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Influence of purines and pyrimidines on circular muscle of the rat proximal stomach

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Abstract

The effects of UTP were examined to characterize the receptor subtypes for UTP in the circular smooth muscle of the rat proximal stomach. The rank order of potency for contraction was 2-methylthio ATP ≫ ATP ≥ UDP = UTP ≥ adenosine 5′-O-(3-thiotriphosphate)(ATP-γ-S) ≫ UMP > CTP = α,β-methylene ATP > adenosine = uridine. In tissues contracted by acetylcholine, ATP, 2-methylthio ATP, α,β-methylene ATP and adenosine each caused relaxation. α,β-Methylene ATP had the most potent effect and UTP caused only a small relaxation. Suramin inhibited ATP- and UTP-induced contractions. The contractile responses to ATP decreased in tissues desensitized with UTP, ATP-γ-S and 2-methylthio ATP, but not with α,β-methylene ATP. However, UTP-induced contraction was not inhibited by desensitization with ATP, α,β-methylene ATP, ATP-γ-S and 2-methylthio ATP. These results suggest that UTP causes contraction via receptors different from common P_2 purinoceptors. These receptors are blocked by suramin in the rat proximal stomach.

Keywords: UTP; ATP; Purinoceptors; Pyrimidinoceptors; Stomach, rat; Suramin

1. Introduction

Adenosine and adenine nucleotides have been shown to evoke various physiological effects via P_1 and P_2 purinoceptors in various tissues (Gordon, 1986; Stone, 1981; El-Moatassim et al., 1992). P_2 purinoceptors are primarily subdivided into P_{2X} (the rank order of potency is α,β-methylene ATP > ATP = 2-methylthio ATP) and P_{2Y} (the rank order of potency is 2-methylthio ATP ≫ ATP > α,β-methylene ATP) purinoceptors (Burnstock and Kennedy, 1985). In general, P_{2X} purinoceptors are transmitter-gated ion channels (Bean, 1992) mediating contraction, while P_{2Y} purinoceptors are GTP-binding protein-coupled receptors (Barnard et al., 1994) mediating relaxation in smooth muscle. In the rat colon muscularis mucosae, however, it has been reported that P_{2Y} purinoceptors are associated with contraction (Bailey and Hourani, 1990).

UTP has also been shown to act via P_{2Y} purinoceptors in the rat colon muscularis mucosae (Hourani et al., 1993) and via P_{2X} purinoceptors in the mouse vas deferens (Von Kügelgen et al., 1990) on the basis of desensitization experiments. Moreover, it has been proposed that UTP acts at purinoceptors, 'P_{2U} purinoceptors' or 'nucleotide receptors', distinct from P_{2X} and P_{2Y} purinoceptors (O'Connor et al., 1991; Conigrave and Jiang, 1995), of which the specific potency order for agonists is UTP ≥ ATP = adenosine 5′-O-(3-thiotriphosphate)(ATP-γ-S) ≫ 2-methylthio ATP = α,β-methylene ATP (Fredholm et al., 1994). It has been reported that UTP causes various responses via receptors different from purinoceptors (Von Kügelgen et al., 1987; Von Kügelgen and Starke, 1990), termed pyrimidinoceptors (Seifert and Schultz, 1989). In the rat duodenum, pyrimidinoceptors have been shown to coexist with purinoceptors and to cause contraction (Johnson and Hourani, 1994).

Suramin, a trypanocidal drug, is the best available antagonist for P_2 purinoceptors, although it is a non-competitive antagonist (Hoyle et al., 1990) and has inhibitory effects on ectonucleotidases (Hourani and Chown, 1989). In PC12 cells, ATP-γ-S and UTP cause the generation of inositol phosphates via P_{2U} purinoceptors sensitive to suramin (Murrin and Boarder, 1992). In vas deferens DDT_1 MF-2 smooth muscle cells (Van der Zee et al., 1992) and human lens epithelial cells (Riach et al., 1995),

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ATP and UTP cause rises in the intracellular Ca^{2+} concentration blocked by suramin. However, in bovine aortic endothelial cells, suramin blocks the formation of inositol triphosphate by the activation of P_{2Y} but not P_{2U} purinoceptors (Wilkinson et al., 1993). The inhibitory effect of suramin on UTP-induced responses seems to vary depending on the tissue.

In the longitudinal smooth muscle of the rat gastric fundus, it has been reported that ATP causes a biphasic response, a relaxation followed by contraction (Burnstock et al., 1970; Lefebvre and Burnstock, 1990), that ATP and related compounds act via P_1 and P_{2X} purinoceptors for relaxation, P_{2Y} purinoceptors for contraction and that suramin inhibits the responses mediated by both P_{2X} and P_{2Y} but not by P_1 purinoceptors (Matharu and Hollingsworth, 1992).

There is no information about the effect of UTP on mechanical responses in the rat stomach. In this study, we aimed to characterize the receptor subtypes for UTP on the circular smooth muscle of the rat proximal stomach. For this purpose, we investigated the mechanical responses to UTP, ATP and related compounds, and the effects of cross-desensitization and suramin on responses to ATP and UTP.

2. Materials and methods

2.1. Preparation

Wistar rats of either sex (200–300 g) were stunned and bled to death. The stomach in the proximal region was excised and cut in a longitudinal direction along the lesser curvature. The tissue was placed in a dissecting chamber filled with physiological solution of the following composition (mM): NaCl 144, KCl 5.8, $CaCl_2$ 2.5, $MgCl_2$ 1.2, HEPES 5, glucose 11.1 (pH 7.4). The circular muscle layer was removed from mucosal and submucosal layers under a binocular microscope. Then circular muscle strips (1.5 mm in width, 5–8 mm in length) were prepared using fine forceps and small knives made from pieces of a razor blade. The tissues were suspended in 3-ml organ baths containing physiological solution at 37°C. A resting tension of 1 g was applied to the tissues.

2.2. Experimental procedures

Tissues were allowed to equilibrate for 1 h and then isometric contractions were recorded. Concentration-dependent curves for contraction were obtained with the non-cumulative addition of each drug. The contraction was expressed as a percentage of the contraction caused by carbachol (1 μM) applied to each tissue at the beginning of the experiments. Agonists were applied for approximately 2 min at 10–15-min intervals. Suramin was applied 1 h before the start of the experiments. Relaxation induced by drugs was obtained with their non-cumulative application during sustained contraction induced by acetylcholine (1 μM) applied at 10–15-min intervals for each concentration of the drugs. Relaxation was expressed as percent inhibition of acetylcholine-induced contraction just before the application of the test drug.

Desensitization with a particular drugs was achieved by continuous application of the drug. It took 5–8 min for the response to the drug to return to near the resting level. Further addition of desensitizing drugs produced no response. Then test drugs were applied without washout of the desensitizing drug. The application of the desensitizing drug was repeated for each concentration of the test drug. α,β-Methylene ATP was added in the same manner as desensitizing drugs (5–8 min).

To examine the effects of external Ca^{2+} removal on ATP- and UTP-induced responses. ATP or UTP was applied 2 min after switching from normal physiological solution to Ca^{2+}-free solution containing 2 mM EGTA. In the presence of nifedipine, which almost abolished the response to 150 mM K^+, test drugs were applied. All experiments were carried out in the presence of indomethacin (10 μM).

2.3. Drugs

The following drugs were used: Adenosine, ADP (adenosine 5′-diphosphate, sodium salt), AMP (adenosine 5′-monophosphate, sodium salt), indomethacin, carbachol (carbamylcholine chloride), α,β-methylene ATP (α,β-methylene adenosine 5′-triphosphate, lithium salt), and uridine were obtained from Sigma (USA), suramin (suramin sodium) was from Biomol. Research Laboratories (USA), 2-methylthio ATP (tetrasodium salt) was from Research Biochemicals (USA), ATP (disodium salt) and ATP-γ-S (adenosine 5′-O-(3-thiotriphosphate), tetralithium salt) were from Boehringer Mannheim (Germany), CTP (cytidine 5′-triphosphate, trisodium salt) and UTP (uridine 5′-triphosphate, trisodium salt) were from Yamasa (Japan), UDP (uridine 5′-diphosphate, disodium salt), UMP (uridine 5′-monophosphate, disodium salt), and nifedipine were from Wako (Japan), acetylcholine (acetylcholine chloride) was obtained from Daiichi (Japan), EGTA and HEPES were from Dojindo (Japan).

2.4. Statistical analysis

Results were expressed as means ± S.E.M. (n = number of observation), and statistical significance was assessed using Student's t-test. P values of less than 0.05 were considered to be significant.

3. Results

3.1. ATP- and UTP-induced responses in circular smooth muscle of the rat proximal stomach

Both ATP and UTP caused contractions in a dose-dependent manner (Fig. 1A). In the tissues which had sponta-

neous basal tone, ATP caused a small phasic relaxation followed by contraction as shown in Fig. 1Aa. In tissues contracted by acetylcholine (ACh, 1 μM), ATP induced a dose-dependent relaxation (Fig. 1Ba). The relaxant response to ATP was followed by a contraction at the high concentration. UTP also caused a small relaxation followed by contraction (Fig. 1Bb). The concentration-dependent curves for ATP and UTP are shown in Fig. 2.

3.2. Effects of purine and pyrimidine compounds

As shown in Fig. 2A, 2-methylthio ATP, ATP-γ-S and UDP each also caused contraction in a dose-dependent manner in the circular smooth muscle of the rat stomach. The mean pD_2 values were 8.89 ± 0.13 ($n = 6$) for 2-methylthio ATP, 6.06 ± 0.14 ($n = 4$) for ATP, 5.84 ± 0.29 ($n = 5$) for UDP, 5.78 ± 0.31 ($n = 5$) for UTP and 5.43 ± 0.27 ($n = 5$) for ATP-γ-S. The maximal contractile responses to 2-methylthio ATP, UDP and UTP were about 40% the of carbachol-induced contraction. The contractile response to ATP or ATP-γ-S was slightly less than that to UTP. α,β-methylene ATP, UMP and CTP caused only a small, if any, or no contraction. Uridine and adenosine failed to cause any contraction at concentrations up to 1 mM. The agonist potency order for contraction was 2-methylthio ATP ≫ ATP ≥ UDP = UTP ≥ ATP-γ-S ≫ UMP > CTP = α,β-methylene ATP > adenosine = uridine = 0.

In acetylcholine (1 μM)-contracted tissues, ATP, ADP, AMP, 2-methylthio ATP, α,β-methylene ATP and adeno-

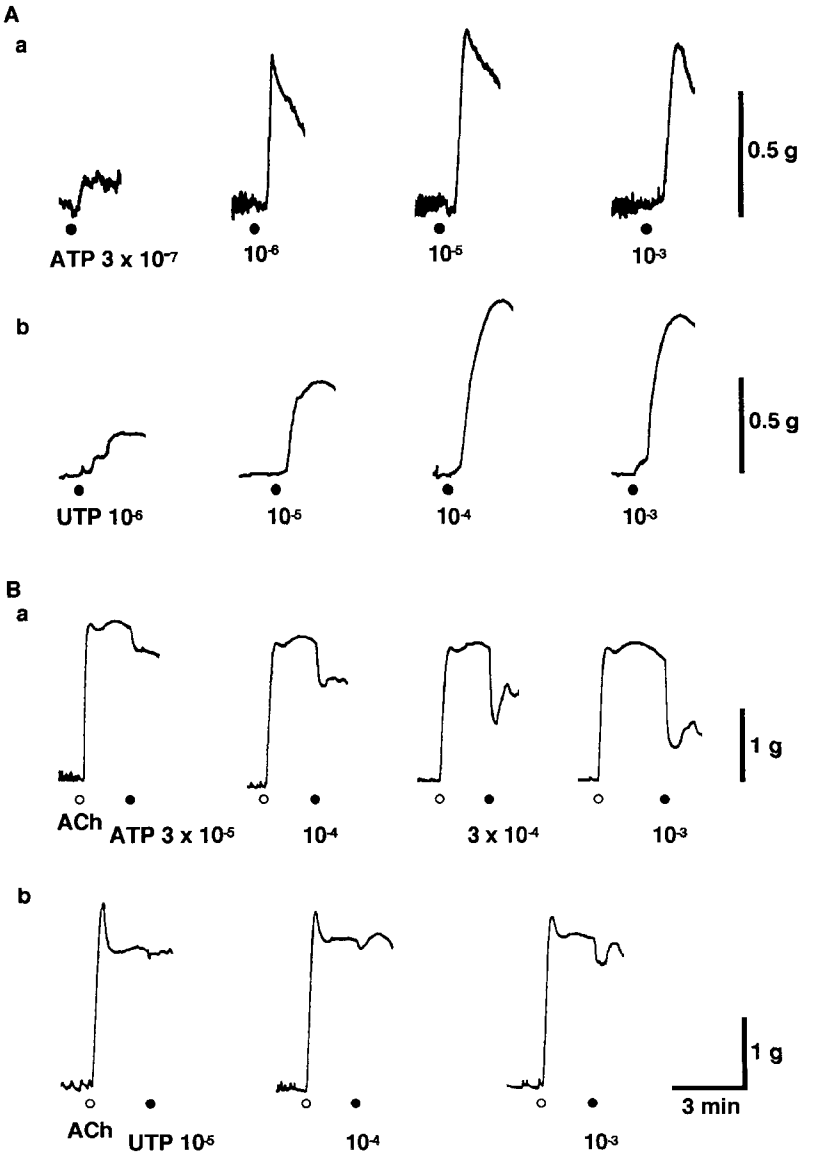

Fig. 1. Representative responses to ATP and UTP in the circular smooth muscle of the rat stomach. (A) Contraction induced by ATP (a) and UTP (b). (B) Relaxation induced by ATP (a) and UTP (b) in tissues contracted by acetylcholine (ACh, 1 μM). Each symbols indicates the time of the application of ATP or UTP (●) at the concentration described and ACh (○). ATP or UTP was applied for approximately 2 min at 10–15-min intervals.

sine each caused relaxation in a dose-dependent manner (Fig. 2B). Relaxation induced by α,β-methylene ATP attained a maximum at around 10 μM. The responses to 2-methylthio ATP, ATP ADP, AMP and adenosine did not reach a maximum at concentrations up to 1 mM. Higher concentrations (> 0.1 mM) of UTP or CTP were required to evoke relaxation.

3.3. Effects of suramin

The effect of suramin on ATP- and UTP-induced contractions is shown in Fig. 3. Suramin (10 and 100 μM) did not affect the contractions induced by acetylcholine (1 μM) or carbachol (1 μM). Suramin at a concentration of 10 μM caused an about 10-fold shift of the concentration-dependent curves for both ATP and UTP to the right and decreased the maximal contraction induced by UTP but not ATP. Suramin at a high concentration (100 μM) greatly suppressed the contractile responses to a high concentration (1 mM) of both agonists.

3.4. Effects of desensitization

As shown in Fig. 4A, desensitization with UTP (100 μM) or ATP-γ-S (100 μM) shifted the concentration-dependent curve for ATP-induced contraction to the right. ATP-γ-S produced a greater inhibition of the response to ATP than did UTP. Desensitization with 2-methylthio ATP (0.1 μM) completely prevented ATP from causing the contraction at all concentrations used. Pre-treatment with α,β-methylene ATP (100 μM) did not inhibit the ATP-induced contraction and slightly increased the responsiveness to ATP ($P < 0.05$, $n = 4$ at 1 mM). The UTP-induced contraction was not significantly inhibited by desensitization with ATP (100 μM), 2-methylthio ATP (100 μM) or ATP-γ-S (100 μM, Fig. 4Ab and Bb). The responsiveness to UTP was slightly increased by ATP ($P < 0.01$, $n = 4$ at 1 μM, $P < 0.05$, $n = 4$ at 3 μM UTP) and ATP-γ-S ($P < 0.01$, $n = 4$ at 1 and 3 μM UTP). Pre-treatment with α,β-methylene ATP (100 μM) did not inhibit the UTP-induced contraction.

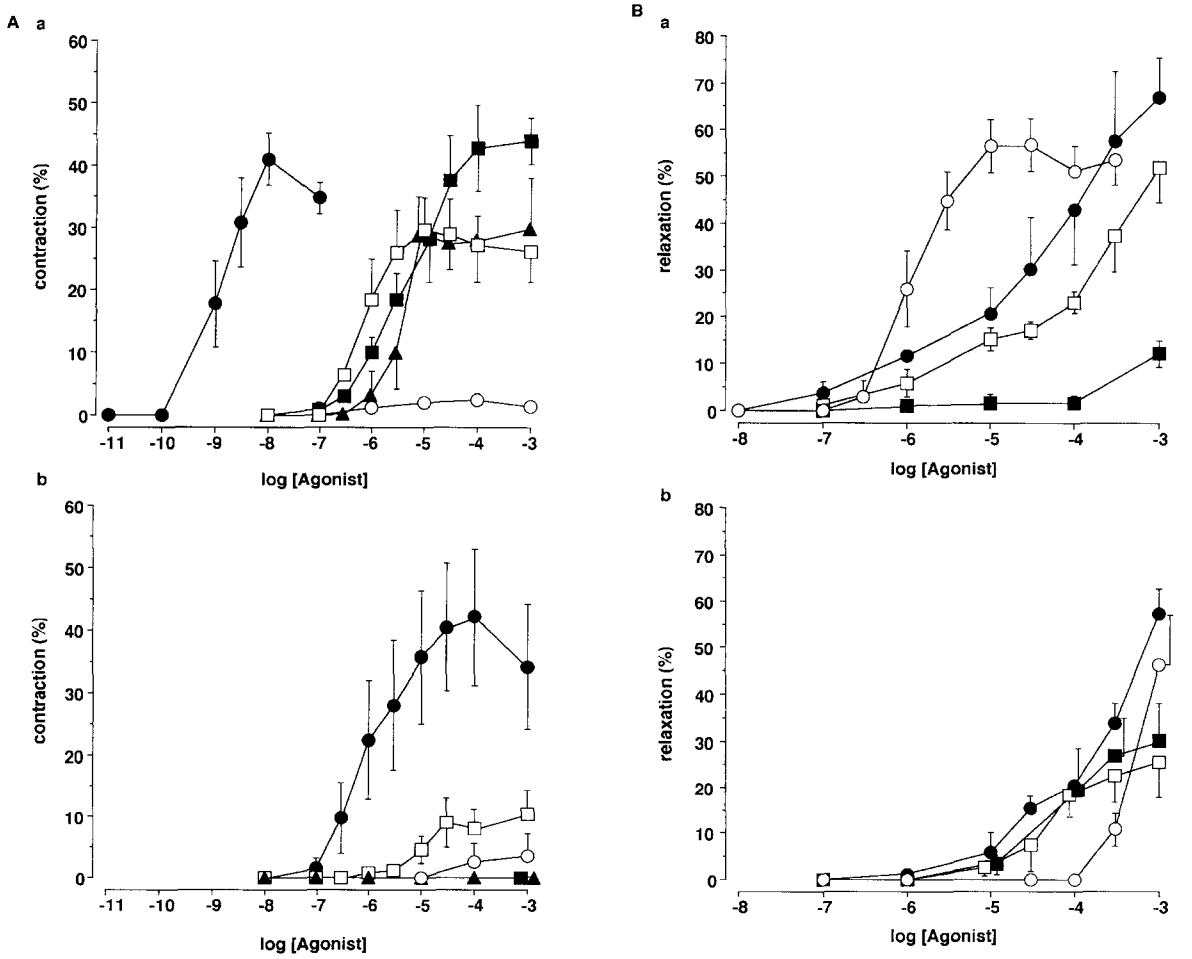

Fig. 2. Concentration-dependent contractile and relaxant responses to UTP, ATP and related compounds. (A) contraction caused by (a) 2-methylthio ATP (●), ATP (□), adenosine 5′-O-(3-thiotriphosphate) (ATP-γ-S, ▲), UTP (■), α,β-methylene ATP (○) and by (b) UDP (●), UMP (□), uridine (▲), adenosine (■) and CTP (○). Each value is mean ± S.E.M. ($n = 3$–8). (B) Relaxation caused by (a) 2-methylthio ATP (●), ATP (□), UTP (■), α,β-methylene ATP (○) and by (b) ADP (●), AMP (□), adenosine (■) and CTP (○). Each value is mean ± S.E.M. ($n = 4$–6).

3.5. Contractile responses to UTP and UDP

The contractile responses to UDP (100 μM) or UTP (100 μM) were studied in the same tissue. As shown in Fig. 5Aa, the UDP-induced contraction (control; 31.5 ± 3.3%, $n = 9$) was not inhibited by desensitization with 100 μM ATP (31.1 ± 6.0%, $n = 9$). However, desensitization with 100 μM UTP significantly ($P < 0.001$) decreased UDP-induced contraction (1.1 ± 0.7%, $n = 9$). Fifteen min after washout of the drugs, the contractile responses to UDP were partially recovered (17.4 ± 4.4%, $n = 9$). Desensitization with 0.1 μM 2-methyltio ATP significantly ($P < 0.01$) decreased the responses to 100 μM ATP (0.3 ± 0.2%, $n = 9$) but not UDP (37.1 ± 7.5, $n = 9$) as compared with the responses before desensitization (ATP; 25.9 ± 6.6% and UTP; 33.6 ± 6.5%, $n = 9$).

Moreover, we investigated the contractile responses to pyrimidines in tissues pre-treated with both 2-methyltio ATP (0.1 μM) and α,β-methylene ATP (100 μM). Carbachol (1 μM)-induced contraction was not affected by pre-treatment with both drugs (data not shown). As shown in Fig. 5B, however, UTP (100 μM)-induced contractions (control; 19.9 ± 5.4%, $n = 4$) were significantly ($P < 0.005$) increased by pre-treatment with these drugs (42.3 ± 8.4%, $n = 4$). Fifteen min after washout of drugs, the contractile responses to UTP was 6.2 ± 2.3% ($n = 4$). In pre-treated tissue, UDP-induced contraction (control; 33.1 ± 6.3%, $n = 4$) was not affected by these drugs (30.6 ± 3.0%, $n = 4$). The responses to UDP was 14.2 ± 2.4% ($n = 4$) after wash out. As described above, UMP and CTP usually caused only a small, if any, or no contraction. The responsiveness to UMP and CTP was not altered under these conditions.

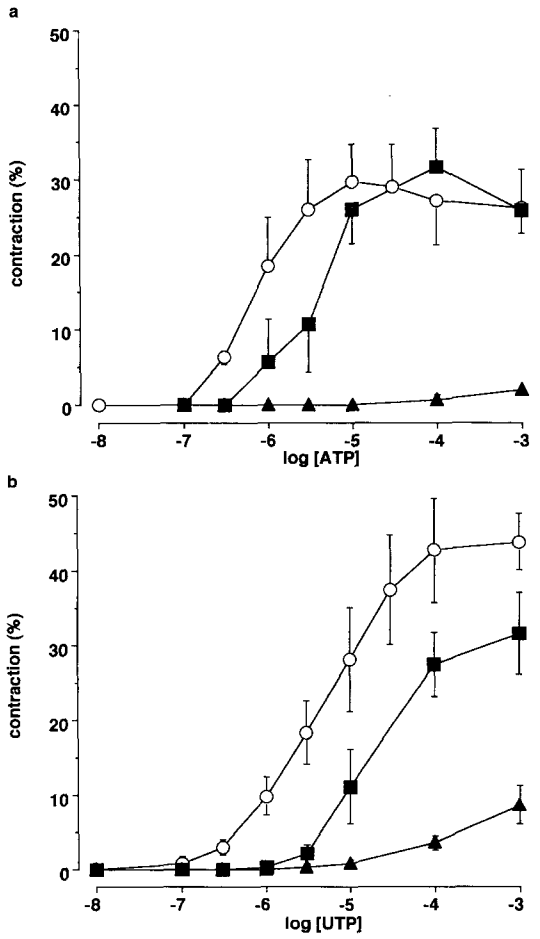

Fig. 3. Effect of suramin on contractions induced by ATP and UTP. Contractions induced by ATP (a) and UTP (b) in the presence of suramin (10 μM, ■; 100 μM, ▲) and its absence (○). Each value is mean ± S.E.M. ($n = 3$–6).

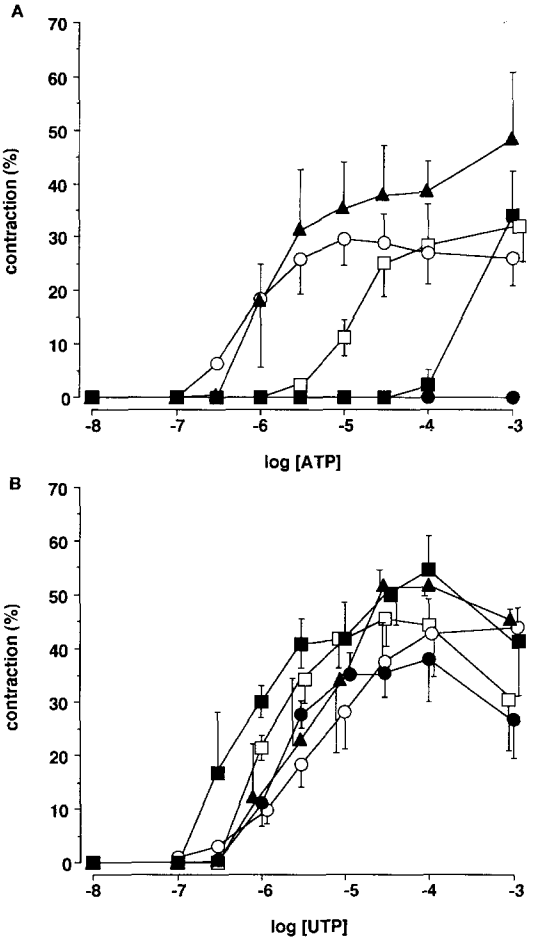

Fig. 4. Effect of desensitization on ATP- and UTP-induced contractions. Contractions induced by ATP (A) and UTP (B) in the control (○) and in tissues pre-treated with 100 μM UTP (□, A), 100 μM ATP (□, B), 100 μM α,β-methylene ATP (▲), 100 μM ATP-γ-S (■) and 0.1 μM 2-methylthio ATP (●). Each value is mean ± S.E.M. ($n = 4$–6).

3.6. Dependency of extracellular Ca^{2+}

The contraction induced by 150 mM K^+ solution was completely abolished in Ca^{2+}-free solution containing 2 mM EGTA. Carbachol (100 μM) caused a small transient

contraction under these conditions. UTP (100 μM) and ATP (100 μM) failed to evoke contractile responses in Ca^{2+}-free solution. Representative data are shown in Fig. 6 and similar results were obtained in 6 other experiments.

Nifedipine (1 μM), a voltage-dependent L-type Ca^{2+} channel blocker, significantly ($P < 0.01$) inhibited contractile responses to 150 mM K^+, ATP and UTP to 5.1 ± 1.3%, 8.4 ± 3.9% and 14.2 ± 1.8%, respectively, as compared with responses in the absence of nifedipine (105.7 ± 8.2%, $n = 4$, 35.0 ± 3.7%, $n = 4$ and 42.0 ± 6.7%, $n = 4$, respectively).

4. Discussion

4.1. P_{2X} and P_{2Y} purinoceptors

In the present study, we investigated the effects of ATP, UTP and related compounds on mechanical responses in the rat proximal stomach. The present experiments were carried out in the presence of indomethacin to prevent the involvement of prostaglandin.

The rank order of contractile potency was 2-methylthio ATP ≫ ATP ≥ UDP = UTP ≥ ATP-γ-S ≫ UMP > CTP

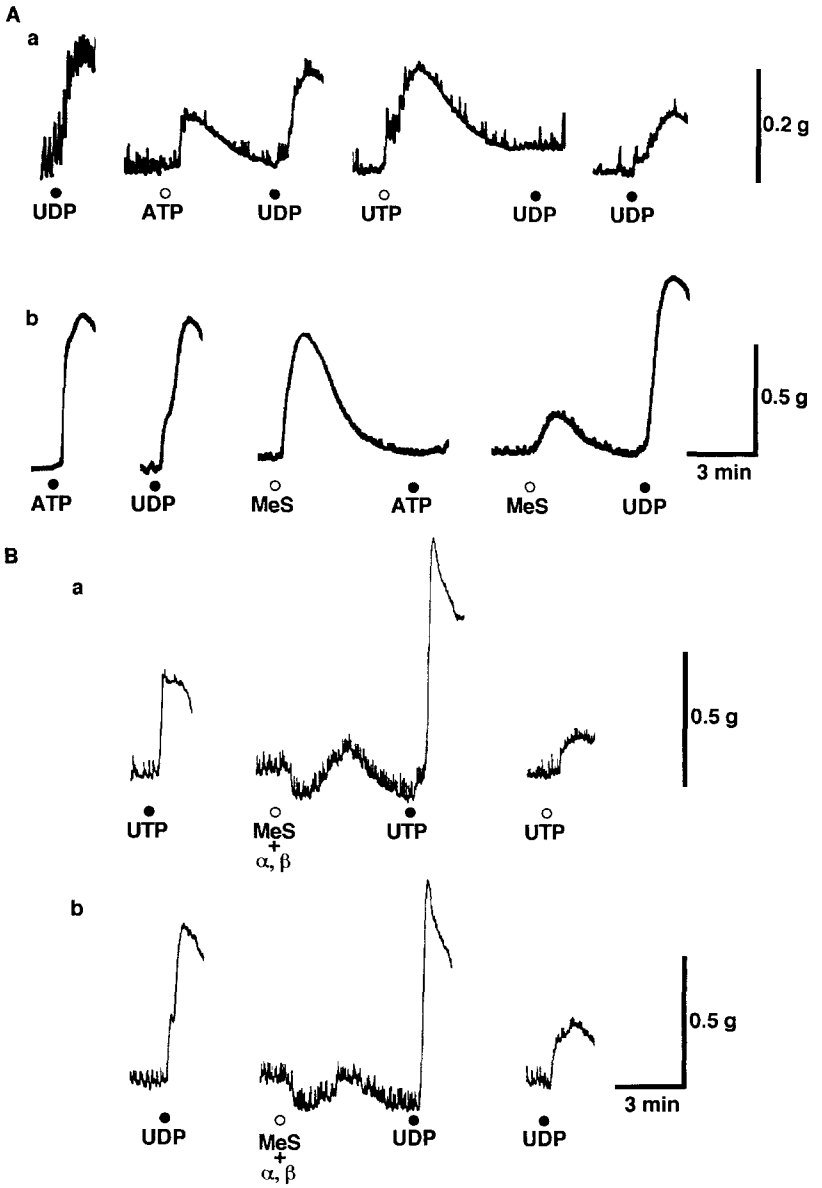

Fig. 5. Effect of desensitization with the related compounds on UTP- and UDP-induced contractions. (A) The original traces of UDP (100 μM)-induced contraction after desensitization with 100 μM ATP, 100 μM UTP (a) and 0.1 μM 2-methyltio ATP (MeS, b). In (a), 15 min after washout of the desensitizing drugs, the contractile responses to UDP were obtained again. The results of (a) and (b) were obtained from different tissues. (B) The original traces of 100 μM UTP-induced (a) and 100 μM UDP-induced (b) contractions after pre-treatment with both 0.1 μM 2-methyltio ATP (MeS) and 100 μM α,β-methylene ATP (α, β). The results of (a) and (b) were obtained from different tissues.

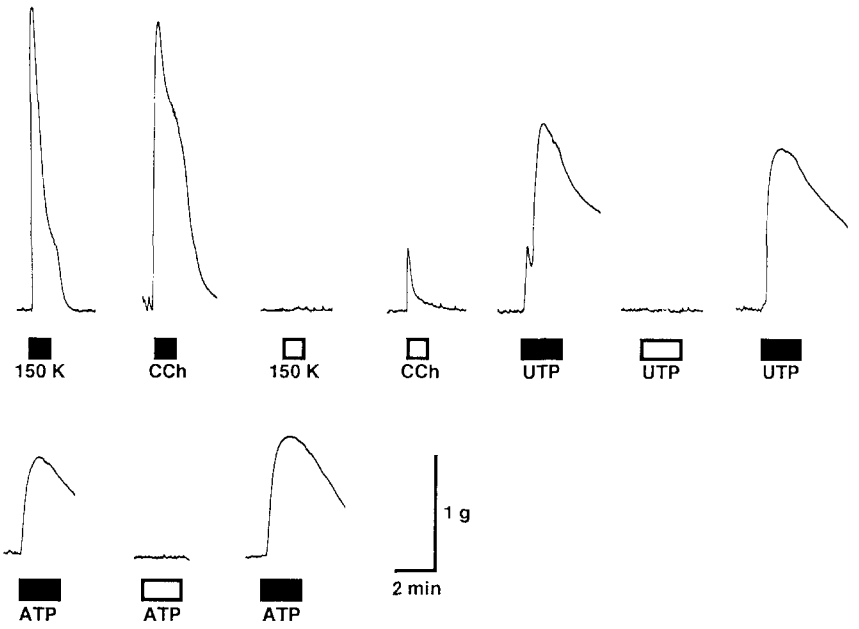

Fig. 6. Representative responses to 150 mM K^+ (150 K), carbachol (CCh, 100 μM), UTP (100 μM) and ATP (100 μM) in the presence (■) or absence of external Ca^{2+} (□). Tissues were incubated for 2 min with Ca^{2+}-free solution containing 2 mM EGTA. Similar results were obtained in 6 other experiments.

= α,β-methylene ATP > adenosine = uridine. According to the criteria for purinoceptor subtypes (Dubyak and El-Moatassim, 1993; Fredholm et al., 1994), these results indicate that the contractile response to ATP is mediated via P_{2Y} purinoceptors in the rat gastric circular muscle. α,β-Methylene ATP was the most potent agonist for producing relaxation, suggesting the involvement of P_{2X} purinoceptors in relaxation. In the longitudinal smooth muscle of the rat gastric fundus, Matharu and Hollingsworth (1992) have reported that the rank order of relaxant potency is 2-methylthio ATP ≫ α,β-methylene ATP > ATP and that ATP causes relaxation via P_{2X} purinoceptors. The order for P_{2X} purinoceptors is reported to vary with tissues and experimental conditions, since ectonucleotidases degrade ATP and 2-methylthio ATP but not α,β-methylene ATP (Welford et al., 1986; Welford et al., 1987; Bailey and Hourani, 1992). Kennedy and Leff (1995) mentioned that the potency order for P_{2X} purinoceptors is 2-methylthio ATP ≥ ATP > α,β-methylene ATP when enzymatic degradation is prevented. In tissues in which P_{2X} and P_{2Y} purinoceptors coexist, the estimation of agonist potency may be more complex. In the mouse vas deferens, Boland et al. (1992) have proposed that the low potency of ATP for contractile P_{2X} purinoceptors is mainly explained by simultaneous activation of relaxant P_{2Y} purinoceptors.

The effects of UTP have been reported to be mediated by P_{2X} purinoceptors in the mouse vas deferens (Von Kügelgen et al., 1990) and P_{2Y} purinoceptors in the rat colon muscularis mucosae (Hourani et al., 1993). The responsiveness to UTP is lower than that to ATP in these tissues. In the present study, a small relaxation induced by UTP seemed to be mediated via P_{2X} purinoceptors in the circular smooth muscle of the rat proximal stomach. The inhibition of ATP-induced contraction by desensitization with UTP suggests that UTP acts on the same P_{2Y} purinoceptors as ATP. However, 2-methylthio ATP, a potent P_{2Y} purinoceptor agonist, could desensitize ATP-induced contraction but not UTP-induced contraction. Furthermore, the inhibitory effects induced by desensitization with UTP on ATP-induced contraction were much smaller than those with 2-methyltio ATP and ATP-γ-S and the desensitization with ATP did not inhibited UTP-induced contraction. The UTP-induced contraction may be partly mediated via P_{2Y} purinoceptors and mainly via the other receptors.

4.2. P_{2U} or nucleotide receptors

The receptors for which the rank order of potency is UTP ≥ ATP = ATP-γ-S > 2-methylthio ATP have been termed 'P_{2U} purinoceptors' or 'nucleotide receptors' (O'Connor et al., 1991; O'Connor, 1992; Fredholm et al., 1994). In the present experiment, however, UTP did not seem to cause contraction via P_{2U} purinoceptors, because desensitization with ATP or ATP-γ-S did not inhibit UTP-induced contraction although ATP-induced contraction was inhibited by desensitization with ATP-γ-S. It seems unlikely that the inhibition of ATP-induced contraction by desensitization with 2-methylthio ATP and ATP-γ-S was a result of relaxation evoked by these agonists, because there was no inhibition of contractile response to ATP in tissues desensitized with α,β-methylene ATP, which was the most potent relaxant. In the desensitized

tissue, significant augmentation was obtained with contraction induced by ATP and UTP. Similar observation has been reported in the rat gastric fundus (Matharu and Hollingsworth, 1992).Although the reason of this augmentation is not clear, it might be due to the desensitization of receptors mediating relaxation.

Suramin, a P_2 purinoceptor antagonist, inhibited UTP-induced contraction in the present study. However, the selectivity of suramin as a P_{2U} antagonist is unclear, because suramin inhibits the responses to UTP via P_{2U} purinoceptors in PC12 cells (Murrin and Boarder, 1992), DDT_1 MF-2 vas deferens cells (Van der Zee et al., 1992) and human lens epithelial cells (Riach et al., 1995) but does not inhibit P_{2U} purinoceptor-mediated responses in bovine aortic endothelial cells (Wilkinson et al., 1993).

4.3. Pyrimidinoceptors

In the perfused rat liver, UTP increases portal pressure via receptors distinct from P_2 purinoceptors and the potency order is UTP > UDP > CTP = UMP (Häussinger et al., 1987). UTP has been proposed to act via pyrimidinoceptors at which it is more effective than UDP, UMP and uracil, and CTP is only a relatively weak agonist (Seifert and Schultz, 1989). It has been shown that ATP, α,β-methylene ATP and UTP cause depolarization and that desensitization with α,β-methylene ATP depresses responses to ATP but not those to UTP, suggesting the presence of pyrimidinoceptors in the rat superior cervical ganglion (Connolly et al., 1993; Connolly, 1994). In the present study, the potency order for contraction among pyrimidines was UDP = UTP ≫ UMP > uridine = CTP and desensitization with ATP, ATP-γ-S and 2-methylthio ATP did not suppress the responses to UTP. These results suggest that the receptors for UTP in the rat stomach have properties similar to pyrimidinoceptors. In the rat duodenum, UTP has been shown to cause contraction via suramin-insensitive pyrimidinoceptors (Johnson and Hourani, 1994). However, suramin blocked UTP-induced contraction in the rat stomach. Therefore, the receptors for UTP presented in rat proximal stomach may have some properties different from those in the rat duodenum.

Recently, P_2 purinoceptors have been classified on the basis of their function and structure (Abbracchio and Burnstock, 1994). However, there is little information about the mechanisms underlying the contraction mediated by pyrimidinoceptors. In the present study, contractions induced by ATP and UTP were significantly inhibited by nifedipine and abolished in the absence of extracellular Ca^{2+}. These results suggest that Ca^{2+} entry via voltage-dependent L-type Ca^{2+} channels is involved in ATP- and UTP-induced contraction. The activation of P_2 purinoceptors induces Ca^{2+} entry from extracellular solution and/or Ca^{2+} release from intracellular Ca^{2+} stores (Dubyak and El-Moatassim, 1993). In the mouse cortical thick ascending limbs (Paulais et al., 1995), the activation of P_{2U} purinoceptors has been reported to elevate the intracellular Ca^{2+} level by triggering Ca^{2+} entry via a nifedipine-sensitive pathway. One of the cloned receptor subtypes has been reported to be GTP-binding protein-coupled P_2 purinoceptor activated by UTP and UDP (Communi et al., 1995; Nguyen et al., 1995). Further experiments are necessary for precise characterization of the receptors activated by ATP and UTP.

In summary, the present results suggest that ATP causes contraction via P_{2Y} purinoceptors and relaxation via P_{2X} purinoceptors based on the potency order of agonists. In addition, UTP induces contraction via receptors different from the common purinoceptors, although the receptors for UTP are antagonized by suramin in a manner similar to P_2 purinoceptors.

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