British Journal of Pharmacology (1997) 120, 631–638

Purinergic facilitation of ATP-sensitive potassium current in rat ventricular myocytes

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1 The effects of different purinergic agonists on the cardiac adenosine 5′-triphosphate (ATP)-sensitive potassium current ($I_{K(ATP)}$), appearing during dialysis of rat isolated ventricular myocytes with a low-ATP (100 μM) internal solution under whole-cell patch-clamp conditions, were examined in the presence of a P_1 purinoceptor antagonist.

2 The extracellular application of ATP in the micromolar range induced, besides known inward currents through cationic and chloride channels, the facilitation of $I_{K(ATP)}$ once $I_{K(ATP)}$ had already been partially activated during the low-ATP dialysis.

3 Analogues of ATP, α,β-methyleneadenosine 5′-triphosphate (α,βmeATP), 2-methylthioadenosine triphosphate (2MeSATP), adenosine 5′-O-3-thiotriphosphate (ATPγS) similarly facilitated $I_{K(ATP)}$. UTP and ADP were very weak agonists while AMP and adenosine had no detectable effect.

4 The half-maximal stimulating concentration (C_{50}) of α,βmeATP, an analogue that did not elicite the interfering inward cationic current was 1.5 μM. Similar apparent C_{50} (1–2 μM) were observed for ATP and analogues tested with somewhat less maximal effect of ATPγS.

5 Suramin, a nonselective P_2-purinoceptor antagonist, altered $I_{K(ATP)}$ at the relatively high concentration required to inhibit purinoceptors. Pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), a supposedly predominantly P_{2X}-purinoceptor antagonist, at micromolar concentration inhibited the transient inward current but did not block the facilitation of $I_{K(ATP)}$.

6 Our results demonstrate that ATP and its analogues facilitate $I_{K(ATP)}$ in rat ventricular myocytes by stimulation of non-P_1-, non-P_{2X}-purinoceptors.

Keywords: Extracellular ATP; purinergic agonists; purinoceptors; ATP-sensitive potassium channel; cardiomyocytes; ATP depletion; suramin; pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS); cardioprotection

Introduction

Adenosine 5′-triphosphate (ATP) is stored in secretory granules of sympathetic or parasympathetic nerve terminals and is co-released together with noradrenaline or acetylcholine. In addition, several cell types such as platelets, vascular endothelial and smooth muscle cells can release ATP under various physiopathological conditions (Gordon, 1986). The effects of ATP on cardiac cells have been generally ascribed to P_2-purinoceptors whose classification was based primarily on rank order of potency of a series of ATP analogues in the absence of specific antagonists (Burnstock & Kennedy, 1985; Dubyak & El-Moatassim, 1993; Fredholm *et al*., 1994). The initial P_2-purinoceptor subclasses are themselves subdivided following the first studies of cloning and expression of ATP receptors. Rather than being differentiated by the activity of a series of ATP analogues, the P_{2X}-purinoceptors, the ligand-gated ion channels/ionotropic receptors can be differentiated from the P_{2Y}-purinoceptors that activate intracellular signalling pathway by their molecular structures. Extracellular ATP, since the pioneer work of Drury and Szent-Gyorgyi (1929), is known to alter both cardiac chronotropy and inotropy. In mammalian cardiac muscle, extracellular ATP modulates several ionic channels and exchangers (Vassort *et al*., 1994).

A number of potassium channels are present in cardiac myocytes that determine action potential shape and frequency of beating. Some observations indicate that several of them are regulated by extracellular ATP (Friel & Bean, 1988; 1990; Kaneda *et al*., 1994; Fu *et al*., 1995; Matsuura *et al*., 1996a,b). In 1983, Noma described an ATP-sensitive potassium (K_{ATP}) channel that activates with decreasing intracellular ATP concentration; such an observation accounts for the shortening of cardiac action potential observed during anoxia (Trautwein *et al*., 1954). The K_{ATP} channel in cardiomyocytes has been characterized by a number of investigators (for a review see Nichols & Lederer, 1991; Terzic *et al*., 1995) and has recently been shown to consist of an inward rectifier subunit plus an ABC protein, the sulphonylurea receptor (Inagaki *et al*., 1995; Sakura *et al*., 1995). Modulation of K_{ATP} channel activity has been the basis of numerous pharmacological studies since these channels are abundant in a variety of tissues and species (Ashcroft & Ashcroft, 1990) including human ventricular cardiomyocytes (Babenko *et al*., 1992). Sulphonylureas are the most selective inhibitors of these channels, they have been used for a long time in the treatment of non-insulin-dependent diabetes mellitus while numerous synthetic K_{ATP} channel openers are currently suggested for their cardioprotective effects (see Terzic *et al*., 1995 for a review). In cardiac cells, adenosine and acetylcholine are reported endogenous modulators of K_{ATP} channel activity. It has been shown that stimulation of adenosine (P_1-purinoceptor) or muscarinic receptors in the presence of guanosine 5′-triphosphate (GTP) at the inner side of sarcolemma fragments excised from ventricular cells mediates an increase in K_{ATP} channel activity via a membrane-delimited signalling pathway that includes a pertussis toxin-sensitive G-protein (Kirsch *et al*., 1990; Ito *et al*., 1994). Recently, the stimulating effect of acetylcholine on $I_{K(ATP)}$ in atrial myocytes was also proposed to be mediated via phosphatidylinositol second messengers (Wang & Lipsius, 1995), while the β-adrenoceptor agonist-induced increase in $I_{K(ATP)}$ is consequent to adenylyl cyclase activation (Shackow & Ten Eick, 1994).

Using whole-cell patch-clamp recordings we have demonstrated that the $I_{K(ATP)}$ appearing in rat isolated ventricular myocytes dialysed with a low-ATP containing solution is facilitated by micromolar concentrations of extracellular ATP and its analogues applied in the presence of a P_1-purinoceptor antagonist.

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Methods

Cell preparation

Adult rat ventricular cardiomyocytes were isolated as described previously (Pucéat *et al*., 1995). Briefly, the heart was rapidly excised from pentobarbitone-anaesthetized (40 mg kg^{-1}), 200–250 g male Wistar rats and perfused according to Langendorff at 37°C, first with a nominally Ca^{2+}-free buffer solution for cell preparation at 6 ml min^{-1} for 5 min and then at 4 ml min^{-1} for 50 min with the same solution supplemented with 1.3 mg ml^{-1} type A collagenase (Boehringer Mannheim GmbH, Mannheim, Germany) and 20 μM Ca^{2+}. Then, ventricles were separated from atria and gently dissociated by pipetting. Cells, filtered through nylon mesh and washed out from collagenase in the enzyme-free solution were allowed to precipitate and supernatant was discarded. Cells from the pellet were resuspended and incubated at 37°C for 15 min. Meantime Ca^{2+} concentration in the solution was increased step by step up to 0.3 mM. Finally, cells were resuspended in a solution containing 1 mM Ca^{2+} and 0.25% bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.). Cells from the dissociations giving not less than 6×10^6 rod-shaped cardiomyocytes were kept at low concentration ($\sim 10^3$ cell ml^{-1}) for 8–10 h at 37°C. Then 30 μl of cell suspension were dropped in a specially designed 0.7 ml perfused bath chamber mounted on a Diaphot 200 inverted Nikon microscope (Nikon Corporation, Tokyo, Japan) 10 min before the electrophysiological experiment; only clearly striated myocytes with a smooth surface were used.

Whole-cell current recording

Whole-cell currents were recorded by the conventional whole-cell configuration of the patch-clamp technique (Hamill *et al*., 1981). Micropipettes manufactured from hard glass capillary tubes (GC120F-10, Clark Electromedical Instruments, Pangbourne, U.K.) by a P-80/PC Flaming-Brown programmable puller (Sutter Instrument Co., CA, U.S.A.) with resistance of 2–3 MΩ when filled with the pipette solution were used. Liquid junction potential between the external and the pipette solutions was less than 2 mV. Whole-cell currents were recorded using a RK-400 cell/patch-clamp amplifier (Biologic, Claix, France) at a holding potential of −40 mV and during repetitive slow (0.05 mV ms^{-1}) voltage-ramp stimuli from +50 to −100 mV (voltage from −40 to +50 mV and from −100 to −40 mV was changed at a voltage-ramp rate of 1 mV ms^{-1}). Command potential generation, acquisition, and on-line analysis of currents elicited during voltage-ramp stimuli were performed with a PCL-718 interface (Advantech Co., Ltd, U.S.A.) on a Pentium computer and the BioQuest software (WC2-6 version, developed by Dr. A. Alekseev). Continuous whole-cell current signal was monitored on a NIC-310 digital oscilloscope (Nicolet Instrument, Plaisir, France) and stored on magnetic tape by a DTR-1800 digital tape recorder (Biologic, Claix, France) for later analysis. The current recordings were taken into account beginning from the 10–15th min after membrane patch rupture if series resistance ranged from 10 to 20 MΩ and was stable (less than 10% increase during the experiment). The series resistance was compensated by 70% through the patch-clamp amplifier circuit. Whole-cell currents were normalized to cell capacitance (C_m) calculated from equation $C_m = Q/V$ where Q is charge estimated by integrating the area defined by the capacitive transient current recorded during a 10 mV hyperpolarizing pulse (V). Long-time current records were replayed from magnetic tape, sampled at 6 or 60 ms-step of digitization and plotted from a file by use of SigmaPlot 5.0 software (Jandel Scientific, San Rafael, CA, U.S.A.).

Under a designed voltage protocol and ionic conditions, the basal current observed soon after the patch rupture was predominantly I_{K1} (through strongly inwardly rectifying potassium channels) that remained relatively constant and was not altered markedly by purinoceptor agonist applications during the 60–80 min recordings in the presence of 10 μM glibenclamide. A potassium, ohmic, voltage-independent, glibenclamide-inhibited current developing slowly during cell dialysis with the 'intracellular' low-ATP-containing glucose-free solution was referred to as $I_{K(ATP)}$. P_2-purinoceptor stimulation of rat ventricular cells mediates substantial inward currents through voltage-independent nonselective cationic (Scamps & Vassort, 1990; 1994) and chloride channels (Kaneda *et al*., 1994) with reversal potential at 0 mV for both currents in the present ionic conditions. Thus, $I_{K(ATP)}$ was estimated from the difference between currents recorded at 0 mV either during voltage ramp stimuli applied at the time of interest or at the beginning of whole-cell current recording. Time course of $I_{K(ATP)}$ at 0 mV was constructed from a sequence of the ramp current traces analysed with the subtraction algorithm. Finally, changes in slope of quasi-linearly increasing $I_{K(ATP)}$ (i.e. 'net-slope' calculated as the difference between the current rises during a minute after, and immediately before application of agonist) were determined to characterize quantitatively the effects of different purines on $I_{K(ATP)}$.

Solutions and reagents

The buffer solution for cell preparation had the following composition (mM): NaCl 123, KCl 5.4, $NaHCO_3$ 5, NaH_2PO_4 2, $MgCl_2$ 1.6, D-(+)-glucose 10, taurine 20 and HEPES 20; pH 7.2 at 23–24°C adjusted with 1 N NaOH. The external control solution contained (mM): NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1 and HEPES 10; pH 7.4 at 23–24°C adjusted with 1 N NaOH and was supplemented with 10 μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a potent adenosine (P_1-purinoceptor) receptor blocker. The 'intracellular' pipette solution contained (mM): KCl 140, EGTA 5, HEPES 5, KOH 10, $MgCl_2$ 1.3, Na_2ATP 0.1 and GTP lithium salt 0.2; pH 7.2 at 23–24°C, adjusted with 1 N KOH. Different nucleotides and other compounds were added to the external solution, according to the experimental protocol described in the Results section, from stock solutions just before use; pH was carefully checked again after dilution of nucleotides. Solutions supplemented by purinergic inhibitors, suramin or pyridoxal-phosphate-6-azophenyl-2′, 4′-disulphonic acid 4-sodium (PPADS) were protected from light.

DPCPX and PPADS were from Research Biochemicals Incorporated (Natick, MA, U.S.A.), 2-methylthioadenosine triphosphate tetrasodium salt (2MeSATP) from ICN Biomedicals Inc. (Aurora, Oh, U.S.A.), suramin (a generous gift from Bayer AG, Leverküssen, Germany), adenosine 5′-O-3-thiotriphosphate (ATPγS) tetralithium salt from Boehringer Mannheim GmbH (Mannheim, Germany) were used. α,β-methyleneadenosine 5′-triphosphate lithium salt (α,βmeATP) and other reagents were obtained from Sigma Chemical Co (St. Louis, MO, U.S.A.), unless otherwise noted.

Different bathing solutions including the control one were applied to the cell with a RSC-100 rapid solution changer system (Biologic, Claix, France) at a flow rate of 100–150 μl min^{-1} and a tube switching time of 100 ms. Electrophysiological experiments were performed at 23–24°C.

Data analysis

All averaged values and error bars represent means ± s.d. Statistical significance was evaluated by Student's unpaired *t* test. Differences with values of $P < 0.05$ were considered to be significant. Fits to the dose-response equation were performed by running SigmaPlot 5.0 software with a non-linear Marquardt-Levenberg curve-fit algorithm.

Results

Effect of extracellular ATP on $I_{K(ATP)}$

By use of the conventional whole-cell patch-clamp technique, rat ventricular cells were dialysed with a low-ATP containing solution (100 μM) that elicited a slowly developing $I_{K(ATP)}$. The nature of this current was revealed by its voltage-independent kinetics, instantaneous activation without any apparent inactivation across a large range of test potentials, its reversal potential close to E_K, the reversal potential of K^+ ions, and its inhibition by glibenclamide. As shown in Figure 1, cells were held at -40 mV and repetitive slow ramps applied from $+50$ to -100 mV. Soon after the patch had been broken, the application of 50 μM ATP induced an inward current with an initial large surge as previously described (Scamps & Vassort, 1990; 1994; Kaneda *et al*., 1994). Within 15 min the holding current started to get more outward; then on top of the inward current, ATP triggered a large outward current that developed slowly and recovered with a similar time course on ATP washout. Outward current was never elicited by ATP applied before some $I_{K(ATP)}$ had occurred. A second ATP application similarly enhanced the outward current that was sustained as long as ATP was present. The late application of glibenclamide inhibited this outward current. Note also that while $I_{K(ATP)}$ developed the tracing became more noisy. In inset of Figure 1, the current tracings elicited by voltage-ramps applied at different times during the occurrence of $I_{K(ATP)}$ and during ATP applications are superimposed. Initially, a strong inward rectifying current, I_{K1} was predominantly elicited; later during the low-ATP dialysis, a quasi-linear relationship was obtained with, in every case, a reversal potential at about -85 mV. In the presence of 5 mM ATP in the pipette, basal outward current was never found to increase over the 60 min of dialysis; the repetitive application of 50 μM ATP within one hour of breaking the cell membrane induced inward currents as already described (Scamps & Vassort, 1990; Kaneda *et al*., 1994) but no outward currents, although following metabolic inhibition with 0.5 μM carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone (FCCP) a large, with magnitude of several nA, glibenclamide-sensitive outward potassium current was produced through $K_{(ATP)}$ channels being in operational state (not shown, 5 cells). These results show that ATP in the presence of a P_1-purinoceptor (adenosine receptor) antagonist mediates a substantial increase in potassium glibenclamide-sensitive current only when $I_{K(ATP)}$ had

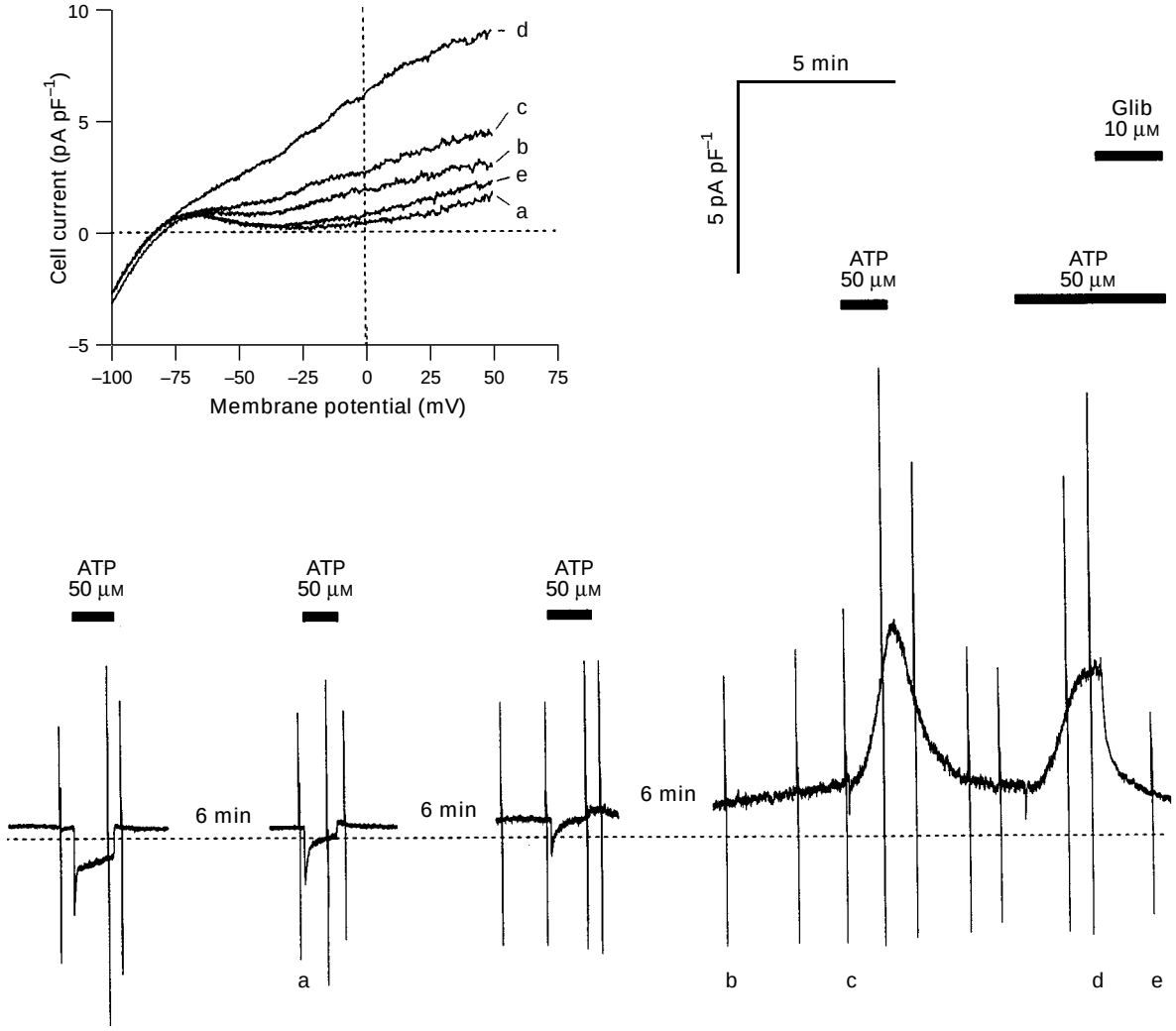

Figure 1 Effects of extracellular ATP application on the holding current in rat ventricular cardiomyocyte. Under whole-cell patch-clamp conditions, a cell dialysed with a low-ATP (100 μM) pipette solution was held at -40 mV. Repetitively applied slow voltage-ramp stimuli are displayed here and in next figures as sharp vertical deviations at this time base. Note that due to low rate of digitization and compression during the current trace plotting, the length of the vertical deflections might not reflect full values of current amplitude during voltage-ramp stimuli. Such a voltage protocol allowed the rapid establishment of the current-voltage relation of quasi-steady-state current appearing at different times in the experiment (traces marked alphabetically are presented in inset). Current recording is presented beginning 18 min after a break in the cell membrane. In this and next figures, currents are normalized to cell capacitance, dashed line indicates zero current level, upward deflection of current trace corresponds to outwardly directed current and agonist applications are indicated by bold horizontal continuous lines; Glib, glibenclamide.

already been partially activated by the low-ATP dialysis. Thus, the ATP-induced change in current may be defined as a facilitation of $I_{K(ATP)}$.

Other purinergic agonists facilitate $I_{K(ATP)}$

Along with $I_{K(ATP)}$ facilitation, ATP activates a substantial non-selective cationic current in rat ventricular cells (Scamps & Vassort, 1990; 1994; see Figure 1). Not only will this current render the analysis of $I_{K(ATP)}$ more difficult but consequent increases in the local concentration of cations are known to alter the behaviour of several ionic channels. The poorly-hydrolysable ATP-analogue, α,βmeATP activates neither the non-selective catonic current nor the Cl^- current (Scamps & Vassort, 1994; Kaneda *et al*., 1994). We thus used this agonist to obtain an insight into the relationship between the purinergic facilitation of $I_{K(ATP)}$ and initial level of $I_{K(ATP)}$. Figure 2 shows a continuous whole-cell recording and exemplifies the effect of repetitive applications of 10 μM α,βmeATP. Clearly, the agonist-facilitated $I_{K(ATP)}$ depended on the previous level of the current with relatively reproducible changes in the facilitation of $I_{K(ATP)}$ when the prevailing level of outward current ranged from about 2 to at least 4 pA pF^{-1}, at a holding potential of -40 mV (observed in all 5 similar experiments).

Taking into account the above observation, the effects of several purinergic agonists applied in the presence of a P_1-purinoceptor antagonist were tested on cells in which the outward holding current developing during cell dialysis with the low-ATP solution had reached an amplitude not less than 2 pA pF^{-1} at -40 mV. Figure 3 presents original recordings showing that at 10 μM all of ATP tested analogues were active in facilitating $I_{K(ATP)}$. Note that $I_{K(ATP)}$ recovered after washout of every agonist like after ATP removal. Even 50 μM uridine 5′-triphosphate (UTP) and 50 μM ADP induced very weak enhancement of $I_{K(ATP)}$. Neither AMP nor adenosine had an effect in 21 similar experiments.

Dose-dependency of purinergic stimulation of $I_{K(ATP)}$

We established that the purinoceptor-mediated facilitation of $I_{K(ATP)}$ was dose-dependent for α,βmeATP, an analogue with

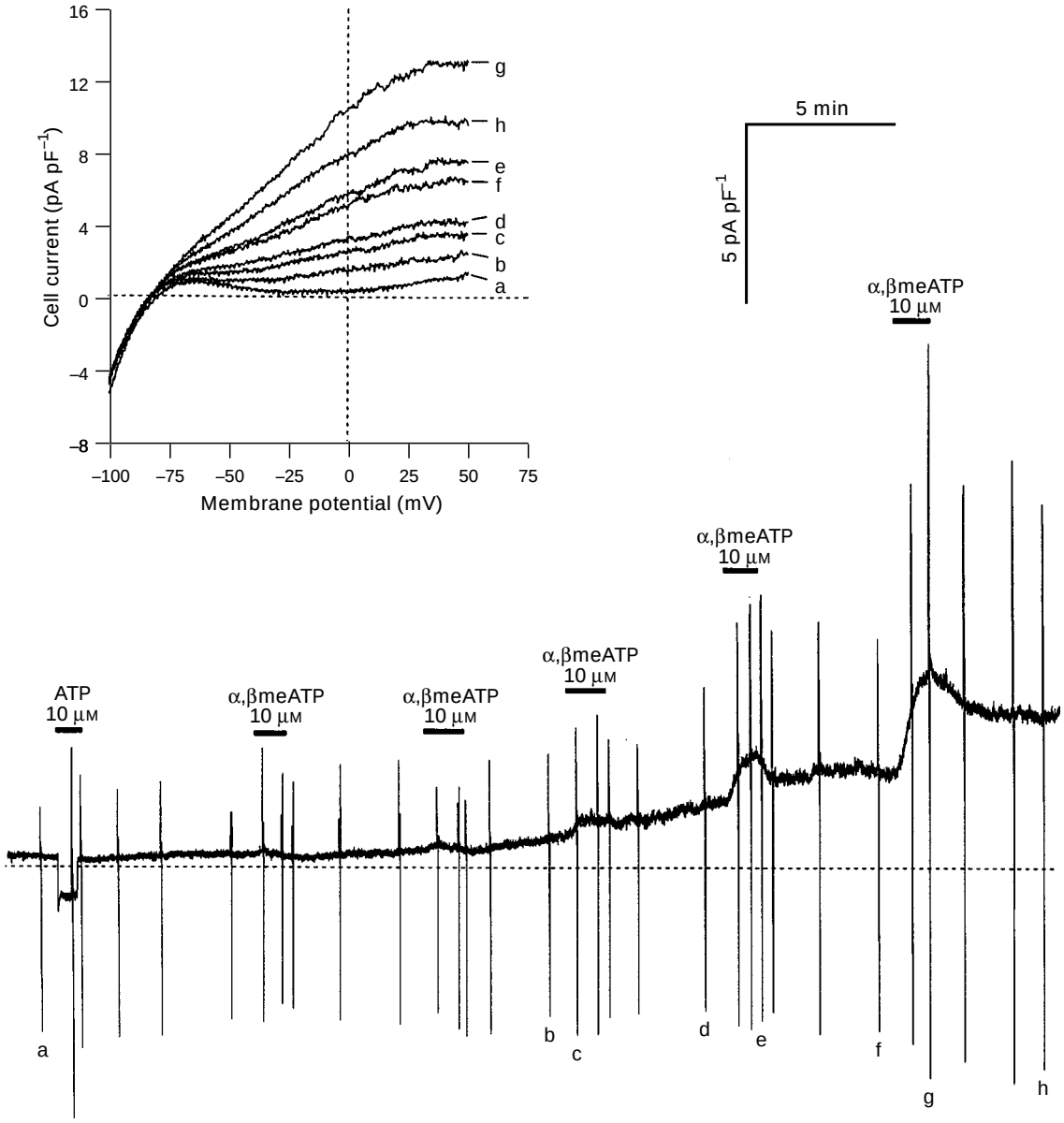

Figure 2 Facilitation of $I_{K(ATP)}$ by purinergic stimulation is dependent upon the previous level of the current. Similar applications of α,βmeATP which, unlike those of ATP, do not induce inward current, progressively facilitate $I_{K(ATP)}$ as the level of the current increases along with the depletion of intracellular ATP during low-ATP dialysis. Continuous current recording is presented beginning 16 min after a break of the cell membrane. Inset: current-voltage relations established by applying voltage-ramp stimuli before and during the successive applications of α,βmeATP as indicated. Experimental conditions and labels as in Figure 1.

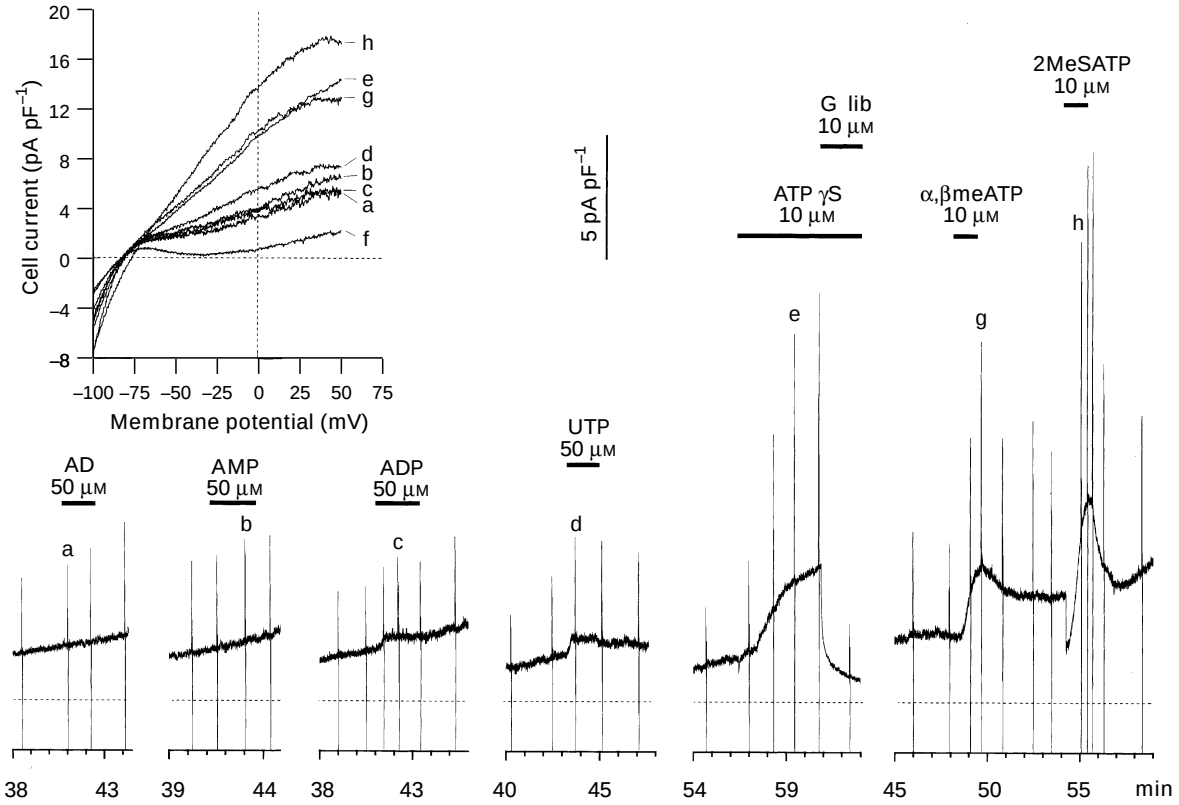

Figure 3 Purinergic agonist-induced facilitation of $I_{K(ATP)}$. Comparison of the effects on $I_{K(ATP)}$ of adenosine (Ad), AMP, ADP, UTP and various ATP analogues applied on different cells in which $I_{K(ATP)}$ had been partially activated during cell dialysis with a low-ATP solution under experimental conditions similar to those of Figure 1; time after the cell membrane rupture is indicated on scales. Inset: current-voltage relations established by applying voltage-ramp stimuli marked alphabetically. Other abbreviations as in Figure 1.

less effects on currents unrelated to $I_{K(ATP)}$ when it was applied on cells at various concentrations but at a given (2–2.5 pA pF^{-1}) previous level of outward current. This is illustrated in Figure 4a. As shown in inset, using equation:

$$I = I_{max}(C^{n_H}(C^{n_H} + C_{50}{}^{n_H})^{-1}),$$

where I and I_{max} are the actual and the maximal increase in quasi-linear rise of $I_{K(ATP)}$, respectively, C and C_{50} the actual and the half-maximal stimulating concentration of α,βmeATP, respectively, and n_H the curvature coefficient, the best fit of averaged experimental data was obtained with C_{50}: 1.5 μM, n_H: 1.3, and I_{max}: 6.4 pA pF^{-1} min^{-1}. Results of similar experiments with other agonists are summarized in Figure 4b. ATPγS was found to be less efficient compared with other adenosine triphosphates tested, and apparent values of the half-maximal stimulating concentration for all the agonists tested were in the range of 1–2 μM. Thus, the agonist potency for the facilitation of $I_{K(ATP)}$ was found to be ATP$\simeq\alpha,\beta$meATP$\simeq$2MeSATP$>$ATPγS$>>$UTP$\simeq$ADP.

Effects of purinoceptor antagonists

Suramin is a broad spectrum non-selective P_2-purinoceptor antagonist whose inhibitory effects are generally checked after pretreatment. Cells which had been pretreated for 30 min at 37°C with 50–100 μM suramin showed a very slow development of $I_{K(ATP)}$ during the low-ATP dialysis. Moreover on these cells, the application of 10 μM ATP was ineffective in facilitating $I_{K(ATP)}$. The effect of ATP was also tentatively checked on cells freshly superfused with suramin. As shown in Figure 5a under similar experimental conditions as with the low-ATP dialysis, the application of 100 μM suramin enhanced the outward current which then slowly decreased. A closer examination of the current trace shows that the current decrease or run-down occurred together with less noise. The latter observations might indicate that less channels remained available to open over the time but with a high open probability. Voltage-ramps applied during the surge of current or after it had partially deactivated were quasi-linear with a reversal potential of about −80 mV. The subsequent application of ATP in the presence of suramin was ineffective despite the remaining potassium current being suppressed by glibenclamide. Note that the transient inward current activated by ATP was not fully inhibited after 10 min of bathing the cell in suramin-containing solution. Similar effects were observed in three other cells. In two other cells, soon after the large outward current had developed on superfusion of suramin, the application of 10 μM glibenclamide inhibited it (not shown).

PPADS generally used at 10–30 μM is considered to be a selective antagonist of P_{2X}-purinoceptors (Lambrecht *et al.*, 1992). Preincubation of cells with 10 μM PPADS for 20 min at 37°C attenuated the development of $I_{K(ATP)}$ during the low-ATP dialysis. Furthermore, the purinergic-mediated facilitation of $I_{K(ATP)}$ was then very weak, and the transient inward current was never observed in PPADS-pretreated cells. As shown in Figure 5b, superfusion of a low-ATP-dialysed cell with 20 μM PPADS, like with 100 μM suramin, induced a bimodal change in the outward current (left part); the latter was also sensitive to 10 μM glibenclamide which markedly inhibited it (2 cells). At a lower concentration, PPADS (2 μM) induced a less marked transient increase in the outward potassium current by itself, inhibited the non-selective cationic current and did not abolish the ATP-induced facilitation of $I_{K(ATP)}$ (right part). Facilitation of $I_{K(ATP)}$ by 10 μM ATP applied on three cells when the outward holding current at −40 mV had reached 2–2.5 pA pF^{-1} was not significantly reduced (2.3$\pm$1.5 vs 4.2$\pm$2.1 in control, see Figure 4b).

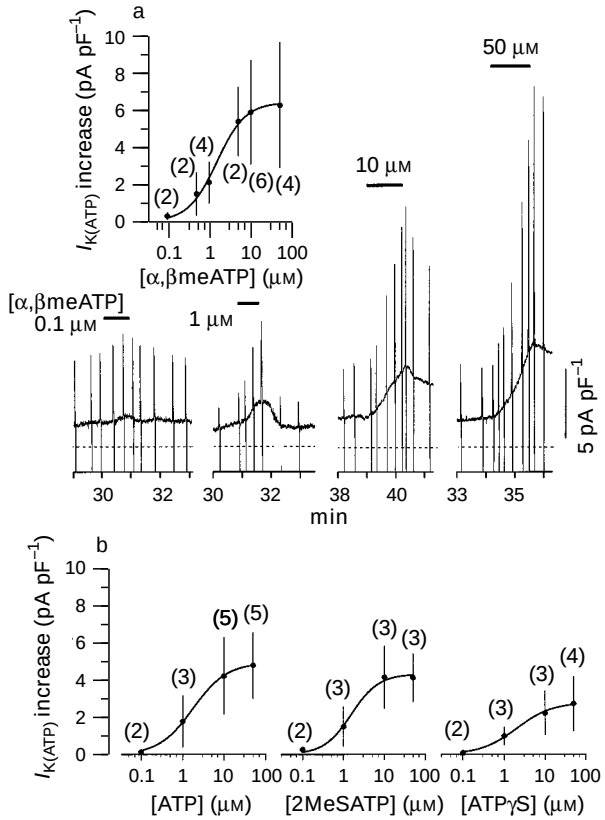

Figure 4 Dose-dependency of the purinergic-induced facilitation of $I_{K(ATP)}$. (a) Representative current recording fragments illustrating the effects of α,βmeATP applied at different concentrations on cells in which a similar level of $I_{K(ATP)}$ had been reached. Duration of cell dialysis with the low-ATP solution is indicated on scales. Other labels and experimental conditions are as in Figure 1. Inset: averaged values of increase in slope of quasi-linearly rising $I_{K(ATP)}$ calculated as described in Methods are plotted against the agonist concentrations and fitted by the pseudo-Hill equation; number of different cells is indicated for each data point in parentheses. The best fit was obtained with $C_{50} = 1.5$; $I_{max} = 6.4$ and $n_H = 1.3$. (b) Results of similar experiments with ATP and its analogues that also activate an inward current. Number of observations is indicated for each data point. The best fits of data were obtained with $C_{50} = 1.8$, 1.6 and 2.0, $I_{max} = 4.9$, 4.3 and 2.8 and $n_H = 1.1$, 1.3 and 1.0, in the presence of ATP, 2MeSATP and α,βmeATP, respectively.

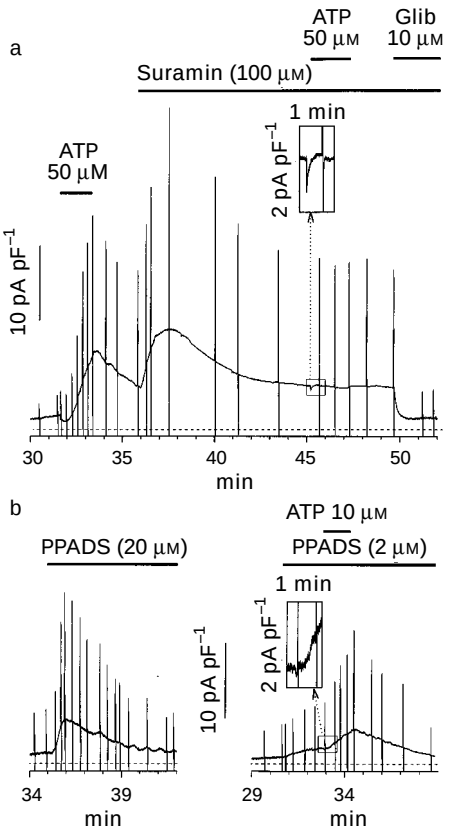

Figure 5 Effects of suramin and PPADS on $I_{K(ATP)}$. (a) A cell dialysed with the low-ATP solution and demonstrating facilitation of $I_{K(ATP)}$ during the first application of ATP was then superfused with a 100 μM suramin-containing solution. This treatment induced a marked increase in the outward potassium current followed by its decline during which ATP did not facilitate $I_{K(ATP)}$ more. Note that the ATP-induced transient inward current was not completely suppressed after about 9 min in the presence of the compound. (b) PPADS at a relatively high concentration (20 μM), similar to suramin, affected by itself the $I_{K(ATP)}$ that had developed during low-ATP dialysis of the cell (left part). At a submicromolar concentration, PPADS (2 μM) was able to prevent the transient inward current induced by ATP but did not abolish the purinergic facilitation of the outward potassium current (right part). Note that $I_{K(ATP)}$ was slightly enhanced and then slowly reduced in the presence of PPADS even at this relatively low concentration. In both (a) and (b) the time after achievement of whole-cell configuration is indicated on scale, and corresponding fragments of the current recording are presented in insets at expanded time and current scales. Other markers and experimental conditions as in Figure 1.

Discussion

The data presented provide the first evidence for a purinergic, not adenosine receptor-mediated, facilitation of cardiac $I_{K(ATP)}$. The experimental conditions were designed, so that cellular ATP was slowly depleted, to simulate a crude model of physiopathological conditions in which a decrease in intracellular ATP is known to occur. Dialysing the cells with a low-ATP solution we showed that purinergic stimulation of $I_{K(ATP)}$ occurs only when it has already been partially activated. The reasons for choosing a 100 μM intracellular ATP dialysing solution were two fold: firstly, it is an ATP concentration that triggers a quasi steady-state activity of $I_{K(ATP)}$ avoiding both its fast activation and its premature run-down; secondly, this concentration is close to the K_m for ATPases, kinases and other ATP-utilizing enzymes. Consequently, such a protocol allowed us to observe reproducible effects of purinergic stimulation on $I_{K(ATP)}$.

$I_{K(ATP)}$ was markedly and similarly enhanced by ATP and its poorly-hydrolysable analogues, although ATPγS demonstrated slightly less efficiency. The apparent C_{50} values to facilitate $I_{K(ATP)}$, in the micromolar range for the various ATP analogues, were similar to those previously found for other purinergic effects (Puceat *et al.*, 1991a, b; Scamps & Vassort, 1994). The guanidyl triphosphate, UTP was as poorly active as ADP. However, the sensitivity of $I_{K(ATP)}$ to purinergic stimulation was different from the stimulation of the Ca^{2+} current for which the rank order of efficacy is 2MeSATP ≃ ATP ≃ ATPγS with α,βmeATP being inactive (Scamps & Vassort, 1994) and for the activation of the Cl^- current that also shows no response to α,βmeATP (Kaneda *et al.*, 1994). The agonists shown to enhance $I_{K(ATP)}$ are the same as those mediating the increase in intracellular free Ca^{2+} concentration or the sarcoplasm acidification, in which ADP and UTP, GTP, CTP and ITP were shown to be ineffective (Pucéat *et al.*, 1991a, b). However, the purinergic-induced facilitation of $I_{K(ATP)}$ could not be attributed to variations in intracellular free Ca^{2+} concentration activating the sarcolemmal Ca^{2+}-ATPase and consequently inducing local ATP-depletion, since experiments performed in the presence of BAPTA, a fast chelating agent, gave similar results as in its absence (not shown, 3 cells). It is difficult to correlate the rank order of efficacy and potency of purinergic agonists to facilitate $I_{K(ATP)}$ with those found for

any of the presently cloned P_2-purinoceptors. However, although P_{2X}- and P_{2Y}-purinoceptors have generally been shown to activate the ligand-operated channels or the phospholipase Cβ, respectively other signal transduction pathways are activated by purinergic agonists. For example, in rat cardiomyocytes, we have recently demonstrated that ATP induces activation of a tyrosine kinase pathway that involves phospholipase $C_{\gamma 1}$ (Pucéat & Vassort, 1996). Furthermore, the ATP- and triphosphate-adenosine derivatives-induced acidification mediated by activation of the Cl^-/HCO_3^- exchanger is associated with a tyrosine-phosphorylation of this exchanger (Pucéat *et al*., 1991b; 1993).

The trypanocidal drug suramin has been shown to inhibit P_{2X}-, P_{2Y}- and P_{2T}-purinoceptor-mediated effects but with relatively low activity; 100 μM are required (Dunn & Blakely, 1988). Moreover, it is now apparent that suramin is a poorly selective antagonist of ATP since it potently inhibits ATPase activity at similar concentrations to those at which it blocks the P_{2X}-purinoceptors (Humphrey *et al*., 1995). In addition among several other non-purinoceptor-mediated antagonistic effects, suramin inhibits ATP breakdown by ectonucleotidases (Hourani & Chown, 1989) and a number of nucleotide-binding enzymes (Voogd *et al*., 1993). Therefore, it is probable that suramin might interact with the nucleotide binding sites of the K_{ATP} channel, assuming the drug reaches the cytoplasmic side of sarcolemma. Intracellular ATP regulates K_{ATP} channels in two ways: it closes the channel (ligand function) and in the presence of Mg^{2+} ions, it maintains the K_{ATP} channel in an operational state presumably through an enzymatic reaction (see for review Ashcroft & Ashcroft, 1990; Nichols & Lederer, 1991; Terzic *et al*., 1995). Suramin, on the one hand, might antagonize intracellular ATP-induced inhibition of channel opening that leads to an increased $I_{K(ATP)}$; on the other hand, suramin might affect a MgATP-dependent mechanism that maintains K_{ATP} channel in a functional state. A rather similar situation might occur with the supposedly selective antagonist of P_{2X}-purinoceptors, PPADS. However, 20% of the inhibition by this compound has been shown to be not concentration-related (Windschief *et al*., 1995), it is known to inhibit ectonucleotidases at a high concentration (Welford *et al*., 1986) and, like suramin, to be an antagonist at cloned P_{2Y}-purinoceptors (Charlton *et al*., 1996). Thus, our results and those of other authors are in general agreement that these compounds interact with different ATP-binding sites apart from the specific purinoceptors. Such observations prevent unequivocal information regarding the inhibitory effect of suramin and PPADS on purinoceptor-mediated modulation of ATP-dependent effector systems from being obtained. However, PPADS, compared with suramin, had a stronger inhibitory effect on the transient non-selective cationic current than on the facilitated $I_{K(ATP)}$. This finding is in agreement with the original proposal that the ATP-induced transient non-selective cationic current is due to a ligand-operated channel (Friel & Bean, 1988), and attributable to one of the P_{2X}-subtype of purinoceptors. The above results also suggest that the ATP-induced facilitation of $I_{K(ATP)}$ is not attributable to the activation of the ionotropic purinoceptors since facilitation is triggered by $\alpha\beta$-meATP and not prevented by PPADS. The inability of $\alpha\beta$-meATP to activate the non-selective currents as well as the high sensitivity of this current to PPADS are in line with corresponding properties of the recently cloned P_{2X5}-receptor expressed in rat heart (Garcia-Guzman *et al*., 1996).

Purinergic-stimulation of $I_{K(ATP)}$ might be of physiopathological significance and could represent an endogenous safety mechanism for cells that are partially deficient in intracellular ATP. As with potassium channel openers, the ATP-facilitated outward $I_{K(ATP)}$ could shorten the action potenial, and thus reduce energy consumption by abbreviating cell contraction. It is well known that ATP is released from the perfused heart particularly during hypoxic or adrenergic stimulation (Vial *et al*., 1987). Although the following hypothesis is currently controversial, at the single cell level it has been proposed (Reisin *et al*., 1994; Schwiebert *et al*., 1995) and denied (Reddy *et al*., 1996; Li *et al*., 1996) that the ABC proteins could carry ATP. Whatever the mechanism of ATP release, under ischaemic conditions $I_{K(ATP)}$ would be activated by intracellular depletion of ATP and facilitated by positive feedback allowing for its modulation. Such a mechanism which leads to cell protection should be differentiated from the effect of ATP which, after its release by damaged cells will diffuse to nearby intact cells and induce spontaneous electrical activity (Scamps & Vassort, 1990; Vassort *et al*., 1994). In this context, one has to consider that such an ATP-induced action potential shortening might contribute to increase tissue imbalance and thus participate in arrhythmias.

In conclusion, extracellular ATP in the micromolar range enhances $I_{K(ATP)}$ in rat isolated ventricular myocytes dialysed under whole-cell patch-clamp conditions with a low-ATP containing solution. Such a facilitation results from stimulation of non-P_1-, non-P_{2X}-purinoceptors, is triggered by ATP analogues (α,βmetATP, 2MeSATP, ATPγS) with ADP and UTP being very weak agonists. This facilitation of $I_{K(ATP)}$ is not inhibited by PPADS at a concentration sufficient to block non-selective cationic channels related to P_{2X}-purinoceptors in other tissues. This is the first time that purinergic modulation of $I_{K(ATP)}$ has been described and this might contribute to adaptation of cardiomyocytes to physiopathological conditions in which the intracellular ATP level is compromised.

The authors would like to acknowledge M. Rongier for cell preparation and M. Pucéat for useful comments. This work was supported in part by grants from Ministère de l'Enseignement Supérieur et de la Recherche (France) and from Russian Foundation for Basic Research 95-04-12056 to A.B.

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(Received May 28, 1996
Revised September 4, 1996
Accepted November 6, 1996)