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PROSTAGLANDIN BIOSYNTHESIS BY GUINEA-PIG SKELETAL MUSCLE *in vitro* AND THE EFFECT OF OESTRADIOL-17β AND PROGESTERONE

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1 The effect of oestradiol and progesterone on prostaglandin biosynthesis by guinea-pig skeletal muscle *in vitro* has been investigated.

2 At high concentrations, oestradiol-17β (100 μg/ml) and progesterone (500 μg/ml) increased prostaglandin E and prostaglandin F synthesis (measured by radioimmunoassay).

3 It is suggested that these steroids are unlikely to be found *in vivo* at levels sufficient to influence prostaglandin synthesis by skeletal muscle in physiological and pathological situations.

Introduction

Oestrogen stimulates uterine prostaglandin synthesis in the guinea-pig both *in vitro* and *in vivo* but progesterone blocks this effect (Naylor & Poyser, 1975).

It is not known whether the effects of these steroids are exerted upon other tissues which are known to synthesize prostaglandins. In a previous investigation, it was shown that subcutaneous skeletal muscle (*M. cutaneus colli*) produces prostaglandins and that this may be an important source of prostaglandins found in wound fluid during mild inflammation (Greenwood & Kerry, 1975; Kerry, 1976).

In this investigation, the effect of oestrogen and progesterone upon prostaglandin biosynthesis of skeletal muscle has been examined.

Methods

Prostaglandin production by skeletal muscle and the effect of oestradiol-17β and progesterone

Male guinea-pigs, 200–400 g in weight, were killed by dislocation of the neck. Blood was removed from the heart into a 5 ml syringe containing about 50 units heparin and was centrifuged at 600 *g* for 25 min to provide a source of plasma. A sheet of skeletal muscle (composed of *M. obliquus abdominis* and *M. transversus abdominis*) was dissected out of the same animal and temporarily stored in cold tissue culture medium (TC199, Gibco-Biocult) containing 25 mM HEPES buffer (N-2-hydroxyethyl-piperazine-N'-ethanesulphonic acid), benzyl penicillin (100 units per ml) and streptomycin (100 μg per ml).

The muscle was cleared of connective tissue and fragments of similar size (approximately 100 mg in weight) were cut, gently blotted dry and carefully weighed in sterile polystyrene universal bottles (Sterilin) to give a measure of wet weight. Ten ml 2.5% v/v autologous plasma diluted with TC199 was added to each muscle sample.

Steroids were added to certain cultures in 200 μl ethanol. Oestradiol-17β was used at a final medium concentration of 10 μg/ml or 100 μg/ml. Progesterone was added to give a final concentration of 10 μg/ml and 500 μg/ml. Control samples contained 200 μl ethanol alone.

All muscle samples were incubated for 18 h at 37°C and in every case visible microbial contamination was absent after this period. The muscle fragments were discarded and any change in the volume of incubation medium was noted. The medium was adjusted to pH 4 with 1.0 M HCl and the prostaglandins extracted with two successive equal volumes of ethyl acetate. The ethyl acetate was evaporated under reduced pressure, the dry extract dissolved in 70% v/v ethanol and then partitioned with two equal volumes of petroleum ether (b.p. 60 to 80°C) to remove neutral lipids. The petroleum ether was discarded and the aqueous ethanol evaporated to dryness.

The residues were dissolved in 5.0 ml ethyl acetate and aliquots from this solution were assayed for prostaglandin E (PGE) and PGF by radioimmuno-

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assay using the double antibody technique (Dighe, Emslie, Henderson, Rutherford & Simon, 1975; Mitchell, Poyser & Wilson, 1976).

Results were analysed by means of the Wilcoxon signed rank test. Oestradiol-17β and progesterone were obtained from Koch-Light Laboratories Limited.

Metabolism of prostaglandins $F_{2\alpha}$ and E_2 by guinea-pig skeletal muscle in vitro

Due to the long incubation time adopted for these experiments, a study was carried out to investigate the degree of prostaglandin metabolism by skeletal muscle in this experimental system. The approach used was similar to that of Maule Walker & Poyser (1978).

PGE_2 2.0 µg plus [^{3}H]-PGE_2 0.5 µCi (160 Ci/mmol) or $PGF_{2\alpha}$ 2.0 µg plus [^{3}H]-$PGF_{2\alpha}$ 0.5 µCi (85 Ci/mmol) were added to muscle fragments of similar size (approximately 100 mg in weight) in 10 ml 2.5% autologous plasma. Samples were incubated for 18 h at 37°C and the prostaglandins extracted as described earlier. Each sample was subjected to thin-layer chromatography using neutral silica gel on 50 mm × 200 mm glass plates (E. Merk, A.G., Darmstadt, F.D.R.). [^{3}H]-PGE_2 and [^{3}H]-$PGF_{2\alpha}$ were run as radioactive marker standards. A double-run development was carried out using the FVI solvent system (Andersen, 1969) first, followed by the GCM solvent system (Millar, 1974). Areas of radioactivity were located by means of a Panax thin-layer chromatographic plate scanner, scraped off and eluted with 2 × 5.0 ml methanol. The two methanol fractions were pooled, taken to dryness and dissolved in 2.0 ml methanol; 100 µl of each sample was added to 10 ml toluene:ethoxyethanol-based scintillant (Hensby, 1974) and counted in a Nuclear Chicago scintillation counter. The quantity of radioactive material recovered from each radioactive zone was calculated as a percentage of the total activity recovered from the plate.

Results

Prostaglandin synthesis by skeletal muscle in vitro *and the effect of oestradiol-17β and progesterone*

Fragments of *M. obliquus abdominis/M. transversus abdominis* produced small but reproducible quantities of both PGE (5.1 ng ± 0.5 s.e. mean/100 mg muscle; $n = 6$) and PGF (6.3 ng ± 1.1/100 mg muscle; $n = 6$) when incubated for 18 h in 2.5% autologous plasma. Oestradiol-17β and progesterone, both at a concentration of 10 µg/ml, exerted no apparent effect on this basal PGE and PGF synthesis.

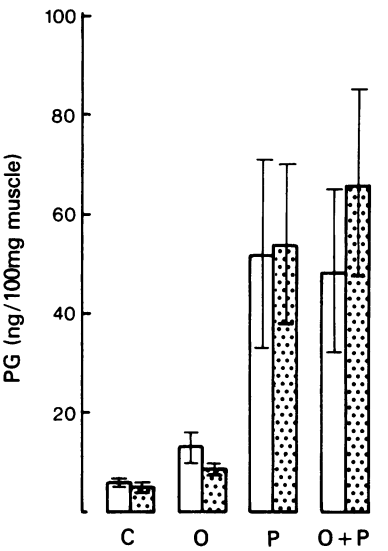

Figure 1 The effect of oestradiol-17β and progesterone on prostaglandin E (stippled columns) and prostaglandin F (open columns) production by guinea-pig skeletal muscle *in vitro*. Vertical lines show s.e. mean. C = control; O = oestradiol-17β (100 µg/ml); P = progesterone (500 µg/ml).

Results from three experiments (six observations) with 100 µg/ml oestradiol and 500 µg/ml progesterone, are shown in Figure 1. Oestradiol alone produced a relatively small but significant increase ($P < 0.05$) in both PGE (79% increase) and PGF (113% increase) biosynthesis. Progesterone alone produced a large variable increase of PGE (mean increase 949%) and PGF (mean increase 664%) over control values (significant at $P < 0.05$) and this increase was also significantly higher than that caused by oestradiol ($P < 0.05$). Oestradiol and progesterone in combination produced prostaglandin levels higher than either the controls or oestradiol alone ($P < 0.05$), but not significantly different from progesterone alone.

Metabolism of prostaglandins $F_{2\alpha}$ and E_2 by skeletal muscle in vitro

Tritiated $PGF_{2\alpha}$ was subjected to only slight metabolism after 18 h in this incubation system. Of the radioactivity, 93% had thin-layer chromatographic properties identical to $PGF_{2\alpha}$ ($R_F = 0.34$); the remaining 7% was recovered as a single, less polar material ($R_F = 0.56$). In the case of PGE_2, 65% of the activity co-chromatographed with authentic material ($R_F = 0.54$) after incubation. The other 35% occurred as a single peak ($R_F = 0.78$). This substance was less

polar than the starting material but has not yet been identified. However, experiments in which 10.0 ml 2.5% plasma in TC199 and 10.0 ml TC199 were incubated with [^{3}H]-PGE_2 0.5 μCi and PGE_2 2.0 μg in the absence of skeletal muscle, also showed isotope degradation (25%).

Discussion

In these experiments, the release of prostaglandins from muscle fragments into incubation medium was examined. The results obtained may therefore not only reflect tissue prostaglandin synthesis, but also the extent to which newly-synthesized prostaglandins were released from the tissue. At the present time, there is no evidence to support one possibility at the expense of the other.

This investigation has established that the steroids, oestradiol-17β and progesterone, can exert an influence on prostaglandin production or release by non-reproductive tissues. Oestradiol caused a small increase in the amounts of PGE and PGF detected but progesterone caused a large increase in the synthesis (or in the amount released) of both these compounds.

Naylor & Poyser (1975), using the same steroid concentrations as those used in this study, showed that oestradiol stimulated PGF synthesis in guinea-pig uterine homogenates by 73%, while progesterone on its own had no effect and inhibited the response to oestradiol.

The differences in the action of the steroids on these two tissues may be attributable to a difference in protocol. Naylor & Poyser (1975), used uterine homogenates, incubated for 90 min at 37°C in plasma-free Krebs solution; in this investigation, skeletal muscle fragments from male guinea-pigs were incubated for 18 h in TC199 containing a 2.5% v/v autologous plasma supplement. Because of these differences, no direct comparison can be made but the similarity in the action of oestradiol and the discrepancy in the action of progesterone is of potential interest.

The doses of the two steroids required to achieve these effects are high when compared to physiological and pathological levels. It may be that the potentiation of prostaglandin synthesis is a non-specific effect at these dose levels, and in the case of skeletal muscle this view could have support from the elevation of both PGE and PGF biosynthesis.

In these experiments, the actual amount of steroid required to exert an effect was certainly less than the doses added to the cultures. The presence of plasma in the culture medium provided a source of protein capable of binding with these agents, and this could diminish the amount of free steroid. The penetration of oestradiol and progesterone into muscle fragments is a further potential factor limiting the quantity reaching the active sites; binding to irrelevant muscle proteins may also serve to reduce steroid availability. Steroid solubility was probably the most important factor governing the effective concentrations of oestradiol-17β and progesterone in these experiments. Oestradiol and progesterone appeared to be completely dissolved at a concentration of 10 μg/ml but the addition of 100 μg/ml oestradiol and 500 μg/ml progesterone to 2.5% plasma in TC199 produced a cloudy solution, indicating only partial solubility at these concentrations. These factors could explain the variability of response in muscle fragments exposed to progesterone.

The degradation of [^{3}H]-PGE_2 in the presence and absence of muscle fragments suggested that this process was essentially non-enzymatic. In view of the relative instability of tritiated prostaglandins (especially PGE_2), it would be of value to repeat this aspect of the investigation with [^{14}C]-PGE_2 and [^{14}C]-$PGF_{2\alpha}$ (both of which are commercially available), in order to obtain a more accurate indication of prostaglandin metabolism in this experimental situation.

Oestradiol-17β is known to influence certain inflammatory processes (Bodel, Dillard, Kaplan & Malawista, 1972) and it seems probable that oestradiol, along with other humoral factors, could modify prostaglandin biosynthesis or release in inflammation. However, there is little possibility of steroid levels reaching local concentrations of the same order of magnitude as those used in these experiments and therefore, any steroidal effect on the inflammatory production of prostaglandins by damaged tissues is likely to be minimal.

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