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Evidence for the presence of P2y and P2x receptors with different functions in mouse stomach

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Abstract

To clarify the function of P2 receptor subtypes in mouse stomach, the motor responses to ATP, α,β-methyleneATP (α,β-MeATP), P2X receptor agonist, 2-methylthioATP (2-MeSATP), P2Y receptor agonist, and the effects of the desensitisation of P2X receptors with α,β-MeATP and of P2Y receptors with ADPβS were analysed recording the endoluminal pressure from whole-organ. ATP-induced relaxation was antagonised by suramin, non-selective P2 receptor antagonist, by desensitisation of P2Y receptors with ADPβS, and increased by desensitisation of P2X receptors with α,β-MeATP. α,β-MeATP produced biphasic responses: relaxation, reduced by P2X- or P2Y desensitisation, and contraction, almost abolished by P2X desensitisation and potentiated by P2Y desensitisation. 2-MeSATP induced relaxation, which was antagonised by P2Y desensitisation and increased by P2X desensitisation. Tetrodotoxin increased the relaxation induced by purines and deeply antagonised the contraction to α,β-MeATP. Our results suggest that in mouse stomach are present muscular P2Y receptors, subserving relaxation, and neuronal presynaptic P2X receptors, mediating contraction.
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Keywords: ATP; P2X receptor; P2Y receptor; Mouse stomach; Contraction; Relaxation

1. Introduction

ATP, in addition to its function as an intracellular energy donor, is recognised as an important signalling molecule that mediates diverse biological effects via cell-surface receptors: the purine receptors (Ralevic and Burnstock, 1998). ATP and related purines act as neurotransmitters in the central, peripheral and enteric nervous system (Bertrand, 2003; Galligan, 2002). In the last instance, ATP is recognised as inhibitory NANC mediator (Jenkinson and Reid, 2000; Manzini et al., 1986; Mulè and Serio, 2003) or as an excitatory neuro-neuronal transmitter (LePard and Galligan, 1999) and, in addition, it can participate in the transduction of sensory stimuli (Bertrand, 2003).

There are two main families of purine receptors, adenosine or P1 receptors, and P2 receptors, recognising primary ATP, ADP and pyrimidines (Ralevic and Burnstock, 1998). Based on differences in molecular structure and signal transduction mechanisms, P2 receptors are divided in two families of ligand-gated ion channels and G-protein-coupled receptors termed P2X and P2Y receptors, respectively (Abbracchio and Burnstock, 1994). Both subtypes of purine receptors can be further subdivided into $P2X_1$ to $P2X_7$ and $P2Y_1$ to $P2Y_8$ by cloning experiments (Burnstock and King, 1996). Full understanding of the functions subserved by P2 receptor subtypes is hindered by general lack of selective agonists and antagonists. Based on functional activities, however, α,β-methylene ATP (α,β-MeATP) is considered to be a typical agonist for P2X receptors, whereas 2-methylthio-ATP (2-MeSATP) or adenosine-5′-O-(2-thiodiphoshate) (ADPβS) are considered typical agonists for P2Y receptors (Abbracchio and Burnstock, 1994; Burnstock and Kennedy, 1985).

Classically, it is accepted that P2X receptors mediate smooth muscle contraction and P2Y mediate muscular

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relaxation (Burnstock and Kennedy, 1985). However, up to date studies have shown that the effects of ATP are species- and region-specific in the gastrointestinal tract. For example, activation of P2X receptors causes contraction in guinea pig ileum (Matsuo et al., 1997), while it induces relaxation in rat ileum (Storr et al., 2000). In dispersed gastric smooth muscle cells $P2Y_2$ and $P2X_1$ receptors mediate contraction (Murthy and Makhlouf, 1998). In rat and guinea pig stomach P2X receptors seem to be involved in the relaxant responses to purines (Ahn et al., 1995; Lefebvre and Burnstock, 1990; Otsuguro et al., 1996), whereas P2Y receptor agonists induce muscle contractions (Ahn et al., 1995; Otsuguro et al., 1996; Zagorodnyuk and Maggi, 1998).

Recently the attention has been focused on the purinergic receptors in the mouse gut, due to the importance of the mouse as model species for investigating the effects that genetic knockouts may have on gastrointestinal motility. ATP, acting on P2Y postjunctional receptors, has been proposed to mediate the neurally-evoked inhibitory responses in mouse stomach and small and large intestine (De Man et al., 2003; Mulè and Serio, 2003; Serio et al., 2003). Indeed, Giaroni et al. (2002) suggested that ATP exerts its inhibitory effects, acting on neuronal P2Y receptors in mouse intestine. Moreover, information on the function and localisation of P2X receptors in mouse gut is still lacking.

The present study was undertaken to investigate the effects induced by the activation of the different P2 receptor subtypes in mouse stomach. We analysed the responses to ATP, the metabolically stable agonist α,β-methylene ATP (α,β-MeATP) and 2-methylthio ATP (2-MeSATP), P2X and P2Y receptor agonists, respectively, and the effects of the desensitisation of P2X with α,β-MeATP and of P2Y with ADPβS on the responses of the different agonists using the whole-organ, which is able to relax in the absence of contractile agents.

2. Material and methods

2.1. General

Experiments were authorized by the Ministero della Sanità (Rome, Italy). Mice (C57BL, 20–35 g) were killed by cervical dislocation. The abdomen was immediately opened, the oesophagus was tied proximal to lower oesophageal sphincter, and the entire stomach was excised. The experimental model was as previously described (Mulè and Serio, 2002). Preparations were mounted in a custom-designed organ bath continuously perfused (1.5 ml/min) with oxygenated (95% O_2 and 5% CO_2) and heated (37 °C) Krebs solution with the following composition (mM): NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.1. The Krebs solution always contained atropine (1 μM) and guanethidine (1 μM) to impose NANC condition. The pyloric end was cannulated and connected to a pressure transducer (Statham Mod. P23XL) in order to detect the changes of intraluminal pressure. The mechanical activity was recorded on ink-writer polygraph (Grass model 7D). The preparations were allowed to equilibrate for at least 60 min before starting the experiment.

2.2. Experimental protocol

At the end of equilibration time, the stable level of pressure reached was considered zero pressure and the responses to increasing concentrations of the various agonists were examined using different preparations. Concentration–response curves to agonists were obtained in a non-cumulative manner. Agonists were therefore added to the bath as single addition, in volume of 50 μl after switching off the perfusion, at intervals of about 30 min. Each concentration was left in contact with the tissue for 4 min and, then the preparation rapidly was washed. Preliminary experiments showed that a second curve to the agonists was reproducible. Curves to ATP were repeated in the presence of the non-selective P2 receptor antagonist, suramin (100 μM for 30 min), after desensitisation of P2Y receptor with ADPβS (10 μM for 30 min) and after desensitisation of P2X receptors with α,β-MeATP (10 μM for 30 min). Curves to α,β-MeATP and 2-MeSATP were repeated after desensitisation of P2X receptors with α,β-MeATP (10 μM) or desensitisation of P2Y receptors with ADPβS (10 μM). In another set of experiments, the effects of a submaximal concentration of ATP (100 μM), α,β-MeATP (30 μM) and 2-MeSATP (10 μM) were evaluated in the absence and in the presence of the neurotoxin, tetrodotoxin (TTX, 1 μM for 30 min).

2.3. Data analysis and statistical tests

Relaxant and contractile effects were measured as the maximal effect obtained during the application period and expressed respectively as a percentage of the response to maximal concentration of the agonist tested in the same preparation. All data are expressed as mean values ± S.E.M. The letter *n* indicates the number of experiments and it is equivalent to the number of experimental animals. For statistical analysis, Student's *t*-test for paired values was used. A probability value of less than 0.05 was regarded as significant.

2.4. Drugs

The following drugs were used: atropine sulphate, guanethidine monosulphate, ATP, α,β-MeATP, ADPβS, 2-MeSATP, tetrodotoxin (TTX) (all purchased from Sigma, St Louis, USA), suramin (Sigma–RBI, Sigma-Aldrich). Stock solutions were prepared by dissolving all drugs in distilled water and kept frozen. The working solutions were prepared

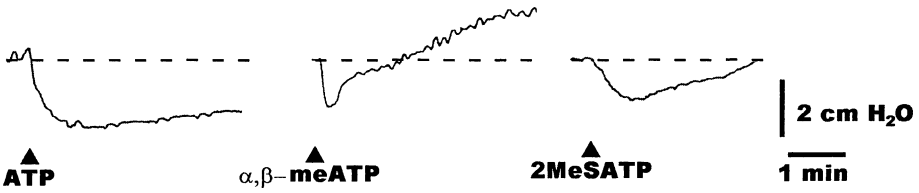

Fig. 1. Representative traces showing the responses to various adenine nucleotides in the mouse gastric preparation. ATP (100 μM) and 2-methylthio-ATP (2MeSATP) (100 μM) caused relaxation, whereas α,β-methyleneATP (α,β-meATP) (100 μM) induced relaxation followed by contractile effect. Dotted line indicates basal pressure (0 pressure) and arrows indicate addition of the agonists.

fresh on the day of the experiment by diluting the stock solutions with Krebs solution.

3. Results

3.1. ATP effects

Under the present experimental conditions, gastric preparations showed small spontaneous phasic contractions. ATP (1 μM–1 mM) induced a concentration-dependent, rapid and well-maintained relaxation with an amplitude of about 3 cm H_2O at 1 mM (Fig. 1). The effect was antagonised by suramin (100 μM), a P2 receptor antagonist (Fig. 2).

In order to clarify the subtype of P2 receptors involved in the response to ATP, the purine was tested after desensitisation of P2Y receptors with ADPβS (10 μM for 30 min) or after desensitisation P2X receptors with α,β-MeATP (10 μM for 30 min). The ATP-induced response was greatly reduced after pre-treatment of gastric preparation with ADPβS (Fig. 3), indicating that P2Y receptors are responsible for the relaxation. By contrast, treatment with α,β-MeATP significantly increased the relaxant response to ATP (Fig. 3). This finding might suggest that ATP acts also on P2X receptors, mediating contractile effects.

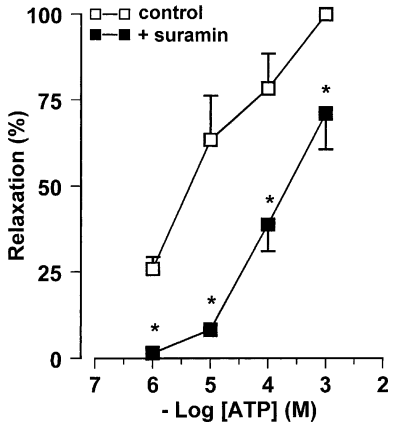

Fig. 2. Concentration–response curves for ATP-induced relaxation in mouse gastric preparations before and after treatment with suramin (100 μM). All values are means ± S.E.M. ($n=5$) and are reported as a percentage of the maximum effect induced by 1 μM ATP. *$P<0.05$ vs. control.

3.2. α,β-MeATP effects

α,β-MeATP (1 μM–100 μM), agonist of P2X receptors, evoked concentration-dependent biphasic responses, characterised by an initial transient relaxation, followed by a rebound contraction (Figs. 1 and 4). After P2X receptor desensitisation by prolonged perfusion with α,β-MeATP (10 μM for 30 min), the relaxant component of the response to α,β-MeATP was decreased and the contractile component was almost abolished. After P2Y receptor desensitisation by prolonged perfusion with ADPβS (10 μM for 30 min), the relaxation evoked by α,β-MeATP was significantly reduced while the contractile component was increased (Fig. 4).

3.3. 2-MeSATP effects

2-MeSATP (0.1 μM–100 μM), agonist of P2Y receptors, produced a concentration-dependent transient relaxation, which slowly returned to the basal level (Figs. 1 and 5). The desensitisation of P2Y receptors with ADPβS (10 μM for 30 min) significantly reduced the relaxation evoked by 2-MeSATP. The desensitisation of P2X receptors with α,β-MeATP (10 μM for 30 min) increased the amplitude and the duration of the relaxation (Fig. 5).

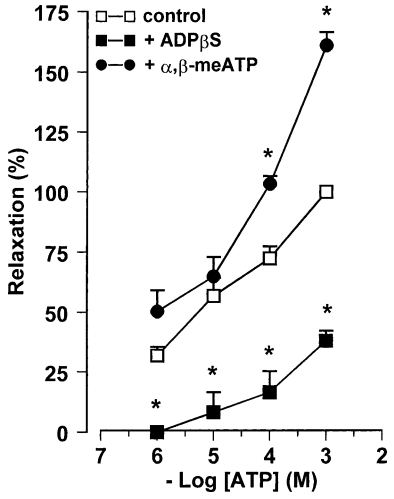

Fig. 3. Concentration–response curves for ATP-induced relaxation in mouse gastric preparations before and after desensitisation of P2Y receptors with adenosine-5′-O-(2-thiodiphoshate) (ADPβS) (10 μM for 30 min) or of P2X receptors with α,β-methyleneATP (α,β-meATP) (10 μM for 30 min). All values are means ± S.E.M. ($n=6$) and are reported as a percentage of the maximum effect induced by 1 μM ATP. *$P<0.05$ vs. control.

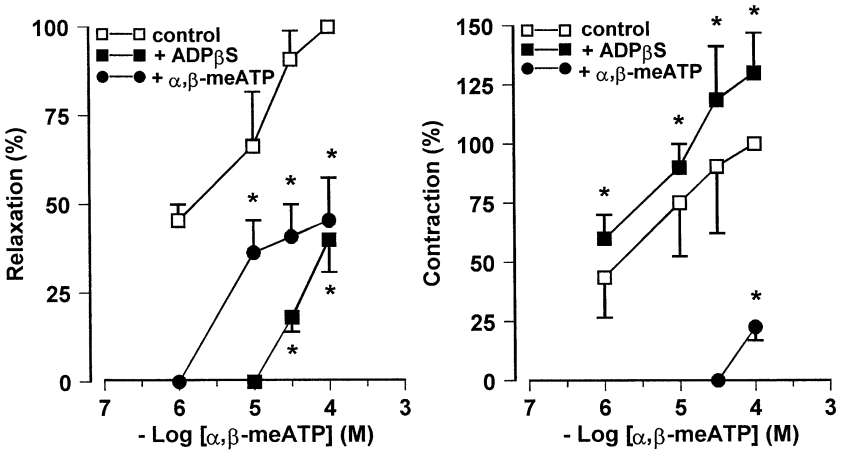

Fig. 4. Concentration–response curves for the relaxant and contractile effects evoked by α,β-methyleneATP (α,β-meATP) in mouse gastric preparations before and after desensitisation of P2Y receptors with adenosine-5′-O-(2-thiodiphoshate) (ADPβS) (10 μM for 30 min) or of P2X receptors with α,β-meATP (10 μM for 30 min). All values are means ± S.E.M. ($n=5$) and are reported as a percentage of the effect induced by 100 μM α,β-meATP. *$P<0.05$ vs. control.

3.4. Effects of TTX on the responses to ATP, α,β-MeATP and 2-MeSATP

TTX (1 μM), which per se did not affect the spontaneous mechanical activity, significantly enhanced the amplitude of the relaxation in response to ATP (100 μM). In addition, TTX increased the amplitude and the duration of relaxation evoked by α,β-MeATP (30 μM) or 2-MeSATP (10 μM). The contractile component evoked by α,β-MeATP (30 μM) was significantly antagonised by TTX (Fig. 6).

4. Discussion

The results of this study suggest that P2Y receptors, activated by ATP and 2-MeSATP, mediate muscular relaxation, while P2X receptors activated by ATP and α,β-MeATP, are both present in mouse gastric preparation, although with different location, the first subserving relaxation and the latter mediating contraction.

In our experiments, suramin, non-selective P2 antagonist, significantly antagonised the response to ATP, confirming the involvement of P2 receptors in the relaxant response evoked by ATP. In order to clarify the subtype of P2 receptors involved in the response, ATP was tested after incubation of the preparation with ADPβS or with α,β-MeATP, which have been used as specific desensitising agents to block P2Y- or P2X receptor-mediated effects (De Man et al., 2003; Serio et al., 2003; O'Connor et al., 1990; Ralevic and Burnstock, 1988). The results obtained suggest that P2Y and P2X receptors are both present in mouse gastric preparation. The relaxation produced by ATP was significantly reduced by P2Y receptor desensitising agent, ADPβS, indicating that P2Y receptors mediate relaxation. On the contrary, the desensitisation of P2X receptors with α,β-MeATP potentiated the relaxation evoked by ATP. This increase could be explained assuming that ATP act also on

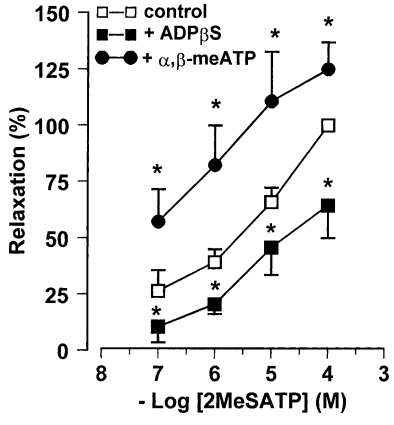

Fig. 5. Concentration–response curves for the relaxation induced by 2-methylthio-ATP (2-MeSATP) in mouse gastric preparations before and after desensitisation of P2Y receptors with adenosine-5′-O-(2-thiodiphoshate) (ADPβS) (10 μM for 30 min) or of P2X receptors with α,β-methyleneATP (α,β-meATP) (10 μM for 30 min). All values are means ± S.E.M. ($n=5$) and are reported as a percentage of the maximum effect induced by 100 μM 2-MeSATP. *$P<0.05$ vs. control.

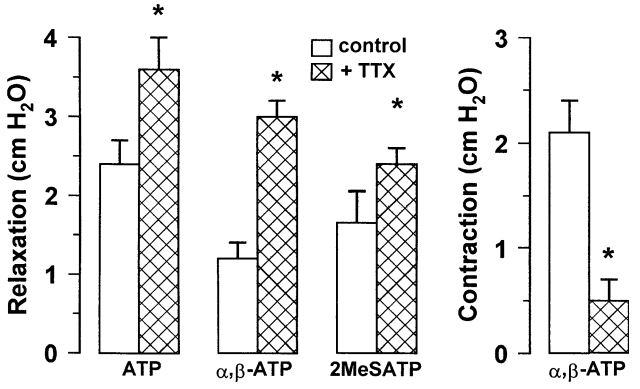

Fig. 6. Effects of tetrodotoxin (TTX) (1 μM) on the response evoked by ATP (100 μM), α,β-methyleneATP (α,β-ATP) (30 μM) and 2-methylthio-ATP (2-MeSATP) (10 μM) in the mouse gastric preparations. All values are means ± S.E.M. ($n=4$). *$P<0.05$ vs. control.

P2X purinoceptors, which mediate contraction and this would counteract its relaxant effects.

To support the hypothesis on the presence of P2X receptors mediating-contraction, we performed experiments using α,β-MeATP as P2X receptor agonist and 2-MeSATP as P2Y receptor agonist. α,β-MeATP had a biphasic effect characterised by an initial short-lasting relaxation followed by contraction. Biphasic responses to similar concentrations of α,β-MeATP have been reported in other intestinal preparations (Daniel, 1985; Storr et al., 2000), including mouse ileum (Vial and Evans, 2001). In our experiments, the observation that desensitisation of P2X receptors reduced the relaxation and almost abolished the contraction to α,β-MeATP suggests that α,β-MeATP activates two different types of receptors: one mediating inhibitory responses and one mediating excitatory responses. Moreover, the incubation of the preparation with ADPβS, which desensitises P2Y receptors, reduced the relaxation and increased the contraction to α,β-MeATP, suggesting that P2X are responsible for contraction, whereas the P2Y receptors might mediate the relaxation. A P2Y receptor agonist activity of α,β-MeATP has been showed in other intestinal preparations (Fredholm et al., 1994).

Moreover, the observation that 2-MeSATP-induced relaxation was antagonised by the desensitisation of P2Y receptors confirms that this subtype of purinoceptors mediates the relaxation. The response to 2-MeSATP was also potentiated by the desensitisation of P2X receptors. Therefore, it is likely that 2-MeSATP is able to interact also with the P2X purinoceptors mediating contraction.

Although the acute effects of the two ATP analogues showed limited selectivity, it is important to underline that desensitisation of α,β-MeATP-sensitive P2X receptors reduced the response to α,β-MeATP, but leaves the relaxant response to 2-MeSATP, while desensitisation of 2-MeSATP-sensitive P2Y receptors reduced the response to 2-MeSATP, leaving the contractile response to α,β-MeATP. Therefore, this would exclude possible cross-desensitisation between P2X and P2Y receptors after the respective desensitisation procedures.

Moreover, our experiments showed that in mouse gastric preparation, the purine muscular relaxant action due to activation of P2Y receptor is independent from neuronal mechanisms, being TTX-insensitive. On the contrary, the observation that contractile component evoked by α,β-MeATP was blocked by TTX suggests that P2X receptors are neuronal, localised at presynaptic level. In other preparations, the contraction evoked by α,β-MeATP is prevented by TTX or atropine, suggesting that it is due to acetylcholine released from cholinergic nerves (Matsuo et al., 1997; Moody and Burnstock, 1982; Sato et al., 1999; Sawyer et al., 2000). However, in mouse stomach, a similar mechanism can be ruled out because all the experiments were performed in the presence of atropine. Therefore, it remains to clarify which transmitter(s) is involved in the contractile response. This appears to be a peculiarity of the mouse gastrointestinal tract because α,β-MeATP-evoked contractions are reduced by TTX but are unaffected by atropine also in the small intestine (De Man et al., 2003; Vial and Evans, 2001). Our data seem in apparent contrast with those of Giaroni et al. (2002), which showed P2X mediating contraction only in mouse colonic smooth muscle. This discrepancy could be to the diverse experimental conditions. In fact, differently from us, they used gastric preparations pre-contracted by carbachol. The activation by carbachol of transduction mechanisms common to purinergic pathways might mask the contractile response to purines. Discrepancy of purinergic action on resting or precontracted tissue has been reported in intestinal preparation (Storr et al., 2000).

In conclusion, our data suggest that in mouse stomach, P2Y receptors activated by ATP and 2-MeSATP mediate muscular relaxation, while P2X receptors activated by ATP and α,β-MeATP mediate muscular contraction. P2X and P2Y receptors have a different localisation, P2X receptors being present at pre-synaptic level on excitatory neurones.

Acknowledgements

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