Interactions between ATP and adenosine on the formation of inositol 1,4,5-trisphosphate (Ins(1,4,5)P₃) and mobilization of intracellular calcium were investigated in the smooth muscle cell line DDT₁ MF-2. Activation of adenosine A₁ receptors with adenosine or cyclopentyladenosine (CPA) or of nucleotide receptors with ATP increased both Ins(1,4,5)P₃ formation and intracellular calcium concentrations.

The A₁ receptor-induced Ins(1,4,5)P₃ formation (EC₅₀ 10 nM) was antagonized by the A₁ agonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and by pretreatment of cells with pertussis toxin (PTX). ATP-stimulated Ins(1,4,5)P₃ formation (EC₅₀ 21 μM) was attenuated, but still present, after PTX treatment. ATP and CPA had supraadditive effects on Ins(1,4,5)P₃ accumulation and CPA increased ATP-induced Ins(1,4,5)P₃ accumulation in a concentration-dependent manner with an EC₅₀ of 3 nM, a concentration which per se had little or no effect on Ins(1,4,5)P₃ accumulation.

ATP (EC₅₀ 4 μM) and CPA (EC₅₀ 4 nM) both increased intracellular calcium levels. The effect of ATP was partially sensitive to PTX treatment, whereas the effect of CPA was blocked both by PTX and by DPCPX. Concentrations of ATP and CPA that by themselves were insufficient to raise intracellular calcium were able to do so when combined. The synergy between ATP and CPA on the mobilization of intracellular calcium was abolished after treatment of cells with PTX or when DPCPX was included in the experiment.

Since ATP was metabolized by ecto-enzymes to ADP, AMP, and adenosine, we also examined whether adenosine formed from ATP could enhance the ATP effect on Ins(1,4,5)P₃ accumulation. Indeed, the addition of the A₁ receptor antagonist DPCPX or removal of endogenous adenosine by inclusion of adenosine deaminase in the experimental medium significantly attenuated the ATP response, and the two treatments did not have additive effects.

The present study thus demonstrates that in a clonal cell line two types of receptors increase phospholipase C activity, but via different pathways; nucleotide receptors appeared to act via partially PTX-insensitive, and A₁ receptors via PTX-sensitive G-proteins. ATP and CPA are not only able per se to induce formation of Ins(1,4,5)P₃ and mobilize intracellular calcium, but they also act synergistically. Finally, it is demonstrated that endogenous adenosine, possibly formed from the rapid breakdown of ATP, can significantly enhance some ATP effects.

ATP is not only a key intracellular energy donor but it also has extracellular signaling functions and acts like a transmitter (1). It acts on specific receptors (P₂ receptors) that are tentatively divided into four subtypes, P₂₁, P₂Y, P₂X, and P₂T (1, 2). Among these, the P₂Y receptor, probably via G-proteins, stimulates phospholipase C (3-5). In addition a so-called nucleotide receptor that is activated equally well by UTP and ATP and is linked to phospholipase C has been postulated (6).

Activation of phospholipase C (7) results in the formation of two second messengers: Ins(1,4,5)P₃, which mobilizes calcium from intracellular stores (8), and diacylglycerol, which activates protein kinase C (9).

Extracellular ATP is rapidly degraded to ADP, AMP, and adenosine (10). One of the metabolites, adenosine, is a ubiquitous modulator in its own right and acts via specific adenosine receptors. These are classified into subtypes, A₁ and A₂, originally depending on whether they stimulate (A₂) or inhibit (A₁) adenylcyclase (11, 12) and later depending on the order of potency of agonists and antagonists in receptor binding studies (13). Adenosine A₁ receptors not only mediate inhibition of adenylcyclase but are also linked to potassium and calcium channels (14, 15).

The reported effects of adenosine on phospholipase C and inositol phosphate formation have been quite variable. In some instances adenosine has demonstrated no effect per se, but has enhanced the effect of phospholipase C-activating receptors including histamine H₁ receptors (16-18), A₁-adrenoceptors (19-21), muscarinic receptors (21), and nucleotide receptor stimulators with GTP (22). In contrast, adenosine receptor activation attenuates histamine H₁-induced (18, 23, 24) and TRH receptor-induced (25) inositol phosphate formation in other systems (for review see Ref. 26). Finally, there

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† To whom correspondence should be addressed: Dept. of Pharmacology, Box 60 400, S-104 01 Stockholm, Sweden. Tel.: 46-8-33-16-53.

1 The abbreviations used are: G-protein, GTP-binding protein; CPA, N'-cyclopentyladenosine; DMEM, Dulbecco's modified Eagle's medium; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; EGTA, (ethylenbis(oxyethylenenitriilo)tetracetetic acid; Pura-2-AM, 1-(2-carboxyoxazol-2-yl)-6-aminohenzofuran-5-oxyl-2,4'-etherphenoxy)-ethane-N,N',N',N'-tetracetic acid-pentaacetoxymethylene; HBSS, Hank's balanced salt solution; HEPES, N-2-hydroxyethylpiperazine-N'-ethansulfonic acid; PTX, pertussis toxin; UTP, uridine 5'-triphosphate; AMP-CPP, adenosine 5'-[(5-methyl)(3'-methylene)triphosphate]; AMP-PCP, adenosine 5'-[(5,3'-methylene)triphosphate]; HPLC, high performance liquid chromatography; PMA, phorbol 12-myristate 13-acetate; 2-methylthio-ATP, 2-methylthioadenosine 5'-triphosphate.
is evidence that in some cell activation of adenosine receptors can directly stimulate phospholipase C (27–30). However, the receptor involved in these effects was not typical for either A1 or A2 receptors, and it has even been proposed that a novel adenosine receptor is involved (30, 31).

Since it is known that ATP is degraded to adenosine and that adenosine via adenosine receptors can modify the formation of inositol phosphates via several receptors, we wanted to directly investigate whether adenosine formed from ATP could, via A1 receptors, influence the ability of ATP, acting on nucleotide receptors, to form Ins(1,4,5)P3 and elevate intracellular calcium. Another purpose of the present study was to investigate if adenosine A1 receptors are able to couple to more than one intracellular signal transduction pathway. We have used a smooth muscle cell line, DDT, MF-2 (32), that is known to express A1 receptors negatively coupled to adenyllylcyclase (33, 34) and some type of receptor for ATP that is coupled to phospholipase C (35).

### EXPERIMENTAL PROCEDURES

**Materials**—Cell culture media, fetal calf serum, and cell culture flasks were from Invitrogen, Breda, The Netherlands. d-myo-[2-14C]inositol 1,4,5-trisphosphate (514 Ci/mmol) was from Amersham Corp. [2,8-3H]Adenosine 3′,5′-cyclic monophosphate (44.5 Ci/mmol) and [2,8-3H]adenosine (25 Ci/mmol) were from Du Pont–New England Nuclear. Isoproterenol, Nω-cyclopentyladenosine, bovine serum albumin, adenosine 3′,5′-cyclic monophosphate, EDTA, EGTA, 8-cyclopentyladenosine 1.3-dipropyl, Fura-2-AM, Hank’s balanced salt solution, AMP-CPP, AMP-PCP, uridine 5′-trisphosphate, guanosine 5′-trisphosphate, adenosine 5′-monophosphate, adenosine 5′-diphosphate, HEPES, and forskolin were all from Sigma. Adenosine deaminase, Ins(1,4,5)P3, and adenosine 5′-triphosphate were purchased from Boehringer, Mannheim, Germany. Pertussis toxin was from List, Campell, CA, and 2-methylthio-adenosine 5′-monophosphate from Research Biochemicals Incorporated, Natick, MA. Rolipram (ZK 62711, 4-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-pyridilondione) was a gift from Schering AG, Berlin, Germany.

**Cell Culture**—DDT, MF-2 smooth muscle cells, originally isolated from a steroid-induced leiomyosarcoma of Syrian hamster vas deferens (32), were obtained from the American Type Culture Collection (ATCC). Cells were grown in suspension, maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 4.5 g of glucose/liter, 60 mM EDTA, 50 mM HEPES, and pH 7.4 and frozen until analyzed.

**CAMP assay**—After being washed once with assay medium (DMEM buffered with 20 mM HEPES, pH 7.4) and resuspended in assay medium to a concentration of 3 × 10⁶ cells/ml, aliquots (0.2 ml) were transferred to test tubes and preincubated for 20 min at 37°C in a water bath before addition of indicated drugs (0.1 ml). Lithium was omitted to avoid any interaction with the formation of inositol phosphates. Reactions were terminated by the addition of perchloric acid to a final concentration of 0.4 M and the tubes placed on ice for 1 h. Samples were neutralized with 4 M KOH, 1 M Tris, 60 mM EDTA and 2 mM EGTA and assayed by reversed-phase HPLC as described elsewhere (36).

**Measurement of Intracellular Concentrations of Free Calcium**—Cells were washed and resuspended in Hank’s balanced salt solution (HBSS, 1.2 mM CaCl₂ supplemented with 0.1% bovine serum albumin and 20 mM HEPES, pH 7.4) to a concentration of 10⁶ cells/ml and loaded with 5 µM Fura-2 AM for 40 min at 37°C. After the loading period cells were washed twice in HBSS and resuspended to a concentration of 10⁶ cells/ml. Prior to the measurements, cells were washed once more and then placed in a cuvette (10⁶ cells in 2 ml of HBSS) and the intracellular calcium concentration determined at 30°C in a dual-wavelength Sigma ZEP 22 fluorometer by using the ratio of excitation wavelengths 334/366 nm with emission cut off at 500 nm. Free calcium concentration was calculated as previously described (38).

**Analysis of Adenine Nucleotides**—After one wash, cells were resuspended in HBSS at a concentration of 2.5 × 10⁶ cells/ml. Aliquots (0.4 ml) of 10⁶ cells were transferred to test tubes and ATP (0.1 ml) added to a final concentration of 10⁻¹⁰ M. Incubations were terminated by the addition of perchloric acid, 0.4 M final concentration. After neutralization with 4 M KOH the amount of adenine nucleotides was determined in the supernatant by reversed-phase HPLC using a 15-cm Nucleosil 5 C18 column. The identities of adenine nucleotides were ascertained by "spiking" of samples with authentic nucleotides and in the case of adenosine by treatment with adenosine deaminase. In experiments where we determined the amount of adenosine released upon stimulation of cells with ATP, cells were prelabeled with [3H]adenosine (5 µCi/ml, 1 h) and activated as described above with the exception that reactions were terminated by rapid centrifugation and aspiration of the supernatants, which were immediately mixed with perchloric acid (0.4 M) and neutralized with KOH prior to HPLC analysis. The amount of released [3H]adenosine was analyzed by measuring the radioactivity in fractions collected after HPLC separation.

**Data Analysis**—Dose–response curves were generated by using the GraphPad (I.S. Software) program. Statistical comparisons between different drug treatments were made using analysis of variance using the Statgraphics (Statistical Graphics Corp.) program with a confidence level of 95% or using Student’s t test (GraphPad InStat; I.S. Software). Data are presented as mean ± S.E.

**RESULTS**

**Formation of Ins(1,4,5)P₃**—ATP increased the formation of Ins(1,4,5)P₃ in a concentration-dependent manner with an EC₅₀ of 21 ± 5 µM (n = 3; Fig. 1). The P₂₅ receptor-selective ATP analogue AMP-CPP was virtually ineffective at 10⁻⁹ M. Similarly, 2-methylthio-ATP, a potent activator of P₂₇ receptors, was almost inactive. By contrast, UTP was at least as potent as ATP (Fig. 1). The ATP-induced increase in Ins(1,4,5)P₃ was very rapid and transient. After an initial peak at 30 s, intracellular levels of Ins(1,4,5)P₃ returned to baseline within 1 min. The use of ATP and adenosine analogues provided evidence that a nucleotide receptor mediating the Ins(1,4,5)P₃ formation in DDT, MF-2 cells. Cells were activated with increasing concentrations of UTP (V), ATP (●), ADP (○), AMP (▲), GTP (△), 2-methylthio-ATP (○), AMP-CPP (○), or AMP-PCP (▲) for 30 s and the amount of Ins(1,4,5)P₃ formed analyzed as described under "Experimental Procedures." Data represent the mean ± S.E. from three experiments, each performed in triplicate.

![Fig. 1. Characterization of a nucleotide receptor mediating Ins(1,4,5)P₃ formation in DDT, MF-2 cells.](image-url)
Adenosine (10 μM) and the selective adenosine A1 receptor agonist CPA (100 nM) also increased intracellular levels of Ins(1,4,5)P3 (Fig. 2). The peak was reached after approximately 1 min, and control values were obtained after 5 min (Fig. 2). Furthermore, when combined, ATP and CPA acted in synergy between 0.5 and 3 min after addition of the two drugs (p < 0.05, Student’s t test; n = 4), and the effect of ATP was reduced by the addition of the A1-specific antagonist DPCPX (Fig. 2).

CPA appears to act via typical A1 receptors since the EC50 value was 10 ± 1 nM (n = 3; Fig. 3). Treatment of cells with pertussis toxin (200 ng/ml, 4 h), which completely prevents subsequent ADP-ribosylation of purified membranes by activated PTX (33), abolished the response to CPA (Fig. 3). The effect of an inactive dose of ATP (1 μM) was not altered by CPA over the concentration range studied, but the effect of a high concentration of ATP (0.1 mM) was enhanced by CPA in a concentration-dependent manner with an EC50 of 3 ± 0.7 nM (n = 3; Fig. 3). This enhancement was blocked by the A1 receptor antagonist DPCPX with a KI of 0.8 nM (Fig. 4), further supporting the idea that the effects are mediated with A1 receptors. PTX pretreatment of the cells markedly attenuated, but did not abolish, ATP-induced Ins(1,4,5)P3 formation (Fig. 5). PTX treatment did not change the EC50 value (29 ± 5 μM; n = 3) for ATP-stimulated Ins(1,4,5)P3 formation (Fig. 5), possibly indicating that a single class of ATP receptors interact both with PTX-sensitive and PTX-insensitive G-proteins to stimulate Ins(1,4,5)P3 formation.

DPCPX did not have any effects on its own on intracellular levels of Ins(1,4,5)P3 (data not shown) but significantly (p < 0.05, analysis of variance) attenuated CPA-induced Ins(1,4,5)P3 formation (Fig. 2). The inclusion of adenosine deaminase (preincubation of cells for 1 h at 37 °C with 3 IU/ml and a maintained concentration of 3 IU/ml during the experiments) by itself reduced the peak Ins(1,4,5)P3 response from 63 ± 2 to 53 ± 5 pmol/10⁶ cells (n = 3) and abolished the ability of DPCPX to attenuate ATP-induced Ins(1,4,5)P3 formation within 10 min (Fig. 2).

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![Fig. 2. Time course for the mobilization of Ins(1,4,5)P3 by ATP, adenosine, and CPA in DDT-1 MF-2 cells. Cells were incubated with vehicle (□), 10 μM adenosine (△), 100 nM CPA (□), 0.1 mM ATP (○), 0.1 mM ATP + 100 nM CPA (△), and 0.1 mM ATP + 100 nM DPCPX (■) for indicated times and the amount of Ins(1,4,5)P3 assayed as described under "Experimental Procedures." All curves, except when comparing CPA and adenosine, differed significantly from each other (analysis of variance, 95% confidence interval). The additive effect, responseCPA + responseATP + DPCPX, is shown with a dotted line (▼). A comparison of the responseCPA + responseATP + DPCPX (▼) with the responseATP + CPA (△) showed that the effect was synergistic and not additive (Student's t test; *, p < 0.05) between 0.5 and 3 min. Points represent mean ± S.E. from four experiments each performed in triplicate.](image)

![Fig. 3. Dose dependence of adenosine A1 receptor-induced Ins(1,4,5)P3 formation in DDT-1 MF-2 cells. Activation of A1 receptors with increasing concentrations of CPA (0.1 mM ATP and 100 nM CPA and increasing concentrations of DPCPX, an adenosine A1 receptor antagonist, for 30 s (left). The calculated KI value for DPCPX was 0.8 nM. To the right is shown the intracellular accumulation of Ins(1,4,5)P3 after a 30-s activation with one of the following: A. 0.1 mM ATP + 100 nM CPA; B, 0.1 mM ATP; C, 0.1 mM ATP + 100 nM DPCPX; D, the additive effect of CPA and ATP in the presence of DPCPX (to block effects of endogenously formed adenosine). The responseATP+CPA (△) was more than additive compared with the responseCPA + responseATP+DPCPX (□) over the concentration range 10⁻⁶ to 10⁻³ M of CPA (Student's t test; *, p < 0.05). Treatment of cells with PTX (200 ng/ml, 4 h) completely prevented CPA from increasing intracellular levels of Ins(1,4,5)P3 (△). Points represent mean ± S.E. from three experiments performed in triplicate.](image)

![Fig. 4. Antagonism of CPA-induced enhancement of ATP-induced Ins(1,4,5)P3 formation by DPCPX. Cells were incubated with the combination of 0.1 mM ATP and 100 nM CPA and increasing concentrations of DPCPX, an adenosine A1 receptor, antagonist, for 30 s (left). The calculated KI value for DPCPX was 0.8 nM. To the right is shown the intracellular accumulation of Ins(1,4,5)P3 after a 30-s activation with one of the following: A, 0.1 mM ATP + 100 nM CPA; B, 0.1 mM ATP; C, 0.1 mM ATP + 100 nM DPCPX; D, the additive effect of CPA and ATP in the presence of DPCPX (to block effects of endogenously formed adenosine). The responseATP+CPA (△) was more than additive when compared to the responseATP+DPCPX + responseCPA (△) (Student's t test; *, p < 0.05). Triplicate determinations from four experiments are shown as mean ± S.E.](image)
Degradation of ATP—In view of the possible formation of adenosine from the added ATP we examined the degradation of ATP by DDT, MF-2 cells. Incubation of cells with ATP showed a time-dependent metabolism of ATP to form ADP, AMP, and adenosine (Fig. 7). Inclusion of adenosine deaminase in the experiments completely prevented the formation of adenosine from ATP (data not shown).

Specificity of the Adenosine/ATP Interaction on Ins(1,4,5)P₃ Formation and Possible Mechanisms—In DDT; MF-2 cells ATP was not influenced by two potassium channel antagonists, 4-aminopyridine (0.3 mM) or tetraethylammonium (5 mM), alone or in combination (data not shown). Indomethacin (10 μM) and dexamethasone (1 μM) alone or in combination were similarly ineffective (not shown). In order to examine the possible involvement of protein kinase C, cells were incubated with PMA (250 nM) 18 h before the experiment to down-regulate protein kinase C. PMA treatment tended to reduce the ATP response in itself, but did not eliminate the synergistic response to CPA and ATP. PMA (250 nM) added at the same time as ATP or CPA was not able to substitute for one or the other of these agonists (not shown). Thus, there is no evidence that cAMP, membrane potential, activation of phospholipase A₂, or protein kinase C is involved in the interaction between ATP and adenosine analogues.
Mobilization of Intracellular Calcium—ATP and CPA independently increased intracellular free calcium with EC$_{50}$ values of 4 ± 0.9 µM and 4 ± 0.9 nM, respectively (n = 4; Fig. 9). After an initial peak, a sustained plateau phase followed (Fig. 10, A and B). The plateau phase was eliminated if extracellular calcium was removed by the addition of 3 mM EGTA (Fig. 10, A and B). The ATP response was partially sensitive to treatment of cells with pertussis toxin (200 ng/ml, 4 h) with no significant change in the dose required for half-maximal response (5 ± 2 µM in treated cells compared to 4 ± 0.9 µM in control cells, p > 0.05, Student’s t test; n = 3; Figs. 9 and 10C). On the other hand, CPA-mediated calcium increase was completely abolished after pertussis toxin treatment (Figs. 9 and 10C). CPA (10 nM) induced calcium mobilization was blocked by the selective A$_1$ receptor antagonist DPCPX (100 nM; Fig. 10D), whereas ATP-induced calcium increase was unaffected by DPCPX (data not shown). Thus, in contrast to the situation with Ins(1,4,5)P$_3$, there is no evidence that endogenous adenosine augments the ATP response on calcium mobilization.

Low doses of ATP (0.3 µM) and CPA (0.3 nM), which were unable to raise intracellular calcium on their own, could when combined cause a clearcut increase in intracellular calcium (Fig. 10E).
When we combined submaximal doses of ATP (1 μM) and CPA (1.8 nM), they acted in concert and gave a recording with a distinctive initial peak, something that was not seen when they were added alone (Fig. 10F).

DISCUSSION

We have confirmed and extended previous findings that ATP increases Ins(1,4,5)P₃ and intracellular calcium in DDT, MF-2 cells (35). The EC₅₀ values (21 and 4 μM) were in agreement with studies in other types of cells (40-45) as well as in DDT, MF-2 cells (35). The receptor involved is best described as a nucleotide receptor rather than as a P₂Y receptor (6) since the potency ratio was UTP = ATP > ADP >> 2-methylothio-ATP. The finding that intracellular calcium was increased at lower concentrations of ATP than was Ins(1,4,5)P₃ indicates that the calcium response is fully activated even at a submaximal increase in Ins(1,4,5)P₃, in agreement with previous reports (35, 41). The time course for Ins(1,4,5)P₃ formation is consistent with results from other cells (42, 44), but differs from an earlier study on DDT, MF-2 cells where a second increase in Ins(1,4,5)P₃ was seen after 5 min (35). A possible explanation for this discrepancy is that we measured the endogenously formed Ins(1,4,5)P₃, whereas in the earlier study Hoiting et al. measured the radioactively labeled Ins(1,4,5)P₃.

ATP caused a rapid rise in intracellular free calcium with a peak at 30 s followed by a sustained plateau phase. The initial peak is probably due to Ins(1,4,5)P₃-mediated calcium mobilization from intracellular pools, since it coincides with the peak in Ins(1,4,5)P₃ formation and is unaffected by removal of extracellular calcium with EGTA. By contrast the plateau phase was dependent on influx of calcium through calcium channels, in agreement with earlier findings (35). ATP responses were partially sensitive to treatment of cells with pertussis toxin, and these data indicate that ATP, via activation of adenosine type A₁ receptors, can increase intracellular Ins(1,4,5)P₃ levels and mobilization of intracellular calcium. Thus, the present results are the most clear demonstration that adenosine, acting at A₁ receptor, can increase calcium per se and enhance the response mediated by other receptors. The synergy was lost after treatment of cells with pertussis toxin, indicating that the PTX-insensitive G-protein is not a target for the adenosine A₁ receptor.

We therefore conclude that adenosine A₁ receptors mediate a synergistic increase in the formation of Ins(1,4,5)P₃ and mobilization of intracellular calcium. The effect was more than additive and CPA caused a concentration-dependent enhancement of ATP-induced Ins(1,4,5)P₃ accumulation with an EC₅₀ value (3 nm) typical for an adenosine A₁ receptor. Perhaps, the present results are the most clear demonstration that adenosine, acting at A₁ receptors, can increase intracellular Ins(1,4,5)P₃ levels and elevate calcium per se and enhance the response mediated by other receptors. The synergy was lost after treatment of cells with pertussis toxin, indicating that the PTX-insensitive G-protein is not a target for the adenosine A₁ receptor.

The synergy on Ins(1,4,5)P₃ formation had functional importance for the mobilization of intracellular calcium, in agreement with a previously reported synergy between adenosine receptors and ATP receptors on the mobilization of intracellular calcium (55). However, in the previous study, no effect of adenosine receptor stimulation on phospholipase C was found, and the effect of a simultaneous activation of adenosine receptors and ATP receptors on the formation of inositol phosphates was not investigated. As mentioned in the introduction there are also several reports that adenosine analogues augment stimulation of phospholipase C by agonists to α₁-adrenergic, histamine H₁, muscarinic, and GTP receptors. In these studies it has not been clearly shown that the effect was mediated via adenosine A₁ receptors. Further, the synergistic effects were generally observed in long term incubations, and none of the authors have directly measured the calcium-mobilizing Ins(1,4,5)P₃ isomer.

ATP-induced Ins(1,4,5)P₃ formation was attenuated in the presence of the adenosine A₁ receptor antagonist DPCPX. Treatment of cells with adenosine deaminase mimicked this effect and abolished the DPCPX-mediated attenuation.

We therefore conclude that adenosine, possibly formed from ATP, can enhance the effect of ATP on Ins(1,4,5)P₃ formation. Rapid breakdown of ATP by ecto-enzymes (10)
has been shown to generate enough adenosine to interact with adenosine receptors and affect signal transduction (56). We show that ATP is metabolized to yield ADP, AMP, and adenosine. After 1 h, adenosine reached a concentration close to 200 nM in the incubation medium. However, in the immediate vicinity of the cell membrane the concentration of adenosine may be sufficient to activate adenosine receptors within 0.5–3 min, i.e. when the enhancement was observed. A local, rapid accumulation of adenosine, formed from ATP, has been postulated by other authors (56). We found no evidence for adenosine release from the cells when activated with ATP.

The fact that adenosine could be formed from ATP and interact with ATP raises a possibility that the observed receptor-receptor interaction may be physiologically important. It is known that ATP may act like a neurotransmitter and it might also affect some other signaling functions (1, 57). The signal molecule is rapidly degraded, leading to a very transient response. If a major metabolite acts synergistically one would expect a larger and more prolonged signal.

The mechanism behind the interaction is not known. The data presented here indicate that it is not dependent on cAMP, on membrane potential, on activation of phospholipase C, on Ins(1,4,5)P3, on calcium, or on adenylate cyclase. We also show that in DDT and MF-2 cells, phospholipase C is coupled both to nucleotide receptors (via pathways involving one pertussis toxin-sensitive and one pertussis toxin-insensitive G-protein) and to pertussis toxin-sensitive adenosine A1 receptors.

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