β-Adrenergic Modulation of Cardiac Ion Channels

Differential Temperature Sensitivity of Potassium and Calcium Currents

KENNETH B. WALSH, TED B. BEGENISICH, and ROBERT S. KASS
From the University of Rochester, School of Medicine and Dentistry, Department of Physiology, Rochester, New York 14642

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-chlorophenylthio 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...
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; Bruin 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-chlorophenylthio (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., 1988a, 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 MgCl₂, 1.0 CaCl₂, 5 dextrose, 5 HEPES, and 5 µM tetrodotoxin, pH 7.4. Electrodes (2–5 MΩ) contained (in millimolar): 50 KCl, 50 K-glutamate, 2 MgCl₂, 1 CaCl₂, 11 EGTA, 3 ATP, and 10 HEPES. This ratio of EGTA/CaCl₂ 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-chlorophenylthio cAMP was purchased from Boehringer Mannheim, Inc. (Indianapolis, IN) and the water-soluble (7-deacetyl-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, ~5 x 10^{-6} cm²/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_{Ca} 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
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
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. 2B). 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 $I_C$, is temperature dependent. Fractional change in current amplitude plotted vs. the temperature of the recording chamber for $I_K$ tails (open circles) and $I_C$ (filled circles). (Inset, left) $I_C$ 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
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

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

<sup>1</sup>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.

<sup>2</sup>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 ($P > 0.5$).

<sup>3</sup>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).
second messenger into the myocytes. The effect of internal dialysis of cAMP on \( I_{\text{Ca}} \) 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 \( I_{\text{Ca}} \) (Kameyama et al., 1985), which in this case reached a peak effect within 4–5 min. A similar enhancement of \( I_{\text{Ca}} \) 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 \( I_{\text{Ca}} \) after application of cAMP. In contrast to the results with \( I_{\text{Ca}} \), augmentation of \( I_k \) occurred only at the warmer temperature (see also Table I). Enhancement of \( I_k \) and \( I_{\text{Ca}} \) 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

both before and during the peak enhancement of \( I_{\text{Ca}} \) by cAMP. Enhancement of \( I_{\text{Ca}} \) and \( I_k \) by cAMP at 32°C followed a similar time course. Cells W17 and WG4. The holding potential in these experiments was \(-30 \text{ mV}\).
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 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 ($\tau$), 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](image)

(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, $\tau$ 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$_{\text{Ca}}$, 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$_{\text{Ca}}$, 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 $\beta$-adrenergic regulation of I$_{\text{Ca}}$, 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 $\beta$-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 Gs, 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 $\beta$-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 \( \beta \)-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 \( \beta \)-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 \( \beta \)-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 \( 11.7 \pm 1.2 \) ms to \( 6.25 \pm 0.7 \) ms (mean \pm 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 \( 32^\circ\)C. On average, the time constant for deactivation at \(-30\) mV was \( 562 \pm 37 \) ms at \( 30-36^\circ\)C (\( n = 9 \)), and \( 1.1 \pm 0.2 \) s at \( 22^\circ\)C (\( n = 8 \)). The average tail amplitude was \( 138 \pm 30 \) pA at \( 22^\circ\)C and \( 290 \pm 38 \) pA at \( 30-36^\circ\)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 adenylyl 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 adenylyl 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 EC50 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). 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.

Original version received 21 March 1988 and accepted version received 27 October 1988.

REFERENCES

Bennett, P., L. McKinney, T. Begenisich, and R. S. Kass. 1986. Adrenergic modulation of the delayed rectifier potassium channel in calf cardiac Purkinje fibers. Biophysical Journal. 49:839-848.

Brum, G., W. Osterrieder, and W. Trautwein. 1984. β-adrenergic increase in the calcium conductance of cardiac myocytes studied with the patch clamp. Pflügers Archiv. 401:111-118.

Caceci, M. S., and W. P. Cacheris. 1984. Fitting curves to data. The simplex algorithm is the answer. Byte. 9:340.

Cachelin, A. B., J. E. de Peyer, S. Kokubun, and H. Reuter. 1983. Ca channel modulation by 8-bromocyclic AMP in cultured heart cells. Nature. 304:462-464.

Cavalie, A., T. F. McDonald, D. Pelzer, and W. Trautwein. 1985. Temperature-induced transitory and steady-state changes in the calcium current of guinea pig ventricular myocytes. Pflügers Archiv. 405:294-296.

Codina, J., Y. Atsuko, D. Grenet, A. M. Brown, and L. Birnbaumer. 1987. The α subunit of the GTP binding protein $G_{s}$ opens atrial potassium channels. Science. 236:442-445.

Corbin, J. D., and S. L. Keely. 1977. Characterization and regulation of heart adenosine 3′:5′-
monophosphate-dependent protein kinase isozymes. *Journal of Biological Chemistry.* 252:910–918.

Goldstein, D. A. 1979. Calculation of the concentrations of free cations and cation-ligand complexes in solutions containing multiple divalent cations and ligands. *Biophysical Journal.* 26:335–42.

Hamill, O. E., A. Marty, E. Neher, B. Sakmann, and J. Sigworth. 1981. Improved patch-clamp techniques for high resolution current recordings from cells and cell-free membrane patches. *Pflügers Archiv.* 391:85–100.

Hofmann, F., J. A. Beavo, P. J. Bechtel, and E. G. Krebs. 1975. Comparison of adenosine 3':5'-monophosphate-dependent protein kinase from rabbit skeletal and bovine heart muscle. *Journal of Biological Chemistry.* 250:7795–7801.

Hoshi, T., S. S. Garber, and R. W. Aldrich. 1988. Direct effect of forskolin on voltage-gated potassium channels in PC12 cells. *Biophysical Journal.* 53:144a. (Abstr.)

Hume, J. R. 1985. Do catecholamines directly modulate the delayed plateau potassium current in frog atrium? *Journal of Molecular and Cellular Cardiology.* 17:813–816.

Kameyama, M., F. Hofmann, and W. Trautwein. 1985. On the mechanism of β-adrenergic regulation of the Ca channel in the guinea-pig heart. *Pflügers Archiv.* 405:285–293.

Logothetis, D. E., Y. Kurachi, J. Galper, E. J. Neer, and D. E. Clapham. 1987. The βi subunits of GTP-binding proteins activate the muscarinic K channel in heart. *Nature.* 325:321–326.

Lux, H. D., and A. M. Brown. 1984. Patch and whole cell calcium currents recorded simultaneously in snail neurons. *Journal of General Physiology.* 83:727–750.

Mitra, R., and M. Morad. 1985. A uniform enzymatic method for dissociation of myocytes from hearts and stomachs of vertebrates. *American Journal of Physiology.* 249:H1056–1060.

Perozo, E., and F. Bezanilla. 1988. Forskolin blocks K-current in squid axons independently of cAMP/ATP levels. *Biophysical Journal.* 53:543a. (Abstr.)

Reuter, H. 1983. Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature.* 301:569–574.

Seamon, K. B., and J. W. Daly. 1985. High-affinity binding for forskolin to rat brain membranes. *Advances in Cyclic Nucleotide Research.* 19:125–135.

Tohse, N., M. Kameyama, and H. Irisawa. 1987. Intracellular Ca and protein kinase C modulate K current in guinea pig heart cells. *American Journal of Physiology.* 253:H1321–H1324.

Tsien, R. W., W. Giles, and P. Greengard. 1972. Cyclic AMP mediates the effects of adrenaline on cardiac Purkinje fibres. *Nature.* 240:181–183.

Walsh, K. B., T. B. Begenisich, and R. S. Kass. 1988a. β-Adrenergic modulation in the heart: independent regulation of K and Ca channels. *Pflügers Archiv.* 411:232–234.

Walsh, K. B., T. B. Begenisich, and R. S. Kass. 1988b. β-Adrenergic modulation in the heart: evidence for independent regulation of K and Ca channels. *Biophysical Journal.* 53:460a. (Abstr.)

Yatani, A., J. Codina, A. M. Brown, and L. Birnbaumer. 1987. Direct activation of mammalian atrial muscarinic potassium channels by GTP regulatory protein Gi. *Science.* 235:207–211.