Inhibitory Action of Extracellular Adenosine 5′-Triphosphate on Parietal Cells Isolated from Rabbit Gastric Mucosa

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Abstract. The effects of the purine nucleotides, adenosine 5′-triphosphate (ATP) and their analogs 2-methylthio ATP and β,γ- methylene ATP, as well as those of the pyrimidine nucleotide, uridine 5′-triphosphate (UTP), on acid production in isolated rabbit gastric parietal cells prepared by enzymatic dispersion and enriched by counterflow elutriation were studied. The (^{14}C)-aminopyrine (AP) accumulation method was used as an index of acid production by the parietal cells. In histamine-stimulated parietal cells, ATP and 2-methylthio ATP, but not β,γ-methylene ATP or UTP, produced significant and concentration-related inhibition of the histamine-stimulated AP uptake. The rank order of potency of these nucleotides in inhibiting histamine-stimulated AP accumulation was 2-methylthio ATP > ATP $\gg$ β,γ-methylene ATP, UTP. In contrast to these results, the AP accumulation responses to secretagogues other than histamine such as carbachol and dibutyryl-cAMP, were not significantly modified by ATP and analogs. Pretreatment of parietal cells with indomethacin, a prostaglandin synthesis inhibitor, led to a significant reduction of the inhibitory responses elicited by ATP on histamine-stimulated AP uptake. These data suggest that ATP selectively inhibits the histamine-stimulated gastric acid secretion in rabbits by acting directly on parietal cells; that a component of this action seems to be related with a stimulation of prostaglandin production; and that the antisecretory effect of ATP on isolated rabbit parietal cells may be mediated via P_{2Y}-purinoceptors.

Key words: ATP–ATP analogs — P_2-purinoceptors — Gastric acid secretion — Rabbit parietal cells

Introduction

Extracellular adenine nucleotides are known to have a number of biological effects on a wide range of tissues via specific cell surface receptors known as P_2-

purinoceptors. These receptors have recently been subdivided into six main classes (Fredholm et al. 1994). The P_{2X} and P_{2Y} receptors are the best characterized ones subserving excitatory and inhibitory responses, respectively. The P_{2T}-purinoceptor is the receptor for endogenous ADP on blood platelets. The P_{2Z}-purinoceptor is the entity mediating responses to ATP^{4-} in mast cells and other immune cells. The P_{2D}-purinoceptor appears to be a newer P_2-purinoceptor subtype sensitive to diadenosinetetraphosphate Ap_4A. There is another class of receptors which has been classified as a P_2-purinoceptor subtype. These receptors are characterized by their ability to respond to adenosine 5′-triphosphate (ATP, purine nucleotide) as well as to uridine 5′-triphosphate (UTP, pyrimidine nucleotide), thus they have been termed in several ways $P_{2U}(P_{2N})$-purinoceptors, "pyrimidinoceptors" and "nucleotide receptors". (for reviews see Gordon 1986; Kennedy 1990; Olsson and Pearson 1990; O'Connor et al. 1991; El-Moatassim et al. 1992; Abbracchio et al. 1993; Fredholm et al. 1994).

The functional response most often attributed to nucleotide receptors (P_{2U}) stimulation has been phospholipase C activation which may be manifest as enhanced inositol triphosphate formation, intracellular calcium release or prostaglandin production (O'Connor et al. 1991; O'Connor 1992). However, P_{2Y}-purinoceptors coupled to stimulation of phospholipase C and inositol triphosphate formation as well as to arachidonic acid mobilization secondary to phospholipase A_2 activation have also been demonstrated (for reviews see O'Connor 1992; Fredholm et al. 1994). Thus, it has been pointed out that some nucleotide receptor (P_{2U}) mediated responses can be incorrectly attributed to P_{2Y}-purinoceptors (O'Connor et al. 1991; O'Connor 1992).

In spite of the broad studies on the biological effects of extracellular ATP, there is little information concerning the influence of this nucleotide on gastric acid secretion. In early works carried out using amphibian gastric mucosa, ATP was found to inhibit histamine-stimulated acid secretion (Kidder 1971; Sanders et al. 1976). In further studies, ATP and analogs were found to inhibit the histamine-stimulated acid secretion in rabbit gastric glands (Ainz et al. 1989; Gil-Rodrigo et al. 1990, 1993), results which confirm in mammalian gastric preparations those previously reported for amphibians (Kidder 1971; Sanders et al. 1976). These lines of evidence indicate that extracellular ATP acts mainly as inhibitor on histamine-stimulated gastric acid secretion.

Furthermore, the studies carried out in isolated rabbit gastric glands have provided good evidence to support the view that ATP is a potent inhibitor of the histamine-stimulated acid secretion and that this is a "purinergic" effect which appears to be partly linked to an activation of prostaglandin production (Gil-Rodrigo et al. 1990, 1993). However, besides the parietal cells, the gastric glands consist of several other cell types such as chief, mucous, endocrine and somatostatin cells; therefore, elucidation of the site of action of ATP was difficult to unravel by

using gastric glands.

In the light of the preceding considerations, the aim of the present work was to study the actions and the rank order of potency of ATP and its analogs, 2-methylthio ATP (2-MeSATP) and β,γ-methylene ATP (β,γ-MeATP), on histamine-stimulated gastric acid secretion, by using parietal cell-enriched fractions of isolated rabbit gastric mucosal cells. These experiments were undertaken to establish the pharmacological profile of these purine nucleotides and to elucidate whether ATP was acting directly (or not) on the parietal cell.

Since ATP/UTP sensitive receptors ("P_{2U}"- or "nucleotide"-receptors) might account for the effect of ATP on gastric acid secretion, the influence of the pyrimidine nucleotide UTP on histamine-stimulated parietal cells was studied and compared to that of ATP. In addition, given that in gastric glands the inhibitory effect of ATP has been described to be selective for the secretory responses elicited by histamine (Gil-Rodrigo et al. 1993), the effects of ATP and analogs on rabbit parietal cells stimulated with secretagogues other than histamine, such as carbachol and dibutyryl-cAMP were also investigated. It has been recognized for years that extracellular ATP induces in several type of cells stimulation of prostaglandin synthesis via P_2-purinoceptors which have recently been classified into the P_{2Y}-purinoceptor family (Fredholm et al. 1994). Since the results reported for ATP in rabbit gastric glands (see above) fit well with this statement, the influence of the known prostaglandin synthesis inhibitor, indomethacin, on the responses to ATP in histamine-stimulated parietal cells was also examined.

Materials and Methods

Materials

Histamine dihydrochloride and adenosine 5′-triphosphate (ATP) were purchased from Merck, Germany; carbamylcholine chloride (carbachol), dibutyryl-cAMP (db-cAMP), β,γ-methylene-ATP (β,γ-MeATP), uridine 5′-triphosphate (UTP), synthetic prostaglandin E_2 (PGE_2), indomethacin, collagenase type I and rabbit albumin were from Sigma, USA; 2-methylthio-ATP (2-MeSATP) was obtained from Research Biochemicals Inc., USA; pronase was from Boehringer, Germany; dimethylamine-(^{14}C)-aminopyrine (AP) was from Amersham, UK. All other chemicals used were of analytical grade.

Cell isolation and parietal cell enrichment

Isolation of mucosal cells from the stomach of New Zealand white rabbits (2.5–4 kg) was performed by sequential pronase and collagenase digestions as described previously (Ainz et al. 1993). The resulting cell preparation was suspended in the respiratory medium used for aminopyrine accumulation studies (composition: 132.4

mmol/l NaCl, 5.4 mmol/l KCl, 5.0 mmol/l Na_2HPO_4, 1.0 mmol/l NaH_2PO_4, 1.2 mmol/l $MgSO_4$, 1.0 mmol/l $CaCl_2$, 1 mg/ml rabbit albumin, 2 mg/ml glucose and 10 mmol/l HEPES, adjusted to pH 7.4 and warmed at 37 °C).

According to our recently described elutriation protocol (Ainz et al. 1993), separation and enrichment of parietal cells were carried out by counterflow elutriation in a Beckman J-6M/E centrifuge equipped with a Beckman JE-6B rotor. The parietal cell enriched fractions used for the present study consisted of 75–80% parietal cells as judged by cell size analysis with a Coulter-Counter Multisizier II and by staining of the cells with a suitable multiple stain solution from Polyscience, USA. The viability, as determined by the trypan blue dye exclusion test, was greater than 85%. In addition, each experimental batch with parietal cells from separate rabbits was assayed with histamine (1 μmol/l – 100 μmol/l) in order to check the secretory responsiveness. All cellular preparations that did not respond to histamine were discarded.

Measurement of acid formation

Acid formation by isolated parietal cells was determined by measuring accumulation of (^{14}C)-aminopyrine (AP). Plastic tubes (10 ml) containing 10^6 cells in 1 ml of respiratory medium with 0.01 μCi of AP and agents being tested in adequate quantities to achieve the desired final concentration were incubated in a shaking incubator (110 cycles/min) at 37 °C for 20 min. After the incubation, aliquots of 0.8 ml from each tube were filtered through Whatman GF/B fiber glass filters under vacuum. Wet filters with the pelleted cells were washed with 20 ml of phosphate buffered solution (composition: 149.6 mmol/l NaCl, 3.0 mmol/l K_2HPO_4, 0.64 mmol/l NaH_2PO_4, pH 7.3) to remove residual free AP, dried, placed in vials containing 5 ml of a suitable scintillation cocktail, and counted.

Since the amount of aminopyrine accumulated in the cells reflects the acid secretory state of the cells, the AP accumulation values were estimated from the cellular cpm values obtained in the absence (basal AP uptake) and the presence of the agents under study, and then expressed as percentages relative to the maximum AP accumulation response obtained in each experiment for 0.1 mmol/l histamine (control, 100%), by applying the expression:

$$(\text{cpm agent} - \text{cpm basal}) \times 100/(\text{cpm 0.1 mmol/l histamine} - \text{cpm basal})$$

In a number of experiments, we compared the results obtained from the cellular content of aminopyrine alone with those obtained from the aminopyrine ratio values determined by dividing the cellular content of AP by the medium content of AP, and we could not observe any significant difference.

In studies with histamine-stimulated parietal cells the peak of maximum AP accumulation to this secretagogue was reached at a concentration of 0.1 mmol/l. The concentration producing a half-maximal effect (EC_{50}) for histamine was calcu-

lated from the regression of the AP accumulation responses to log of histamine concentration. Likewise, linear regression of the individual log concentration-response curves to histamine in the presence of each nucleotide at a given concentration was used to estimate the apparent concentrations of histamine required to produce the half-maximal effect observed with histamine alone. The influence of the purine nucleotides ATP, 2-MeSATP, β,γ-MeATP and the pyrimidine UTP on histamine EC_{50}, first reflecting their potencies, was expressed as the ratio of EC_{50} to histamine in the presence and the absence of the nucleotide.

Maximum inhibition of histamine-raised AP accumulation by parietal cells for a particular nucleotide was not attained at the concentrations tested, therefore IC_{50} values could not be calculated. Instead, drug potencies were expressed as the ratio of the concentrations of nucleotides required to produce 50% of inhibitory response reached with the reference inhibitor PGE_2 (1 nmol/l, taken as 100%) on parietal cells stimulated with 0.1 mmol/l histamine. All experiments were carried out in triplicate for each data point. The data, expressed as means ± S.E.M. (n = number of experiments performend with cellular preparations from different rabbits,) were evaluated for differences by Student's unpaired t-test. Values of $P < 0.05$ were considered significant.

Results

Effects of ATP, 2-MeSATP, β,γ-MeATP and UTP on histamine-stimulated AP accumulation.

Histamine concentration-dependently increased AP uptake. Maximum AP accumulation was observed at 0.1 mmol/l. Higher concentrations of histamine did not further increase AP accumulation. Histamine concentration producing 50% maximal effect (EC_{50}) was estimated to be 3.4 ± 0.03 μmol/l. These results are compatible with previous reports using rabbit gastric glands (Berglindh et al. 1976) or rabbit parietal cells (Soll and Berglindh 1987; Ota et al. 1989; Ainz et al. 1993).

The log concentration-response curves to histamine (1 μmol/l–0.1 mmol/l) in the absence and the presence of increasing concentrations of ATP (1 μmol/l–1 mmol/l), 2-MeSATP (1 μmol/l–100 μmol/l, higher concentrations could not be assayed due to solubility problems in the incubation saline), β,γ-MeATP (1 μmol/l–1 mmol/l) and UTP (1 μmol/l–1 mmol/l) are shown in Figure 1. As can be seen, the AP accumulation responses to histamine were significantly inhibited ($P < 0.05$) in a concentration-related fashion by both ATP and 2-MeSATP at all concentrations tested (Fig. 1*a* and *b*). The selective P_{2X} agonist, β,γ-MeATP, failed to produce any significant inhibition of the histamine-stimulated AP accumulation (Fig. 1*c*). Likewise, the log concentration-response curve to histamine was neither significantly affected by the pyrimidine nucleotide UTP (Fig. 1*d*). At these concentrations, none

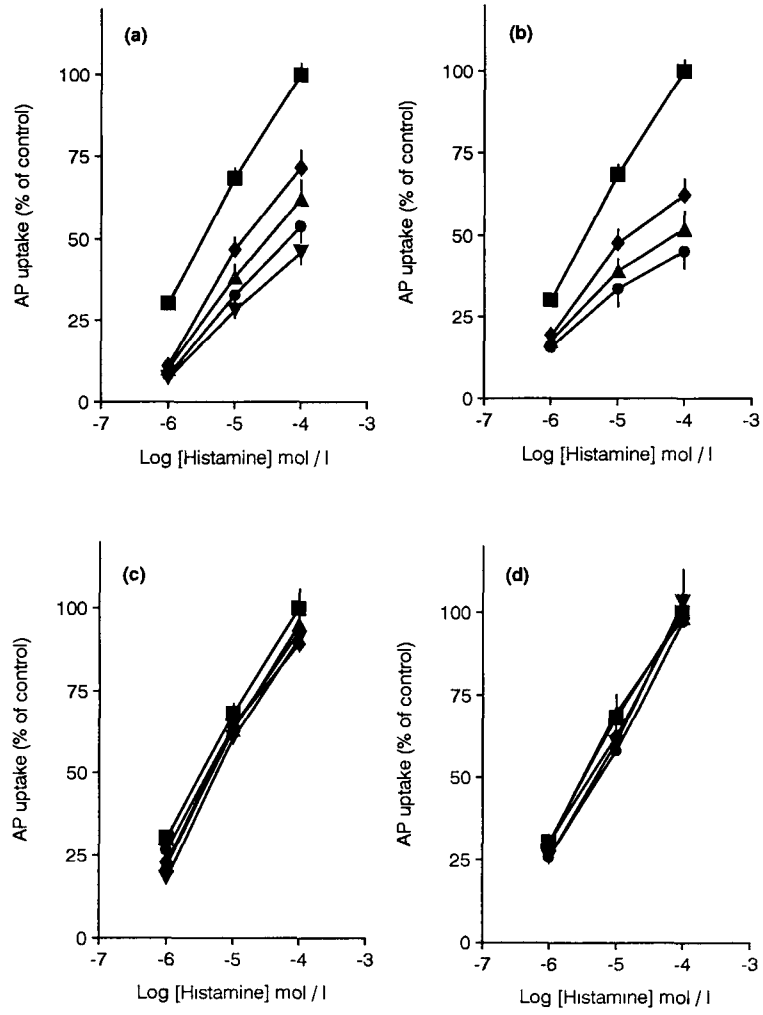

Figure 1. Effects of increasing concentrations of (*a*) ATP, (*b*) 2-MeSATP, (*c*) β,γ-MeATP and (*d*) UTP: 1 μmol/l (♦); 10 μmol/l (▲); 100 μmol/l (●); and 1 mmol/l (▼) on the log concentration-response curve to histamine (■) in rabbit parietal cells. Responses are expressed as percentages relative to maximum (^{14}C)-aminopyrine (AP) uptake obtained in each experiment for 0.1 mmol/l histamine (control, 100%). Each point represents the mean of triplicate determinations ± S.E.M. from at least four experiments (45 for histamine alone).

of them had significant effects on the basal AP accumulation rate.

2-MeSATP and ATP each caused significant increases in the histamine EC_{50},

whereas β,γ-MeATP or UTP did not. Equations of the regression lines of the log concentration-response curves shown in Figure 1 were used to estimate histamine concentrations required to produce half-maximal AP accumulation responses in the presence and the absence of either 2-MeSATP or ATP. The corresponding dose-ratios were: 10.29 (4.93), 18.47 (9.34) and 47.27 (14.19) in the presence of 1 μmol/l, 10 μmol/l and 100 μmol/l of 2-MeSATP and in the presence of the same concentrations of ATP (numbers in parentheses), respectively. 2-MeSATP was, therefore, clearly more potent than ATP ($P < 0.05$) in inhibiting histamine-stimulated AP accumulation responses.

In maximum histamine (0.1 mmol/l)-stimulated rabbit parietal cells, the maximum inhibitory response, being approximately 60% of the total possible inhibition, was reached with PGE_2 at a concentration of 1 nmol/l, and was taken as 100%. The inhibitory effects of either 2-MeSATP (100 μmol/l) or ATP (100 μmol/l) expressed now as percentages relative to 1 nmol/l PGE_2-induced inhibition were close to 90% and 70%, respectively. The inhibitory potency of both purine nucleotides estimated as the ratio of ATP to 2-MeSATP concentration required to produce 50% maximum inhibition obtained with 1 nmol/l PGE_2 was 170. Hence, 2-MeSATP was 170 times more potent than ATP in inhibiting maximum histamine (0.1 mmol/l)-stimulated AP accumulation.

From all these data, the order of potency of these nucleotides in producing inhibition of the histamine-stimulated AP uptake in rabbit parietal cells was: 2-MeSATP > ATP $\gg$ β,γ-MeATP, UTP (Fig. 1), being consistent with that conventionally described for P_{2Y}-purinoceptors.

Lack of effects of ATP and analogs on AP accumulation responses to carbachol and dibutyryl-cAMP.

In accordance with the pattern of effectiveness reported for the classical secretagogues histamine, carbachol or dibutyryl-cAMP in rabbit gastric glands and parietal cells (Soll and Berglindh 1987; Ota et al. 1989; Gil-Rodrigo et al. 1993), the enhancement of AP accumulation brought about by 0.1 mmol/l histamine was much stronger than that produced by 0.1 mmol/l carbachol or 0.1 mmol/l db-cAMP (Fig. 2*a*, *b* and *c*).

The log concentration-response curves to ATP (1 μmol/l–1 mmol/l), 2-MeSATP (1 μmol/l–100 μmol/l) and β,γ-MeATP (1 μmol/l–1 mmol/l) on 0.1 mmol/l histamine-stimulated parietal cells (Fig. 2*a*) show that the inhibitory profiles to ATP and 2-MeSATP were similar, the latter one being more potent. Again, the ATP analog, β,γ-MeATP was ineffective.

In contrast, AP accumulation responses to 0.1 mmol/l carbachol or 0.1 mmol/l db-cAMP were not affected by ATP (1 μmol/l–1 mmol/l), 2-MeSATP (1 μmol/l–100 μmol/l) or β,γ-MeATP (1 μmol/l–1 mmol/l). The log concentration-response curves in Figure 2*b* and *c*, show the lack of effects of ATP and analogs on either 0.1

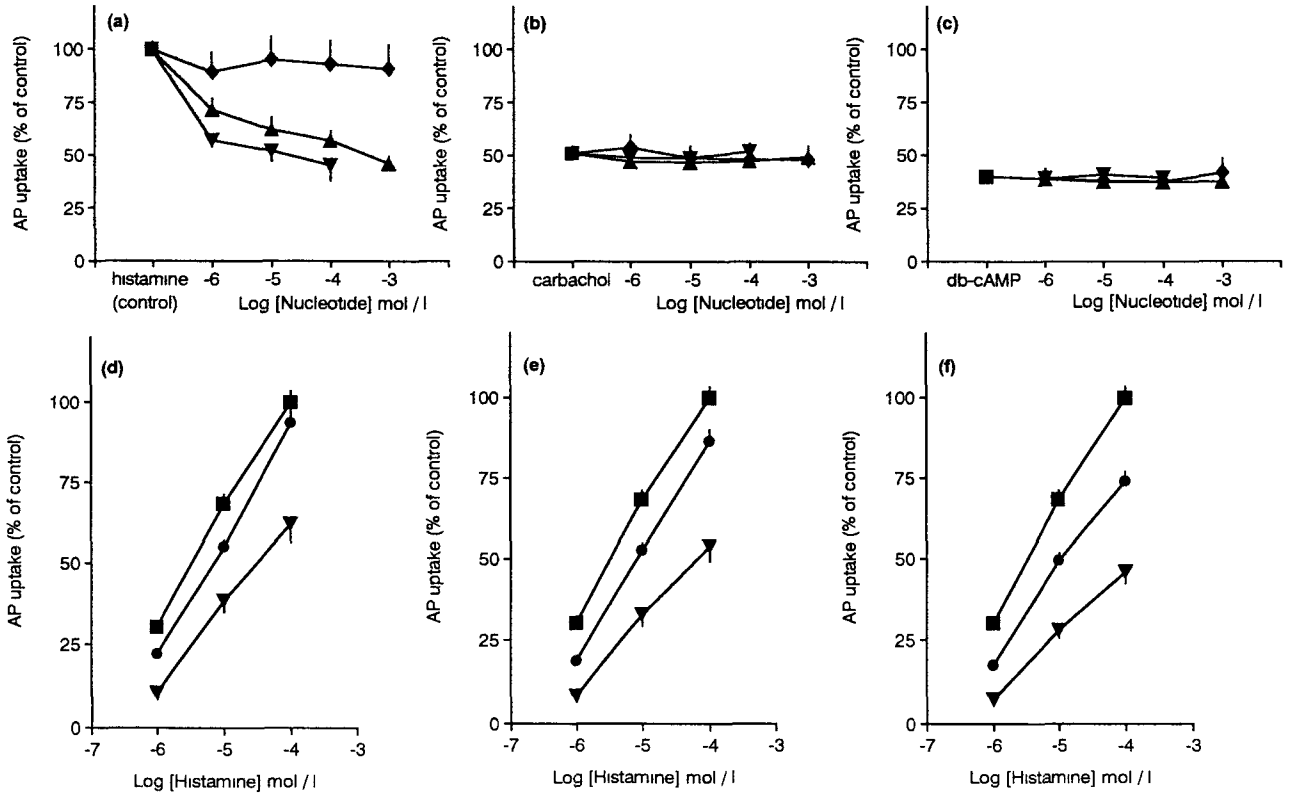

Figure 2. Upper set: effects of ATP (▲), 2-MeSATP (▼) and β,γ-MeATP (♦) on (^{14}C)-aminopyrine (AP) uptake obtained in rabbit parietal cells stimulated with 0.1 mmol/l of (*a*) histamine, (*b*) carbachol and (*c*) db-cAMP. **Lower set:** concentration-response curves to histamine alone (■), and to histamine plus ATP 10 μmol/l (*d*), 0.1 mmol/l (*e*) and 1 mmol/l (*f*) in the absence (▼) and the presence (●) of indomethacin, 10 μmol/l. All responses are expressed as percentages relative to the maximum (^{14}C)-aminopyrine (AP) uptake obtained in each experiment for 0.1 mmol/l histamine (control, 100%). Each point represents the mean of triplicate determinations ± S.E.M. from at least four experiments (45 for histamine alone).

mmol/l carbachol- or 0.1 mmol/l db-cAMP-stimulated rabbit parietal cells. These data indicate that the inhibitory effect of ATP was selective for histamine.

Action of indomethacin on the ATP-induced inhibition of histamine-stimulated AP accumulation.

Parietal cells were pretreated with 10 μmol/l indomethacin for 10 min before histamine and ATP additions. Indomethacin, at the concentration tested, did not significantly modify either basal or histamine-stimulated AP accumulation by parietal cells.

The set of Figures 2 *d*, *e* and *f*, illustrates the log concentration-response curves to histamine alone and to histamine plus 10 μmol/l, 100 μmol/l and 1 mmol/l ATP, respectively, in the absence and the presence of 10 μmol/l indomethacin. As can be seen, in the presence of 10 μmol/l indomethacin, the log concentration-response curves to histamine plus ATP, at the above concentrations, were markedly shifted towards that of histamine alone. This indicates that the inhibitory effects elicited by ATP on the AP responses to histamine (1 μmol/l–0.1 mmol/l) were significantly reduced ($P < 0.05$, in all cases) in the presence of 10 μmol/l indomethacin.

Discussion

Previous studies have shown that extracellular ATP inhibits histamine-stimulated gastric acid secretion in frog gastric mucosa (Kidder 1971; Sanders et al. 1976) and rabbit gastric glands (Ainz et al. 1989; Gil-Rodrigo et al. 1990, 1993). From our present results it can be concluded that extracellular ATP also inhibits histamine-stimulated acid secretion in enriched isolated rabbit parietal cells.

Recent studies indicate that there are receptors that respond to ATP (purine nucleotide) and UTP (pyrimidine nucleotide) but not to 2-MeSATP or α,β-MeATP, which are commonly considered to be the key agonists in the definition of P_{2Y}- and P_{2X}-purinoceptors, respectively (O'Connor et al. 1991; Fredholm et al. 1994). Thus, the ATP/UTP-sensitive receptors have been defined as a separate class of receptors, so-called "nucleotide receptors" or P_{2U}-receptors, which are characterized by the following agonist potency order: UTP $\geq$ ATP $\gg$ 2-MeSATP, α,β-, β,γ-MeATP (O'Connor et al. 1991; Fredholm et al. 1994).

In order to examine whether nucleotide receptors were involved in the antisecretory effect of ATP, experiments were carried out using ATP, UTP and the ATP analogs 2-MeSATP and β,γ-MeATP. From the results, their order of potency in producing inhibition of the histamine-stimulated AP accumulation in rabbit parietal cells was: 2-MeSATP > ATP $\gg$ β,γ-MeATP, UTP. This pattern of activity, with 2-MeSATP being more potent than ATP and with the absence of a detectable inhibitory effect of UTP, is consistent with that conventionally described for P_{2Y}-purinoceptors (Fredholm et al. 1994), and leads to the conclusion that the anti-

secretory effect of ATP on histamine-stimulated parietal cells may be mediated via P_{2Y}-purinoceptors. In addition, since the experiments were performed using enriched parietal cell preparations, it can be also concluded that ATP inhibits the stimulatory action of histamine by acting directly on the parietal cell.

In support of these conclusions, P_{2Y}-purinoceptors have recently been characterized in crude plasma membranes from isolated rabbit gastric glands and subsequently in isolated basolateral membrane enriched fractions from rabbit parietal cells by means of binding assays using the ATP analog [^{35}S]dATP as radioligand (Vallejo et al. 1994).

Although the major emphasis of the present study was to characterize ATP action on isolated rabbit parietal cells, some information regarding the cellular mechanism of action of ATP can be derived from our experimental approach. As it has recently been reviewed by the IUPHAR Purinoceptor Classification Subcommittee (Fredholm et al. 1994), P_{2Y}-purinoceptors constitute G-protein-linked receptors which are often coupled to phopholipase C (PLC) activation and inositol triphosphate (IP_3) formation but, as pointed out in this compilation, additional transduction mechanisms, including modulation of cyclic AMP generation as well as arachidonic acid mobilization and enhancement of prostaglandin production, have also been demonstrated. With this in mind, it seems reasonable to think that one (or a combination) of these transduction pathways can be involved in the mechanism through which ATP and its analog 2-MeSATP inhibits the histamine-stimulated AP uptake by parietal cells.

The first transduction mechanism seems unlikely, since there are important evidences supporting the view that cholinergic muscarinic agonists as well as gastrin, agents that stimulate H^+ secretion by parietal cells, bind to specific receptors on parietal cells coupled via GTP-binding proteins to PLC activation, IP_3 production and release of calcium from intracellular stores, leading to stimulation of the parietal cell function (Hersey and Sachs 1995). On the other hand, protein kinase C (PKC) activation by diacylglycerol (DAG, the other messenger generated in response to PLC stimulation) and phorbol esters (exogenous PKC activators) appears to be related to inhibition of acid secretion by parietal cells (Anderson and Hanson 1985; Soll and Berglindh 1987; Nandi et al. 1994) and not to stimulation of parietal cell function. There is, however, controversy as to whether phorbol esters stimulate or inhibit acid secretion (Hersey and Sachs 1995). Based on this and on our results showing the lack of effect of ATP and analogs on carbachol-stimulated parietal cells, it appears improbable that the histamine-selective antisecretory effect of ATP could involve the PLC-IP_3-DAG transduction pathway. Additionally, the absence of any effect of the ATP analog β,γ-MeATP on either basal or secretagogue-stimulated, particularly on carbachol-stimulated, AP accumulation suggests that there are no P_{2X}-purinoceptors in rabbit parietal cells.

With respect to the second possibility, inhibition of cAMP production in re-

sponse to extracellular ATP has been described in some type of cells (Okajima et al. 1989; Anderson et al. 1991; El-Moatassim et al. 1992; Boyer et al. 1993). This effect has been attributed to P_{2Y}-purinoceptors coupled via a Gi or Gi-like protein to adenylate cyclase inhibition and reduction of intracellular level of cAMP (Anderson et al. 1991; Boyer et al. 1993; Fredholm et al. 1994). Many of the present results, including the antisecretory effect of ATP and the inhibitory profile of purines on histamine-stimulated parietal cells as well as the secretagogue selectivity found for ATP, are consistent with such a mechanism of action. However, on the basis of this transduction pathway, we are not able to explain the indomethacin-induced reduction of the inhibitory responses elicited by increasing concentrations of ATP on parietal histamine-stimulated AP accumulation.

This problem can be overcome when the third ATP signal transduction system is taken into account. It has been recognized that extracellular ATP stimulates endogenous production of prostaglandins via P_{2Y}-purinoceptors in many cell types (Felder et al. 1991; El-Moatassim et al. 1992; Abbracchio et al. 1993; Fredholm et al. 1994). Phospholipase A_2 (PLA_2) activity has been found in parietal cells (Olaisson et al. 1990). The hydrolysis of phosphatidylcholine by PLA_2 is an important source of arachidonic acid for prostaglandin production. Prostaglandins have been reported to inhibit histamine-stimulated acid production in rabbit parietal cells by acting at a high-affinity specific receptor localized on the parietal cell surface (Seidler et al. 1989; Schubert and Shamburek 1990; Hersey and Sachs 1995). Also, prostanoid inhibition has been reported to be highly specific for histamine given that, in isolated parietal cells, the stimulation of the hydrogen ion generation by secretagogues other than histamine, including carbachol, gastrin or db-cAMP is not inhibited by these compounds (Soll and Berglindh 1987; Wolfe and Soll 1988; Choquet et al. 1990; Schepp et al. 1992).

In order to explain this specificity, prostaglandins have been proposed to act through a Gi protein which specifically inhibits the activity of the adenylate cyclase linked to histamine H_2 receptors attenuating histamine-stimulated cAMP generation and acid formation by parietal cells (Soll and Berglindh 1987, Wolfe and Soll 1988, Choquet et al. 1990; Schepp et al. 1992). Although the results presented herein are consistent with this hypothesis, it should be noted that indomethacin did not completely abolish the inhibitory responses to increasing concentrations of ATP. Thus, we speculate that in rabbit parietal cells ATP responsive receptors might be coupled to both inhibition of cAMP production and stimulation of prostaglandin production. This suggestion is based on evidence from other cells in which ATP receptors, particularly P_{2Y}-purinoceptors, appear coupled to more than one signal transduction system (Anderson et al. 1991).

Data in the present work showing the inhibitory effect of extracellular ATP on histamine-stimulated acid production and the possible presence of inhibitory P_{2Y}-purinoceptors on parietal cells suggest that ATP, the endogenous ligand at

P_{2Y}-purinoceptors, can be involved in the physiological regulation of the gastric acid secretory process. Certainly, additional studies are necessary to unambiguously determine the cellular mechanisms through which ATP and related purine nucleotides modulate gastric acid secretion. The present characterization of ATP inhibition of acid secretory responses in isolated rabbit parietal cells will hopefully provide a basis for future studies of the cellular mechanism of this important purine nucleotide as extracellular effector.

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