

ORIGINAL PAPER

Roles of P2X receptors and Ca^{2+} sensitization in extracellular adenosine triphosphate-induced hyperresponsiveness in airway smooth muscle

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Clinical and
Experimental
Allergy

Summary

Background The release of adenosine triphosphate (ATP) from the airway epithelial cells during the inflammatory process is considered to play an important role in the pathophysiology of asthma and chronic obstructive pulmonary disease.

Objective This study was designed to determine whether extracellular ATP is involved in the bronchial hyperresponsiveness as an interaction between epithelium and smooth muscle in the airways.

Methods We examined the contractile response to methacholine (MCh) before and after exposure to low concentrations ($\leq 10 \mu\text{M}$) of ATP in isolated, epithelium-denuded guinea-pig tracheal smooth muscle by measuring isometric tension. Intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) were assessed by fluorescent intensities of fura-2.

Results MCh-induced contractile force was increased with no change in $[\text{Ca}^{2+}]_i$ after exposure to $10 \mu\text{M}$ ATP for 15 min. The ability of ATP to enhance the MCh-induced contraction was markedly attenuated by suramin, a non-selective P2 receptor inhibitor. Pre-incubation with ATP γ S, a non-hydrolysable analogue of ATP and α, β -meATP, a P2X agonist, also enhanced the MCh-induced contraction. In contrast, uracil triphosphate, a P2Y agonist, did not affect the MCh-induced contraction. Y-27632, a Rho-kinase inhibitor, suppressed the ability of ATP to enhance the MCh-induced contraction. Moreover, PP1 and PP2, Src tyrosin kinase inhibitors, suppressed the enhancement of MCh-induced contraction by ATP.

Conclusion Pre-treatment with ATP induces hyperresponsiveness to MCh mediated by Ca^{2+} sensitization via the P2X receptor in airway smooth muscle. The present findings suggest the possible involvement of both the Rho-kinase and Src pathways in the intracellular mechanism of this phenomenon.

Keywords asthma, Ca^{2+} sensitivity, P2 receptor, Rho, Src, COPD

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Introduction

Extracellular purine nucleotides such as adenosine triphosphate (ATP) and uracil triphosphate (UTP) induce a wide range of biological responses in various organs including the respiratory system [1–4]. It is known that ATP causes anion secretion in airway epithelial cells [4, 5] and stimulates surfactant secretion from type II alveolar epithelial cells [6]. Moreover, it has been reported that extracellular ATP directly mobilizes intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) and induces contraction of airway smooth muscle [7–12]. Thus, these purine nucleotides, which are released from the epithelial cells during

the inflammatory process, may play an important role in the pathophysiology of asthma and chronic obstructive pulmonary disease (COPD) [13].

The effects of extracellular purine nucleotides are mediated by their specific receptors, on the plasma membrane, P2 receptors, subclassified as P2X and P2Y receptors both of which have been identified in the lung [2, 14]. P2X receptors are members of Ca^{2+} -permeable ion channels [15] whereas P2Y receptors are coupled to a G protein that links to the RhoA/Rho-kinase pathway [16]. Moreover, a recent report has demonstrated that Src, a member of non-receptor tyrosin kinases, is involved in the downstream signals by extracellular ATP in vascular smooth muscle cells [17].

Nevertheless, the post-receptor intracellular signal pathways responsible for regulating the muscle tone by extracellular ATP are still uncertain in airway smooth muscle.

This study was designed to determine whether pretreatment with ATP causes hyperresponsiveness to cholinergic stimulation in airway smooth muscle. We focused on the up-regulation of methacholine (MCh)-induced force generation in response to low concentrations ($\leq 10 \mu\text{M}$) of ATP exposure, which did not directly develop the contractile response *per se* at the concentrations used.

Methods

Isolation of tracheal smooth muscle tissues

The tracheae were excised from male Hartley guinea-pigs (250–350 g) after intra-peritoneal injection of pentobarbital (50 mg/body). The tracheal rings were opened by cutting them longitudinally in the cartilaginous region. The airway epithelium was denuded as previous reports have demonstrated that the airway smooth muscle tone in response to ATP is affected by the epithelium [9, 18, 19]. All animal procedures were approved by the Animal Care and Use Committee of Nagoya University Graduate School of Medicine.

Isometric force recording and solution

Freshly isolated muscle tissue strips containing two cartilaginous rings were prepared [20, 21]. One end of the cartilaginous ring was fixed to the wall of a tissue bath chamber, and the other end was connected to a force-displacement transducer to monitor isometric force in the organ bath (0.6 mL of volume). The normal solution was composed of (in mM): NaCl (137), KHCO_3 (5.9), CaCl_2 (2.4), MgCl_2 (1.2), and glucose (11.8), bubbled with a mixture of 99% O_2 and 1% CO_2 (pH 7.4). The Ca^{2+} -free solution was prepared by replacing 2.4 mM of CaCl_2 in the normal solution with 2.2 mM of NaCl and 0.2 mM of EGTA. The bathing solution was perfused in the organ bath at a constant flow of 3 mL/min. The temperature of the organ bath was maintained at 37 °C. To achieve complete relaxation, the Ca^{2+} -free solution was applied to the tissues at the end of each measurement. The resting tone was abolished by addition of indomethacin (2 μM) throughout the experiments. The force levels obtained after equilibration in the Ca^{2+} -free solution and 1 μM MCh were defined as 0% and 100% of contraction, respectively. The level of force corresponding to a particular contraction was expressed on a normalized scale between 0% and 100% contraction as defined above.

Measurement of intracellular Ca^{2+} concentrations

Under some experimental conditions, the force and $[\text{Ca}^{2+}]_i$ of airway smooth muscle tissues were simulta-

neously recorded as described previously [22, 23]. The preparation containing two cartilaginous rings was mounted horizontally in a tissue bath. The tissue strip was treated with fura-2/AM (10 μM , containing 0.01% of pluronic F-127) for 4 h at room temperature (22–24 °C). After loading, the normal solution was perfused at 37 °C for 50 min to wash out the extracellular fura-2/AM. The mucosal side of the muscle strips was exposed to the excitation light, and the light emitted from the strip was collected in a photomultiplier through a band-pass filter centred at 500 nm. The intensity of fura-2 fluorescence due to excitation at 340 nm (F_{340}) and at 380 nm (F_{380}) was measured after background subtraction using a spectrofluorometer (CAF-110, Japan Spectroscopic, Tokyo, Japan). The absolute amount of $[\text{Ca}^{2+}]_i$ was not calculated because the dissociation constant of fura-2 for Ca^{2+} in smooth muscle cytoplasm is different from that obtained *in vitro* [24]. Thus, the ratio of F_{340} to F_{380} (F_{340}/F_{380}) was used as an indicator of the relative level of $[\text{Ca}^{2+}]_i$. The values of F_{340}/F_{380} in response to 1 μM MCh and the Ca^{2+} -free solutions were defined as 100% and 0%, respectively.

Experimental protocols

The effects of P2 receptor agonists on the contraction and $[\text{Ca}^{2+}]_i$ elicited by MCh (1 μM) were assessed with ATP (a P2X and P2Y agonist), $\text{ATP}\gamma\text{S}$ (a non-hydrolysable analogue of ATP), $\alpha,\beta\text{-meATP}$ (a metabolically stable ATP analogue, a P2X receptor agonist), and UTP (a selective P2Y receptor agonist). MCh (1 μM) was applied for 10 min before and after exposure to these P2 receptor agonists. The normal solution was applied for 15–20 min to wash out each purinergic agonist. The contractile force and F_{340}/F_{380} levels by 1 μM MCh were approximately 60–80% and 80–90% of the maximum values, respectively [21, 22].

To assess the roles of the post-receptor signal pathways in the ATP-induced airway hyperresponsiveness (AHR) to MCh, either agent, a non-selective P2 receptor antagonist (suramin), a Rho-kinase inhibitor (Y-27632), or Src inhibitor (PP1 and PP2), was applied 3 min before ATP application. After each inhibitor and ATP were washed out for 15–20 min, 1 μM MCh was applied again to the identical tissues.

Reagents

ATP, UTP, $\alpha,\beta\text{-meATP}$, $\text{ATP}\gamma\text{S}$, indomethacin, MCh and verapamil were obtained from Sigma (St Louis, MO, USA). Y-27632, PP1 and PP2 were from Calbiochem (La Jolla, CA, USA). Fura-2/AM was from Dojin (Kumamoto, Japan).

Statistical analysis

All data are expressed as means $\pm$ SD. Student's *t*-test or analysis of variance, followed by Bonferroni's test and Tukey's test for *post hoc* analysis were used to evaluate the

statistical significance. $P < 0.05$ was considered to be statistically significant.

Results

Pre-treatment with adenosine triphosphate enhances airway smooth muscle contraction by methacholine

MCh ($1 \mu\text{M}$) was applied to the tissues for 10 min before and after exposure to ATP (0.1 – $10 \mu\text{M}$) for 15 min. Representative simultaneous recordings of F_{340}/F_{380} and force are shown in Fig. 1a. Application of $10 \mu\text{M}$ ATP led to modest increase in F_{340}/F_{380} with no contractile response. This augmented F_{340}/F_{380} by ATP was returned to the basal level when ATP was washed out for 20 min (Fig. 1a). ATP-induced contraction was not detectable until the concentration of this agent was increased up to $30 \mu\text{M}$ (data not shown).

When $1 \mu\text{M}$ MCh was applied to the identical tissues again after exposure to $10 \mu\text{M}$ ATP, the contraction was

significantly augmented; in contrast, the level of F_{340}/F_{380} was not affected (Fig. 1a). Pre-exposure to ATP (0.1 – $10 \mu\text{M}$) increased the subsequent contraction in response to MCh in a concentration-dependent manner (Fig. 1b). The values of the percent contraction and F_{340}/F_{380} by $1 \mu\text{M}$ MCh after exposure to $10 \mu\text{M}$ of ATP were $120.9 \pm 7.5\%$ ($P < 0.05$) and $95.5 \pm 3.7\%$ (not significant), respectively ($n = 6$), of the time-matched control values (Figs 1b and c).

Next, MCh (0.01 – $10 \mu\text{M}$) was cumulatively applied before and after incubation with $10 \mu\text{M}$ ATP for an equivalent min. The concentration–contraction curve for MCh was significantly shifted upward by ATP ($n = 6$) ($P < 0.05$) (Fig. 2).

Effects of pre-treatment with adenosine triphosphate analogue on the hyperresponsiveness to methacholine

The contraction elicited by MCh ($1 \mu\text{M}$) was also significantly enhanced by pre-exposure to ATP γ S (0.1 – $10 \mu\text{M}$), a

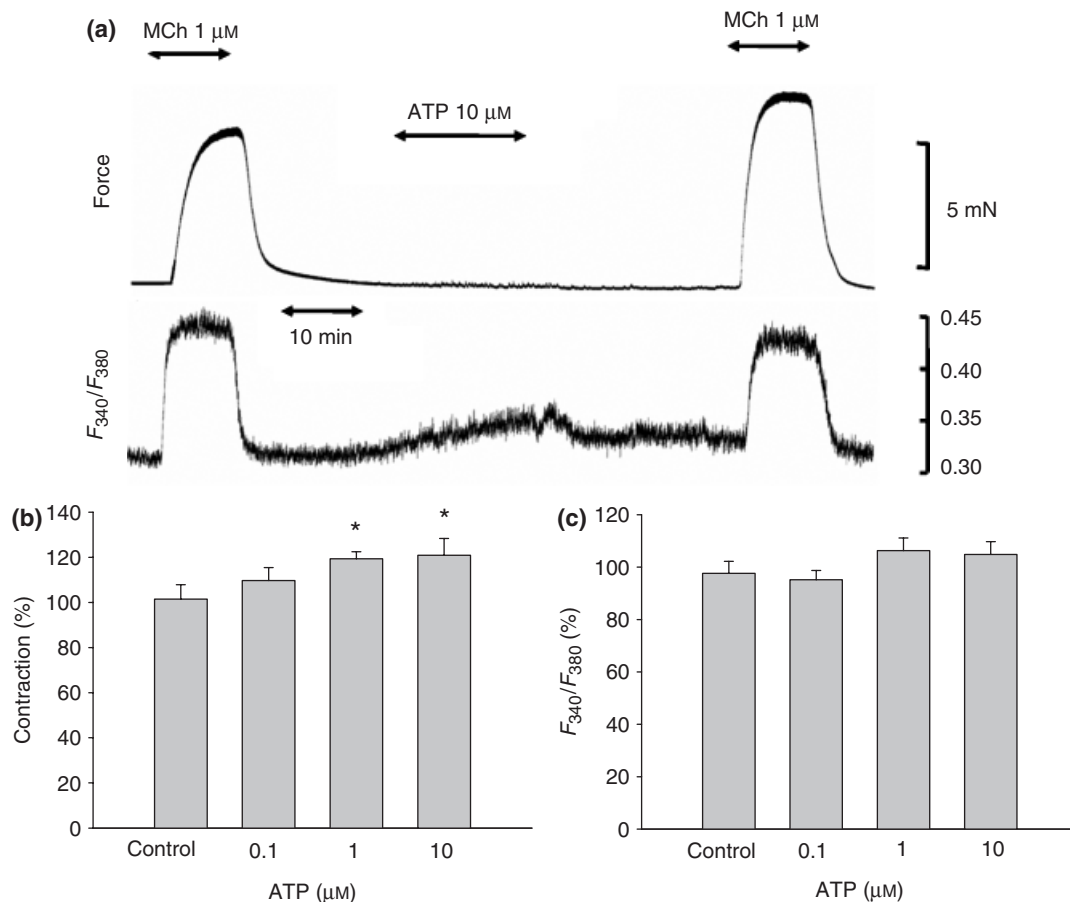


Fig. 1. (a) Representative recordings of the contractile force development (upper trace) and the corresponding change in fura-2 fluorescence signals (F_{340}/F_{380}), an index of intracellular Ca^{2+} concentrations (lower trace), induced by $1 \mu\text{M}$ methacholine (MCh) before and after application of $10 \mu\text{M}$ of adenosine triphosphate (ATP). Concentration-dependent effects of ATP (0.1 – $10 \mu\text{M}$) ($n = 6$) on the MCh-induced contraction (b) and the F_{340}/F_{380} (c). The values of the force and the F_{340}/F_{380} in response to MCh ($1 \mu\text{M}$) and in the normal solution were defined as 100% and 0%, respectively. Bars represent means $\pm$ SD. *Significantly different from the time-matched control values ($P < 0.05$).

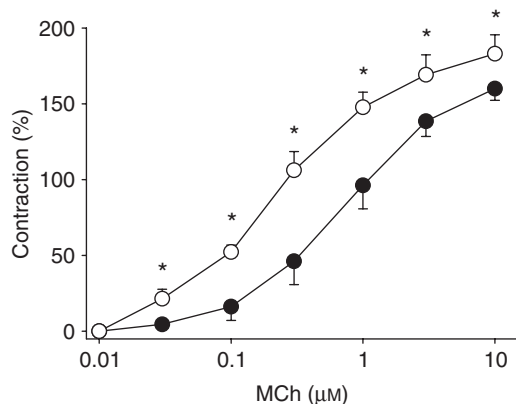


Fig. 2. Concentration-contractile force relation curves in response to methacholine (MCh) (0.01–10 μ M) in the presence (open circles) or absence (closed circles) of adenosine triphosphate (ATP) (10 μ M) ($n = 4$). The values of the force in response to MCh (1 μ M) and in the normal solution were defined as 100% and 0%, respectively. Bars represent means $\pm$ SD. *Significantly different from the values in the absence of ATP ($P < 0.05$).

non-hydrolysable analogue of ATP and both a P2X and P2Y agonist, for 15 min in a concentration-dependent manner (Fig. 3a). In contrast, F_{340}/F_{380} was not elevated (Fig. 3b), similar to the results shown in Fig. 1c. The values of the percent contraction and F_{340}/F_{380} with 10 μ M of ATP γ S were $116.0 \pm 8.0\%$ ($P < 0.05$ vs. the time-matched control values) and $103.6 \pm 8.4\%$ (not significant), respectively ($n = 4$).

Effects of a P2 receptor antagonist on adenosine triphosphate-induced hyperresponsiveness to methacholine

When ATP (10 μ M) was applied with suramin (10 μ M), a non-selective P2 receptor antagonist, the augmentation in 1 μ M MCh-induced contraction after exposure to ATP was significantly inhibited (Fig. 4). The level of F_{340}/F_{380} was not affected by suramin (Fig. 4). The values of the percent contraction and F_{340}/F_{380} after exposure to ATP with suramin were $106.1 \pm 4.1\%$ ($P < 0.05$ vs. the values without suramin) and $98.0 \pm 4.2\%$ (not significant), respectively ($n = 4$).

Involvement of the P2X receptor in the adenosine triphosphate-induced airway hyperresponsiveness

MCh (1 μ M) was applied to the tissues for 10 min before and after exposure to α, β -meATP (0.1–10 μ M), a selective P2X agonist, for 15 min. Representative simultaneous recordings of F_{340}/F_{380} and force are shown in Fig. 5a. Application of α, β -meATP (10 μ M) did not affect F_{340}/F_{380} directly. Pre-incubation with α, β -meATP (0.1–10 μ M) significantly increased MCh-induced contraction in a concentration-dependent manner, with no significant

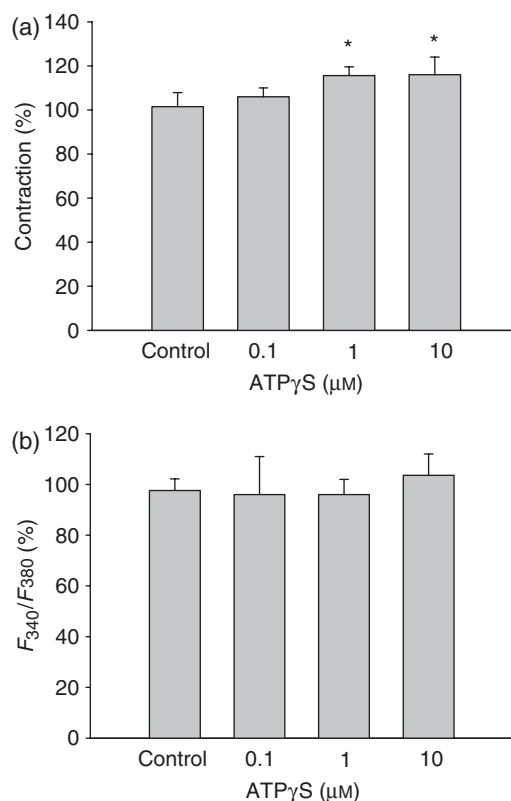


Fig. 3. Concentration-dependent effects of adenosine triphosphate (ATP) γ S (0.1–10 μ M) ($n = 4$) on the 1 μ M methacholine-induced contraction (a) and the F_{340}/F_{380} (b). Bars represent means $\pm$ SD. *Significantly different from the time-matched control values ($P < 0.05$).

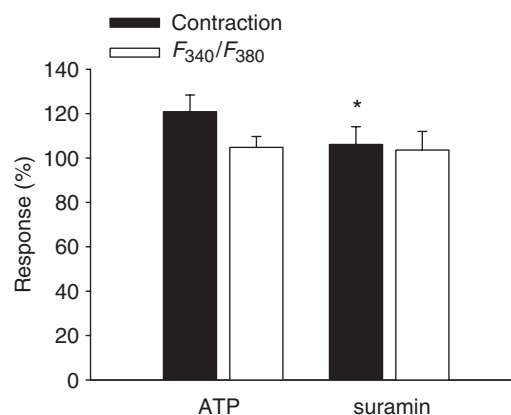


Fig. 4. Effects of suramin (10 μ M), a non-selective P2 receptor antagonist, on the 1 μ M methacholine-induced contraction (closed column) and the F_{340}/F_{380} (open column) after exposure to 10 μ M adenosine triphosphate (ATP). Bars represent the means $\pm$ SD. *Significantly different from the values in the absence of suramin ($P < 0.05$).

changes in the F_{340}/F_{380} (Figs 5b and c). The values of the percent contraction and F_{340}/F_{380} with 10 μ M of α, β -meATP were $117.0 \pm 8.1\%$ ($P < 0.05$) and $94.0 \pm 8.5\%$ (not significant), respectively ($n = 4$).

In contrast, neither contraction nor F_{340}/F_{380} elicited by MCh (1 μ M) were affected by exposure to UTP (10 μ M), a

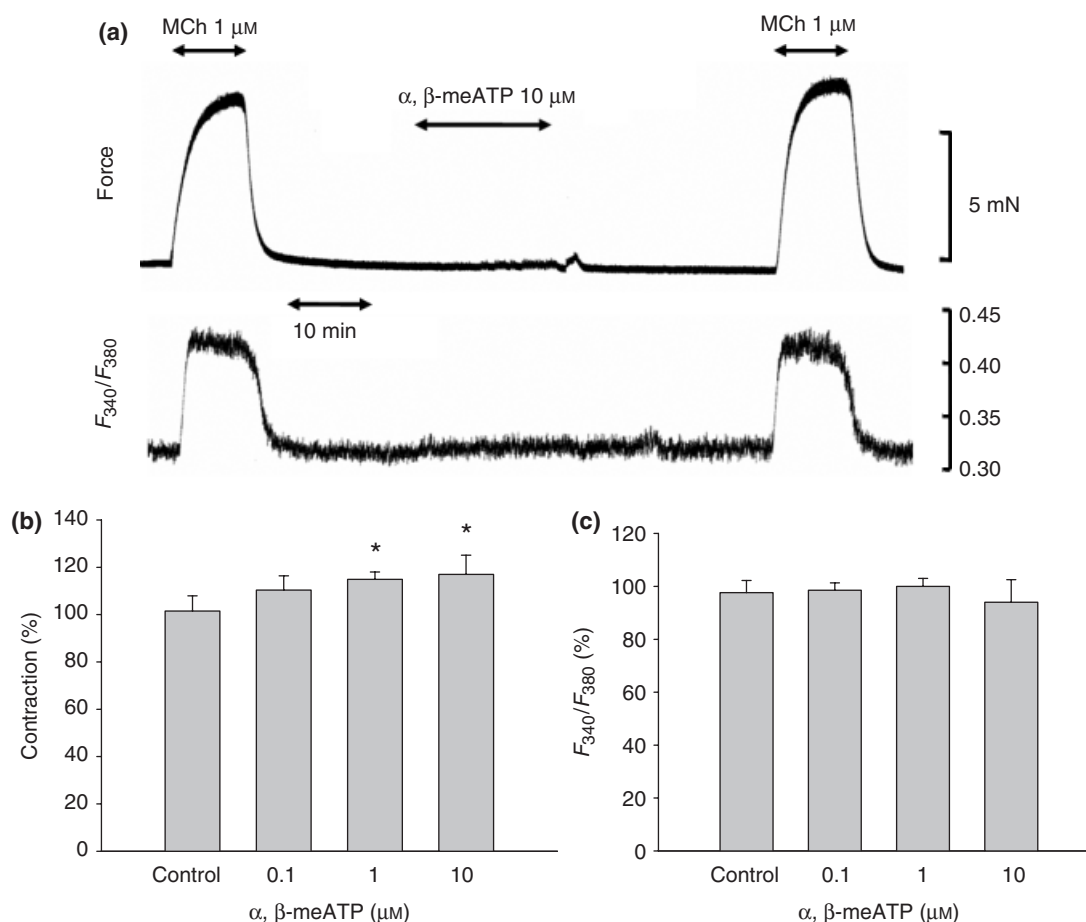


Fig. 5. (a) Representative recordings of the contractile force development (upper trace) and the corresponding change in F_{340}/F_{380} (lower trace) induced by 1 μM methacholine (MCh) before and after application of 10 μM of α, β -me adenosine triphosphate (ATP). Concentration-dependent effects of α, β -meATP (0.1–10 μM) ($n = 4$) on the 1 μM MCh-induced contraction (b) and the F_{340}/F_{380} (c). Bars represent means $\pm$ SD. *Significantly different from the time-matched control values ($P < 0.05$).

selective P2Y agonist, for an equivalent min (Fig. 6). The values of the percent contraction and F_{340}/F_{380} with 10 μM of UTP were $108.3 \pm 1.1\%$ and $99.7 \pm 2.1\%$, respectively ($n = 4$) (not significant).

Involvement of Rho-kinase in adenosine triphosphate-induced airway hyperresponsiveness

The contraction induced by 1 μM MCh was measured before and after exposure to 10 μM ATP in the presence of Y-27632 (0.1–10 μM), a Rho-kinase inhibitor. The augmentation in MCh-induced contraction by pre-exposure to ATP was significantly inhibited by Y-27632 in a concentration-dependent manner (Fig. 7a). The values of the percent contraction for MCh after exposure to ATP with 10 μM Y-27632 were $93.4 \pm 3.4\%$ ($n = 4$) ($P < 0.001$ vs. the values without Y-27632). To examine the effect of pre-treatment with Y-27632 on the subsequent MCh-induced contraction, 1 μM MCh was applied before and after treatment with 10 μM Y-27632 for 15 min. After

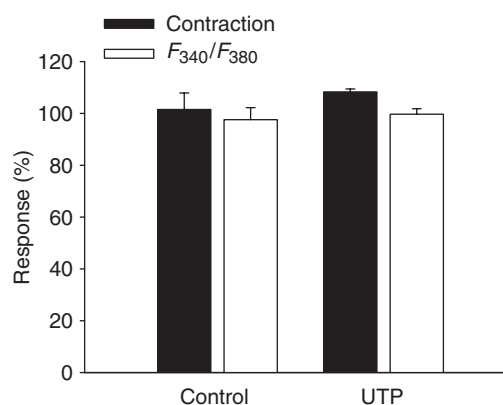


Fig. 6. Effect of uracil triphosphate (UTP) (10 μM) on the 1 μM methacholine-induced contraction (closed column) and the F_{340}/F_{380} (open column) ($n = 4$). Bars represent means $\pm$ SD. *Significantly different from the time-matched control values ($P < 0.05$).

Y-27632 was washed out for 15–20 min, the subsequent MCh-induced contraction was not significantly affected ($97.1 \pm 5.4\%$, $n = 4$).

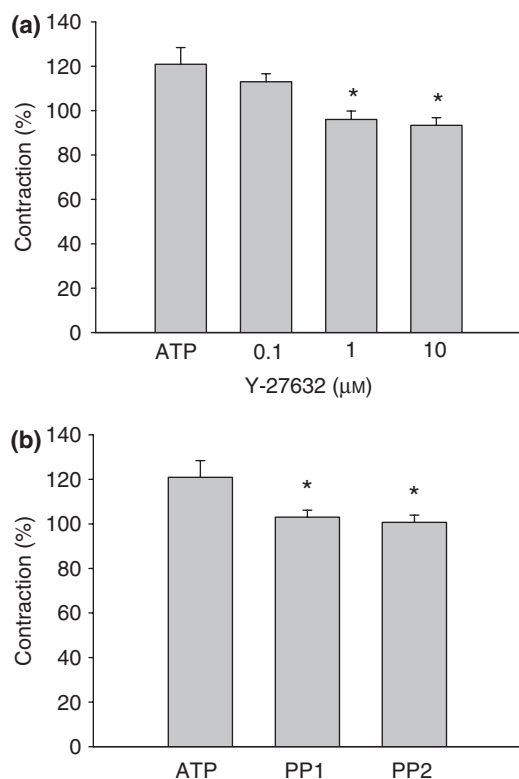


Fig. 7. Effects of Y-27632 (0.1–10 μM) ($n = 4$) (a) and Src inhibitors PP1 (10 μM) and PP2 (10 μM) ($n = 4$) (b) on the 1 μM methacholine-induced contraction after exposure to 10 μM adenosine triphosphate. Bars represent means $\pm$ SD. *Significantly different from the values in the absence of each inhibitor ($P < 0.05$).

Roles of Src tyrosin kinase in adenosine triphosphate-induced airway hyperresponsiveness

Next, the role of Src in the ATP-induced AHR was examined. After incubation with ATP (10 μM) in the presence of PP1 (10 μM) or PP2 (10 μM), Src inhibitors, 1 μM MCh-induced contraction was not enhanced (Fig. 7b). The values of the percent contraction for MCh after exposure to ATP with 10 μM PP1 and 10 μM PP2 were $103.0 \pm 3.1\%$ ($n = 4$) ($P < 0.001$) and $100.7 \pm 3.2\%$ ($n = 4$) ($P < 0.001$), respectively. MCh-induced contraction was not affected by pre-incubation with an equi-molar of PP1 or PP2 in the absence of ATP (data not shown).

Discussion

The main findings of the present pharmacological study are that in guinea-pig airway smooth muscle, (1) pre-incubation with low concentrations ($\leq 10 \mu\text{M}$) of ATP and its analogue ATP γ S increased the contractile force in response to subsequent MCh application via Ca^{2+} sensitization, (2) this ability of ATP to enhance MCh-induced contraction was mimicked by a P2X receptor agonist α, β -meATP but was not affected by a P2Y agonist UTP, and (3) the ATP-induced hyperresponsiveness to MCh was

blocked by Rho-kinase and Src inhibitors. The present study demonstrates for the first time that pre-exposure to low concentrations of ATP, concentrations at which ATP failed to cause contraction *per se*, significantly enhanced the MCh-induced contraction. Hence, extracellular ATP released from the epithelium may contribute to AHR.

The augmented MCh-induced contraction after exposure to ATP was reversed by suramin, a nonspecific inhibitor of P2 receptors (Fig. 4), demonstrating that extracellular ATP elicits the cellular response of airway smooth muscle via P2 receptor activation. Next, we sought to examine the involvement of subtypes of P2 receptors in the hyperresponsiveness to MCh after exposure to ATP. Pre-exposure to low concentrations of ATP enhanced the MCh-induced contraction without significant changes in $[\text{Ca}^{2+}]_i$ (Fig. 1), suggesting that ATP increases the subsequent contraction induced by MCh via up-regulating Ca^{2+} sensitivity. Moreover, α, β -meATP, a P2X receptor agonist, as well as ATP γ S caused significant augmentation in MCh-induced contraction without elevating $[\text{Ca}^{2+}]_i$ (Fig. 5), similar to ATP. In contrast, pre-treatment with UTP, a P2Y receptor agonist, did not affect subsequent response to MCh (Fig. 6). These results demonstrate that P2X receptors rather than P2Y receptors play a key role in the ability of ATP to enhance MCh-induced contraction possibly via the Ca^{2+} -sensitizing mechanism in guinea-pig airway smooth muscle.

As contraction of airway smooth muscle is regulated by the sensitivity to Ca^{2+} of the contractile elements mediated by the RhoA/Rho-kinase activation [22, 25–28], it is considered that this pathway may play an important role in the AHR in asthma [29–31]. The increase in contractile response to MCh after exposure to ATP was significantly inhibited by Y-27632, a Rho-kinase inhibitor (Fig. 7a). Furthermore, when the tissues were incubated with ATP in the presence of 10 μM HA-1077, another inhibitor of Rho-kinase, MCh-induced contraction was not enhanced by pre-exposure to ATP either, similar to the observation by Y-27632 (data not shown). We note that the contractile force elicited by MCh was not affected after either Y-27632 or HA-1077 was washed out for 15–20 min (data not shown). It is not likely that pre-incubation with these Rho-kinase inhibitors directly inhibited the subsequent MCh-induced contraction under the present experimental conditions. Therefore, Ca^{2+} sensitization by Rho-kinase is likely involved in the ATP-induced AHR to MCh. P2X receptors are not coupled to G proteins different from P2Y receptors [15]. Our findings, unexpectedly, suggest the involvement of the Rho/Rho-kinase in the downstream pathway of P2X receptors. Consistent with our findings, previous reports have indicated that P2X receptor-mediated contraction was attenuated by the Rho-kinase inhibition with Y-27632 in urinary bladder smooth muscle [32] and vas deferens [33]. Moreover, Rho-kinase inhibitors directly inhibit KCl-induced contraction in

airway smooth muscle in various species [34], indicating that Rho-kinase acts on smooth muscle contraction independently of G-protein-related pathways. However, the mechanisms of the functional relationship between P2X and the Rho/Rho-kinase pathway are still uncertain in airway smooth muscle.

The phenomenon of agonist synergism in airway smooth muscle has been reported previously [21, 34–36]. This agonist synergism is important for the development of AHR because the smooth muscle in the airways is expected to be continually exposed to various chemical mediators and cytokines during the airway inflammatory process in asthma. Gerthoffer [35] has demonstrated the significant role of Ca^{2+} sensitization in the agonist synergism in airway smooth muscle. Recently, Liu et al. [36] found that isoprostane significantly enhances a muscarinic agonist-induced contraction, which is attenuated by a Rho-kinase inhibition with Y-27632 in bovine tracheal smooth muscle. Moreover, we have demonstrated that a sphingosine 1-phosphate up-regulates the MCh-induced contraction via inactivation of myosin phosphatase by Rho-kinase rather than enhancing $[\text{Ca}^{2+}]_i$ [21]. Consistent with these recent studies, the present findings suggest that the Rho/Rho-kinase pathway might be involved in agonist synergism in airway smooth muscle.

It is generally known that the tone of airway smooth muscle is also regulated by $[\text{Ca}^{2+}]_i$ [22, 25, 37]. Bergner and Sanderson [7] have demonstrated that in mouse lung slices containing airway smooth muscle cells, ATP directly stimulates Ca^{2+} oscillations via activating the phospholipase C/inositol 1,4,5-triphosphate pathway with contractile force development. In this study, extracellular ATP (Fig. 1a) and ATP γ S (data not shown) caused modest increases in $[\text{Ca}^{2+}]_i$ with no detectable contraction. In contrast, α,β -meATP had no direct effect on $[\text{Ca}^{2+}]_i$ (Fig. 5a). Although ATP directly mobilizes intracellular Ca^{2+} , the $[\text{Ca}^{2+}]_i$ returned to the basal level after ATP was washed out for 15–20 min (Fig. 1a). Pre-exposure to these three P2 agonists (ATP, ATP γ S, and α,β -meATP) led to augmentation in MCh-induced contraction without a significant change in $[\text{Ca}^{2+}]_i$ (Figs 1, 3, and 5). Taken together, these results indicate that intracellular Ca^{2+} mobilization may not be involved in the ATP-induced AHR to MCh. ATP-induced contraction is likely related to the Ca^{2+} oscillations in airway smooth muscle of mouse lung slices [7]. However, the frequency of Ca^{2+} oscillations caused by ATP is gradually reduced and roughly disappeared during a 2-min exposure to 100 μM ATP. Although our experimental apparatus is not available for detecting Ca^{2+} oscillations different from previous studies using a confocal microscopy, we guess that Ca^{2+} oscillations do not occur after ATP is washed out for 15 min. Thus, it is possible that the Ca^{2+} oscillations are not involved in the ATP-induced hyperresponsiveness to MCh in this study.

Pre-treatment with Src inhibitors, both PP1 and PP2, reversed the hyperresponsiveness to MCh induced by ATP in airway smooth muscle (Fig. 7b). These findings indicate that Src is involved in the ATP-induced hyperresponsiveness to MCh. In vascular smooth muscle, extracellular ATP activates Src family kinases [17]. Both ATP and Src accelerate DNA synthesis and proliferation in airway smooth muscle cells [38, 39]. However, the relationship between extracellular ATP and Src activation in airway smooth muscle is still unclear. Further studies would be helpful to clarify the mechanisms underlying the role of Src in the AHR induced by ATP.

In conclusion, pre-exposure of airway smooth muscle to low concentrations of ATP causes augmentation in MCh-induced contraction mediated by P2X receptors. Our results suggest that both Ca^{2+} sensitization via the Rho/Rho-kinase pathway and Src family activation play an important functional role in the ATP-induced AHR. These findings may provide the evidence that extracellular ATP released from epithelium enhances contractile ability of airway smooth muscle, which leads to AHR in patients with asthma and COPD.

Acknowledgements

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