Antagonism of an Adenosine/ATP Receptor in Follicular Xenopus Oocytes

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ABSTRACT

Follicular Xenopus oocytes possess a novel receptor where both adenosine and ATP activate a cAMP-dependent, nonrectifying K⁺-current. Five compounds, α,β-methylene ATP (α,β-meATP), 8-(p-sulphophenyl)theophylline (8-SPT), theophylline, 2,2'-pyridilisatogen tosylate (PIT) and suramin, were tested as antagonists of adenosine- and ATP-activated K⁺-currents. The descending order of activity (pIC50 values) against adenosine responses was: α,β-meATP (6.72) = 8-SPT (6.68) > theophylline (5.32) > PIT (4.58), whereas suramin was relatively inactive. The blocking actions of α,β-meATP and alkylxanthine compounds were reversible with washout, whereas blockade by PIT was irreversible. These antagonists showed similar blocking activity against ATP responses, except for PIT which was more effective at ATP responses than at adenosine responses. The selectivity of antagonists was tested against cAMP-dependent K⁺-currents evoked by forskolin and follicle-stimulating hormone (FSH), 8-SPT and theophylline did not inhibit but instead augmented forskolin and FSH responses; this augmentation may be caused by inhibition of phosphodiesterase activity inside follicle cells. On the other hand, α,β-MeATP and PIT inhibited forskolin and FSH responses; both compounds apparently are nonselective antagonists. Thus, only alkylxanthine derivatives (8-SPT and theophylline) were selective antagonists of the novel adenosine/ATP receptor in Xenopus oocytes, whereas α,β-meATP and PIT were nonselective in their blocking actions and suramin was relatively inactive.

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The pharmacology of this adenosine/ATP receptor in follicular Xenopus oocytes has been characterized extensively. Adenosine and ATP are equipotent agonists (EC50 values, 1.9 ± 0.3 μM and 1.7 ± 0.3 μM, respectively), although ATP activity is spared by adenosine deaminase whereas adenosine activity is abolished in the presence of this enzyme (King et al., 1996b). The receptor also is activated by β,γ-met ATP which is as potent as ATP (King et al., 1996b). Full blockade of adenosine and ATP responses was seen with high concentrations of alkylxanthine derivatives (8-SPT and theophylline) and α,β-meATP (King et al., 1996b). Other adenosine/ATP receptors have been reported on nerve terminals of rat sympathetic nerves (Shinozuka et al., 1988, 1990; Forsyth et al., 1991; Todorov et al., 1994) and in rabbit brain cortex (von Kügelgen et al., 1992) and show a pharmacological profile similar to this oocyte receptor. These atypical adenosine/ATP receptors have been called P3 receptors by some investigators, although von Kügelgen and colleagues (1992) preferred the term “novel P1 receptor” because, in their hands, adeno-

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sine is the most active of naturally occurring agonists and the receptor is blocked selectively by alkylxanthine derivatives.

In the present study, we have examined the activity and selectivity of potential antagonists of this novel P1/P3 receptor in follicular *Xenopus* oocytes. This work precedes ongoing experiments in our laboratory on expression cloning of this adenosine/ATP receptor with fractionated pools of oocyte mRNA. Thus, information on agonist activity (see King et al., 1996b) and antagonist activity (present article) should help in the process of characterizing the recombinant P1/P3 receptor. Previously identified antagonists of the P1/P3 receptor in mammalian tissues and oocytes include 8-t-p-sulphophenyltheophylline and theophylline (Lotan et al., 1982, 1985, 1986; von Kügelgen et al., 1992; King et al., 1996b) and α,β-meATP (Shinozuka et al., 1990; von Kügelgen et al., 1992; King et al., 1996b). These compounds generally have been tested only at single doses which gave full blockade. Here, these compounds were tested extensively on adenosine- and ATP-activated K⁺-current in follicular oocytes. Two P2 receptor-selective antagonists also were tested, namely PIT (Spedding and Weetman, 1976) and suramin (Dunn and Blakeley, 1988), to help discriminate between P2 and P1/P3 receptor pharmacology. The selectivity of antagonists also was tested against forskolin- and FSH-activated cAMP-dependent K⁺-current in follicular oocytes. Part of this study was presented to the British Pharmacological Society (King et al., 1997).

**Methods**

*X. laevis* were anesthetized with Tricaine (0.1% w/v), decapitated and ovaries surgically removed. Mature oocytes (stages V and VI) were removed from the inner lining of ovarian sacs and stored at (4°C) in Barth’s solution (pH 7.4) containing (mM): NaCl, 110; KCl, 1; NaHCO₃, 2.4; Tris-HCl, 7.5; Ca(NO₃)₂, 0.33; CaCl₂, 0.41; MgSO₄, 0.82; gentamycin sulfate, 50 μg/l.

Follicular oocytes were studied under voltage-clamp conditions with a twin-electrode amplifier (Axoclamp 2A), as described previously (King et al., 1996a, b). Oocytes were placed in an electrophysiological chamber (0.5 ml volume) and superfused (at 5 ml/min) with a Ringer’s solution (at 18°C) containing (mM): NaCl, 110; KCl, 2.5; HEPES, 5; CaCl₂, 1.8, adjusted to pH 7.4. The voltage-recording (1–2 megohm tip resistance) and current-recording (2–5 megohm tip resistance) microelectrodes were filled with 0.6 M K₂SO₄ and 3.0 M KCl, respectively. Oocytes were studied if their resting membrane potential was greater than −40 mV and input resistance greater than 0.5 meq/m. The reversal potential (Eᵥₑ) of agonist-evoked K⁺-currents was determined from the current/voltage (I/V) relationship to brief voltage commands (from 0 mV to −100 mV, in 10-mV steps for 1 s, Vᵥₑ = −40 mV) These brief voltage steps were applied before agonist superfusion to determine the leakage current and reapplied at the peak of agonist responses; leakage currents were subtracted from agonist-evoked currents. Electrophysiological data were stored on magnetic tape using a DAT recorder (Sony 1000ES) and displayed by a pen recorder (Gould, Ilford, UK).

Adenosine, ATP and other agonists were added to the superfuse at the concentrations mentioned in the text. Nucleosides and nucleotides were applied for 120 s at 20 min apart, whereas forskolin and FSH were applied for 300 s at 60 min apart. The maximum amplitude of drug-evoked K⁺-currents was highly variable (100–1000 nA, at −40 mV) among follicular oocytes from the same donor. This variability is caused by differences in oocyte size, maturation stage, receptor density and extent of intercellular coupling between the oocyte and follicle cells. Therefore, data from I/V relationships were normalized to the K⁺-current evoked at 0 mV in each experiment.

The activity of antagonists were tested primarily against adenosine responses (10 μM). Cumulative concentrations of antagonist were added to the superfuse, where each concentration was applied for 20 min before testing the activity of adenosine. The antagonist concentration that reduced adenosine responses by 50% (IC₅₀ value) was determined from linear plots of data, where the transform log (Iₓᵢₓₑᵢₓ, − I) was used (I representing the percentage inhibition caused by each antagonist concentration). The slope of inhibition curves was also taken from these linear plots. Determinations are expressed in terms of mean ± S.E.M. for four sets of data per antagonist; activity indices are given as pIC₅₀ values (−logIC₅₀ value). With a concentration near the mean pIC₅₀ value, antagonists were retested against ATP responses (10 μM) and adenosine responses (10 μM) and the degree of inhibition compared statistically by Student’s unpaired t-test. The selectivity of antagonist was also tested against forskolin (10 μM) and FSH (30 nM)-activated K⁺-currents. In these experiments, antagonists were applied for 60 min at a concentration that gave 100% inhibition of adenosine responses and their effects assessed from changes in chord conductance and Eᵥₑ of I/V plots for forskolin- and FSH-evoked K⁺-currents.

The [³H]adenosine-prelabeling technique (Donaldson et al., 1988) was used to measure the production of [³H]cAMP from [³H]ATP in follicular *Xenopus* oocytes stimulated by activators of cAMP-dependent K⁺-currents. Five oocytes per well were incubated in 1 ml of L-15 medium (Sigma, Poole, Dorset, UK) containing 2 μCi [³H]adenine (2 h at 25°C). Oocytes were washed three times in L-15 medium, placed in 1 ml of L-15 medium containing either adenosine, ATP or forskolin (10 μM, for 10 min) or FSH (30 nM, for 10 min), then lysed by adding 50 μl concentrated HCI (12 N). Experiments were repeated in the presence of each antagonist (used at its IC₅₀ level), preincubated for 20 min before the addition of an agonist. Control oocytes were washed three times in L-15 medium, placed in 1 ml of L-15 medium (10 min) and lysed with HCI to assess the level [³H]adenine uptake. Cell lysate (50 μl) was placed in 5 ml of scintillant (HiSafe, Fisons, Loughborough, UK) and counted for 60 s in a scintillation counter (Beckman LS6000 IC).

All compounds were obtained from Sigma Chemicals (Poole, Dorset, UK), except for 8-SPT (RBI, Natick, MA), equine FSH (Intervet, Cambridge, UK) and PIT (Servier, Croissy-Sur-Seine, France). Suramin (Germaniant) was a gift from Bayer plc (Newbury, UK). [³H]8-Adenine was obtained from Amersham (Amersham, UK). Compounds were dissolved in Ringer’s solution, except for PIT in 0.1 N HCl, then adjusted to pH 7.0 with 0.1 N NaOH and FSH in sterile phosphate-buffered saline.

**Results**

Both ATP and adenosine (10 μM) evoked an outward K⁺-current in follicular *Xenopus* oocytes (fig. 1A). The ATP-activated K⁺-current was preceded by a small inward current (Iᵤᵢᵢᵲ, Iᵢᵢᵲ) which is caused by ATP activation of P2 receptors on the follicle cell layer (King et al., 1996c). Other adenosine analogs (NECA, 2-p-(2-carboxyethyl)phenethylamino-5’-N-ethylcarboxamidoadenosine and R(-)-N⁶-(2-phenylisopropyl)adenosine) also activated the K⁺-current (fig. 1A), as did the adenylyl cyclase activator forskolin (fig. 1B) and the gonadotrophin FSH (fig. 1C). The relative potencies of nucleosides and nucleotides have been described in detail elsewhere (King et al., 1996b), as have the activity of forskolin (Stinmarke and Van Renterghem, 1986) and FSH (Greenfield et al., 1990a, b) on follicular oocytes. Agonist-evoked K⁺-current was a nonrectifying outward current which reversed to inward current at −90 mV and was inhibited by adding 20 mM TEA to the superfuse. Extracellular TEA reduced the K⁺-current evoked by adenosine, ATP and forskolin (fig. 2), as well as FSH (data not shown), without.
altered $E_{\text{rev}}$, which indicates that TEA reduced the number of opened cAMP-dependent $K^+$-channels. Thus, on physiological grounds, the membrane responses to extracellular adenosine, ATP, forskolin and FSH were considered to be identical.

The activity of five potential antagonists of agonist-activated $K^+$-currents are described in figure 3 and table 1. $\alpha,\beta$-MeATP was the most effective antagonist against adenosine (10 $\mu$M)-evoked responses, with a threshold concentration for blockade at 10 nM and full blockade at 10 $\mu$M. Blockade was reversible by washout (1 h). 8-SPT was as potent as $\alpha,\beta$-meATP, with a threshold for blockade at 30 nM and full blockade at 10 $\mu$M. The slope of the inhibition curve for 8-SPT was significantly steeper than the curve for $\alpha,\beta$-meATP. Blockade by 8-SPT was slowly reversible with washout (2 h). Theophylline was effective across a concentration range of 100 nM to 100 $\mu$M, whereas PIT was effective across a range of 1 $\mu$M to 100 $\mu$M. Blockade by theophylline was slowly reversible with washout (2 h) whereas blockade by PIT was irreversible (>3 h). Suramin was ineffective at low concentrations (0.1–30 $\mu$M) and caused only a slight inhibition (8 ± 2%) at 100 $\mu$M. IC$_{50}$ values for antagonists were: $\alpha,\beta$-MeATP, 0.19 ± 0.03 $\mu$M ($n = 4$); 8-SPT, 0.21 ± 0.01 $\mu$M ($n = 4$); theophylline, 4.8 ± 3.3 $\mu$M ($n = 4$); PIT, 26.1 ± 4.5 $\mu$M ($n = 4$); suramin, >100 $\mu$M ($n = 4$). Equivalent pIC$_{50}$ values are given in table 1.

The blocking activity of four antagonists was compared against ATP and adenosine responses (fig. 4). Each antagonist was tested at a concentration close to its IC$_{50}$ level against both agonists (10 $\mu$M). Except for PIT, the blocking activity of other antagonists were not significantly different at ATP and adenosine responses. The greater activity of PIT at ATP responses remains to be fully resolved (see “Discussion”) but seemed unimportant in light of the nonselective blocking actions of the drug (see below).

The slopes of inhibition curves for the four antagonists varied considerably (see fig. 3, table 1), which indicated that some or all of these compounds either exerted nonspecific intracellular effects on receptor-operated AC activity or had nonselective effects on cAMP-activated $K^+$-channels. Thus, each antagonist was tested against cAMP-dependent $K^+$-current evoked by either the intracellular AC activator forskolin (10 $\mu$M) or via extracellular gonadotrophin receptors by FSH (30 nM). 8-SPT (10 $\mu$M) and theophylline (300 $\mu$M) (both used at the IC$_{100}$ level) potentiated forskolin-activated $K^+$-current, increasing chord conductance 5-fold and 3-fold, respectively, without altering $E_{\text{rev}}$ (fig. 5). On the other hand, $\alpha,\beta$-meATP (10 $\mu$M) and PIT (100 $\mu$M) (both used at the IC$_{100}$ level) inhibited forskolin-activated $K^+$-current, decreasing chord conductance by 4-fold and 2-fold without altering $E_{\text{rev}}$ (fig. 5). A similar pattern of results occurred when antagonists were tested against FSH responses. 8-SPT and theophylline increased (4-fold and 3-fold, respectively) whereas $\alpha,\beta$-meATP and PIT decreased (4-fold and 3-fold, respectively) the chord conductance for FSH-activated $K^+$-current without altering $E_{\text{rev}}$ (fig. 6).

Experiments were performed to measure cAMP production in follicular oocytes, for two reasons: 1) to confirm that all stimulators of $K^+$-current do elevate cytosolic cAMP levels and 2) to explain the potentiating or inhibiting effects of antagonists on forskolin- and FSH-stimulated $K^+$-current. The [3H]8-adenine prelabeling technique (Donaldson et al., 1988) was chosen to measure the conversion of [3H]ATP to [3H]cAMP by AC, rather than measurements of total cAMP, because the oocyte maintains a significantly higher cAMP concentration than either basal or stimulated cAMP levels found in the enveloping follicle cell layer (Thibier et al., 1982).

The uptake of [3H]8-adenine was very low with fewer than 500 dpm obtained after 2 h at 25°C. Different assay conditions were tested, including incubation at 30°C or 37°C in the presence or absence of 5% CO$_2$, incubation for 4 h, 8 h or overnight and use of different incubation media (L-15, Hanks’ Buffered Saline Solution, HEPES-buffered Ringer’s solution), without significant [3H]8-adenine uptake. Xenopus oocytes apparently lack a membrane transport system for adenosine, a finding corroborated by other investigators who have used oocytes [S.P.H. Alexander (Nottingham University) and J. Mowbray (University College London); personal communications]. The effects of antagonists on cAMP production remain to be determined.

Discussion
cAMP-dependent $K^+$-currents are activated by adenosine and ATP in follicular oocytes via a receptor positively coupled to adenylate cyclase (Lotan et al., 1982, 1985, 1986; Stinnarke and Van Renterghem, 1986; Miledi and Woodward, 1989; Greenfield et al., 1990a, b; King et al., 1996b). This novel adenosine/ATP receptor has a pharmacological profile (King et al., 1996b) similar to adenosine/ATP receptors found on rat sympathetic nerve terminals (Shinozuka et al., 1988, 1990; Forsyth et al., 1991; Todorov et al., 1994) and rabbit cortical neurons (von Kügelgen et al., 1992). However, the case is clearest for the oocyte receptor that it is unnecessary for ATP to be degraded to adenosine before activating a cAMP-dependent $K^+$-current. Adenosine deaminase abolishes only adenosine responses but not ATP responses at follicular oocytes (King et al., 1996b). Also, the rate of ATP breakdown by oocyte ecto-ATPase is too low to generate sufficient adenosine to activate the oocyte receptor (King et al., 1996b). Several antagonists of adenosine/ATP receptors have been described briefly, including 8-SPT and theophyll-
line (Lotan et al., 1982, 1985, 1986; von Kügelgen et al., 1992; King et al., 1996b) and a,β-MeATP (Shinozuka et al., 1990; von Kügelgen et al., 1992; King et al., 1996b). Here, the activity, potency and selectivity of these antagonists have been studied at the oocyte receptor and compared against the activity of two P2 receptor antagonists, PIT and suramin.

α,β-MeATP and 8-SPT were equipotent in blocking adenosine-activated cAMP-dependent K⁺-currents in follicular oocytes, some 25-fold more potent than theophylline and 135-fold more potent than PIT, whereas suramin was relatively inactive. α,β-MeATP is not an agonist of the oocyte receptor (King et al., 1996b), and therefore, its blocking activity was unrelated to a desensitization of the receptor to other agonists. Other ATP analogs have been shown to be competitive or noncompetitive antagonists of ATP receptors, including oxidized ATP (P2Z) (Wiley et al., 1994), trinitrophenyl-ATP (P2X) (Mockett et al., 1994) and adenosine-3’-phosphate-5’-phosphosulfate (P2Y 1) (Boyer et al., 1996).

8-SPT and theophylline are commonly used antagonists of adenosine receptors at which 8-SPT is 4-fold (A1), 2-fold (A2A) and 4-fold (A2B) more potent than theophylline in binding studies and functional assays (Bruns et al., 1985; Daly et al., 1986). The higher potency (25-fold) of 8-SPT versus theophylline at the oocyte receptor may help distinguish this receptor from adenosine (P1) receptor subtypes on the basis of relative potency. PIT originally was shown to discriminate

### TABLE 1

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>IC₅₀ value (µM)</th>
<th>pIC₅₀ value</th>
<th>Slope (nH)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>α,β-MeATP</td>
<td>0.19 ± 0.03</td>
<td>6.72 ± 0.07</td>
<td>0.83 ± 0.15</td>
<td>Reversible</td>
</tr>
<tr>
<td>8-SPT</td>
<td>0.21 ± 0.01</td>
<td>6.68 ± 0.02</td>
<td>1.71 ± 0.13</td>
<td>Reversible</td>
</tr>
<tr>
<td>Theophylline</td>
<td>4.8 ± 3.3</td>
<td>5.32 ± 0.37</td>
<td>0.57 ± 0.06</td>
<td>Reversible</td>
</tr>
<tr>
<td>PIT</td>
<td>26.1 ± 4.5</td>
<td>4.58 ± 0.07</td>
<td>1.47 ± 0.09</td>
<td>Irreversible</td>
</tr>
<tr>
<td>Suramin</td>
<td>&gt;100</td>
<td>–</td>
<td>–</td>
<td>Inactive</td>
</tr>
</tbody>
</table>
between ATP- and adenosine-mediated responses in gut smooth muscle (Spedding and Weetman, 1976) and was considered a selective antagonist for ATP (P2) receptors. However, its activity at the oocyte receptor and low affinity (pKi 5.3) for an adenosine (A1) receptor in rat brain (King et al., 1996a) has cast doubt on this selectivity for P2 receptor subtypes. Suramin is competitive antagonist at ATP (P2) receptors (Dunn and Blakeley, 1988) and is thought to be inactive at adenosine (P1) receptors. The weak activity (8%) of suramin (100 mM) observed at the oocyte P1/P3 receptor may be related to its ability to inhibit G proteins, including Gs (Beindl et al., 1996; Freissmuth et al., 1996).

8-SPT, theophylline and α,β-meATP were as effective on ATP responses as on adenosine responses, but PIT was more effective on ATP responses. In the follicular oocyte, ATP responses involve a small inward current ICl, Ca, which is activated by P2Y and P2U subtypes (King et al., 1996c), followed by the outward K1-current activated by the ATP/adenosine receptor. The impact of the inward current on the amplitude of outward current can be minimized by adjusting the voltage clamp to the Erev for the P2Y response (-25 mV) and P2U response (-10 mV). Such adjustments failed to alter the level of PIT blockade and suggest that PIT did not enhance the activity of ATP at the P2 receptors, as it otherwise does at the recombinant P2Y1 receptor (King et al., 1996a), to help obscure the outward current. However, PIT was found to be a nonselective blocker for nonnucleotide agonists of CAMP-dependent K+-currents (see below), and its greater activity at ATP responses versus adenosine responses was not investigated further.

When used at the IC100 concentration, 8-SPT (10 μM) and theophylline (100 μM) potentiated forskolin- and FSH-activated K+-currents. Although inhibition of intracellular PDE by both compounds offers the simplest explanation for their potentiating effect, we were unable to measure cAMP production in the follicular cells to confirm this hypothesis. Alkylxanthines show weak activity as PDE inhibitors (Smellie et al., 1979), with pIC50 values for 8-SPT and theophylline at PDE isozymes Ib, II, III, IV and V in the range of 3.2 to 4.7 and 3.2 to 3.8, respectively (Ukena et al., 1993). The concentrations of alkylxanthines used in the present study were just below their pIC50 values for some PDE isozymes and, so, they might be expected to cause enzyme inhibition in the follicular oocyte. Reeves et al. (1995) found that 8-SPT (at the same concentration used in our experiments, 10 μM) and the non-selective PDE inhibitor isobutylmethylxanthine (1 μM) almost doubled the maximum contraction to NECA at an adenosine receptor on rat colonic muscularis mucosa. Thus, it is feasible that 8-SPT and theophylline potentiated forskolin and FSH responses by inhibiting an intracellular PDE.

When used at the IC100 concentration, α,β-meATP (10 μM) and PIT (100 μM) inhibited forskolin- and FSH-activated K+-currents. Many studies have shown that α,β-meATP affects AC activity by either a competitive inhibition of ATP-induced cAMP accumulation in liver cells, adipocytes (Krug et al., 1973a, b), and anterior pituitary gland cells (Hertendy and Yeh, 1976) but not pancreatic acini (Heisler, 1976) or a noncompetitive inhibition of a feedback regulator of AC activity (Ho and Sutherland, 1975; Rossomando and Hesla, 1976). In these studies, high concentrations (0.1–5.0 mM) of
α,β-meATP were necessary to show activity, with $K_i$ values of 0.5 mM (liver), 1.2 mM (adipocytes) and 0.2 mM (pituitary) for competitive inhibition (Krug et al., 1973b; Hertelendy and Yeh, 1976). However, Hertelendy and Yeh (1976) reported a 24 ± 6% (mean ± S.E.M.) inhibition of cAMP levels in anterior pituitary cells with only 5 μM α,β-meATP. Thus, it is not unreasonable to suppose that α,β-meATP (10 μM) would significantly decrease forskolin- and FSH-stimulated cAMP levels and associated $K^+$-current in follicular oocytes. However, it is unlikely that AC inhibition alone could account for α,β-meATP blockade of adenosine and ATP responses which occurred between 0.03 and 10 μM. The nonselective inhibition of forskolin- and FSH-stimulated cAMP levels and associated $K^+$-current by PIT was probably caused by its strong alkylating effect on surface proteins where it can bind covalently to leucine and valine residues and react strongly with sulphhydryl groups on cysteine and methionine residues (Hooper and Robertson, 1971). Thus, PIT probably denatured $K^+$-channel subunits to reduce $K^+$-currents. PIT also increased the leakage conductance in follicular oocytes (data not shown) and may have entered follicle cells to denature $G_s$ and adenylate cyclase as well.

It has proved inordinately difficult to measure changes in cAMP levels in follicle cells because the oocyte maintains a significantly higher cAMP concentration than either basal or stimulated cAMP levels found in the enveloping follicle cell layer (Thibier et al., 1982). To overcome this problem, Greenfield and colleagues (1990a, b) used “follicle ghosts” to measure cAMP accumulation to adenosine and FSH stimulation. However, these investigators still found no detectable increases in cAMP to agonists (100 μM) or forskolin (100 μM) alone, even in the presence of the PDE inhibitor Ro7–2956, and only obtained a significant response when agonists and forskolin were applied together. The ineffectiveness of forskolin alone is surprising because many investigators, including Greenfield et al. and ourselves, have shown that forskolin (10 μM) evokes larger $K^+$-currents than do maximal concentrations of either adenosine or ATP. The efficacy of forskolin implies that it does increase cAMP significantly to activate $K^+$-channels but these levels are below the limits of detection of most assay systems. We chose the $[^3H]$adenine pre-labeling technique to measure de novo synthesis of $[^3H]$cAMP in follicle cells and to explore the relationship between cAMP levels and increased $K^+$-conductance. However, follicular oocytes represent an atypical cell type that fails to take up significant amounts of $[^3H]$adenine. Accordingly, we were unable to verify the stimulatory and inhibitory actions of alkylxanthines, α,β-meATP and PIT on AC activity.

In summary, only alkylxanthine derivatives (8-SPT and theophylline) proved to be selective antagonists of the novel P1/P3 receptor in Xenopus oocytes, whereas α,β-meATP and PIT were non selective antagonists of this receptor subtype. Together with existing data on agonist activity, this information on antagonist activity may be useful in the future to help characterize the recombinant form of the P1/P3 receptor and to expedite expression-cloning experiments.

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