Activation of Chloride Current by Purinergic Stimulation in Guinea Pig Heart Cells

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Single atrial cells from guinea pig heart were voltage-clamped using the whole-cell configuration of the patch-clamp technique under conditions in which most of the ionic and exchange currents known in cardiac cells were minimized. Extracellular 5 or 50 \( \mu \)M ATP activated a Cl\(^{\text{-}} \) current, in addition to a rapidly desensitizing cation-selective current. A nonhydrolyzable ATP analogue, adenosine-5'-O-(3-thiotriphosphate) (50 \( \mu \)M), also evoked these two currents, indicating involvement of purinoceptors rather than ecto-ATPase on the membrane. ADP, AMP, and adenosine were also effective in inducing the Cl\(^{\text{-}} \) current, showing no clear order of potency for the purinoceptor subtypes involved. The purinoceptor-activated Cl\(^{\text{-}} \) current, like the \( \beta \)-catecholamine–cAMP–dependent cardiac Cl\(^{\text{-}} \) current, showed outward rectification and time independence. (Circulation Research 1992;70:851-855)

**KEY WORDS**  • purinergic receptor  • chloride current  • cation-selective current  • adenine nucleotide  • ATP

Agonist-dependent, nonsynaptic chloride channels have been found in several types of epithelial cells\(^1\) and blood cells.\(^2\) They are mostly cAMP dependent, and activation of these channels is thought to play an important role in cellular functions such as secretion and volume regulation. Recently, similar Cl\(^{\text{-}} \) channels that are regulated by the \( \beta \)-catecholamine–cAMP system have been identified in cardiac cells,\(^3-6\) and their roles in the cardiac electrical activity under physiological and pathophysiological conditions have been given attention.\(^7\) On the other hand, a different regulatory mechanism exists in oocytes, in which Cl\(^{\text{-}} \) channels are activated by ATP, presumably via P2-purinoceptor stimulation.\(^8\) We report here an activation of cardiac Cl\(^{\text{-}} \) current by purinoceptor stimulation with little discrimination among the broad spectrum of adenine nucleotides. Since ATP is known to be a cotransmitter with catecholamine in sympathetic nerves,\(^9\) sympathetic control of the cardiac Cl\(^{\text{-}} \) current may involve two types of receptor–channel mechanisms.

**Materials and Methods**

**Cell Preparation**

Single atrial cells were obtained from hearts of guinea pigs (250–400 g body weight) using an enzymatic dissociation procedure similar to that described previously.\(^10,11\) Briefly, hearts were retrogradely perfused with a modified Ca\(^{2+}\)-free Tyrode’s solution containing collagenase (0.06 mg/ml, Yakuruto, Tokyo) for 10–15 mins through the coronary artery. After the enzyme treatment, the cells were dissociated in high-K\(^{\text{+}} \), low-Cl\(^{-} \) storage solution\(^12\) and stored in a refrigerator (~4°C).

**Voltage-Clamp Technique**

The cells were voltage-clamped using the whole-cell version of the patch-clamp technique\(^13\) with an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt, FRG). Patch electrodes were made from glass capillaries (1.5 mm o.d., 1.0 mm i.d.) using a horizontal micropipette puller (model P-80/PC, Sutter Instrument Co., Novato, Calif.), and electrode tips were then fire-polished with a microforge. Electrodes had a resistance of 1.2–2.0 MΩ when filled with the internal solution. Either square pulses or ramp voltages (dv/dt = 1 V/sec) were used to record the whole-cell current. The ramp voltage protocols consisted of three phases: an initial +100-mV depolarizing phase from the holding potential of ~40 mV, a second hyperpolarizing phase of 200 mV, and then a third phase returning to the holding potential. The current–voltage relation was measured during the second hyperpolarizing phase.

**Solutions**

The compositions of the external solutions are as follows: Normal Tyrode’s solution contained (mM) NaCl 140, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 0.5, glucose 5.5, and HEPES 5.0 (pH 7.4 with NaOH). Modified Ca\(^{2+}\)-free Tyrode’s solution contained (mM) NaCl 100, KCl 10, KH\(_2\)PO\(_4\) 1, MgCl\(_2\) 2, glucose 30, taurine 50, and HEPES 5 (pH 7.4 with NaOH). The external solution used for recording the ATP-induced current was Ca\(^{2+}\)-free, K\(^{+}\)-free Tyrode’s solution containing (mM) NaCl 140, MgCl\(_2\) 2, and HEPES 10 (pH 7.4 with NaOH). As blockers in this solution, 10 \( \mu \)M ouabain (Sigma Chemical Co., St. Louis, Mo.) was used to block the Na\(^{+}\)-K\(^{+}\) pump, 2 mM BaCl\(_2\) was used to block the K\(^{+}\) channel, and 1 \( \mu \)M nicardipine was used to block Ca\(^{2+}\) channels.

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When ionic selectivity of ATP-induced currents was studied, external NaCl was totally or partially replaced with equimolar sodium aspartate, LiCl, CsCl, Tris-HCl, or tetraethylammonium chloride (TEA-Cl). In some experiments, NaCl was totally substituted with 93 mM CaCl₂. The pipette solution (mM) Cs⁺ 126, to replace internal K⁺, aspartate 90, Tris-ATP 5, MgCl₂ 2, and EGTA 5 (pH 7.2 with CsOH). This solution also contained 20 mM TEA-Cl to block K⁺ channels. Total internal and external Cl⁻ concentration was 54 mM and 148 mM, respectively. All experiments were performed at 36±1°C.

**Results**

Under our experimental conditions with selected external and pipette solutions, membrane currents elicited by square voltage pulses (200 msec in duration) of various amplitude (up to ±80 mV) applied from the holding potential of −40 mV were practically time independent (not shown, see Reference 6). Therefore, to measure the current–voltage relation of the remaining background currents, ramp pulses were applied to the cell every 2 or 6 seconds.

Bath application of 50 μM ATP consistently induced a transient increase in inward current, whereas in ~40% of the cells examined, this response was followed by a gradual increase in both the inward and outward currents (Figure 1A). The rapidly activated inward current, which desensitized in several seconds, showed a prominent inward rectification, and its outward component was very small (Figure 1C). This current component was not affected by substitution of external NaCl with equimolar CsCl, LiCl, or sodium aspartate, but it disappeared after substitution with isotonic CaCl₂, equimolar Tris-HCl, or TEA-Cl solution (not shown), indicating involvement of a class of nonspecific monovalent cation channels. Similar ATP-activated, rapidly desensitizing cationic conductances have also been observed in cardiac cells by other investigators.14,15

The conductance component that was gradually activated by ATP reached a maximum 40 seconds to 1 minute after starting the ATP application, suggesting involvement of intracellular metabolic pathways. This response tended to decline thereafter in spite of the maintained exposure to ATP (slow desensitization). The current–voltage relation of this ATP-induced current component exhibited outward rectification (Figure 1E) and had a reversal potential of −27 mV on average (n = 5). This value was close to the predicted Cl⁻ equilibrium potential (E_0) under our experimental conditions: E_0 = 61 × log(54/148) = −26.7 mV.

We measured the reversal potential of the above current component at various [Cl⁻] levels. In the experiment shown in Figures 2A–2C, [Cl⁻] was reduced from 148 to 18 mM during ATP application, by substituting 130 mM NaCl with equimolar sodium aspartate. This led to an inward shift of the holding current, accompanied by a marked decrease in the outward current during ramp pulses (Figure 2A). The reversal potential of the ATP-induced current changed from −26 mV in Cl⁻-rich solution to +20 mV in low-Cl⁻ solution in this case (Figure 2C). Figure 2D summarizes the results of the experiments in which the reversal potential was determined at three different [Cl⁻] levels.

The reversal potential −log[Cl⁻], relation had a slope of 52.6 mV per 10-fold change in [Cl⁻], indicating that Cl⁻ ions are the main charge carrier of this current component.

Membrane currents were also measured by the square-pulse method, using 200-msec pulses of various amplitude, to detect any time dependence of the agonist-induced current. In these experiments, the ATP-induced Cl⁻ current, obtained by subtracting the control current from the current after ATP, showed little time dependence at any voltage (not shown), indicating that this current acts as a background current in the cell excitation. This property, as well as the outwardly rectifying property, has also been noted for the cAMP-dependent Cl⁻ current in cardiac ventricular cells.3–6

Hydrolysis of external ATP by ecto-MgATPase, without purinoceptor stimulation, has been reported to be involved in the translocation of ions across the cell membrane associated with changes in membrane conductance in cardiac cells.16–18 However, in our study, ATP hydrolysis did not appear to be necessary for both the rapidly desensitizing and maintained responses, since 50 μM of the nonhydrolyzable ATP analogue,
Adenosine-5′-O-(3-thiotriphosphate), could also elicit the membrane responses very similar to those generated by ATP (not shown). This finding strongly suggests that the ATP-induced conductance changes in guinea pig atrial cells are mediated by purinoceptors.

If the purinoceptors operate in the observed responses, adenine nucleotides other than ATP such as ADP, AMP, and adenosine are expected to more or less mimic the action of ATP, depending on the purinoceptor subtypes involved. Therefore, we examined the effects of these compounds. ADP (5 or 50 μM) was found to be effective in producing both the rapidly desensitizing cationic current and Cl− current (not shown). However, AMP (50 μM, not shown) and adenosine (50 and 100 μM, Figure 3) activated only Cl− current, failing to evoke the rapidly desensitizing cationic current. Thus, the agonist potency at least for the generation of cationic conductance seemed to coincide well with that proposed for the P2X-purinoceptor, as in the case of the purinoceptor-activated transient cationic current observed in other cell types. For the late response, it is evident in Figure 3E that the adenosine-induced current, like the ATP-induced one shown in Figure 1E or Figure 2C, shows outward rectification with a reversal potential near the predicted Cl− equilibrium potential (~26.7 mV).

To further quantify the agonist potency for generation of the Cl− current, we measured the increase in slope conductance produced by 50 μM ATP, ADP, AMP, and adenosine, respectively, from the current–voltage relation. The conductance was measured at the reversal potential (~27 mV), and its agonist-induced increase was normalized with respect to the capacitive area of the cell membrane. In this comparison, there was no significant difference in the conductance increases produced by the four agonists: ATP, 100.2±57.8 (mean±SD) pS/pF, n=7; ADP, 66.6±43.9 pS/pF, n=4; AMP, 36.9±12.6 pS/pF, n=3; adenosine, 92.9±74.9 pS/pF, n=4. These calculations were made in the cells that clearly responded to the agonists. It should be noted, however, that the proportion of agonist-sensitive cells was almost the same (40–50%) for the four agonists. Thus, we could obtain no clear order of the agonist potency for the Cl− current response.

**Discussion**

Purinoceptor stimulation induced a Cl− current in atrial cells, in addition to the transient nonelective cation current that has been well documented in a variety of excitable cells, including cardiac cells. Agonist-dependent Cl− channels exist in several types of cells. However, they are mostly activated by a cAMP-dependent mechanism, and regulation involving purinoceptors has been known only for the Cl− channels in frog oocytes. Our results provide evidence, for well-differentiated cardiac cells, that ATP can activate a Cl− current via a purinergic mechanism, though the specific purinoceptor subtype was not clear in our study. The ATP-induced Cl− current was observed in ~40% of the atrial cells examined. We consider that the response might depend on the cell conditions, which may be influenced by the experimental manipulations such as cell isolation or cell dialysis.

Scamps and Vassort observed an ATP-activated, Cl−-sensitive current in rat ventricular cells. They concluded that this current is a nonselective cation current that develops in response to a rise in intracellular Ca2+. The latter is thought to be caused by an impairment of the cellular acid–base balance, which is sensitive to ATP-dependent Cl−-HCO3− exchange. However, their study does not appear to exclude the possibility that the

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**FIGURE 2.** Dependence of the reversal potential (Er) of ATP-induced current on [Cl−]o. Panel A: Chart recording of the membrane current in response to ramp pulses. ATP (50 μM) was first applied to the cell in NaCl ([Cl−]o=148 mM) solution, and then external solution was changed to sodium aspartate (Na-aspart) ([Cl−]o=18 mM) solution when the background conductance was increased by ATP. Finally ATP was washed off. Panel B: Current–voltage relations recorded in Na-aspart or NaCl solution, in the absence and presence of ATP. Averages of each two consecutive current–voltage relations indicated by the symbols in panel A are shown with the same symbol: ○, control in NaCl solution; • and ▲, during ATP application in NaCl and Na-aspart solution, respectively; △, after ATP was washed off in Na-aspart solution. Panel C: Current–voltage relations of the ATP-induced current (difference current) recorded in NaCl solution (●−○) and Na-aspart solution (▲−△). Panel D: Graph showing the relation between the Er of ATP-induced current and [Cl−]o, obtained with internal solution containing 54 mM Cl−. The linear line was fitted to the data according to regression analysis with the least-squares method and had a slope of 52.6 mV per 10-fold change in [Cl−]o.
Figure 3. Effects of adenosine (100 μM) on background conductance. Panel A: Chart recording of membrane current in response to the voltage ramp. Adenosine was applied to the cell for a period indicated by the bar. The current tracings indicated by the symbols in the chart recording are shown in panels B–E. Panel B: Current–voltage relations recorded before adenosine (○) and 6 seconds after exposure to adenosine (●). Panel C: Adenosine-sensitive current obtained from panel A by subtraction. Panel D: Current–voltage relation recorded before adenosine (○), 4 minutes after exposure to adenosine (●), and after washing off adenosine (△). Panel E: Adenosine-induced current obtained from panel D by subtraction.

observed current contained a Cl− current that was not well discriminated from the large cationic current; the low bath temperature (22°C) they used could have been a factor.

It may be of interest to see whether Cl− current is activated by β-adrenergic stimulation in the atrium, as has been shown in the ventricle. In our preliminary experiments on guinea pig atrial cells, β-adrenergic stimulation induced a Cl− current in ~10% of the cells examined, and this current also showed outward rectification and time independence (authors’ unpublished observations, March/April 1991). However, it remains to be elucidated whether the same population of Cl− channels are involved in the purinergic and β-adrenergic responses.

The intermediate steps linking the purinoceptor stimulation and the Cl− channel activation remain to be determined. In rat myocardium, activation of the P2-purinoceptor leads to changes in cAMP only if the cAMP level has been previously elevated by β-adrenergic stimulation. Basal intracellular cAMP content in rat and dog ventricular myocytes is not influenced by P2-purinoceptor stimulation. The intracellular second messenger activated after P2-purinoceptor stimulation is unknown in cardiac muscle. However, in smooth muscle cells, P2-purinoceptor stimulation does not lead to changes in cAMP. In rat heart cells, ATP, a most potent P2-purinoceptor agonist, can further increase the Ca2+ current after a maximal β-adrenergic or cAMP stimulation, suggesting that this effect is independent of the cAMP system. All these findings are in favor of the idea that stimulation of cardiac purinoceptors alone does not change the cAMP level. Further studies are necessary to ascertain this point as well as to identify the purinoceptor subtype involved in the activation of Cl− current.

A relevant previous finding that merits discussion is that adenosine, which was a stimulant of Cl− current in our study, suppresses the histamine-induced, cAMP-dependent Cl− current in guinea pig ventricular cells. This suppression is attributed to a purinoceptor-mediated inhibition of the cAMP formation that is enhanced by histamine. The disparity between this observation and ours might be explained if the receptor type and/or the population of channel involved were different in the two responses. Alternatively, purinoceptor stimulation might not only affect the cAMP system but also trigger another channel-regulating mechanism yet to be defined.

On the other hand, in frog oocytes, which possess ATP-dependent Cl− channels, a Cl− current can also be activated by an increased [Ca2+]i (see Reference 8). Some of the cAMP-dependent Cl− channels in epithelial cells are also Ca2+ dependent. It may be important to note that purinoceptor activation seems to increase [Ca2+]i in a variety of cell types. This effect may involve a production of inositol 1,4,5-triphosphate or an enhanced Ca2+ influx. It has recently been demonstrated in cultured smooth muscle cells that ATP causes a brief (<10-second) rise in [Ca2+]i, which leads to a transient activation of Ca2+-dependent Cl− current.

Under our experimental conditions, however, the internal solution contained 5 mM EGTA, which was expected to maintain a low level of [Ca2+]i. Moreover, ATP could induce the Cl− current even when internal 4 mM EGTA or 20 mM 1,2-bis-(2-aminoxyethyl)tetraethylene-N,N,N′,N′-tetraacetic acid was used (authors’ unpublished observations, August/September 1991). Thus, it seems unlikely that an increased [Ca2+]i is involved in the activation of cardiac Cl− current by purinoceptor stimulation. Elucidation of the whole mechanism as well as identification of the purinoceptor-dependent single Cl− channel must await further investigation.

Since ATP is thought to be coreleased together with norepinephrine from sympathetic nerve fibers, the cardiac Cl− current is likely to be controlled by two sets of agonist–receptor interactions. The physiological implication of this feature is unclear, but purinergic regulation of this current may become more effective under some pathological conditions. It is known that β-adrenergic stimulation and ischemia lead to an increase in the release of adenine nucleotides and adenosine from the myocardium, with the concentrations of both purine derivatives reaching biologically effective ones (10−6 to 10−5 M). Activation of the outward Cl− current by ATP is expected to shorten the action potential duration, and this will cut off the Ca2+ influx through the Ca2+ channel that is enhanced by β-adrenergic stimulation associated with an increased sympathetic tone. This effect may, in turn, result
in a protection of the heart from arrhythmogenic and cardiotoxic effects of excess sympathetic activity.

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