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The Effects of K^+ Channels Modulators Terikalant and Glibenclamide on Membrane Potential Changes Induced by Hypotonic Challenge of Guinea Pig Ventricular Myocytes

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Abstract. Contribution of inward rectifier K^+ currents (I_{K1}) and ATP-sensitive K^+ currents (I_{KATP}) to membrane potential changes of ventricular myocytes appearing during hypotonic challenge is unclear. We used here the whole cell patch clamp technique, voltage and current clamp modes, to record membrane potentials and ionic currents in isolated guinea pig ventricular myocytes under isotonic or hypotonic perfusion. The difference in osmolarity between iso- and hypotonic solutions was about 100 mOsm. Exposure to hypotonic solution for 60 s induced initial prolongation of action potential duration at 90% of repolarization (APD_{90}) (from 176 ± 10 to 189 ± 11 ms, $P<0.05$, $n = 13$). Further perfusion for the next 300 s shortened APD_{90} to 135 ± 9 ms ($P<0.01$, in comparison with control values, $n = 13$) and depolarized resting potential from -79.2 ± 1.5 to -75.0 ± 0.9 mV, ($P<0.05$, $n = 13$). Neither pretreatment with a blocker of I_{K1} channels, terikalant at 10 μM, nor with a blocker of I_{KATP} channels, glibenclamide at 1 μM, prevented the above-mentioned changes in membrane potential induced by hypotonic challenge when a pipette solution containing 5 mM ATP was used. Also, glibenclamide and terikalant did not affect the hypotonic-sensitive current, obtained by ramp or voltage-step protocols, respectively. Additionally, the current-voltage relationship (I-V curve) of the whole cell hypotonic-sensitive current shifted from an isotonic I-V curve in a parallel way. Our results indicate that I_{K1} and I_{KATP} do not participate in membrane potential changes induced by hypotonic solution at least in the guinea pig ventricular myocytes with sufficient intracellular ATP.

Keywords: patch clamp, ventricular myocyte, hypotonic swelling, ATP-sensitive K^+ channel, inward rectifier K^+ channel

Introduction

The swelling of the cardiomyocytes is a well-documented phenomenon observed in ischemic heart regions due to the lower osmolarity of the environment (1 – 3). Membrane potential changes induced by hypotonic stress as initial prolongation and secondary shorthening of action potential duration (APD) and depolarization of resting potential (RP) have already been reported (4 – 6). Activation of chloride current ($I_{Cl,swell}$), non-selective cation current (I_{NSC}) and slow component of delayed rectifier K^+ current (I_{Ks}) by hypotonic solution were also described (7 – 9). However, the observed ionic current changes could not explain all membrane potential changes. Two important events appearing during hypotonic stress of guinea pig ventricular myocytes, APD shortening and depolarization of RP, have not yet been explained in terms of ionic mechanisms. Activation of ATP-sensitive K^+ currents (I_{KATP}) and inward rectifier K^+ currents (I_{K1}) could contribute to APD shortening and RP depolarization, respectively, but their roles in the above-mentioned phenomena have not been clarified (4, 7, 10). For instance, Du and Sorota (4) have shown that depolarization of resting potential during hypotonic challenge in canine atrial myocytes was due to activation

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of $I_{Cl,swell}$ current. They also examined participation of I_{K1} in the above-mentioned changes of resting potential using barium (Ba^{2+}) as a blocker. However, we have recently shown that activation of $I_{Cl,swell}$ current does not cause depolarization of guinea pig ventricular myocytes. According to Vandenberg et al. (5) and our previous data (Kocic et al. (7)), shortening of APD observed in the ventricular myocytes exposed to hypotonic challenge was related to the activation of $I_{Cl,swell}$ and I_{Ks} currents, but in about 50% of examined cells only. On the other hand, Priebe and Beuckelmann reported that activation of I_{KATP} current produced APD shortening in guinea pig ventricular myocytes during hypotonic stress (10). Hence, we undertook our experiments in order to find out whether activation of I_{KATP} and I_{K1} currents contribute to APD shortening and RP depolarization of guinea pig ventricular myocytes treated by hypotonic solution, employing pharmacological tools and the patch clamp technique under the same experimental conditions as previously (7).

Material and Methods

All experiments were performed in accordance with the US National Institute of Health's Guide for the Care and Use of Laboratory Animals and approved by the Animal Research Committee of Tokyo Medical and Dental University.

Animals

Both sexes of guinea pigs, weighing between 300 and 500 g, were used.

Myocytes isolation

Anesthetized animals (pentobarbital sodium 30 mg /kg, i.p.) were under artificial respiration. The thorax was opened and the heart carefully prepared for the enzymatic dissociation procedure, as described elsewhere (11). Briefly, the heart was hung on a Langendorff apparatus and successively perfused by normal Tyrode solution for 5 to 10 min and then with nominally Ca^{2+}-free solution until arrest of heart beating, after which collagenase was added (60 mg%, type II; Worthington Biochemical Lakewood, NJ, USA) for about 20 min. Next, the heart was gently minced to small pieces, and the suspended cells were washed, filtered, and stored in high-K^+, low-Cl^- solution at room temperature. The cells were used within 9 h of isolation. The isotonic bath solution contained 64.0 mM NaCl, 4.0 mM KCl, 1.8 mM $CaCl_2$, 0.53 mM $MgCl_2$, 5.5 mM glucose, 5.0 mM HEPES, and 150 mM mannitol. The hypotonic bath solution had the same components, but the mannitol concentration was 50 mM, resulting in a decrease in osmolarity by about 100 mOsm (Vapor Pressure Osmometer; Wescor, Logan, UT, USA). The pipette solution contained 20 mM KCl, 0.02 mM $CaCl_2$, 5.0 mM HEPES, 100.0 mM K^+-aspartate, 5.0 mM Mg^{2+}APT, 5.0 mM K^+ creatine phosphate, and 0.05 mM EGTA.

Drugs

Glibenclamide was purchased from RBI (Natick, MA, USA). Terikalant was a kind gift from Dr. Icilo Cavero (Rhone Poulenc Rorer, France). Glibenclamide was dissolved in DMSO, and terikalant was dissolved in distilled water. Concentration of DMSO in the solutions was less than 0.05% and had no effects on the measured electrophysiological parameters.

Electrophysiology

The borosilicate electrodes were pulled (PP-83; Narishige, Tokyo) and polished to have a resistance between 1 and 2 MΩ. An agar bridge filled with 2 M KCl was used to reduce junction potential. Obtained data were filtered at 2 kHz, observed by an oscilloscope, amplified by a patch clamp amplifier (Axopatch 200B; Axon Instrument, Foster City, CA, USA), converted (Digidata 1200, Axon Instruments), and stored in a personal computer. Action potentials were recorded in a current clamp mode and elicited by injection of supra-threshold current, the duration of which was 1 – 3 ms. Whole cell currents were recorded in a voltage clamp mode. We applied ramp and voltage-steps protocols. The descending ramp protocol from +60 to −110 mV was applied for 4.5 s. Voltage was changed in 10 mV steps from −110 to −40 mV, every 2 s, from a holding potential of −40 mV. This protocol allows us to assess the values of I_{K1} currents. The pCLAMP software (Version 8, Axon Instruments) was used to prepare the protocols and to collect the data. The Origin software was used for data analysis.

Experimental protocols

Our preliminary test has shown that membrane potential changes induced during first exposure to hypotonic solution reappeared during the second exposure in all examined ventricular myocytes. Therefore, only the cells reacted by typical changes of membrane potential (initial prolongation of APD after about 60 s in hypotonic solution and secondary shortening of APD accompanied by depolarization of RP) were pretreated with terikalant or glibenclamide before the second exposure to hypotonic solution. Also, we have previously found that action potentials maintain a stable shape for at least 15 min (7) in isotonic solution (duration of the standard experiment in isotonic + hypotonic solutions

was about 12 min). When disturbances occurred, the action potential suddenly changed its shape and usually completely disappeared; thereby, the experiment was stopped.

Statistics

One-way ANOVA followed by the multiple comparison test and a Student *t*-test for paired data were used. $P<0.05$ was considered as a significant difference.

Results

I_{K1} and hypotonic changes of membrane potentials

We measured steady-state I_{K1} at the end of 2 s pulses applied between −110 and −40 mV, in 10-mV steps from a holding potential of −40 mV. Exposure of guinea pig ventricular myocytes to hypotonic solution produced currents whose I-V curve was shifted to the right as compared with an isotonic solution (Fig. 1: A and B). Terikalant at concentration of 10 μM significantly decreased both inward and outward I_{K1} in isotonic solution (Fig. 1C), but did not prevent shifting of the I-V curve obtained in hypotonic solution (Fig. 1D).

Typical changes of membrane potentials during hypotonic challenge presented in Fig. 2A are initial prolongation after 60 s in hypotonic solution, secondary shorthening of APD after 300 s, and simultaneous depolarization of resting potentials. Average values are summarized in Table 1. Addition of terikalant at concentration 10 μM prolonged APD and decreased RP (Table 1) in isotonic solution. However, exposure of the cells pretreated with terikalant to hypotonic solution

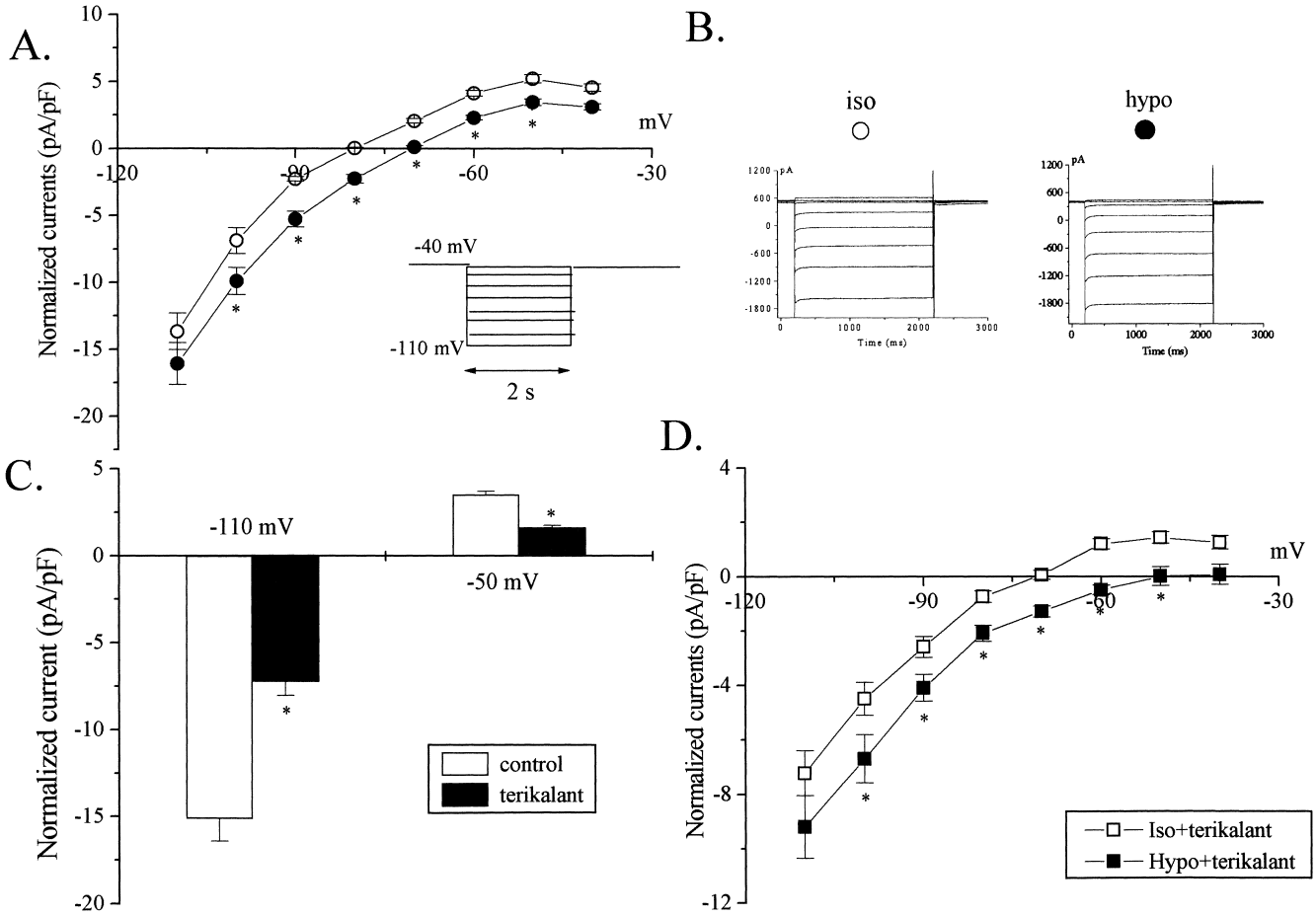


Fig. 1. The effects of terikalant on I_{K1} changes in isotonic and hypotonic solutions. A: Average current-voltage relationship in isotonic (open circle) and hypotonic (closed circle) solutions. Inset: Voltage protocol. B: Original traces obtained from one typical cell in isotonic and hypotonic solutions. Mean ± S.E.M. from 6 cells. *$P<0.05$, significant differences between corresponding values obtained in hypotonic and isotonic solutions. Student *t*-test for paired data. C: Effects of 10 μM of terikalant on I_{K1} in isotonic solution obtained at −110 mV (maximal inward component) and −50 mV (maximal outward component). Mean ± S.E.M. from 5 cells. *$P<0.05$, significant differences between corresponding values. D: Average current-voltage relationship in isotonic and hypotonic solutions obtained in the presence of 10 μM of terikalant. Mean ± S.E.M. from 5 cells. *$P<0.05$, significant differences between corresponding values obtained in hypotonic and isotonic solutions.

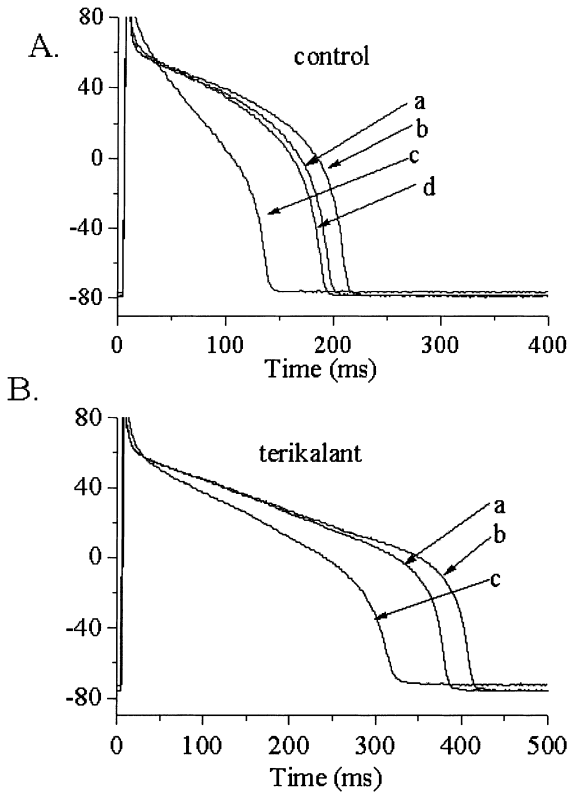


Fig. 2. Original traces of action potentials recorded in the absence (A) and in the presence (B) of terikalant (10 μM). A: a, isotonic solution; b, after 60 s in hypotonic solution; c, after 300 s in hypotonic solution; d, washout in isotonic solution. B: a, isotonic solution + terikalant; b, after 60 s in hypotonic solution + terikalant; c, after 300 s in hypotonic solution + terikalant. Similar results were obtained in 7 control cells and 7 cells pretreated with terikalant.

induced the same changes as in the absence of terikalant: initial prolongation of APD followed by secondary shortening and depolarization of resting potential (Fig. 2B).

I_{KATP} and hypotonic changes of membrane potential

Figure 3 presents whole cell currents, the average values obtained from 6 cells (3A) and original traces from one typical cell (3B), obtained by the fast-ramp protocol in isotonic and hypotonic solutions. Hypotonic-sensitive current was not changed after application of 1 μM glibenclamide, a blocker of KATP channels (Fig. 3B). Accordingly, pretreatment with glibenclamide did not prevent membrane potential changes in hypotonic solution. There appeared early prolongation and late shortening of APD accompanied by depolarization of resting potential in both, control and pretreated by glibenclamide, group of cells superfused with hypotonic solution, (Fig. 4: A, B and Table 1).

Discussion

The major findings of this study are that shortening of APD and depolarization of RP observed in guinea pig ventricular myocytes during hypotonic challenge can not by explained by activation of I_{KATP} and I_{K1}, respectively. It has been shown previously that hypotonic swelling activates inward and outward currents. Most of those currents were identified as I_{Ks} (slow component of delayed rectifier potassium current), $I_{Cl,swell}$ (swelling-induced chloride current), and I_{NSC} (non-selective cation current) (4 – 6). There have been also described typical membrane potential changes under such a condition: initial prolongation with a secondary shortening of APD and simultaneous depolarization of RP (7). However, the mechanism of depolarization of resting potential observed in all ventricular myocytes exposed to hypotonic solution was not elucidated. A possible participation of I_{K1} to the above-mentioned phenomenon was not completely excluded (4, 7). In this study we measured whole cell currents at voltages slightly negative (−80 to −110 mV) or positive (−80 to −40 mV) in relation to the RP. Those currents are mainly related to I_{K1} changes. The obtained values have shown parallel shifting of the I-V curve in hypotonic solution, which argues against the participation of I_{K1} in the described changes of RP. It is well known that an increase in I_{K1} caused by

Table 1. Membrane potential changes in guinea pig ventricular myocytes in the absence or in the presence of terikalant and glibenclamide

	N	APD_{90} (ms)			RP (mV)	
		Iso	Hypo 60 s	Hypo 300 s	Iso	Hypo 300 s
Control for terikalant group	7	182 ± 11	194 ± 11*	151 ± 9**	−80.1 ± 1.3	$-76.0 \pm 1.2^{\#}$
Terikalant group, 10 μM	7	382 ± 35	431 ± 41*	335 ± 29**	$-78.0 \pm 0.7^{\&}$	$-74.0 \pm 0.8^{\#}$
Control for glibenclamide group	6	171 ± 9	186 ± 10*	132 ± 7**	−79.5 ± 0.7	$-75.1 \pm 0.6^{\#}$
Glibenclamide group, 1 μM	6	166 ± 9	183 ± 10*	125 ± 9**	−79.0 ± 0.8	$-74.5 \pm 0.7^{\#}$

*P<0.05, **P<0.01, significant differences of APD_{90} compared to the value in isotonic solution. $^{\#}P$<0.05, significant differences of RP compared to values in isotonic solution. $^{\&}P$<0.05, significant difference in comparison to the value before addition of terikalant. Mean ± S.E.M. APD_{90} = action potential duration at 90% of repolarization, RP = resting potential, Iso = isotonic solution, Hypo = hypotonic solution.

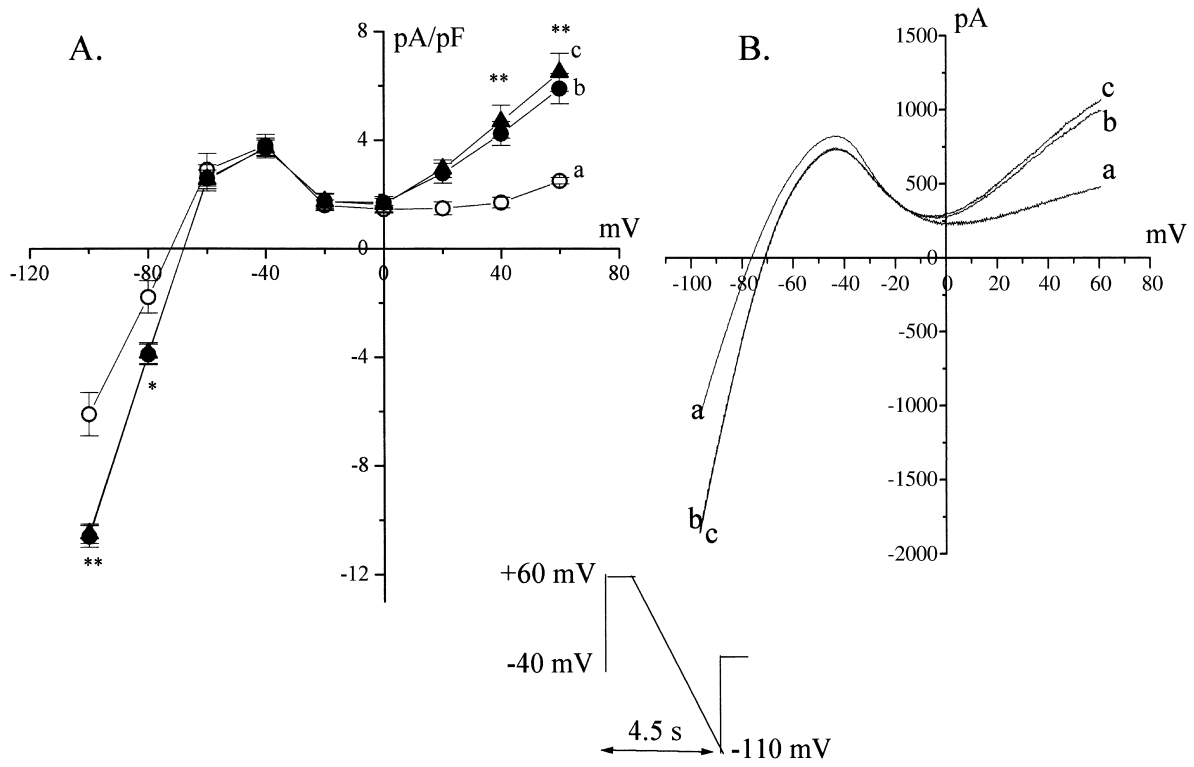
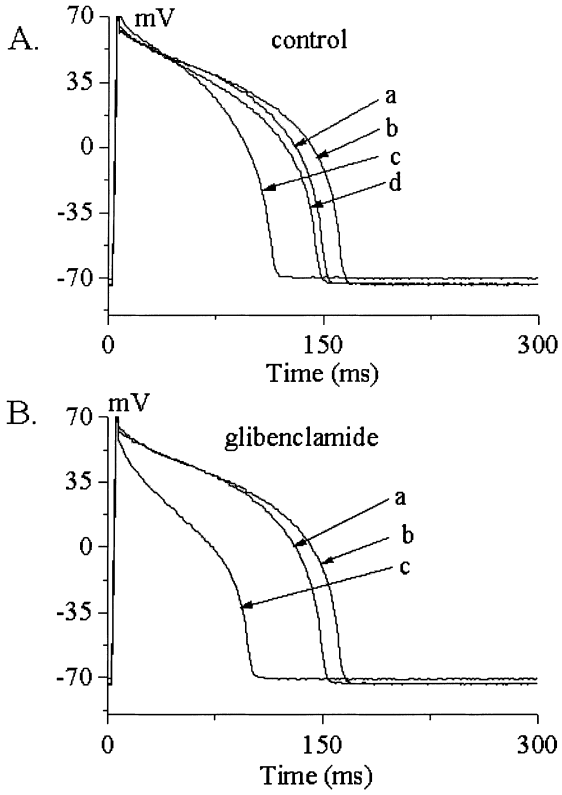


Fig. 3. Normalized whole cell currents obtained by ramp protocol presented in the *Inset* (A) and original traces recorded from one typical cell (B). a, isotonic solution; b, after 300 s in hypotonic solution; c, after additional 200 s in hypotonic solution in the presence of glibenclamide (1 μM). **P*<0.05, ***P*<0.01, significant differences compared to the values obtained in isotonic solution.



increased K^+ gives the characteristic cross-over phenomenon (12), which is not present in our experiments (Fig. 1A). Moreover, we used terikalant, one of the most selective blockers of I_{K1}, which did not affect either hypotonic currents obtained by voltage-steps protocol between −110 and −40 mV or membrane potential changes during hypotonic challenge of guinea pig ventricular myocytes. Terikalant is a relatively new compound, structurally it is (*s*)(−)1-[2-(3,4-dihydro-2*H*-1-benzopyran-4-yl)ethyl]-4-(3,4-dimethoxyphenyl) piperidine (13). In fact, this compound can block not only I_{K1}, but also the rapid component of delayed rectifier potassium current (I_{Kr}) and transient outward current (I_{to}) (14, 15). A significant prolongation of APD after application of terikalant gives evidence for

Fig. 4. Original traces of action potentials in control cells (A) and cells pretreated with glibenclamide at 1 μM (B). A: a, isotonic solution; b, after 60 s in hypotonic solution; c, after 300 s in hypotonic solution; d, washout in isotonic solution. B: a, isotonic solution + glibenclamide; b, after 60 s in hypotonic solution + glibenclamide; c, after 300 s in hypotonic solution + glibenclamide. Similar results were recorded in 6 cells.

inhibition of I_{Kr}, as I_{to} does not have an important role in guinea pig heart (16). Moreover, slight but significant depolarization of resting potential and decreasing of both inward and outward I_{K1} steady-state current after addition of 10 μM terikalant confirmed that I_{K1} was inhibited by this compound (Fig. 1: C and D, Table 1). Previously, we excluded participation of I_{Kr} current in membrane potential changes during hypotonic stress in guinea pig ventricular myocytes (7). In this study, we confirmed pharmacologically and electrophysiologically that I_{K1} indeed does not participate in depolarization of resting potential that appeared in guinea pig ventricular myocytes exposed to hypotonic solution.

On the other hand, abbreviation of APD appearing regularly in all guinea pig ventricular myocytes exposed to hypotonic solution still requires explanation. Activation of $I_{Cl,swell}$ and I_{Ks} currents contribute to the abovementioned phenomenon in about 50% of examined cells (7). It was postulated that activation of I_{KATP} could participate in described APD shortening during hypotonic stress. Indeed, the results obtained previously (10) indicated that hypotonic stress shortened APD solely by activation of I_{KATP}. The ATP-sensitive K^+ channels play an important protective role in ischemic heart tissue. Hypotonic swelling of cardiomyocytes appearing in the ischemic heart area could contribute to activation of the mentioned channels (10), although the authors reported that the described changes of membrane potentials and activation of I_{KATP} occurred only when ATP concentration in the pipette solution was decreased to 2 mM or less. Here, we clearly demonstrated that in the presence of 5 mM ATP concentration in the pipette solution, the same condition as used previously when APD shortening was observed in all examined ventricular myocytes (7), hypotonic swelling did not activate I_{KATP} channels. Although, it is difficult to expect activation of K_{ATP} channels in the presence of 5 mM ATP, it is possible under specific conditions (17). However, we did not detect any potassium time-independent current sensitive to hypotonic solution, and secondly, glibenclamide, a known blocker of I_{KATP} currents, did not affect membrane potential changes induced by hypotonic challenge (Figs. 3 and 4). Therefore, lack of time-independent K^+ current during hypotonic swelling and lack of effect of glibenclamide on the membrane potential changes evoked by hypotonic stress are strong arguments against contribution of I_{KATP} to the APD shortening under such a condition.

In conclusion, we have shown here that I_{K1} and I_{KATP} are not involved in the hypotonic stress induced membrane potential changes in guinea pig ventricular myocytes with sufficient intracellular ATP.

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