Catecholamine stimulation of cyclic AMP and progesterone production in rat corpora lutea of different ages

Ensio Norjavaara, Gunnar Selstam and Kurt Ahrén

Departments of Physiology, University of Göteborg and University of Umeå, Sweden

Abstract. In vitro effects of catecholamines (adrenaline and noradrenaline) and adrenergic antagonists on adenosine 3',5'-cyclic monophosphate (cAMP) and progesterone production by rat corpora lutea (CL) of different ages (1–8 days old) were studied. To obtain defined ages of CL a pregnant mare's serum gonadotrophin (PMSG) model was used. The effect of catecholamines on cAMP decreased with luteal age while the effect on progesterone production was maximal on 5 day old CL. The β-blocker propranolol inhibited the effects of catecholamines in concentrations around 10⁻⁵ M. The effects of LH could only be inhibited with higher doses of propranolol known to exert unspecific effects. These results support the theory that LH and catecholamine effects on rat corpora lutea are mediated through different receptors.

Catecholamines have been shown to increase cAMP and progesterone production in corpora lutea (CL) of different species: bovine CL (Condon & Black 1976; Godkin et al. 1977), dispersed ovine luteal cells (Jordan et al. 1978), luteal dominated rat ovaries (Ratner et al. 1980) and dispersed cells from luteinized rat ovaries (Harwood et al. 1980a). Of the different possible sources for catecholamines, the most likely route by which catecholamines could influence ovarian cells is by release from peripheral nerve endings. Such adrenergic nerve endings in the ovary are associated with vascular and non-vascular tissues. One structure in the ovary that is well innervated is the fibromuscular theca layer of the follicle (Bahr et al. 1974; Burden 1978; Sjöberg et al. 1979). This functional unit has been hypothesized to be involved in the ovulatory process. Also the stroma is richly innervated (Jacobowitz & Wallach 1967; Burden 1972; Walles et al. 1975). Interstitial cells have been shown to produce steroids (Rice & Savard 1966; Marsh et al. 1976), but the function, the degree of contribution to the total ovarian steroid production from these cells as well as if the nerve supply to these cells is of importance are at present uncertain.

Few studies have dealt with the question whether the CL has a nervous supply. For the cat CL, such an indication of an adrenergic nervous supply has been shown (Jacobowitz & Wallach 1967; Fink & Schofield 1971) with the method of Falck and Hillarp (Falck et al. 1962; Falck & Owman 1965). In comparison between CL from normal cycling rats and guinea pigs, it was found that the 'cortical components' of rat CL were sparsely innervated, while those of guinea pig CL were not (Burden 1972). Unsicker (1974) could not find nerves within the CL of rat and pig, but the surrounding vascular as well as fibromuscular components of at least the rat CL were innervated. Thus the anatomical basis for an ovarian release of catecholamines influencing the CL seems to exist. There are some discrepancies in the effects of catecholamines on progesterone and cAMP formation on luteal tissue of 'luteal dominated ovaries'. While Ratner et al. (1980), and Richardson & Masson (1980) had no effect on progesterone production as well as only a minor increase of the cAMP content as compared to gonadotrophic stimulation (Ratner et al. 1980),
other authors showed large increments on cAMP content (Godkin et al. 1977; Jordan et al. 1978; Harwood et al. 1980a) and progesterone production (Condon & Black 1976; Jordan et al. 1978; Harwood et al. 1980b). The possibility exists that many or all of the catecholamine effects are dependent on CL-age as has been shown for LH (Herlitz et al. 1974). The aim of the present study was to test effects of catecholamines on CL of different ages and if these effects are mediated through specific catecholamine receptors.

Materials and Methods

Immature rats of the Sprague-Dawley strain, purchased from Anticimex Ltd., Sweden, were kept under controlled conditions (light between 05.00–19.00 h).

To obtain well defined ages of CL ovulations were induced by 8 IU pregnant mare's serum gonadotrophin (PMSG, Organon, The Netherlands or Sigma Co., USA) in the morning of day 28 of life. This treatment results in ovulation in the morning of day 31 and subsequent formation of a physiological number of CL (Herlitz et al. 1976; Khan et al. 1979). The CL were isolated around noon time from 31 to 38 days old rats and designated 1 to 8 day old, respectively.

The rats were killed by cervical dislocation and the ovaries excised. CL were isolated by free hand dissection under a stereomicroscope, and 4–5 CL transferred to a flask containing 1 ml Krebs bicarbonate buffer but with 1.25 mM of Ca²⁺. The buffer contained 5.6 mM glucose and 1 mg bovine serum albumin per ml. The CL were preincubated for 30 min at 37°C under a gas phase of 95% O₂ and 5% CO₂. CL were incubated for indicated periods of time with or without adrenaline (ACO, Sweden), noradrenaline (Apoteksbolaget, Sweden), isoproterenol (Apoteksbolaget, Sweden), LH (NIH-LH-B9, generously supplied by NIH, Bethesda), propranolol (ICI, USA) or phenoxybenzamine (= PBA; Smith-Kline-labs. England). When propranolol and PBA were used they were also added to the preincubation media.

After incubation the CL were homogenized in 1.5 ml 5% trichloroacetic acid. The protein content was determined according to Lowry et al. (1951) and cAMP according to Gilman (1970) but with small modifications (Khan 1979). Progesterone in medium was analysed as described by Hillensjö et al. (1976). When tissue content

![Graph](image)

**Fig. 1.**

Effect of 5 μg/ml adrenaline on cAMP content (a) and progesterone accumulation in incubation medium (b) of 1–8 days old corpora lutea (CL). CL were preincubated in plain buffer and then incubated for 30 min in modified Krebs buffer containing glucose and bovine serum albumin with or without adrenaline (see Materials and Methods). The content of cAMP in the CL and progesterone accumulation in the media were measured at the end of incubation. Values are given as mean ± SEM based on 6–31 observations. Significance as tested with variance of analysis followed by Student Neuman-Keul’s test were for cyclic AMP and progesterone stimulation $P < 0.05$ for 1, 3 and 5 days CL but not significant for 8 days CL.
Table 1.
Effects of adrenaline (5 μg/ml), noradrenaline (5 μg/ml), isoproterenol (5 μg/ml), PBA (10^{-4} M to 10^{-7} M) and LH (5 μg/ml) on cAMP content of tissue and medium and progesterone accumulation in media.

<table>
<thead>
<tr>
<th>Luteal age (days)</th>
<th>Group</th>
<th>Cyclic AMP (pmol/mg protein)</th>
<th>Progesterone (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tissue</td>
<td>Medium</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>100 ± 23</td>
<td>4 ± 2</td>
</tr>
<tr>
<td></td>
<td>Adrenaline</td>
<td>763 ± 108</td>
<td>37 ± 13</td>
</tr>
<tr>
<td></td>
<td>Noradrenaline</td>
<td>625 ± 100</td>
<td>54 ± 12</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>26 ± 6</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>PBA (1^{-4} M)</td>
<td>28 ± 2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Adrenaline</td>
<td>144 ± 24</td>
<td>29 ± 7</td>
</tr>
<tr>
<td></td>
<td>Adrenaline + PBA (10^{-3} M)</td>
<td>184 ± 25</td>
<td>9 ± 5</td>
</tr>
<tr>
<td></td>
<td>Adrenaline + PBA (10^{-7} M)</td>
<td>208 ± 25</td>
<td>12 ± 8</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>105 ± 19</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LH</td>
<td>459 ± 47</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LH + PBA (10^{-4} M)</td>
<td>525 ± 53</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>13 ± 2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Adrenaline</td>
<td>37 ± 9</td>
<td>13 ± 10</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>30 ± 5</td>
<td>4 ± 3</td>
</tr>
<tr>
<td></td>
<td>Adrenaline</td>
<td>32 ± 8</td>
<td>5 ± 5</td>
</tr>
</tbody>
</table>

Experimental conditions as in Fig. 1. Values are mean ± SEM of 4–6 observations. Adrenaline, noradrenaline, isoproterenol and LH significantly increased cAMP in tissue and medium, and medium content of progesterone for 1, 3 and 5 days old corpora lutea (P < 0.05). PBA did not influence cAMP in tissue and medium or medium content of progesterone.

Table 2.
Effect of adrenaline (5 μg/ml) on progesterone content in luteal tissue and accumulation in incubation media of CL of different ages.

<table>
<thead>
<tr>
<th>Group</th>
<th>Incubation time min</th>
<th>Progesterone (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tissue</td>
</tr>
<tr>
<td>1 day old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>120</td>
<td>–</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>120</td>
<td>–</td>
</tr>
<tr>
<td>3 days old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>276 ± 48</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>30</td>
<td>882 ± 137</td>
</tr>
<tr>
<td>Control</td>
<td>120</td>
<td>356 ± 77</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>120</td>
<td>843 ± 180</td>
</tr>
<tr>
<td>5 days old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>120</td>
<td>–</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>120</td>
<td>–</td>
</tr>
</tbody>
</table>

Experimental conditions as in Fig. 1. Values are ± SEM of 4–8 observations. Adrenaline significantly increased tissue content and accumulation in media of progesterone in all groups studied (P < 0.05).
of progesterone was measured, CL were homogenized in 1.5 ml Tris buffer (50 mM Tric-HCl, 146 mM NaCl and 15 mMNa3; pH 8.0). Progesterone was extracted with petroleum ether and analysed as for incubation media. All values are given as mean ± standard error of the mean. Differences between groups were tested with t-test or analysis of variance followed by Student Newman-Keul's test (Woolf 1968). A P-value of less than 0.05 was considered significant.

Results

The influence of adrenaline (5 μg/ml) on CL of different ages is shown in Fig. 1. It shows that the maximal luteal cAMP content after an adrenaline stimulation decreased with increasing luteal age. It is also seen that the cAMP content in the control CL decreased with increasing CL age, particularly between 1 and 3 days of age. The effect of stimula-
tion is approximately the same in 1 and 3 days old CL, while it is clearly lower in older CL. Adrenaline also caused a release of cAMP into the incubation medium (Table 1). Progesterone accumulation in incubation media was measured after 30 min of adrenaline stimulation (Fig. 1B). Despite the short incubation time a significant increase in progesterone accumulation was noticed for most ages of CL. Values after 120 min of incubation are depicted in Table 2. The maximal stimulation for progesterone was noticed for 5 days old CL. The luteal content of progesterone was also increased after adrenaline stimulation (Table 2). When the dose response curves of adrenaline on cAMP and progesterone were compared a conspicuous similarity was noticed (Fig. 2). For all doses the effect of cAMP content decreased with increasing age of the CL, while the effect of progesterone increased. On both parameters maximal effect for all luteal ages were seen at 5 and 50 μg/ml of adrenaline. The time relationships for cAMP are shown in Fig. 3. An increase was seen already after 1 min of exposure to adrenaline (Fig. 3B). Maximal peak values were seen within the first 15 min on cAMP content.

In order to test whether the response of catecholamines and LH are mediated via α- or β-receptor activity, inhibition by PBA and propranolol were tested. Propranolol completely inhibited the effect of catecholamines on cAMP production in concentrations 10^-5 M or higher for all luteal ages (Fig. 4A). For 3 and 5 days old CL also 10^-3 M was effective. Propranolol by itself did not significantly change the control levels of cAMP. Propranolol in a concentration of 10^-3 M also inhibited the effect of LH (Fig. 4B). A small reduction of the effect of LH in 5 days old CL was seen with 10^-3 M. Inhibitory effect of propranolol on progesterone production was tested on 3 days old CL (Fig. 5). Catecholamine-stimulated progesterone production was inhibited by propranolol in concentrations 10^-3 to 10^-5 M (Fig. 5A). The LH-stimulated progesterone production was only inhibited by 10^-3 M propranolol. Since commercially available propranolol represents equal amounts of β- and t-forms, a propranolol compound consisting only of the β-form and thus having weaker β-blocking properties was tested in lower doses. This compound did not block the adrenaline effect on cAMP and progesterone in 3 days old CL in a concentration of 10^-3 M, while a concentration of 10^-1 M had the same blocking effect as the D,.l-form (not shown in figures). Several doses of PBA were tested together with adrenaline and LH. No inhibition of cyclic AMP or progesterone production was seen (Table 2).

Other β-stimulators were tested. Noradrenaline and isoproterenol (Table 2) gave a response on

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**Fig. 3.**

Effects of 5 μg/ml adrenaline on cAMP content in 1 (a) and 3 (b) days old CL. Experimental conditions as in Fig. 1. Values are mean ± SEM of 5 observations.
Experimental adrenaline with mean was was 3
For inhibition similar age
The accumulation of cAMP and progesterone on 3 days old CL, which was equivalent to the effect of adrenaline. The effects of noradrenaline could be inhibited by propranolol (Fig. 5).

Discussion

The effect of catecholamines on the cAMP production in vitro of the CL is highest in 1 and 3 days old CL and then the effect decreases with increasing age of CL. This age-dependent effect is very similar to that of LH as shown by Hertilz et al. (1974). Possible reasons for changed sensitivity to hormones in vitro with luteal age has been discussed earlier (Ahrén et al. 1981). In the ovary, an increase of cAMP is always accompanied or quickly followed by a release of cAMP. This release is sometimes of considerable magnitude and has been studied in vitro and in vivo as release into the follicular fluid or ovarian venous effluent (Selstam et al. 1974, 1976; Selstam & Weiss 1979). The decreased effect of catecholamines with increasing CL age in this study cannot be explained on the basis of differences in release, since the release was noticed to be, if anything, larger from incubated young CL (Table 2). It seems therefore that also in the CL the magnitude of release primarily is a function of cellular concentration. Other possible factors to explain different effects with CL age are changed adenylyl cyclase activity, change in receptor characteristics, functional change of cellular energy metabolism or cellular availability of substrates and hormones.

The effects of catecholamines on cAMP production were maximal after 5–15 min of incubation, which is a considerably shorter time than for LH (Ahrén et al. 1981). It is likely that, at least partly, this difference can be explained by a more rapid diffusion of catecholamines into the tissue. Since the time-relationships were different no attempts were made to study additivity between gonadotro-
phins and catecholamines on cAMP increments (Selstam et al. 1974).

Maximal levels on progesterone production after catecholamine stimulation was also dependent on luteal age. It increased up to day 5. Also the non-stimulated basal production increased concomitantly. A similar increase with luteal age is seen in plasma progesterone of the rat (e.g. Ahrén et al. 1980). This increase in progesterone production with luteal age seems to be due to increased steroidogenic capacity, since luteal blood flow is not changed during this period (Pang & Behrman 1979; Damber et al. 1981) and the cAMP system can be stimulated at all ages. The quantitatively small effect on the cAMP system and lack of the effect on progesterone production in the study of Ratner et al. (1980) may perhaps be assigned to the luteal model used. Dispersed human luteal cells have been reported to be unresponsive to β-stimulators when progesterone production was measured (Richardson & Masson 1980). Catecholamine may, however, potentiate the effects of gonadotropins in human luteal tissue (Hamberger et al. 1980). In addition, dispersed luteal cells from the rat and the sheep are responsive to catecholamines in terms of cAMP and progesterone production (Jordan et al. 1978; Harwood et al. 1980a).

In order to test the specificity of the catecholamine effect α- and β-blockers were used. Propranolol which blocks both β1- and β2-receptors was tested in a wide range of doses. Lower doses (10−5 M or less) inhibited only the effects of catecholamines, while these concentrations did not inhibit the LH effects, which is in agreement with other studies (Jordan et al. 1978; Harwood et al. 1980a). Higher concentrations of propranolol could inhibit also the effects of LH. Higher concentrations, however, do exert quinidine like effects (Marshall et al. 1975).

The inhibitory effect of high concentrations of propranolol on the production of cAMP and progesterone is most likely attributable to this unspecific effect of the drug. The same inhibition was found by Condon & Black (1976), Godkin et al. (1977) and Jordan et al. (1978). In some cases propranolol also suppressed basal progesterone levels. Care must, however, be taken when analysing agonist-antagonist studies of the luteal β-receptor, since some studies indicate that a separation of classical β1 or β2 receptors do not exist in the CL (Coleman et al. 1979).

PBA gave no significant change of catecholamine effects on cAMP and progesterone production. In bovine and rat CL (Condon & Black 1976; Harwood et al. 1980a) likewise no change could be seen. Although α-receptor activity can exert effects on the cAMP system in other tissues (Jacobs 1979), so far this has not been shown to be the case for the CL.

Although it is tempting to propose a functional role of catecholamines for the CL, further studies are needed to investigate possible sources for catecholamines. Particularly are studies of nerves in and around the CL with reference to luteal age needed. Catecholamines themselves may perhaps not have direct effects but may be necessary for gonadotrophic or other hormonal effects or may interfere with degradation of catechol-oestrogens. Thus, many possibilities for a role of catecholamines in luteal function remain open.

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