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ATP-induced contraction in smooth muscle of chicken anterior mesenteric artery involves both pharmacomechanical and electromechanical couplings *via* activation of P2X receptor

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Abstract

The relationship between ATP-induced membrane potential and contraction was inestigated in the smooth muscle of chicken anterior mesenteric artery using micro-electrode and tension recording techniques respectively. Application of ATP to endothelium-denuded arterial preparations developed concentration-dependent, slow depolarization which started only at concentrations not less than 10 μM. On the other hand the tension recording experiment revealed that ATP produced concentration-dependent contractions, which were evident at concentrations lower than those evoked depolarizing response to ATP (as lower 100 nM). Both depolarizing & contracting responses were abolished by the P2X receptor blocker, PPADS (50 μM) but not by P2Y receptor blocker CBF3GA (100 μM), indicating that the excitatory responses of ATP were mediated via activation of P2X receptor. These findings suggest that ATP-mediated contraction is not dependent on membrane depolarization only, at least at low concentrations in the first order branches of chicken anterior mesenteric artery.

Keywords: ATP; Chicken; Mesenteric artery; Membrane potential; Contraction

Introduction

ATP has been proven to be the main nonadrenergic noncholinergic (NANC) transmitter, co-localized with NE in the perivascular sympathetic nerves, where it produces either excitatory or inhibitory response according to the receptor set that is activated, P2X or P2Y

(Burnstock, 1987; Abbracchio & Burnstock, 1994). A depolarizing response termed excitatory junction potential is evoked when the ionotropic P2X receptor is activated (Stjarne, 1986). However, a hyperpolarizing response is evoked when ATP acts on the endothelial G-protein coupled P2Y receptor (Keef *et al*., 1992). In a previous study done in our laboratory (Draid *et al.*, 2005), we reported that in the first order branches of chicken anterior mesenteric artery, ATP released by EFS or that exogenously applied to the adventitial side, produced hyperpolarization and relaxation that were endothelium-dependent. Both membrane and muscle inhibitory responses were parallel regarding ATP concentrations used. Removal of endothelium reversed the inhibitory responses to excitatory ones in most of preparations.

However, and this is the point of this report, we present that the membrane and muscle excitatory responses to exogenously applied ATP are not parallel in contrast to the inhibitory responses. Data of effects of ATP on endothelium denuded preparation were presented and discussed.

Materials and methods

Preparation

First order branches of anterior mesenteric artery from female white leghorn chickens aged 10-14 weeks old were used. Ethics and experimental procedures were approved by Gifu University Animal Care and Use Committee and were in accordance with the Japanese Department of Agriculture guidelines and all efforts were made to minimize animal suffering and to reduce the number of animals used. The chickens were killed by cervical dislocation. First order branches of the mesenteric artery were carefully dissected from the ileal tissue and placed in a physiological salt solution (PSS) containing the following (in mM): NaCl 118, KCl 4.6, $CaCl_2$ 2.7, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11. The solution in the supply reservoir was gassed continuously with 95% O_2 - 5% CO_2 gas mixture creating a pH of 7.4, at room temperature. The connective tissue was removed and vessels were cannulated at their proximal ends with glass micropipettes (200 μm tip diameter) attached to a gravity-driven perfusion apparatus that perfused the vessel with warmed (35^{o}C) PSS to remove the clotted blood in the vessels. The endothelium was removed mechanically. Branches of the mesenteric arteries were placed in warmed (35^{o}C).

Membrane recordings

The preparations which have been cleaned were placed in a partition chamber in which large extracellular silver-silver chloride plates were used to elicit nerve stimulation as described previously (Bolton et al., 1984). The preparations were perfused at constant flow rate (3 ml/min) with prewarmed (35oC) PSS containing the cholinergic blocker atropine (0.5 ?M) and the adrenergic blockers, prasozin (5 ?M) and propranolol (1 ?M) to establish the NANC condition. Tissue preparations were allowed to equilibrate for approximately 1 h before experiments were undertaken. Membrane potentials were recorded with conventional glass capillary microelectrodes, filled with 3 M KCl with tip resistances ranging from 50-80 MΩ. The microelectrode insertions were made into the circular muscle cells through the adventitial side within 2 mm of the stimulating plate (Takewaki & Ohashi, 1977). Electrical activity was monitored on an oscilloscope (CS 4026, Kenwood, Japan) and recorded on a

thermal-array recorder (RD - 111T, TEAC, Japan). Electrical field stimulation (EFS; 15 V, 1 ms pulse width) with single stimuli and variable numbers of stimuli at 20 Hz was applied with a SEN-3301 stimulator (Nihon Kohden, Japan). Recordings were done from preparations with and without endothelial cells. Endothelial removal was done mechanically and considered successful when no hyperpolarization was elicited by acetylcholine (ACh; 5 μM). Integrity of the smooth muscle cells after mechanical removal of endothelium was confirmed by successful recording of the hyperpolarizing effect of the ATP-sensitive K^+ channel opener, cromakalim (1 μM) (data not shown).

Tension recordings

A vascular segment of 3-5 mm length were cut and mounted for isometric tension recording in a 5 ml organ bath containing PSS at 35^{o}C that was gassed with 95% O_2 - 5% CO_2. Two fine, stainless steel pins 100 ?m in diameter were introduced through the lumen of the segment. One pin was fixed to the organ bath floor whilst the other was connected to a force transducer (AD instrument MLT 050/D, Australia) for tension recording. An initial resting tension of 0.7 g was applied to the arterial rings which were subsequently left to equilibrate for 60-90 min. At the end of this period, the tension on the vessel was taken as the resting tension and no further mechanical adjustment was made during experimentation. Changes in isometric force were recorded using a PowerLab 2/25 data acquisition system (Chart software, version 5.0.2, AD instruments, Australia).

The experiment was done only using preparations with denuded endothelia. ATP was used in concentration range (100 nM - 1 mM). Contracting effects were expressed as percentage of NE-induced contraction. After exposure to a drug, the preparation was washed with PSS and left for at least 20 min before further experimentation.

Drugs

The following drugs have been used in the present study: 2-methylthio ATP (2-MeSATP), α,β-Methylene ATP (α,β-MeATP), Acetylcholine (ACh), adenosine triphosphate (ATP), atropine, Cibacron blue F3GA (CBF3GA), cromakalim, guanethidine, Nor-epinepherine (NE), pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), prazosin, propranolol; All drugs have been purchased from Sigma, St. Louis, USA.

Prazosin (10 mM) was dissolved in methanol (100 %) NE was dissolved in 10 μM ascorbic acid, while all other drugs were dissolved in distilled water. Drugs were applied at required concentrations by their addition to the superfusing PSS (microelectrode recording) or directly to the bath (mechanical recording).

Statistics

Data are expressed as mean ± S.E.M.; *n* represents the number of chickens from which the tissues were isolated. Statistical analysis was performed with student's unpaired *t*-test, and *P* values <0.05 were considered statistically significant.

Results

Membrane responses

In the first order branches of chicken anterior mesenteric artery, resting membrane potential in circular smooth muscle cells averaged -61.8 ± 0.4 mV (n = 21). Unlike most of mammalian blood vessels which are quiescent, circular smooth muscle cells of chicken anterior mesenteric artery exhibited either electrical quiescence or spontaneous electrical arrhythmic or rhythmic potentials. Adventitial application of ATP on preparations with intact endothelia showed slowly developing hyperpolarization in a dose-dependent manner (data not shown). In case of endothelium-denuded preparations, no change in membrane potential occurred at concentrations of ATP as low as 10 μM. However, ATP concentration at and higher than 10 μM resulted in concentration-dependent depolarization being 19 ± 1.6 mV at 1 mM ATP, (Fig 1A). Similar response were produced by α,β-MeATP but not by 2-MeSATP The depolarizing effect of ATP was completely blocked by the P2X receptor blocker PPADS (100 μM) (Fig. 1B). Desensitization of P2X receptor by α,β-MeATP (10 μM, for 30 min) produced also complete blocking effect to ATP as well as α,β-MeATP depolarizing responses, (Fig 1B).

Mechanical responses

Similarly, circular smooth muscle of chicken anterior mesenteric artery shows spontaneous activity at low frequency (repeated every 10 min). Removal of endothelium didn't aff-

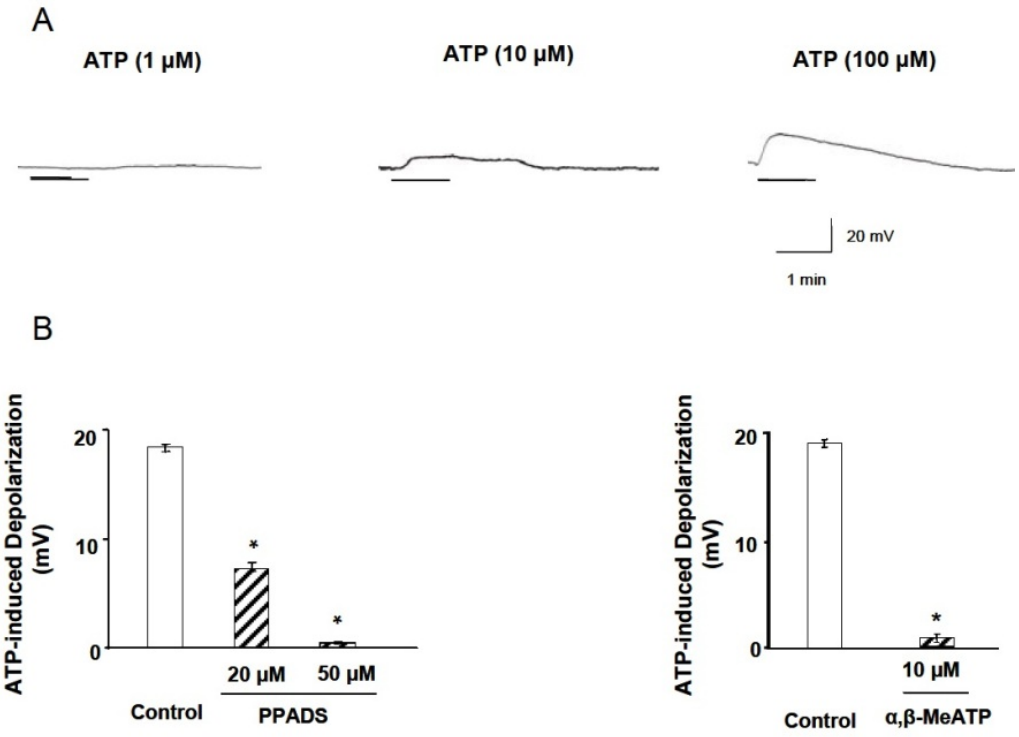

Figure 1. Effect of exogenously applied ATP on membrane potential of chicken mesenteric artery. **A)** A typical recording showing concentration-dependent membrane responses of ATP (1-100 μM; n=6). **B)** Summary graph showing the concentration-dependent effect of PPADS (n=5) and the effect of α,β-MeATP desensitization (10μM for 30 min; n=5) on the amplitude of ATP induced-depolarization.

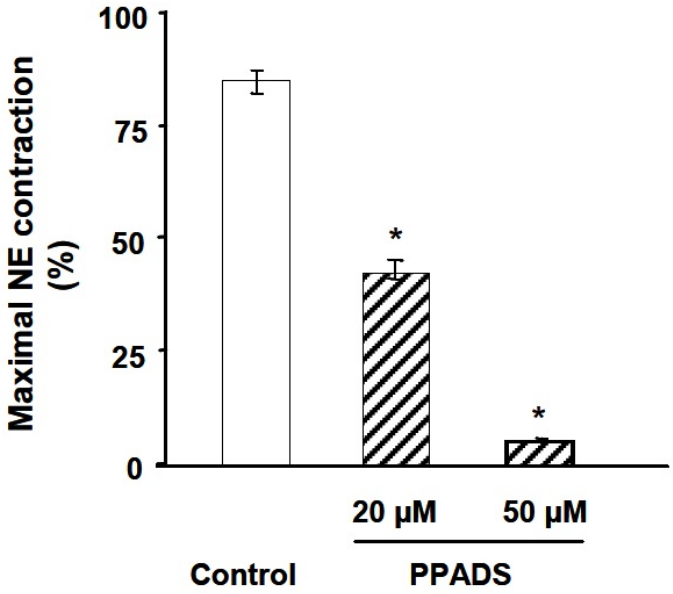

Figure 2. Summary graph showing the effect of PPADS (n=5) on the amplitude of ATP induced-contraction

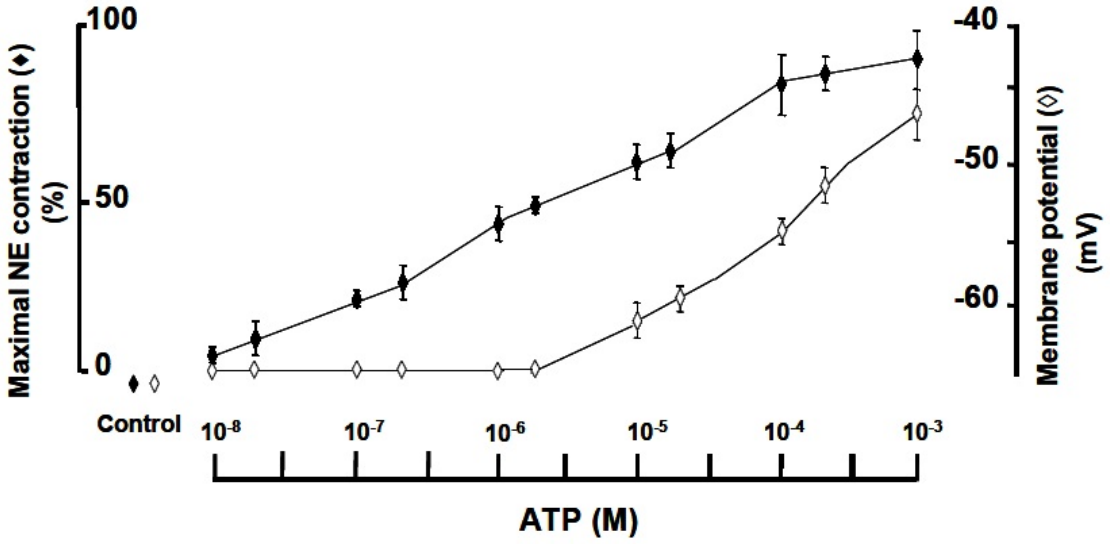

Figure 3. Summary graph showing relationship between membrane potential (◊) or contraction (♦) and the concentration of ATP (10 nM – 1 mM) (n= 6).

ect the pattern of such spontaneous activity. All contractions induced by ATP were sensitive to blocking or desensitizing P2X receptor by PPADS or α,β-MeATP, respectively (Fig 2). Application of ATP to ring preparations which are endothelium-denuded produced concentration-dependent contractions being 90 ± 6 % at 1 mM ATP (Fig 3). Interestingly and in contrast to membrane potential results the rings were responsive even to the low concentrations of ATP to which the membrane was resistant. Figure 3 is a summary graph showing relationship between membrane potential or contraction and the concentration of ATP (10 nM – 1 mM).

Discussion

The relationship between muscle tension and membrane potential has already been measured by Hodgkin and Horowicz (1960); he stated that hyperpolarization is followed by relaxation and depolarization followed by contraction. Data of the present study showed an exception for this established rule. Exogenous application of ATP in endothelium-denuded preparations mediated depolarization only at high concentrations starting from 10 μM. However, tension-recording experiments revealed that ATP mediates contractile responses at concentrations of nanomolar level. It is well documented that ATP produces its excitatory response via P2X receptor (Thapaliya *et al.,* 1999). Our data is consistent with this, where the depolarizing & contracting responses have been inhibited by PPADS indicating that they are mediated via P2X receptor as expected. The difference in concentrations of ATP mediating depolarizing & contracting responses may suggest that a much lower sensitivity of P2X receptors located on vascular smooth muscle of chicken anterior mesenteric arteries comparing with that of P2Y1 receptor that is located on the endothelium. That is because we found in our previous report (Draid *et al*, 2005), that both hyperpolarizing and relaxing effects of ATP were parallel & both are evident even at nanomolar levels in endothelium-intact preparations; and it was indicated that the inhibitory responses were mediated via P2Y1 receptor located on the endothelium as the responses were completely blocked by the specific P2Y1 specific antagonists MRS 2179 and A3P5PS.

The difference in concentrations of ATP in mediating depolarizing and contracting responses my also suggest that the ATP-mediated contractile response is mainly nondependent on membrane depolarization at least at low concentrations. Therefore, it is assumed that the contracting effects of ATP that are mediated at low concentrations are mediated *via* pharmaco-mechanical coupling mechanism without change in the membrane potential. This unkown pharmacomechanical coupling mechanism may end with an increase in intracellular Ca^{++} level with the result of a contractile response.

The finding of the present study that ATP at small concentrations induces vasoconstriction *via* pharmacomechanical rather then electromechanical coupling; and this action is mediated via P2X1 receptor that is the principal P2X receptor subtype expressed on most vascular smooth muscles (Valera *et al*, 1994; Collo *et al*,1996); could be explained on the basis of three hypotheses. First is that ATP may act indirectly *via* enhancing the release of NA presynaptically and then the released NA mediates the recorded contraction (Angus *et al.*, 1988; Gitterman and Evans, 2001; Stephens *et al.*, 1991). The second hypothesis is that ATP stimulates P2X1 receptor on vascular smooth muscle, however this P2X receptor doesn't induce any intracellular signals but does activate an associated P2Y receptor that is cAMP-linked. The later produces vasoconstriction in a similar manner like NA. The third hypothesis, that is relatively weak, is that P2X receptor in chicken mesenteric arteries may be not only ion-channel gated but-also associated somehow with a stimulatory G-protein.

The first hypothesis could be excluded depending on other experiments that revealed that the contractile effect of ATP or α,β-meATP was not affected by prazosin (data not shown). The second hypothesis is the most accepted one as it's established in some other tissues as blood platelets (Vial *et al.*, 2002) where ATP stimulates P2X1 receptor, then this activation potentials subsequent P2Y1 receptor-mediated intracellular Ca^{++} increase, and this synergy

may be important in the regulation of blood clotting (Hechler *et al.*, 2003). However, farther detailed studies are needed for confirmation of this pathway in chicken mesenteric arteries.

Conflict of interest

There is no conflict of competing interest associated with the authors of this paper.

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