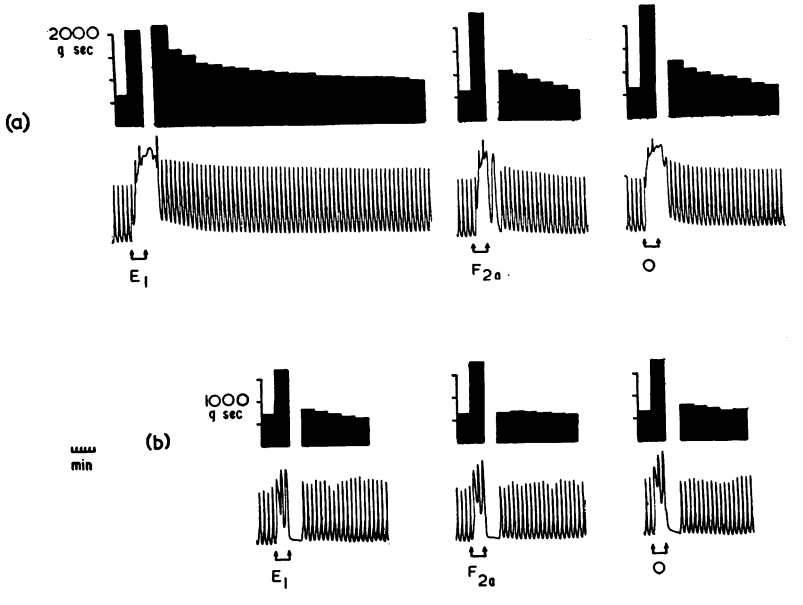


Fig. 2. Effect of approximately equi-active concentrations of prostaglandins E_1 and $F_{2\alpha}$ and of oxytocin (O) on the response of (*a*) guinea-pig uterus and (*b*) rat uterus to electrical stimulation. The histograms above the kymograph tracings record integrated tension measured continuously for successive three min periods, except during the period of washing out a dose, when electrical stimulation was stopped. In (*a*) $E_1=5$ ng prostaglandin E_1/ml.; $F_{2\alpha}=50$ ng prostaglandin $F_{2\alpha}$/ml. and O=0.4 mU oxytocin/ml. In (*b*) $E_1=100$ ng prostaglandin E_1/ml.; $F_{2\alpha}=20$ ng prostaglandin $F_{2\alpha}$/ml. and O=1.0 mU oxytocin/ml.

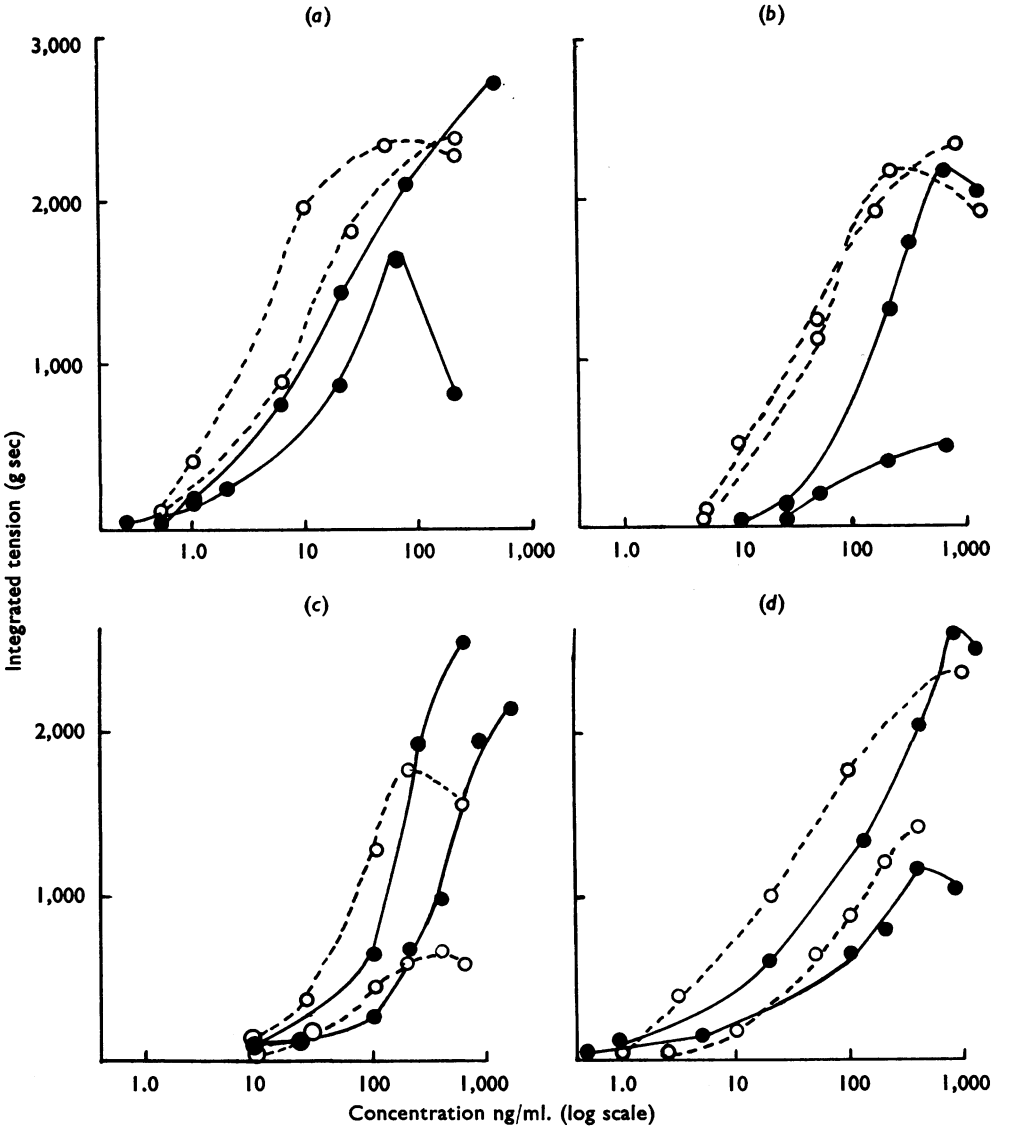


Fig. 3. Log concentration-response curves for prostaglandins using uteri from dioestrous animals (dotted lines and open circles) and animals in oestrous pre-treated with oestradiol benzoate (continuous lines and dots): (*a*) guinea-pig, E_1; (*b*) guinea-pig, $F_{2\alpha}$; (*c*) rat, E_1; (*d*) rat, $F_{2\alpha}$.

response was proportional to the concentration of E_1 in the organ bath. In Fig. 2 kymograph tracings are shown of the responses of rat and guinea-pig dioestrous uteri to concentrations of prostaglandins E_1 and $F_2\alpha$ and to oxytocin from about the middle of their dose-response curves. The relative concentrations used were such that they produced approximately equal effects on the myometrium. On the rat uterus the concentrations were 100 ng E_1/ml., 20 ng $F_2\alpha$/ml. and 1.0 mU oxytocin/ml., and on the guinea-pig uterus they were 5 ng E_1/ml., 50 ng $F_2\alpha$/ml. and 0.4 mU oxytocin/ml. The histo-

grams above the tracings record the integrated tension measured continuously for 3 min periods, except during the washing out period when electrical stimulation was stopped. It will be seen that though there was a prolonged increase in the response of the guinea-pig uterus to electrical stimulation after washing out prostaglandin E_1, this did not occur after $F_{2\alpha}$ or oxytocin. This result was repeatedly observed in twelve experiments using the dioestrous uterus. The oestrogen dominated uterus gave the same response. In the case of the rat uterus, though repeated attempts were made to reproduce this effect, it was observed in only one experiment out of fifteen when a very high concentration of 1.6 μg E_1/ml. was used.

The uterine horn of a guinea-pig is larger and thicker than that of a rat, and this being so it might be argued that it would be easier to wash out a dose of prostaglandin E_1 from the latter than from the former. This might account for the persistent after-effects of E_1 being observed with the guinea-pig uterus but not with the rat uterus. In order

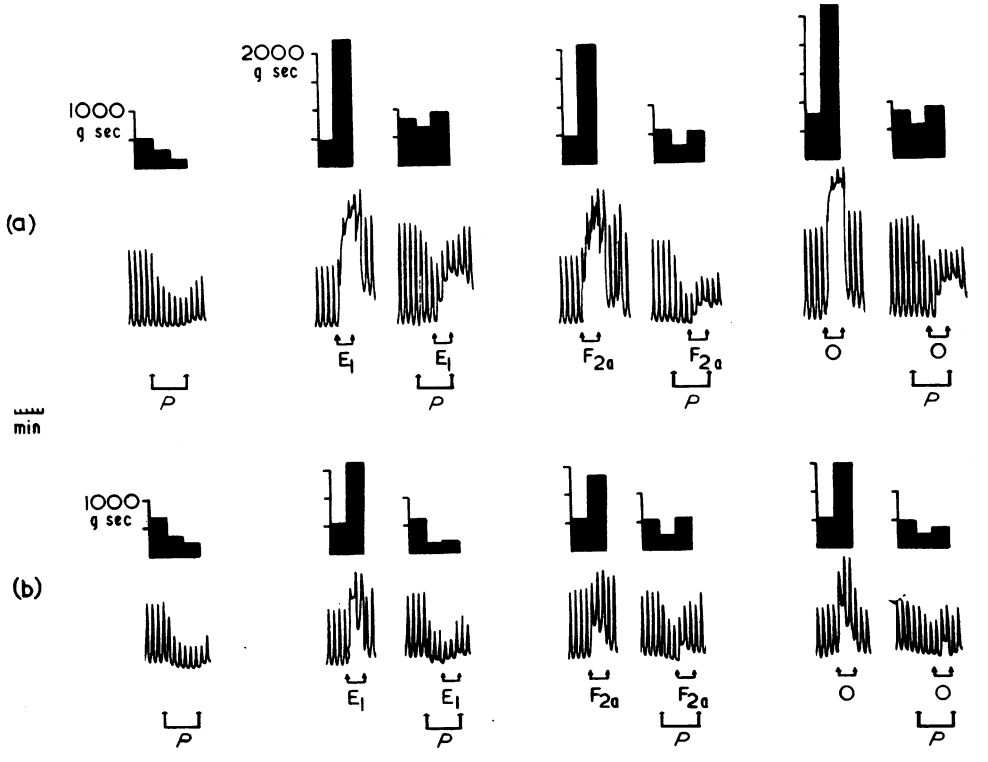


Fig. 4. Effect of progesterone on the response of (*a*) guinea-pig uterus and (*b*) rat uterus. Histograms above the kymograph tracings show the integrated tension recorded for periods of 3 min. The effect on the response of the uterus to electrical stimulation of leaving progesterone (P) in the organ bath for two 3 min periods is shown on the left. The remaining kymograph tracings show the effects of prostaglandins E_1 and $F_{2\alpha}$ and of oxytocin (O) added to the organ bath before and in the presence of progesterone. In (*a*) P=40 μg progesterone/ml.; E_1=5 ng prostaglandin E_1/ml.; $F_{2\alpha}$=50 ng prostaglandin $F_{2\alpha}$/ml. and O=0.4 mU oxytocin/ml. In (*b*) P=20 μg progesterone/ml.; E_1=100 ng prostaglandin E_1/ml., $F_{2\alpha}$=20 ng prostaglandin $F_{2\alpha}$/ml. and O=1.0 mU oxytocin/ml.

to test this possibility, the response of the guinea-pig uterus to E_1 was studied after trimming the horns to approximately the same weight and dimensions as a rat uterine horn. The phenomenon was still present exactly as with the intact horn.

Influence of oestrogen on the response to prostaglandins

Using rat and guinea-pig uteri both in oestrous and dioestrous, dose-response curves were constructed for prostaglandins E_1 and $F_{2\alpha}$ (Fig. 3). In case the control response to electrical stimulation of the dioestrous uterus differed consistently from that of the oestrogen treated uterus, this was always measured immediately before adding a dose of prostaglandin to the organ bath. The figures for integrated tension in response to prostaglandins plotted on the graphs were obtained by subtracting the control figure immediately before each dose from that recorded with the dose in the organ bath, thus eliminating from the results any differences between control responses. In retrospect this was not strictly necessary, since the mean integrated tension during control periods $\pm$S.E. using oestrogen treated guinea-pig uteri was 655 ± 55 g sec, and using dioestrous horns 555 ± 50 g sec. With rat uteri, the mean control using oestrogen treated uteri was 155 ± 15 g sec and with dioestrous uteri 160 ± 15 g sec. It will be seen that the guinea-pig uterus was more sensitive to prostaglandin E_1 than to $F_{2\alpha}$, the threshold concentration of the former being 0.25 or 0.5 ng/ml. and of the latter about 5 ng/ml. The reverse was the case when the rat uterus was used, the threshold concentration of E_1 being about 10 ng/ml. and of $F_{2\alpha}$ 0.5 or 1 ng/ml. There were wide variations in the dose-response curves obtained, and it was not possible to ascertain whether the slopes for E_1 and $F_{2\alpha}$ differed significantly. Further experiments, though desirable, could not be undertaken owing to the scarcity of samples of prostaglandins available for experiment. The influence of oestrogens upon the response of the myometrium to the prostaglandins appeared to be only marginal, though in the case of the guinea-pig the dioestrous uterus may have been more sensitive than the oestrogen dominated uterus, especially to prostaglandin $F_{2\alpha}$.

Influence of progesterone on the response to prostaglandins

Progesterone was added to the organ bath in sufficient concentration to cause depression, but not abolition, of the response of the dioestrous uterus to electrical stimulation. In the case of the guinea-pig uterus the concentration used was 40 μg/ml.; with the rat uterus 20 μg/ml. Using these concentrations of progesterone, the responses to prostaglandins E_1 and $F_{2\alpha}$ and to oxytocin for comparison were measured before and in the presence of progesterone. The results of two such experiments, one with each species, are shown in Fig. 4. When progesterone was left in the organ bath for two 3 min periods, the response of the uterus to electrical stimulation was successively depressed compared to the preceding control period of 3 min. Approximately equi-active doses of the prostaglandins and oxytocin were added to the organ bath before and during the second 3 min period with progesterone in the organ bath. It will be seen that in the presence of progesterone the responses to all the agents added to the organ bath were substantially depressed. Four experiments were done with the uteri of each species, and the results were similar. Taking the results of all experiments into consideration, there did not appear to be any difference in the degree to which the responses of prostaglandins and oxytocin were depressed.

DISCUSSION

In these experiments the electrically stimulated, rather than the spontaneously acting, uterus was used because it is often difficult to distinguish between the effects of small doses of drugs and spontaneous contractions. Furthermore, after washing out a dose it is easier to assess when the myometrium has returned to a steady state.

It is difficult to explain the persistent enhancement of the response of the guinea-pig uterus to electrical stimulation after washing-out doses of prostaglandin E_1. Clegg, Hall & Pickles (1965) have reported enhancement of the response to vasopressin after washing out doses of prostaglandins E_1 and E_2 from the guinea-pig uterus. They found that during the period in which enhancement occurred the myometrium showed no consistent changes in electrical activity or resting tension, such as might have been expected if the simplest explanation is true ; namely that E_1, though not $F_{2\alpha}$, persists at its site of action and continues to excite the cell membrane during the period of enhancement. They postulated that in some non-specific way prostaglandins of the E series might facilitate the coupling of excitation of the cell membrane and intracellular contraction of actomyosin. There appears to be no explanation as to why this phenomenon should appear with the guinea-pig but not the rat uterus. Mechanical causes due to the differing sizes of the uterine horns in these species appear to have been excluded by the experiments described above.

Differences in the relative sensitivity of the rat and guinea-pig uterus to prostaglandins of the E and F series similar to those observed above have been reported by other workers (Bergström, Eliasson, Euler & Sjövall, 1959 ; Pickles & Hall, 1963 ; Horton & Main, 1965). The relative threshold concentration reported above for E_1 and $F_{2\alpha}$ on the uteri of the two species are in general agreement with those found by Swedish workers (Änggård & Bergström, 1963 ; Eliasson, 1963), though Horton & Main (1965) were unable to confirm a threshold concentration for $F_{2\alpha}$ as low as 1 ng/ml., using the rat uterus. They reported a threshold concentration of 25–50 ng/ml. and suggested that the exact stage of the oestrous cycle might be critical for high sensitivity.

Änggård & Bergström (1963) reported that pre-treatment of the rat with oestrogen increased the sensitivity of the uterus to prostaglandin $F_{2\alpha}$ from ten to fifty times. On the other hand Best & Pickles (1963) found that oestrogen tended, if anything, to diminish the sensitivity of the guinea-pig uterus to prostaglandins of the E series. Bygdeman (1964), using isolated strips of human myometrium, reported that oestrogen had no significant effect upon the response to prostaglandin E_1, which exerts an inhibitory action upon the human myometrium however, whereas it has a spasmogenic effect on the rat and guinea-pig uterus. In the experiments described above the effect upon the response of the uterus to prostaglandins of pre-treating the animals with oestrogen appears to have been negligible. In this respect the prostaglandins do not necessarily differ from other uterine stimulants. It has been shown, for instance, that the sensitivity of the rat uterus to 5-hydroxytryptamine is greatly increased by oestrogens (Robson, Trounce & Didcock, 1954). They found that ovariectomy diminished the sensitivity of the rat uterus to 5-hydroxytryptamine, but that it could be fully restored by treating the animals with oestrogens. Fitzpatrick (1961), however, reported that pre-treatment of ovariectomized rats with oestrogens did not induce adequate uterine sensitivity for oxytocin assays. He

obtained greater sensitivity by pre-treating intact rats with oestrogens, but even so more than half of the uteri were rejected as being too insensitive.

There is general agreement that progesterone reduces or abolishes uterine activity and the response to oxytocin (Pose & Fielitz, 1961). It has been shown by Marshall & Csapo (1961) that progesterone hyperpolarizes the cell membrane and lowers its excitability, thus reducing uterine sensitivity to stimulants, which would account for the depression of the response to prostaglandins described above. The only other report found in the literature describing the effect of progesterone upon the response to prostaglandins is that of Bygdeman (1964). He found that the effect of prostaglandin E_1 on strips of isolated human myometrium might be reduced or increased by adding progesterone to the organ bath, according to the stage of the menstrual cycle. But since prostaglandin E_1 has an inhibitory action on the human uterus, his results may not be comparable to those reported in this paper.

SUMMARY

1. The response of the electrically stimulated rat and guinea-pig uterus *in vitro* to prostaglandins E_1 and $F_{2\alpha}$ was studied under differing hormonal conditions.

2. In both species there was no significant difference in sensitivity to the prostaglandins between the uterus from an animal in dioestrous and one from an animal in oestrous pre-treated with oestrogen.

3. In both species addition of progesterone to the organ bath caused depression of the excitatory effects of both prostaglandins and of oxytocin.

4. After washing out a dose of prostaglandin E_1 there was a persistent increase in the response of the guinea-pig uterus to electrical stimulation, which was not found after $F_{2\alpha}$ and which did not occur with the rat uterus.

5. The observation of other workers that the guinea-pig uterus is more sensitive to prostaglandin E_1 than to $F_{2\alpha}$, while the reverse is true of the rat uterus, was confirmed.

I should like to express my gratitude to Dr. V. R. Pickles of the Department of Physiology, the University of Sheffield, for samples of the prostaglandins, and to Professor R. S. Stacey for helpful advice and criticism during the preparation of this paper.

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