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Dual effects of extracellular ATP on the muscarinic acetylcholine receptor-operated K^+ current in guinea-pig atrial cells

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Abstract

Adenosine 5′-triphosphate (ATP) is stored in sympathetic and parasympathetic nerve terminals and co-released with norepinephrine and acetylcholine during nerve stimulation. In the heart in situ parasympathetic nerve is tonically stimulated and the activated muscarinic acetylcholine-receptor-operated K^+ current ($I_{K.ACh}$) plays an important role in the repolarization of the atrial action potential, the sinoatrial node automaticity and the atrioventricular conduction. In the present study, effects of extracellular ATP on the $I_{K.ACh}$ activated by carbachol or adenosine were examined in isolated guinea-pig atrial cells by use of the patch-clamp technique. ATP (10 μM) per se produced a transient activation of $I_{K.ACh}$ in atrial cells held at −40 mV. When $I_{K.ACh}$ was preactivated by 1 μM carbachol or 10 μM adenosine, ATP (1–100 μM) produced a transient increase followed by a sustained decrease of the current. These ATP-induced biphasic changes of $I_{K.ACh}$ were abolished by suramin (100 μM) or reactive blue-2 (30 μM), but not by theophylline (500 μM), indicating the involvement of P_2 purinoceptors. ATP also enhanced and then partially reversed the action potential shortening induced by carbachol or adenosine in current-clamped atrial cells. Extracellular ATP did not increase but decreased the openings of the single K_{ACh} channel that were recorded by use of a pipette solution containing 1 μM carbachol in the cell-attached mode. Thus, P_2 purinoceptor stimulation produces dual effects of ATP on the pre-activated $I_{K.ACh}$ and may modulate the chronotropic and inotropic responses during autonomic nerve stimulation. © 1997 Elsevier Science B.V.

Keywords: ATP, extracellular; Muscarinic acetylcholine receptor-operated K^+ current; Atrial cell; Purinoceptor; Action potential duration

1. Introduction

Adenosine 5′-triphosphate (ATP) is a neurotransmitter which is stored in synaptic vesicles of the autonomic nerves. It is co-released with noradrenaline or acetylcholine in response to nerve stimulation and elicits diverse biological responses (Gordon, 1986; Richardson and Brown, 1987). Acetylcholine, ATP and adenosine activate muscarinic receptor, P_2 and P_1 purinoceptors, respectively, and they commonly decrease the firing rate of the sinus node and cardiac contractility (Burnstock and Meghji, 1981; Chiba and Himori, 1975; Fleetwood and Gordon, 1987). However, positive inotropic and chronotropic responses to adenine nucleotides including ATP have been also reported in frog (Burnstock and Meghji, 1981) and mammalian heart (Chiba et al., 1983; Legssyer et al., 1988). More recently, a biphasic inotropic effect, an initial rapid decrease followed by an increase in contractile tension, of adenine compounds in rat atrial preparations was also observed (Froldi et al., 1994). Thus, cardiac effects of adenine nucleotides including ATP appear to be complex and the underlying mechanisms have not been fully understood.

The negative chronotropic effect produced by extracellular ATP in beating hearts has generally been assumed to stem from stimulation of adenosine A_1 receptors. Since ATP can be metabolized to adenosine by ectonucleotidase present in the plasma membrane, it has been suggested that the effect of ATP is through degradation to adenosine, which then binds to adenosine A_1 receptors and activates the muscarinic acetylcholine receptor-operated K^+ (K_{ACh}) channels (Belardinelli et al., 1989). On the other hand, recent reports have indicated that ATP per se activates the K_{ACh} channel through the activation of P_2 purinoceptors in mammalian atrial cells (Friel and Bean, 1990; Matsuura et

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al., 1996a). However, in the hearts in situ parasympathetic nerve is tonically stimulated, and the activated $I_{K.ACh}$ may play an important role in the regulation of cardiac function, such as the sinoatrial node automaticity, the atrio-ventricular conduction and the repolarization of atrial action potential. Therefore, it may be also important to examine effects of ATP on the pre-activated $I_{K.ACh}$.

In this study, effects of ATP on the $I_{K.ACh}$ activated by muscarinic M_2 receptor stimulation were examined in isolated guinea-pig atrial cells. The findings presented here indicate that extracellular ATP possesses dual effects, stimulatory and inhibitory effects, on the pre-activated $I_{K.ACh}$ through P_2 purinoceptor activation.

2. Materials and methods

2.1. Cell preparations

Guinea pigs, weighing 200–350 g, were used in these experiments. Single atrial cells were isolated by an enzymatic dispersion, as described previously (Mori et al., 1995). Briefly, the heart was removed from the open-chest guinea pig anesthetized with pentobarbital sodium, and mounted on a modified Langendorff perfusion system for retrograde perfusion of the coronary circulation with a normal HEPES-Tyrode solution. The perfusion medium was then changed to a nominally Ca^{2+}-free Tyrode solution and then to a solution containing 0.02% (w/v) collagenase (Wako, Osaka, Japan). After digestion, the heart was perfused with a high K^+ and low Cl^- solution (modified Kraft-Brühe (KB) solution) (Isenberg and Klockner, 1982; Hara and Nakaya, 1995). Atrial tissue was cut into small pieces in the modified KB solution and gently shaken up to isolate cells. The cell suspension was stored in a refrigerator (4°C) and used within 12 h. The composition of the normal HEPES-Tyrode solution was (mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5 and HEPES-NaOH buffer (pH 7.4) 5.0. The composition of the modified KB solution was (mM): KOH 70, L-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10 and HEPES-KOH buffer (pH 7.4) 10.

2.2. Whole-cell current recordings

Whole-cell membrane currents were recorded by the patch-clamp method (Hamill et al., 1981). Single atrial cells were placed in a recording chamber (1 ml volume) attached to an inverted microscope (Olympus IMT-2, Tokyo, Japan) and superfused with the HEPES-Tyrode solution at a rate of 3 ml/min. The temperature of the external solution was kept constant at 36 ± 1°C. Glass patch pipettes with a diameter of 1.5 mm were filled with an internal solution. The composition of the standard pipette solution was (mM): K-aspartate 110, KCl 20, $MgCl_2$ 1.0, ATP-K_2 5.0, EGTA 10 and HEPES-KOH buffer (pH 7.4) 5.0. The free Ca^{2+} concentration in the pipette solution was adjusted to pCa 8 according to the calculation by Fabiato and Fabiato (1979) with the correction of Tsien and Rink (1980). The resistance of the patch pipette filled with the internal solution was 2–3 MΩ. The tight-seal, whole-cell voltage-clamp technique was used. After the gigaohm-seal between the tip of the electrode and the cell membrane was established, the membrane patch was disrupted by applying more negative pressure to make the whole-cell voltage-clamp mode. The electrode was connected to a patch-clamp amplifier (Nihon Kohden CEZ-2300, Tokyo, Japan). Command pulses were generated by a 12-bit digital-to-analog converter controlled by PCLAMP software (Axon Instruments, Foster City, CA, USA). Current signals were digitized and stored on the hard disk of an IBM-compatible computer (Compaq Prolinea 4/50 with a 200 M byte hard disc, Houston, TX, USA). A liquid junctional potential between the internal solution and the bath solution of −8 mV was corrected.

Effects of ATP on the muscarinic acetylcholine receptor-operated potassium current ($I_{K.ACh}$) were examined in the cells held at −40 mV. The $I_{K.ACh}$ was activated by the extracellular application of 1 μM carbachol or 10 μM adenosine in the GTP (100 μM)-loaded cells or by the intracellular loading of GTPγS (100 μM), a nonhydrolysable GTP analogue. To calculate percent change of $I_{K.ACh}$ by ATP, the difference between the steady-state current in the solution containing either 1 μM carbachol or 10 μM adenosine and the current level in the absence of any agonist was taken as 100% in the GTP-loaded cells. In the GTPγS-loaded cells, the difference between the persistent outward current in the absence of agonist and the initial current level just after the break of the patch membrane in the pipette was taken as 100%. In glibenclamide (10 μM)-treated cells, effects of ATP on the outward current in the absence and presence of muscarinic stimulation were examined.

In part of the experiments, a ramp-pulse protocol was used to record the quasi-steady-state membrane current. The membrane potential was held at −40 mV and depolarized first to +50 mV at a rate of 1.2 mV/ms. It was then repolarized or hyperpolarized to −100 mV with a slope of −1.2 mV/ms, during which time the change in the membrane current was automatically plotted against the membrane potential. The current-voltage relation was measured during the repolarized or hyperpolarizing phase. The ramp-voltage pulses were applied at appropriate timing.

Action potential of single myocytes was recorded by switching to current-clamp mode after the establishment of whole-cell voltage-clamp mode. External and pipette solutions were the same as those used in the whole-cell voltage-clamp experiments. Current pulses of a suprathreshold were applied to the cells through the pipette

every 5 s. After establishment of action potential configuration, effects of various drugs on the action potential were evaluated.

2.3. Unitary current recordings

Unitary current recordings were performed in the cell-attached configuration of the patch-clamp techniques. Patch pipettes were silicone-coated and filled with a solution containing (in mM) KCl 140, $CaCl_2$ 1.8, HEPES-KOH buffer (pH 7.4) 5 and carbachol 0.001. After the gigaohm seal between the patch electrode and the cell membrane was formed in the HEPES-Tyrode solution, cells were exposed to an external solution containing (in mM) KCl 140, $MgCl_2$ 1.8, EGTA 0.1 and HEPES-KOH buffer (pH 7.4) 5. The holding potential was clamped at −80 mV, and the inward K_{ACh} channel current was recorded at room temperature (20–25°C). When the activity of K_{ACh} channels was stabilized, ATP was added to the bath solution. These signals were obtained by the same patch-clamp amplifier and stored on a video cassette recorder (Hitachi VT-F20, Tokyo, Japan) through a pulse-code modulator (VR10-B, Instrutech, New York, NY, USA). Later, the data were filtered at 1.5 kHz, transferred to the hard disc of the computer at a sampling rate of 10 kHz and analyzed by PCLAMP software.

2.4. Drugs

Drugs used in this study are as follows: ATP-Na_2 (Kowa, Nagoya, Japan), carbachol chloride, suramin, theophylline (Wako, Osaka, Japan), GTP-Na_3, adenosine, GTPγS-Li_4, adenosine, glibenclamide (Sigma, St. Louis, MO, USA), reactive blue-2 (Fluka, Buchs, Switzerland) and pertussis toxin (Kaken Pharmaceutical, Tokyo, Japan).

2.5. Statistics

All values are presented in terms of mean ± S.E. Student's *t*-tests and analysis of variance were used for statistical analysis of the data. *P* values of less than 0.05 were considered significant.

3. Results

3.1. Effects of extracellular ATP on the whole-cell current

As shown in Fig. 1A, ATP (10 μM) per se induced a transient outward current in atrial cells held at −40 mV. A similar increase in the outward current was observed in response to 10 μM ATP in not only normal cells ($n = 5$) but also glibenclamide (10 μM)-treated cells ($n = 4$).

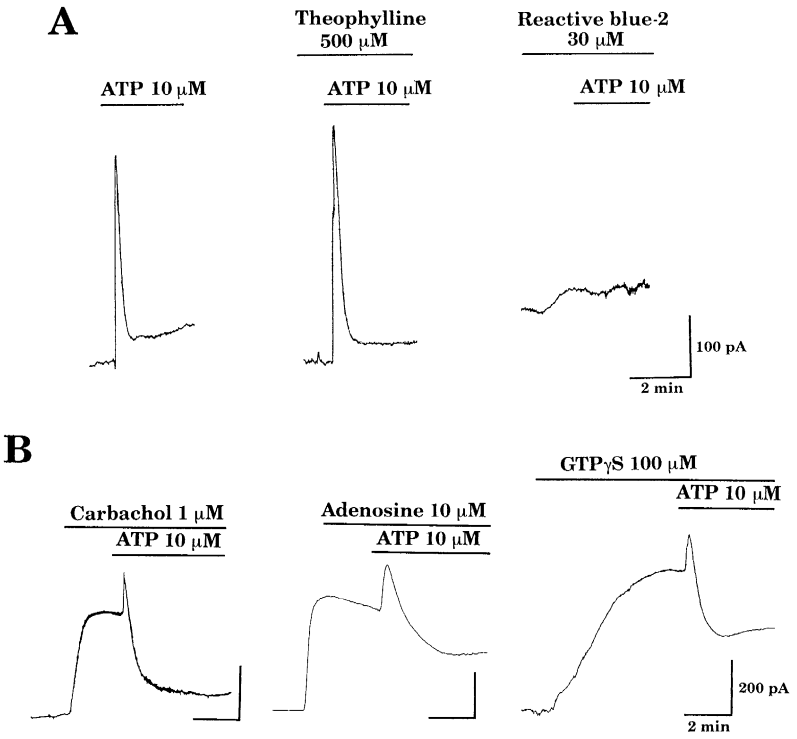

Fig. 1. Effects of extracellular ATP on the holding current in the absence (A) and presence of carbachol (1 μM), adenosine (10 μM) or intracellular loading of GTPγS (100 μM) (B) in guinea-pig atrial cells clamped at −40 mV. Influences of purinoceptor antagonists on the effect of ATP on the holding current in the absence of muscarinic or adenosine receptor stimulation were also examined (A). Drug applications are shown by the bars above each original current trace.

However, in atrial cells preincubated with pertussis toxin (5 μg/ml, 36°C, 120 min) neither 1 μM carbachol nor 10 μM ATP could produce the increase in the outward current. The transient increase in the outward current after 10 μM ATP was observed in the presence of 500 μM theophylline, a P_1 purinoceptor antagonist, but not in the presence of 30 μM reactive blue-2, a P_2 purinoceptor antagonist (Fig. 1A). Moreover, extracellular ATP produced a transient potentiation followed by a sustained inhibition of the outward current when the muscarinic acetylcholine receptor-operated K^+ current ($I_{K.ACh}$) was pre-activated by an application of carbachol (1 μM), as shown in Fig. 1B. ATP at a concentration of 10 μM transiently increased the carbachol (1 μM)-induced $I_{K.ACh}$ by 38.1 ± 5.2% ($P < 0.05$) and then decreased it by 46.7 ± 11.5% ($P < 0.05$) in 7 cells. Similar biphasic changes of the pre-activated $I_{K.ACh}$ were observed with a lower (1 μM) and a higher concentration (100 μM) of ATP although a clear concentration-response relationship could not be found. Extracellular ATP at concentrations of 1 and 100 μM produced increases of 27.8 ± 5.2% ($P < 0.05$) and 16.9 ± 4.1% ($P < 0.05$) and decreases of 52.3 ± 11.7%

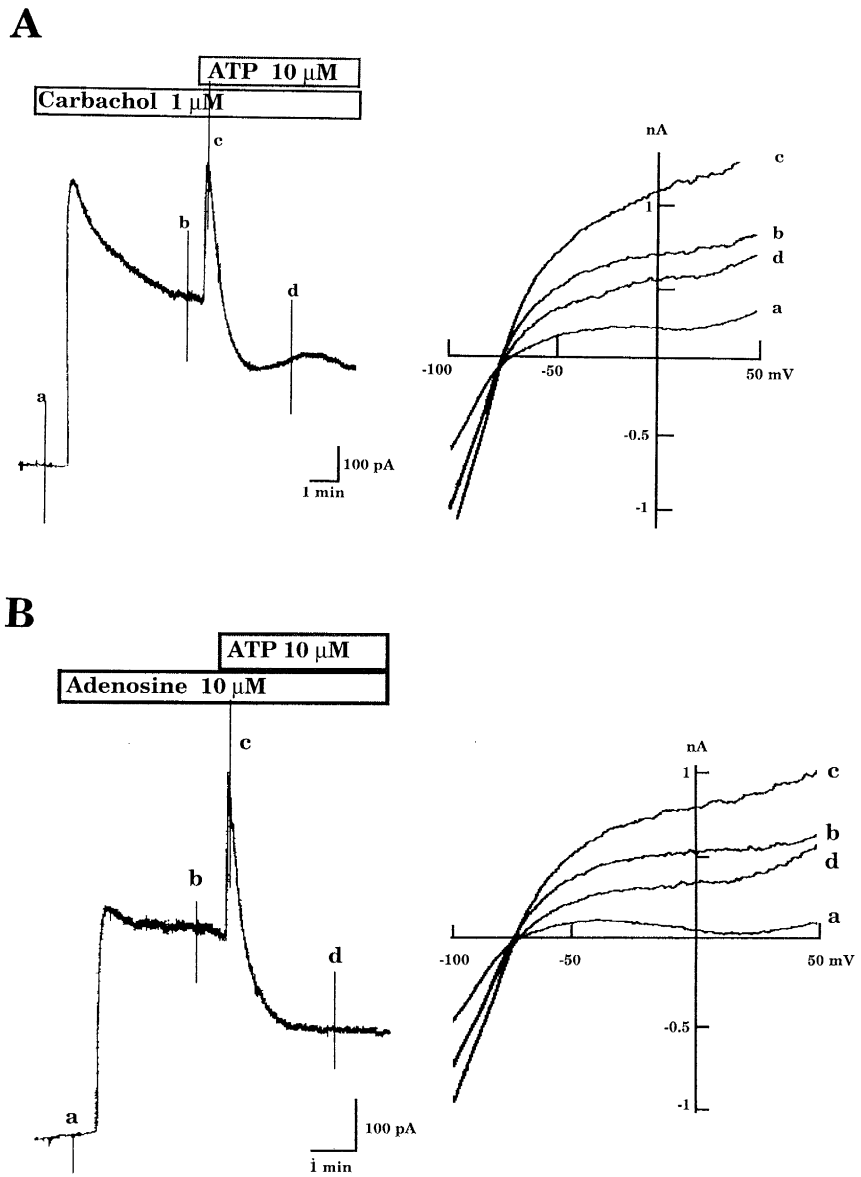

Fig. 2. Effects of extracellular ATP on the quasi-steady-state membrane current recorded by a ramp-pulse protocol in the presence of carbachol (1 μM, A) or adenosine (10 μM, B). Membrane currents were recorded applying ramp-voltage pulses with appropriate timing from the holding potential of −40 mV. The membrane potential was first depolarized to +50 mV at a rate of 1.2 mV/ms and then repolarized or hyperpolarized to −100 mV with a slope of −1.2 mV/ms, during which time the change in the membrane current was automatically plotted against the membrane potential. The current-voltage relation was measured during the repolarized or hyperpolarizing phase. The current tracings indicated by the symbols (a, b, c and d) in the chart record are shown in the right panels. Note that extracellular ATP produced a biphasic change in the quasi-steady-state membrane current, an initial activation phase and a late inhibition phase. Almost no change in the reversal potentials was observed among quasi-steady-state membrane currents.

($P < 0.05$) and 31.7 ± 9.0% ($P < 0.05$) in 6 and 8 atrial cells, respectively. The inhibitory effect of ATP on the pre-activated $I_{K.ACh}$ was sustained and little recovery of the outward current was observed upon washout of ATP. No significant change of the carbachol-induced $I_{K.ACh}$ was observed after an application of 0.1 μM ATP in 5 cells. Biphasic changes of the pre-activated $I_{K.ACh}$ were also observed in response to 10 μM ATP in 4 glibenclamide (10 μM)-treated atrial cells (data not shown).

It is well known that adenosine can also activate $I_{K.ACh}$ through the activation of the adenosine A_1 receptor-pertussis toxin-sensitive G protein pathway in atrial cells (Kurachi et al., 1986). Therefore, effects of extracellular ATP on the adenosine-induced $I_{K.ACh}$ were also evaluated. Similarly ATP produced biphasic effects on the adenosine-induced outward current, as shown in Fig. 1B. ATP at a concentration of 10 μM potentiated the adenosine-induced $I_{K.ACh}$ by 96.6 ± 20.5% ($P < 0.05$) and then inhibited it by 41.8 ± 7.2% ($P < 0.05$) in 9 atrial cells. Intracellular loading of GTPγS, a non-hydrolysable GTP analogue, could activate directly the GTP-binding proteins and evoke persistent activation of $I_{K.ACh}$, as reported by Breitwieser and Szabo (1985). External ATP (10 μM) produced similar biphasic effects on the GTPγS (100 μM)-induced $I_{K.ACh}$ (Fig. 1B); an initial increase of 28.6 ± 6.4% ($P < 0.05$, $n = 5$) followed by a late decrease of 51.8 ± 9.7% ($P < 0.05$).

In order to evaluate the reversal potentials of the outward current activated and inhibited by extracellular ATP, the quasi-steady-state membrane current was recorded by a ramp-pulse protocol of 125 ms from +50 to −100 mV. Either carbachol (1 μM) or adenosine (10 μM) produced a marked increase in the quasi-steady-state outward current at potentials positive to −75 mV (Fig. 2). In the presence of 1 μM carbachol, addition of 10 μM ATP transiently increased the outward current at 0 mV by 50.3 ± 16.5% ($P < 0.05$) and then decreased it by 31.8 ± 5.6% ($P < 0.05$, $n = 6$). In the presence of 10 μM adenosine, ATP at the same concentration transiently increased the outward current at 0 mV by 49.8 ± 7.4% ($P < 0.05$) and then decreased it by 30.0 ± 4.0% ($P < 0.05$, $n = 7$). The reversal potentials of carbachol- and adenosine-induced current were −78.9 ± 4.8 mV ($n = 6$) and −76.3 ± 1.0 mV ($n = 7$), respectively. These potentials were close to the calculated equilibrium potential of K^+. Addition of ATP did not

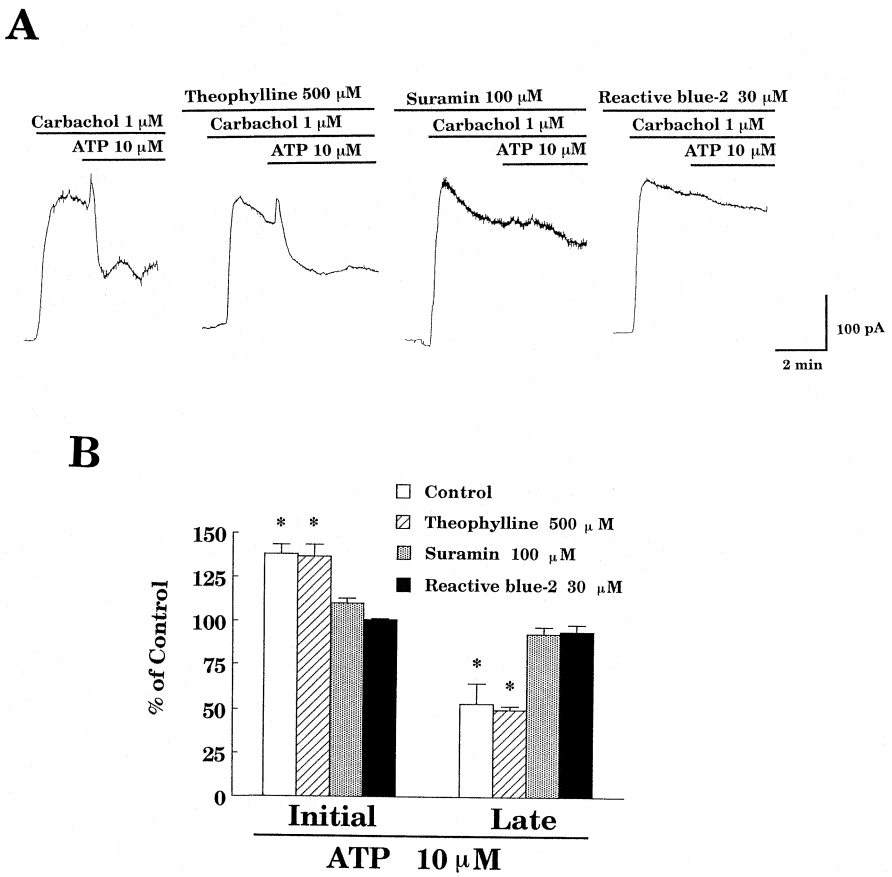

Fig. 3. Influences of theophylline (500 μM), suramin (100 μM) and reactive blue-2 (30 μM) on the ATP (10 μM)-induced biphasic changes of the $I_{K.ACh}$ pre-activated by 1 μM of carbachol. Actual records of ATP-induced changes of the pre-activated $I_{K.ACh}$ in the absence and presence of various purinoceptor antagonist are shown in panel A. The current changes during the initial and late phases after 10 μM ATP in the absence and presence of various purinoceptor antagonists are summarized in panel B. The amplitude of the carbachol-induced current before ATP is expressed as 100%. Values are expressed as mean ± S.E. of 5–7 experiments. * $P < 0.05$ vs. control.

significantly affect the reversal potentials of the quasi-steady-state membrane current during both the initial activation phase and the late inhibition phase, indicating that the current modulated by ATP would be a K^+ current, i.e., $I_{K.ACh}$.

In the following experiments influences of various purinoceptor antagonists on the ATP-induced modulation of $I_{K.ACh}$ were examined. In the presence of 500 μM theophylline, a P_1 purinoceptor antagonist (Burnstock, 1972), ATP still produced the dual effects on the $I_{K.ACh}$ pre-activated by 1 μM carbachol (Fig. 3). However, the biphasic changes of $I_{K.ACh}$ after ATP were abolished by both suramin (100 μM) and reactive blue-2 (30 μM), P_2 purinoceptor antagonists (Dunn and Blakeley, 1988; Hourani et al., 1992; Burnstock and Warland, 1987) (Fig. 3). These results suggest that ATP-induced biphasic changes of the pre-activated $I_{K.ACh}$ are mediated by P_2 purinoceptors.

3.2. Effects of extracellular ATP on the single-channel current

The unitary K_{ACh} channel current was recorded from cell-attached patches by use of patch pipettes containing 1 μM carbachol. In these experiments, both bath solution and pipette solution contained 140 mM potassium and the pipette potential was clamped at various potentials. The current-voltage relation for the single-channel current was determined in 4 cells and the slope conductance was 45.0 ± 1.5 pS, and displayed inward rectification. These electrophysiological properties of the K^+ channel are consistent with previous reports (Sakmann et al., 1983; Kurachi et al., 1986). When the pipette potential was positive, the unitary K_{ACh} channel current was recorded as an inward current, which is shown as a downward deflection (Fig. 4). After establishment of a stable channel activity (more than 6 min after establishment of cell-attached configuration), ATP (100 μM) was added to the bath solution. Extracellular ATP failed to increase the channel activity and gradually decreased the open probability without affecting the amplitude of the unitary current, as shown in Fig. 4. The open probability (NP_o), where N is the number of K_{ACh} channels in the patch pipette and P_o is the probability of the channel being open, was significantly decreased from 0.333 ± 0.057 to 0.136 ± 0.032 ($P < 0.05$, $n = 7$) at 3 min after 100 μM ATP (Fig. 4C). The amplitude of unitary current before and after ATP was 3.65 ± 0.13 and 3.81 ± 0.17 pA, respectively, being not significantly different from each other.

3.3. Effects of extracellular ATP on the action potential

Action potentials of guinea-pig atrial cells stimulated at 0.2 Hz were recorded in the whole-cell current-clamp mode. The baseline characteristics of the action potentials were as follows: the resting membrane potential, $-72.3 \pm$

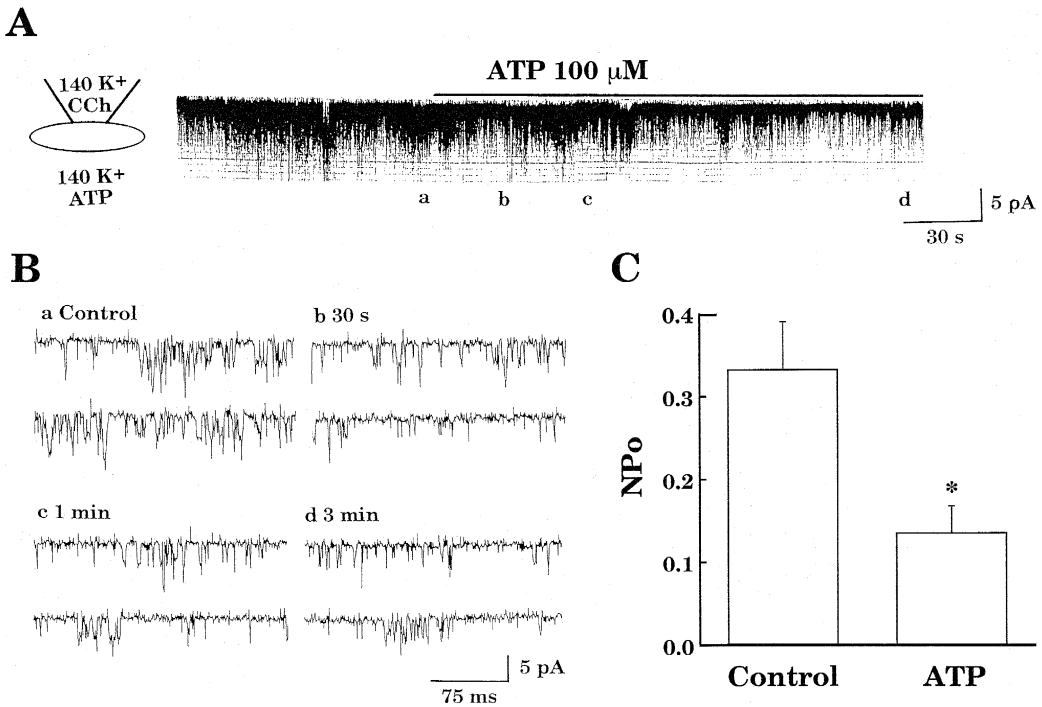

Fig. 4. Effects of extracellular ATP (100 μM) on the single K_{ACh} channel current recorded from cell-attached patches by use of a patch-pipette solution containing 1 μM carbachol in a guinea-pig atrial cell. The holding potential was −80 mV. Expanded current traces in B are obtained at the points marked by a, b, c and d in the upper chart recording (A). Note that application of ATP to the bath gradually inhibited the activity of the single-channel current. Bar graph in C indicates the open probability (NP_o) measured before and after 100 μM ATP. Values are expressed as mean ± S.E. of 7 experiments. * $P < 0.05$ vs. control.

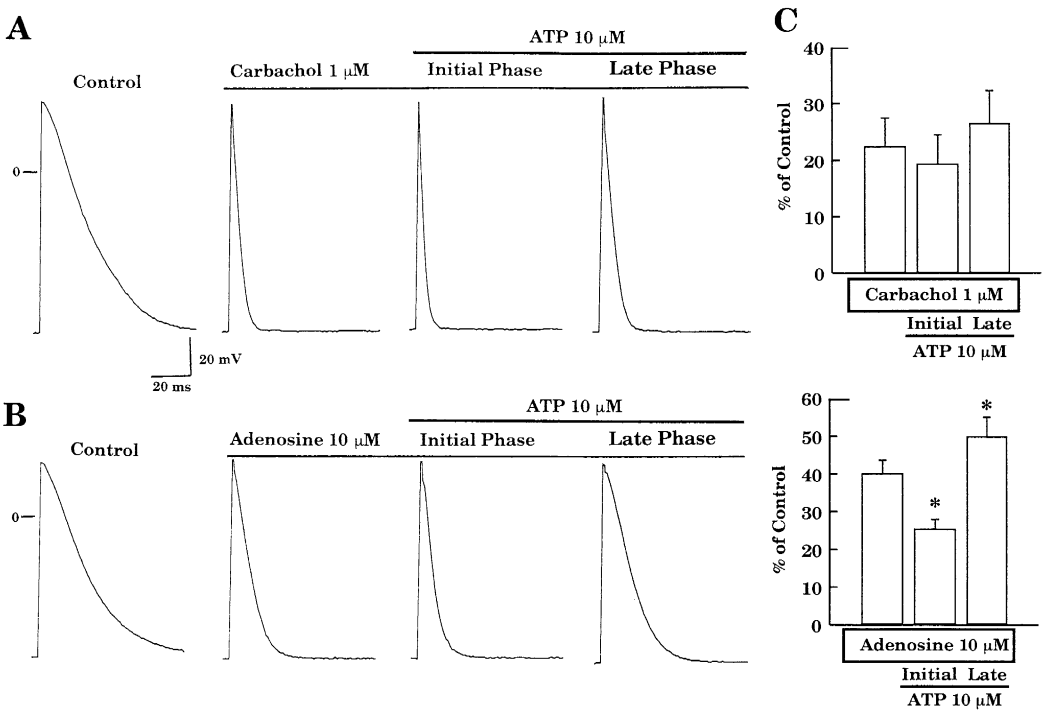

Fig. 5. Effects of extracellular ATP (10 μM) on the carbachol (1 μM, A)- and adenosine (10 μM, B)-induced action potential shortening in guinea-pig atrial cells. Action potentials were recorded in the current-clamp mode. ATP transiently potentiated and then attenuated the action potential shortening. Summarized data of changes in action potential duration at 90% repolarization level (APD_{90}) are shown in C. Values are expressed as mean ± S.E. of 5 and 8 experiments. * $P < 0.05$ vs. adenosine alone.

1.7 mV; action potential amplitude, 107.4 ± 2.2 mV; action potential duration (APD) at 50% repolarization level (APD_{50}), 27.2 ± 2.6 ms; APD at 90% repolarization level (APD_{90}), 60.8 ± 5.4 ms ($n = 17$). ATP (10 μM) per se shortened APD_{90} by 39.9 ± 13.7% in 4 cells. When the action potential was shortened by 1 μM carbachol or 10 μM adenosine, ATP transiently shortened APD further and then partly reversed the shortening of the action potential, as shown in Fig. 5. APD_{90} after 1 μM carbachol and 10 μM adenosine was 22.5 ± 5.0% ($n = 5$) and 40.3 ± 3.4% ($n = 8$) of the control, respectively. In the presence of 1 μM carbachol, ATP (10 μM) further shortened APD_{90} to 19.3 ± 5.1% of the control during the initial phase and then prolonged it to 26.6 ± 5.7% during the late phase (Fig. 5A). In the presence of 10 μM adenosine, ATP (10 μM) further shortened APD_{90} to 25.4 ± 2.5% ($P < 0.05$) of the control and then prolonged it to 50.5 ± 5.0% ($P < 0.05$) (Fig. 5B). Thus, ATP produced biphasic changes of action potential duration under the muscarinic M_2 or adenosine A_1 receptor stimulation.

4. Discussion

ATP has been shown to produce intricate effects on the heart, which depend on the animal species, cardiac tissues and experimental conditions employed. ATP has been reported to produce positive inotropic response (Legssyer et al., 1988; Mantelli et al., 1993), negative inotropic response (Burnstock and Meghji, 1981, 1983), positive chronotropic response (Chiba et al., 1983; Takikawa et al., 1990) and negative chronotropic response (James, 1965; Chiba et al., 1983). ATP has been also shown to modulate several cardiac ion currents such as the L-type Ca^{2+} current (Scamps et al., 1990; Qu et al., 1993a,b; Scamps and Vassort, 1994a; Song and Belardinelli, 1994), the Na^+ current (Scamps and Vassort, 1994b), the delayed rectifier K^+ current (Matsuura et al., 1996b), the non-specific cationic current (Zheng et al., 1993; Scamps and Vassort, 1994a; Parker and Scarpa, 1995) and the Cl^- current (Matsuura and Ehara, 1992). Thus, extracellular ATP exerts various electromechanical responses in the heart.

Since ATP can be metabolized to adenosine by ectonucleotidase, the negative chronotropic and inotropic responses to ATP in atrial tissues had been ascribed to the stimulation of adenosine A_1 receptors by adenosine (Belardinelli et al., 1989). However, it had not been determined with certainty whether the effect of extracellular ATP could be entirely attributed to the degraded metabolite adenosine. Friel and Bean (1990) reported that extracellular ATP activates an inwardly rectifying K^+ current in bovine atrial myocytes, which is similar to that activated by acetylcholine or adenosine. More recently, Matsuura et al. (1996a) have reported that ATP per se activates the K_{ACh} channel through the stimulation of the P_2 purinoceptor-pertussis toxin-sensitive G protein pathway in guinea-

pig atrial cells. Consistent with these studies, ATP in concentrations of 1–100 μM produced a transient increase in the outward current which disappeared in pertussis toxin-treated atrial cells, in the present study. Since the activation of the outward current was abolished by suramin, a non-selective P_2 purinoceptor antagonist (Dunn and Blakeley, 1988) or reactive blue-2, a selective P_{2Y} purinoceptor antagonist (Burnstock and Warland, 1987), but not by theophylline, a P_1 purinoceptor antagonist (Burnstock, 1972), these results may suggest that the response is mediated by P_{2Y} purinoceptors. However, reactive blue-2 might not be selective enough to discriminate P_{2Y} purinoceptors from other purinoceptors (Uneyama et al., 1994), and further studies are needed to substantiate the receptor involved.

When $I_{K.ACh}$ was pre-activated by muscarinic or adenosine receptor stimulation, extracellular ATP produced dual effects on the $I_{K.ACh}$. Again both the initial activation phase and the late inhibition phase were abolished by suramin or reactive blue-2 but not by theophylline, indicating the involvement of P_2 purinoceptors. These changes of the outward current were reflected in the biphasic changes of the action potential duration. There may be tonic stimulation of the parasympathetic nerve in the in situ heart. In addition, there are spontaneous openings of the K_{ACh} channel without any muscarinic receptor occupation and the background $I_{K.ACh}$ appears to be important for the repolarization of the action potential as well as the maintenance of the resting membrane potential in atrial cells (Kaibara et al., 1991). Therefore, the inhibitory effect of ATP on $I_{K.ACh}$ may be important for the regulation of electromechanical activity of atrial, sinoatrial nodal and atrioventricular node tissues of the heart in situ.

In the present study, we did not examine the intracellular mechanism by which extracellular ATP produced dual effects on the pre-activated $I_{K.ACh}$. Matsuura et al. (1996a) have demonstrated that the ATP-induced activation of $I_{K.ACh}$ is due to the interaction of pertussis toxin-sensitive G proteins and the K_{ACh} channel, which is analogous to the muscarinic M_2 receptor- or adenosine A_1 receptor-mediated activation of $I_{K.ACh}$. In this study extracellular ATP gradually inhibited the K_{ACh} channel activity recorded in the cell-attached mode without transient increase in the activity. Therefore, activation of the outward current is mediated by a membrane-delimited process, probably activation of pertussis toxin-sensitive G proteins. On the other hand, P_2 purinoceptor-mediated inhibition of the K_{ACh} channel involves some intracellular second messenger(s) but not membrane-delimited components. P_2 purinoceptors have been shown to couple with various intracellular signal transduction systems. Stimulation of P_2 purinoceptors in cardiac myocyte has been shown to cause phosphoinositide hydrolysis through the activation of phospholipase C (Yamada et al., 1992). In addition, P_2 purinoceptor stimulation is reported to increase the production of prostaglandins (Needham et al., 1987) and to activate phospholipase D in vascular endothelial cells (Martin and Michaelis, 1989). Some of the soluble intracellular products might inhibit the openings of the K_{ACh} channel. Further studies are needed to define the intracellular mechanism(s).

This study has demonstrated that extracellular ATP produces dual effects, i.e. stimulatory and inhibitory effects, on the pre-activated $I_{K.ACh}$ through the activation of cardiac P_2 purinoceptors. ATP is stored in sympathetic and parasympathetic nerve terminals and is co-released with norepinephrine and acetylcholine in response to nerve stimulation (Gordon, 1986). When sympathetic nerve is stimulated, co-released ATP may partly counteract the positive chronotropic effect of norepinephrine by activating $I_{K.ACh}$. When parasympathetic nerve is stimulated, co-released ATP may gradually attenuate the excessive negative chronotropic response to acetylcholine by the sustained inhibition of $I_{K.ACh}$. In addition, the dual effect of ATP on $I_{K.ACh}$ may at least in part explain the biphasic inotropic response, rapid negative inotropism followed by slow positive inotropism, in isolated atrial preparations (Froldi et al., 1994). Further studies are needed to clarify the physiological significance of the dual effects of ATP on $I_{K.ACh}$ in vivo and the intracellular signal transduction systems involved.

After the revision this manuscript, Matsuura and Ehara have also reported that external ATP gives an inhibition of the K_{Ach} channel in guinea-pig atrial myocytes (Matsuura and Ehara, 1996, J. Physiol. 497, 379).

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