β-Adrenergic Modulation of Cardiac Ion Channels

Differential Temperature Sensitivity of Potassium and Calcium Currents

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ABSTRACT β-Adrenergic stimulation of ventricular heart cells results in the enhancement of two important ion currents that regulate the plateau phase of the action potential: the delayed rectifier potassium channel current (I_K) and L-type calcium channel current (I_{Ca}). The temperature dependence of β-adrenergic modulation of these two currents was examined in patch-clamped guinea pig ventricular myocytes at various steps in the β-receptor/cyclic AMP-dependent protein kinase pathway. External applications of isoproterenol and forskolin were used to activate the β-receptor and the enzyme adenylate cyclase, respectively. Internal dialysis of cyclic 3',5'-adenosine monophosphate (cAMP) or the catalytic subunit of cAMP-dependent protein kinase (CS), as well as the external addition of 8-chlorphenylthio cAMP (CPT-cAMP) was applied to increase intracellular levels of cAMP and CS. Isoproterenol-mediated increases in I_K, but not I_{Ca}, were found to be very temperature dependent over the range of 20–37°C. At room temperature (20–22°C) isoproterenol produced a large (threefold) enhancement of I_{Ca} but had no effect on I_K. In contrast, at warmer temperatures (30–37°C) both currents increased in the presence of this agonist and the kinetics of I_K were slowed at −30 mV. A similar temperature sensitivity also existed after exposure to forskolin, CPT-cAMP, cAMP, and CS, suggesting that this temperature sensitivity of I_K may arise at the channel protein level. Modulation of I_K during each of these interventions was accompanied by a slowing in I_K kinetics. Thus, regulation of cardiac potassium channels but not calcium channels involves a temperature-dependent step that occurs after activation of the catalytic subunit of cAMP-dependent protein kinase.

INTRODUCTION

β-Receptor stimulation in mammalian ventricular muscle and Purkinje fiber cells enhances currents through two ion channels that have opposing effects on cellular

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electrical activity during the plateau phase of the action potential: L-type Ca channel current (I_{Ca}) and delayed rectifier potassium channel current (I_K) (Reuter, 1983; Bennett et al., 1986). In heart as in other tissues, agonist binding to β-receptors stimulates adenylate cyclase, which increases intracellular levels of cyclic AMP (cAMP). Previous investigations have provided data suggesting that ion channel currents may be increased as a result of channel protein phosphorylation mediated by the catalytic subunit of cAMP-dependent protein kinase (CS) (Tsien et al., 1972; Cachelin et al., 1983; Brum et al., 1984). These studies have not addressed the issue of whether the regulation of these Ca and K channels is identical nor whether their modulation can be separated.

In the present study, the temperature dependence of β-adrenergic modulation of I_K and I_{Ca} was examined in patch-clamped guinea pig ventricular myocytes at various steps in the β-receptor/cAMP-dependent protein kinase pathway. This was achieved through external application of isoproterenol, forskolin, or the membrane-soluble cAMP analogue 8-chlorphenylthio (CPT) cAMP, as well as by internal dialysis with cAMP or CS. We found that temperature can separate the modulation of I_K and I_{Ca} brought about through stimulation at four different levels: the β-receptor (using isoproterenol), adenylate cyclase (with forskolin), protein kinase (with cAMP), and the channel (using CS). Furthermore, the modulation of I_K appears similar at each of these steps. Our results show that β-adrenergic regulation of these two channels differs at a step that occurs after activation of CS and that the modulation of I_K is mediated primarily via this subunit.

These results have been reported in preliminary form (Walsh et al., 1988*a*, *b*).

MATERIALS AND METHODS

Preparation of Cells and Solutions

Isolated ventricular cells were obtained from adult guinea pig hearts following a procedure similar to that of Mitra and Morad (1985). Briefly, hearts were removed from guinea pigs of 250–350 g body weight, mounted on a Langendorf-type column, and perfused for 10 min with a Ca-free Tyrode's solution containing collagenase (type 2; Worthington Biochemical Corp., Freehold, NJ) and protease (type 14; Sigma Chemical Co., St. Louis, MO). After 20 min of perfusion with a 200 μM Ca-containing Tyrode's, the heart was dissected into small pieces and single cells were obtained by gentle agitation.

Recordings of membrane currents were made using the whole-cell arrangement of the patch-clamp technique (Hamill et al., 1981), in a chamber containing normal external solution consisting of (in millimolar): 138 NaCl, 4.8 KCl, 1.2 $MgCl_2$, 1.0 $CaCl_2$, 5 dextrose, 5 HEPES, and 5 μM tetrodotoxin, pH 7.4. Electrodes (2–5 MΩ) contained (in millimolar): 50 KCl, 50 K-glutamate, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, 3 ATP, and 10 HEPES. This ratio of EGTA/$CaCl_2$ sets the free intracellular Ca concentration to ~10 nM (Goldstein, 1979). The pH of this solution was adjusted to 7.3 with KOH, bringing the total K concentration to 140 mM. The temperature of the recording chamber was varied between 20 and 37°C. Forskolin, L(−)isoproterenol, cAMP, and CS were obtained from Sigma Chemical Co. 8-chlorphenylthio cAMP was purchased from Boehringer Mannheim, Inc. (Indianapolis, IN) and the water-soluble (7-deaceyl-7-[4-methylpiperazine]-butyryl-forskolin) and inactive (1,9-dideoxy forskolin) isomers of forskolin were obtained from Calbiochem Behring Corp. (San Diego, CA).

Current Measurement and Cell Dialysis

To study I_{Ca} and I_K, cell membrane potential was held at −30 mV to inactivate the fast sodium current. I_{Ca} was elicited by test pulses of 40-ms duration to potentials of −10 to +10 mV, and I_K deactivating tails were recorded on return to the holding potential after 1.5-s prepulses to +30–40 mV. I_K tails can be measured under these conditions because deactivation of I_{Ca} is orders of magnitude faster (Bennett et al., 1986). I_K and I_{Ca} were sampled at 167 Hz and 5 kHz, respectively, and filtered at 50 Hz and 2 kHz. Capacity transients were removed using an analogue blanking circuit. For the dialysis experiments cAMP (50 μM) and CS (1 μM) were added directly to the patch pipette. After disruption of the cell membrane the chemicals move into the cell by diffusion. Using a simple compartmental model, Kameyama et al. (1985) have predicted that substances like GTP and cAMP (molecular masses, 400–500 D, diffusion coefficients, $\sim 5 \times 10^{-6}$ cm^2/s) reach 90% of the pipette concentration in ~5 min. However, during the first minute the intracellular concentration of these compounds is <5% of the pipette concentration, thus allowing the measurement of control currents. A large molecular weight substance such as CS (molecular mass, ~40,000 D) would be expected to reach equilibrium at a much slower rate (6–10 min).

Spontaneous rundown of membrane currents measured with the whole-cell arrangement of the patch clamp is a problem that can introduce errors in the interpretation of our experimental results, particularly if the rundown rate changes with temperature. We therefore carried out control experiments designed to monitor the stability of I_K and I_{Ca} in the absence of β-adrenergic stimulation over the temperature range of our experiments. Under the conditions used in these studies we observed minimal changes in the amplitude of I_K and I_{Ca} over the normal time course (14–15 min) of our experiments and no difference between rundown at room temperature and at 32°C. In five control experiments performed at room temperature (22°C), the fractional amplitude of I_K declined to 0.94 ± 0.05 (mean ± SE). This was not statistically different ($P > 0.3$ using an independent t test) from the fractional change (1.00 ± 0.03) observed in I_K at 32°C. Over the same time period, I_{Ca} declined by 0.87 ± 0.04 ($n = 4$) at 22°C and 0.88 ± 0.03 ($n = 2$) at 32°C. Thus the temperature-dependent modulation reported in this study should be minimally affected by current rundown.

Experimental Design

Fig. 1 briefly summarizes the β-receptor/cAMP-dependent protein kinase pathway and outlines the experimental approach we used to probe temperature-dependent regulation of I_K and I_{Ca} at different steps in this pathway. The β-agonist isoproterenol and the diterpene compound forskolin were chosen to directly stimulate the membrane-bound β-receptor and the enzyme adenylate cyclase, respectively. To enhance the release of CS from the protein kinase holoenzyme, we increased intracellular levels of cAMP either by direct dialysis of this second messenger or by external application of CPT-cAMP. Finally, to determine if temperature sensitivity arises at the level of the channel proteins, cells were dialyzed with CS.

RESULTS

Temperature-dependent Regulation of I_K in the Same Cell

Fig. 2 illustrates the results of an experiment in which we examined the temperature dependence of I_K modulation by isoproterenol in a single cell. Addition of isoproterenol caused almost no change in the amplitude of the I_K tail recorded at 21°C (Fig. 2 *A*). Isoproterenol was then quickly washed out of the recording chamber and

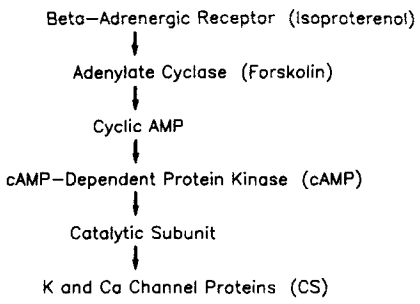

FIGURE 1. Summary of β-receptor/cAMP-dependent protein kinase pathway and an outline of our experimental approach. In this cascade, binding of an agonist such as isoproterenol to the β-receptor stimulates the enzyme adenylate cyclase, which leads to an increase in intracellular levels of cAMP. Binding of cAMP to the regulatory subunit of the protein kinase causes a release of CS, which is then free to phosphorylate ion channel proteins. To probe the temperature dependence at different levels, the β-agonist isoproterenol and the diterpene compound forskolin were chosen to directly stimulate the membrane-bound β-receptor and the enzyme adenylate cyclase, respectively. Dialysis with cAMP and CS, as well as exposure to CPT-cAMP, were used to enhance the release of CS from the protein kinase holoenzyme, and to produce direct phosphorylation of the channel proteins, respectively.

the temperature of the bath was warmed to 32°C. The temperature change affected both the amplitude and decay rate of the I_K tail. The amplitude of the tail was increased by a factor of 3.8, and the time constant of decay decreased from 2.03 s at 22°C to 680 ms at 32°C. The increase in amplitude was due in part to the more rapid kinetics of I_K during the 1.5-s activation pulse, but both changes represent large temperature coefficients (Q_{10}'s) (see Discussion). Application of isoproterenol

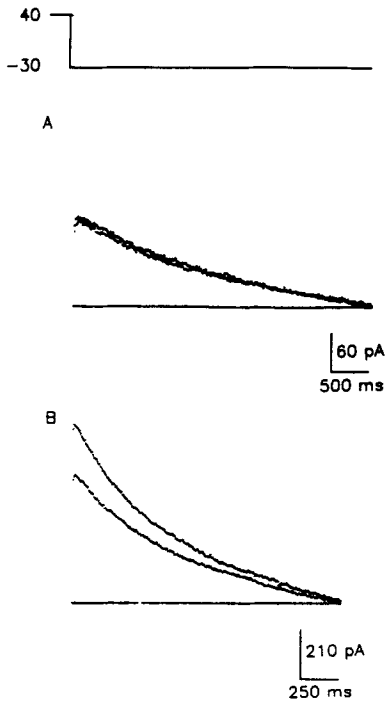

FIGURE 2. Temperature-dependent regulation of I_K in a single cell. I_K tails recorded upon return to the holding potential of −30 mV after a 1.5-s voltage step to +40 mV. Each panel shows currents recorded from the same cell in the presence and absence of 1 μM isoproterenol at 22°C (*A*) and 32°C (*B*). Addition of 1 μM isoproterenol resulted in a fractional increase in I_K of 1.04 and 22°C and 1.38 at 32°C. Measurements were first carried out in the absence and presence of isoproterenol at 22°C, and then repeated after washout of the agonist at 32°C. Cell WGJ.

at 32°C resulted in an additional increase in I_K. The I_K tail amplitude was now enhanced by a factor of 1.38 (Fig. 2 *B*). Thus, stimulation of the β-receptor by isoproterenol resulted in a temperature-dependent increase in I_K. The effects of isoproterenol on I_K tail kinetics are described later in this paper.

In the following sections of this paper we examined the temperature-dependent regulation of I_K at various steps in the β-receptor/cAMP-dependent protein kinase pathway and compared this with regulation of I_{Ca}.

Modulation of I_K, but Not of I_{Ca}, by Isoproterenol Is Temperature Dependent

β-Receptor–mediated increases in I_K, but not in I_{Ca}, were found to be very temperature dependent over the range of 20–37°C. In Fig. 3 the fractional current change in the amplitude of I_K tails after exposure to 1 μM isoproterenol is plotted as a function of the temperature of the recording chamber. The inset shows examples of I_{Ca} and I_K recorded at 22 and 36°C in the absence and presence of isoproterenol. In eight experiments at temperatures between 30 and 32°C, I_K in the presence of agonist was 1.51 ± 0.05 (mean ± SE) times the current without agonist. This same treatment at 20–22°C produced a significantly smaller change in the amplitude of I_K to 1.02 ± 0.02 ($n = 8$). At a warmer temperature range (34–37°C) even larger increases in I_K were produced by isoproterenol. The fractional increase of 2.5 obtained at 37°C approaches that previously obtained in multicellular Purkinje fibers exposed to 500 nM norepinephrine at this temperature, but is somewhat lower than the maximal response in those preparations (Bennett et al., 1986). In contrast to the results with I_K, the increase in I_{Ca} brought on by isoproterenol was independent of temperature: there was an average increase at 20–22°C of 2.4 ± 0.2 ($n = 5$) and 2.7 ± 0.4 ($n = 5$) at 30–36°C. Exposure to the specific β-antagonist propranolol prevented the enhancement of both I_K and I_{Ca} produced by isoproterenol.

Temperature-dependent Increase in I_K during Direct Adenylate Cyclase Stimulation

The separation of agonist-induced modulation of I_K and I_{Ca} by temperature suggests some difference in the underlying regulatory mechanisms. We used forskolin (1 μM) to directly activate adenylate cyclase (Seamon and Daly, 1985). This eliminated the agonist-receptor binding step and the subsequent coupling of this binding step to stimulation of the enzyme adenylate cyclase as possible sites for temperature-dependent regulation. As was the case with isoproterenol, forskolin enhanced I_{Ca} at both 22 and 30°C, but I_K was increased only at the higher temperature (Fig. 4, Table I). Even with forskolin concentrations as high as 50 μM, no significant increase in I_K could be observed at 22°C, despite the fact that there was a maximal enhancement in I_{Ca}. In fact, in most experiments at this temperature, there was a forskolin-induced reduction of I_K (Table I). Overall, these results demonstrate that enhancement of I_K by forskolin is pronounced at warm temperatures, and suggest that the temperature-dependent step in the regulation of I_K occurs after elevation of intracellular cAMP.

To clarify the effects of forskolin at various temperatures we used two analogues of this compound. The water-soluble analogue of forskolin (7-deaceyl-7-[4-methylpiperazine]-butyryl-forskolin) produced the same temperature-dependent action on I_K

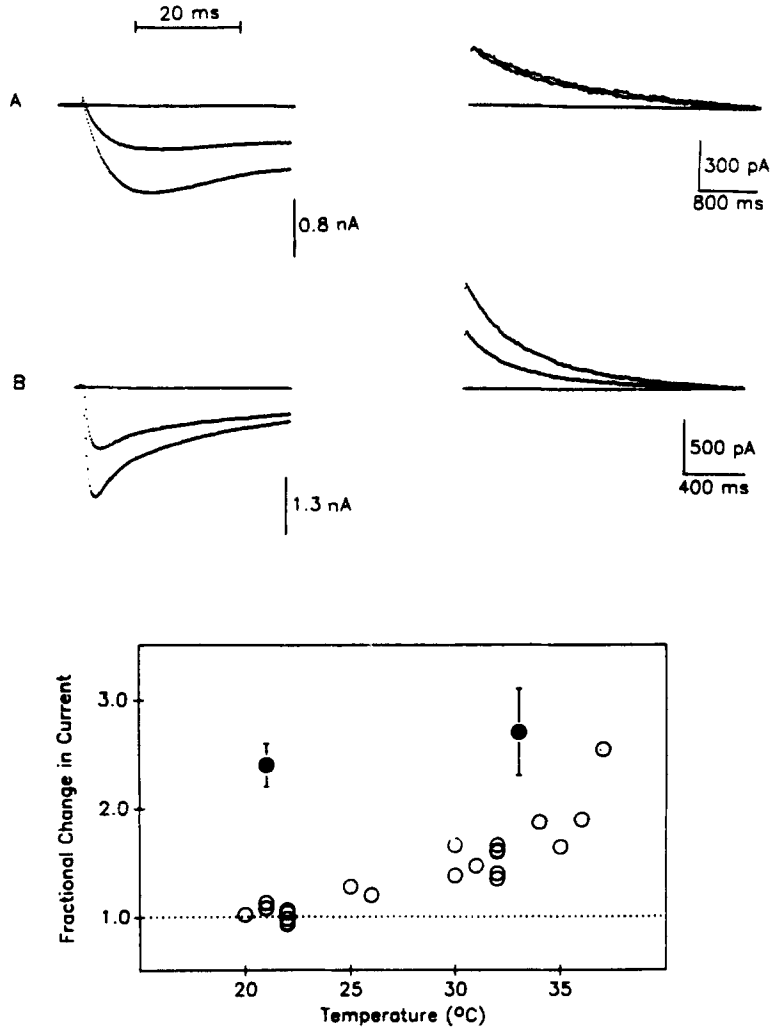

FIGURE 3. Isoproterenol-induced enhancement of I_K, but not I_{Ca}, is temperature dependent. Fractional change in current amplitude plotted vs. the temperature of the recording chamber for I_K tails (open circles) and I_{Ca} (filled circles). (*Inset, left*) I_{Ca} recorded during a 40-ms voltage step to 0 mV. (*Inset, right*) I_K recorded after a 1.5-s voltage step to +40 mV. The holding potential was −30 mV. Each pair of records shows currents obtained before and after addition of 1 μM isoproterenol. (*A*) Recordings obtained at 22°C in cell WH. (*B*) Currents obtained from cell WEG at 36°C. Time-independent currents have been subtracted in these and other records.

as those caused by forskolin dissolved in dimethyl sulfoxide (Fig. 5 *A*). This result provided evidence against a solvent-induced increase in this ionic current, but it did not explain the reduction of current observed at the lower temperature range. To test for direct actions of forskolin on I_K that may occur in addition to stimulating adenylate cyclase, we used an analogue of forskolin (1,9-dideoxy forskolin), that does not activate adenylate cyclase (Seamon and Daly, 1985). This analogue failed to

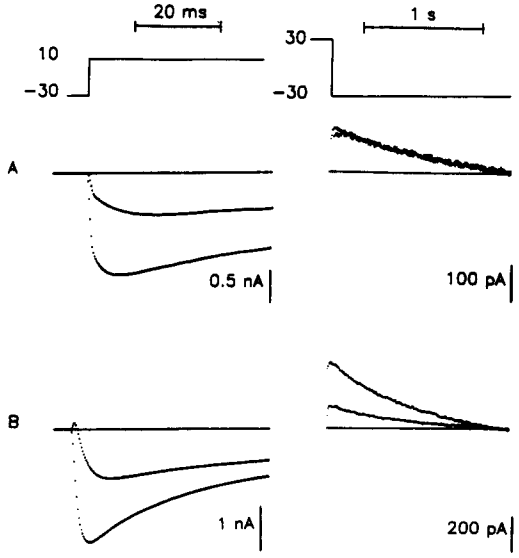

FIGURE 4. Increase in I_K during direct adenylate cyclase stimulation is temperature dependent. I_{Ca} recorded during a 40-ms voltage step to +10 mV (*left*) and I_K recorded after a 1.5-s voltage step to +30 mV (*right*). The holding potential was −30 mV. Each pair of records shows currents obtained before and after the addition of 1 μM forskolin (in 0.2% dimethyl sulfoxide). (*A*) Currents recorded at 22°C. Cell WB11. (*B*) Currents recorded at 30°C. Cell WC1.

enhance I_{Ca} at 32°C, which indicates that this compound did not stimulate adenylate cyclase. However, as shown in Fig. 5 *B*, a 20-μM concentration of 1,9-dideoxy forskolin reduced the amplitude of I_K by 0.56. A similar reduction in I_K was observed with both forskolin and 1,9-dideoxy forskolin at room temperature (see Fig. 5 *A* and Table I). Thus, in addition to stimulating adenylate cyclase, forskolin may produce a partial block of the I_K channel.

Temperature-dependent Modulation of I_K Is Mediated through cAMP

Temperature sensitivity might arise during stimulation of adenylate cyclase. To bypass this step we added cAMP to our patch electrodes and directly injected this

TABLE I

*Summary of Changes in I_K and I_{Ca}**

Condition	Fractional change‡			
	I_K		I_{Ca}	
	19–22°C	28–32°C	19–22°C	28–32°C
1–10 μM forskolin§	0.59 ± 0.06 (n = 4)	2.08 ± 0.29 (n = 4)	2.27 ± 0.17 (n = 3)	2.36 ± 0.40 (n = 3)
50 μM cAMP	1.04 ± 0.06 (n = 4)	1.34 ± 0.06 (n = 4)	1.80 ± 0.32 (n = 4)	1.67 ± 0.36 (n = 3)
1 μM CS	1.40 ± 0.04 (n = 3)	2.49 ± 0.36 (n = 3)	1.65 ± 0.31 (n = 3)	1.40 ± 0.09 (n = 3)

*All values represent the mean ± SE. I_K tails were measured on return to −30 mV after a 1.5-s voltage step to 40 mV. I_{Ca} was recorded during a 40-ms voltage step to 0 mV.

‡Under each condition used, increases in I_K occurring at warm temperatures were found to be statistically different ($P < 0.05$) from those obtained at room temperature using an independent t test. In contrast, there was no significant change in I_{Ca} over the two temperature ranges ($P > 0.5$).

§The decrease in I_K produced by forskolin at room temperature may result from a direct action on I_K channels (Hoshi et al., 1988; Perozo and Bezanilla, 1988).

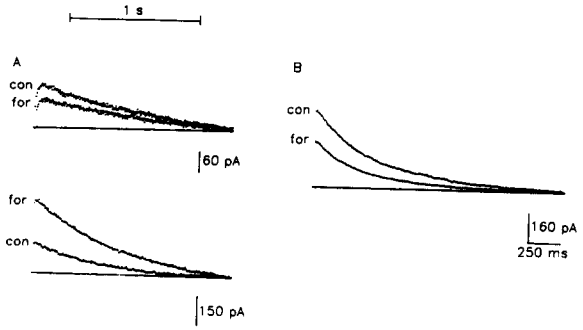

FIGURE 5. Temperature-dependent actions of forskolin analogues on I_K. I_K recorded after a 1.5-s voltage step to +30 mV. (*A*) Currents obtained in the presence and absence of 50 μM of the water-soluble analogue of forskolin (7-deaceyl-7-[methylpiperazine]-butyryl-forskolin) at 20°C (*top*) and 32°C (*bottom*). At 20°C forskolin reduced the fractional current amplitude of I_K to 0.68, but increased I_K by 2.42 at 32°C. Cells WH5 and WF5. (*B*) Currents obtained before and after the addition of 20 μM of the inactive analogue of forskolin (1,9-dideoxy forskolin) at 32°C. 1,9-dideoxy reduced I_K by 0.56 in this experiment. Con, control; for, forskolin. Cell WGM.

second messenger into the myocytes. The effect of internal dialysis of cAMP on I_{Ca} at 22°C is illustrated in Fig. 6 *A*. Currents were recorded at various times after the rupture of the cell membrane by the tip of a pipette containing 50 μM cAMP. As reported in previous studies, introduction of exogenous cAMP produced a rapid increase in I_{Ca} (Kameyama et al., 1985), which in this case reached a peak effect within 4–5 min. A similar enhancement of I_{Ca} was also obtained at 32°C (see Table I).

Fig. 6 *B* shows I_K tails obtained at 22 and 32°C, both before and during the peak enhancement of I_{Ca} after application of cAMP. In contrast to the results with I_{Ca}, augmentation of I_K occurred only at the warmer temperature (see also Table I). Enhancement of I_K and I_{Ca} by cAMP at 32°C followed a similar time course.

One possible explanation for the lack of effect of cAMP on I_K at room temperature could be that the onset of the modulation of the current occurs so quickly, that the current is fully enhanced by the time the first record is obtained. This might also

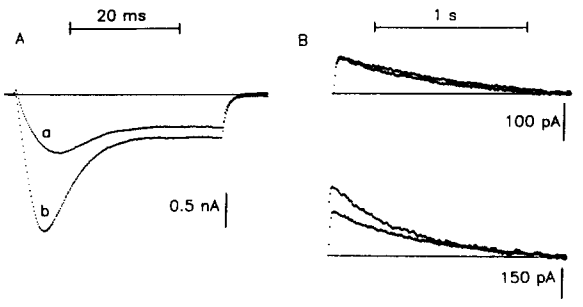

FIGURE 6. Temperature-dependent modulation of I_K is mediated through cAMP. (*A*) I_{Ca} recorded at 22°C during a 40-ms voltage step to −10 mV. Records were obtained during the first (*a*) and fifth (*b*) minute after rupture of the cell membrane by a patch electrode containing 50 μM cAMP. Cell WI5. (*B*) I_K recorded after a 1.5-s voltage step to +40 mV at 22°C (*top*) and 32°C (*bottom*) both before and during the peak enhancement of I_{Ca} by cAMP. Enhancement of I_{Ca} and I_K by cAMP at 32°C followed a similar time course. Cells WI7 and WG4. The holding potential in these experiments was −30 mV.

explain the small changes observed in I_K and I_{Ca} in the presence of cAMP as compared with those recorded with isoproterenol and forskolin. To eliminate this possibility we studied the action of external application of CPT-cAMP, a membrane-soluble cAMP analogue. Although we observed little change in I_K at 21°C, CPT-cAMP produced a consistent increase (50–100%) in I_K at 32°C (see Fig. 8 *B*). From these results, we concluded that the temperature-dependent step in the modulation of I_K occurs after agonist-induced elevation of cellular cAMP.

Temperature-dependent Regulation of I_K Is Mediated through CS

Differential regulation of I_K and I_{Ca} could result if these channels are phosphorylated by two different protein kinases. Indeed, two isozymes of cAMP-dependent protein kinase with different regulatory, but identical catalytic, subunits have been isolated from heart (Hofmann et al., 1975; Corbin and Keely, 1977). If binding of

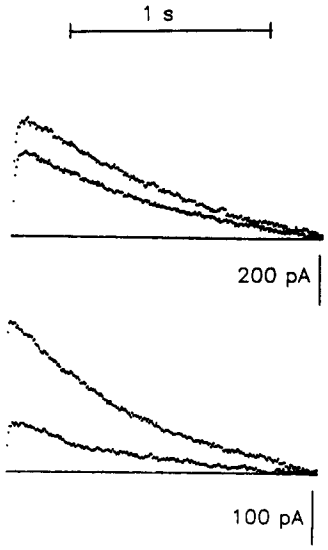

FIGURE 7. Temperature-dependent regulation of I_K is mediated through the catalytic subunit of cAMP-dependent protein kinase. I_K recorded after a 1.5-s voltage step to +40 mV at 22°C (*top*) and 32°C (*bottom*). The holding potential was −30 mV. The first record in each pair was recorded during the first minute after rupture of the cell membrane by a patch electrode containing 1 μM CS. The second record demonstrates the peak enhancement of I_K obtained during the 10th (*bottom*) and 12th (*top*) min after breakthrough. Cells WJ7 and WJ2.

cAMP to the regulatory subunit of the protein kinase involved in regulating I_K was temperature dependent, this might account for our results. To answer this question we dialyzed the cells with CS. A lack of a temperature-dependent effect of CS on I_K would lend support to the hypothesis that I_K and I_{Ca} are regulated by two different kinases. As can be seen in Fig. 7, enhancement of I_K by CS was much more pronounced at warmer temperatures. However, in contrast to our results with isoproterenol, forskolin, and cAMP, small but consistent increases in I_K were obtained at 22°C in the presence of the CS (Table I). In addition, enhancement of I_K by CS at warm temperatures was far more pronounced than that observed in I_{Ca} at either temperature (Table I).

Kinetic Changes in I_K Are Similar at Each Stage of β-Adrenergic Regulation

Stimulation of β-receptors by norepinephrine has been shown to alter I_K kinetics in intact Purkinje fiber cells (Bennett et al., 1986). We were interested in determining

whether similar kinetic changes occurred in isolated myocytes and whether differences in kinetic modulation could be detected at distinct levels in the β-receptor/cAMP-dependent protein kinase pathway.

Fig. 8 shows kinetic changes in I_K tails caused by exposure of a cell to isoproterenol (*A*), CPT-cAMP (*B*), and during internal dialysis with CS (*C*). Deactivation of I_K was slowed by each agent. For the experiment plotted in Fig. 8 *A*, the time constant of decay (τ), derived from single-exponential fits to the I_K tails, increased from 584 to 647 ms in the presence of isoproterenol. This was equal to a fractional increase of 1.10 and was close to the mean change observed with isoproterenol

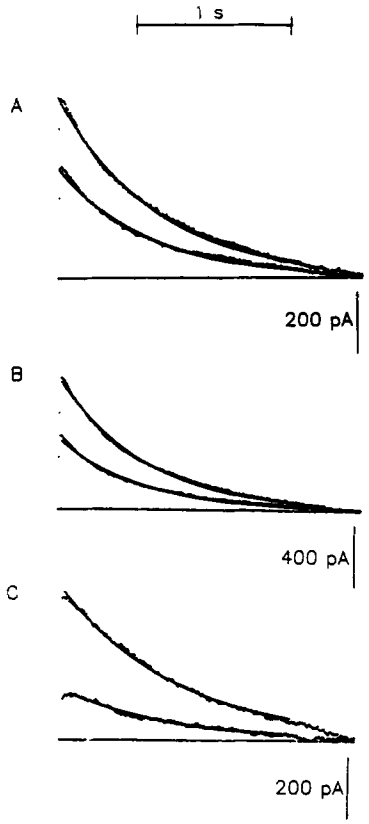

FIGURE 8. β-Adrenergic stimulation causes a slowing in the decay of I_K. Records obtained at 32°C in the presence and absence of (*A*) isoproterenol (*B*) CPT-cAMP, (*C*) or during dialysis of CS. I_K recorded after a 1.5-s voltage step to +40 mV. Superimposed on the raw data are single exponential fits obtained using a nonlinear fitting algorithm called "simplex" (Caceci and Cacheris, 1984). The time constant of decay (τ), increased from 584 to 647 ms in the presence of 1 μM isoproterenol. Cell WE6. After addition of 150 μM CPT-cAMP τ increased from 515 to 549 ms. Cell WDM4. Dialysis with CS increased τ from 544 to 606 ms. Cell WJ7.

(mean ± SE = 1.08 ± 0.05; n = 7). Modulation of I_K by CPT-cAMP and by CS produced similar effects on I_K kinetics. Overall in the presence of forskolin, cAMP, and CS, τ increased by 1.22 ± 0.03 (n = 4), 1.18 ± 0.02 (n = 4), and 1.22 ± 0.04 (n = 4), respectively. These results suggest that the primary step in modulation of I_K is activation of CS.

DISCUSSION

Temperature-dependent Regulation of I_K during β-Adrenergic Stimulation

The principal finding of this study is that treatment of isolated ventricular myocytes with agents that produce increases in intracellular levels of CS, enhanced I_K, but not

I_{Ca}, in a temperature-dependent fashion. The enhancement of I_K that we observed in the presence of isoproterenol is in agreement with previous studies of adrenergic regulation of I_K in Purkinje fiber preparations (Tsein et al., 1972; Bennett et al., 1986), but contrasts with the results of Hume (1985), who reported that epinephrine and isoproterenol produce large increases in I_{Ca}, but have no detectable effect on I_K in isolated frog atrial cells at room temperature. In light of the present studies, it is reasonable to speculate that adrenergic modulation of I_K channels in frog atrial cells may also occur only at warm temperatures. Alternatively, I_K channels in atrial cells, unlike those in ventricular cells, may be unresponsive to adrenergic stimulation.

The observation that temperature-dependent regulation of I_K occurred in the presence of CS implies that temperature dependence arises at some step either preceding, during, or following the CS-induced phosphorylation of the I_K channel protein(s). A similar temperature-dependent step does not appear to be involved in the β-adrenergic regulation of I_{Ca}, which suggests that cardiac K and Ca channels may be under different mechanistic regulation. There are a number of mechanisms that could account for this temperature-dependent regulation of I_K. Binding of CS to the phosphorylation site on the I_K channel may be physically restricted at room temperature. Accessibility of this site for CS may increase at warm temperatures after a temperature-dependent conformational change in the channel protein(s). Alternatively, a transition phase in the membrane lipids might have to be reached to expose this site. Another possible mechanism consistent with our results is that phosphorylation of the I_K channel occurs at both temperatures, but increased K channel activity results from this phosphorylation is expressed to a greater extent at warm temperatures. For example, if β-adrenergic stimulation results in an increase in the number of functional K channels, it might be imagined that activation of these extra channels is temperature dependent. In this case the process linking phosphorylation with an increase in active channels is temperature dependent. Finally, I_K channels might be both phosphorylated and active at both temperatures but dephosphorylation of the channel by a cellular phosphatase may be more dominant at room temperature.

Our results clearly show that enhancement of I_K in guinea pig ventricular cells occurs in a temperature-dependent manner at the level of CS. Thus, it is clear that this potassium channel is not primarily activated by a regulatory protein as is the case for muscarinic potassium channels in atrial cells (Codina et al., 1987; Logothetis et al., 1987; Yatani et al., 1987), but instead is modulated via the cAMP second-messenger system. However, a secondary role for GTP-binding proteins (G-proteins) in this regulation can not yet be ruled out since Yatani et al., (1987) have reported that purified G_S, the G-protein involved in activating adenylate cyclase, can increase Ca channel activity in isolated cardiac myocytes.

Kinetic Changes in I_K

In addition to studying changes in the amplitude of I_K, we also examined the kinetic effects of β-adrenergic regulation of I_K. Enhancement of I_K by isoproterenol, forskolin, cAMP, or CS was accompanied by a slowing in the rate of tail decay measured at the −30 mV holding potential. The agents that produced the largest increases in the amplitude of I_K (forskolin and CS) also caused the greatest slowing

in the kinetics. These results demonstrate that stimulation at any level in the β-receptor/cAMP-dependent protein kinase pathway produces a similar change in the kinetic properties of the I_K channel, which further supports the view that the principal regulatory step for I_K occurs via cAMP-dependent protein kinase.

In multicellular Purkinje fiber preparations, norepinephrine-induced changes in I_K kinetics have been reported over a broad voltage range (Bennett et al., 1986). In the Purkinje fiber, I_K tails are best described by the sum of two exponentials at most voltages, and norepinephrine increases or diminishes the slower time constant depending upon the recording voltage (Bennett et al., 1986). Our results, obtained over a limited voltage range, indicate that the decay of I_K in isolated guinea pig myocytes is well described by a single exponential process at −30 mV, and that β-stimulation prolongs the time course of this process. More data are needed over a wider voltage range to determine whether I_K is fundamentally different in these two cell types.

In the absence of stimulation of the β-receptor/cAMP-dependent protein kinase pathway, changes in temperature have marked effects on both I_K and I_{Ca}. Cavalie et al. (1985) previously investigated temperature-induced changes in I_{Ca} in guinea pig myocytes and reported Q_{10}'s of 2.87 and 2.52 for the amplitude and time to peak, respectively. These investigators suggested that the high temperature coefficients obtained for I_{Ca} are a reflection of possible metabolic regulation of calcium channel function and availability. We observed similar temperature-induced changes in I_{Ca} properties. In nine experiments, the time to peak (measured between −10 and +10 mV) changed from 11.7 ± 1.2 ms to 6.25 ± 0.7 ms (mean ± SE), values which are within the range of times expected for Ca channel activation at these voltages (Lux and Brown, 1984).

The amplitude and kinetics of I_K were found to have a similar strong dependence on temperature. For the experiment conducted in the same cell (Fig. 2), the time constant for deactivation at −30 mV decreased by a factor of 0.33 and the tail amplitude increased by a factor of 3.8 when the temperature was changed from 22 to 32°C. On average, the time constant for deactivation at −30 mV was 562 ± 37 ms at 30–36°C (n = 9), and 1.1 ± 0.2 s at 22°C (n = 8). The average tail amplitude was 138 ± 30 pA at 22°C and 290 ± 38 pA at 30–36°C (n = 9). Although part of the increase in tail amplitude was due to the faster activation kinetics, and thus to the greater number of channels opened during the conditioning pulse, it is clear that both I_K amplitude and kinetics have Q_{10}'s >2. This provides additional evidence that I_K activity is coupled to metabolic regulation, and suggests the possibility of a basal level of regulatory control.

Actions of Forskolin Analogues on I_K

In Table I we have summarized our results with forskolin, cAMP, and CS. In addition to the clear temperature dependence of each of these agents on I_K, a number of other observations warrant discussion. At room temperature (19–22°C), forskolin produced a reduction in the amplitude of I_K. A similar reduction in potassium currents by forskolin has been reported by Perozo and Bezanilla (1988) in squid axons and Hoshi et al. (1988) in pheochromocytoma cells. Since these effects also developed in the presence of forskolin analogues such as 1,9-dideoxy forskolin, which do

not stimulate adenylate cyclase, it has been suggested that the reductions observed may result from a direct action of this compound on the potassium channels. We have also found that 1,9-dideoxy forskolin reduces I_K at both 22 and 32°C. Thus, the enhancement of I_K that occurs during the forskolin-induced stimulation of adenylate cyclase at warm temperatures, may be an underestimate of the full adrenergic effect.

Additional Differences in the Regulation of I_K and I_{Ca}

Another important observation concerns the effect of CS dialysis on I_K and I_{Ca}. Our results in Table I may suggest that I_K is more sensitive to modulation by internally applied CS than I_{Ca}, since we observed large increases in I_K with a concentration of CS that produced minimal changes in I_{Ca}. Consistent with this finding, Kameyama et al., (1985) have reported that CS enhances I_{Ca} in guinea pig ventricular cells with an EC_{50} of 3–4 μM, a concentration approximately three to four times higher than that used in our study. However, the greater sensitivity of I_K to CS must be reconciled with our finding that modulation produced by isoproterenol, forskolin, and cAMP, which all ultimately act by increasing intracellular levels of CS, was more effective in enhancing I_{Ca}.

In addition to the independent regulation of ventricular Ca and K channels observed in this study during β-adrenergic stimulation, I_{Ca} and I_K may be differentially regulated by other cellular enzymes. We have recently observed that the phorbol ester 12,13 phorbol dibutyrate, a potent activator of protein kinase C, enhances the amplitude of I_K in heart cells at concentrations that have little effect on I_{Ca} (Walsh et al., 1988*b*). Tohse et al., (1987) have reported a similar enhancement in I_K using the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate. This may suggest that cardiac I_K channels, but not I_{Ca} channels, can be regulated by a diverse group of phosphorylating enzymes.

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