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Evidence for Adenosine Triphosphate (ATP) as an Excitatory Neurotransmitter in Guinea-Pig Gastric Antrum

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We explore the question of whether adenosine 5'-triphosphate (ATP) acts as an excitatory neurotransmitter in guinea-pig gastric smooth muscle. In an organ bath system, isometric force of the circular smooth muscle of guinea-pig gastric antrum was measured in the presence of atropine and guanethidine. Under electrical field stimulation (EFS) at high frequencies (>20 Hz), NO-mediated relaxation during EFS was followed by a strong contraction after the cessation of EFS (a "rebound-contraction"). Exogenous ATP mimicked the rebound-contraction. A known P_{2Y}-purinoceptor antagonist, reactive blue 2 (RB-2), blocked the rebound-contraction while selective desensitization of P_{2X}-purinoceptor with α,β-MeATP did not affect it. ATP and 2-MeSATP induced smooth muscle contraction, which was effectively blocked by RB-2 and suramin, a nonselective P_2-purinoceptor antagonist. Particularly, in the presence of RB-2, exogenous ATP and 2-MeSATP inhibited spontaneous phasic contractions, suggesting the existence of different populations of purinoceptors. Both the rebound-contraction and the agonist-induced contraction were not inhibited by indomethacin. The rank orders of agonists' potency were 2-MeSATP > ATP $\geq$ UTP for contraction and α,β-MeATP $\geq$ β,γ-MeATP for inhibition of the phasic contraction, that accord with the commonly accepted rank order of the classical P_{2Y}-purinoceptor subtypes. Electrical activities of smooth muscles were only slightly influenced by ATP and 2-MeSATP, whereas α,β-MeATP attenuated slow waves with membrane hyperpolarization. From the above results, it is suggested that ATP acts as an excitatory neurotransmitter, which mediates the rebound-contraction via P_{2Y}-purinoceptor in guinea-pig gastric antrum.

Key Words: ATP, Smooth musle, Purinoceptors, Stomach

INTRODUCTION

Gastrointestinal (GI) smooth muscles generally become relaxed when non-adrenergic non-cholinergic (NANC) nerves are stimulated. It has been considered that the relaxation is mediated by inhibitory NANC neurotransmitters such as nitric oxide (NO), vasointestinal polypeptide (VIP), and adenosine 5'-triphosphate (ATP), which are released from enteric nerves of GI tract (Bult et al, 1990; Crist et al, 1992). Among them, ATP is now well established as an endogenous purinergic neurotransmitter in the GI tract and the urinary bladder and as a cotransmitter of noradrenaline in sympathetic nerve terminals (Sneddon & Westfall, 1984; Fujii, 1988; Hoyle et al, 1990; Crist et al, 1992). Until now, ATP has been considered the most likely inhibitory NANC neurotransmitter in mammalian GI smooth muscles. Especially in the gastric fundus, ATP has been proposed as a major inhibitory neurotransmitter responsible for the receptive relaxation after meal (Beck et al, 1988). However, GI smooth muscles showed excitatory re-

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sponses to ATP and its analogues in the guinea-pig ileum (Wiklund & Gustafsson, 1988) and in the rat colon muscalaris mucosae (Bailey & Hourani, 1990). In the rat gastric fundus, exogenous ATP evoked a biphasic response, *i.e.*, transient relaxation followed by contraction (Matharu & Hollingsworth, 1992). Consequently, these results imply that ATP acts not only as an inhibitory neurotransmitter but also as an excitatory transmitter in GI smooth muscles. Preliminary experiments in our laboratory showed that the smooth muscle of the guinea-pig stomach contracted when challenged with exogenous ATP (Ahn et al, 1995). Therefore, this study was designed to find evidence that ATP acts as an endogenous excitatory neurotransmitter and to determine receptor populations expressed in this preparation by using pharmacological tools.

METHODS

Measurement of contractile force in isolated smooth muscle strips

Isometric contractions were measured in isolated circular muscle strips of guinea-pig gastric antrum. Muscle strips (2~3 mm wide, 10~12 mm long) from the antral part were cut parallel to the inner circular muscle layers. Muscle strips were placed in a vertical chamber (50 ml) containing bicarbonate-buffered Tyrode solution maintained at 36.5°C and gassed with 5% CO_2/95% O_2. Bicarbonate-buffered Tyrode solution contained (in mM): NaCl 116, KCl 5.4, $NaHCO_3$ 24, NaH_2PO_4 1, $MgCl_2$ 1, $CaCl_2$ 2, glucose 5.6 (pH 7.35 with HCl). One end of the strip was fixed on platinum steel tissue hook and the other end was connected to the force transducer (Harvard, U.K.) to measure contractile forces. A 1 g load was placed on each preparation and the tissues were equilibrated for more than 60 min. Electrical field stimulation (EFS; 5 sec, 0.5 ms pulse duration) with supramaximal voltages (>70 V) was applied via a pair of platinum plate-electrodes designed parallel to the preparation. NANC nerves of the preparation were stimulated with EFS in the presence of atropine (1 μM) and guanethidine (10 μM). Under control condition, the circular smooth muscles of guinea-pig gastric antrum showed spontaneous phasic contractions at a relatively constant magnitude. In a series of experiments, a non-cumulative concentration-response curve to ATP and its analogues were obtained. In most cases, however, ATP and analogues of ATP showed marked tachyphylaxis. Therefore, we compared the potency order of several analogues of ATP within a limited range (1~10 μM). After obtaining control responses to an agonist, strips were rapidly washed several times with fresh Tyrode solution, and the next experiment with another agonist was conducted at least 20 min after the first trial. To block P_{2Y} receptors, reactive blue 2 (RB-2) was treated for 10~15 min. Suramin (100 μM), a known nonselective P_2 receptor blocker was pretreated for more than 30 min, and the responses to ATP and its analogues were obtained again in the presence of the antagonists. To desensitize the P_{2X} receptors (blockade of P_{2X} receptors), α,β-methylene ATP (α,β-MeATP; 100 μM) was pretreated for more than 20 min. Suppression of spontaneous phasic contraction was observed after the treatment with α,β-MeATP, and it was recovered to the control level about 20 min later. When desensitization was successfully achieved, muscle strips did not further respond to the same concentration of α,β-MeATP. To suppress the generation of prostaglandins, indomethacin (10~20 μM) was treated for more than 20 min before the application of experimental stimuli. To compare maximal contractile responses in each tissue during EFS, magnitudes of contractions provoked by EFS were normalized to the mean amplitudes of the phasic contractions of strips which were not electrically stimulated. To compare the contractile responses to several agonists for purinoceptors, magnitudes of contractions were normalized to that of the acetylcholine (1 μM) in the absence of atropine. From the preliminary experiments, it was verified that atropine had no influence on the contractile responses induced by purinergic agonists.

Measurement of electrical activity of the muscle

Muscle strips (2~3 mm wide, 10~12 mm long) were mounted on a silicon rubber in a 2 ml horizontal chamber. Strips were pinned out at one end with tiny pins and the other end was connected to the isometric force transducer (Harvard, U.K.). Strips were constantly perfused at a rate of 2~3 ml/min with a bicarbonate-buffered Tyrode solution. Electrical activity of the muscle was recorded using conventional intracellular microelectrode filled with 3 M KCl (tip resistance of 40~80 MΩ) and drawn by a chart recorder.

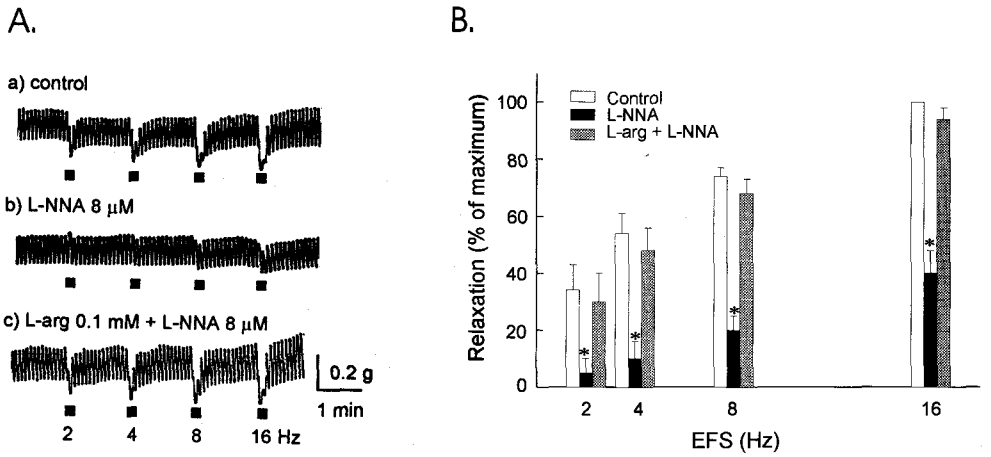

Fig. 1. NO-mediated relaxation produced by the stimulation of non-adrenergic non-cholinergic (NANC) nerves of the circular smooth muscle of guinea-pig gastric antrum. Relaxations were induced by trains of electrical field stimulation (EFS; 2～16 Hz, 5 sec, 0.5 ms pulse duration, supramaximal voltages) in the presence of atropine (1 μM) and guanethidine (10 μM). A. NO-synthase antagonist (L-NNA, 8 μM) inhibited the relaxation which is reversed by the pretreatment of L-arginine (L-arg, 0.1 mM). B. Magnitude of the relaxation was normalized to the response at 16 Hz. n=12～16, $P<0.01$.

Chemicals

Tetrodotoxin (TTX), acetylcholine, atropine, guanethidine, L-arginine, N_W-nitro-L-arginine (L-NNA), adenosine 5'-triphosphate (ATP), α,β-methylene ATP (α,β-MeATP), β,γ-methylene ATP (β,γ-MeATP), 2-methylthio ATP (2-MeSATP), uridine 5'-triphosphate (UTP), reactive blue 2 (Cibacrone blue 3GA; RB-2), and indomethacin were all purchased from Sigma Chemicals Co. (St. Louis, MO, U.S.A.). Suramin was generously donated by Professor H. Suzuki (Department of Physiology, Nagoya City University School of Medicine, Nagoya, Japan).

Data analysis

Data are presented as mean±S.E.M with n, the sample size. Statistic significance was estimated by Student's paired or unpaired t-test. P values less than 0.05 were considered statistically significant.

RESULTS

Relaxation by nitric oxide (NO)

Low frequencies (2～16 Hz) of EFS relaxed muscles in a frequency-dependent manner (Fig. 1). Magnitudes of NANC nerves-mediated relaxation peaked at around 16 Hz (Fig. 1B) and the response was completely blocked by tetrodotoxin (TTX) (*see* Fig. 2B). Treatment with L-NNA (8 μM), well-known antagonist for nitric oxide synthase, markedly suppressed the NANC-nerve mediated relaxation. NANC nerves-mediated relaxations were completely recovered by the preincubation of the strips with L-arginine (0.1 mM), indicating that the relaxation is due to the release of nitric oxide.

Rebound-contraction and its modification by purinoceptor antagonists

Under EFS at high frequencies (>～20 Hz), NO-mediated relaxation during the EFS was followed by a strong contraction after the cessation of EFS, so we named it 'rebound-contraction'. The rebound-contraction lasted relatively long, and its amplitudes were dependent on the frequency of EFS (Fig. 2A). The rebound-contraction was reversibly blocked by 0.2 μM TTX (40 Hz; 375±57% *vs.* 104±12%, $P<$ 0.01, n=14), suggesting the NANC nerves-mediated responses (Figs. 2B & 3B). Both phentolamine and substance P receptor antagonist (N-acetyl-L-tryptophan 3,5-bis (trifluoromethyl)-benzyl ester) could not modify the rebound-contraction (data not shown). Exogenous ATP mimicked the rebound-contraction

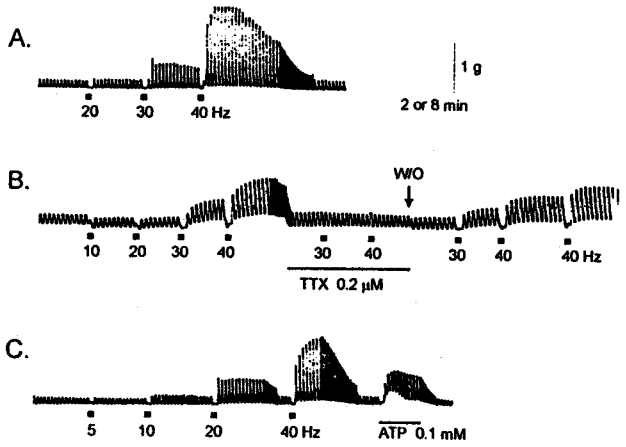

Fig. 2. A high frequency of EFS induced the rebound-contraction. The same experimental protocols, which described in *Fig. 1,* were applied. A. NO-mediated relaxation during the strong EFS (>20 Hz) was followed by the rebound-contraction. Magnitude of the rebound-contraction was dependent on the frequency of stimulus. B. TTX (0.2 μM) reversibly blocked the rebound-contraction. C. Exogenous application of ATP (100 μM) produced muscle contraction.

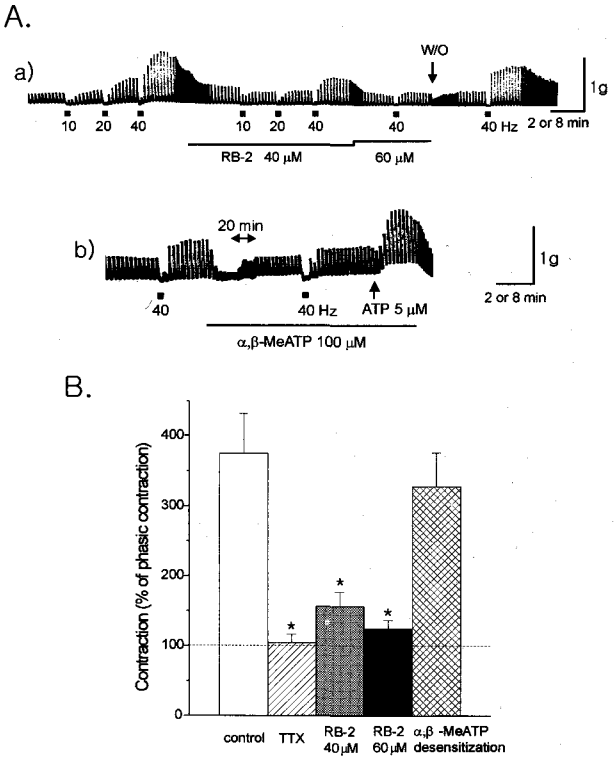

Fig. 3. Effects of purinoceptor antagonists on the rebound contraction. A. Reactive blue 2 (RB-2), a known P_{2Y}-purinoceptors antagonist, reversibly and dose-dependently inhibited the rebound-contraction (a). The rebound-contraction as well as ATP-induced contraction was not inhibited by the desensitization of P_{2X}-purinoceptors by α,β-methylene ATP (α,β-MeATP) (b). B. Amplitude of the rebound-contraction at 40 Hz was compared each other. Control, n=23; TTX, n=14; RB-2, n=9; α,β-MeATP desensitization, n=5; $P<0.01$.

and provoked strong contraction (Fig. 2C). So we tested whether ATP mediated the rebound-contraction. For the purpose, we used two different purinoceptor antagonists, *i.e.*, reactive blue 2 (RB-2) as a selective P_{2Y}-purinoceptor antagonist, and the strong desensitization of P_{2X}-purinoceptors with α,β-MeATP. Results were summarized in Fig. 3. P_{2Y}-receptor antagonist, RB-2 inhibited the EFS (40 Hz)-induced rebound-contraction dose-dependently (control=375±57%, 40 μM RB-2=156±21%, 60 μM RB-2=124±12%, $P<0.01$, n=9). In contrast to the results with RB-2, blockade of P_{2X}-receptor with α,β-MeATP desensitization showed no significant effect on the rebound-contraction (control=375±57%, α,β-MeATP =328±48%, n=5).

Effects of purinoceptor antagonists on the contractile responses of purinergic agonists

In the absence of purinoceptors antagonists, both ATP (5 μM) and the selective P_{2Y} agonist 2-MeSATP (5 μM) produced prominent contraction (Fig. 4Aa, b). Blockade of P_{2X}-receptor with α,β-MeATP-induced desensitization slightly attenuated the contraction provoked by ATP (270±22% *vs.* 228±30%, n=5) and 2-MeSATP (365±32% *vs.* 317±29%, n=5) to a statistically insignificant degree (Figs. 3Ab & 5). The selective P_{2Y} antagonist RB-2 (20 μM) completely blocked the contraction induced by ATP and 2-MeSATP. Moreover, both ATP and 2-MeSATP produced inhibitory force responses in the presence of RB-2 (Fig. 4Ba for ATP). After the blockade of P_{2Y}-receptor by RB-2, ATP and 2-MeSATP inhibited the spontaneous phasic contraction of the muscle up to 74.4±7.4% and 76±8.0% of the control, respectively (Fig. 5). The nonselective P_2-receptor antagonist suramin (100 μM) significantly ($P<0.01$) antagonized the contraction induced by ATP (270±22% *vs.* 110±5%, n=5) and 2-MeSATP (365±32% *vs.* 139±12%, n=5) (Figs. 4C & 5).

The selective agonist for P_{2X}-receptor, α,β-MeATP (5 μM) inhibited the spontaneous phasic contraction (Fig. 4Ac). In the presence of 100 μM

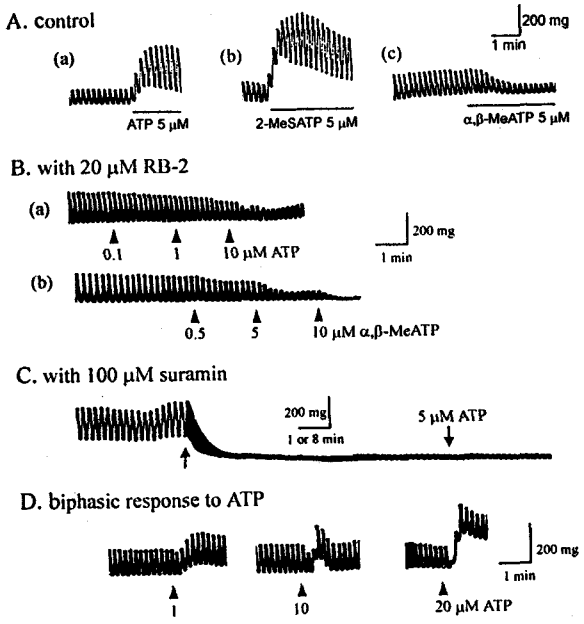

Fig. 4. Nucleotides-induced contractions and their modification by purinoceptors antagonists. A. ATP and 2-methylthio ATP (2-MeSATP) provoked contraction, while α,β-MeATP inhibited spontaneous phasic contraction. To compare the potency among nucleotides, the same concentration (5 μM) was applied. B. In the presence of RB-2, a P_{2Y}-purinoceptors antagonist, ATP and 2-MeSATP inhibited spontaneous phasic contraction in a dose-dependent manner. C. Nonselective P_2-purinoceptors blocker, suramin (100 μM) completely blocked the ATP-induced contraction. D. ATP produced biphasic contractile response over 10 μM.

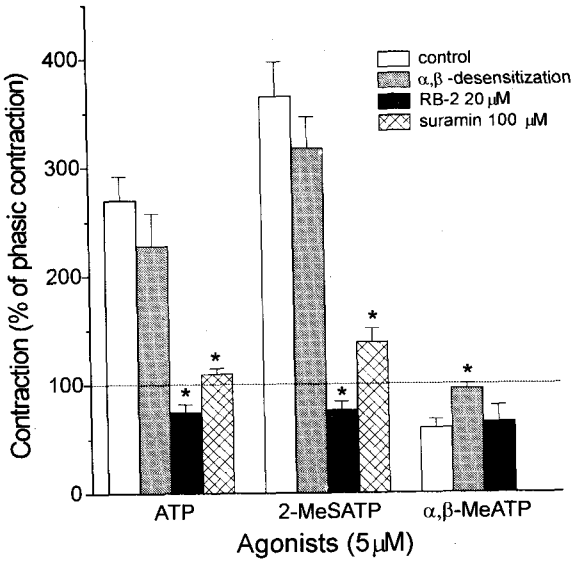

Fig. 5. Comparison of blocking effects of purinoceptor antagonists on the contractions produced by exogenous ATP and its analogues. ATP- and 2-MeSATP-induced contractions were markedly antagonized by RB-2 (20 μM; n=12 for each agonist, $P<0.01$), and suramin (100 μM; n=5 for each agonist, $P<0.01$). α,β-MeATP-induced relaxation was not modified by RB-2 (n=6).

α,β-MeATP, the second application of α,β-MeATP produced no contractile response (60±8% *vs.* 96±4%, n=10), suggesting a successful desensitization of P_{2X}-receptor (Fig. 5). However, the inhibition of the spontaneous phasic contraction by α,β-MeATP was not modified by RB-2 (60±8% *vs.* 65±15%, n=6) (Figs. 4Bb & 5).

The muscle showed biphasic contractile responses to the exogenous ATP and 2-MeSATP (especially upon >10 μM), *i.e.*, a transitory inhibition of the phasic contraction was followed by a contraction (Fig. 4D). At present, we can not explain the mechanism, but the involvement of enteric nerves was excluded because the responses were not modulated by TTX (data not shown). The possible mechanism will be described in *Discussion*.

The rank order of potency of agonists on the contractile force

Traditionally, a division of the P_2-purinoceptor into the P_{2X} and P_{2Y} subtypes was largely based on the rank order of purinergic agonists' potency in a number of tissues (Burnstock & Kennedy, 1985). In order to elucidate the purinoceptor subtypes expressed in smooth muscle of guinea-pig gastric antrum, we compared the rank order of the potency of several nucleotide analogues (Fig. 6). As described in *Methods*, a non-cumulative concentration-response curves were obtained with ATP, 2-MeSATP, UTP, β,γ-MeATP or α,β-MeATP within a limited range of concentrations (1~10 μM). ATP and 2-MeSATP produced contraction in a dose-dependent manner. 2-MeSATP produced stronger contraction than ATP. The magnitude of contraction induced by 1, 5 and 10 μM of 2-MeSATP and ATP was 57±7, 102±12 and 124±9 %, and 37±6, 73±12 and 76±13% of the ACh (1 μM)-induced contraction, respectively. In addition to the above purine nucleotides, UTP, which has a pyrimidine-based structure, also contracted the muscle. Potency of UTP (34±6, 66±7 and 68±7% of

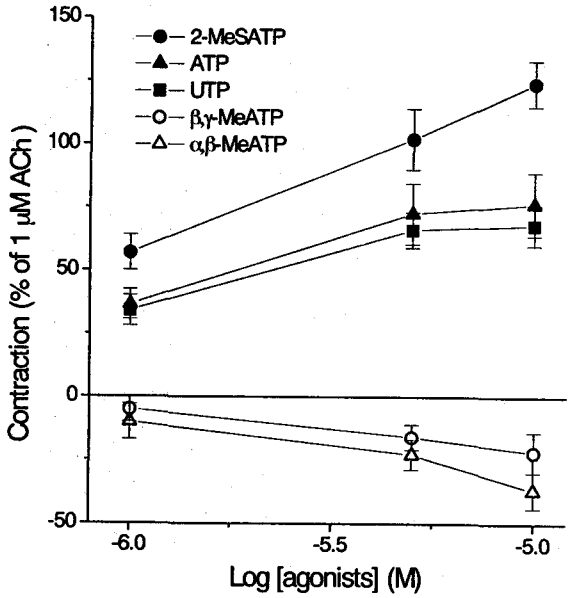

Fig. 6. The rank order of potency of agonists. 2-Methylthio ATP (2-MeSATP), ATP, and uridine 5'-triphosphate (UTP) produced contraction dose-dependently, whereas α,β-methylene ATP (α,β-MeATP) and β,γ-methylene ATP (β,γ-MeATP) relaxed muscle. Magnitude of the agonists-induced contractions were normalized to the acetylcholine (1 μM)-induced contraction. n=6~10.

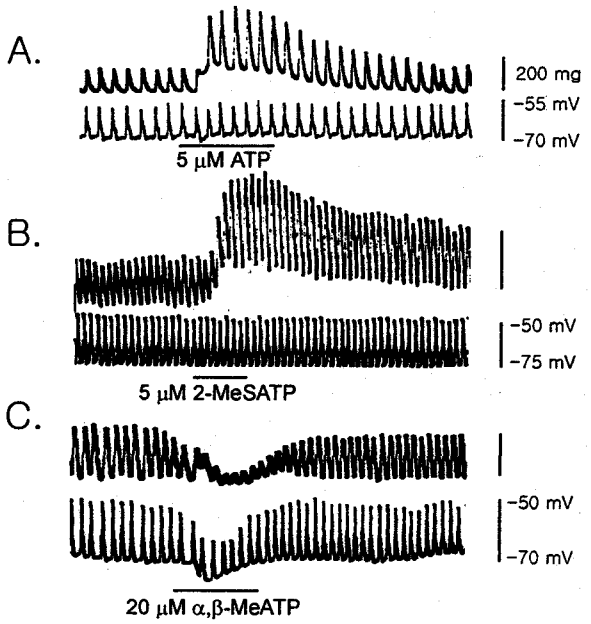

Fig. 7. Effects of ATP and its analogues on the electrical activities of the guinea-pig gastric antral smooth muscle. A, B. ATP and 2-methylthio ATP (2-MeSATP) had no marked influence on the slow waves. C. α,β-Methylene ATP (α,β-MeATP) attenuated the amplitude of slow waves with membrane hyperpolarization. Similar responses were recorded from other smooth muscle preparations. Number of muscle preparations recorded: 9, 5, and 4 for ATP, 2-MeSATP, and α,β-MeATP, respectively.

the ACh (1 μM)-induced contraction for 1, 5 and 10 μM UTP) was slightly weaker than that of ATP. Both β,γ-MeATP and α,β-MeATP inhibited the spontaneous phasic contraction. The inhibitory potency of β,γ-MeATP was slightly weaker than that of α,β-MeATP. The magnitudes of relaxation induced by 1, 5 and 10 μM of β,γ-MeATP and α,β-MeATP was -5 ± 5, -16 ± 5 and -22 ± 8 %, and -10 ± 7, -23 ± 6 and -37 ± 7% of the ACh (1 μM)-induced contraction, respectively.

As a result, the rank order of nucleotides' potency in producing force response of guinea-pig gastric antral smooth muscle was 2-MeSATP > ATP ≥ UTP for contraction, and α,β-MeATP≥ β,γ-MeATP for the inhibition of spontaneous contraction (Fig. 6). This accords with the commonly accepted rank order for classical P_{2Y}-purinoceptor subtypes (Burnstock & Kennedy, 1985).

The action mechanisms of purine nucleotides for contractility

The effects of excitatory (ATP and 2-MeSATP) and inhibitory (α,β-MeATP) purine nucleotides on the electrical activities (slow waves) of the smooth muscles were investigated. Five micromole of ATP and 2-MeSATP produced contraction with no significant changes in slow waves (Fig. 7A, B). Although transitory slight hyperpolarization could be recorded in some preparations (3 of 12 preparations, data not shown), the majority showed no change in slow waves. Inhibitory purine nucleotide, α,β-MeATP (20 μM) decreased the amplitude of slow waves with membrane hyperpolarization (7.3 ± 0.9 mV, n=4) (Fig. 7C).

The results shown in Figs. 2~7 suggested that ATP, which was released from enteric nerves, provoked the rebound-contraction through P_{2Y} receptors. In many smooth muscles, it has been suggested that the activation of P_{2Y} receptor produced prostaglandins from the smooth muscles, thus finally provoking contraction indirectly (Anderson, 1982; Carter et al, 1988). So we tested whether prostaglandins are involved in both the rebound-contraction and the ATP-

induced contraction. Incubation of the muscle strips with indomethacin (10~20 μM) had no influence on the rebound-contraction and the contraction provoked by ATP (5 μM) or 2-MeSATP (5 μM) (data not shown). In the same preparation, as expected, RB-2 (20~40 μM) completely blocked the rebound-contraction (data not shown). These results indicates that prostaglandins are not involved in P_{2Y}-mediated contraction in smooth muscle of guinea-pig gastric antrum.

DISCUSSION

The results presented in this paper suggest that NO and ATP are endogenous NANC neurotransmitters in the smooth muscle of guinea-pig gastric antrum. As observed in other visceral smooth muscles (Bult et al, 1990), NO behaves as an inhibitory neurotransmitter in this tissue. Under EFS at high frequencies ($> \sim 20$ Hz), the NO-mediated relaxation was followed by the rebound-contraction after the cessation of EFS. From the pharmacological experiments, the followings were obtained: (1) The rebound-contraction is completely blocked by TTX in the presence of atropine and guanethidine; (2) Exogenous ATP contracts the muscle; (3) The P_{2Y} receptor antagonist but not the blockade of P_{2X} receptor antagonized the rebound-contraction; (4) The rank order of nucleotides' potency in the contraction is in accordance with the classical subdivision of P_{2Y}-purinoceptor (2-MeSATP > ATP $\geq$ UTP > β,γ-MeATP $\geq$ α,β-MeATP). These results, therefore, suggest that ATP is an endogenous excitatory NANC neurotransmitter, which produces the rebound-contraction via P_{2Y} purinergic receptors.

The pharmacological effects of extracellular adenosine and adenosine nucleotides are mediated through two distinct receptor classes named P_1 and P_2 purinoceptor. At the P_1 purinoceptor, adenosine acts with greater potency than ATP, whereas at the P_2 purinoceptor, ATP is more potent than adenosine (Burnstock & Kennedy, 1985). In the classic work on classification of P_2 purinoceptor subtypes in mammalian smooth muscle, Burnstock & Kennedy (1985) proposed a subdivision of the P_2-purinoceptor into P_{2X}- and P_{2Y}-subtypes on the basis of the rank order of agonist potency for contractile response. In general, the order of potency at the P_{2X}-purinoceptor is α,β-MeATP, β,γ-MeATP > ATP = 2-MeSATP, while at the P_{2Y}-purinoceptor, the order is reverse with 2-MeSATP >> ATP > β,γ-MeATP, α,β-MeATP. Although the deficiencies of agonist-based receptor characterization are recently recognized (O'Connor et al, 1991; Kennedy & Leff, 1995), these classical analyses have proved a satisfactory basis for further investigation. Even if a more definitive characterization of P_2-purinoceptors continued to be hindered by the lack of selective and competitive receptor antagonists, some kinds of purinergic antagonists have been proved to be useful in the evaluation of the receptor classification. Suramin was reported to be a competitive antagonist of the P_2 purinoceptor, but it does not appear to distinguish between P_{2X} and P_{2Y} purinoceptors (Bao et al, 1993; Ohno et al, 1993; Uneyama et al, 1994). Even if α,β-MeATP is an well known P_{2X}-selective agonist, prolonged treatment of cells with a high concentration of α,β-MeATP selectively desensitizes P_{2X} purinoceptor (Kasakov & Burnstock, 1983). Although its nonspecific effects have also been reported in some types of smooth muscles (Choo, 1981), reactive blue 2 (RB-2) was found to possess selectivity for the P_{2Y} receptor without effects at P_{2X}-purinoceptor or other receptors (Burnstock & Warland, 1987). α,β-MeATP desensitization and RB-2 were therefore commonly used as a useful tool to discriminate P_{2X}-purinoceptor from P_{2Y}-purinoceptor. To elucidate purinoceptors which cause contraction in the smooth muscle of guinea-pig gastric antrum, we tested the antagonistic profiles of the three putative P_2 receptor antagonists of suramin, α,β-MeATP and RB-2. RB-2 dose-dependently blocked the rebound-contraction and the contraction induced by purinergic agonists, while the desensitization with α,β-MeATP exert no influence on it. These results, therefore, strongly suggested P_{2Y}-mediated contraction in guinea-pig gastric antral smooth muscle. As we have suggested, P_{2Y}-mediated contractions were founded in other tissues such as rat colon (Bailey & Hourani, 1990) and rat gastric fundus (Matharu & Hollingsworth, 1992). These cases are considered unusual among GI smooth muscles. In contrast to the above suggestion, P_{2X}-mediated contraction was reported frequently in other smooth muscles such as urinary bladder (Fujii, 1988; Hoyle et al, 1990), vas deferens (Sneddon & Westfall, 1984), and mesenteric artery (Burnstock & Warland, 1987). These contradictory results, therefore, indicate a difference among species or tissues.

Although it is only tentative at present, recent molecular biological studies (Abbracchio & Burn-

stock, 1994) suggest many new P_{2Y} subclasses (P_{2Y1}-P_{2Y7}). Among them, subtypes which are sensitive to UTP are P_{2Y2} (originally named as P_{2U}) and P_{2Y4}-P_{2Y6} (O'Connor et al, 1991; Abbracchio & Burnstock, 1994). The P_{2U}/P_{2Y2} receptor is activated by UTP and ATP with similar potency and is not activated by nucleoside diphosphates. The P_{2Y4} receptor is highly selective for UTP over ATP, while the P_{2Y6} receptor is activated most potently by UDP and weakly by UTP, ADP, and ATP (Abbracchio & Burnstock, 1994; Nicholas et al, 1996). The agonist potency order of original P_{2U} receptor may be characterized as UTP=ATP > ADP > α,β-MeATP=2-MeSATP (O'Connor et al, 1991). UTP contracted muscle with similar potency to ATP in the present study. However, the rank order of nucleotides' potency obtained (2-MeSATP > ATP $\geq$ UTP > β,γ-MeATP $\geq$ α,β-MeATP) does not accord with that of P_{2U}/P_{2Y2} receptor, especially in case of 2-MeSATP. Although it is not conclusive at present, the different potency order may exclude the mediation of P_{2U}/P_{2Y2} receptor and P_{2Y4}-P_{2Y6} receptors in our tissue. Rather than P_{2U}/P_{2Y2} receptors, the obtained rank order in the present experiments is more similar to that of P_{2Y1} receptor (Webb et al, 1993; Abbracchio & Burnstock, 1994; Palmer et al, 1998). Therefore, it seems that P_{2Y1} receptor is the most probable receptor which mediates the nucleotide-induced contraction of guinea-pig gastric antral smooth muscle.

The smooth muscle of guinea-pig gastric antrum showed biphasic contractile responses to the exogenous ATP and 2-MeSATP, *i.e.*, a transitory inhibition of the spontaneous phasic contraction was followed by a contraction (*see* Fig. 4D). This result may suggest that different populations of purinoceptor subtypes are present in guinea-pig stomach. Such a biphasic contractile response to ATP has previously been reported in the guinea-pig trachea (Brown & Burnstock, 1981) and in contracted vascular rings (Ralevic & Burnstock, 1991). In the present study, after the blockade of P_{2Y} receptor by RB-2, ATP or 2-MeSATP inhibited the spontaneous contraction of the muscle with no detectable contraction. These responses strongly support the idea that ATP acts on different types of purinoceptors at the same time. Among those purinoceptors, one is certainly the P_{2Y} receptor which mediates the rebound-contraction. In this study, a known agonist for P_{2X} purinoceptor, α,β-MeATP elicited a pronounced relaxation without an ensuing contraction. Both α,β-MeATP and β,γ-MeATP relaxed muscles with the similar potency, and the relaxation evoked by α,β-MeATP was completely inhibited by the desensitization with α,β-MeATP. At glance, these results seem to indicate a P_{2X}-mediated relaxation. However, it needs a prudential interpretation, because it was proved that the P_{2X} purinoceptors are cation-selective receptor-ion channel complex which open on binding to extracellular ATP (Benham & Tsien, 1987; Evans et al, 1995). The cation channels play a role in fast synaptic transmission between neurons and from autonomic nerves to smooth muscles. When the channels are open by ATP, inward cationic current which is mainly carried by extracellular Na^+ and Ca^{2+} depolarizes membrane and thus leads muscle to contract (Benham & Tsien, 1987). The experiments on the simultaneous recording of the mechanical force and the membrane potential reveals the acting mechanisms of α,β-MeATP, *i.e.*, it relaxed the muscle with membrane hyperpolarization, which excludes P_{2X}-mediated cation channel opening. In contrast to α,β-MeATP, both ATP and 2-MeSATP contracted the muscle with no significant change in the membrane potential. These results indicate P_{2X} receptors are not involved in ATP- or 2-MeSATP-induced contraction.

P_{2Y} receptors are now well established as a G-protein coupled receptor (O'Connor et al, 1991). Biochemical and functional responses generally attributed to P_{2Y} receptor stimulation include phospholipase C activation and subsequent inositol phosphate production and resultant elevation of intracellular calcium level (O'Connor et al, 1991). In the previous study which used isolated single smooth muscle cells of guinea-pig gastric antrum (Ahn et al, 1995), it has been observed that ATP enhanced the activity of the spontaneous transient outward potassium current (STOCs), which is a reflection of sporadic release of Ca^{2+} from the intracellular calcium stores. This change of the STOCs by ATP might be explained by the increase of Ca^{2+} release from the internal calcium stores (Benham & Bolton, 1986). In our previous study (Ahn et al, 1995), Ca^{2+} release by ATP was prevented by the intracellular application of heparin which can block the Ca^{2+} release from the IP_3-sensitive Ca^{2+} stores. Pharmacological evidences obtained both from the present study and the previous result (Ahn et al, 1995) indicate that ATP produces contraction through the P_{2Y} receptor-phospholipase C cascade. In the present study, the dual force response upon ATP seems to be the result of two opposite

effects in the preparation expressing both the P_{2Y} and the other type of purinoceptors. It could be considered that ATP acts on P_{2Y}-purinoceptor and produces the contraction overcoming the relaxation, which is mediated by the other type of purinoceptors.

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