Purine Amplification of Luteinizing Hormone Action in Ovarian Luteal Cells*

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Adenosine produced a severalfold amplification of luteinizing hormone (LH)-stimulated cAMP accumulation and progesterone secretion in rat luteal cells, but showed no similar effect on LH-stimulated cAMP accumulation and androgen secretion in Leydig cells. In the absence of LH, adenosine produced a small but significant stimulation of cAMP accumulation and steroid secretion in both luteal and Leydig cells. In isolated luteal membranes, adenosine increased LH-stimulated adenylyl cyclase activity, but this response was small compared to the effect seen in intact cells. The ED50 of adenosine for amplification of LH-stimulated cAMP accumulation in luteal cells was 22 μM; deoxycoformycin, an inhibitor of adenosine deaminase, increased adenosine potency (ED50 ~ 10 μM) and treatment with adenosine deaminase decreased maximum adenosine activity by 25%. ATP, ADP, and AMP were equipotent with adenosine; inosine and adenine showed 75 and 30%, respectively, of the maximum activity of adenosine. Adenosine deaminase treatment reduced activity of ATP, ADP, AMP, and adenosine to a level similar to that of inosine but had no effect on the activity seen with inosine or adenine. It was concluded that the activity of the adenine nucleotides was probably due to adenosine formation. The relative potency of the adenine-derivative purines was adenosine > inosine > adenine. Guanosine, guanine, cytidine, cytosine, thymidine, and thymine were inactive. None of the purines inhibited LH-stimulated cAMP accumulation, LH receptor binding activity, or progesterone secretion. An adenosine analog, 2-chloroadenosine, which is resistant to metabolism, mimicked the effect of adenosine but with 80% less activity. Dipyridamole, a drug which inhibits cellular transport of purines, inhibited luteal cell uptake of adenosine (IC50 ~ 5 μM) and amplification of LH-stimulated cAMP accumulation produced by adenosine (IC50 ~ 3 μM). Maximal inhibition of this effect of adenosine by dipyridamole was about 75%. Dipyridamole had no effect on 2-chloroadenosine-dependent amplification of LH-stimulated cAMP accumulation, but completely blocked the response to inosine. Theophylline also inhibited both cellular uptake of adenosine (IC50 ~ 7 mM) and adenosine amplification of cAMP accumulation. It is suggested that a major component (~80%) of amplification by adenosine of LH-stimulated cAMP accumulation is dependent on cellular transport of the purine, a conclusion based on inhibition of uptake of adenosine by dipyridamole and theophylline. Since dipyridamole maximally reduced adenosine amplification to a level similar to that of 2-chloroadenosine, but had no effect on the amplification produced by the analog, it is suggested that an additional component of adenosine action may involve interaction with a catalytic site on the luteal cell to amplify the response to LH.

Since the observation of Sattin and Rall (1) that adenosine stimulated cAMP accumulation in brain slices, a variety of other tissues has been shown to respond to purines with stimulatory and/or inhibitory effects on cAMP accumulation or adenylyl cyclase activity (2). In adrenal and Leydig tumor cells, adenosine stimulated steroidogenesis in intact cells and stimulated adenylyl cyclase activity in membrane preparations from the same cells (3). No information is presently available on the effect of purines in normal adrenal, Leydig, or ovarian cells. It has been suggested that adenosine interacts with specific membrane receptors in brain slices (1) and in adrenal and Leydig tumor cells (3). A high and low affinity binding site was shown for adenosine in fat cell membranes, and binding of the high affinity site was antagonized by theophylline (4). However, it is not known whether membrane binding mediates the effects of adenosine in the various cell types.

Both the ovarian luteal and testicular Leydig cells respond to LH with a rapid rise in cAMP accumulation and steroid synthesis and secretion (5, 6). Considerable evidence indicates that LH, by interaction with specific receptors (7), activates adenylyl cyclase, which results in an increase in intracellular cAMP (8-10). In the intact luteal cell, LH stimulation of cAMP accumulation is subject to rapid inhibition by prosta- glandin F2α (11) and by gonadotropin-releasing hormone (12, 13). Both inhibitory agents appear to act through specific membrane receptors (14, 15). More recently, we reported that rat luteal cells in culture showed a marked time-dependent amplification of cAMP accumulation in response to LH (16). Since adenosine had previously been shown to elicit both stimulation and inhibition of cAMP accumulation, we, therefore, examined the possibility that adenosine may affect LH-stimulated processes in the luteal cell. We also assessed the effect of adenosine on similar parameters of Leydig cell function to determine the specificity of the cellular response to adenosine in a separate, but LH-dependent system.

MATERIALS AND METHODS
Multiple ovulation and ovarian luteinization were induced in im-

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1 The abbreviations used are: LH, luteinizing hormone; hCG, human chorionic gonadotropin.

10390
Adenosine Amplification of LH Action in Luteal Cells

RESULTS

Effect of Adenosine on the Dose-Response to LH—The effect of adenosine on the dose-response of luteal cells to LH with cAMP accumulation is shown in Fig. 1. Both basal and LH-stimulated cAMP levels were significantly increased by adenosine. The increase in basal cAMP levels produced by adenosine was about 2-fold (p < 0.01) (see inset in Fig. 1) and a marked increase in LH-stimulated cAMP accumulation occurred. This potentiation of LH-stimulated cAMP accumulation increased from 2.5-fold at 10 ng/ml of LH to more than 34-fold at 1000 ng/ml of LH. In other studies, an extended dose-response to LH was examined (data not shown). Maximum cAMP accumulation occurred at 1000 ng/ml of LH in the absence (7.6-fold, p < 0.01) and in the presence of 50 μM adenosine (90-fold, p < 0.01), and the half-maximal dose (EDso) of LH (about 100 ng/ml) appeared not to be changed by 50 μM adenosine.

LH, in the absence of adenosine, produced a dose-dependent increase in progesterone secretion with an EDso of about 25 ng/ml (Fig. 2) and a maximal response was seen at about 100 ng/ml of LH. However, in the presence of adenosine, the magnitude of LH-stimulated progesterone secretion was sharply increased; adenosine reduced the EDso for LH from 25 to 10 ng/ml and increased the maximum response to LH by about 30%. In the absence of LH, adenosine produced a slight, but significant, increase in progesterone secretion.

Dose-Response Effect of Adenosine—Adenosine produced a significant (p < 0.01) dose-dependent increase in cAMP accumulation and progesterone secretion in the absence of LH (Table I). This effect was seen at 100 and 1000 μM adenosine but not at 10 μM adenosine. The magnitude of stimulation by adenosine alone was small in comparison to the effect seen with LH or a combination of adenosine and LH. In the presence of LH, a significant increase (p < 0.01) in cAMP accumulation was seen at 10 μM adenosine, and this response was amplified with higher concentrations of the purine. The concentration of LH (200 ng/ml) used in these studies was maximal for progesterone secretion; however, 100 and 1000 μM adenosine significantly increased (p < 0.05) the maximum progesterone secretory response over that seen with LH alone. These data are consistent with the effect of aden-
Adenosine Amplification of LH Action in Luteal Cells

Luteal cells were incubated for 90 min and each treatment group contained 5 replicates. See "Materials and Methods."
ulated cAMP accumulation. Adenosine deaminase significantly reduced basal cAMP levels from 0.51 ± 0.03 to 0.38 ± 0.03 pmol/10^5 cells, but no effect was seen on LH-stimulated cAMP levels. Adenosine deaminase had no effect on the response of luteal cells to adenine. Adenine amplified LH-stimulated cAMP accumulation to about 10% of that seen with 100 μM adenosine, but no activity was seen following incubation of guanosine with adenosine deaminase. Guanine, thymine, cytosine, cytidine, and thymidine were inactive.

Based on the studies with deoxycoformycin and with adenosine deaminase, adenosine was the most potent of the compounds examined. The activity of purine nucleotides appeared to be due to either contamination with adenosine or, more probably, conversion to adenosine following incubation with the cells. Inosine and adenine were significantly less active than adenosine. The relative potency based on maximal amplification was adenosine > inosine > adenine. None of the compounds inhibited LH-stimulated progesterone secretion.

**Time Course for Purine Augmentation of LH-stimulated cAMP Accumulation**—An increase in cAMP accumulation by adenosine was evident after activation of the cells by LH (Fig. 4). Following an initial lag period of about 5 min, adenosine produced a linear increase in cAMP levels for 60 min. No apparent difference in the lag period was seen between adenosine, inosine, and adenine or a combination of adenosine and adenosine deaminase. However, the rate of cAMP accumulation with adenosine was 2-fold greater than that for inosine or a combination of adenosine and adenosine deaminase and 4.5-fold greater than that for adenine.

**Effect of 2-Chloroadenosine**—In the absence of LH, 10 and 100 μM 2-chloroadenosine increased basal cAMP levels by 1.8- and 3.5-fold (p < 0.01), respectively; basal progesterone secretion was increased by 1.2- and 1.3-fold (p < 0.05), respectively (Fig. 5). 1 μM 2-chloroadenosine was inactive, data not shown. In the presence of increasing doses of LH, both cyclic AMP accumulation and progesterone secretion were increased in a dose-dependent manner by 2-chloroadenosine. This effect of the analog was similar in effect, but smaller in magnitude, than that seen for adenosine. For example, the ED₅₀ for LH on cAMP accumulation was not affected by 2-chloroadenosine, but the maximum response to LH was increased about 1.5-fold (p < 0.01) by 100 μM concentration of 2-chloroadenosine. The effect of 2-chloroadenosine on progesterone secretion was to decrease the ED₅₀ for LH from about 20 to 5 ng/ml; no change in the maximum response to LH was seen that was statistically significant. In other studies, the potency of 2-chloroadenosine on LH-stimulated cAMP accumulation was compared directly to that of adenosine and was found to be about one-fifth as active.

**Effect of Dipyridamole**—To examine the possibility that purines may have an intracellular site of action, dipyridamole, a known inhibitor of purine uptake by cells, was tested (Figs. 6 and 7). In the absence of LH or purines, dipyridamole at 10 and 50 μM increased cAMP accumulation by 1.7-fold (p < 0.05), but no significant effect was seen at 5 μM (Fig. 6). Adenosine (50 μM) in the absence of LH increased cAMP accumulation by 2-fold (p < 0.05), but no dose-dependent effect of dipyridamole on this response to adenosine was seen. The increase in cAMP accumulation produced by LH in the absence of adenosine was not significantly changed by 5 μM dipyridamole, but at 10 and 50 μM concentration of drug, this response was significantly reduced by 30% (p < 0.05) and 70% (p < 0.01), respectively. The increase in cAMP levels produced by a combination of LH and adenosine was markedly reduced in a dose-dependent manner by dipyridamole; half-maximal inhibition was seen at about 3 μM dipyridamole.

A clear dose-dependent increase in basal progesterone se-
Levels were 0.4 pmol/10^5 cells and 0.8 pmol/10^5 cells and 0.8 pmol/10^5 cells, respectively. Each treatment group contained 4 replicates. Adenosine 200 ng/d of LH luteal cells (mean 5010^394 for 90 min with dipyridamole parallel to that seen in the absence of adenosine. Progesterone secretion in response to LH in the absence of adenosine increase in progesterone secretion produced by adenosine presence of LH and deoxycoformycin; the latter was added to appear to be slightly reduced by dipyridamole, but no clear dose-response effect was evident. On the other hand, progesterone secretion in response to a combination of adenosine and LH was decreased by dipyridamole.

In an additional study, the effect of dipyridamole was examined on adenosine-stimulated cAMP accumulation in the presence of LH and deoxycoformycin; the latter was added to inhibit endogenous adenosine deaminase activity (Fig. 8). In addition, the effect of dipyridamole was tested in combination with inosine and 2-chloroadenosine both of which were co-incubated with LH and adenosine deaminase. A highly significant and dose-dependent decrease in cAMP accumulation was seen with dipyridamole. Maximum inhibition was seen at 5 μM dipyridamole for both adenosine and inosine; at this dose, cAMP levels were decreased about 75 and 90%, respectively. At 5 μM dipyridamole, cAMP accumulation in response to inosine was not significantly different from that seen with LH alone. On the other hand, the response to adenosine and

**TABLE III**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Adenosine</th>
<th>Dipyridamole</th>
<th>Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>38.5 ± 7.6</td>
<td>NT^a</td>
<td>NT</td>
</tr>
<tr>
<td>1.0</td>
<td>NT</td>
<td>28.2 ± 7.5</td>
<td>NT</td>
</tr>
<tr>
<td>5.0</td>
<td>64.0 ± 1.4</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>10.0</td>
<td>NT</td>
<td>62.0 ± 3.7</td>
<td>NT</td>
</tr>
<tr>
<td>25.0</td>
<td>79.5 ± 2.0</td>
<td>74.1 ± 1.0</td>
<td>NT</td>
</tr>
<tr>
<td>50.0</td>
<td>88.6 ± 0.2</td>
<td>84.3 ± 1.7</td>
<td>NT</td>
</tr>
<tr>
<td>500.0</td>
<td>NT</td>
<td>NT</td>
<td>10.6 ± 3.5</td>
</tr>
<tr>
<td>1,000.0</td>
<td>NT</td>
<td>NT</td>
<td>26.4 ± 6.9</td>
</tr>
<tr>
<td>5,000.0</td>
<td>NT</td>
<td>NT</td>
<td>37.4 ± 8.6</td>
</tr>
<tr>
<td>10,000.0</td>
<td>NT</td>
<td>NT</td>
<td>70.8 ± 2.8</td>
</tr>
<tr>
<td>IC_50</td>
<td>2 μM</td>
<td>5 μM</td>
<td>7 mM</td>
</tr>
</tbody>
</table>

^a Not tested.
LH was significantly greater at both 5 and 10 μM dipyridamole than the response to LH alone. In contrast to the effect on adenosine- and inosine-stimulated cAMP accumulation, dipyridamole showed no dose-response effect on 2-chloroadenosine-stimulated cAMP accumulation in the presence of LH. It was interesting that the potency of adenosine in the presence of dipyridamole was about the same as that seen with 2-chloroadenosine (Figs. 5 and 8).

To assess the action of dipyridamole on uptake of adenosine, cells were incubated in the presence of variable concentrations of radiolabeled adenosine and with dipyridamole for 5 min at room temperature (Table III). Uptake of radiolabeled adenosine was linear over the first 5 min of incubation and a linear relationship between cell number and adenosine uptake was evident. Increasing concentrations of nonradioactive adenosine produced a clear dose-dependent inhibition of [3H]adenosine uptake; the concentration of adenosine which produced 50% inhibition (IC50) of uptake was about 2 μM. Dipyridamole and theophylline also produced a dose-dependent inhibition of [3H]adenosine uptake by the cells with an IC50 of 5 μM and 7 mM, respectively. Thus, 1 site of dipyridamole action in the luteal cell may be inhibition of adenosine uptake which may be the basis for inhibition by this drug of adenosine amplification of cAMP accumulation.

**Effect of Theophylline**—Since theophylline has been shown to inhibit adenosine-stimulated cAMP accumulation in other tissues, this action of theophylline was examined in luteal cells (Fig. 9). In 1 study, 1 and 10 mM theophylline was examined in the presence of LH and in combination with LH and 10 or

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**Fig. 9.** Effect of theophylline on adenosine amplification of LH-stimulated cAMP accumulation and progesterone secretion in luteal cells (mean ± S.E.). Luteal cells (10^5 cells/flask) were incubated for 90 min with LH (200 ng/ml). Theophylline was tested at 0 (■), 1 (△), and 10 (▲) mM in the presence of the indicated concentrations of adenosine. Basal cAMP and progesterone levels were 0.2 ± 0.1 pmol/10^5 cells and 58 ± 1 ng/10^5 cells, respectively. Each treatment group contained 5 replicates.

**Fig. 10.** Effect of adenosine on basal and LH-stimulated cAMP accumulation and androgen secretion in Leydig cells (mean ± S.E.). Leydig cells (10^6 cells/flask) were incubated for 90 min with 0 (■), 10 (△), and 100 μM adenosine (▲) in the absence and presence of LH (see "Materials and Methods"). Each treatment group contained 4 replicates.

**Table IV**

<table>
<thead>
<tr>
<th>Theophylline (mM)</th>
<th>0</th>
<th>0.01</th>
<th>0.1</th>
<th>1.0</th>
<th>5.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP (pmol)</td>
<td>9.9 ± 0.4</td>
<td>9.9 ± 0.3</td>
<td>12.1 ± 0.6*</td>
<td>12.8 ± 1.1*</td>
<td>9.2 ± 0.2</td>
<td>5.4 ± 0.2*</td>
</tr>
<tr>
<td>Progesterone (ng)</td>
<td>53.6 ± 1.4</td>
<td>54.3 ± 1.9</td>
<td>58.4 ± 3.1</td>
<td>46.9 ± 1.5*</td>
<td>48.1 ± 3.4*</td>
<td>35.2 ± 3.2*</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to the no theophylline control.
Adenosine Amplification of LH Action in Luteal Cells

**Effect of Adenosine on Adenylate Cyclase Activity in Ovarian Luteal Membranes**

Each treatment group contained 10 replicates (see "Materials and Methods"). No LH versus adenosine (p < 0.10); control versus LH (p < 0.01); LH versus LH + adenosine (p < 0.01).

<table>
<thead>
<tr>
<th>Adenosine</th>
<th>0</th>
<th>10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pμmol cAMP/10 min/6 mg protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.78 ± 0.08</td>
<td>0.61 ± 0.08</td>
</tr>
<tr>
<td>5 μg/ml</td>
<td>1.34 ± 0.08</td>
<td>1.73 ± 0.07</td>
</tr>
</tbody>
</table>

100 μM adenosine. Both 1 and 10 mM theophylline significantly increased, by about 2-fold (p < 0.01), cAMP accumulation in response to LH, but progesterone secretion was significantly reduced at each dose of theophylline by about 40% (p < 0.01). Adenosine at 10 and 100 μM produced a dose-dependent increase in LH-stimulated cAMP accumulation; at 10 μM adenosine the increase in cAMP levels was greater than that seen with theophylline alone. Theophylline at 1 mM had no significant effect on adenosine-stimulated cAMP accumulation but 10 mM theophylline decreased the stimulatory effect of adenosine by about 20 and 60%, respectively. Inhibition of LH-stimulated progesterone produced by 1 mM theophylline was partially prevented, in a dose-dependent manner, by increasing doses of adenosine. At 10 mM theophylline, increasing doses of adenosine had no effect on progesterone secretion.

In another study, an extended dose-response to theophylline in the presence of 50 μM adenosine and LH was examined (Table IV). Accumulation of cAMP was significantly increased with 0.1 and 1 mM theophylline, but no effect was seen with 0.01 mM theophylline. However, at 5 and 10 mM theophylline, a significant and dose-dependent decrease in cAMP accumulation occurred. Progesterone secretion in response to adenosine and LH was not affected by 0.01 or 0.1 mM theophylline, but a significant decrease in progesterone secretion was seen at 1, 5, and 10 mM theophylline. The decrease in progesterone secretion seen at 5 and 10 mM theophylline reflected a similar but more pronounced decrease in cAMP levels at the same doses of drug; at 1 mM theophylline, cAMP accumulation was increased, whereas progesterone secretion was inhibited. This discrepancy may be due to an inhibitory action of theophylline on progesterone secretion independent of adenosine antagonism. Nonetheless, increased levels of adenosine appeared to overcome the inhibitory action of 1 mM theophylline (Fig. 9).

Since the response of luteal cells to theophylline was clearly different from that seen with adenosine, it is unlikely that adenosine augmented LH-stimulated cAMP accumulation and progesterone secretion by inhibition of cAMP phosphodiesterase activity.

In other studies, theophylline was found to produce a significant inhibition of adenosine uptake by luteal cells (Table III). The IC₅₀ for this effect of theophylline was about 7 mM, but inhibition was clearly evident at a dose of 1 and 5 mM. Thus, the inhibitory effects of theophylline on LH-stimulated responses of the luteal cell may be due, in part, to inhibition of adenosine uptake.

**Effect of Adenosine on Leydig Cells**—The effect of adenosine on Leydig cell cAMP accumulation and androgen production in the absence and presence of variable concentrations of LH is shown in Fig. 10. In contrast to the effect in luteal cells, 1 (not shown), 10, and 100 μM adenosine had little or no effect on LH-stimulated cAMP accumulation and androgen secretion in Leydig cells, but basal cAMP accumulation and androgen secretion were slightly, but significantly, increased (p < 0.05) with 100 μM adenosine. The dose-response of testicular cells to LH with cAMP accumulation was qualitatively similar to that seen with ovarian luteal cells with a half-maximal response at a concentration of LH between 10 and 100 ng/ml. However, androgen secretion in response to LH was near maximal at about 10 ng/ml of LH in testicular cells but about 25 ng/ml of LH for progesterone secretion in luteal cells.

In other studies, testicular cells were incubated with deoxycoformycin (2 μM) to examine the possibility that the lack of response of these cells to adenosine may have occurred due to deamination of endogenous adenosine to inosine. Although deoxycoformycin increased the response to adenosine in luteal cells, no effect was seen in testicular cells by adenosine alone or in combination with deoxycoformycin in an experimental protocol identical with that shown in Fig. 10.

**Effect of Adenosine on LH-Receptor Binding**—To assess the possibility that adenosine and other nucleosides may affect binding activity of the ovarian luteal LH receptor, membranes from this tissue were incubated in the presence of 10 nM to 100 μM concentration of each nucleoside in the presence of 125I-hCG (Fig. 11). No effect of adenosine, inosine, guanosine, or cytidine was seen. In addition, co-incubation of adenosine (10 and 100 μM) with variable concentrations of LH had no effect on the magnitude of inhibition of binding of 125I-hCG by LH. Thus, it was concluded that adenosine did not affect binding activity of the luteal LH receptor.

**Effect of Adenosine on Adenylate Cyclase Activity in Luteal Membranes**—The effect of adenosine on adenylate cyclase activity in membranes prepared from ovarian luteal tissue is shown in Table V. In the absence of LH, 10 μM adenosine had no effect on enzyme activity. In the presence of LH, 5 μM adenosine had no effect on enzyme activity. LH (5 μg/ml) stimulated adenylate cyclase activity about 1.7-fold (p < 0.001), and this response was significantly increased by 30% (p < 0.01) with 10 μM adenosine. In other studies, the increase in adenylate cyclase with higher doses of adenosine was never greater than about 30%.
DISCUSSION

The present studies show that adenosine produced a remarkable increase in LH-stimulated cAMP accumulation in luteal cells and this effect was reflected in a marked increase in progesterone secretion, the functional response of these cells. To a much lesser extent, adenosine also increased basal cAMP accumulation and progesterone secretion in the absence of LH; the latter effect was variable and seen only at high concentrations of adenosine. Although a similar effect of adenosine on cAMP accumulation has been seen with other tissues and cells in response to catecholamine (1, 25, 26), this appears to be the first report of an interaction of adenosine with a polypeptide hormone. In the luteal cell, the effect of adenosine on cAMP accumulation was a marked increase in the maximum response to LH but with little effect on the EDSO of the hormone. Progesterone secretion showed only a modest and variable increase in response to adenosine at maximal concentrations of LH, but the EDSO for LH was markedly decreased. These effects of adenosine may have physiological significance since the luteal cell in situ is normally exposed to very low levels of LH. For example, in the present studies, 10 ng/mL of LH had no effect on progesterone secretion, but in the presence of adenosine, the same dose of LH produced a near maximal stimulation of progesterone secretion. Although no evidence presently exists to show that adenosine plays a role in modulating LH action in vivo, these studies show that the luteal cell can rapidly increase its functional response to LH by severalfold without a change in LH concentration or binding of gonadotropin to the LH receptor. Conversely, a decrease in LH-stimulated cAMP accumulation and progesterone secretion in luteal cells incubated with prostaglandin F2α (11) and gonadotropin-releasing hormone (12, 13) has been shown. Thus, the functional response of the luteal cell may be modulated as much by local paracrine effectors (e.g., prostaglandin F2α and adenosine) as by the concentration of LH.

Amplification by adenosine of LH-stimulated processes showed cell specificity. Leydig cell cAMP accumulation and androgen secretion in response to LH was not significantly affected by adenosine. Incubation of Leydig cells with adenosine and an adenosine deaminase inhibitor, deoxycoformycin, also showed no effect. On the other hand, Leydig cells responded to LH with maximal steroid secretion at much lower doses than did the luteal cells. However, in the presence of adenosine, the steroid secretory response of luteal cells to LH more closely resembled the Leydig cell with regard to sensitivity to LH. It is possible that the Leydig cell functional response may be more efficiently coupled to LH than in the luteal cell. This difference in cellular response to LH may be relevant to the cyclical loss of function of the corpus luteum which occurs in each cycle and which is associated with a loss of response to LH before a decrease in LH receptor binding occurs (27). In contrast, no cyclical and coordinated loss of Leydig cell function is seen.

In the absence of LH, adenosine increased both Leydig cell cAMP accumulation and androgen secretion. Previous evidence of adenosine-stimulated cAMP accumulation and steroid secretion in adrenal and Leydig tumor cells has been reported (3). From those studies, it appears that adenosine may stimulate or inhibit the cell response (28). In the present studies, both luteal and Leydig cells showed an increase in response to adenosine in the absence of LH, but the major effect of adenosine in the luteal cell was clearly an amplification of LH action which was not evident in the Leydig cell. No inhibition of LH-stimulated cAMP accumulation or androgen secretion was seen with adenosine.

Amplification of LH-stimulated cAMP accumulation by adenosine showed a dose-dependent effect and the EDSO for this response was about 5 μM. However, this effect of adenosine was enhanced by co-incubation of luteal cells with both adenosine and deoxycoformycin, an inhibitor of adenosine deaminase (29). Under these circumstances, the EDSO for adenosine was reduced to about 10 μM which indirectly indicates that adenosine-augmented cAMP accumulation was seen at about 5 μM. At this dose of dipyridamole, amplification of LH-stimulated cAMP accumulation was about 75%. Dipyridamole has been shown to inhibit cellular transport or by inhibition of adenosine at an extracellular site. In other systems such as brain slices (31), dipyridamole has been shown to inhibit cellular transport of adenosine, but the same drug increased cAMP accumulation in response to a combination of adenosine and biogenic amines. If an extracellular, catalytic site of adenosine action is involved, this site in the brain appears not to be inhibited to the same extent as the site involved in urine transport. In addition, the high affinity binding site of adenosine in fat cell membranes was not inhibited by dipyridamole (4). In adrenal tumor cells, 40 μM dipyridamole increased adrenal steroidogenesis in response to adenosine yet this dose of drug was shown to inhibit adenosine transport by 95% (3). Thus, it appears that dipyridamole, in several systems, may block cellular transport of adenosine but enhance stimulation of cAMP accumulation produced by this
purine. Since dipyridamole showed a marked and dose-dependent inhibition of adenosine on LH-stimulated cAMP accumulation and on adenosine uptake (IC\textsubscript{50} = 5 mM) in the present studies, it is suggested that a major site of adenosine action in the luteal cell may be dependent on adenosine transport. This conclusion is consistent with the apparent lack of specificity of the purine transport system and the ability of inosine and adenine to mimic adenosine actions in the luteal cell, albeit at reduced potency.

An extracellular site of adenosine action in the luteal cell may also occur. Evidence for this conclusion is indirect but based on several experiments. First, dipyridamole maximally inhibited adenosine-augmented cAMP accumulation by about 80%. The residual activity of adenosine in the presence of dipyridamole may have been due to an extracellular site of action or to cellular uptake of adenosine by a process which was not inhibited by dipyridamole. Second, 2-chloroadenosine amplified LH-stimulated cAMP accumulation, and this response was not affected by incubation of the analog or the cells with adenosine deaminase. Since dipyridamole had no effect on the response to 2-chloroadenosine and 2-chloroadenosine appears not to be an effective substrate for cellular adenine uptake in brain slices (32), it is possible that potentiation by this analog of LH-stimulated cAMP was due to an extracellular site of action. It is interesting that maximum inhibition by dipyridamole of adenosine-augmented cAMP accumulation was about 75%, whereas 2-chloroadenosine was about 10 to 20% as active as adenosine. Third, adenosine augmented cAMP accumulation in the presence of LH in isolated membranes from luteal tissue in which an ATP-generating system was present to provide excess substrate (22). Fourth, theophylline produced a dose-dependent inhibition of adenosine-augmented cAMP accumulation; this effect of theophylline was evident at doses between 1 and 10 mM, but lower doses increased the response to adenosine presumably due to cAMP-phosphodiesterase inhibition. The antagonistic effect of theophylline on adenosine amplification of cAMP accumulation is consistent with reports from other investigators (3, 31) in which adenosine was suggested to have an extracellular site of action. Furthermore, increasing the dose of adenosine reversed the inhibition produced by theophylline on LH-stimulated progesterone secretion. However, theophylline inhibited uptake of adenosine in the present studies with an IC\textsubscript{50} of 7 mM and other studies showed that 1 mM theophylline inhibited adenosine uptake in brain slices (31).

Based on studies in other tissues, cellular transport of adenosine is rapidly followed by phosphorylation to nucleotide metabolites (33). Although adenosine (or some product) may produce an intracellular catalytic effect to amplify LH-stimulated cAMP accumulation, it is equally possible that cellular transport of adenosine may lead to increased substrate levels of ATP in the luteal cell for production of cAMP by an LH-activated adenylate cyclase complex. An effect of adenosine on cAMP phosphodiesterase activity appears unlikely in view of the results with theophylline in this and in other studies (31, 34). Independent of the mechanism or the site of action of adenosine, the magnitude of increase in cAMP accumulation and the increase in functional response to low levels of LH, to which the luteal cell is usually exposed, raise the possibility that adenosine may be a physiological amplifier of LH action in vivo.

REFERENCES