MODULATION OF CYCLIC AMP IN ISOLATED RAT UTERINE TISSUE SLICES BY PORCINE RELAXIN

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SUMMARY

Porcine relaxin produced a rapid, dose-related rise of cyclic AMP values in rat uterine tissue incubated in vitro. In time-course experiments, peak cyclic AMP concentrations were observed in the uterine slices at 5 min; subsequently the values fell, at first rapidly and then more slowly with the tissue concentration remaining significantly raised at 15 min. Levels of cyclic GMP in the same tissue slices were not significantly altered by relaxin. Furthermore, no increase in basal cyclic AMP values was measured in control slices prepared from the rat heart or jejunum. An increase in cyclic AMP concentration comparable to that found in the rat uterus was observed in slices of porcine uterus and cervix but not of vagina when they were stimulated with porcine relaxin. Our results suggest that the hormonal action of relaxin on the uterus and cervix is mediated through receptors linked to the enzyme, adenylate cyclase.

INTRODUCTION

Relaxin (Fevold, Hisaw & Leonard, 1932) is a polypeptide (Schwabe, McDonald, Steinetz, 1976, 1977) thought to be involved in cervical dilatation and to play an important role in the separation of the pubic symphysis during parturition in some species (Hisaw, 1927; Porter, 1979). The action of relaxin on the pubic symphysis of the mouse has been shown to be associated with an increase in the concentration of cyclic AMP (Braddon, 1978). In addition, relaxin causes uterine relaxation, both in vivo (Felton, Frieden & Bryant, 1953; Porter, Downing & Bradshaw, 1979) and in vitro (Griss, Keck, Engelhorn & Tuppy, 1967). Myometrial relaxation induced by β-adrenoceptor agonists (Johansson & Andersson, 1978) and histamine (Nitznegg, Schubert & Fuchs, 1975) are associated with an increase in the tissue concentration of cyclic AMP. We therefore examined the postulate that myometrial relaxation produced by relaxin may be linked to activation of adenylate cyclase and formation of cyclic AMP. In the present study we demonstrate that basal cyclic nucleotide concentrations increase in rat and porcine uterine slices after incubation with porcine relaxin in vitro.

MATERIALS AND METHODS

Animals

Tissue was obtained from adult female rats, weighing 200–300 g, at oestrus (M.R.C. Porton strain). Oestrus was detected from vaginal smears and only rats with predominantly cornified epithelial cells and few, if any, nucleated cells or lymphocytes, were used. The reproductive organs from pigs with vaginal smears showing predominantly nucleated epithelial cells were obtained from an abattoir.
Hormones and chemicals

Porcine relaxin (mol. wt ~5800) purified by Sephadex G-50 gel filtration and with a biological potency of 500 guinea-pig units/mg (20–25% purity), was a gift of Professor D. G. Porter and Dr J. Bradshaw (University of Bristol). Tritium-labelled cyclic nucleotides, [8-3H]adenosine 3':5'-cyclic phosphate (30 Ci/mmole) and ribose [8-3H]guanosine 3':5'-cyclic phosphate (31 Ci/mmole), were obtained from The Radiochemical Centre (Amersham). Cyclic AMP, cyclic GMP, 4-isobutyl-methyl xanthine, isoprenaline and bacitracin were obtained from Sigma Chemical Co. (London). All other chemicals were obtained from British Drug Houses (Poole, Dorset) and were of Analar quality.

Incubation procedure

The uteri of three or more rats (killed by cervical dislocation) were rapidly removed and placed in Krebs–Ringer bicarbonate buffer containing in mmol/l: NaCl, 121; KCl, 5-0; NaHCO3, 25-2; CaCl2, 2-6; glucose, 5-6; β-hydroxybutyric acid, 6-1; Na glutamate, 3-7; Na pyruvate, 3-7; Na fumarate, 2-7; at pH 7-4. The uterine horns were dissected free of fat and connective tissue, slit longitudinaly and then cut transversely. Porcine reproductive organs were obtained as soon as possible after slaughter and placed in ice-cold Krebs–Ringer bicarbonate buffer, and slices were prepared in the same manner as for rat tissues. The pooled slices (each weighing about 5–10 mg) of both rats and pigs were initially incubated for 30 min in a Grant shaking water bath at 140 strokes per min at 37 °C. Throughout the incubation, the slices were continuously gassed with a mixture of 95% O2 and 5% CO2. At the end of the first incubation the slices were removed, dried on filter paper and weighed on a torsion balance. Slices with a total weight ranging from 50 to 100 mg were placed in single Erhlenmeyer flasks (capacity 15 ml), containing 2 ml Krebs–Ringer bicarbonate buffer to which had been added isobutyl-methylxanthine (0-1 mmol/l), bovine serum albumin (0-1%) and bacitracin (50 μg/ml). The slices were incubated for a further 20 min before the addition of 50 μl isoprenaline or relaxin (dissolved in 0-9% (w/v) NaCl) or 50 μl 0-9% NaCl (control tissue). For time-course experiments the incubations ranged from 0-5 to 30 min. At the end of each incubation, tissue was removed, immediately frozen in an aluminium clamp cooled in liquid nitrogen and subsequently extracted for cyclic nucleotides. Each tissue incubation was performed in either triplicate or quadruplicate.

Measurement of cyclic AMP and cyclic GMP

Each frozen tissue sample was boiled (100 °C) for 10 min in 1 ml theophylline solution (6 mmol/l). The denatured tissue was homogenized and cyclic nucleotides extracted as described by Albano, Bhoola & Harvey (1976). Cyclic AMP was measured by competitive protein binding, utilizing the cyclic AMP binding protein from bovine adrenal cortex as reported by Brown, Albano, Ekins, Sgherzi & Tampion (1971). Cyclic GMP was determined by radioimmunoassay, using specific antibodies raised in rabbits to succinylated cyclic GMP by Dr J. Albano (University of Bristol), following the procedure described by Steiner, Parker, Ward & Kipnis (1972). The cyclic nucleotides were measured according to the method published by Albano, Bhoola, Heap & Lemon (1976). The concentration of cyclic nucleotides in each sample was determined in triplicate and the mean of the values for each sample was calculated. Results are expressed as pmol cyclic nucleotide per mg wet tissue. The S.E.M. (n = 3 or 4) was determined and significant differences analysed using Student’s t-test.

RESULTS

Action of relaxin on cyclic AMP levels in rat uterine slices

Time-course

The effect of relaxin (1 μg/ml) on levels of cyclic AMP in the rat uterus was investigated using various periods of incubation (Fig. 1). It produced a significant increase in the
Fig. 1. Time-course of changes in cyclic AMP concentrations in rat uterine tissue slices after incubation for various periods with relaxin (1 µg/ml; ●) or 0·9% saline (○). Each point represents the mean of three or four determinations ± S.E.M.

Fig. 2. Effect of either 0·9% saline or various doses of relaxin on cyclic AMP levels in rat uterine tissue slices after incubation for 5 min. Each bar represents the mean of three or four determinations ± S.E.M.
concentration of cyclic AMP at the shortest incubation time of 30 s. The concentration rose rapidly and almost linearly, reaching peak values at 5 min by which time the concentration of cyclic AMP had approximately doubled. After 5 min, the values fell, at first rapidly and then more slowly, with the concentration still significantly \((P < 0.05)\) raised at 10 and 15 min.

Dose-dependency
The action of various doses of relaxin (0–125–5 \(\mu\)g/ml) on rat uterine slices was examined (Fig. 2). Because peak increases in the time-course experiments were obtained at 5 min, incubations for the dose-related effect were performed for 5 min. All the doses used caused a significant \((P < 0.05)\) rise in the uterine concentration of cyclic AMP. A maximal increase of about 2.5-fold was obtained with a dose of 1 \(\mu\)g/ml.

Size of effect of relaxin and isoprenaline on cyclic AMP levels
The action of relaxin on basal cyclic AMP concentration was compared with that of isoprenaline in uterine slices incubated for 5 min. A maximal increase above basal values of 2.4-fold was obtained with 1 \(\mu\)g relaxin/ml (\(\sim 0.18\) \(\mu\)mol/l). Although a comparable increase was measured with 20 \(\mu\)M-isoprenaline, a maximal increase of 11.1-fold was observed with 200 \(\mu\)M-isoprenaline. It seemed that whereas relaxin was more potent, isoprenaline produced a much greater maximal effect. Since both relaxin and isoprenaline produce relaxation of the
Table 1. *Time-course of the action of relaxin (1µg/ml) on cyclic GMP concentrations in rat uterine tissue slices incubated for 5 min* (Values represent the mean ± s.E.M.; n = 3–4)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Basal</th>
<th>Relaxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>0.0108±0.0017</td>
<td>0.0134±0.0023</td>
</tr>
<tr>
<td>1</td>
<td>0.0101±0.0034</td>
<td>0.0075±0.0014</td>
</tr>
<tr>
<td>2</td>
<td>0.0142±0.0053</td>
<td>0.0117±0.0048</td>
</tr>
<tr>
<td>5</td>
<td>0.0115±0.0039</td>
<td>0.0106±0.0026</td>
</tr>
<tr>
<td>15</td>
<td>0.0104±0.0034</td>
<td>0.0087±0.0015</td>
</tr>
<tr>
<td>30</td>
<td>0.0243±0.0037</td>
<td>0.0212±0.0062</td>
</tr>
</tbody>
</table>

Fig. 4. Concentrations of cyclic AMP in various tissues from (a) rat and (b) pig after incubation for 5 min in control buffer (open bars), or buffer containing 1 µg relaxin/ml (vertically hatched bars) or 20 µmol isoprenaline/l (cross-hatched bars). Each bar represents the mean of three determinations ± s.E.M.
uterus it therefore appeared that the activation of adenylate cyclase by relaxin might be mediated indirectly through release of endogenous catecholamines. Propranolol (50 μmol/l), a β-adrenoreceptor antagonist, significantly inhibited the rise produced by isoprenaline (200 μmol/l) but did not affect the increase caused by relaxin (1 μg/ml) (Fig. 3).

Action of relaxin on cyclic GMP levels in rat uterine slices

The concentration of cyclic GMP was measured in the same samples as those used to determine cyclic AMP values when incubated with relaxin (1 μg/ml). No significant differences were observed between control and relaxin-treated samples over a range of incubations from 0.5 to 30 min (Table 1). Although the basal values for cyclic GMP were low, they were within the sensitivity limits of the assay.

Comparison of the action of relaxin on cyclic AMP levels in rat uterine, ventricular and jejunal slices

Relaxin (1 μg/ml) raised the basal concentration of cyclic AMP in tissue slices prepared from the uterus after incubation for 5 min but not those prepared from ventricles and jejunum. In contrast, isoprenaline clearly increased the basal concentrations of cyclic AMP in all three tissues after a similar period of incubation (Fig. 4a).

Action of relaxin on cyclic AMP levels in porcine uterus, cervix and vagina

Both relaxin (1 μg/ml) and isoprenaline (20 μmol/l) caused significant increases in the basal concentration of cyclic AMP in slices of porcine uterus and cervix incubated for 5 min (Fig. 4b). Isoprenaline but not relaxin also produced a clear increase in levels of cyclic AMP in the vagina.

DISCUSSION

Activation of relaxin receptors was associated with an intracellular rise in the basal concentration of cyclic AMP but not of cyclic GMP. Preliminary experiments suggested that the changes in cyclic AMP were mediated through activation of adenylate cyclase. A detailed study of the relaxin-activated adenylate cyclase may provide a useful method for the assay and characterization of relaxin receptors. Specific, high-affinity binding sites have been demonstrated only in tissues recognized as target organs for relaxin (McMurty, Kwok & Bryant-Greenwood, 1978). When injected intravenously into rats, 125I-labelled relaxin (2500–3500 guinea-pig units/mg) binds specifically to the uterine horns and the cervix (Cheah & Sherwood, 1979). Similarly, we have demonstrated an action of relaxin on cyclic AMP levels only in tissues known to respond physiologically to the hormone. McMurty et al. (1978) identified relaxin receptors in the cervix and our results showed that this hormone increased cyclic AMP concentration in the porcine cervix. Furthermore, Braddon (1978) reported an in-vivo increase of cyclic AMP levels in response to relaxin in the mouse pubic symphysis but not in non-target tissues such as the liver. Our present results confirm the results presented in two recent reports (Cheah & Sherwood, 1979; Sanborn, Kuo, Weisbrodt & Sherwood, 1979). Of particular importance are the results of Sanborn et al. (1979) in which they demonstrate that the ability of an impure preparation of relaxin (442 guinea-pig units/mg comparable to the 500 guinea-pig units/mg preparation used in this study) to raise cyclic AMP levels in the uterus and cervix is retained by the purified hormone (2500–3500 guinea-pig units/mg).

A similarity may exist between the cellular mode of action of relaxin and isoprenaline, as both relax the uterus and both are associated with a rise in the basal concentration of cyclic AMP as shown in this study. At present, this analogy cannot be extended because there appear to be differences in the nature of myometrial inhibition produced by relaxin and
isoprenaline. Whereas relaxin inhibits spontaneous myometrial activity, but not myometrial contractions in response to oxytocin (Porter et al. 1979), isoprenaline appears to produce a greater myometrial inhibitory effect and to inhibit both spontaneous and oxytocin-induced contractions (Mahon, Reid & Daly, 1967). It is possible that separate mechanisms may be involved in initiating spontaneous and hormone- or drug-stimulated uterine activity. The precise intracellular role, therefore, of cyclic AMP in modulating the relaxin-induced inhibition of spontaneous uterine contractions cannot at present be precisely delineated.

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REFERENCES


