High Glucose Regulates the Activity of Cardiac Sarcolemmal ATP-Sensitive K^+ Channels via 1,3-Bisphosphoglycerate

A Novel Link Between Cardiac Membrane Excitability and Glucose Metabolism

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Because we were interested in assessing glucose-mediated regulation of the activity of sarcolemmal ATP-sensitive K^+ channels (K_{ATP} channels) (which are closed by physiological levels of intracellular ATP and serve to couple intracellular metabolism with the membrane excitability in the heart) during ischemia, we performed experiments designed to test whether high extracellular glucose would have effects on sarcolemmal K_{ATP} channels per se. Surprisingly, we found that high extracellular glucose (50 mmol/l) activates sarcolemmal K_{ATP} channels in isolated guinea pig cardiomyocytes. To activate K_{ATP} channels, glucose had to be transported into cardiomyocytes and subjected to glycolysis. The activation of these channels was independent of ATP production and intracellular ATP levels. The effect of glucose on sarcolemmal K_{ATP} channels was mediated by the catalytic activity of glyceraldehyde-3-phosphate dehydrogenase and consequent generation of 1,3-bisphosphoglycerate. The 1,3-bisphosphoglycerate (20 mmol/l), an intermediate product of glycolysis, directly targeted and activated K_{ATP} channels, despite physiological levels of intracellular ATP (5 mmol/l). We conclude that glucose, so far exclusively viewed as a metabolic fuel in the heart important only during ischemia/hypoxia, may serve a signaling role in the nonstressed myocardium by producing an agent that regulates cardiac membrane excitability independently of high-energy phosphates. *Diabetes* 54:383–393, 2005

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Received for publication 28 July 2004 and accepted in revised form 21 October 2004.

cGMP, guanosine 3′,5′-cyclic monophosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HERG, human ether-a-go-go–related gene; K_{ATP} channel, ATP-sensitive K^+ channel; NAD, nicotinamide dinucleotide; 8-*p*-CPT-cGMP, 8-(4-chlorophenylthio)-cGMP.

It is generally accepted that diabetes is associated with an increased risk of coronary heart disease. Although this appears to be explained in part by the well-known association of diabetes with hypertension, dyslipidemia, and coronary atherosclerosis, additional pathophysiological mechanisms linking diabetes and heart disease have recently been suggested. These include the potentially adverse effects of hyperglycemia on endothelial function and redox state, effects of excess circulating glucose and fatty acids on cardiomyocyte ultrastructure, intracellular signaling and gene expression, and the possibility that diabetes may impair recruitment of the myocardial insulin-responsive glucose transport system in response to ischemia (rev. in 1,2). In support of the idea that hyperglycemia may be directly harmful to the heart are experimental studies demonstrating that glucose may have a range of effects on cardiac membrane excitability and excitation-contraction coupling. In this regard, it has been shown that hyperglycemia impairs function of the human ether-a-go-go–related gene (HERG) K^+ channel, reduces transient outward K^+ current, increases intracellular concentration of Ca^{2+}, and impairs excitation-contraction coupling in the heart (3–6). However, as opposed to the consensus view concerning deleterious consequences of diabetes on anatomy and physiology of the heart, the acute effect of hyperglycemia on cardiac function is more controversial, in particular with regard to cardiac resistance to ischemia. Some reports have suggested that hyperglycemia exacerbates the outcome of myocardial infarction (7,8) and inhibits ischemic preconditioning (9), whereas others have reported that acute hyperglycemia may decrease intracellular Ca^{2+} in cardiomyocytes and promote cardiac viability and resistance during ischemia (10–13).

Considering the controversy in the effect of acute hyperglycemia on cardiac myocytes exposed to ischemia, we have aimed to address the effect of high levels of extracellular glucose on cardiac response to ischemia using our newly developed experimental model that utilizes adult beating cardiomyocytes (14). Because we were interested in assessing glucose-mediated regulation of the activity of sarcolemmal ATP-sensitive K^+ channels (K_{ATP} channels)

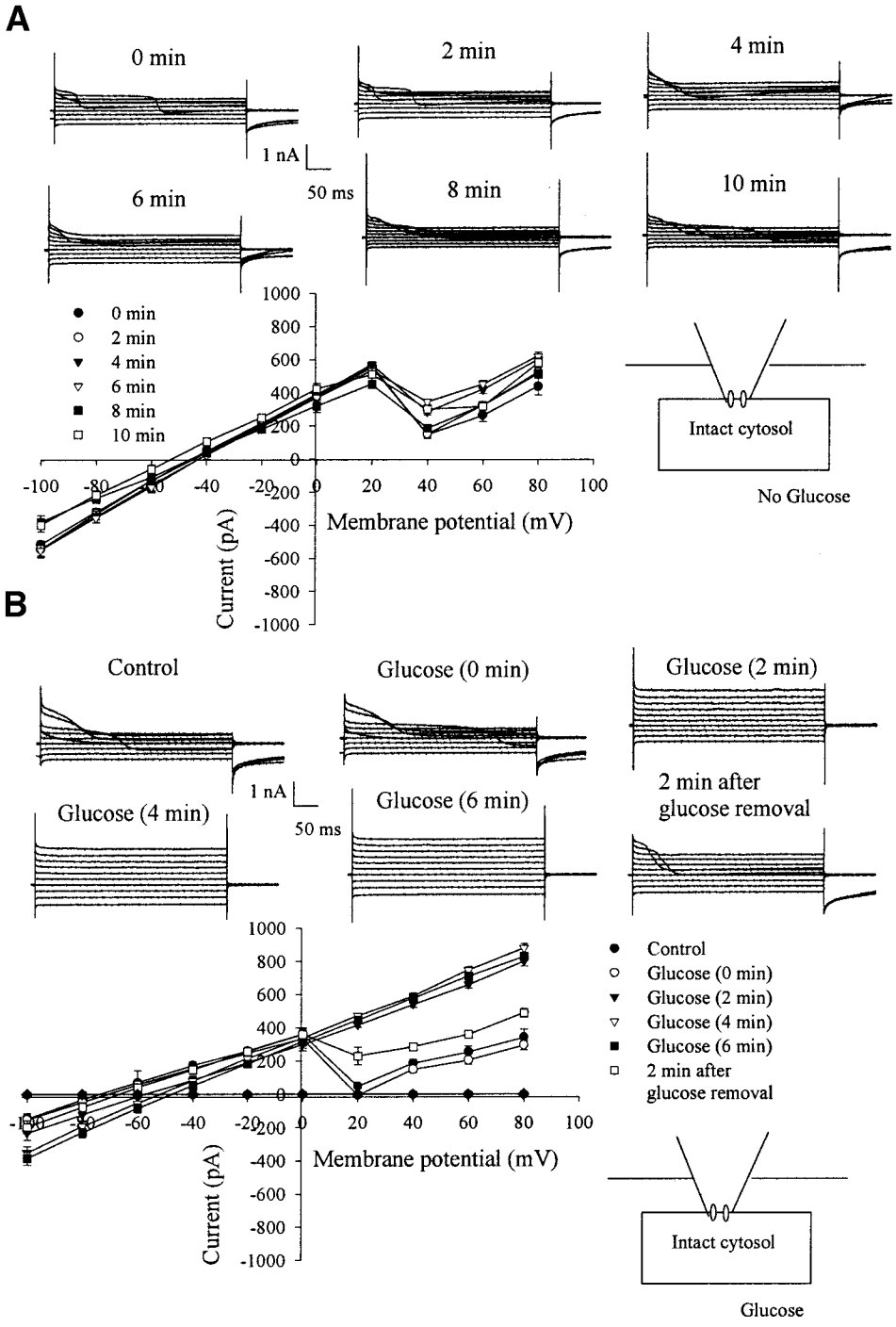


FIG. 1. Glucose induces outward K^+ current in cardiomyocytes. *A* and *B*: Membrane currents recorded in response to 400 ms–long pulses (from −100 to +80 mV) using perforated patch-clamp electrophysiology (this technique is schematically presented on the figure as a rectangular shape (depicting a cardiomyocyte) in contact with a tip of the patch pipette while the sarcolemma, apart from ion-permeable holes, as well as the cytosol remained largely intact) and corresponding I-V relationships. Each point is the means ± SE (n = 6 for each). Cardiomyocytes were bathed with Tyrode solution without (*A*) and with (*B*) glucose (50 mmol/l). Time point 0 min refers to the recording made immediately after the whole-cell configuration was established. *C*: Current density at +80 mV under conditions in *A* and *B*. Each bar represents the means ± SE (n = 6).

(ion channels coupling the metabolic condition of the heart with its membrane excitability) (15) during ischemia, we have performed experiments designed to verify that high extracellular glucose would not have effects on sarcolemmal K_{ATP} channels. However, contrary to our expectations, we have obtained results suggesting that high glucose activates sarcolemmal K_{ATP} channels. We have further elucidated this phenomenon and, apparently, uncovered a previously unrecognized signaling pathway that links glucose with sarcolemmal K_{ATP} channel activity.

RESEARCH DESIGN AND METHODS

Ventricular cardiomyocytes were dissociated from guinea pig hearts, as previously described (16). In brief, hearts were retrogradely perfused (at 37°C) with medium 199, followed by Ca^{2+}-EGTA–buffered low-Ca^{2+} medium (pCa = 7) and finally low-Ca^{2+} medium containing pronase E (8 mg/100 ml),

FIG. 1.—*Continued*

proteinase K (1.7 mg/100 ml), bovine albumin (0.1 g/100 ml, fraction V), and 200 μmol/l $CaCl_2$. Ventricles were cut into fragments in the low-Ca^{2+} medium enriched with 200 μmol/l $CaCl_2$. Single cells were isolated by stirring the tissue (at 37°C) in a solution containing pronase E and proteinase K supplemented with collagenase (5 mg/10 ml).

Patch-clamp electrophysiology. For conventional whole-cell electrophysiology, cells were superfused with Tyrode solution without glucose (in mmol/l: 136.5 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.53 $MgCl_2$, and 5.5 HEPES-NaOH, pH 7.4). Pipettes (resistance 3–5 mol/lΩ) were filled with (in mmol/l) 140 KCl, 1 $MgCl_2$, 5 ATP, and 5 HEPES-KOH, pH 7.3. In some cells the whole-cell K^+ current was measured using the perforated patch-clamp technique with essentially the same pipette solution as above, except ATP was omitted and amphotericin B (240 mg/ml; Sigma) was added. In the majority of experiments, we added (either in Tyrode or pipette solution, as stated in the text when appropriate) glucose or other substrates of glycolytic enzymes: glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-bisphosphate, glyceraldehyde 3-phosphate, nicotinamide dinucleotide (NAD), 1,3-bisphosphoglycerate, and 3-phosphoglycerate (all from Sigma, Dorset, U.K.) and 1,3-bisphosphoglycerate, which was made as previously described by Graciet et al. (17). For all cells monitored, the membrane potential was normally held at −40 mV, and the currents evoked by a series of 400-ms depolarizing and hyperpolarizing current steps (−100 to +80 mV in 20-mV steps) were recorded directly to hard disk using an Axopatch-200B amplifier, Digidata-1321 interface, and pClamp8 software (Axon Instruments, Foster City, CA). The capacitance compensation was adjusted to null the additional whole-cell capacitative current. The slow capacitance component measured by this procedure was used as an approximation of the cell surface area and allowed normalization of current amplitude (i.e., current density). Currents were low-pass filtered at 2 kHz and sampled at 100-ms intervals. To monitor on-line behavior of single-channel molecules, the giagaohm seal patch-clamp technique was applied in the inside-out configuration. Cells were superfused with Ca^{2+}-free and glucose-free Tyrode solution. Fire-polished pipettes, coated with Sylgard (resistance 5–7 mol/lΩ), were filled with (in mmol/l) 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES-KOH, pH 7.3. Recordings were made at room temperature (22°C) using a patch-clamp amplifier (Axopatch-200B). Single-channel activity was monitored on-line and stored on a personal computer. Channel activity, assayed by digitizing segments of current recordings and forming histograms of baseline and open level data points, were expressed as NP_o, where N is the number of channels in the patch and P_o is the probability of each channel to be open. Data were reproduced, low-pass filtered at 1 KHz (−3 dB), and sampled at the 100-μs rate for further analysis with pClamp8 software (18).

Statistical analysis. Data are presented as the means ± SE, with n representing the number of patched cells. Mean values were compared by the paired or unpaired Student's t test or rank tests where appropriate. All statistical tests were performed using the SigmaStat program (Jandel Scientific). $P < 0.05$ was considered statistically significant.

RESULTS

High extracellular glucose activates the whole-cell K^+ current in cardiomyocytes. In the current study, we considered whether glucose has acute effect on whole-cell K^+ currents. Therefore, we measured the whole-cell K^+ current in cardiomyocytes, using perforated patch whole-cell recording. In perforated patch-clamp mode, intracellular milieu remains largely undisturbed because amphotericin B creates holes in the sarcolemma that are permeable to ions but impermeable to larger molecules (19). Under these conditions the steady-state voltage-current (I-V) relationship (Fig. 1*A*) was in an N shape presumably because of inward rectification of I_{k1} channels and the absence of active K_{ATP} channels blocked by physiological levels of intracellular ATP (Fig. 1*A*). The whole-cell K^+ current was largely steady during 10 min of recording because the current density at 80 mV was 3.6 ± 0.6 and 4.5 ± 0.5 pA/pF immediately after and 10 min after, respectively, the moment when whole-cell configuration was established ($P = 0.26$, $n = 6$) (Fig. 1*A–C*). When glucose (50 mmol/l) was added to the extracellular solution, after 2 min of lag period, it induced outward K^+ currents that increased at potentials more positive than −70 mV (Fig. 1*B–C*). Specifically, the current density at 80 mV was 3.9 ± 0.6 pA/pF in the absence of glucose and 10.0 ± 0.8 pA/pF after a 2-min incubation with glucose ($P = 0.0003$, $n = 6$) (Fig. 1*B–C*). Extracellular glucose applied at a concentration of 30 mmol/l also induced a statistically significant increase in the whole-cell K^+ current (the current density at 80 mV was 3.2 ± 0.6 pA/pF in the absence of glucose and 7.1.0 ± 0.9 pA/pF after a 2-min incubation with glucose, $P = 0.03$, $n = 4$). The inward rectification of the I-V relationship was much weaker in the presence of glucose, as reflected by the loss of N shape (Fig. 1*B*). The effect of glucose was reversible because 2 min after glucose removal, the N shape of I-V relationship was restored and the current density at 80 mV returned to 6.1 ± 0.6 pA/pF, which was significantly lower than that obtained just before glucose removal (at time point 6 min; 10.4 ± 0.9 pA/pF, $n = 6$, $P = 0.0003$) (Fig. 1).

K^+ current in cardiomyocytes activated by high glucose is mediated by the sarcolemmal K_{ATP} channels. Properties of glucose-induced outward K^+ current resembled those observed when K^+ current is conducted by the opening of sarcolemmal K_{ATP} channels in the heart (18). To further test the hypothesis that high glucose activates sarcolemmal K_{ATP} channels, we used HMR 1098, a selective antagonist of these channels (20). HMR 1098 (30 μmol/l) blocked the glucose-induced increase in outward K^+ current (Fig. 2). Specifically, in this series of experiments, current density at 80 mV was 2.8 ± 0.2 pA/pF under control conditions. In the presence of glucose (50 mmol/l), current density at 80 mV rose to 5.3 ± 0.6 pA/pF ($n = 5$, $P = 0.01$ when compared with control) (Fig. 2), whereas in the presence of both glucose (50 mmol/l) and HMR 1098 (30 μmol/l), it was 3.3 ± 0.4 pA/pF ($P = 0.02$, $n = 5$) (Fig. 2).

Glucose transport and glycolysis is required for the activation of sarcolemmal K_{ATP} channels. It has been reported that the membrane-permeant guanosine 3′,5′-cyclic monophosphate (cGMP) analog 8-(4-chlorophenylthio)-cGMP (8-*p*-CPT-cGMP) causes a significant decrease in glucose transport in cardiomyocytes (21). Pretreatment of cardiomyocytes for 10 min with 200 μmol/l 8-*p*-CPT-cGMP abolished the glucose-induced K^+ current ($n = 4$) (Fig. 3). In addition, 15-min pretreatment with 2-deoxyglu-

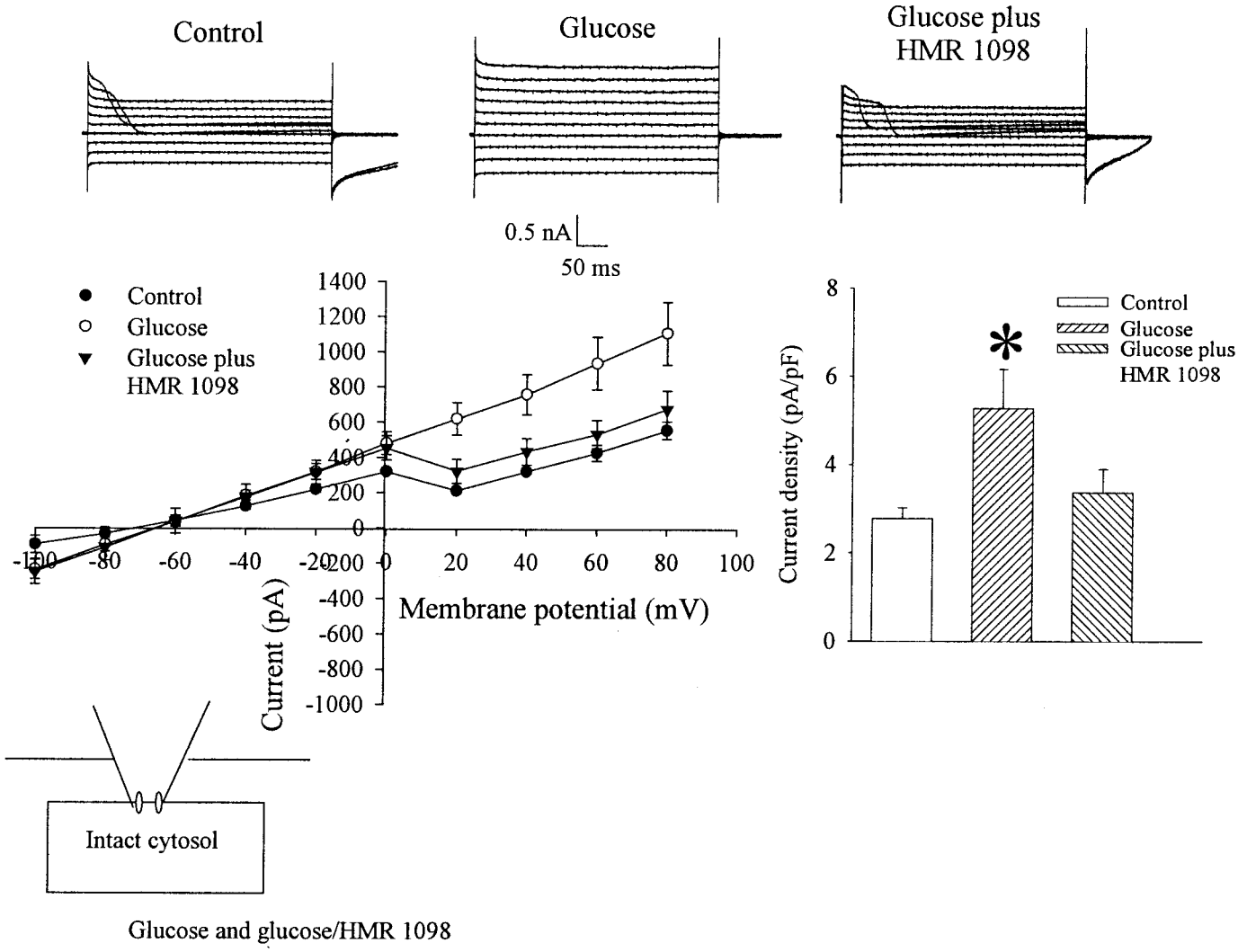


FIG. 2. Glucose-induced outward K^+ current flows through sarcolemmal K_{ATP} channels. Membrane currents were recorded using perforated patch-clamp electrophysiology, and the figure shows the corresponding I-V relationships (each point is the means ± SE, $n = 5$) and bar graph (representing current density at +80 mV) in the absence of glucose (control), presence of glucose (50 mmol/l; after 4 min of exposure), and presence of glucose (50 mmol/l) plus HMR 1098 (30 μmol/l; after 4 min of exposure). Each bar represents the means ± SE ($n = 5$). *$P < 0.01$.

cose (50 mmol/l), an inhibitor of glycolysis (22), also inhibited glucose-induced K^+ current ($n = 4$) (Fig. 3).

Intracellular presence of substrates of hexokinase, phosphoglucose isomerase, and phosphofructokinase do not affect the whole-cell K^+ current. Findings that glucose transport and glycolysis is required for the activation of sarcolemmal K_{ATP} channels prompted us to hypothesize that some intermediate product of glycolysis may regulate the activity of K_{ATP} channels. To test this hypothesis we have measured membrane currents from a guinea pig ventricular myocyte using a conventional whole-cell configuration. In the whole-cell configuration of a patch-clamp technique, pipette solution replaces intracellular environment. which allowed us to fully control the composition of the intracellular environment. When physiological levels of ATP (5 mmol/l) were present in the patch pipette solution, the steady-state voltage-current (I-V) relationship (Fig. 4*A*) was in an N shape because of the strong inward rectification of I_{k1} channels and the absence of active K_{ATP} channels blocked by high intracellular ATP (Fig. 4*A*). From the moment of establishing the whole-cell configuration, currents were measured every 2 min. A progressive decline in the magnitude of the whole-cell K^+ current without changes in the shape of the I-V relationship was observed in every patch recorded under these conditions ($n = 11$) (Fig. 4*A* and *C*). The addition of 20 mmol/l glucose into the patch pipette did not significantly change the whole-cell K^+ current in cardiomyocytes in terms of magnitude, shape, or time-dependent magnitude decline ($n = 6$) (Fig. 4*B* and *C*). Because these results suggested that glucose does not have direct effects on K^+ currents, we hypothesized that glucose-induced activation of K_{ATP} channels might be mediated by some of the glycolytic products. Therefore, we measured the whole-cell K^+ current in cells with intracellular environments containing only pipette solution, substrates of glycolytic enzymes, and, when ATP was not applied as a substrate, a physiological level of ATP (5 mmol/l). Specifically, we tested the effects of the following substrates on the whole-cell K^+ current: glucose-6-phosphate/ATP (20 mmol/l each), fructose-6-phosphate/ATP (20 mmol/l each), or fructose-1,6-bisphosphate (20 mmol/l fructose 1,6-

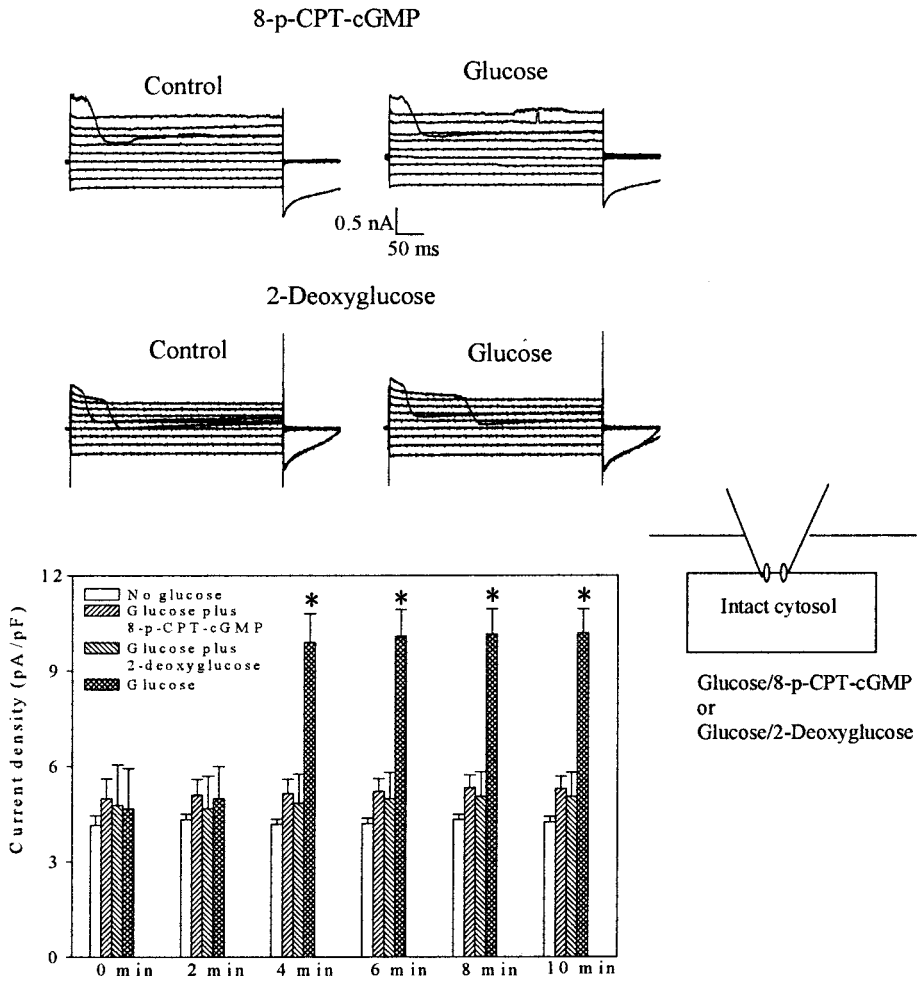


FIG. 3. Glucose transport and glycolysis are required for glucose-induced activation of K_{ATP} channels. Membrane currents in the absence and presence of glucose (50 mmol/l; 4 min of exposure) were recorded using perforated patch-clamp electrophysiology in cardiomyocytes pretreated with 8-*p*-CPT-cGMP (200 μmol/l) or 2-deoxyglucose (50 mmol/l). The bar graph shows current density at +80 mV in cells under depicted conditions and time points. Time points refer to the time from the moment of establishing the whole-cell configuration. Each bar represents the means ± SE (n = 4–6). *P < 0.01 when compared with the control.

bisphosphate and 5 mmol/l ATP; this concentration of ATP was applied because fructose-1,6-bisphosphate is the sole substrate for aldolase, and ATP was applied not as a substrate but as a K_{ATP} channel ligand to assess whether the product would have a direct effect on the channels) (Fig. 5). None of these substrates had a significant effect on the whole-cell K^+ current (n = 5 for each) (Fig. 5).

Substrates of glyceraldehyde-3-phosphate dehydrogenase activate sarcolemmal K_{ATP} channels. When substrates of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were concomitantly present (glyceraldehide-3-phosphate and NAD, 20 mmol/l each) in the pipette solution containing 5 mmol/l ATP, K_{ATP} channel–mediated current was observed starting from the 2-min time point, and recordings revealed that outward K^+ currents associated with a much weaker inward rectification of the I-V relationship (n = 5) (Fig. 6). The current density at 80 mV rose from 3.1 ± 1.0 pA/pF at time 0 to 14.2 ± 3.0 pA/pF after a 2-min incubation with GAPDH substrates (P = 0.0003) (Fig. 6). This increase in K^+ current was maintained by the end of the experiment (Fig. 6). Glyceraldehide-3-phosphate (20 mmol/l) and NAD (20 mmol/l) added into pipette solution (containing 5 mmol/l ATP) alone did not have any effect on the whole-cell K^+ current in cardiomyocytes (n = 4 for each) (Fig. 6).

A product of GAPDH catalytic activity,1,3-bisphosphoglycerate, is an activator of sarcolemmal K_{ATP} channels. Because the opening of sarcolemmal K_{ATP} channels by GAPDH substrates was detected, it was possible that the GAPDH-catalyzed reaction product 1,3-bisphosphoglycerate activates K_{ATP} channels. This hypothesis was tested using both whole-cell and single-channel electrophysiology. We have added 1,3-bisphosphoglycerate (20 mmol/l) into the pipette solution containing ATP (5 mmol/l) and measured the whole-cell K^+ current. Under these conditions, from the beginning of the experiment, whole-cell current had properties of the K^+ current flowing through K_{ATP} channels, i.e., the N shape of I-V relationship—typical for the whole-cell K^+ current in cardiomyocytes when K_{ATP} channels are closed—was lost (Fig. 7*A*). The addition of 1,3-bisphosphoglycerate (20 mmol/l) on the intracellular face of excised membrane patches induced vigorous opening of K_{ATP} channels in the presence of 5 mmol/l ATP (Fig. 7*C*).

DISCUSSION

In the current study, we have suggested that high glucose may activate sarcolemmal K_{ATP} channels in cardiomyocytes. The glucose-induced activation seems to be mediated by glycolysis and the production of 1,3-bisphos- phoglycerate, which directly targets and activates sarcolemmal K_{ATP} channels.

In previous studies, the effect of high glucose on cardiac

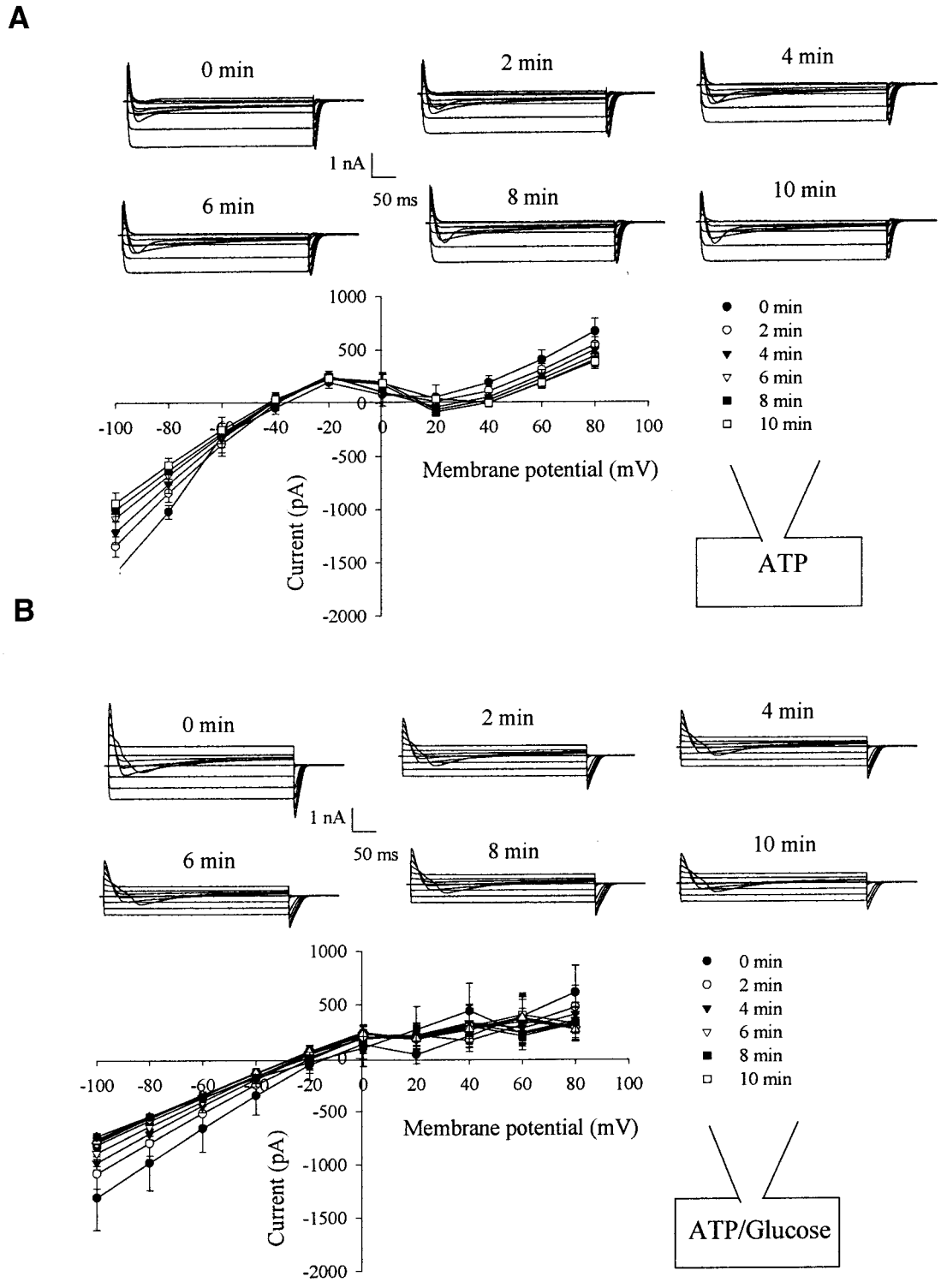


FIG. 4. Intracellular glucose has no effect on whole-cell K^+ current measured by conventional patch-clamp electrophysiology. *A* and *B*: Membrane currents recorded in response to 400 ms–long pulses (from −100 to +80 mV) using conventional whole-cell patch-clamp electrophysiology (this technique is schematically presented on the figure as a rectangular shape (depicting a cardiomyocyte) in contact with a tip of the patch pipette while a part of the sarcolemma is disrupted, allowing pipette solution to replace cytosol) and corresponding I-V relationships (each point is the means ± SE, n = 6–11) at depicted time points. Cardiomyocytes were filled up with pipette solution without (*A*) or with (*B*) glucose (20 mmol/l). Time point 0 min refers to the recording made immediately after the whole-cell configuration was established. *C*: Current density at +80 mV under conditions in *A* and *B*. Each bar represents the means ± SE (n = 6–11).

membrane excitability has been rarely investigated under nonstress conditions. In genetically and streptozocin-induced diabetic rats, transient outward K^+ current is greatly reduced (3,23), and acute hyperglycemia inhibits the opening of HERG K^+ channels, which are ion channels controlling the rapid component of the delayed rectifier K^+ current in the heart, which is the major repolarizing current in the plateau voltage range of cardiac action

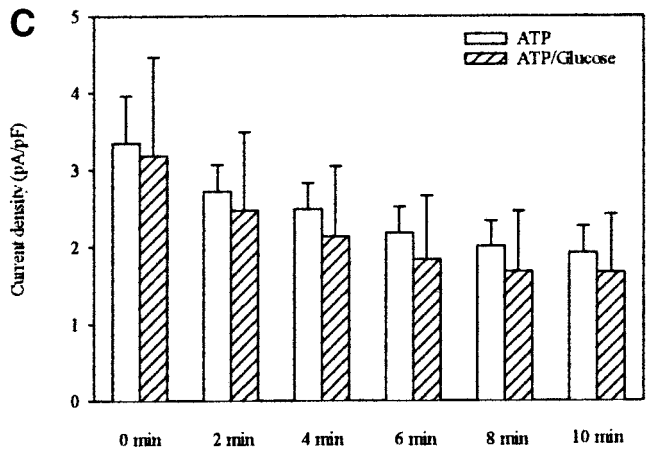


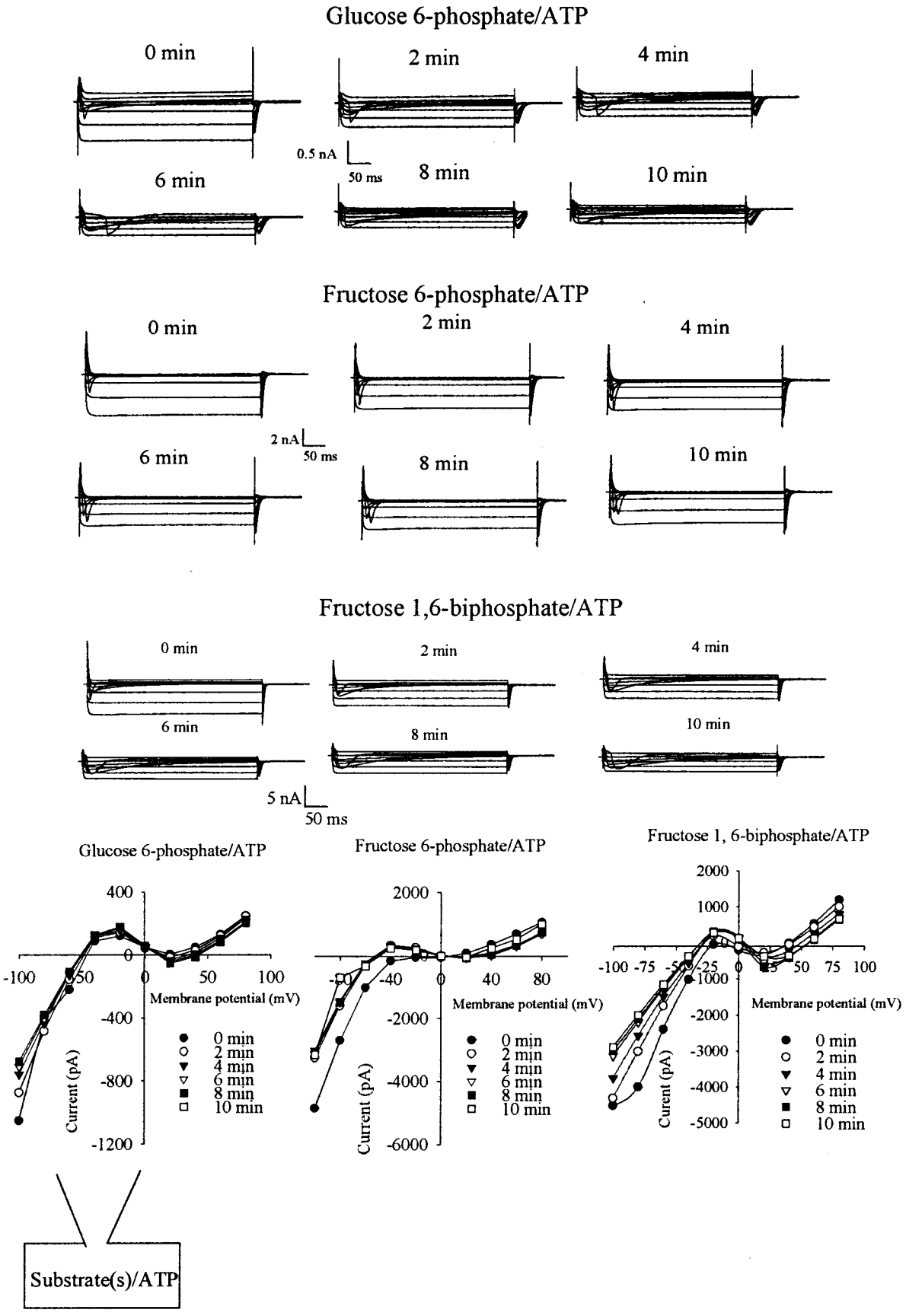
FIG. 4.—*Continued*

potential (6). It has been established since the 1970s that elevating extracellular glucose dramatically attenuates action potential duration shortening during hypoxia in intact cardiac tissue, and that action potential duration shortening is even more rapid in the absence of glucose (24–26). However, it should be said that, as opposed to hearts exposed to ischemia/hypoxia, in hearts under physiological conditions, glycolysis is only a minor source of intracellular ATP (27). Considering that the bioenergetic status of the heart is linked with its membrane excitability via K_{ATP} channels, the effect that hyperglycemia would have in normoxic and hypoxic hearts may be quite different. More specifically, intracellular ATP inhibits K_{ATP} channels (15), and it is believed that ischemia/hypoxia-induced decreases in cytosolic ATP concentration activate these channels, leading to efflux of K^+ and shortening of the action membrane potential. During hypoxia, glycolysis takes over fatty acids in producing ATP, which in turn may lead to glycolysis-mediated inhibition of sarcolemmal K_{ATP} channels (indirectly via ATP production) and attenuation of action potential duration shortening during hypoxia. In contrast, in normoxic hearts K_{ATP} channels are closed anyway, and in any case, glucose is not an important source of ATP, so hyperglycemia may not have such dramatic effects on action potential duration. Because hyperglycemia per se inhibits HERG K^+ channels (6), it should significantly increase the action potential duration in normoxic hearts. However, it has been recently reported that hyperglycemia has no significant effects on action potential duration in nonstressed hearts (28), which may be explained by the activation of K^+ currents (such as K_{ATP} channel–mediated currents), which would counteract the effect of hyperglycemia on HERG K^+ channels. Thus, although on first sight our results seem to contradict the established dogma that hyperglycemia increases action potential duration, they actually fit very well into the most recent findings showing that although hyperglycemia blocks HERG K^+ channels, it still does not affect action potential duration in the heart.

The K_{ATP} channel–opening effect of glucose was not observed when conventional whole-cell instead of perforated-patch electrophysiology was used. This would suggest that an intact nondialyzed intracellular environment was essential for glucose-induced activation of K_{ATP} channels. 8-*p*-CPT-cGMP and 2-deoxyglucose, inhibitors of glucose transport and glycolysis, respectively (21,22), inhibited the effect of glucose, suggesting that the transport of glucose into cells and glycolysis are required for the opening of sarcolemmal K_{ATP} channels. Because pipette solution replaces cytosol during whole-cell patch-clamp recordings, enzymes that are not attached to the cellular cytoskeleton and other structural proteins are being removed from their physiological sites of action. This, in turn, may explain why glucose was without any effect when K^+ current was measured using conventional whole-cell electrophysiology.

Results with inhibitors of glucose transport and glycolysis allowed a supposition that one of the intermediary products of the glycolytic pathway might possess previously unrecognized K_{ATP} channel–opening properties. To examine this hypothesis, we have measured the whole-cell K^+ current in cells with intracellular environments containing only pipette solution, substrates of glycolytic enzymes, and physiological levels of ATP (5 mmol/l). Under these conditions, glucose-6-phosphate (a substrate of phosphoglucose isomerase generating fructose-6-phosphate), fructose-6-phosphate/ATP (substrates of phosphofructokinase generating fructose-1,6-bisphosphate), and fructose-1,6-bisphosphate (a substrate of aldolase generating glyceraldehide-3-phosphate) did not have any effect on the whole-cell K^+ current. This would stand against the possibility that some of these substrates, either directly or via generated product(s), regulate the activity of sarcolemmal K_{ATP} channels. However, substrates of GAPDH (glyceraldehyde-3-phosphate and NAD) activated K_{ATP} channels. Because this was observed only when glyceraldehyde-3-phosphate and NAD were applied together, it was likely that the opening of K_{ATP} channels was associated with the GAPDH catalytic activity, and that the product of GAPDH-catalyzed reaction, 1,3-bisphosphoglycerate, may be responsible for the activation of sarcolemmal K_{ATP} channels. Whether 1,3-bisphosphoglycerate is an opener of sarcolemmal K_{ATP} channels was tested using both whole-cell and single-channel electrophysiology. When present in pipette solution, 1,3-bisphosphoglycerate induced whole-cell outward K^+ currents, despite the concomitant presence of millimolar ATP, suggesting that this compound opens sarcolemmal K_{ATP} channels. Direct application of 1,3-bisphosphoglycerate on the intracellular side of excised membrane patches activated K_{ATP} channels, and this further supports the notion that this intermediary product of glycolysis targets and activates K_{ATP} channels.

The consequences of opening the sarcolemmal K_{ATP} channels for cardiac function are complex, and they may depend on the extent of channel activation and probably other as yet unknown factors. On one hand, the opening of the channels increases the resistance of cardiomyocytes against ischemia/hypoxia and protects the heart against myocardial infarction (29,30). On the other hand, the activation of K_{ATP} channels may be associated with the appearance of arrhythmias. It has been suggested that the activation of sarcolemmal K_{ATP} channels could be both pro- and antiarrhythmogenic (31,32). Increased K^+ conductance shifts resting membrane potential toward K^+ equilibrium, which should reduce ectopic pacemaker activity. However, the activation of K_{ATP} channels accelerates the depolarization of the action potential, reducing



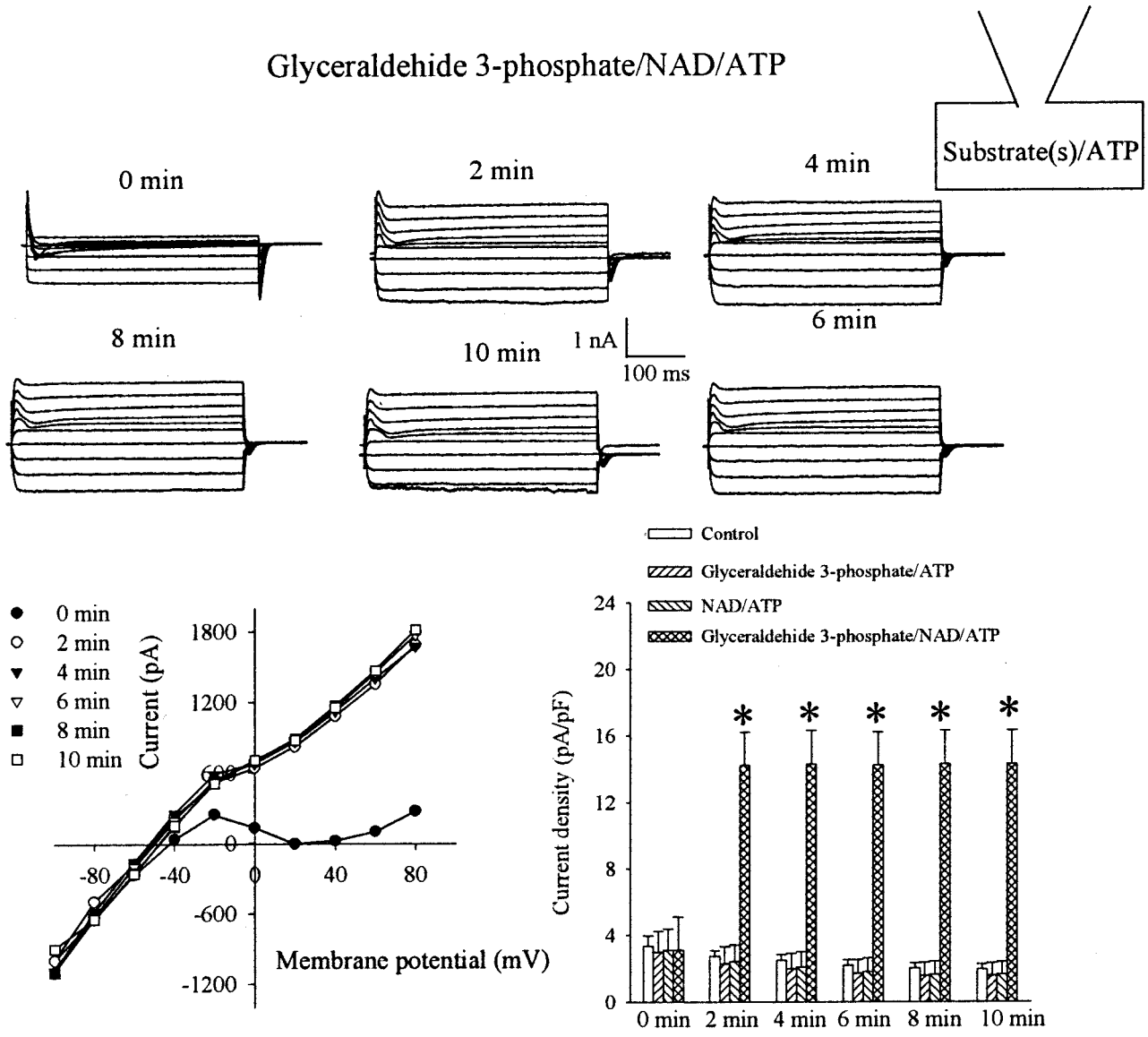


FIG. 6. Substrates of GAPDH regulate the activity of sarcolemmal K_{ATP} channels. Membrane currents were recorded using conventional patch-clamp electrophysiology and corresponding I-V relationships (each point is the means ± SE, $n = 5$) at depicted conditions (each substrate was added to the pipette solution at a concentration of 20 mmol/l, whereas ATP was kept at 5 mmol/l). Time point 0 min refers to the recording made immediately after the whole-cell configuration was established. The bar graph shows current density at +80 mV in cells under depicted conditions and time points. Each bar represents the means ± SE ($n = 4$–11). *$P < 0.01$ when compared with the control.

the refractory period of the cell, which may increase the probability of reentrant arrhythmias (33). It has been shown that action potential duration is much longer in diabetic that nondiabetic hearts (28). It has been suggested that this effect of diabetes is a consequence of downregulation of ion channels controlling transient outward K^+ currents (3,23). However, although this diabetes-induced change of action potential duration in the heart is chronic, it can be acutely corrected with insulin (28). Because insulin does not directly activate K^+-selective conductance in the heart (34,35), our data would explain very well how insulin, by stimulating glucose transport, glycolysis, and 1,3-bisphosphoglycerate production, shortens/corrects action potential duration in diabetic hearts.

Previously, it was shown that lipids/fatty acids, which are the major source of ATP in the heart, are directly linked with heart membrane excitability via phosphatidylinositol phosphates and long-chain acyl-CoA esters (36,37). Analogously to the role that phosphatidylinositol phosphates and long-chain acyl-CoA esters play in linking fatty acids with K_{ATP} channels, 1,3-bisphosphoglycerate seems to play a role in linking glucose metabolism with cardiac electrophysiology. The existence of direct, ATP-independent signaling pathways in regulating the K_{ATP}

FIG. 5. Substrates of hexokinase, phosphoglucose isomerase, and phosphofructokinase do not affect whole-cell K^+ current. The figure shows membrane currents recorded using conventional patch-clamp electrophysiology and corresponding I-V relationships (each point is the means ± SE, $n = 5$) at depicted conditions (glucose 6-phosphate/ATP [20 mmol/l each], fructose 6-phosphate/ATP [20 mmol/l each], or 20 mmol/l fructose 1,6-bisphosphate and 5 mmol/l ATP). Time point 0 min refers to the recording made immediately after the whole-cell configuration was established.

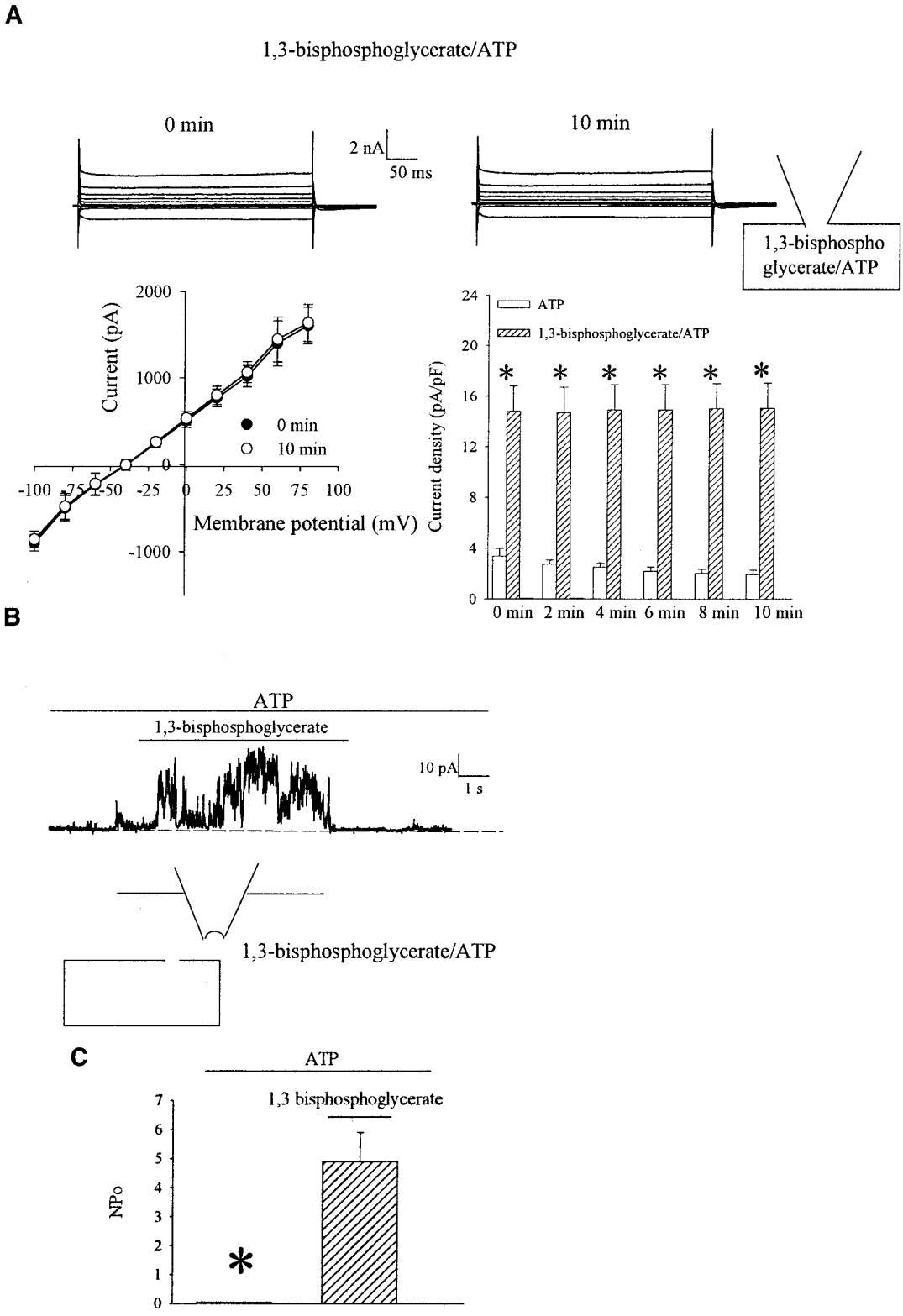


FIG. 7. 1,3-Bisphosphoglycerate, a product of GAPDH catalytic activity, directly regulates the activity of sarcolemmal K_{ATP}, despite high levels of intracellular ATP. *A*: Membrane currents were recorded using conventional whole-cell patch-clamp electrophysiology and corresponding I-V relationships (each point is means ± SE, $n = 6$) under depicted conditions (1,3-bisphosphoglycerate was added at a concentration of 20 mmol/l, whereas ATP was kept at 5 mmol/l). Time point 0 min refers to the recording made immediately after the whole-cell configuration was established. The bar graph shows current density at +80 mV in cells under depicted conditions and time points. Each bar represents the means ± SE ($n = 6–11$). *B*: Inside-out single-channel recording of K_{ATP} channel activity in membrane patch treated with ATP (5 mmol/l) alone, ATP (5 mmol/l) plus 1,3-bisphosphoglycerate (20 mmol/l), and again ATP (5 mmol/l) alone. Holding potential: 0 mV. Dotted lines correspond to 0 current levels. C: Channel activity expressed as NP_o (where N is the number of channels in the patch and P_o is the probability of each channel to be open) under the conditions in *B* ($n = 5$). *$P < 0.01$ when compared with the control.

channels of metabolites that produce ATP probably allows subtlety in the regulation of these channels. As an example, because glucose is not the only source of ATP in the heart, ATP-sensing by K_{ATP} channels would be reflective of the overall metabolic status of cardiac cells but not of the status of glucose metabolism. As opposed to ATP, levels of 1,3-bisphosphoglycerate depend solely on glucose levels and metabolism. This means that glucose metabolism, independently of ATP production or any other factors, is transduced into changes in membrane excitability that make the heart responsive to changes in glucose levels, irrespective of the conditions of other metabolic pathways and/or total energy status. This type of link between K_{ATP} channel activity and glucose metabolism may be important not only for cardiac cells but also for other cell types with functions regulated by K_{ATP} channels, including insulin-secreting β-cells.

ACKNOWLEDGMENTS

This research was supported by grants from the British Heart Foundation, the BBSRC (Biotechnology and Biological Sciences Research Council), the MRC (Medical Research Council), Wellcome Trust, Anonymous Trust, and Tenovus-Scotland. We thank Aventis Pharma (Frankfurt, Germany) for HMR 1098.

A majority of the cardiomyocytes used in this study were isolated by G.R. Budas.

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