BOVINE LUTEAL CYCLIC AMP AND CYCLIC GMP PHOSPHODIESTERASE ACTIVITY; SENSITIVITY TO VARIOUS DRUGS AND HORMONES

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1. Introduction

Previous studies have shown the presence of two forms of cyclic AMP phosphodiesterase activity in bovine corpus luteum tissue [1]. These enzymes have K_M values of approx. 2×10^{-4} M and 1×10^{-6} M, similar to those of other tissues studied [2]. However to our knowledge the presence of cyclic GMP phosphodiesterase activity has not been demonstrated in ovarian tissue. This study indicates that a cyclic GMP phosphodiesterase is present in bovine luteal tissue.

The effects of known inhibitors of phosphodiesterase activity were studied to establish whether they had selective effects in inhibiting either the cyclic AMP or cyclic GMP phosphodiesterase from ovarian tissue. It has been previously reported that many inhibitors of phosphodiesterase are more effective in preventing cleavage of cyclic AMP than cyclic GMP [2], although this varies from tissue to tissue [3].

The effects of various drugs and hormones were determined with a view to finding doses at which different effects might be dissociated. Of special interest were the effect of indomethacin, which is a potent inhibitor of prostaglandin biosynthesis [4] and flufenamic acid which antagonises the effect of prostaglandin $F_{2\alpha}$ [5] since these compounds have been reported to also have the ability to inhibit phosphodiesterase activity in other tissues [6,7].

2. Materials and methods

2.1. *Chemicals*

Cyclic AMP, cyclic GMP, snake venom (*Crotalus atrox*), indomethacin, histamine, 17β-estradiol, progesterone, 20α-hydroxy-4-pregnen-3-one were purchased from Sigma Chemical Company; anion exchange resin (AGl-X2 400 mesh) was supplied by Bio-Rad; RO 07-2956 and RO 20-1724 were given by Roche; ICI 63, 197 was supplied by I.C.I.; nupercaine was obtained from Ciba; ascorbic acid was obtained from Eastman Organic Chemicals; flufenamic acid (ARLEF) was supplied by Parke-Davis and prostaglandins $F_{2\alpha}$ and E_2 were supplied by the Upjohn Company. Adenosine [8–^{3}H] 3′ 5′ cyclic phosphate ammonium salt (spec. act. 20.7 Ci/mmol) and guanosine [8-^{3}H] 3′ 5′ cyclic phosphate ammonium salt (spec. act. 13 Ci/mmol) came from the Radiochemical Centre, Amersham. Other chemicals were supplied by British Drug Houses.

2.2. *Enzyme prepration*

A crude enzyme preparation was made by homogenizing approx. 1 g of corpus luteum tissue in 12 ml Tris/Mg^{2+} buffer, pH 8 (8 ml 0.05 M Tris, pH 8 and 4 ml 10 mM $MgCl_2$). The homogenate was centrifuged at 5000 *g* for 30 min at 4°C, and the supernatant fraction was dispensed into 0.5 ml aliquots and stored at −20°C until required.

No separation or purification of the different isoenzymes was undertaken, however the low concentration used should reflect the activity of the low K_M enzyme, which is thought to be of the greatest physiological importance.

2.3. *Determination of cyclic AMP and cyclic GMP phosphodiesterase activities*

A modification of the radiodisplacement assay of Brooker, Thomas and Appleman [8] was used. The

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reaction mixture contained 70 mM Tris-HCl buffer, pH 8, 40 mM magnesium chloride; 0.8 mM EDTA; 0.05 μCi [^{3}H] cyclic AMP or cyclic GMP; unlabelled cyclic AMP or cyclic GMP at various concentrations and enzymatic preparation containing 1 mg/ml snake venom. All the assays were carried out in linearity conditions with respect to time and protein concentration, allowing measurement of the initial rates of reaction. Protein concentrations were determined by the method of Lowry et al. [9] using bovine serum albumin as standard.

3. Results

Kinetic analysis of both cyclic AMP and cyclic GMP phosphodiesterase activity using Lineweaver–Burke plots (fig.1.) shows that both phosphodiesterases have similar values for the K_M. Over the range of 3.3. $\times 10^{-7}$ M to 5×10^{-6} M an apparent K_M of $2.80 \pm 0.31 \times 10^{-6}$ M and a V_{max} of 5.18 ± 0.32 pmol/10 min/μg protein was obtained for cyclic AMP, and an apparent K_M of $4.09 \pm 0.72 \times 10^{-6}$ M and a V_{max} of 8.89 ± 0.83 pmol/10 min/μg protein was obtained for cyclic GMP phosphodiesterase.

The effects of various known inhibitors of phosphodiesterase activity on ovarian cyclic AMP and cyclic GMP phosphodiesterase activities are shown in table 1. Both the Roche compounds (RO 07–2956 and RO 20–1724) and ICI 63, 197 appear to be more specific for the cyclic AMP phosphodiesterase than the cyclic GMP phosphodiesterase. However theophylline and papaverine were able to inhibit both the

Table 1
Effect of some inhibitors of phosphodiesterase activity on bovine luteal cyclic AMP and cyclic GMP phosphodiesterase activity at low substrate concentrations

I_{50} (μM)		
	Cyclic AMP phosphodiesterase	Cyclic GMP phosphodiesterase
RO 20-1724	8.2	1800
RO 07-2956	130	3300
ICI 63,197	10	580
Theophylline	750	360
Papaverine	5.8	7.2

I_{50} represents the concentration of drug required to cause a 50% inhibition of enzyme activity. Cyclic nucleotide substrate concentration of 5×10^{-7} M was used in the assays.

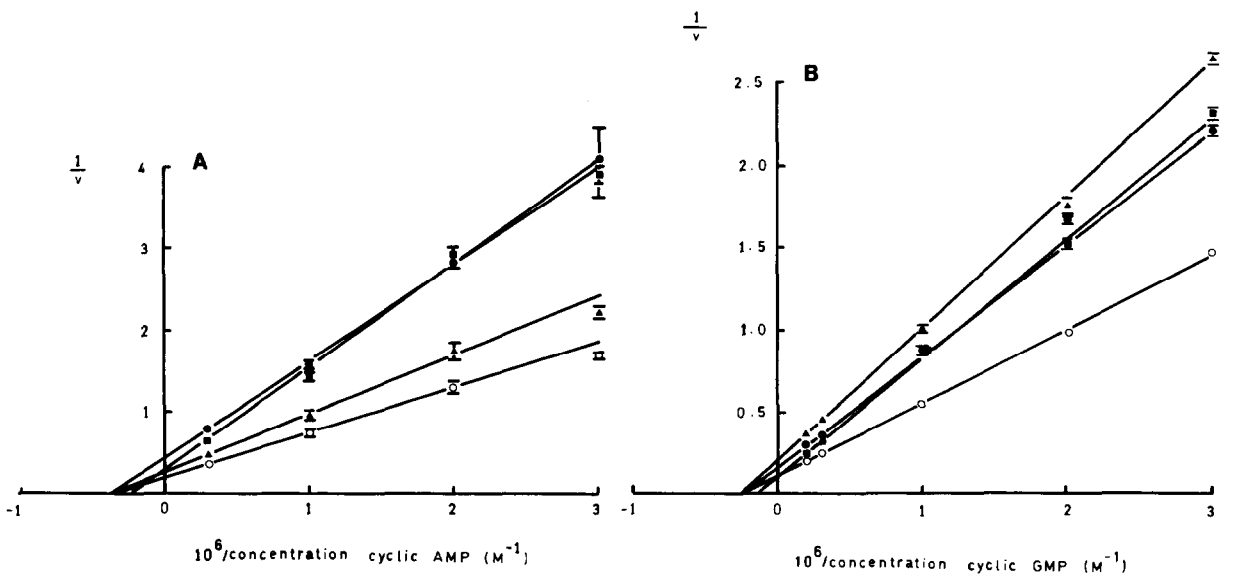

Fig.1. Lineweaver–Burke plots of, (A) bovine luteal cyclic AMP phosphodiesterase assayed over the range 5×10^{-6} M–3.3×10^{-7} M. (○) Controls; (●) effect of 1×10^{-4} M indomethacin; (■) effect of 3×10^{-6} M papaverine; (▲) effect of 1.3×10^{-4} M theophylline. (B) Bovine luteal cyclic GMP phosphodiesterase assayed over the range 5×10^{-6} M–3.3×10^{-7} M. (○) Controls; (▲) effect of 3.3×10^{-4} M theophylline; (■) effect of 3×10^{-6} M papaverine; (●) effect of 2×10^{-5} M indomethacin. Each point is the mean of four determinations, bars represent S.E.M. v = pmoles cyclic nucleotide hydrolyzed/10 min/μg protein.

Table 2
Effect of various drugs and hormones on bovine luteal cyclic AMP and cyclic GMP phosphodiesterase activities at low substrate concentrations

	I_{50} (μM)	
	Cyclic AMP phosphodiesterase	Cyclic GMP phosphodiesterase
Indomethacin	500	100
Flufenamic acid	280	400
Nupercaine	800	500
Human chorionic gonadotrophin (HCG)	No effect	No effect
$CaCl_2$	No effect	No effect
Histamine	No effect	No effect
Prostaglandin E_2	No effect	No effect
Prostaglandin $F_{2\alpha}$	No effect	No effect
17β-estradiol	2000	800
Progesterone	90	160
20α-hydroxy-4-pregnen-3-one	5500	6500
Ascorbic acid	No effect	No effect
Cyclic AMP	–	2500
Cyclic GMP	1300	–

I_{50} represents the concentration of drug required to cause a 50% inhibition of enzyme activity. Cyclic nucleotide substrate concentration of 5×10^{-7} M was used in the assays.

cyclic AMP and cyclic GMP phosphodiesterases to the same extent; papaverine being the most potent substance tested.

The effects of the other drugs and hormones on phosphodiesterase activity are shown in table 2. All three antagonists of prostaglandin synthesis or action (indomethacin; flufenamic acid and nupercaine) were found to be fairly potent inhibitors of phosphodiesterase activity, having I_{50}s comparable with theophylline. Indomethacin had a similar I_{50} in corpus luteum tissue to that found in human synoviocytes [7]. The results of Ferre et al. [6] also show that indomethacin and flufenamic acid are of equal potency in inhibiting cyclic AMP phosphodiesterase from human placenta. HCG, calcium chloride, histamine, prostaglandins E_2, $F_{2\alpha}$ and ascorbic acid at concentrations ranging from 10^{-8} M – 10^{-3} M had no detectable effect on the phosphodiesterase activity. However the three steroid hormones tested were able to inhibit phosphodiesterase activity, the most potent of these was progesterone. This is in agreement with the results of Ferre et al. [6] who found that progesterone had a strong inhibitory action whereas β-estradiol was less effective. Cyclic AMP was able to inhibit cyclic GMP phosphodiesterase and cyclic GMP was able to inhibit cyclic AMP phosphodiesterase, but only at relatively high concentrations.

The type of inhibition exhibited by some of the drugs used was also investigated. Fig.1 shows the effect of theophylline, papaverine and indomethacin on Lineweaver–Burke plots for cyclic AMP and cyclic GMP phosphodiesterase activity. Theophylline and indomethacin appear to exhibit a non-competitive type of inhibition for both cyclic nucleotide phosphodiesterases, whereas papaverine exhibited a competitive type for cyclic GMP phosphodiesterase but a mixed type for cyclic AMP phosphodiesterase. The type of inhibition exhibited by the two Roche compounds and ICI 63,197 were also investigated. For cyclic AMP phosphodiesterase, RO 20-1724 inhibited non-competitively whereas RO 07-2956 and ICI 63, 197 inhibited competitively. For cyclic GMP phosphodiesterase, RO 20-1724 inhibited competitively whereas the ICI 63, 197 and RO 07-2956 exhibited a mixed type of inhibition.

4. Discussion

This study has shown that bovine corpus luteum tissue contains soluble phosphodiesterases which are able to hydrolyse both cyclic AMP and cyclic GMP. These phosphodiesterases have similar K_M values at the low substrate concentrations used. The cyclic AMP phosphodiesterase appears to be distinct from the cyclic GMP phosphodiesterase. There are two reasons for this; (a) the activity of the two cyclic nucleotide phosphodiesterases are affected differently by some of the drugs tested. For example, RO 20-1724 at a concentration of only 8 μM was necessary to cause a 50% inhibition of cyclic AMP phosphodiesterase, whereas a concentration of 1800 μM was necessary to cause a 50% inhibition of cyclic GMP phosphodiesterase; (b) cyclic AMP only inhibits cyclic GMP phosphodiesterase activity at relatively high concentrations (I_{50} = 2 mM) and vice versa. If the same enzyme was hydrolysing both cyclic nucleotides then a lower I_{50} would be expected.

It has been found that in various tissues each of these cyclic nucleotides is able to influence the rate of hydrolysis of the other [10] either stimulating or inhibiting the rate of hydrolysis of the other depending on its concentration. However we found no ability of either cyclic nucleotide to stimulate the rate of hydrolysis of the other. On the other hand both cyclic nucleotides were able to inhibit the hydrolysis of the other, but the I_{50} is far greater than the physiological concentration, so it is doubtful whether this has any physiological significance.

An interesting observation was the fact that progesterone, 20α-hydroxy-4-pregnen-3-one and 17β-estradiol were able to inhibit both cyclic AMP and cyclic GMP phosphodiesterases. Similar observations have been noted in human placental tissue [6] and it seems likely that this phenomenon has a physiological significance. There was a surprising difference between the I_{50} for progesterone and the I_{50} for 20α-hydroxy-4-pregnen-3-one, since there is only a slight difference in the chemical structure of these two steroids.

Calcium ions, in low concentrations, stimulate phosphodiesterase activity in rat brain in the presence of Mg^{2+} but such a stimulation was not found in other rat tissues [11]. In rat pancreas [12] Ca^{2+} does not stimulate phosphodiesterase but has an inhibitory effect when added in concentrations above 0.1 mM. In bovine corpus luteum tissue, calcium appears to have no effect at all on phosphodiesterase activity.

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