ELSEVIER

European Journal of Pharmacology 281 (1995) 213–217

ejp

Short communication

# Potentiating and inhibitory effects of periodate-oxidized ATP analogs on contractions of vas deferens to ATP

Jeffrey S. Fedan [a,*], L. Jameel R. Grant [b]

[a] *Physiology Section, Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, USA*

[b] *Department of Pharmacology and Toxicology, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, WV 26506, USA*

Received 7 June 1995; accepted 9 June 1995

**Abstract**

Previous studies have shown that treatment of guinea-pig isolated vas deferens with the affinity label periodate-oxidized ATP (2′,3′-dialdehyde ATP), results in two irreversible effects on biphasic contractile responses to ATP, i.e., potentiation of the $P_{2X}$ purinoceptor-mediated first phase and inhibition of the ecto-kinase-mediated second phase. The present experiments were designed to evaluate whether periodate-oxidized ADP, periodate-oxidized AMP, and periodate-oxidized adenosine, produce similar effects. Periodate-oxidized ATP and periodate-oxidized ADP ($10^{-2}$ M) elicited contraction of the vas deferens (periodate-oxidized ATP > periodate-oxidized ADP; periodate-oxidized AMP and periodate-oxidized adenosine had no agonist activity. After incubation of the preparations for 5 min with $10^{-2}$ M periodate-oxidized ATP, periodate-oxidized ADP, periodate-oxidized AMP or periodate-oxidized adenosine, the first phase of contraction to submaximal ATP concentrations was potentiated. Simultaneously, periodate-oxidized ATP, periodate-oxidized ADP and periodate-oxidized AMP inhibited the second contractile phase, whereas periodate-oxidized adenosine did not. The results indicate that the requirement for 5′-phosphate to produce potentiation and inhibition is different: 5′-phosphate is not needed to potentiate the first phase of contraction to ATP, but at least one 5′-phosphate is required to inhibit the second phase of contraction.

*Keywords:* $P_2$ purinoceptor; Vas deferens, guinea-pig; Periodate-oxidized nucleotide; ATP; 2′,3′-Dialdehyde ATP analog

## 1. Introduction

Contractile responses of the smooth muscle of the guinea-pig isolated vas deferens to adenine nucleotides involve activation of $P_{2X}$ purinoceptors (Fedan et al., 1982; Abbracchio and Burnstock, 1994), but several mechanisms appear to be involved. In concentrations below ca. $10^{-4}$ M, ATP and 5′-*O*-(3-thiotriphosphate) (ATPγS) elicit fast, monophasic contractions. At concentrations $\geq 10^{-4}$ M, the contractions acquire a second, longer-lasting phase. The second phase of contraction to ATPγS is greatly prolonged compared to ATP (Fedan et al., 1982).

The first phase of the contractile response of guinea-pig vas deferens to ATP and ATPγS is mediated by $P_{2X}$ purinoceptors. This phase is inhibited selectively and covalently by the photoaffinity label antagonist, $ANAPP_3$ (Hogaboom et al., 1980; Fedan et al., 1982, 1985; Fedan and Lamport, 1990; Lamport-Vrana et al., 1991). The second phase of response to ATP is associated with ecto-phosphorylation (or a longer-lasting ecto-thiophosphorylation in response to ATPγS) within seconds of a ca. 21 kDa protein. This mechanism, probably involving an ecto-kinase, is inhibited selectively by the affinity label, periodate-oxidized ATP (2′,3′-dialdehyde ATP; Fedan and Lamport, 1990; Lamport-Vrana et al., 1991). Periodate-oxidized ATP incorporates covalently into several, non-21 kDa proteins (Lamport-Vrana et al., 1991). It has subsequently been reported that a 21 kDa protein in endothelial cells also is phosphorylated in response to ATP, albeit more slowly (Pirotton et al., 1992). More recently, periodate-oxidized ATP was reported to inhibit irreversibly an initial $P_{2Z}$ purinoceptor-mediated permeabilization of macrophages without affecting a sec-

* Corresponding author. Physiology Section, NIOSH, 1095 Willowdale Road, Morgantown, WV 26505-2888, USA. Tel. 304-285-5766, fax 304-285-5820, e-mail jsf2@niords1.EM.CDC.GOV.


*SSDI* 0014-2999(95)00352-5

ondary intracellular $Ca^{2+}$ release mediated by $P_{2Y}$ purinoceptors (Murgia et al., 1993), i.e., periodate-oxidized ATP showed phase-selectivity in its inhibitory effects.

Periodate-oxidized ATP has other interesting properties in the guinea-pig isolated vas deferens (Fedan and Lamport, 1990). It is initially a contractile agonist. While treatment with periodate-oxidized ATP selectively inhibits the second phase of contraction to ATP as discussed above, periodate-oxidized ATP also potentiates the first phase of contraction to submaximal concentrations of ATP via a mechanism thought to involve inhibition of degradative ecto-ATPase activity (Fedan and Lamport, 1990).

To obtain information on the structural determinants important to the various actions of periodate-oxidized ATP, the present study was designed to compare the periodate-oxidized analogs of ADP, AMP and adenosine against periodate-oxidized ATP for their ability to initiate contractile responses, to potentiate the first phase of response to ATP, and to inhibit the second phase of the response.

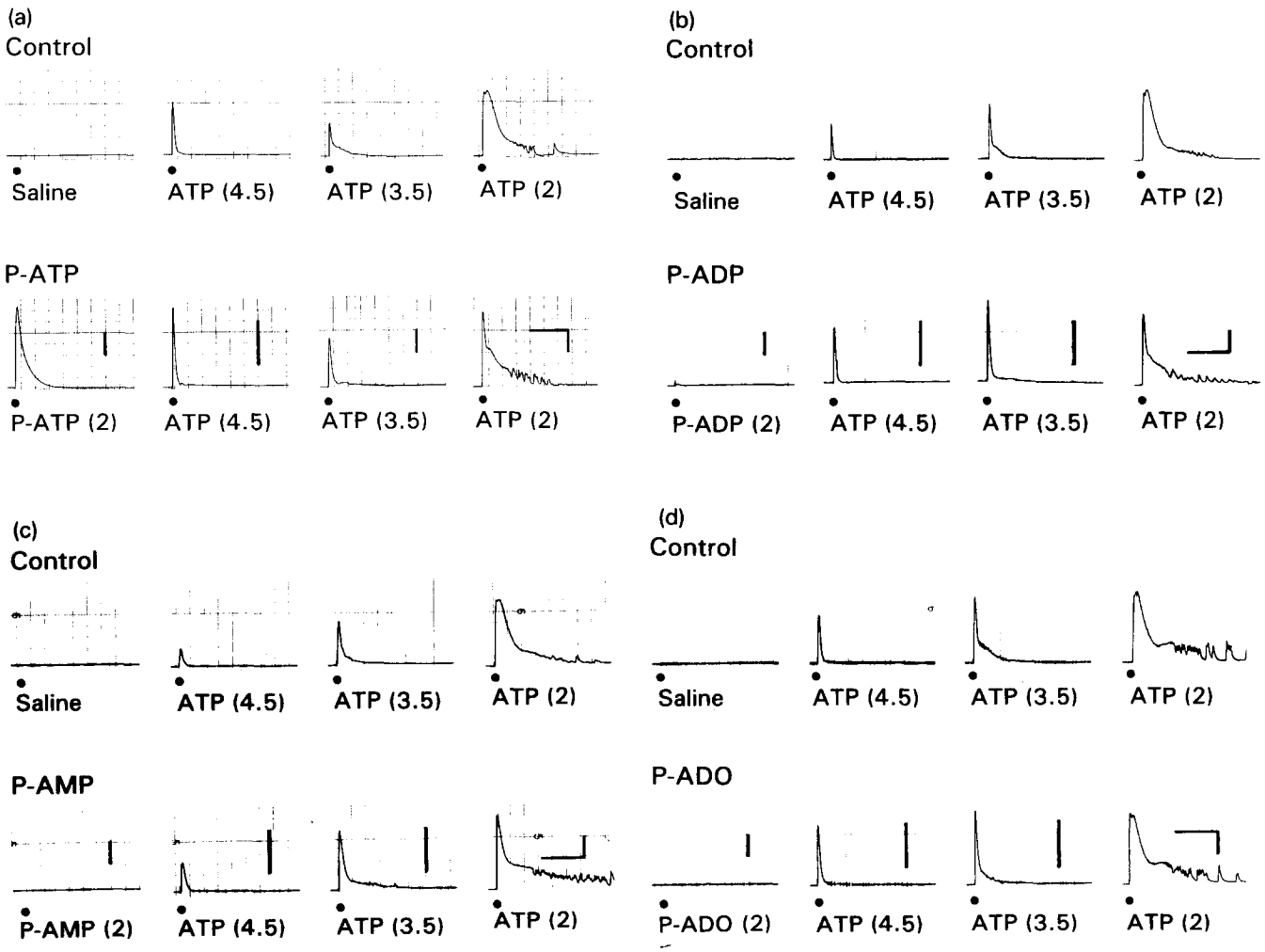


Fig. 1. Effects of (a) periodate-oxidized ATP (P-ATP), (b) periodate-oxidized ADP (P-ADP), (c) periodate-oxidized AMP (P-AMP), and (d) periodate-oxidized adenosine (P-ADO) on guinea-pig isolated vas deferens. The upper row shows responses from control preparations, which received saline, while the bottom row shows the initial responses to the periodate-oxidized compounds, if any, and the effects of the compounds ($10^{-2}$ M for 5 min) on the first and second phases of contraction to ATP. The concentrations of the periodate-oxidized analogs and ATP are indicated as $-\log$ molar (in parentheses). Responses to three ATP concentrations are shown which illustrate purely first-phase monophasic twitches ($3 \times 10^{-5}$ M ATP is '4.5'), responses in which the second phase has appeared ($3 \times 10^{-4}$ M ATP is '3.5'), and responses in which the second phase is fully developed ($10^{-2}$ M ATP is '2'). The results are representative of 4 separate experiments with each periodate-oxidized compound. The horizontal calibration bar (30 s) applies to all responses; the vertical calibration bar (2 g) applies to the labelled panel and the one above.

## 2. Materials and methods

### 2.1. Preparation of guinea-pig isolated vasa deferentia for contraction studies

English short-hair guinea pigs (450–500 g; Harlan Sprague Dawley, Indianapolis, IN, USA) were killed by a blow to the head, exsanguination and thoracotomy. The vasa deferentia were removed, the seminal fluid rinsed from the lumen with 1 ml of modified Krebs-Henseleit (MKH) solution (composition below), and the tissues were cleaned of connective tissue. Each vas deferens was attached to a holder, placed in a 1-ml organ bath containing MKH solution at 37°C and attached to a force-displacement transducer for the measurement of isometric contractions. A preload of 0.2–0.4 g was applied, and the preparations were equilibrated for 1 h with renewal of MKH solution at 15-min intervals before experimental procedures were initiated.

MKH solution contained (mM): NaCl, 113.0; KCl, 4.8; $CaCl_2$, 2.5; $KH_2PO_4$, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; glucose 5.7 (pH 7.4, 37°C); it was gassed with 95% $O_2$ + 5% $CO_2$.

### 2.2. Treatment with periodate-oxidized analogs

One tissue of a pair was incubated in the organ bath with periodate-oxidized ATP, periodate-oxidized ADP, periodate-oxidized AMP or periodate-oxidized adenosine (in concentrations of $10^{-2}$ M) for 5 min, followed by two washes at 5-min intervals with fresh MKH solution. The contralateral, control preparation received a volume equivalent of saline.

### 2.3. Concentration-response curves

Stepwise-increasing, non-cumulative ATP concentration-response curves were obtained using a 13-min addition cycle. The preparation was exposed to a given concentration of ATP for 3 min. The ATP was then removed from the bath with two washes with MKH solution at 5-min intervals before the next higher concentration of ATP was added.

### 2.4. Repetitive challenge with ATP

Three control responses to $10^{-2}$ M ATP (13-min addition cycle) were obtained, by which time the responses were constant. One preparation of a pair was incubated with periodate-oxidized analogs as described above, while the contralateral control vas deferens received saline. After treatment, the preparations were challenged repetitively with $10^{-2}$ M ATP using the 13-min addition cycle.

### 2.5. 120 mM KCl

At the conclusion of nucleotide additions, the vasa deferentia were contracted with 120 mM KCl to provide an internal reference response against which responses to ATP were normalized.

### 2.6. Statistical analysis

Each vas deferens was used to obtain only one concentration-response curve, or for one ATP repetitive challenge experiment. The results are presented as mean ± S.E.M.; $n$ is the number of separate experiments. The data were analyzed for differences using Student's $t$-test for paired samples. $P < 0.05$ was considered significant.

### 2.7. Drugs

ATP sodium (vanadate-free), periodate-oxidized ATP sodium, periodate-oxidized ADP sodium, periodate-oxidized AMP free base and periodate-oxidized adenosine free base were from Sigma Chemical Co. (St. Louis, MO, USA). Each agent was dissolved freshly in saline for use.

## 3. Results

Periodate-oxidized ATP ($10^{-2}$ M) contracted (5.36 ± 0.39 g) the vas deferens upon addition to the bath (Fig. 1a). Periodate-oxidized ADP ($10^{-2}$ M) evoked a contraction (2.52 ± 0.28 g) which was smaller ($P < 0.05$) than that to periodate-oxidized ATP (Fig. 1b); in some preparations (not shown), spontaneous contractions developed during incubation with periodate-oxidized ADP. Neither periodate-oxidized AMP nor periodate-oxidized adenosine ($10^{-2}$ M) had agonist activity (Fig. 1c and d). However, a contraction occurred at the moment of washout of periodate-oxidized adenosine (not shown).

As illustrated in Fig. 1, in low ATP concentrations, the contractile responses consisted of rapidly developing and transient monophasic twitches. With increasing ATP concentration, a second phase appeared in the response which ascended in magnitude in relation to the first phase. The peak force generated at maximal ATP concentrations was usually, but not always, attributable to the second contractile phase.

Incubation with periodate-oxidized ATP, periodate-oxidized ADP, periodate-oxidized AMP and periodate-oxidized adenosine ($10^{-2}$ M for 5 min) resulted in potentiation of responses to ATP (Figs. 1 and 2). This potentiation generally occurred over the range of $10^{-6}$ to $10^{-3}$ M ATP, and it reflected the effect of the affinity labels on the first phase of contraction. The

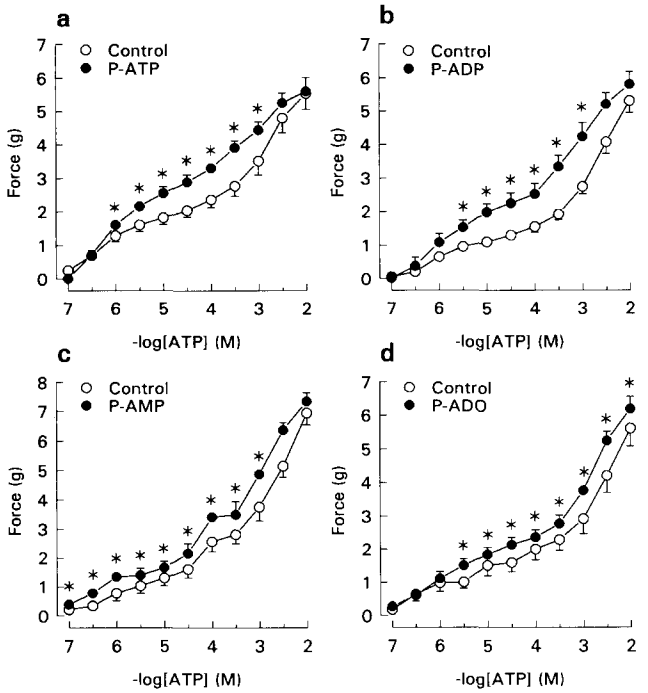


Fig. 2. Effects of incubation with (a) periodate-oxidized ATP (P-ATP), (b) periodate-oxidized ADP (P-ADP), (c) periodate-oxidized AMP (P-AMP) and (d) periodate-oxidized adenosine (P-ADO) on ATP concentration-response curves. The results are given as mean ± S.E.M.; $n = 4$ separate experiments for each periodate-oxidized compound. * Significantly larger than saline-treated, contralateral control preparations.

maximum response was not affected, except following periodate-oxidized adenosine exposure, in which case the first phase of contraction was potentiated.

Concomitant with potentiation of the first contractile phase, the second phase of response to ATP was inhibited following incubation with periodate-oxidized ATP, periodate-oxidized ADP and periodate-oxidized AMP (Fig. 1). This effect was not observed after periodate-oxidized adenosine treatment.

The effects of the periodate-oxidized analogs were irreversible in that, after washout, they persisted throughout the ca. 2.5-h period of concentration-response curve construction. In preparations repetitively challenged with ATP ($10^{-2}$ M), the second phase of contraction to ATP remained inhibited following incubation with periodate-oxidized ATP, periodate-oxidized ADP and periodate-oxidized AMP, in the manner shown in Fig. 1, during a ca. 1.5-h examination period. In contrast, periodate-oxidized adenosine treatment had no effect on the second phase of the responses ($n = 4$ separate experiments for each periodate-oxidized compound; data not shown).

Under no condition did incubation with a periodate-oxidized compound affect the contraction to 120 mM KCl ($P > 0.05$).

## 4. Discussion

The present results have demonstrated that periodate-oxidized ADP, periodate-oxidized AMP, and periodate-oxidized adenosine share with periodate-oxidized ATP the ability to potentiate the first phase of contraction of the vas deferens to ATP after the smooth muscle is incubated with the compounds. The fact that this effect was produced by periodate-oxidized adenosine indicates that the potentiation did not require the presence or cleavage of 5′-phosphate.

The mechanism of potentiation was not investigated in this study, but its irreversibility suggests that it occurred following covalent interactions. In the first report on the effects of periodate-oxidized ATP on nucleotide-induced responses (Fedan and Lamport, 1990), we speculated that the potentiation by periodate-oxidized ATP could be due to inhibition of ecto-phosphohydrolase, i.e., ATPase, activity. This hypothesis remains very plausible in view of the recent report that periodate-oxidized ATP, an inhibitor of ATPases (Colman, 1982), inhibits ecto-ATPase activity in macrophages (Murgia et al., 1993). Other explanations can be considered, however. For example, incubation of vas deferens with AMP or adenosine 5′-monophosphorothioate (AMP$\alpha$S) results in a transient potentiation of ATP-induced contractions (Fedan, 1987). This particular potentiating effect has different structural determinants than the one produced by the periodate-oxidized analogs, i.e., it was not produced by ATP, ATP$\gamma$S, ADP or adenosine 5′-*O*-(2-thiodiphosphate) (ADP$\beta$S), all of which contract the smooth muscle. Clearly, the periodate-oxidized analogs behaved differently.

These experiments also extended our earlier observations with periodate-oxidized ATP (Fedan and Lamport, 1990) and showed that periodate-oxidized ADP and periodate-oxidized AMP inhibited the second phase of contraction to ATP. The inhibitory effect of the three compounds was irreversible and indistinguishable. On the other hand, periodate-oxidized adenosine did not inhibit the second phase of contraction to ATP. [$^3$H]Periodate-oxidized ATP covalently labels vas deferens proteins (Lamport-Vrana et al., 1991) under the same conditions in which the second phase of contraction to ATP is irreversibly inhibited (Fedan and Lamport, 1990). The present findings suggest that the same sites may be affinity-labelled by periodate-oxidized ADP and periodate-oxidized AMP, with ecto-phosphoryl transfer and the second contractile phase inhibited as a result. Inasmuch as periodate-oxidized adenosine was incapable of producing this effect, it may be concluded that at least one 5′-phosphate needs to be present to inhibit this mechanism. This does not necessarily imply that cleavage of the 5′-phosphate chain is obligatory for inhibition of the

second phase. The absence of 5′-phosphate could interfere with the binding of a 2′-3′-dialdehyde-substituted compound to a regulatory site in or near the ecto-kinase. The possibility that periodate-oxidized adenosine had no inhibitory effect because it had no agonist activity is ruled out by the observation that periodate-oxidized AMP also did not evoke contraction; however, periodate-oxidized AMP inhibited the second phase. Our results provide no insight into whether periodate-oxidized AMP formed from the degradation of periodate-oxidized ATP and periodate-oxidized ADP is actually the inhibiting ligand.

None of the observations made in this study were produced following incubation of vas deferens with ATP (Fedan and Lamport, 1990).

It is becoming apparent that more than one transduction mechanism may be involved in a cell's multiphasic response to extracellular ATP. In addition, the involvement of one or more of the mechanisms may depend on the concentration of the nucleotide. For example, multi-component responses have been described in mouse vas deferens (Von Kügelgen et al., 1990), thymocytes (Matkó et al., 1991), parotid acinar cells (McMillian et al., 1993) and macrophages (Murgia et al., 1993). Three purinoceptors are thought to be involved in responses of FRTL-5 (thyroid) cells (Sato et al., 1992) and rat vas deferens (Bültmann and Starke, 1994) to ATP. Periodate-oxidized analogs of ATP and adenosine may be useful for identifying multiple mechanisms of response to ATP in other cell types.

In summary, our experiments have demonstrated that periodate-oxidized analogs of ATP, ADP, AMP and adenosine potentiate the first phase of contraction to ATP, and, except for periodate-oxidized adenosine, inhibit the second phase of contraction. These actions are irreversible, and are interpreted to result from covalent inhibition of ecto-phosphohydrolase and ecto-phosphoryl transfer, respectively.

## Acknowledgements

These studies were made possible through a Minority High School Student Research Apprentice Award to L.J.R.G., and were supported, in part, by NIH S03RR03445-07. We thank Terry Stewart for expert secretarial assistance, and Lyndell L. Millecchia and Kathleen B. Kinsley for comments on the manuscript. Mention of brand name does not constitute product endorsement.

## References

Abbracchio, M.P. and G. Burnstock, 1994, Purinoceptors: are there families of $P_{2X}$ and $P_{2Y}$ purinoceptors?, Pharmacol. Ther. 64, 445.

Bültmann, R. and K. Starke, 1994, $P_2$-Purinoceptor antagonists discriminate three contraction-mediating receptors for ATP in rat vas deferens, Naunyn-Schmied. Arch. Pharmacol. 349, 74.

Colman, R.F., 1982, Affinity labeling of purine nucleotide sites in proteins, Annu. Rev. Biochem. 52, 67.

Fedan, J.S., 1987, Potentiation of adenosine triphosphate-induced contractile responses of the guinea-pig isolated vas deferens by adenosine monophosphate and adenosine 5′-monophosphorothioate, Br. J. Pharmacol. 91, 633.

Fedan, J.S. and S.J. Lamport, 1990, Two dissociable phases in the contractile response of the guinea pig vas deferens to adenosine triphosphate, J. Pharmacol. Exp. Ther. 253, 993.

Fedan, J.S., G.K. Hogaboom, D.P. Westfall and J.P. O'Donnell, 1982, Comparison of the effects of arylazido aminopropionyl ATP ($ANAPP_3$), an ATP antagonist, on responses of the smooth muscle of the guinea-pig vas deferens to ATP and related nucleotides, Eur. J. Pharmacol. 85, 77.

Fedan, J.S., G.K. Hogaboom, J.P. O'Donnell, S.J. Jeng and R.J. Guillory, 1985, Photoaffinity labeling and characterization of $P_2$-purinergic receptors of the smooth muscle of the guinea-pig vas deferens with [$^3$H]arylazido aminopropionyl ATP ([$^3$H]$ANAPP_3$), Eur. J. Pharmacol. 108, 49.

Hogaboom, G.K., J.P. O'Donnell and J.S. Fedan, 1980, Purinergic receptors: photoaffinity analog of adenosine triphosphate is a specific pharmacological adenosine triphosphate antagonist, Science 208, 1273.

Lamport-Vrana, S.J., K.E. Vrana and J.S. Fedan, 1991, Involvement of ecto-phosphoryl transfer in contractions of the smooth muscle of the guinea pig vas deferens to adenosine 5′-triphosphate, J. Pharmacol. Exp. Ther. 258, 339.

Matkó, J., P. Nagy, G. Panyi, G. Vereb, L. Bene, L. Mátyus and S. Damjanovich, 1991, Biphasic effect of extracellular ATP on the membrane potential of mouse thymocytes, Br. J. Pharmacol. 191, 378.

McMillian, M.K., S.P. Soltoff, L.C. Cantley, R. Rudel and B.R. Talamo, 1993, Two distinct cytosolic calcium responses to extracellular ATP in rat parotid acinar cells, Br. J. Pharmacol. 108, 453.

Murgia, M., S. Hanau, M. Pizzo and F. Di Virgilio, 1993, Oxidized ATP. An irreversible inhibitor of the macrophage purinergic $P_{2Z}$ receptor, J. Biol. Chem. 268, 8199.

Pirotton, S., O. Boutherin-Falson, B. Robaye and J.-M. Boeynaems, 1992, Ecto-phosphorylation on aortic endothelial cells. Exquisite sensitivity to staurosporine, Biochem. J. 285, 585.

Sato, K., F. Okajima and Y. Kondo, 1992, Extracellular ATP stimulates three different receptor-signal transduction systems in FRTL-5 thyroid cells. Activation of phospholipase C, and inhibition and activation of adenylate cyclase, Biochem. J. 283, 281.

Von Kügelgen, I., R. Bültmann and K. Starke, 1990, Interaction of adenine nucleotides, UTP and suramin in mouse vas deferens: suramin-sensitive and suramin-insensitive components in the contractile effect of ATP, Naunyn-Schmied. Arch. Pharmacol. 342, 198.