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Differential sensitivity of atrial and ventricular K_{ATP} channels to metabolic inhibition

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Abstract

Objective: The aim is to compare the activation of ATP-sensitive potassium channels (K_{ATP} channels) in intact and metabolically impaired atrial and ventricular myocytes. **Methods**: The K_{ATP} channel current is measured by whole cell and gramicidin-perforated patch clamp recordings in 164 cultured neonate rat cardiomyocytes. **Results:** In whole cell recordings with 84 μmol/l ADP in pipette, spontaneous activity is significantly higher in atrium than ventricle, and EC_{50} for the K_{ATP} channel opener diazoxide is 0.13 μmol/l (atrium) versus 3.1 μmol/l (ventricle). With an ATP-regenerating system in pipette, EC_{50} for diazoxide is 19.7 μmol/l (atrium) versus 54.9 μmol/l (ventricle). In gramicidin-perforated patch recordings, atrial myocytes respond significantly to 100 nmol/l of the mitochondrial protonophore CCCP, while ventricular myocytes do not. EC_{50} for diazoxide is 129 μmol/l (atrium) versus >2500 μmol/l (ventricle) for myocytes exposed to CCCP, and 676 versus >2500 μmol/l, respectively, without CCCP. **Conclusions**: (1) K_{ATP} channels are significantly more sensitive to metabolic inhibition in atrial than ventricular myocytes. (2) Sensitivity of atrium versus ventricle to the channel opener diazoxide increases from 3:1 to ≥24:1 with ADP or metabolic inhibition. If extended to intact hearts, the results would predict a higher atrial sensitivity to ischemia, and a high sensitivity of the ischemic atrium to K_{ATP} channel openers.
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Keywords: Atrial function; K-ATP channel; Membrane currents; Myocytes

1. Introduction

Sarcolemmal K_{ATP} channels were first discovered in heart [1] and subsequently in most other organs. They couple the membrane potential to the metabolic state of the cell [2–5]. In pancreatic β-cells, the normally open K_{ATP} channels close in response to increased glucose influx, causing membrane depolarization and calcium-induced exocytosis of insulin secretory vesicles [3]. In cardiac myocytes, the normally closed K_{ATP} channels open in response to metabolic distress, and thus reduce the action potential duration, calcium influx, force of contraction and possibly the ATP demand [5]. In heart as well as kidney and brain the sarcolemmal K_{ATP} channels thus help protect the tissues from ischemia, though mitochondrial K_{ATP} channels are also likely to be involved [6].

Sarcolemmal K_{ATP} channels are composed of a potassium channel pore, formed by four potassium inward rectifier subunits (KIR6.1 or KIR6.2), and by an association of four regulatory sulfonylurea receptor subunits (SUR1, SUR2A or SUR2B) [2–5]. There is convincing evidence from both native ventricular and artificially expressed K_{ATP} channels that the 'cardiac' K_{ATP} channel is a combination of KIR6.2 and SUR2A subunits [7]. These channels typically open in response to the K_{ATP} channel opener pinacidil but not to diazoxide [2,4,5]. In contrast, atrial K_{ATP} channels open in response to both pinacidil and diazoxide [8], though species differences exist [9]. Furthermore, the K_{ATP} channel blocker glibenclamide is highly effective in closing atrial but not ventricular K_{ATP} channels [8]. These differences in pharmacological characteristics are important, since K_{ATP} channel modulators used in the treatment of diabetes (sulfonylureas) or other diseases

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(diazoxide) could affect atrial myocytes, even though they little affect the ventricular myocytes.

Interestingly, in the presence of 100 μmol Mg-ADP, ventricular myocytes do become sensitive to diazoxide [10]. Such ADP levels can be reached in hypoxia [11] and presumably in ischemic heart disease. Interactions between diazoxide and Mg-ADP may be explained by the absolute requirement, in SUR, of the second nucleotide binding fold for the action of both diazoxide and Mg-ADP [10,12,13], though other SUR domains are also required for diazoxide action [14]. Mg-ADP antagonizes the ATP-induced inhibition of the K_{ATP} channel [15]. The low sensitivity of ventricular myocytes to diazoxide may be explained by an interaction of the C-terminal tail of SUR2A with the second nucleotide binding fold [13]. Interactions between diazoxide and Mg-ADP on the atrial K_{ATP} channel are still unknown, but the high sensitivity to diazoxide [8] raises two questions. First, is this sensitivity mediated by an atrium-specific K_{ATP}-channel, or was it due to the presence of ADP in the patch pipette? ATP-containing solutions are usually contaminated by ADP. Second, is the atrial K_{ATP} current more sensitive to ADP, and thus more sensitive to hypoxia and ischemia than the ventricular K_{ATP} current? Answering these question should help to better understand the excitability of the normal and ischemic heart.

This study is conducted on neonate cultured myocytes, since the startling diazoxide sensitivity has been discovered in neonate atrial myocytes in culture [8]. The first aim is to compare the sensitivity of atrial and ventricular K_{ATP} currents to ADP and diazoxide in conventional whole cell recordings, using the same patch pipette solutions on either type of myocyte. The second aim is to compare the sensitivity of atrial and ventricular myocytes to CCCP and diazoxide in gramicidin-perforated patch clamp recordings, an experimental method that best preserves the physiology of intact cells [16,17]. The results indicate that atrial myocytes loaded with 84 μmol/l ADP are 24-times more sensitive to diazoxide than ADP-loaded ventricular myocytes. Furthermore, intact atrial myocytes respond to simulated mild ischemia (100 nmol/l CCCP) while ventricular myocytes do not. The results support the hypothesis of an atrium-specific K_{ATP} channel with high sensitivity to metabolic inhibition and diazoxide.

2. Methods

2.1. Cell culture

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Atrial myocytes were dissociated from atrial appendages of 2–3-day-old rats and cultured for up to 3 days in a 5% CO_2 incubator, as described previously [18]. Ventricular myocytes were dissociated from the ventricular apex of 2–3-day-old rats and cultured [19] similarly to the method of Sadoshima et al. [20]. The cells were plated at low density (10 000–50 000 cells/dish) on fibronectin–gelatin coated 8-mm glass slides. On the second and third day of culture, when the recordings were made, at least two out of three cells are myocytes. Only initially contracting myocytes devoid of contact to neighboring cells were examined.

2.2. Patchclamp recordings of K_{ATP} current

Whole cell recordings of the K_{ATP} current were obtained similarly as described previously [8]. From a holding potential of −40 mV voltage ramps were imposed every 30 s from −80 to +90 mV over a 10-s period. This resulted in quasi-steady-state current–voltage curves. Membrane potential was measured in current-clamp mode at 0 pA at the end of each ramp.

For whole cell recordings, the pipette solution contained (in mmol/l) 120 KCl, 1.3 $CaCl_2$, 1.3 $MgCl_2$, 10 HEPES, 10 glucose, 10 BAPTA and either 1 mmol/l K_2-ATP and 10 μmol/l K-ADP (to partly simulate metabolic impairment), or an ATP-regenerating system with 1 mmol/l K_2-ATP, 3 mmol/l creatine-phosphate, 5 U/ml creatine kinase (to minimize the ADP concentration). BAPTA was chosen rather than EGTA to minimize chelation of Mg^{2+}. The pH was adjusted to 7.3 with KOH, and osmolality to 290 mOsm/kg with KCl. Nucleotide containing solutions were aliquoted, frozen and thawed just before use, and kept on ice for a maximum of 2–4 h. The ATP and ADP concentrations were assayed by HPLC in thawed aliquoted samples. Patch pipette solutions supplemented with 10 μmol/l ADP and 1 mmol/l ATP showed HPLC assay values of 84 μmol/l ADP and 1.08 mmol/l ATP. To minimize the ADP contamination, ATP was kept at around 1 mmol/l. Patch pipette solutions supplemented with the ATP-regenerating system showed HPLC assay values of 0.0 μmol/l ADP and 1.16 mmol/l ATP. The bath solution contained (in mmol/l): 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 118 NaCl, 10 HEPES, and 10 glucose. The pH was adjusted to 7.4 with NaOH, and osmolality to 290 mOsmol/kg with sucrose.

For gramicidin-perforated patch recordings, the pipette contained (in mmol/l) 120 KCl, 1.3 $CaCl_2$, 1.3 $MgCl_2$, 10 HEPES, 10 glucose, 10 BAPTA and 1 K_2-ATP, and was supplemented with gramicidin D at a concentration of 5 μg/ml [16]. The bath solution was as described above. The recording was made in the cell attached mode. Several minutes elapsed until the gramicidin established a low resistance path between pipette and cytoplasm. The series resistance (mean±S.E.M.) was 35.2±2.9 MΩ (n=41) in atrial myocytes and 36.6±4.8 MΩ (n=16) in ventricular myocytes. The resistance compensation range extends to 100 MΩ in the Axopatch 200B amplifier, and is sufficient for a precise voltage clamp control during maximal K_{ATP} channel activation. This perforated patch procedure allows

to measure the K_{ATP} current and membrane potential while largely preserving the cellular protein and ion content [16,17]. Membrane potentials (means±S.E.M.) reached −75.8±0.8 mV in atrial myocytes and −78.2±1.8 mV in ventricular myocytes, attesting to the capability of these cells of maintaining normal potassium gradients.

2.3. General protocols

In whole cell recordings on 107 myocytes, spontaneous activation of the K_{ATP} current was measured during a control period of 8–12 min. Incremental concentrations of diazoxide were then applied (from 0.01 to 100 μmol/l) during periods of 6–10 min, followed by a maximal stimulation with co-added 100 μmol/l pinacidil and by an inhibition test with co-added 0.1–1 μmol/l glibenclamide. Not all concentrations were applied to all cells, as the total recording period was limited to 45–50 min. Four groups were examined: (1) and (2) atrial and ventricular myocytes, respectively, with 84 μmol/l ADP and 1.08 mmol/l ATP (measured values) in the pipette; (3) and (4) atrial and ventricular myocytes, respectively, with an ATP-regenerating system in pipette. The rationale was to simulate mild hypoxic conditions with moderately elevated ADP [11], and to compare these with conditions where cytoplasmic ADP levels were minimized by the ATP-regenerating system. An 8–12-min control period was deemed sufficient to dialyze ADP, ATP and creatine kinase into the cytoplasm. From the measurements of access resistance and cell capacitance of this study, the calculated time constant of cell dialysis [21] for ADP was 148±9 s (n=89) in atrial myocytes and 137±13 s (n=18) in ventricular myocytes. The calculated cytoplasmic concentration of creatine kinase reached 60% of the pipette concentration within a 10-min period.

The general protocol was the same in gramicidin perforated patch recordings on 57 myocytes, with the following exceptions. In groups 5–8, atrial and ventricular myocytes were stimulated at the end of the experiment with the mitochondrial protonophore CCCP (1 μmol/l) in order to evoke a maximal activation. In groups 7 and 8, atrial and ventricular myocytes were exposed, after the control period and throughout the experiment, to 100 nmol/l CCCP. The rationale was to compare channel activation by diazoxide in the presence or absence of a mild metabolic impairment. Unlike the metabolic inhibitor oligomycin, CCCP increases the ATP consumption by the F1F0-ATPase. Concentrations of diazoxide and CCCP were similar to those used in previous studies [8,22,23], and finalized by trial and error.

2.4. Chemicals and drugs

CCCP, diazoxide, glibenclamide, gramicidin D, creatine phosphate, creatine kinase, K_2-ATP; K-ADP, and BAPTA came from SIGMA, and pinacidil from Alexis. CCCP was stored in ethanol as a 2 mmol/l stock solution, yielding a maximal ethanol concentration of 0.05% in the external buffer. Diazoxide, pinacidil and glibenclamide were stored in DMSO as 100, 100 and 10 mmol/l stock solution, respectively, yielding a maximal DMSO concentration of 0.1%. These concentrations of ethanol and DMSO were found previously to have no effect on K_{ATP} channel activity ([8,22]; and unpublished).

2.5. Data analysis

In order to exclude the possible contribution of chloride channels, the K_{ATP} current obtained during the voltage ramp was measured at 0 mV, close to the chloride equilibrium potential. The steady state current was obtained for the control period and each period of drug application and each myocyte. Steady state is defined here, for each cell and each drug application, as a current varying less than ±1% of the maximal current per min. During diazoxide stimulation, steady state was reached in most cases for the gramicidin recordings in both cell types, in 78.7% of all cases for whole cell atrial K_{ATP} current recordings, and in 64.9% of all cases for whole cell ventricular K_{ATP} current recordings. Overshoot with subsequent lower K_{ATP} current was usually produced in the cases where no steady state was achieved. The maximal current was measured in those situations. In a first analysis, these currents (in pA) were averaged over all cells for each group and plotted as means±S.E.M. in Figs. 2 and 5. Significance of the means and differences between means were analyzed by ANOVA for repeated measures on ranks (nonparametric statistics), with software from the SAS Institute (Carey, NC, USA). To obtain the change in current density (in pA/pF), the current at the end of the control period was subtracted and the difference divided by the cell capacitance. Averages were obtained over all cells for each group and plotted as means±S.E.M. in Figs. 3 and 6. The EC_{50} and maximal slope of the resulting eight sigmoid dose–response curves for diazoxide were analyzed by Microcal ORIGIN software (version 5), and the results are listed in Table 1.

3. Results

3.1. Comparison of atrial and ventricular K_{ATP} channels in whole cell recordings

Using the same patch pipette solutions and recording conditions, atrial and ventricular myocytes were recorded either with 84 μmol/l ADP and 1.08 mmol/l ATP (example Fig. 1) or with an ATP-regenerating cocktail in the pipette. The slow ramp protocol (Fig. 1A) ensures that the voltage-dependent potassium channels are only minimally activated if at all. Activation by pinacidil and diazoxide and inhibition by glibenclamide provide pharma-

Table 1
Statistical summary for dose–response curves of K_{ATP} channel opener diazoxide[a]

Recording	EC_{50} (μmol/l)		EC_{50} log (μmol/l)		Maximal slope (pA/pF)/decade		*n*	
	A	V	A	V	A	V	A	V
Whole-cell with ADP	0.13	3.1	−0.89±0.13	0.49±0.28	63.6±16.0	29.4±8.9	30	11
Whole-cell with CrP	19.7	54.9	1.29±0.17	1.74±0.16	20.8±3.5	41.8±9.2	59	7
Gramicidin with CCCP-7	129	3715	2.11±0.11	3.57±0.79	29.6±3.4	30.3±12.0	18	9
Gramicidin without CCCP	676	2570	2.83±0.01	3.41±0.06	67.6±0.2	71.2±3.2	23	7

[a] Sigmoids were fitted to the dose–response curves to yield the EC_{50} and maximal slope. In whole cell patch clamp recordings the pipette contained either 1.08 mmol/l ATP and 84 μmol/l ADP (ADP) or an ATP-regenerating system (CrP). In gramicidin-perforated patch recordings, the bath contained either 100 nmol/l or no CCCP. Note large differences in EC_{50} between atrial (A) and ventricular (V) myocytes under the same recording conditions; *n*, number of myocytes.

cological evidence that the channels are K_{ATP}-channels (Fig. 1C) (see also [8]). In contrast to ventricular myocytes, atrial myocytes display at rest a significant K_{ATP} channel activation in the presence of Mg-ADP (Fig. 2), and a remarkable sensitivity to diazoxide with a threshold concentration of 10 nmol/l. Under the same recording conditions, the ventricular myocytes display a threshold concentration of 1 μmol/l. The ATP-regenerating cocktail decreases the diazoxide sensitivity in both cell types, as the threshold concentration increases to 0.1–1 μmol/l in atrial myocytes and to 10 μmol/l in ventricular myocytes. It abolishes the spontaneous activation in the atrial myocytes. In order to more precisely define the diazoxide sensitivity, the change in current density was calculated, mean values fitted by sigmoid dose–response curves (Fig. 3), and EC_{50} and maximal slopes determined and shown in Table 1. In atrial myocytes, the presence of 84 μmol/l ADP increases the maximal slope and dramatically decreases the EC_{50} (in μmol/l) by 150-fold. In ventricular myocytes, the presence of Mg-ADP shifts the dose–response curve to the left, but the decrease of EC_{50} is only 18-fold. Under identical recording conditions, atrial myocytes display 24-fold

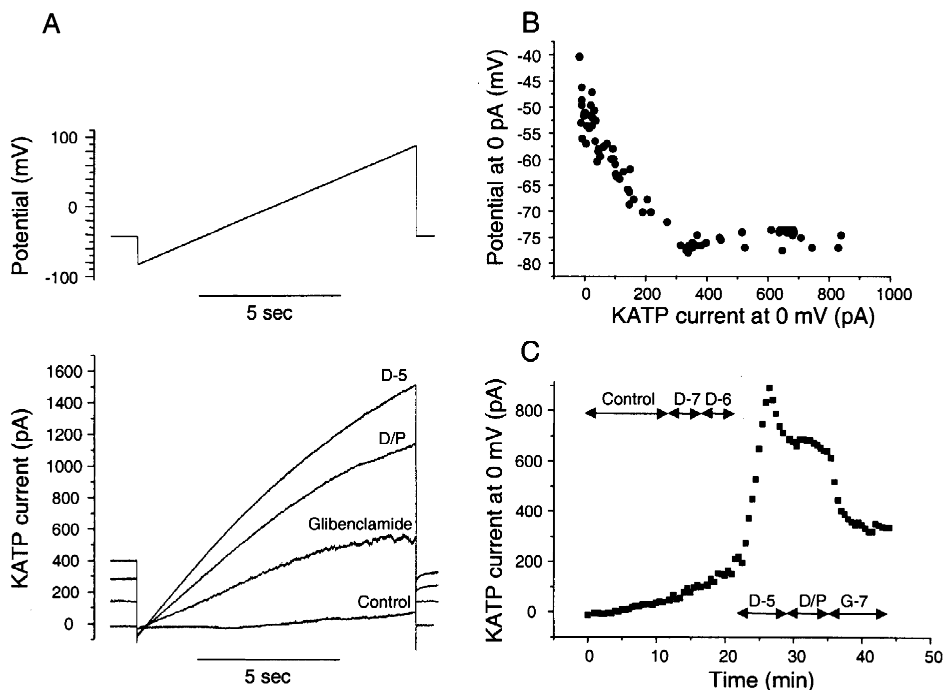

Fig. 1. Example of whole cell recording on a ventricular myocyte with 84 μmol/l ADP and 1.08 mmol/l ATP in pipette. (A) Ramp protocol (10 s) of voltage clamp with current traces below; (B) relationship between membrane potential at 0 pA and current at 0 mV; (C) K_{ATP} current at 0 mV as function of time. Concentrations in log molar, D, diazoxide; D/P, D with 100 μmol/l pinacidil; G-7, 100 nmol/l glibenclamide on top of D/P.

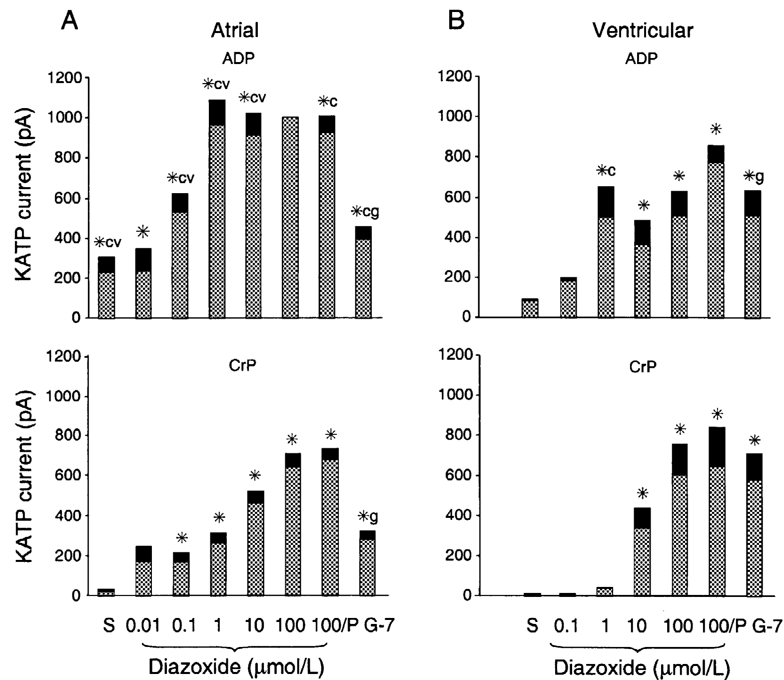

Fig. 2. Statistical summary of all whole cell recordings of K_{ATP} current at 0 mV. Means are shown as light columns, and S.E.M. as black bars on top. (A) Atrial myocytes; (B) ventricular myocytes. ADP, 84 μmol/l ADP and 1.08 mmol/l ATP in pipette; CrP, ATP-regenerating system in pipette. *, $P<0.05$ relative to 0; c, $P<0.05$ relative to CrP; v, $P<0.05$ relative to ventricle; g, $P<0.05$ relative to no glibenclamide. S, spontaneous activity; for other abbreviations see Fig. 1.

(ADP) and 2.8-fold (ATP regenerating cocktail) lower EC_{50} than ventricular myocytes.

3.2. Comparison of atrial and ventricular K_{ATP} channels in gramicidin-perforated patch recordings

Both atrial and ventricular myocytes become remarkably insensitive to diazoxide when recorded in the gramicidin-perforated patch mode. Thus mild metabolic impairment was simulated by exposing the myocytes to low dose CCCP (100 nmol/l) (example Fig. 4). This significantly activates the atrial but not the ventricular myocytes. Threshold concentrations of diazoxide are 1 and 10 μmol/l for atrial and ventricular myocytes, respectively, in the presence of 100 nmol/l CCCP (Fig. 5), and 10–100 μmol/l in its absence. Maximal concentrations of 100 μmol/l diazoxide and pinacidil were used in an attempt to maximally stimulate the K_{ATP} current, but result in only half-maximal activation or less when compared to values obtained under whole cell recordings. Maximal activation

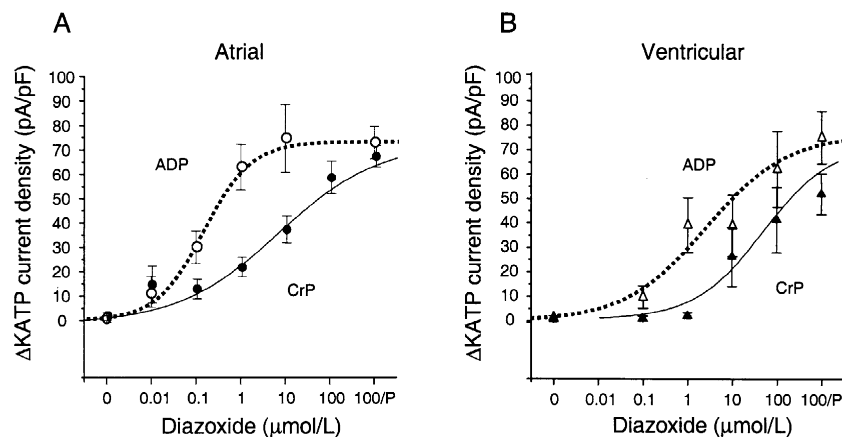

Fig. 3. Dose–response curves for increase in K_{ATP} current density at 0 mV (current normalized by cell capacitance). (A) Atrial myocytes; (B) ventricular myocytes. For statistics see Table 1; 100/P=100 μmol/l diazoxide with 100 μmol/l pinacidil (not included in sigmoid regression curves). For abbreviations see Fig. 2.

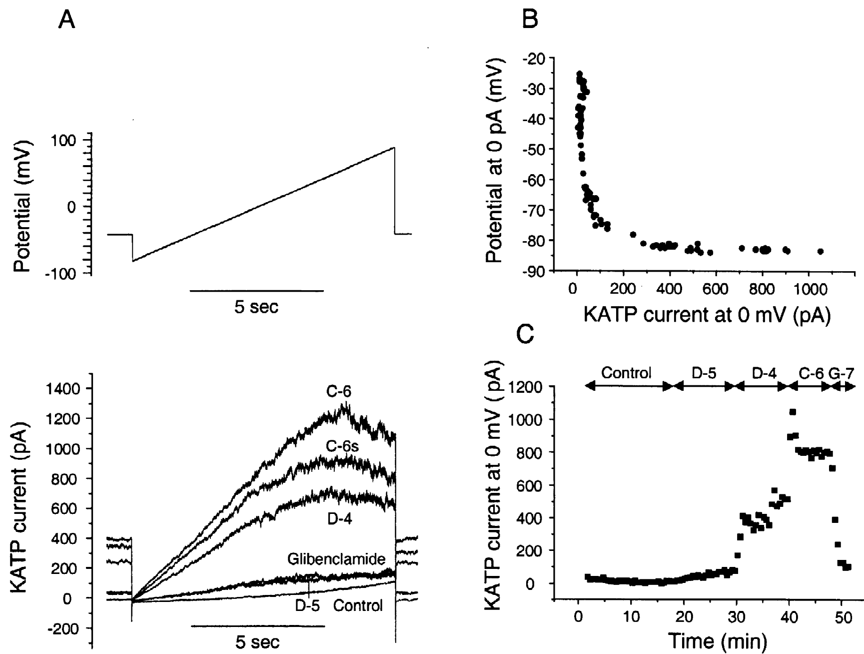

Fig. 4. Example of gramicidin-perforated patch recording on an atrial myocyte. (A) Ramp protocol (10 s) of voltage clamp with current traces below; (B) relationship between membrane potential at 0 pA and current at 0 mV; (C) K_{ATP} current at 0 mV as function of time. For abbreviations see Fig. 1. C-6, C-6s=1 μmol/l CCCP at peak and steady-state.

is attained at the end of the experiment with 1 μmol/l CCCP (Fig. 5).

In atrial myocytes that are mildly impaired with 100 nmol/l CCCP, sigmoid dose–response curves for the current densities (Fig. 6) show a 5-fold lower EC_{50} for diazoxide, relative to no CCCP (Table 1). In contrast, the EC_{50} for diazoxide in ventricular myocytes is not significantly changed by CCCP. When metabolically impaired by 100 nmol/l CCCP, the atrial myocytes are 28-times more sensitive to diazoxide than ventricular myocytes.

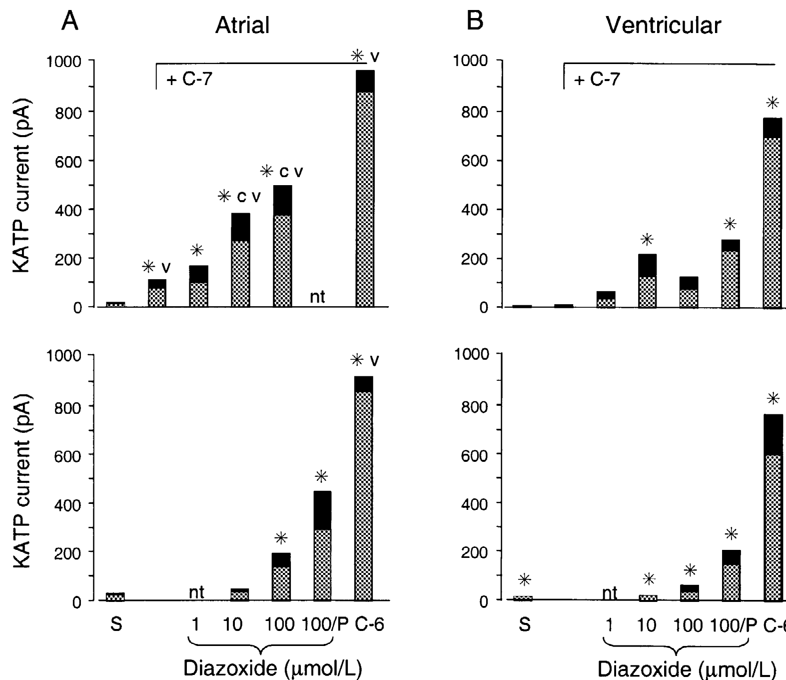

Fig. 5. Statistical summary of all gramicidin-perforated patch recordings of K_{ATP} current at 0 mV. Means are shown as light columns, and S.E.M. as black bars on top. (A) Atrial myocytes; (B) ventricular myocytes. nt, not tested; for other abbreviations see previous figures. Note leftward shift of atrial K_{ATP} current activation by diazoxide when myocytes are exposed to 100 nmol/l CCCP.

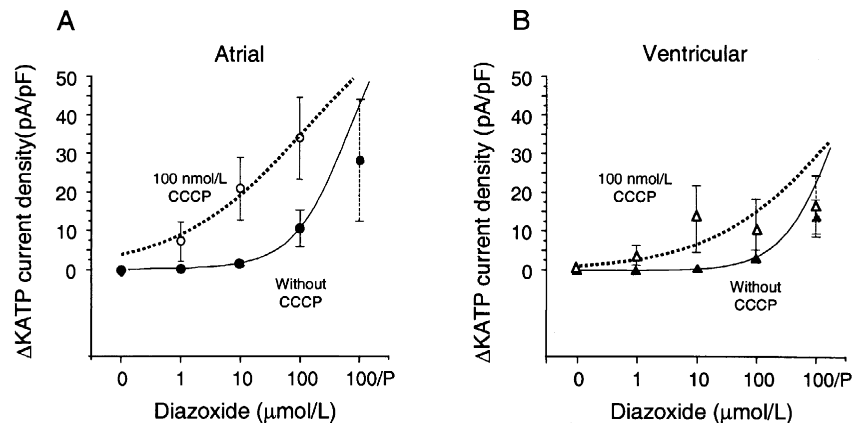

Fig. 6. Dose–response curves for increase in K_{ATP} current density at 0 mV. (A) Atrial myocytes; (B) ventricular myocytes. Sigmoid curves were fitted without including response to 100/P. For statistics see Table 1. For abbreviations see previous figures.

When not metabolically impaired the atrial myocytes are 3.8-times more sensitive than the ventricular myocytes. Interestingly, the exposure to CCCP decreases the maximal slope of the dose response curves (Table 1). Presumably, in intact cells local gradients of ADP and other soluble cytoplasmic factors may contribute to the overall responsiveness of the sarcolemmal K_{ATP} channels.

Thus both electrophysiological approaches yield similar ratios of sensitivity to diazoxide of atrial relative to ventricular K_{ATP} channels. These ratios are particularly large (>24:1) when the myocytes are metabolically impaired.

4. Discussion

This study compares—in the same species and under identical experimental conditions—the activation of atrial and ventricular K_{ATP} currents by metabolic inhibition and diazoxide. The major new findings are: (i) the presence of Mg-ADP in the patch pipette, or low dose CCCP in the extracellular medium, activates an atrial but not a ventricular K_{ATP} current; (ii) in the presence of Mg-ADP in the patch pipette the atrial K_{ATP} current is 24-times more sensitive to diazoxide than the ventricular K_{ATP} current; (iii) in the presence of low dose CCCP in the extracellular medium the atrial K_{ATP} current is 28-times more sensitive to diazoxide than the ventricular K_{ATP} current; (iv) in the absence of ADP or CCCP, the atrial K_{ATP} current is 3- to 4-times more sensitive to diazoxide than the ventricular K_{ATP} current.

4.1. Evidence for an atrium-specific K_{ATP} channel in cell culture

In a previous study we have shown that the K_{ATP} current in cultured rat atrial myocytes is highly sensitive to diazoxide [8] as compared to published sensitivities of ventricular myocytes [2,4,5]. However, the application of ADP sharply increases the sensitivity to diazoxide of native ventricular as well as KIR6.2/SUR2A K_{ATP} channels [10], raising the question if contaminating ADP in the patch pipette confounds the interpretation of patch clamp experiments. Are the atrial and ventricular K_{ATP} channels one and the same, represented by KIR6.2/SUR2A [5], and do they respond to diazoxide as a function of intracellular ADP? The present study was designed to answer these questions.

ADP could be the confounding factor, since the ADP concentration measured by HPLC (84 μmol/l) is significantly higher than the concentration added (10 μmol/l) to the ATP-containing pipette solution. Presumably, the additional ADP is a hydrolytic product of ATP. This factor is now controlled for by applying the same pipette solutions to both atrial and ventricular myocytes. Under these identical recording conditions, the atrial myocytes respond to mild metabolic inhibition while ventricular myocytes do not (see i) above). The atrial myocytes are highly sensitive to the interaction between diazoxide and intracellular Mg-ADP, while the ventricular myocytes are not (see ii) above). A critical test with gramicidin-perforated patch recordings (see iii) above), where the myocytes are left largely intact [16,17], confirms the results from whole cell recordings. Could other methodological differences be involved? The myocytes stem from the same species (rat), strain (Sprague–Dawley), and age (2–3 days), are dispersed on the same day and recorded on the same days, and exposed to similar culture media. Although the serum concentration is 10% for the atrial and 5% for the ventricular culture medium [19], the cells undergo the same hypertrophy as measured by β-actin mRNA [Schmidt et al., submitted for publication, 2002]. Thus hypertrophy per se, due to serum stimulation [24], could not have been a factor either.

Five other factors conceivably contribute to the overall responsiveness of K_{ATP} channels: (1) cell geometry; (2) local pH; (3) cytosolic Mg-ATP and free Mg^{2+} concentration; (4) nucleotide dialysis and (5) nucleotide

hydrolysis. Regarding geometrical factors, the reticular network and myofibrils are far less developed in neonatal than adult myocytes, thus it is unlikely that morphological differences could explain the large difference in diazoxide sensitivity of neonate atrial and ventricular myocytes. Regarding local pH, CCCP may perhaps form proton leaks in the plasmalemma. Since membranes have relatively uniform characteristics, a potential proton leak in plasmalemma or other membranes should be similar in both cell types. The very low concentrations of CCCP used in this study to prime the cells (100 nmol/l) should therefore have very little differential effects on both cell types. Regarding cytosolic Mg-ATP and free Mg^{2+}, the same concentrations were applied to both cell types. They were >150-times higher than in another study [10] where ADP and diazoxide sensitivities were examined in guinea pig ventricular myocytes. The reason is that our pipette solution contained BAPTA—which chelates mainly Ca^{2+}, vs. EGTA [10]—which chelates both Ca^{2+} and Mg^{2+}. It is possible, therefore, that Mg-ATPase activity on or near the K_{ATP} channels somewhat enhanced the sensitivity to diazoxide. Regarding nucleotide dialysis in whole cell recordings, the time constants were similar in both types of myocytes (see Methods), and short (<150 s) relative to the 10-min control period. However, the Mg-ATPase activity of SUR would differ, if the K_{ATP} channel composition differed between atrial and ventricular myocytes. Thus, the present study indeed favors the existence of an atrium-specific K_{ATP} channel in myocyte culture.

4.2. Evidence for SUR1, SUR2B and/or KIR6.1 subunits in the atrial K_{ATP} channel

In the presence of 84 μmol/l intracellular ADP, a significant basal activity exists in atrial K_{ATP} channels, while the ventricular K_{ATP} channels are totally inactive. It is already known that KIR6.2/SUR1 in β-cells and KIR6.1/SUR2B in smooth muscle cells show significant basal activity, while KIR6.2/SUR2A channels in striated muscle are virtually silent (reviewed in [5]). Possible reasons include a higher hydrolytic activity of SUR1 and SUR2B relative to SUR2A, and a higher sensitivity to Mg-ADP of KIR6.1/SUR relative to KIR6.2/SUR [25]. Furthermore, there is a significant difference in diazoxide sensitivity between atrial and ventricular K_{ATP} channels (Table 1) that cannot be explained on methodological grounds (see above). This further suggests the involvement of SUR1 and SUR2B, as these subunits are known to confer the diazoxide sensitivity to pancreatic KIR6.2/SUR1 and smooth muscle KIR6.1/SUR2B channels [2–5]. Finally, the results demonstrate a striking synergy between ADP and diazoxide in the activation of the atrial K_{ATP} channels. This is shown in whole cell recordings by the sharp increase in the maximal slope of the diazoxide dose response curves for atrial myocytes, while there is a parallel left-ward shift of the dose–response curves for ventricular myocytes (Fig. 3; Table 1). A parallel shift of the dose–response curve would have been expected for the atrial K_{ATP} channel if it was identical to KIR6.2/SUR2A. Overall, these results suggest that the atrial K_{ATP} channels include SUR1, SUR2B and possibly KIR6.1 subunits. The determination of the exact composition of the atrial K_{ATP} channel will require immunoprecipitation techniques for the small amounts of channel protein available from atrial myocyte cultures.

4.3. Significance

Whole cell recordings allow for a precise characterization of the K_{ATP} channels, since the myocytes are dialyzed with known ion and nucleotide concentrations. However, soluble cytoplasmic messengers escape into the patch pipette solution, and local gradients in ADP or other factors are almost totally dissipated. The results from gramicidin-perforated patch recordings are more representative for predicting the behavior of intact cells [16,17]. Mild metabolic inhibition significantly increases the K_{ATP} current in atrial but not ventricular intact myocytes, and small increases in potassium current are sufficient to hyperpolarize the cell. One would predict that mild metabolic inhibition could increase the risk of arrhythmia in atrial but not ventricular myocytes. These experiments on cultured neonate myocytes need to be extended to freshly isolated myocytes, in vivo, and to humans to further test whether the atrium is more sensitive to ischemia than ventricle. Regional differences in the sensitivity of the K_{ATP} channels to Mg-ADP could have a profound influence on impulse conduction in ischemic hearts.

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