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Rise in cytosolic Ca^{2+} concentration induced by P_2-purinoceptor activation in isolated myocytes from the rat gastrointestinal tract

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1 The changes in the free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in response to agonists of P_2-purinoceptors were studied in myocytes isolated from the longitudinal muscle layer of different regions of the rat gastrointestinal tract (stomach, jejunum, ileum, caecum and colon). $[Ca^{2+}]_i$ was estimated by emission from the fluorescent dye, indo-1.

2 ATP and the P_{2Y}-purinoceptor agonist, 2-methylthio-ATP (2-MeSATP), transiently increased $[Ca^{2+}]_i$ in single myocytes from all segments of the gastrointestinal tract, whereas α,β-methylene-ATP, a P_{2X}-purinoceptor agonist, had no effect.

3 The rise in $[Ca^{2+}]_i$ induced by ATP and 2-MeSATP was maintained in Ca^{2+}-free solution but was abolished by depletion of the intracellular store with thapsigargin (1 μM).

4 Single myocytes from stomach, caecum and colon also responded to UTP by a transient increase in $[Ca^{2+}]_i$.

5 Individual myocytes responded to ATP, 2-MeSATP and UTP in a nearly all-or-nothing manner. The increasing of agonist concentration enhanced the number of responding cells but did not increase the amplitude of the $[Ca^{2+}]_i$ rise.

6 These results suggest that myocytes from the longitudinal layer of gastrointestinal muscle do not possess functional P_{2X}-purinoceptors and that agonists of P_{2Y} and P_{2U}-purinoceptors induced a rise in $[Ca^{2+}]_i$, probably via an all-or-nothing mobilization of Ca^{2+} from intracellular stores.

Keywords: Intestinal smooth muscle; P_2-purinoceptors; ATP; free cytosolic calcium

Introduction

The motility of gastrointestinal tract is ensured by the contractile activity of smooth muscle cells which is modulated by neurotransmitters released from autonomic nerves. In addition to well known transmitters, adenosine 5'-triphosphate (ATP) may play an important role in the neurogenic control of gastrointestinal motility. The most convincing evidence in support of such a role has been obtained from studies of autonomic neurotransmission that was resistant to antagonists of cholinergic or noradrenergic transmission. In a variety of smooth muscles, including gastrointestinal preparations (Westfall *et al.*, 1990), ATP has been shown to be co-released at nerve terminals as (1) ATP receptor antagonists or desensitizing ATP receptor agonists attenuate nerve stimulation-evoked responses in smooth muscle and (2) ATP receptor agonists mimicked the responses evoked by nerve stimulation in smooth muscle cells.

Exogenous ATP has been shown to contract smooth muscle from stomach, ileum or colon (Moody & Burnstock, 1982; Wiklund & Gustafsson, 1988; Kennedy & Humphrey, 1994; Bailey & Hourani, 1990), but relaxation induced by application of external ATP has also been reported in duodenum and stomach (Manzini *et al.*, 1985; Matharu & Hollingsworth, 1992). In considering these differences, it is important to note that the study of the responses to extracellular ATP is often complicated by (1) the presence of functionally distinct ATP receptor subtypes on the different cell types comprised in the tissue and (2) the rapid catabolism of extracellular ATP to produce adenosine and the subsequent stimulation of adenosine receptors. Thus, exposure of smooth muscle strips to extracellular ATP could produce complex biological responses that render difficult a clear identification of the ATP receptor subtypes located in the membrane of smooth muscle cells.

ATP receptors have been termed P_2-purinoceptors while adenosine receptors were called P_1-purinoceptors (Burnstock, 1978). P_2-purinoceptors are not homogeneous and are now subdivided into different subtypes, depending on the potencies of some analogues of ATP as agonists. In smooth muscles, P_{2X}, P_{2Y} and P_{2U}-purinoceptor subtypes have been identified (Fredholm *et al.*, 1994). At the P_{2X}-purinoceptor, the agonist potency order was originally defined as α,β-methylene-ATP (α,β-MeATP) > ATP > 2-methylthio-ATP (2-MeSATP) > > uridine 5'-triphosphate (UTP), while it is 2-MeSATP > > ATP > $\alpha\beta$-MeATP > > UTP at the P_{2Y}-purinoceptor. However, it has recently been shown that when agonist breakdown is prevented, a potency order of 2-MeSATP $\geqslant$ ATP > α,β-MeATP is seen at the P_{2X}-purinoceptor (Kennedy & Leff, 1995). At the P_{2U}-purinoceptor, the rank order of the agonist potency is UTP $\geqslant$ ATP > > 2-MeSATP = α,β-MeATP. The P_{2X}-purinoceptor belongs to the class of ion-gating P_2-purinoceptors, whereas the P_{2Y} and P_{2U}-purinoceptors are members of the G protein-linked P_2-purinoceptors.

The purpose of the present experiments was to identify the P_2-purinoceptor subtypes located in the membrane of gastrointestinal smooth muscle cells by measuring the changes in the free cytosolic calcium concentration ($[Ca^{2+}]_i$) induced by P_2-purinoceptor agonists in freshly isolated myocytes from the longitudinal muscle layer of different regions of rat gastrointestinal tract (stomach, jejunum, ileum, caecum and colon).

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Methods

Cell preparation

Wistar rats (150 g) were stunned and then killed by cervical dislocation. The longitudinal muscle layer of stomach, jejunum, ileum, caecum and colon segments was peeled from the underlying circular muscle in the physiological saline solution (PSS), (composition given below) and cut in small pieces. Smooth muscle cells were isolated from the tissues taken in the different regions of the gastrointestinal tract, following a protocol derived from that used by Pacaud & Bolton (1991). Fragments were washed for 10 min in Ca^{2+}-free phosphate-buffered saline (PBS), then incubated in PBS containing 1 mg ml^{-1} collagenase, 0.1 mg ml^{-1} pronase and 20 mg ml^{-1} bovine serum albumin at 37°C for 30 min. After this time, the solution was removed and the pieces of tissues were incubated again in a fresh enzyme solution at 37°C for 30 min. Tissues were then placed in enzyme-free solution and triturated using a fire polished Pasteur pipette to release cells. Cells were stored on glass cover-slips at 4°C in PSS containing 0.8 mM Ca^{2+} and used on the same day. About 80–90% of the cells excluded trypan blue, and contracted in response to various agonists (acetylcholine, ATP) and membrane depolarization.

Fluorescence measurement and estimation of $[Ca^{2+}]_i$

Changes in $[Ca^{2+}]_i$ were monitored fluorometrically by use of the Ca^{2+}-sensitive probe indo-1 as described previously (Pacaud *et al.*, 1991; 1993). Briefly, cells were loaded with indo-1 by incubation in PSS containing 1 μM indo-1 penta-acetoxymethyl ester (indo-1 AM) for 25 min at room temperature and then washed in PSS for 25 min. The coverslip with attached cells was then mounted in a perfusion chamber and continuously perfused. The recording system included a Nikon Diaphot inverted microscope fitted with epifluorescence (Nikon France, Charenton-le-pont, France). The cell studied was illuminated at 360 nm. Emitted light from a window slightly larger than the cell was counted simultaneously at 405 nm and 480 nm by two photomultipliers (P100, Nikon). Voltage signals at each wavelength were stored in an IBM-PC computer for subsequent analysis. The ratio (405/480) was calculated online and displayed with the two voltage signals on a monitor. $[Ca^{2+}]_i$ was estimated from the 405/480 ratio (Grynkiewicz *et al.*, 1985) using a calibration for indo-1 determined within cells (Pacaud *et al.*, 1991).

Solutions

The normal physiological saline solution (PSS) contained (in mM): NaCl 130, KCl 5.6, $MgCl_2$ 1, $CaCl_2$ 2, glucose 11, HEPES 10, pH 7.4 with NaOH. Ca^{2+}-free PSS was prepared by omitting $CaCl_2$ and by adding 0.5 mM ethyleneglycol-*bis*[β-aminoethyl ether] *N,N,N',N'*-tetraacetic acid (EGTA). ATP and the other purinoceptor agonists were applied to the recorded cell by pressure ejection from a glass pipette for the period indicated on the records. Repetitive stimulation caused a decrease in the amplitude of the response. Therefore, each record of $[Ca^{2+}]_i$ response to purinoceptor agonists was obtained from a different cell.

Activation of P_2-purinoceptors could produce membrane depolarization and thus, could indirectly activate Ca^{2+} entry through voltage-dependent Ca^{2+} channels. Therefore, all experiments were done at room temperature (20–22°C) and in the presence of 10 μM of the voltage-dependent Ca^{2+} channel blocker, gallopamil-hydrochloride (D600), to observe the changes in $[Ca^{2+}]_i$ directly activated by agonists of P_2-purinoceptors.

Chemicals and drugs

Collagenase was from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.). PBS was from Biochrom KG (Berlin, Germany). Pronase (type E), bovine serum albumin, ATP disodium salt, α,β-MeATP, UTP, D600, and thapsigargin were purchased from Sigma (Saint Quentin Fallavier, France). Indo-1 AM was from Calbiochem (France Biochem, Meudon, France). Caffeine was from Merck (Darmstadt, Germany) and 2-MeSATP was from Research Biochemical Incorporated (Natick, MA).

Statistical analysis

Results are expressed as the mean ± standard error of the mean (s.e.mean) with n the sample size. Significance was tested by means of Student's t test.

Results

Effects of P_2-purinoceptor agonists on $[Ca^{2+}]_i$ in ileal and jejunal myocytes

In all ileal myocytes tested, ATP (10 μM) transiently increased $[Ca^{2+}]_i$ from the resting level (139 ± 5 nM, n = 39) to 541 ± 82 nM (n = 7) (Figure 1). The peak of the response was obtained 2–4 s after the beginning of ATP ejection and ejections of ATP for durations comprised between 4 and 15 s produced similar responses. Therefore, ATP and the other P_2-purinoceptor agonists were applied for 6 s. Similarly, all the cells tested responded to 2-MeSATP (10 μM) by a transient rise in $[Ca^{2+}]_i$ to 475 ± 47 nM (n = 13), whereas α,β-MeATP (10 μM, n = 12) and UTP (10 μM, n = 9) were virtually inactive. The 2-MeSATP-induced $[Ca^{2+}]_i$ rise was still recorded in Ca^{2+}-free PSS but was completely abolished when intracellular Ca^{2+} store had been depleted by thapsigargin (1 μM) (Figure 2). The ATP-induced $[Ca^{2+}]_i$ rise as well as the small increase in $[Ca^{2+}]_i$ that could be observed upon application of α,β-MeATP displayed a similar behaviour (not shown). The concentration-dependency of 2-MeSATP and ATP on $[Ca^{2+}]_i$ in ileal myocytes is shown in Figure 3. Individual ileal cells responded to these agonists in a nearly all-or-nothing manner but, the number of responding cells increased with agonist concentration. For example, stimulation with 0.1 μM ATP produced a transient increase in $[Ca^{2+}]_i$ of maximal amplitude in only 2 out of 8 cells tested and there was no significant change in $[Ca^{2+}]_i$ in the remaining cells. Similar results were obtained for

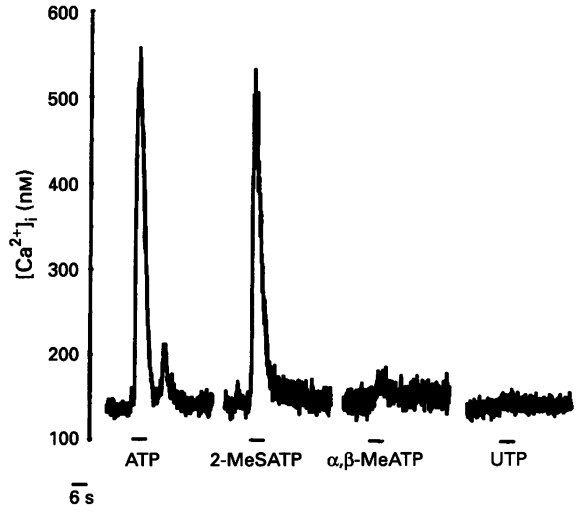

Figure 1 Effect of P_2-purinoceptor agonists (10 μM) in ileal myocytes. Under control conditions (PSS 2 mM Ca^{2+}, 10 μM D600), ATP and 2-methylthio-ATP (2-MeSATP) induced a transient rise in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) while α,β-methylene-ATP (α,β-MeATP) and UTP were virtually ineffective. Each response was obtained from a different cell.

the four agonists in jejunal myocytes. $[Ca^{2+}]_i$ was transiently increased to 560±64 nM ($n=10$) and 455±74 nM ($n=6$) upon application of 2-MeSATP (10 μM) and ATP (10 μM), respectively.

Effects of P_2-purinoceptor agonists on $[Ca^{2+}]_i$ in gastric myocytes

Stimulation of gastric smooth muscle cells with ATP (10 μM) or 2-MeSATP (10 μM) was efficient in each cell tested, producing a transient increase in $[Ca^{2+}]_i$ to 530±59 nM ($n=10$) and 440±30 nM ($n=10$), respectively (Figure 4). UTP seems to be less potent as only 4 out of 12 cells responded to 10 μM UTP with a transient increase in $[Ca^{2+}]_i$ to 405±29 nM ($n=4$). α,β-MeATP (10 μM) was ineffective ($n=10$). The peak $[Ca^{2+}]_i$ reached in response to 2-MeSATP and UTP was plotted against the log concentration of agonist showing that the amplitude of the responses was almost independent of the concentration (Figure 5).

Effects of P_2-purinoceptor agonists on $[Ca^{2+}]_i$ in myocytes isolated from the caecum

Transient increases in $[Ca^{2+}]_i$ to 561±54 nM ($n=10$ out of 10 cells tested), 502±46 nM ($n=11$ out of 11 cells tested) and 405±21 nM ($n=17$ out of 20 cells tested) were induced in smooth muscle cells from caecum stimulated with ATP (10 μM), 2-MeSATP (10 μM), and UTP (10 μM), respectively (Figure 6). In the half of the cells tested, α,β-MeATP (10 μM) induced a small and transient increase in $[Ca^{2+}]_i$ to 233±25 nM ($n=4$ out of 8 cells tested) that was not suppressed by removal of external Ca^{2+}. As shown in Figure 7, the amplitude of the peak $[Ca^{2+}]_i$ rise was not significantly different for concentrations of 2-MeSATP and UTP at 10^{-9} M to

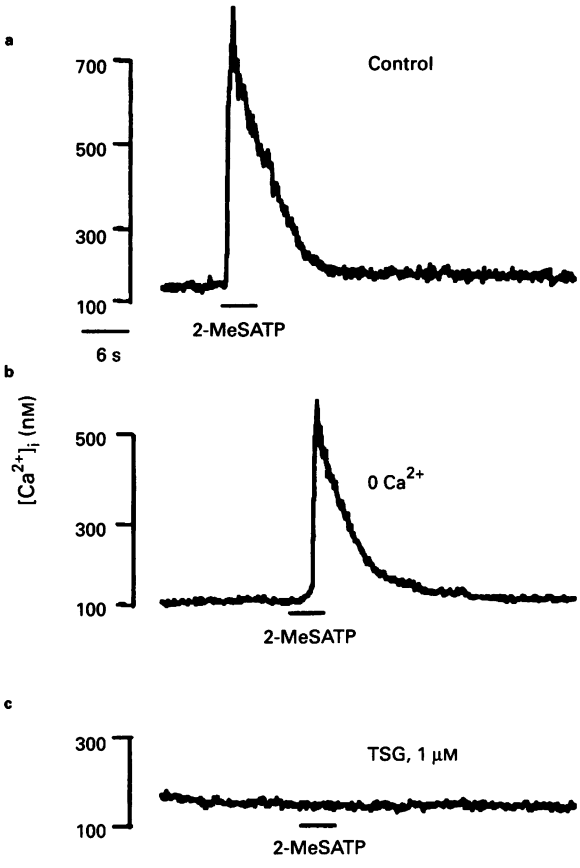

Figure 2 Characterization of the response induced by the P_{2Y}-purinoceptor agonist, 2-methylthio-ATP (2-MeSATP, 10 μM) in freshly isolated cells from ileum (3 different cells). 2-MeSATP induced a transient increase in $[Ca^{2+}]_i$ (a) which was not inhibited in Ca^{2+}-free PSS (b), but was completely suppressed after depletion of intracellular Ca^{2+} store by thapsigargin (TSG, 1 μM) (c).

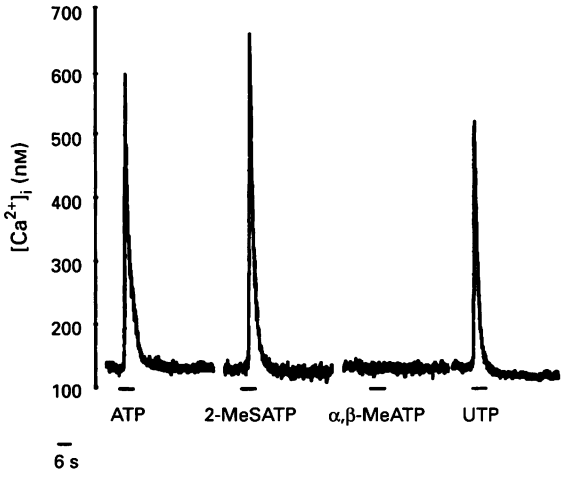

Figure 4 Effect of P_2-purinoceptor agonists (10 μM) in gastric myocytes (4 different cells). Under control conditions (PSS 2 mM Ca^{2+}, 10 μM D600), ATP, 2-MeSATP and UTP induced a transient rise in $[Ca^{2+}]_i$, while α,β-MeATP was ineffective.

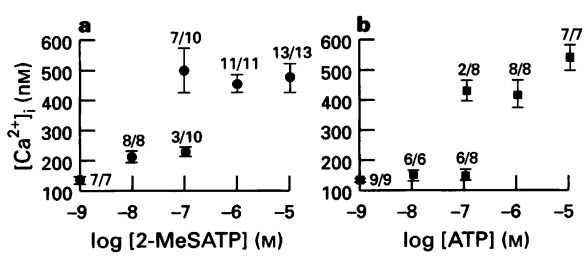

Figure 3 Concentration-dependence of the $[Ca^{2+}]_i$ rise induced by 2-MeSATP (●) and ATP (■) in freshly isolated cells from ileum. The peak of the agonist-induced $[Ca^{2+}]_i$ rise was plotted against agonist concentration. Each point represents a mean value±s.e.mean and the number of cells used to determine the mean value over the number of cells tested is indicated near the symbol. The number of responding cells increased with the agonist concentration, but not the amplitude of the response.

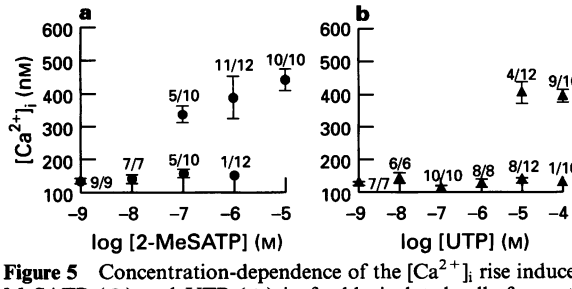

Figure 5 Concentration-dependence of the $[Ca^{2+}]_i$ rise induced by 2-MeSATP (●) and UTP (▲) in freshly isolated cells from stomach. The peak of the agonist-induced $[Ca^{2+}]_i$ rise was plotted against agonist concentration. Each point represents a mean value±s.e.mean and the number of cells used to determine the mean value over the number of cells tested is indicated near the symbol. The number of responding cells increased with the agonist concentration but the amplitude of the responses was almost independent of the concentration.

10^{-7} M, respectively. However, the number of responding cells increased with agonist concentration.

Effects of P_2-purinoceptor agonists on $[Ca^{2+}]_i$ in colonic myocytes

Application of ATP (10 μM) and 2-MeSATP (10 μM) transiently raised $[Ca^{2+}]_i$ to 605±75 nM ($n=4$ out of 4 cells tested) and 509±69 nM ($n=3$ out of 3 cells tested), respectively (Figure 8a). Stimulation with α,β-MeATP (10 μM) failed to increase $[Ca^{2+}]_i$ ($n=7$). There was no concentration-dependent increase in the peak $[Ca^{2+}]_i$ rise with concentrations of 2-MeSATP over 10^{-8} M, only the number of responding cells increased (Figure 8b). Colonic myocytes were insensitive to low concentrations of UTP (≤ 1 μM); however, an increase in $[Ca^{2+}]_i$ was observed in response to UTP (10 μM) in 2 out of 6 cells tested.

Discussion

More than two decade ago, ATP was reported as an inhibitory neurotransmitter in the gut muscle (Burnstock *et al.*, 1970). Nevertheless, due to the ease of its metabolism to adenosine and adenine nucleotides, and to the complexity of purinoceptors, its role still remains unclear. Indeed, both relaxation and contraction have been reported (Moody & Burnstock, 1982; Manzini *et al.*, 1985; Wiklund & Gustafsson, 1988; Bailey & Hourani, 1990; Satchell, 1990; Matharu & Hollingsworth, 1992; Kennedy & Humphrey, 1994). However, most studies were performed on gastrointestinal tract muscle strips which renders the interpretation of the results more complicated. We show that in single myocytes isolated from the longitudinal muscle layer of different regions of the gastrointestinal tract, ATP induced an increase in $[Ca^{2+}]_i$.

α,β-MeATP, 2-MeSATP and UTP, agonists of the P_{2X}, P_{2Y} and P_{2U}-purinoceptors respectively, have been used to identify the receptors involved in the action of ATP. P_{2X}-purinoceptors do not appear to be present in these cells. The small transient rise in $[Ca^{2+}]_i$ that could be recorded upon α,β-MeATP application was not modified by the removal of external Ca^{2+} but was suppressed after depletion of the intracellular Ca^{2+} store. This observation is not consistent with the involvement of the P_{2X}-purinoceptor which corresponds to a Ca^{2+}-permeable ligand-gated ion channel (Benham & Tsien, 1987; Valera *et al.*, 1994; Brake *et al.*, 1994), but is likely to be due to activation of P_{2Y}-purinoceptors. This result suggests that cells from the longitudinal layer of the different regions of the gastrointestinal tract tested do not possess functional P_{2X}-purinoceptors. In this way, intestinal myocytes appear to be very different from vascular myocytes as the presence of P_{2X}-purinoceptor has been described in myocytes from different vessels such as rabbit ear artery, rat aorta, rat portal vein and human saphenous vein (Benham & Tsien, 1987; Pacaud *et al.*, 1994; 1995; Loirand & Pacaud, 1995). The absence of a P_{2X}-purinoceptor-induced response in isolated myocytes is not consistent with previous reports suggesting that ATP and α,β-MeATP relax longitudinal muscle strips from gastric fundus (Matharu & Hollingsworth, 1992) and caecum (Wiklund & Gustafsson, 1988), possibly by involving P_{2X}-purinoceptors. On the contrary, our results are consistent with the data showing that the contraction of longitudinal muscle strips of ileum induced by α,β-MeATP involved P_{2X} or P_{2X}-like purinoceptors located at the cholinergic nerve terminals (Kennedy & Humphrey, 1994).

Unlike α,β-MeATP, 2-MeSATP induced a rise in $[Ca^{2+}]_i$ in isolated cells from the longitudinal muscle layer all along the gastrointestinal tract. Caecal and colonic myocytes were highly sensitive to 2-MeSATP as responses to 10^{-8} M were observed in 6 and 5 out of 10 cells, respectively. Cells from the other portions (ileum-jejunum and stomach) were approximately 10 times less sensitive. The $[Ca^{2+}]_i$ rise induced by 2-MeSATP was not suppressed in Ca^{2+}-free solution but was abolished by the depletion of intracellular Ca^{2+} store by thapsigargin. This re-

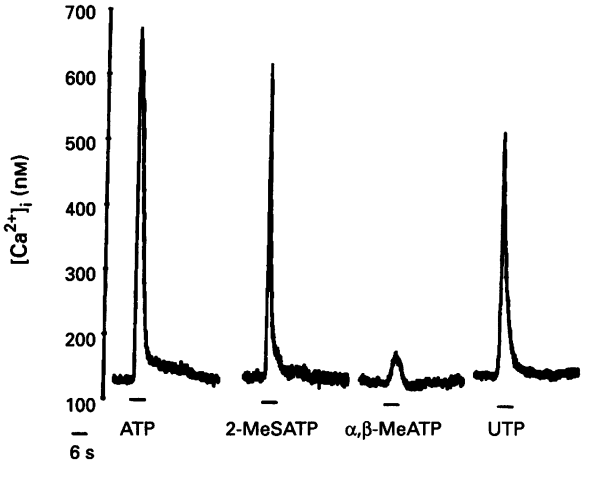

Figure 6 Effect of P_2-purinoceptor agonists (10 μM) in myocytes isolated from the caecum (4 different cells). Under control conditions (PSS 2 mM Ca^{2+}, 10 μM D600), ATP, 2-MeSATP and UTP induced a transient rise in $[Ca^{2+}]_i$. In 50% of the cells tested, α,β-MeATP induced a small increase in $[Ca^{2+}]_i$.

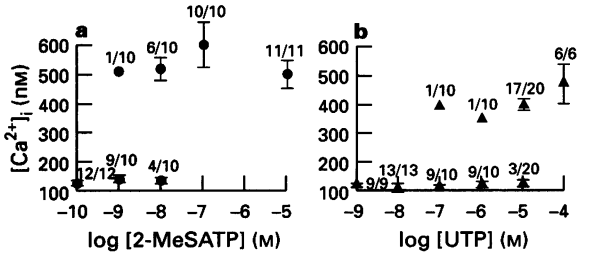

Figure 7 Concentration-dependence of the $[Ca^{2+}]_i$ rise induced by 2-MeSATP (●) and UTP (▲) in freshly isolated cells from the caecum. The peak of the agonist-induced $[Ca^{2+}]_i$ rise was plotted against concentration. Each point represents a mean value±s.e.mean and the number of cells used to determine the mean value over the number of cells tested is indicated near the symbol. The number of responding cells increased with the agonist concentration but not the amplitude of the responses.

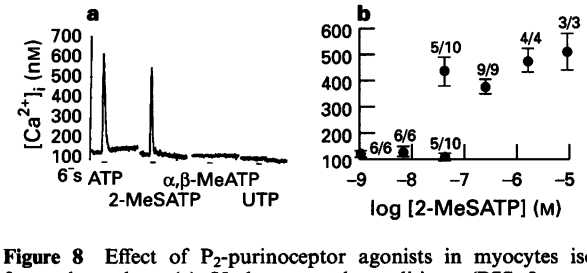

Figure 8 Effect of P_2-purinoceptor agonists in myocytes isolated from the colon. (a) Under control conditions (PSS 2 mM Ca^{2+}, 10 μM D600), ATP (10 μM) and 2-MeSATP (10 μM) induced a transient rise in $[Ca^{2+}]_i$, while α,β-MeATP (10 μM) and UTP (10 μM) were ineffective. Responses shown came from 4 different cells. (b) Concentration-dependence of the $[Ca^{2+}]_i$ rise induced by 2-MeSATP. The peak of the agonist-induced $[Ca^{2+}]_i$ rise was plotted against agonist concentration. Each point represents a mean value±s.e.mean and the number of cells used to determine the mean value over the number of cells tested is indicated near the symbol. The number of responding cells increased with the agonist concentration but not the amplitude of the responses.

sult indicates that 2-MeSATP-induced $[Ca^{2+}]_i$ rise was due to Ca^{2+} store release and was thus consistent with the activation of P_{2Y}-purinoceptors, known to be associated with the stimulation of phospholipase C activity, inositol 1,4,5-trisphosphate production and intracellular Ca^{2+} mobilization (Dubyak & El-Moatassim, 1993). It seems difficult to reconcile our results showing that the activation of P_{2Y}-purinoceptors leads to an increase in $[Ca^{2+}]_i$ in isolated intestinal myocytes with previous studies that ascribed the relaxant effect of ATP to the activation of P_{2Y}-purinoceptor in longitudinal muscle from duodenum (Furukawa & Nomoto, 1989; Nicholls *et al.*, 1990). However, we are working on single cells whereas segments were used in the other studies (Furukawa & Nomoto, 1989; Nicholls *et al.*, 1990). The presence of P_{2Y}-purinoceptors on plexus nerve may be responsible for this discrepancy. An alternative explanation would be that the rise in $[Ca^{2+}]_i$ induced by P_{2Y}-purinoceptors activation opens Ca^{2+}-dependent K^+ channel and thus, leads to relaxation.

Apart from myocytes from the ileum-jejunum segment, myocytes from other segments of the gut responded to application of UTP by a transient increase in $[Ca^{2+}]_i$. As (i) the agonist potency order at P_{2Y}-purinoceptors is 2-MeSATP > > ATP > α,β-MeATP > > UTP and (ii) α,β-MeATP is nearly ineffective, it seems that the rise in $[Ca^{2+}]_i$ induced by UTP was not due to activation of P_{2Y}-purinoceptors. This interpretation is also consistent with the absence of effect of UTP in myocytes from jejunum and ileum, although they are sensitive to 2-MeSATP. These results thus suggest the presence of functional P_{2U}-purinoceptors in the membrane of myocytes from stomach, caecum and colon. P_{2U}-purinoceptors are also coupled to phospholipase C activation, inositol 1,4,5-trisphosphate production and intracellular Ca^{2+} mobilization (Dubyak & El-Moatassim, 1993). However, cells were one hundred to one thousand times less sensitive to UTP than to 2-MeSATP (Figures 5 and 7), suggesting that the ATP-induced rise in $[Ca^{2+}]_i$ was mainly due to activation of P_{2Y}-purinoceptors. Recently, UTP has been shown to induce contraction in the rat duodenum, but the effective concentrations were high as 55 μM UTP was necessary to obtain 50% of the maximum response to 0.1 μM carbachol (Johnson & Hourani, 1994). Furthermore, it is not absolutely certain that this effect was mediated via activation of a receptor by UTP in the membrane of duodenal myocytes.

Our results also show that there was no concentration-dependent increase in the amplitude of the $[Ca^{2+}]_i$ rise evoked by P_2-purinoceptor agonists in single myocytes from the longitudinal layer of the gastrointestinal muscle, but the threshold concentration varied from the cell to cell. Therefore increase in the agonist concentration enhanced the number of responding cells. Similarly, it has been recently reported that the carbachol-induced $[Ca^{2+}]_i$ rise and membrane current take place in an all-or-nothing fashion in single intestinal myocytes (Ohta *et al.*, 1994). This behaviour may be explained by the regenerative all-or-nothing Ca^{2+} release induced by inositol 1,4,5-trisphosphate and ascribed to the Ca^{2+}-dependent feedback control of the inositol 1,4,5-trisphosphate receptor channel (Iino *et al.*, 1993).

As a conclusion, we found that agonists of P_{2Y} and occasionally P_{2U}-purinoceptors induced a rise in $[Ca^{2+}]_i$ in rat myocytes from the longitudinal muscle layer of the gastrointestinal tract. These effects were caused by an all-or-nothing mobilization of Ca^{2+} from intracellular stores. Our study will facilitate the understanding of purinoceptor-mediated physiological mechanisms; however, a similar study remains to be performed on circular smooth muscle cells.

References

BAILEY, S.J. & HOURANI, S.M.O. (1990). A study of purinoceptors mediating contraction in the rat colon. *Br. J. Pharmacol.*, **100**, 753–756.

BENHAM, C.D. & TSIEN, R.W. (1987). A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature*, **328**, 275–278.

BRAKE, A.D., WAGENBACH, M.D. & JULIUS, D. (1994). New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. *Nature*, **371**, 519–523.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: a Multidisciplinary Approach.* ed. Straub, R.W. & Bolis, L. pp. 107–118. New York: Raven.

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D. & SMYTHE, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br. J. Pharmacol.*, **40**, 668–688.

DUBYAK, G.R. & EL-MOATASSIM, C. (1993). Signal transduction via P_2-purinergic receptors for extracellular ATP and other nucleotides. *Am. J. Physiol.*, **265**, C577–C606.

FREDHOLM, B.B., ABBRACHIO, M.P., BURNSTOCK, G., DALY, J.W., HARDEN, T.K., JACOBSON, K.A., LEFF, P. & WILLIAMS, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.*, **46**, 143–156.

FURUKAWA, K. & NOMOTO, T. (1989). Postnatal changes in response to adenosine and adenine nucleotides in rat duodenum. *Br. J. Pharmacol.*, **97**, 1111–1118.

GRYNKIEWICZ, G., POENIE, M. & TSIEN, R.Y. (1985). A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.*, **260**, 3440–3450.

IINO, M., YAMAZAWA, T., MIYASHITA, Y., ENDO, M. & KASAI, H. (1993). Critical intracellular Ca^{2+} concentration for all-or-none Ca^{2+} spiking in single smooth muscle cells. *EMBO J.*, **12**, 5287–5291.

JOHNSON, C.R. & HOURANI, S.M.O. (1994). Contractile effects of uridine 5′-triphosphate in the rat duodenum. *Br. J. Pharmacol.*, **113**, 1191–1196.

KENNEDY, I. & HUMPHREY, P.P.A. (1994). Evidence for the presence of two types of P_2 purinoceptor in the guinea-pig ileal longitudinal smooth muscle preparation. *Eur. J. Pharmacol.*, **261**, 273–280.

KENNEDY, I. & LEFF, P. (1995). How should P_{2X}-purinoceptors be classified pharmacologically? *Trends Pharmacol. Sci.*, **16**, 168–174.

LOIRAND, G. & PACAUD, P. (1995). Mechanism of ATP-induced rise in cytosolic Ca^{2+} in freshly isolated smooth muscle cells from human saphenous vein. *Pflügers Arch.*, **430**, 429–436.

MANZINI, S., MAGGI, C.A. & MELI, A. (1985). Further evidence for involvement of adenosine 5′ triphosphate in non-adrenergic non-cholinergic relaxation of the isolated duodenum. *Eur. J. Pharmacol.*, **113**, 399–408.

MATHARU, S.M. & HOLLINGSWORTH, M. (1992). Purinoceptors mediating relaxation and spasm in the rat gastric fundus. *Br. J. Pharmacol.*, **106**, 395–403.

MOODY, C.J. & BURNSTOCK, G. (1982). Evidence for the presence of P_1-purinoceptors on cholinergic terminals in the guinea-pig ileum. *Eur. J. Pharmacol.*, **77**, 1–9.

NICHOLLS, J., HOURANI, S.M.O. & KITCHEN, I. (1990). The ontogeny of purinoceptors in rat urinary bladder and duodenum. *Br. J. Pharmacol.*, **100**, 874–878.

OHTA, T., ITO, S. & NAKAZATO, Y. (1994). All-or-nothing responses to carbachol in single intestinal smooth muscle cells of rat. *Br. J. Pharmacol.*, **112**, 972–976.

PACAUD, P. & BOLTON, T.B. (1991). Relation between muscarinic receptor cationic current and internal calcium in guinea-pig jejunal smooth muscle cells. *J. Physiol.*, **441**, 477–499.

PACAUD, P., GREGOIRE, G. & LOIRAND, G. (1994). ATP-induced Ca^{2+} entry releases Ca^{2+} from intracellular store in smooth muscle cells of rat portal vein. *Br. J. Pharmacol.*, **113**, 457–462.

PACAUD, P., LOIRAND, G., BARON, A., MIRONNEAU, C. & MIRONNEAU, J. (1991). Ca^{2+} channel activation and membrane depolarization mediated by Cl^- channels in response to noradrenaline in vascular myocytes. *Br. J. Pharmacol.*, **104**, 1000–1006.

PACAUD, P., LOIRAND, G., GREGOIRE, G., MIRONNEAU, C. & MIRONNEAU, J. (1993). Noradrenaline-activated heparin-sensitive Ca^{2+} entry after depletion of intracellular Ca^{2+} store in portal vein smooth muscle cells. *J. Biol. Chem.*, **268**, 3866–3872.

PACAUD, P., MALAM-SOULEY, R., LOIRAND, G. & DESGRANGES, C. (1995). ATP raises $[Ca^{2+}]_i$ via different P_2-receptor subtypes in freshly isolated and cultured aortic myocytes. *Am. J. Physiol.*, **269**, H30–H36.

SATCHELL, D. (1990). The effects of ATP and related nucleotides on visceral smooth muscle. *Ann. N.Y. Acad. Sci.*, **603**, 53–63.

VALERA, S., HUSSY, N., EVANS, R.J., ADAMI, N., NORTH, R.A., SURPRENANT, A. & BUELL, G. (1994). A new class of ligand-gated ion channel defined by P_{2X} receptor for extracellular ATP. *Nature*, **371**, 516–519.

WESTFALL, D.P., SEDAA, K.O., SHINOZUKA, K., BJUR, R.A. & BUXTON, I.L. (1990). ATP as a cotransmitter. *Ann. N.Y. Acad. Sci.*, **603**, 300–310.

WIKLUND, N.P. & GUSTAFSSON, L.E. (1988). Indications for P_2-purinoceptor subtypes in guinea-pig smooth muscle. *Eur. J. Pharmacol.*, **148**, 361–270.

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