

Effect of Adenosine 5'-Triphosphate on Secretagogue-Stimulated (^{14}C)-Aminopyrine Accumulation by Rabbit Isolated Gastric Glands

C. E. GIL-RODRIGO, R. GOMEZ, J. M. GANDARIAS, B. GALDIZ, M. CAROU, I. BERGARETXE, A. VALLEJO and L. F. AINZ *

*Departamento de Fisiología, Facultad de Medicina,
Universidad del País Vasco (UPV-EHU),
P.O. Box 699, 48080-Bilbao, Spain*

Abstract. The effect of adenosine 5'-triphosphate (ATP) on gastric acid secretion stimulated by histamine, carbachol and dibutyryl-cAMP (db-cAMP) was studied using glands isolated from rabbit gastric mucosa. The (^{14}C)-aminopyrine (AP) accumulation method was used as an index of acid production by the gastric glands. Histamine-stimulated AP accumulation was significantly inhibited by ATP (10 $\mu\text{mol/l}$ –1 mmol/l). The inhibitory action of ATP appeared to be specific, inasmuch as this nucleotide had no significant effect on basal secretion or secretion stimulated by carbachol or db-cAMP. The antisecretory effect of ATP on histamine-stimulated glands was not affected by the P_1 -purinoceptor antagonist, theophylline. Pretreatment of glands with indomethacin, a well known prostaglandin synthesis inhibitor, led to a significant reduction of the inhibitory responses to ATP. These results show that ATP inhibits the histamine-stimulated AP accumulation by rabbit isolated gastric glands and suggest that this effect is not due to an ectoenzymatic conversion of ATP into adenosine but to a direct effect of ATP which may be mediated via a P_2 -purinoceptor subtype linked to prostaglandin production.

Key words: ATP — Secretagogues — Purinoceptors — Acid secretion — Rabbit gastric glands

Introduction

Extracellular adenosine 5'-triphosphate (ATP) exhibits many biological effects (Gordon 1986). Most of them are thought to be mediated via specific purinergic receptors at the target cell (Gordon 1986; Williams 1987). Purinergic receptors were classified by Burnstock (1978) into a P_1 - and P_2 -type, preferentially activated

* To whom correspondence should be addressed

by adenosine/AMP and ATP/ADP, respectively. The P_2 -purinoceptors have been further subdivided into at least two major subtypes P_{2x} and P_{2y} (Burnstock and Kennedy 1985; Williams 1987).

Whereas there are numerous studies on the effects of ATP in physiological systems such as nervous, cardiovascular, respiratory, urogenital and gastrointestinal smooth muscle systems (Gordon 1986; Olsson and Pearson 1990), little information is available on the effects of ATP 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 designed to search for possible mechanisms mediating the effects displayed by a water-soluble splenic material on mammalian gastrointestinal preparations (Gandarias et al. 1978; Ainz et al. 1983; Gandarias et al. 1984) we found that adenosine and adenine nucleotides caused significant changes on acid secretion in isolated whole rat stomachs and based on this, a regulatory role for purines in the mammalian gastric acid secretory process was proposed (Gandarias et al. 1982; 1985). In addition, we have recently presented evidence that adenosine and ATP stimulates and inhibits, respectively, acid secretion in rabbit gastric glands (Gil-Rodrigo et al. 1990). Thus, our results with ATP in mammalian gastric preparations agree to those previously reported in amphibian (see above), and therefore ATP appears to act mainly as inhibitor on histamine-stimulated gastric acid secretion.

Based on this, the aim of the present study has been to examine the effect of ATP on rabbit gastric glands stimulated not only with histamine but also with secretagogues other than histamine, such as carbachol and db-cAMP. In addition, the influence of theophylline, a selective competitive antagonist to adenosine receptors, was studied since the possibility of an indirect effect of ATP via ectoenzymatic breakdown into adenosine exists. On the other hand, ATP has been described to induce in some cases prostaglandin synthesis (Olsson and Pearson 1990); therefore, the influence of indomethacin, a well known prostaglandin synthesis inhibitor, on the responses elicited by ATP was also studied.

Materials and Methods

Fed New Zealand white rabbits weighing 2.5–4 kg were killed by cervical fracture/dislocation and the stomach was perfused as described by Berglinth and Öbrink (1976). The buffer solutions for perfusion, disaggregation of glands and AP accumulation studies (respiratory medium), and the general assay procedure were performed essentially as reported by Sack and Spenney (1982). Briefly, aliquots of 0.1 ml of the stock gland suspension were placed in 1.5 ml preweighed sealed conical polypropylene Eppendorf tubes containing 0.01 μ Ci of AP, agents to be tested in adequate quantity to achieve the desired final concentration, and respiratory medium up to complete a volume of 1 ml. The tubes were then sealed and incubated by submersion and horizontal mixing in a shaking incubator at 37°C for 30 min. After the incubation, the tubes were centrifuged for 5 min.

Aliquots of 0.1 ml of the supernatant from each tube were placed in vials containing 10 ml of a suitable scintillation cocktail and counted. The remaining supernatant was discarded and the gland pellets were dried at 90 °C for 60 min. The tubes containing dry pellets were then reweighed, resuspended in 0.1 ml of 1 mol/l KOH, dissolved by heating at 90 °C for 15 min and quantitatively transferred and counted in 10 ml of suitable scintillation cocktail. The AP accumulation values were estimated as the ratio, $Rap = AP \text{ intraglandular} / AP \text{ medium}$, applying the expression (Sack and Spenny 1982):

$$Rap = \text{pellet cpm} / (2 \mu\text{l/mg}) (\text{mg dry wt}) (\text{medium cpm}/\mu\text{l})$$

Each preparation was tested for viability by trypan blue dye exclusion. Normally, a cellular viability greater than 90% was obtained. In addition, each experimental batch with gastric glands from separate rabbits was assayed with histamine (1 $\mu\text{mol/l}$ –100 $\mu\text{mol/l}$) in order to check the secretory responsiveness. Glandular preparations that did not respond to histamine were discarded.

Concentrations of agents given in the text are expressed as final molar tube concentrations. Data are expressed in terms of the quotient between the Rap values ($QRap$) obtained as follows: $QRap = Rap(\text{agent})/Rap(\text{basal})$. Thus, in the absence of any drug $QRap = 1$. All experiments were carried out in triplicate for each data point. Statistical evaluation of the results, expressed as means \pm S.E.M. (n = number of experiments performed with glandular preparations from different rabbits), was calculated by Student's t test. The agents used were: histamine, carbachol, dibutyryl-cAMP, adenosine 5'-triphosphate (ATP) and indomethacin purchased from "Merck"; theophylline from "Sigma"; and [¹⁴C]-aminopyrine from "Amersham". All other chemicals used were of analytical grade.

Results

Effects of ATP on secretagogue-stimulated AP accumulation

Histamine (0.1 mmol/l), carbachol (0.1 mmol/l) and dibutyryl-cAMP (0.1 mmol/l) caused potent increases in AP accumulation by rabbit gastric glands. Considerable species differences have been reported in the magnitude of the responses to these secretagogues (Soll and Berglinth 1987).

In agreement with the pattern of effectiveness reported for these secretagogues in rabbit gastric glands and parietal cells (Berglinth et al. 1976; Soll and Berglinth 1987; Ota et al. 1989), the results presented here show that the enhancement in AP uptake elicited by histamine was clearly higher than that by carbachol or db-cAMP (Fig. 1a). In fact, the AP accumulation responses to 0.1 mmol/l carbachol and to 0.1 mmol/l db-cAMP, expressed as percentages relative to the response obtained with 0.1 mmol/l histamine, were about 52% and 43%, respectively.

Exogenous ATP (10 $\mu\text{mol/l}$ – 1 mmol/l) caused significant and progressive reduction of the AP response to 0.1 mmol/l histamine (Fig. 1b). The histamine-stimulated AP uptake was inhibited by 34.1%, 43.1% and 49.8%, in the presence of 10 $\mu\text{mol/l}$, 100 $\mu\text{mol/l}$ and 1 mmol/l ATP, respectively.

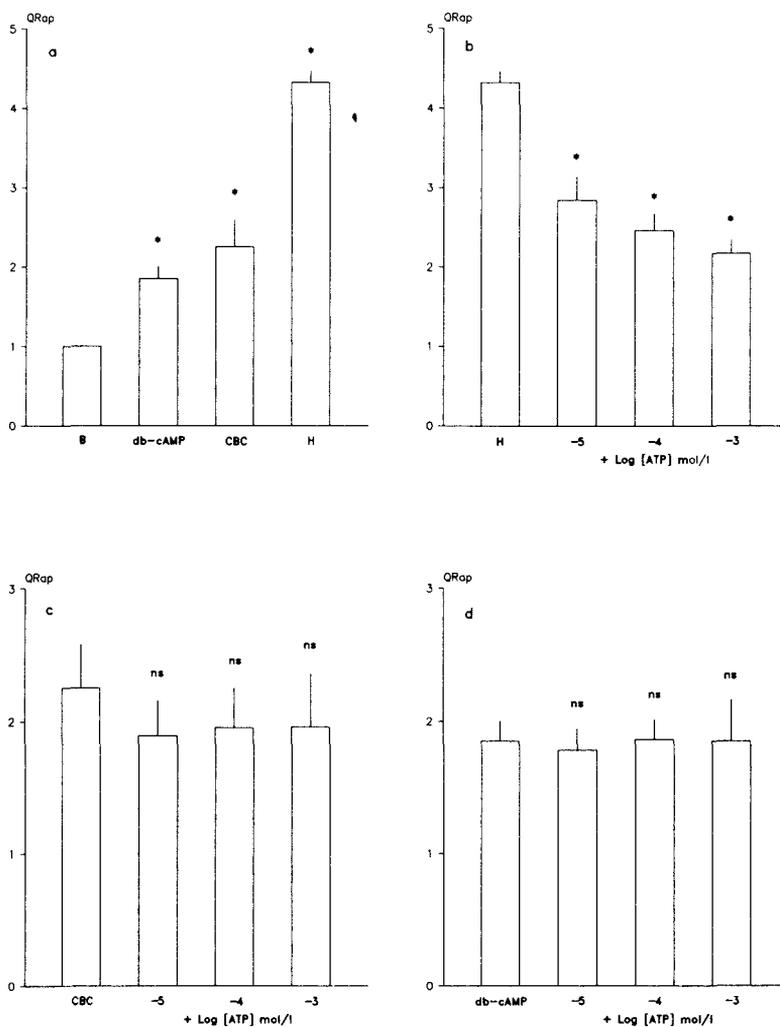


Figure 1. a) Effects of 0.1 mmol/l dibutyl-cAMP (db-cAMP), 0.1 mmol/l carbachol (CBC) and 0.1 mmol/l histamine (H) on basal (B) aminopyrine accumulation by rabbit gastric glands. Graphs b), c) and d) show, respectively, the effects of increasing concentrations (10 μ mol/l, 100 μ mol/l and 1 mmol/l) of ATP on 0.1 mmol/l histamine (H), 0.1 mmol/l carbachol (CBC) and 0.1 mmol/l dibutyl-cAMP (db-cAMP) stimulated rabbit gastric glands. The aminopyrine (AP) accumulation responses are expressed as $QRap$ [quotient between AP ratios (Rap)] obtained by dividing Rap agents/ Rap basal, thus $QRap$ basal = 1. Means from at least four experiments, the vertical lines show s.e. * denotes $p < 0.05$ and ns denotes $p > 0.05$ vs. respective control: B, basal, H, histamine, CBC, carbachol and db-cAMP, dibutyl-cAMP.

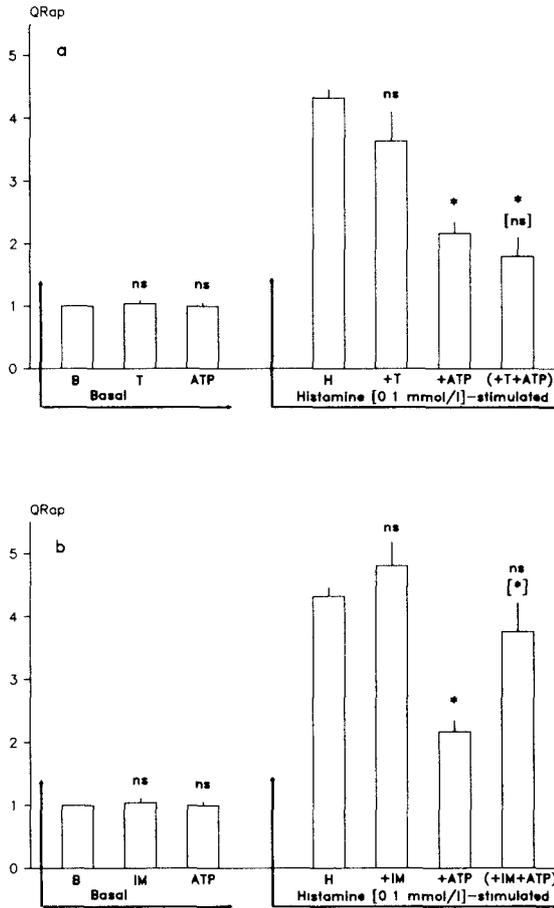


Figure 2. a) Effects of 1 μmol/l theophylline (T) and 1 mmol/l ATP (ATP) on basal (B) aminopyrine accumulation by rabbit gastric glands, and effects of 1 μmol/l theophylline (+T) and that of ATP in the absence (+ATP) and the presence of 1 μmol/l theophylline (+T+ATP) on 0.1 mmol/l histamine (H) stimulated rabbit gastric glands. b) Effects of 0.1 mmol/l indomethacin (IM) and 1 mmol/l ATP (ATP) on basal (B) aminopyrine accumulation by rabbit gastric glands and effects of 0.1 mmol/l indomethacin (+IM) and that of ATP in the absence (+ATP) and the presence of 0.1 mmol/l indomethacin (+IM+ATP) on 0.1 mmol/l histamine (H) stimulated rabbit gastric glands. The aminopyrine (AP) accumulation responses are expressed as *QRap* [quotient between AP ratios (*Rap*)] obtained by dividing *Rap* agents/*Rap* basal, thus *QRap* basal = 1. Means from at least four experiments, the vertical lines show s.e. * denotes $p < 0.05$ and ns denotes $p > 0.05$ vs. control basal (B) or control 0.1 mmol/l histamine (H). Symbols in square brackets denote [*] $p < 0.05$ and [ns] $p \geq 0.05$ vs. 0.1 mmol/l histamine plus 1 mmol/l ATP (+ATP).

The inhibitory effect of ATP was selective for histamine since at the concentrations tested (see above) this nucleotide had no significant effect on either carbachol (0.1 mmol/l)- or db-cAMP (0.1 mmol/l)-stimulated gastric glands (Fig. 1c and d). In addition, at the concentrations used ATP did not modify basal AP accumulation by glands (the effect of 1 mmol/l ATP on basal AP uptake is shown in Fig. 2a and b).

Effect of theophylline on the ATP-induced inhibition of histamine-stimulated AP accumulation

Theophylline (1 $\mu\text{mol/l}$) did not significantly modify either basal or histamine (0.1 mmol/l)-stimulated AP accumulation by gastric glands (Fig. 2a).

In the presence of 1 $\mu\text{mol/l}$ theophylline, a concentration at which this methylxanthine has been reported to antagonize the effect of adenosine on acid secretion in rabbit gastric glands (Gil-Rodrigo et al. 1990), the inhibitory effect of 1 mmol/l ATP on histamine-stimulated AP uptake was unaltered (Fig. 2a).

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

Gastric glands were pretreated with 0.1 mmol/l indomethacin, a known prostaglandin synthesis inhibitor, for 10 min before 0.1 mmol/l histamine and 1 mmol/l ATP additions. Under our experimental conditions, 0.1 mmol/l indomethacin alone did not significantly modify either basal or histamine (0.1 mmol/l)-stimulated AP accumulation by gastric glands (Fig. 2b).

As shown in Figure 2b, the inhibitory effect of 1 mmol/l ATP on the AP response to 0.1 mmol/l histamine was almost completely abolished in the presence of 0.1 mmol/l indomethacin

Discussion

In confirmation of previous results in frog gastric mucosa (Kidder 1971; Sanders et al. 1976) and rabbit gastric glands (Ainz et al. 1989; Gil-Rodrigo et al. 1990), the results presented above show that ATP inhibits histamine-stimulated acid secretion. In the present work we have attempted to characterize this inhibitory action of ATP and to provide some information regarding the mechanism of action of ATP. Many of the extracellular effects of ATP are thought to be mediated via P_2 -purinoceptors at the target cells (Gordon 1986; Williams 1987). Some of them, however, might be mediated via P_1 -purinoceptors since extracellular ATP suffers a rapid ectoenzymatic breakdown to adenosine (Burnstock and Buckley 1985; Gordon 1986). In order to examine whether the antisecretory effect of ATP was direct or indirect (via degradation to adenosine), experiments were carried out using theophylline, an antagonist to P_1 -purinoceptors. Theophylline has been described not

only to antagonize P₁-purinoceptors but also to inhibit phosphodiesterase activity (Burnstock and Buckley 1985; Choi et al. 1988). At high concentrations (close to milimolar) theophylline acts mainly as phosphodiesterase inhibitor, while at lower concentrations (micromolar) it mainly acts as antagonist to P₁ receptors (Burnstock and Buckley 1985; Choi et al. 1988). We have recently reported (Gil-Rodrigo et al. 1990) that in rabbit gastric glands, theophylline inhibits the AP uptake responses to adenosine and that the inhibition is more potent for theophylline at 1 μmol/l than higher. Therefore, in the present study 1 μmol/l theophylline was used. As can be seen from the results, 1 μmol/l theophylline: 1) appeared to have no influence on phosphodiesterase activity since it did not modify either basal or histamine-stimulated AP uptake, 2) failed to antagonize the inhibitory effect of ATP on histamine-stimulated AP accumulation by rabbit gastric glands. If ATP were acting indirectly via conversion to adenosine, then the inhibitory effect would be antagonized by theophylline.

This observation coupled with the knowledge that adenosine and/or analogues enhance both basal and histamine-stimulated AP uptake by gastric glands and parietal cells isolated from rabbits (Ainz et al. 1989, 1992; Ota et al. 1989; Gil-Rodrigo et al. 1990) strongly support the view that ATP is acting directly and not via its ectoenzymatic conversion to adenosine.

Results from assays with ATP on the AP accumulation responses to secretagogues other than histamine have shown that this nucleotide did not significantly inhibit AP accumulation stimulated by carbachol or db-cAMP, indicating that the inhibitory effect of ATP was selective for histamine.

Because the secretagogues tested stimulate acid formation by different intracellular mechanisms and ATP only inhibits AP uptake stimulated by histamine, it is clear that ATP does not block a common distal step in the stimulus-secretion coupling pathway. If ATP did have such an action, then acid secretion would be suppressed irrespective of the type of secretagogue used.

The results with db-cAMP, which presumably mimics histamine (an agent that increases intracellular cAMP content by interacting with an H₂-receptor-adenylate cyclase complex present in parietal cells (Soll and Berglindh 1987)), suggests that ATP inhibits histamine-stimulated acid formation at a site proximal to the cAMP step.

Since 1) ATP appears to act directly via P₂-purinoceptors rather than via P₁-purinoceptor following ectoenzymatic breakdown to adenosine (see above), 2) the specificity of the inhibitory effect of ATP on histamine-stimulated accumulation by gastric glands resembles that previously reported for prostaglandins, well known inhibitors of gastric acid secretion (Soll and Berglindh 1987; Seidler et al. 1989), and 3) ATP acts in some tissues, including the gastric fundus (Lefebvre and Burnstock 1990), through P₂-purinoceptors linked to prostaglandin biosynthesis (Olsson and Pearson 1990), experiments were carried out to examine whether

the effect of ATP on histamine-stimulated AP accumulation by gastric glands was somehow prostaglandin mediated

The results from assays with indomethacin, a known prostaglandin synthesis inhibitor, show that in the presence of this agent the inhibitory effect of ATP on the AP responses to histamine was almost completely abolished. This finding suggests the involvement of prostaglandins in the antisecretory action of ATP.

There is some important evidence in support that prostaglandins inhibit acid production by gastric parietal cells via specific membrane receptor (Seidler et al 1989) coupled to adenylate cyclase through a protein G_i, which inhibits the activity of this enzyme attenuating histamine-stimulated cAMP generation and acid formation by parietal cells (Soll and Berglindh 1987, Wolfe and Soll 1988). It is conceivable that ATP inhibits AP accumulation by a following mechanism. ATP, acting via P₂-purinoceptors linked to prostaglandin biosynthesis, enhances endogenous production of these substances. Prostaglandins acting as described above lead to attenuation of cAMP generation and acid production by parietal cells. The results presented herein are consistent with such a mechanism of action for ATP. In addition, the mechanism proposed explains most of the questions considered along this discussion.

In summary, the present study shows that extracellular ATP inhibits histamine-stimulated acid secretion in rabbit gastric glands. This antisecretory effect is not mediated by P₁-purinoceptors following ectoenzymatic breakdown of ATP to adenosine and is selective for histamine. In addition, evidence has been provided for prostaglandins being involved in the mechanism by which ATP modulates gastric acid production in rabbits.

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