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Ontogeny of P2-purinoceptors in the longitudinal muscle and muscularis mucosae of the rat isolated duodenum

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1 The ontogeny of P2-purinoceptors in the longitudinal muscle and the muscularis mucosae of the rat isolated duodenum was investigated by use of functional assays in tissues from neonatal animals. The degradation of purinoceptor agonists by the rat duodenum muscularis mucosae was also investigated.

2 In the rat duodenum muscularis mucosae adenosine 5′-(α,β-methylene)triphosphonate (AMPCPP), adenosine 5′-triphosphate (ATP), uridine 5′-triphosphate (UTP) and 2-methylthioadenosine 5′-triphosphate (2-Me-S-ATP) all caused a contraction from day 10 to day 40, day 10 being the earliest age it could be tested. The potency order of agonists above day 25 was AMPCPP > ATP = UTP > 2-Me-S-ATP and this is similar to the potency order previously obtained for the adult tissue. However, in the neonatal tissues below day 20, 2-Me-S-ATP was the most potent agonist and at days 10 and 15 the order was 2-Me-S-ATP > AMPCPP > ATP = UTP.

3 In the rat duodenum muscularis mucosae desensitization was observed with AMPCPP at day 30 but not at day 15. At day 30, cross-desensitization was also observed between AMPCPP and 2-Me-S-ATP but not between AMPCPP and ATP or UTP, whereas no cross-desensitization was observed at day 15 with AMPCPP and any of the agonists. At day 15 and below AMPCPP and 2-Me-S-ATP may therefore both activate P2Y-receptors (2-Me-S-ATP > AMPCPP, no desensitization with AMPCPP) whereas above day 20 the agonists activate P2X-receptors (AMPCPP > 2-Me-S-ATP, desensitization with AMPCPP) which is similar to the adult tissue. Since ATP and UTP were equipotent in the muscularis mucosae and as no cross-desensitization was observed with AMPCPP and UTP or ATP at days 15 or 30, it is likely that ATP and UTP both activate P2U-receptors throughout the ages, as in the adult.

4 The potency of all the agonists in causing contraction in the rat duodenum muscularis mucosae decreased with age. The potency of AMPCPP and 2-Me-S-ATP in causing contractions was highest in the neonates before day 25, and reached values not significantly different from adult by day 30, and the potency of ATP and UTP causing contractions in this tissue was also highest in the neonates at days 10 and 15, and reached values not significantly different from adult by day 20. This suggests either that the receptor populations mediating contraction are highest in the neonates below day 20 or that the agonists are degraded by the muscularis mucosae to a greater extent after day 20.

5 In the rat duodenum muscularis mucosae the degradation of ATP, UTP, 2-Me-S-ATP and AMPCPP was followed by high pressure liquid chromatography at days 15 and 30. ATP was degraded to adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP) and inosine with no adenosine being detected, 2-Me-S-ATP was degraded to 2-methylthioadenosine 5′-diphosphate (2-Me-S-ADP), 2-methylthioadenosine 5′-monophosphate (2-Me-S-AMP) and 2-methylthioadenosine (2-Me-S-adenosine), and UTP was degraded to uridine 5′-diphosphate (UDP), uridine 5′-monophosphate (UMP) and uridine. The rate of degradation of these agonists was much faster at day 30 than at day 15, probably due to the increase in the size of the tissue. AMPCPP was also degraded with adenosine 5′-(α,β-methylene)diphosphonate (AMPCP) being detected at both ages. However, at day 30 the rate of degradation of AMPCPP was much slower than for ATP, UTP or 2-Me-S-ATP.

6 In the rat duodenum longitudinal muscle 2-Me-S-ATP and AMPCPP both caused a relaxation with a potency order of 2-Me-S-ATP > AMPCPP, suggesting the activation of P2Y-receptors, as previously found for the adult tissue. Weak relaxations were observed to both the agonists at day 15 (the earliest age it could be studied), and the potency of the agonists reached values not significantly different from adult tissues by day 25.

7 Overall, these results suggest that in the neonatal rat duodenum longitudinal muscle there are P2Y-receptors mediating relaxation and that the receptor population is fully developed by day 25. In the neonatal rat duodenum muscularis mucosae before day 20 there are P2Y-receptors mediating contraction, while after day 20 P2X-receptors mediate this effect. P2U-receptors also mediate contraction throughout the ages. The results also indicate that the ectonucleotidase activity in the rat duodenum muscularis mucosae increases with age, and the potency of agonists in the rat duodenum may therefore reflect both the number and nature of the receptors involved and the activity of the ectonucleotidases in the tissue.

Keywords: Rat duodenum longitudinal muscle; rat duodenum muscularis mucosae; P2-purinoceptors; development; ontogeny; ATP; UTP; ectonucleotidases

Introduction

Pharmacological actions of adenosine and adenosine 5′-triphosphate (ATP) on smooth muscle are mediated via specific receptors known as P1- and P2-purinoceptors, respectively. P1-purinoceptors have been sub-divided into A_1, A_{2a}, A_{2b} and

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A_3 and P2-purinoceptors have been sub-divided on the basis of functional studies into a number of subtypes including P2X, P2Y and P2U (for reviews see Collis & Hourani, 1993; Fredholm *et al*., 1994). The potency order of agonists at the P2X subtype in causing contraction in smooth muscle preparations is adenosine 5′-(α,β-methylene)triphosphonate (AMPCPP) > ATP = 2-methylthioadenosine 5′-triphosphate (2-Me-S-ATP) (Burnstock & Kennedy, 1985). However, recent cloning studies have shown that there are several subtypes of P2X-purinoceptor, and that the smooth muscle type $P2X_1$-receptor (cloned from the rat vas deferens) has a potency order of 2-Me-S-ATP > ATP > AMPCPP (for review see Burnstock & King, 1996). The difference in the potency orders between the cloned receptor and the smooth muscle preparations has been attributed to the greater influence in smooth muscle of the breakdown of agonists by ectonucleotidases (see Kennedy & Leff, 1995; Surprenant *et al*., 1995). Analogues of ATP with substitutions in the triphosphate chain, such as AMPCPP, are much more resistant to ectonucleotidases than is ATP itself or analogues substituted on the purine ring, such as 2-Me-S-ATP, and at P2X-purinoceptors in smooth muscle the apparent potency of ATP and 2-Me-S-ATP is therefore reduced (Welford *et al*., 1986; 1987). The potency order of agonists at the P2Y subtype in smooth muscle is 2-Me-S-ATP > ATP > AMPCPP (Burnstock & Kennedy, 1985), whereas at the P2U subtype the pyrimidine nucleotide uridine 5′-triphosphate (UTP) is equipotent with ATP with a potency order of UTP = ATP >> 2-Me-S-ATP = AMPCPP (O'Connor *et al*., 1991; Dubyak & El-Moatassim, 1993). Although P2Y receptors are generally associated with relaxation of smooth muscle, in some tissues such as the rat colon muscularis mucosae they cause contraction (Bailey & Hourani, 1990).

As there are no selective potent antagonists at the different subtypes of P2-purinoceptors, desensitization has been used to discriminate between them. P2X-receptors can be readily desensitized to AMPCPP and this is characteristic of this subtype (Burnstock & Kennedy, 1985), whereas P2Y or P2U receptors do not generally show marked desensitization to agonists. However, in the rat colon muscularis mucosae desensitization has been observed to 2-Me-S-ATP acting at P2Y-receptors and in bovine aortic endothelial cells responses to UTP acting via P2U-receptors show desensitization (Hourani *et al*., 1993; Wilkinson *et al*., 1994).

With the recent cloning of members in the P2-purinoceptor family a revision of the current nomenclature has been made with all P2-purinoceptors now being assigned to either the G protein coupled P2Y family (which includes the P2U-purinoceptor) or the ion channel P2X family (see Burnstock & King, 1996). However, in this paper the current IUPHAR recommendations for purinoceptor nomenclature will be used (Fredholm *et al*., 1994); these account adequately for the observed potency orders in smooth muscle preparations where, in any case, it is not usually known which of the many cloned subtypes are present and responsible for the functional responses.

The location of P1- and P2-purinoceptors in the separated layers of the duodenum from adult rats has been shown in our previous studies. In the longitudinal muscle there are A_1-, A_{2b}- and P2Y-receptors all mediating relaxation and in the muscularis mucosae there are A_{2b}-, P2X- and P2U-receptors all mediating contraction (Nicholls *et al*., 1996; Johnson *et al*., 1996). We have also followed the development of P1-purinoceptors in the separated layers of the rat duodenum by use of functional studies and (for the A_1 receptor) radioligand binding studies. Here the relaxant A_1-receptor on the longitudinal muscle was only present and functional after day 20, whereas A_{2b}-receptors both on the muscularis mucosae (causing contraction) and on the longitudinal muscle (causing relaxation) were functional as early as they could be detected (days 10 and 15, respectively) (Brownhill *et al*., 1996; Peachey *et al*., 1996). The aim of the present study was to investigate the development of P2-purinoceptors in the separated layers of the rat duodenum to determine which subtypes of P2-purinoceptors are present and functional in neonatal tissues and whether they develop at different times. ATP has been shown to be rapidly degraded by the adult rat duodenum muscularis mucosae (Johnson *et al*., 1996), and as discussed earlier the presence of ectonucleotidases may complicate the interpretation of potency orders. Where appropriate the activity of the ectonucleotidases in the neonatal tissues was therefore also investigated.

Methods

Pharmacological studies

Adult male Wistar rats (>60 day) and male neonatal rats 10, 15, 20, 25, 30, or 40 days old were killed by cervical dislocation. The day of birth was designated as day 1, and animals were culled to litters of ten rats per mother to maintain a standard litter size. Neonatal rats were weaned at day 20.

The duodenum was dissected out by cutting at the base of the pyloris and a length of 1.5 cm (adult) or 1.0 cm (neonates) was used, cleared of any connective tissue and placed over a glass rod. The longitudinal muscle was removed by making a longitudinal cut and gently rubbing the layer with moist cotton wool, and the remaining thick walled tube contained the muscularis mucosae. The wet weights of the longitudinal muscle in mg at the various ages were as follows: 15 days, 7.5 ± 0.1; 20 days, 9.5 ± 0.2; 25 days, 11.5 ± 1.0; 30 days, 15.1 ± 2.0; 40 days, 20.0 ± 2.0; adult, 35.0 ± 3.5, and the wet weights of the muscularis mucosae in mg at the various ages were as follows: 10 days, 7.5 ± 2; 15 days, 23 ± 2; 20 days, 36 ± 3; 25 days, 68 ± 4; 30 days, 101 ± 6; 40 days, 150 ± 7; adult 237 ± 10. The tissues were mounted in 4 ml organ baths containing Krebs of the following composition (mM): $MgSO_4$ 1.2, NaCl 118, $NaHCO_3$ 25, KH_2PO_4 1.2, KCl 4.8, $CaCl_2$ 2.5 and glucose 11, gassed with 95% O_2:5% CO_2, and maintained at 35–36°C. A resting tension of 1 g (adult), 0.5 g (20–40 days) or 0.3 g (10–15 days) was applied to the tissues, and responses were measured isometrically with a Grass FT03 transducer and recorded on a Grass model 79D polygraph after incubation for 60 min. Concentration-response curves were obtained non-cumulatively, with a 10 min dose cycle. Responses of the longitudinal muscle were quantified by precontracting the tissue with carbachol (0.3 μM) before challenge with the drugs and the relaxations are expressed as % reduction of the carbachol contraction. The dose of carbachol (0.3 μM) was submaximal giving 70% of the maximum contraction in the neonates (day 20) and in the adult. Contractions to carbachol were measured from the top of the spontaneous activity to the highest point of the carbachol contraction and relaxations were measured as a reduction in the height of the rhythmic contraction induced by carbachol. Responses of the muscularis mucosae were quantified by adding the drug directly to the bath and contractions are expressed as % of contraction induced by KCl (35 mM).

In the muscularis mucosae AMPCPP was used in an attempt to desensitize the receptors. A dose of 100 μM AMPCPP was added, left in contact with the tissue for 10 min, and the dose was repeated 5 min after washout at which time the tension had returned to baseline. Contractions are expressed as % of the response obtained to the initial dose of AMPCPP. Statistical analysis was carried out by one-way ANOVA followed by Dunnett's two-tailed *post-hoc* test. Cross-desensitization between AMPCPP and 2-Me-S-ATP, ATP or UTP was also investigated. A dose of 2-Me-S-ATP (100 or 3 μM), ATP (100 or 10 μM) or UTP (100 or 10 μM) (control response) was first administered followed by the desensitizing dose of AMPCPP (100 μM) for 10 min, after which the tissue was challenged with AMPCPP (100 μM) and the test agonists 2-Me-S-ATP, ATP or UTP, and contractions are expressed as % of the control response. For cross-desensitization studies the results were compared by use of Student's *t* test.

The potency of agonists in causing relaxations in the longitudinal muscle was expressed as the negative $\log_{10}$ of the molar concentration of the agonist producing 30% reduction of carbachol contraction (pEC_{30}), calculated by regression analysis of the individual log concentration-response curves. The potency of agonists causing contractions in the muscularis mucosae was expressed as the negative $\log_{10}$ of the molar concentration of the agonist producing 60% of the KCl response (pEC_{60}) calculated as before. For 2-Me-S-ATP, which did not reach 60% contraction in the muscularis mucosae at days 30, 40 and adult, pEC_{60} values were calculated by extrapolation of the individual log concentration-response curves. In each case pEC_{30} or pEC_{60} values were used as these values were appropriate to make a comparison of the potency of agonists with age. Comparison of results across the ages was carried out by one-way ANOVA followed by Duncan's New Multiple Range *post-hoc* test.

Degradation studies in the rat duodenum muscularis mucosae

The degradation of ATP, UTP, 2-Me-S-ATP and AMPCPP by the muscularis mucosae from animals aged day 15 and 30 was followed by high pressure liquid chromatography (h.p.l.c.) as previously described for the adult tissue (Johnson *et al*., 1996), but using a Shimadzu LC-10A h.p.l.c. system. Briefly, tissues were mounted in 4 ml organ baths as in functional studies, 100 μM ATP, UTP, AMPCPP and 2-Me-S-ATP were added individually to the baths, 100 μl aliquots were taken at 0, 2, 5, 10, 15, 20 min, stored at -20°C and analysed by h.p.l.c. at a later date. To compare the rate of degradation of the agonists, a half-life was calculated for each tissue from a semi-log plot of $\log_{10}$ concentration of agonist versus time, extrapolating if necessary. Statistical analysis was carried out by use of Student's *t* test (for comparison of half lives for agonists between the two ages) or a one-way ANOVA followed by Duncan's New Multiple Range *post-hoc* test (for comparison of half lives across the agonists).

Materials

2-Me-S-ATP was obtained from Research Biochemicals Inc. (Natick, MA, U.S.A.) and all other drugs were obtained from Sigma Chemical Co. (Poole, U.K.). The buffer salts were of analytical or h.p.l.c. grade and were obtained from BDH (Poole, U.K.).

Results

Functional studies on the rat duodenum longitudinal muscle

2-Me-S-ATP and AMPCPP both relaxed the carbachol contracted tissue from day 15 onwards (Figure 1), the earliest age which could be tested due to the fragility of the tissue. Weak relaxations were observed to both the agonists at day 15, but only at high concentrations of agonists (>3 μM) (Figure 1). In most cases a maximal response to the agonists could not be achieved, but the concentration-response curves to 2-Me-S-ATP were generally less steep than those for AMPCPP in the neonates (Figure 1), as previously observed in the adult tissue (Johnson *et al*., 1996). The potency of 2-Me-S-ATP and AMPCPP in causing relaxations (expressed as pEC_{30}) increased with an increase in age, being lowest at day 15 and reaching values not significantly different from adult by day 25 ($P>0.05$) (Figure 2a). The potency order of agonists was 2-Me-S-ATP > AMPCPP at all ages except at day 15 when the agonists were equipotent (Table 1).

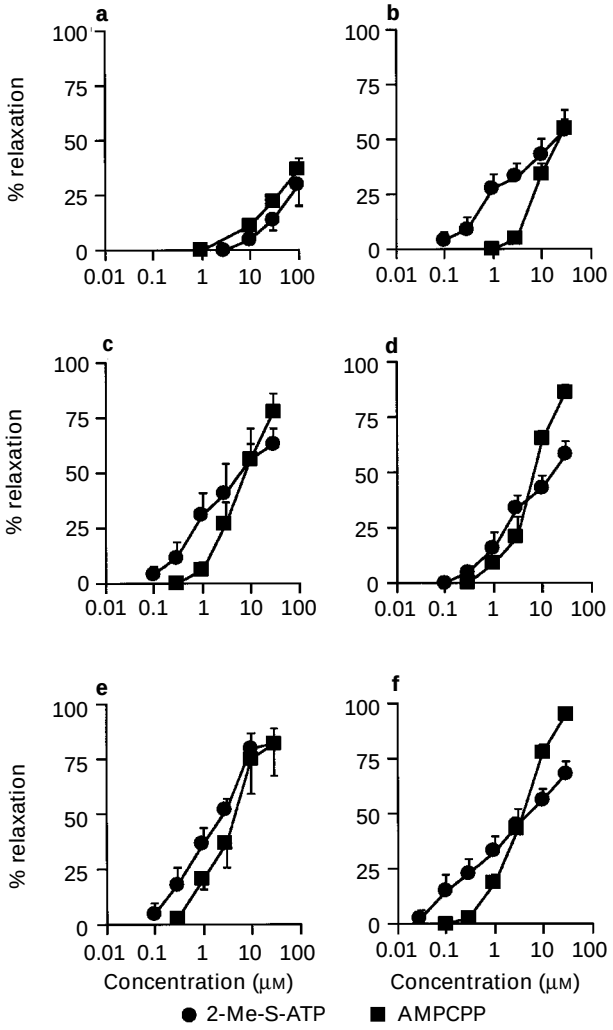


Figure 1 Relaxant responses induced by AMPCPP and 2-Me-S-ATP in the rat duodenum longitudinal muscle at various ages; (a) day 15, (b) day 20, (c) day 25, (d) day 30, (e) day 40 and (f) adult (>60 day). Each point is the mean of at least four determinations and the vertical lines show s.e.mean where larger than the symbol. For abbreviations see text.

Functional studies on the rat duodenum muscularis mucosae

Contractions to AMPCPP, 2-Me-S-ATP, ATP and UTP were observed in the rat duodenum muscularis mucosae from day 10 onwards (Figures 3 and 4), the earliest age which could be tested due to the fragility of the tissue. The potency of ATP and UTP in causing contractions (expressed as pEC_{60}) decreased with age, being highest at days 10 and 15 and reaching values not significantly different from adult by day 20 ($P>0.05$) (Figure 2b). The potency of AMPCPP and 2-Me-S-ATP in causing contractions (expressed as pEC_{60}) also decreased with age being highest between days 10–25 and reaching values not significantly different from adult by day 30 ($P>0.05$) (Figure 2c). At days 10 and 15 2-Me-S-ATP was more potent than AMPCPP, but this order of potency was reversed from day 20. UTP and ATP were equipotent throughout the ages. The pEC_{60} values for the agonists and the potency orders at all the ages are summarized in Table 2. Repeated administrations of AMPCPP (100 μM) did not desensitize the response to AMPCPP at day 15. However, a significant reduction of the initial response to AMPCPP was observed at day 30 ($P<0.05$) (Figure 5a). Cross-desensitization between 100 μM AMPCPP and 2-Me-S-ATP (3 μM), ATP (10 μM) or UTP (10 μM) was not observed at day 15 (Figure 5b), whereas at day 30 a significant reduction of the control response to 100 μM 2-Me-S-ATP was observed ($P<0.05$) while

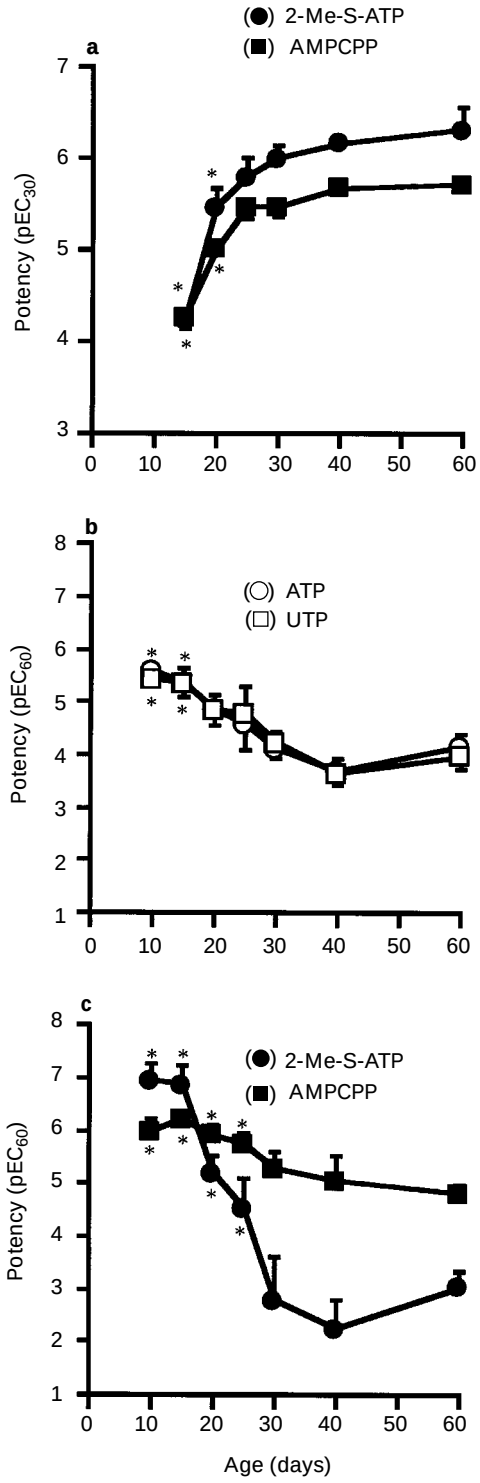


Figure 2 Variation in potency of (a) AMPCPP and 2-Me-S-ATP in causing relaxations in the rat duodenum longitudinal muscle, (b) UTP and ATP in causing contractions in the rat duodenum muscularis mucosae and (c) AMPCPP and 2-Me-S-ATP in causing contractions in the rat duodenum muscularis mucosae. The potency of 2-Me-S-ATP and AMPCPP in causing relaxations in the longitudinal muscle was expressed as the negative $\log_{10}$ of the molar concentration of the agonist producing 30% reduction of contractions induced by carbachol (0.3 μM) (pEC_{30}). The potency of 2-Me-S-ATP, AMPCPP, ATP and UTP in causing contractions in the muscularis mucosae was expressed as the negative $\log_{10}$ of the molar concentration of the agonist producing 60% of the contractions induced by KCl (35 mM) (pEC_{60}). Each point is the mean of at least four determinations and the vertical lines show s.e.mean where larger than the symbol. $^*P<0.05$ significantly different from adult values (ANOVA followed by Duncan's New Multiple Range *post-hoc* test). For abbreviations see text.

contractions to 100 μM ATP and UTP were unaffected ($P>0.05$) (Figure 5c).

Degradation studies on the rat duodenum muscularis mucosae

ATP (100 μM) was rapidly degraded by the muscularis mucosae to adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP) and inosine with no adenosine being

Table 1 Potencies of agonists (pEC_{30}) in causing relaxation of the rat duodenum longitudinal muscle at various ages

Age (days)	*Potency* (pEC_{30} values) *2-Me-S-ATP*	*AMPCPP*
15	4.2±0.1	4.3±0.1
20	5.5±0.2	5.0±0.1
25	5.8±0.2	5.5±0.1
30	6.0±0.2	5.5±0.1
40	6.2±0.2	5.7±0.1
Adult (>60)	6.3±0.2	5.7±0.1

Data are the mean and s.e.mean of the pEC_{30} values ($n=4$). For abbreviations see text.

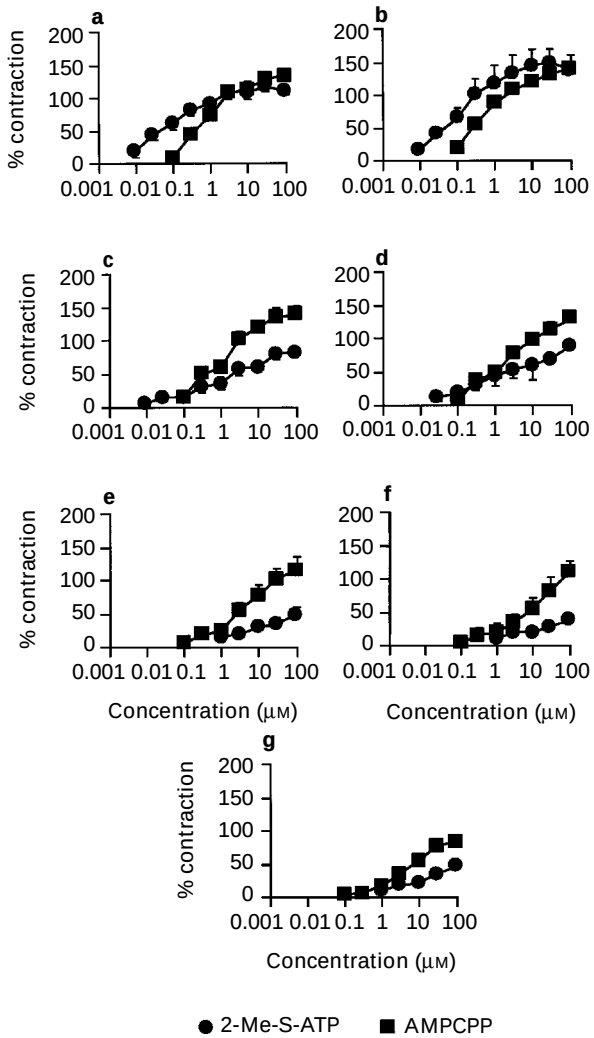


Figure 3 Contractile responses induced by AMPCPP and 2-Me-S-ATP in the rat duodenum muscularis mucosae at various ages; (a) day 10, (b) day 15, (c) day 20, (d) day 25, (e) day 30, (f) day 40 and (g) adult (>60 day). Each point is the mean of at least four determinations and the vertical lines show s.e.mean where larger than the symbol. For abbreviations see text.

detected at days 15 and 30 (Figure 6a,e). However, at day 15 around 60% of ATP remained after 2 min with a half-life for ATP of 8.9±3.0 min (Figure 6a), whereas at day 30 significantly less ATP (17%) remained after 2 min ($P<0.05$), with a half-life for ATP of 0.4±0.8 min (Figure 6e). A similar rate of degradation was seen for UTP and 2-Me-S-ATP at days 15 and 30. UTP was degraded to uridine 5′-diphosphate (UDP), uridine 5′-monophosphate (UMP) and uridine (Figure 6b,f), and 2-Me-S-ATP was degraded to 2-methylthioadenosine 5′-diphosphate (2-Me-S-ADP), 2-methylthioadenosine 5′-monophosphate (2-Me-S-AMP) and 2-methylthioadenosine (2-Me-S-adenosine) (Figure 6c,g). At day 15 around 60% of UTP and 80% of 2-Me-S-ATP remained after 2 min with a half-life for UTP and 2-Me-S-ATP of 11.4±3.8 min and 13.5±3.9 min, respectively (Figure 6b,c). However, at day 30 significantly less UTP (14%) and 2-Me-S-ATP (12%) remained after 2 min

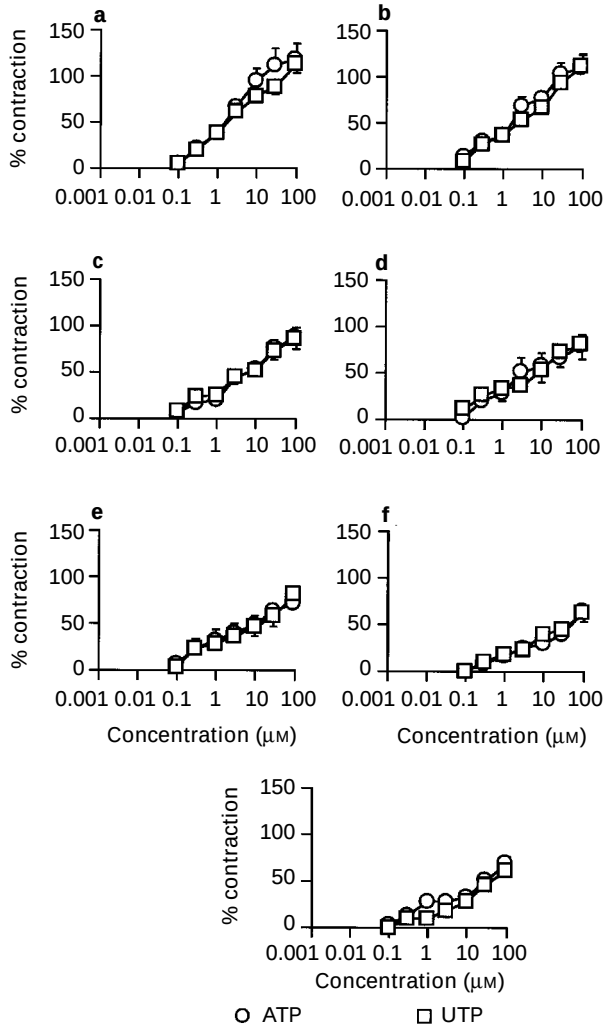


Figure 4 Contractile responses induced by UTP and ATP in the rat duodenum muscularis mucosae at various ages; (a) day 10, (b) day 15, (c) day 20, (d) day 25, (e) day 30, (f) day 40 and (g) adult (>60 day). Each point is the mean of at least four determinations and the vertical lines show s.e.mean where larger than the symbol. For abbreviations see text.

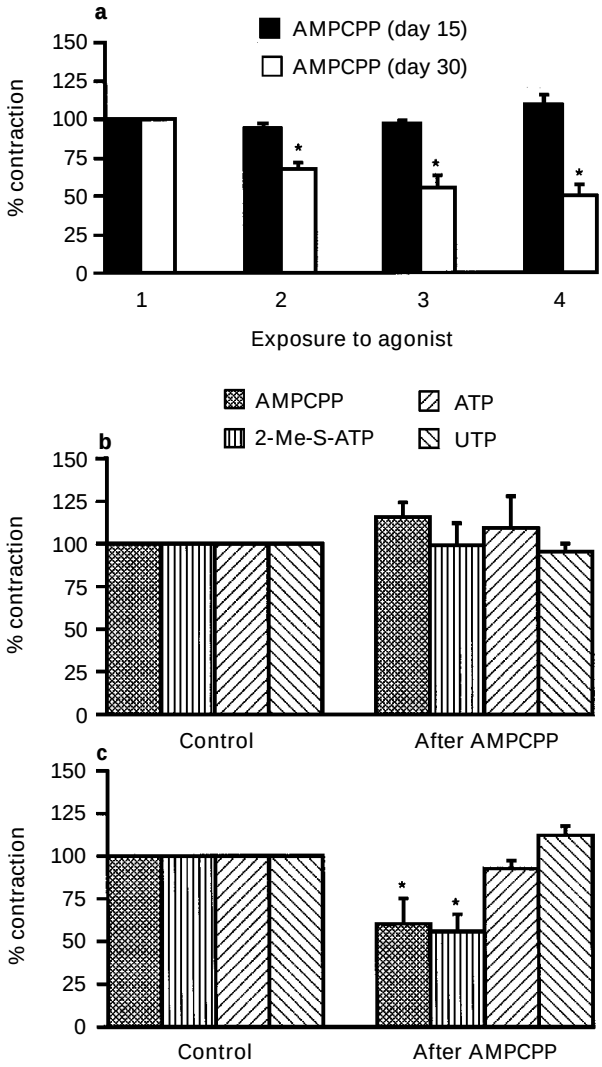
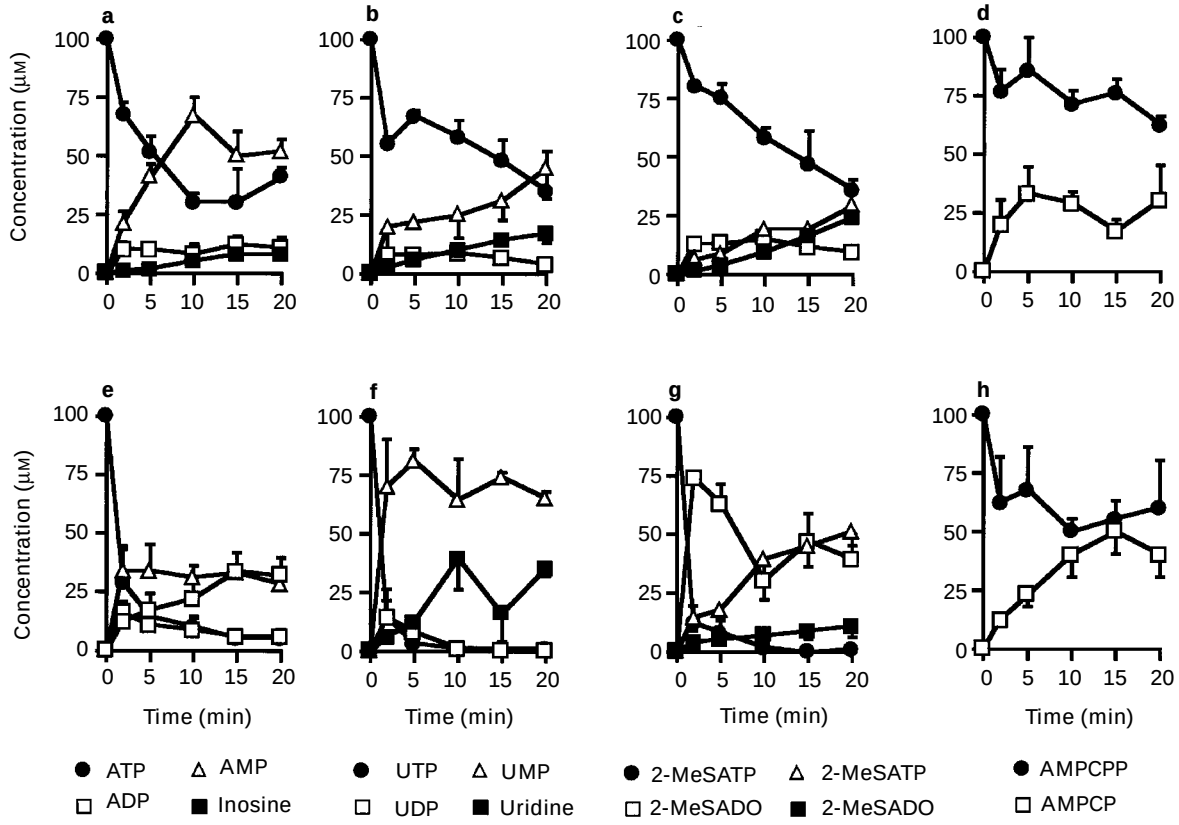


Figure 5 Contractions of the rat duodenum muscularis mucosae (a) to repeated administrations of AMPCPP (100 μM) at day 15 and at day 30, (b) to 2-Me-S-ATP (3 μM), ATP (10 μM) and UTP (10 μM) following exposure to a desensitizing dose of AMPCPP (100 μM) at day 15 and (c) to 2-Me-S-ATP (100 μM), ATP (100 μM) and UTP (100 μM) following exposure to a desensitizing dose of AMPCPP (100 μM) at day 30. Contractions are expressed as % response to initial dose of agonist. Each column is the mean of at least four determinations and the vertical lines show s.e.mean. *$P<0.05$ significantly different from control value (Figure 5a, ANOVA followed by Dunnett's two-tailed *post-hoc* test; Figure 5b,c Student's *t* tests). For abbreviations see text.

Table 2 Potency order of agonists in causing contraction of the rat duodenum muscularis mucosae at various ages

Age (days)	*Potency order* (pEC_{60} values in parentheses)
10	2-Me-S-ATP (7.0±0.3) > AMPCPP (6.0±0.2) > ATP (5.6±0.1) = UTP (5.4±0.1)
15	2-Me-S-ATP (6.9±0.2) > AMPCPP (6.2±0.1) > ATP (5.4±0.3) = UTP (5.4±0.2)
20	AMPCPP (5.9±0.1) > 2-Me-S-ATP (5.2±0.3) ≥ ATP (4.8±0.3) = UTP (4.8±0.3)
25	AMPCPP (5.7±0.2) > 2-Me-S-ATP (4.5±0.5) = ATP (4.6±0.7) = UTP (4.8±0.5)
30	AMPCPP (5.3±0.3) > ATP (4.2±0.3) = UTP (4.2±0.2) ≥ 2-Me-S-ATP (2.8±0.8)
40	AMPCPP (5.0±0.5) > ATP (3.7±0.2) = UTP (3.7±0.6) > 2-Me-S-ATP (2.3±0.5)
Adult (>60)	AMPCPP (4.8±0.1) > ATP (4.2±0.2) = UTP (4.0±0.1) > 2-Me-S-ATP (3.0±0.3)

Data are the mean and s.e.mean of the pEC_{60} values ($n=3-4$). For abbreviations see text.



Degradation by the rat duodenum muscularis mucosae of (a and e) ATP (100 μM) to ADP, AMP and inosine; (b and f) UTP (100 μM) to UDP, UMP and uridine; (c and g) 2-Me-S-ATP (100 μM) to 2-Me-S-ADP, 2-Me-S-AMP and 2-Me-S-adenosine and (d and h) AMPCPP (100 μM) to AMPCP. Degradation in (a), (b), (c) and (d) are by 15 day tissues and in (e), (f), (g) and (h) are by 30 day tissues. Each point is the mean of three determinations and the vertical lines show s.e.mean where larger than the symbol. For abbreviations see text.

($P<0.05$), with a half-life for UTP and 2-Me-S-ATP of 0.2 ± 0.4 min and 0.3 ± 0.1 min, respectively (Figure 6f,g). The levels of ATP, UTP or 2-Me-S-ATP remaining after 2 min and the half-lives for the agonists were not significantly different from each other either at day 30 or at day 15 ($P>0.05$), whereas the levels of ATP, UTP and 2-Me-S-ATP remaining after 2 min at day 30 and the half lives for the agonists at this age were significantly greater than those obtained at day 15 ($P<0.05$). AMPCPP was also degraded by the muscularis mucosae at days 15 and 30 with only adenosine 5′-(α,β-methylene)diphosphonate (AMPCP) being detected (Figure 6d,h). At day 15 around 75% of AMPCPP remained after 2 min (Figure 6d), whereas at day 30 around 65% of AMPCPP remained after 2 min (Figure 6h). These values were not significantly different ($P>0.05$). The half-life for AMPCPP at days 30 and 15 were 12.0 ± 1.5 min and 26.0 ± 7.0 min, respectively, and again these values were not significantly different ($P>0.05$). The half-life for AMPCPP at day 30 was significantly higher than the half-lives for ATP, UTP, or 2-Me-S-ATP at that age ($P<0.05$), whereas the half-lives at day 15 for all the agonists were not significantly different from each other ($P>0.05$).

Discussion

Potent relaxations were observed in the rat duodenum longitudinal muscle to 2-Me-S-ATP and AMPCPP from day 20 onwards, with a potency order of 2-Me-S-ATP > AMPCPP, whereas only weak relaxations were observed at day 15. The potency order of 2-Me-S-ATP > AMPCPP in the neonatal tissues from day 20 onwards is similar to that obtained for the adult tissue (Johnson *et al*., 1996), suggesting the activation of P2Y-receptors. The potency of the agonists in causing relaxation increased with age being lowest at days 15 and 20, and reaching values not significantly different from adult by day 25, suggesting that the P2Y-receptor population becomes fully developed by day 25. Relaxations have also been observed in the whole rat duodenum to ATP and 2-Me-S-ATP acting via P2Y-receptors, and in the whole tissue the potency of agonists was also lowest at day 20 and was similar to the adult by day 25 (Nicholls *et al*., 1990).

Contractile responses were observed in the muscularis mucosae of the rat duodenum to ATP, UTP, 2-Me-S-ATP and AMPCPP. The potency order of agonists at day 30 and above was AMPCPP > ATP = UTP > 2-Me-S-ATP, which is similar to that observed in the adult tissue (Johnson *et al*., 1996). In that study it was concluded that AMPCPP and 2-Me-S-ATP act via P2X-purinoceptors and that UTP and ATP act via P2U-purinoceptors, and it seems likely that this is also the case in the neonatal tissues at day 30 and above. The fact that responses to ATP were apparently mediated via P2U-purinoceptors, rather than P2X-purinoceptors as ATP is known to activate P2X-purinoceptors, probably reflects the relative numbers of the two subtypes and the potency of ATP at these receptors, rather than suggesting the presence of a novel P2X-purinoceptor subtype unresponsive to ATP. As in the adult, responses to AMPCPP were desensitized at day 30 and cross-desensitization was observed between AMPCPP and 2-Me-S-ATP, also suggesting that both agonists activate P2X-receptors. On the other hand, no cross-desensitization was observed at day 30 between AMPCPP and ATP or UTP, suggesting that the latter agonists were not acting via P2X-receptors, but via P2U-receptors as in the adult. However below day 20, 2-Me-S-ATP was more potent in eliciting contractions than AMPCPP, and at days 10 and 15 the order was 2-Me-S-ATP

> AMPCPP > ATP = UTP. At day 15, unlike at day 30, responses to AMPCPP were not desensitized and no cross-desensitization was observed with AMPCPP and 2-Me-S-ATP. This suggests that AMPCPP and 2-Me-S-ATP were not acting via P2X-receptors and as 2-Me-S-ATP was more potent than AMPCPP in eliciting contractions in this tissue, the contractions may be mediated via P2Y-receptors at this age. In contrast ATP was equipotent with UTP at day 15 and again no cross-desensitization was observed between AMPCPP and ATP or UTP, suggesting the activation of P2U-receptors, as observed at day 30. Therefore in the rat duodenum muscularis mucosae, different P2-purinoceptor subtypes are activated in the neonates compared to the adult, but mediate the same response. Indeed in our previous ontogenetic study in the whole rat duodenum, contractile responses mediated via P2Y-receptors were shown before day 20, while only relaxations via P2Y-receptors could be detected after day 20 (Nicholls *et al*., 1990). This is probably because the relaxant response in the rat duodenum longitudinal muscle is fully developed by day 20 and begins to predominate, thus masking the contractile response of the muscularis mucosae.

The potency of all the agonists in eliciting contractions in the rat duodenum muscularis mucosae decreased with age. The potency of AMPCPP and 2-Me-S-ATP in causing contractions in this tissue was greatest in the neonates below day 25 and reached values similar to adult by day 30. The potency of ATP and UTP in causing contractions also decreased with age, being greatest at days 10 and 15 and reached values similar to adult by day 20. This drop in potency of agonists may be due to the breakdown of compounds by ectonucleotidases present on the muscularis mucosae. In the adult rat duodenum muscularis mucosae we have shown that the rate of breakdown of ATP is similar to that of UTP, whereas the breakdown of AMPCPP is much slower (Johnson *et al*., 1996). Therefore the activity of ectonucleotidases in the rat duodenum muscularis mucosae was investigated in tissue preparations from rats aged 15 and 30 days, in order to investigate the reason for the drop in potency of agonists.

At day 30 ATP, 2-Me-S-ATP and UTP were broken down at a similar rate and the half-lives were not significantly different from each other. However, the half-lives of ATP, UTP or 2-Me-S-ATP were significantly lower at day 15 than at day 30, indicating that the ectonucleotidase activity is lower in the neonatal rat duodenum muscularis mucosae at day 15 than at day 30. This increase in observed ectonucleotidase activity is probably attributable to the increase in the size of the tissue, as the average wet weight increases from 23.3 mg at day 15 to 100.7 mg at day 30. The major breakdown products of ATP, UTP and 2-Me-S-ATP at both ages were AMP, UMP and 2-Me-S-AMP, respectively. This suggests that the levels of ecto 5′-nucleotidase which converts AMP to adenosine may be lower than the levels of other degradative enzymes found on the muscularis mucosae. In the case of ATP breakdown at 30 days the sum of the concentrations of metabolites does not equal the reduction in the ATP concentration. This is commonly observed in experiments of this type (see eg Welford *et al*., 1986; 1987; Bailey & Hourani, 1990), and probably reflects the uptake of adenosine into the tissue. AMPCPP was the most stable agonist at day 30 with the half-life for AMPCPP being significantly greater than that for ATP, UTP or AMPCPP. This is similar to the adult rat duodenum muscularis mucosae (Johnson *et al*., 1996), where AMPCPP is also the most stable agonist. At day 15 AMPCPP was also degraded by the muscularis mucosae, but there was no significant difference in the half-life of AMPCPP compared to those of ATP, UTP or 2-Me-S-ATP.

The decrease in potency of ATP and UTP in eliciting contraction in the muscularis mucosae was approximately 1.5 log units, whereas the decrease in potency for 2-Me-S-ATP (also eliciting contraction) was greater than 3 log units. Since the relative rates of degradation of ATP, UTP and 2-Me-S-ATP are similar at days 15 and 30, the difference in the drop in the potency of 2-Me-S-ATP compared to ATP or UTP cannot be explained by the greater rate of breakdown of 2-Me-S-ATP at day 30. Therefore, it is likely that the reason for the high potency of 2-Me-S-ATP observed at days 10 and 15 compared to adult or day 30 tissue preparations is indeed due to the activation of P2Y-receptors (at which 2-Me-S-ATP has high potency) in the neonates but P2X-receptors after day 20. As there are low levels of ectonucleotidase activity in the neonatal tissue preparations compared to the adult tissues the drop in potency of ATP and UTP may be partly due to the increase in the rate of breakdown. However, as there was no significant difference in the breakdown of AMPCPP in 15 day tissues compared to 30 day tissues, and as there was also a drop in potency of AMPCPP with age, it seems likely that a change in receptor number is also involved in the drop in potency of this agonist in the muscularis mucosae.

In conclusion, this work shows the presence of a functional P2Y-receptor mediating relaxation in the rat duodenum longitudinal muscle as early as day 15 (the earliest age it could be studied), and that this receptor is fully developed by day 25. In the adult rat duodenum muscularis mucosae there are P2X- and P2U-receptors both mediating contractions. The contractile P2U-receptor in the muscularis mucosae is functional as early as day 10 (the earliest age it could be studied) and the receptor population may be highest in the neonates before day 20. However, the results presented here suggest that a P2Y-receptor mediates contractions in this tissue before day 20, whereas a P2X-receptor mediates the same response after day 20, implying a switch in the P2-purinoceptors with age from a G-protein coupled receptor (P2Y) to an ion channel receptor (P2X), both mediating the same response. A switch in receptors (albeit both G protein-coupled) has also been noted in the development of opioid receptors, with stress-induced antinociception being mediated via μ-receptors in rats before day 20 but via δ-receptors after that age (Muhammad & Kitchen, 1993). The physiological importance of the switch in the purinergic component in the rat duodenum is unclear, but it occurs at a time when there are marked changes in the gastrointestinal tract associated with weaning from maternal milk to solid food. There are also other changes in the purinoceptor population of the rat duodenum at this time, such as the development of A_1-adenosine receptors in the longitudinal muscle (Peachey *et al*., 1996; Brownhill *et al*., 1996). Finally, the ectonucleotidase activity in the rat duodenum muscularis mucosae increases with age and this may at least partly explain the drop in potency of agonists in this tissue with age.

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