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Potentiation of slow component of delayed rectifier K^+ current by cGMP *via* two distinct mechanisms: inhibition of phosphodiesterase 3 and activation of protein kinase G

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1 Regulation of the slowly activating component of delayed rectifier K^+ current (I_{Ks}) by intracellular guanosine 3′5′ cyclic monophosphate (cGMP) was investigated in guinea-pig sino-atrial (SA) node cells using the whole-cell patch-clamp method.

2 When a cell was dialyzed with pipette solution containing 100 μM cGMP, I_{Ks} started to gradually increase and reached a maximum increase of a factor of 2.37 ± 0.39 ($n=4$) about 10–15 min after rupture of patch membrane. Atrial natriuretic peptide (ANP, 100 nM) also potentiated I_{Ks}, consistent with intracellular cGMP-induced enhancement of I_{Ks}.

3 Bath application of a selective blocker of the cGMP-inhibited phosphodiesterase (PDE3) milrinone (100 μM) enhanced I_{Ks} by a factor of 1.50 ± 0.09 ($n=4$) but failed to further enhance I_{Ks} after a maximum stimulation by intracellular cGMP (100 μM), suggesting that blockade of PDE3 activity is involved in the enhancement of I_{Ks}. A potent but nonspecific PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX, 100 μM) further increased I_{Ks} stimulated by 100 μM milrinone, indicating that PDE subtypes other than PDE3 are also involved in the regulation of basal I_{Ks} in guinea-pig SA node cells.

4 Bath application of 100 μM 8-bromoguanosine 3′5′ cyclic monophosphate (8-Br-cGMP) increased I_{Ks} by a factor of 1.48 ± 0.11 ($n=5$) and this stimulatory effect was totally abolished by cGMP-dependent protein kinase (PKG) inhibitor KT-5823 (500 nM), suggesting that the activation of PKG also mediates cGMP-induced potentiation of I_{Ks}.

5 These results strongly suggest that intracellular cGMP potentiates I_{Ks} not only by blocking PDE3 but also by activating PKG in guinea-pig SA node cells.

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Abbreviations: AC, adenylyl cyclase, ANP, atrial natriuretic peptide; 8-Br-cGMP, 8-bromoguanosine 3′5′ cyclic monophosphate; 293B, trans-6-cyano-4-(*N*-ethylsulphonyl-*N*-methylamino)-3-hydroxy-2,2-dimethyl-chromane; cAMP, adenosine 3′5′ cyclic monophosphate; CCh, carbachol; CFTR, cystic fibrosis transmembrane conductance regulator; DMSO, dimethyl sulphoxide; E-4031, *N*-(4-((1-(2-(6-methyl-2-pyridinyl)ethyl)-4-piperidinyl)carbonyl)phenyl)-methanesulphonamide dihydrochloride dihydrate; GC, guanylyl cyclase; HERG, human ether-á-go-go related gene; IBMX, 3-isobutyl-1-methylxanthine; I_K, delayed rectifier K^+ current; I_{Kr}, rapidly activating component of delayed rectifier K^+ current; I_{Ks}, slowly activating component of delayed rectifier K^+ current; cGMP, guanosine 3′5′ cyclic monophosphate; KB solution, Kraft–Brühe solution; PDE, phosphodiesterase; PDE2, cGMP-stimulated PDE; PDE3, cGMP-inhibited PDE; PKA, adenosine 3′5′ cyclic monophosphate-dependent protein kinase; PKG, guanosine 3′5′ cyclic monophosphate-dependent protein kinase; Rp-8-Br-cAMPS, *R*p-8-bromoadenosine 3′5′ cyclic monophosphorothioate; SA node, sino-atrial node

Introduction

Activation of the delayed rectifier K^+ current (I_K) plays an important role in initiating the repolarization process of the action potential in most types of cardiac cells. In sino-atrial (SA) node pacemaker cells, deactivation of I_K at negative potentials, together with the activation of inward currents, contributes to the development of the slow diastolic depolarization (pacemaker potential; for a review see Irisawa *et al*., 1993). Sanguinetti & Jurkiewicz (1990; 1991) identified two kinetically and pharmacologically distinct components of I_K in guinea-pig cardiac myocytes; namely, the rapidly and slowly activating components of I_K (I_{Kr} and I_{Ks}, respectively). I_{Kr} is characterized by its marked inward rectification at positive potentials and its sensitivity to inhibition by methanesulphonanilide class III anti-arrhythmic drugs such as E-4031, sotalol and dofetilide, whereas I_{Ks} exhibits a minimal inward rectification and is resistant to these drugs. These two components of I_K have since been shown to exist in cardiac cells of a variety of mammalian species including

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humans (Wang *et al*., 1994; Li *et al*., 1996). The activation of adenosine 3′5′ cyclic monophosphate (cAMP)-dependent protein kinase (PKA) potentiates I_{Ks} in cardiac cells (Harvey & Hume, 1989b; Yazawa & Kameyama, 1990; Sanguinetti *et al*., 1991; Wang *et al*., 1994).

Molecular biological studies have shown that *KCNQ1* gene encodes the pore-forming α-subunit KvLQT1 that coassembles with an accessory β subunit minK protein (encoded by *KCNE1* gene) to produce the I_{Ks} channels (Barhanin *et al*., 1996; Sanguinetti *et al*., 1996), whereas human ether-á-go-go related gene (*HERG*) encodes the pore-forming subunit of the channel that underlies cardiac I_{Kr} (Curran *et al*., 1995; Sanguinetti *et al*., 1995; Trudeau *et al*., 1995). Mutations in *KCNQ1, KCNE1* and *HERG* are responsible for the long QT syndrome in humans (Curran *et al*., 1995; Wang *et al*., 1996; Splawski *et al*., 1997), an inherited disorder characterized by a prolongation of the QT interval on electrocardiograms (ECG) arising from abnormal cardiac repolarization.

Intracellular guanosine 3′5′ cyclic monophosphate (cGMP), which is synthesized from guanosine triphosphate (GTP) through the activation of soluble and particulate guanylyl cyclases (sGC and pCG, respectively), has been demonstrated to modulate activities of ion channels in various species and tissue types (for a review see Lucas *et al*., 2000). In the heart, most studies investigating the regulation of ion channels by intracellular cGMP have been conducted on the L-type Ca^{2+} channels ($I_{Ca,L}$). Intracellular cGMP stimulates the cGMP-stimulated phosphodiesterase (PDE2) and facilitates the degradation of cAMP into 5′-AMP, which results in depression of $I_{Ca,L}$ (Hartzell & Fischmeister, 1986; Vandecasteele *et al*., 2001). On the other hand, cGMP inhibits the cGMP-inhibited phosphodiesterase (PDE3), which can elevate cAMP levels and thereby potentiate $I_{Ca,L}$ (Ono & Trautwein, 1991; Shirayama & Pappano, 1996; Vandecasteele *et al*., 2001). Species- or tissue-dependent expression of phosphodiesterase (PDE) subtypes has been suggested to account for these opposite actions of cGMP on cardiac $I_{Ca,L}$ (for a review see Beavo, 1995).

Intracellular cGMP was also shown to activate cGMP-dependent protein kinase (PKG), leading to the modulation of $I_{Ca,L}$ in cardiac myocytes. A number of studies have revealed that the activation of PKG depresses $I_{Ca,L}$ in ventricular myocytes from rat (Méry *et al*., 1991; Sumii & Sperelakis, 1995), guinea-pig (Ono & Trautwein, 1991; Shirayama & Pappano, 1996) and rabbit (Tohse *et al*., 1995) heart. Jiang *et al*. (2000) have identified a PKG phosphorylation site at serine 533 of the α_{1C} subunit in association with depression of $I_{Ca,L}$. In recent years, however, studies using rabbit cardiac cells have demonstrated a stimulatory action of PKG activation on $I_{Ca,L}$ (Kumar *et al*., 1997; Han *et al*., 1998; Wang *et al*., 2000). Intracellular cGMP was thus found to either stimulate or depress $I_{Ca,L}$ not only by altering cAMP concentrations through regulation of PDEs (PDE2 and PDE3) but also by activating PKG.

There is, however, little information concerning the regulation of ionic currents other than $I_{Ca,L}$ by intracellular cGMP in cardiac cells. We herein report that in guinea-pig SA node cells, cGMP potentiates I_{Ks} by interacting with two distinct target proteins; namely, activation of PKG and inhibition of PDE3.

Methods

Cell preparation

Single SA node cells were isolated from hearts of 5- to 8-week-old female guinea-pigs (body weight, 250–400 g) using an enzymatic dissociation procedure as described previously (Guo *et al*., 1997). Briefly, the guinea-pigs were deeply anaesthetized with sodium pentobarbitone (80 mg kg^{-1}, i.p.), and the chest was opened under artificial respiration. The ascending aorta was cannulated *in situ* to start coronary perfusion of the heart. The heart was then excised and retrogradely perfused through the aortic cannula on a Langendorff perfusion apparatus at 37°C, initially for 4 min with normal Tyrode solution, then for 4 min with nominally Ca^{2+}-free Tyrode solution, finally for 7–10 min with nominally Ca^{2+}-free Tyrode solution containing 0.4 mg ml^{-1} collagenase (Wako Pure Chemical Industries, Osaka, Japan). All the above solutions were oxygenated. The digested heart was then removed from the Langendorff perfusion apparatus and the SA node region was dissected out and was cut perpendicular to the crista terminalis into small strips measuring about 0.5 mm in width. The SA node tissue was further incubated for 16–20 min at 37°C in nominally Ca^{2+}-free Tyrode solution supplemented with 1.0 mg ml^{-1} collagenase and 0.1 mg ml^{-1} elastase (Roche, Mannheim, Germany). Finally, the enzyme-digested SA node strips were mechanically agitated in a high-K^+, low-Cl^- Kraftbrühe (KB) solution (Isenberg & Klöckner, 1982) to disperse the cells. Isolated cells thus obtained were then stored at 4°C in the KB solution until required for experimental use.

All these experimental procedures were reviewed and approved by the Shiga University of Medical Science Animal Care Committee, Japan.

Voltage-clamp technique and data analysis

Isolated SA node cells were voltage-clamped using the whole-cell configuration of the patch-clamp technique (Hamill *et al*., 1981) with a patch-clamp amplifier (CEZ-2400; Nihon Kohden, Tokyo, Japan). Patch electrodes were fabricated from glass capillaries (outside diameter, 1.5 mm; inside diameter, 0.9 mm; Narishige Scientific Instrument Laboratory, Tokyo, Japan) using a four-stage horizontal micropipette puller (P-97; Sutter Instrument Co., Novato, CA, U.S.A.), and the tips were then fire-polished using a microforge. Patch electrodes had a resistance of 2.0–2.5 MΩ when filled with the control pipette solution. An aliquot of cells was allowed to settle onto the glass bottom of a recording chamber (0.5 ml in volume) mounted on the stage of an inverted Nikon Diaphot microscope (Tokyo, Japan). The chamber was maintained at 34–36°C and was continuously perfused at a rate of 2 ml min^{-1} with normal Tyrode solution. A tight seal (resistance, 5–50 GΩ) was formed between the electrode tip and the cell membrane by gentle suction (−20 to −40 cm H_2O). The patch membrane was then ruptured by a brief period of more vigorous suction, controlled manually with a 2.5 ml syringe.

When superfused with normal Tyrode solution, an SA node cell was characterized by its spontaneous and regular contraction at a rate of more than 150 min^{-1} (Guo *et al*., 1997; Matsuura *et al*., 2002). Whole-cell voltage-clamp

experiments from these cells confirmed the presence of the hyperpolarization-activated inward current (I_f) in response to hyperpolarizing voltage steps (see Figure 1). Cell membrane capacitance (C_m) was calculated from the capacitive transients elicited by 20 ms voltage-clamp steps (± 5 mV) according to the relationship (Bénitah *et al*., 1993): $C_m = \tau_C I_0 / \Delta V_m \ (1 - I_\infty / I_0)$, where τ_C is the time constant of the capacitive transient (0.290 ± 0.041 ms, $n = 9$), I_0 is the initial peak current amplitude, ΔV_m is the amplitude of voltage step (± 5 mV) and I_∞ is the steady-state current value. The average capacitance value for SA node cells used in the present study was 35.8 ± 2.8 pF (mean $\pm$ s.e.mean, $n = 9$).

I_{Ks} was recorded from SA node cells during depolarizing voltage steps, under conditions where the Na^+ current (I_{Na}) was inactivated by setting the holding potential to -50 mV, and $I_{Ca,L}$ and I_{Kr} were respectively inhibited by the addition of 0.4 μM nisoldipine and 5 μM E-4031 to normal Tyrode solution. Isolated SA node cells were usually exposed to this external solution for whole-cell recordings of I_{Ks} either prior to (data shown in Figures 2 and 4D) or 1–2 min after (data shown in Figures 3, 4A–C and 5–8) a rupture of the patch membrane; however, in either case I_{Ks} was found to reach a stable level (about 60–80% of initial value) within about 5–7 min of the patch membrane rupture, when evaluated by measuring the amplitudes of outward tail currents elicited upon repolarization to a holding potential of -50 mV following a 2 s depolarizing step to $+30$ mV every 15 or 30 s (see Figure 2A). Accordingly, experiments designed to examine the effects of extracellularly applied agents were started after this stable baseline current level had been established (see Figures 3, 4A–C and 5–8), while measurements of I_{Ks} were initiated immediately after a rupture of the patch membrane in experiments designed to study the time course of changes in the amplitude of I_{Ks} during dialysis of the cell interior with the pipette solution containing cGMP (see Figures 2 and 4D).

Current and voltage signals were stored on digital audiotape (DM120, Hitachi Maxell, Tokyo, Japan) using a PCM data recorder (RD-120TE, TEAC, Tokyo, Japan). Current and voltage records were fed to a computer (PC98RL, NEC, Tokyo, Japan) every 0.2–1 ms through a low-pass filter (48 dB per octave, E-3201A, NF, Tokyo, Japan) at an appropriate cut-off frequency (usually 3 kHz) and then were analysed using in-house programs.

In all current recordings demonstrated in the figures, the zero-current level is indicated to the left of the current records by a horizontal line.

Solutions and drugs

Normal Tyrode solution contained (mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5 and HEPES 5.0 (pH adjusted to 7.4 with NaOH). The nominally Ca^{2+}-free Tyrode solution used for the cell isolation procedure was prepared by simply omitting $CaCl_2$ from the normal Tyrode solution. The external bath solution used for measuring whole-cell I_{Ks} was normal Tyrode solution supplemented with 0.4 μM nisoldipine (a generous gift from Bayer, Germany) and 5 μM E-4031 (*N*-(4-((1-(2-(6-methyl-2-pyridinyl)ethyl)-4-piperidinyl)carbonyl)phenyl)methanesulphonamide dihydrochloride dihydrate; Wako Pure Chemical Industries, Osaka, Japan). Nisoldipine was prepared as a 1 mM stock solution in ethanol and then was added to the normal Tyrode solution to achieve a final concentration of 0.4 μM. Nisoldipine was previously shown to have no effect on cardiac I_K at this concentration (Sanguinetti & Jurkiewicz, 1991). E-4031 was dissolved in distilled water as a 1 mM stock solution and was diluted to give a concentration of 5 μM. The block of I_{Kr} by 5 μM E-4031 developed rapidly and usually reached a full block within 30 s of superfusion (data not shown; see Sanguinetti & Jurkiewicz, 1990).

Other agents added to the external solution included atrial natriuretic peptide (human, hANP, Sigma Chemical Co., MO, U.S.A.), milrinone (a generous gift from Yamanouchi Pharmaceutical Co., Tokyo, Japan), 3-isobutyl-1-methylxanthine (IBMX, Sigma), 8-bromoguanosine 3′5′ cyclic monophosphate (8-Br-cGMP, sodium salt; Sigma), KT-5823 (Calbiochem, CA, U.S.A.), *R*p-8-bromoadenosine 3′5′ cyclic monophosphorothioate (*R*p-8-Br-cAMPS; Biolog Life Science Institute, Bremen, Germany) and the chromanol derivative 293B (trans-6-cyano-4-(*N*-ethylsulphonyl-*N*-methylamino)-3-hydroxy-2,2-dimethyl-chromane; a generous gift from Aventis Pharma Deutschland GmbH, Frankfurt, Germany). Milrinone, IBMX, 8-Br-cGMP and *R*p-8-Br-cAMPS were directly dissolved into the external solution prior to use. IBMX was used at 100 μM, a concentration which was shown to produce a maximal stimulatory effect on basal $I_{Ca,L}$ by inhibiting nonselectively all PDE subtypes (PDE1-4; Shirayama & Pappano, 1996). 8-Br-cGMP was used at 100 μM, a concentration which was shown to maximally activate PKG in rat ventricular cells (Méry *et al*., 1991). *R*p-8-Br-cAMPS competitively binds to the regulatory subunits of PKA and thereby specifically prevents the cAMP-dependent PKA activation (De Wit *et al*., 1984). We added *R*p-8-Br-cAMPS to the bath at 200 μM, a concentration shown to potently inhibit PKA activation in many cell types (Liu *et al*., 2001). hANP was dissolved as a 1 mM stock solution in distilled water and was diluted to achieve a final bath concentration of 100 nM. ANP at this concentration was shown to significantly reduce the amplitude of $I_{Ca,L}$ through the activation of PKG in rat ventricular cells (Tohse *et al*., 1995). 293B was dissolved in dimethyl sulphoxide (DMSO, Sigma) to make a 100 mM stock solution and then was diluted at a final concentration of 50 μM in the external solution. In order to confirm that I_K stimulated by internal dialysis with cGMP is entirely due to I_{Ks}, we need to use 293B at concentrations that are enough to totally block I_{Ks} while minimally affecting I_{Kr}. We therefore used 293B at 50 μM, a concentration which was demonstrated to produce a practically full inhibition of I_{Ks} without a significant effect on I_{Kr} in guinea-pig ventricular cells (Bosch *et al*., 1998). KT-5823 was dissolved in DMSO as a 1 mM stock solution and then was diluted at a final bath concentration of 500 nM. The inhibitory constant (K_i) of KT-5823 for PKG was shown to be 234 nM whereas the value for PKA was more than 10 μM (Kase *et al*., 1987; Hidaka & Kobayashi, 1992). KT-5823 was therefore used at 500 nM, which is expected to potently inhibit PKG activity while minimally affecting PKA (Wang *et al*., 2000).

The control pipette solution contained (mM): potassium aspartate 70, KCl 50, KH_2PO_4 10, $MgSO_4$ 1, Na_2-ATP (Sigma) 3, Li_2-GTP (Roche) 0.1, EGTA 5 and HEPES 5 (pH adjusted to 7.2 with KOH). Since the amount of KOH required for titration was found to be 24 mM on average, the

final K^+ concentration in the control pipette solution was 154 mM. The concentration of free Ca^{2+} and Mg^{2+} in the pipette solution was estimated to be approximately 6.0×10^{-11} M (pCa = 10.2) and 3.7×10^{-5} M (pMg = 4.4), respectively (Fabiato & Fabiato, 1979; Tsien & Rink, 1980). In experiments using cGMP (sodium salt, Nacalai Tesque, Kyoto, Japan), a 100 mM stock solution made in distilled water was added to the control pipette solution (final concentration, 100 μM). It has been suggested in guinea-pig ventricular cells that cGMP at a concentration of $\sim$100 μM is required to maximally activate PKG when loaded through a recording pipette, although cGMP at a concentration of $\sim$10 μM is maximally effective in inhibiting PDE3 activity (Ono & Trautwein, 1991). We therefore decided to dialyze the cells with cGMP at a concentration of 100 μM to potently activate PKG as well as to inhibit PDE3 activity. The KB solution for cell preservation contained (mM): potassium glutamate 70, KCl 30, KH_2PO_4 10, $MgCl_2$ 1, taurine 20, EGTA 0.3, glucose 10 and HEPES 10 (pH adjusted to 7.2 with KOH).

Data analysis and statistics

Results are expressed as mean $\pm$ s.e.mean, and n indicates the number of cells studied. Statistical comparisons were made using Student's paired or unpaired t-tests as appropriate, and differences were considered to be significant at $P < 0.05$.

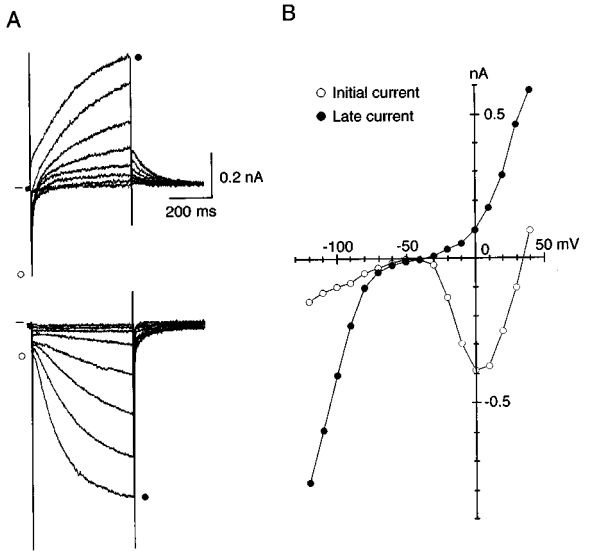

Figure 1 Whole-cell membrane currents in an isolated guinea-pig SA node cell superfused with normal Tyrode solution. (A) Superimposed current traces during 500 ms voltage-clamp steps to membrane potentials of -30 to $+40$ mV (upper panel) and -50 to -120 mV (lower panel) in 10 mV steps applied from a holding potential of -40 mV. (B) Current-voltage ($I-V$) relationships for the initial and late currents. Initial current was measured at peak of $I_{Ca,L}$ or 5–10 ms into hyperpolarizing test pulse. Late current was measured near end of 500 ms clamp pulse.

Results

Whole-cell membrane currents in guinea-pig SA node cells

Figure 1A shows a representative example of whole-cell membrane currents recorded from a spontaneously contracting SA node cell in normal Tyrode solution when the cell membrane was stepped from a holding potential of -40 mV to test potentials of -30 to $+40$ mV (upper panel) and -50 to -120 mV (lower panel) for 500 ms. Depolarizing test steps initially activated an inward current which reached its maximum at a test potential of 0 mV (Figure 1B, open circles) and exhibited a sensitivity to inhibition by nisoldipine (data not shown), suggesting that the inward current was mostly attributable to an activation of $I_{Ca,L}$. The time-dependent increase in outward current during depolarization and decaying outward tail current upon return to the holding potential appear to reflect the activation and deactivation of the delayed rectifier K^+ current (I_K), respectively. A small part of I_K was found to be sensitive to block by 5 μM E-4031 (Matsuura *et al*., 2002), thus suggesting that I_K in guinea-pig SA node cells is largely composed of I_{Ks} with I_{Kr} also present. Changes in membrane current during hyperpolarizing voltage steps were characterized by the activation of the hyperpolarization-activated inward current (I_f), which is evident at potentials $\leqslant -70$ mV (Figure 1B).

Enhancement of I_{Ks} by intracellular cGMP

We examined the regulation of I_{Ks} by intracellular cGMP in guinea-pig SA node cells, under conditions in which $I_{Ca,L}$ and I_{Kr} were respectively blocked by the addition of 0.4 μM nisoldipine and 5 μM E-4031 to normal Tyrode solution. Time course of changes in I_{Ks} after rupture of the patch membrane was first tested in cells dialyzed with control pipette solution (Figure 2A). After gaining access to the cell interior (rupture of the patch membrane), the cell was hyperpolarized from a holding potential of -50 to -130 mV, which detected the presence of I_f (data not shown), and was then repetitively depolarized every 30 s to $+30$ mV for 2 s to monitor the influence of cell dialysis on I_{Ks}. The amplitude of I_{Ks} tail current elicited upon repolarization to -50 mV usually declined (rundown) within approximately 5 to 7 min of patch rupture but stabilized thereafter for a period of more than 10 min. The degree of the current rundown, when evaluated by the decrease in the amplitude of tail current elicited upon repolarization to -50 mV following 2 s depolarization to $+30$ mV, ranged between approximately 20 and 40% of the initial value measured shortly after establishment of whole-cell configuration.

When an SA node cell was dialyzed with a pipette solution containing 100 μM cGMP, the amplitude of I_{Ks} tail current, measured using the same voltage-clamp protocol (2 s depolarization to $+30$ mV from a holding potential of -50 mV applied at a 30 s interval), was gradually increased and reached a maximum response about 12 min after a rupture of the patch membrane (Figure 2B). To confirm that internal dialysis with cGMP potentiated I_{Ks} but not I_{Kr}, we tested the effect of the chromanol derivative 293B (Busch *et al*., 1996). It has been demonstrated in guinea-pig ventricular cells that 293B at a concentration of 50 μM almost completely blocks I_{Ks} while minimally affecting I_{Kr} (Bosch *et al*., 1998). As demonstrated in Figure 2B, bath application of 50 μM 293B rapidly and totally abolished both the time-dependent outward current during depolarization and the decaying outward tail current upon repolarization, thus supporting the

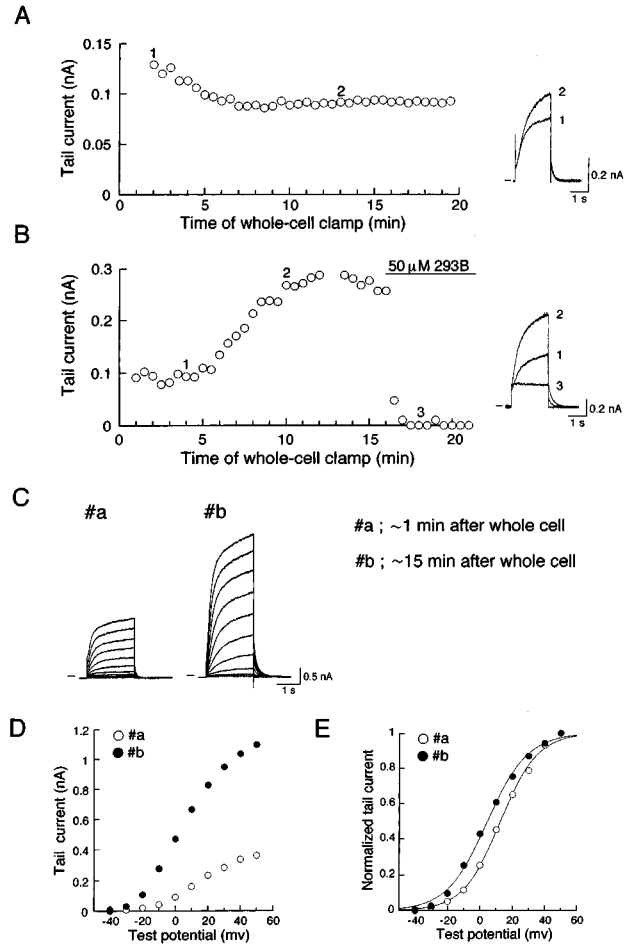

Figure 2 Enhancement of I_{Ks} by internal dialysis with cGMP. (A) Time course of changes in the amplitude of I_{Ks} tail current recorded from an isolated SA node cell dialyzed with control pipette solution. After establishing the whole-cell configuration, the cell was initially hyperpolarized to confirm the presence of an obvious I_f, and then was depolarized every 30 s from a holding potential of -50 mV to $+30$ mV for 2 s. The amplitude of I_{Ks} tail current, measured upon return to a holding potential of -50 mV, is plotted as a function of time after rupture of the patch membrane. (B) Time course of I_{Ks} response to dialysis of the cell interior with a pipette solution containing 100 μM cGMP. The voltage-clamp protocol was the same as in the experiment shown in (A). After I_{Ks} response to internal dialysis with cGMP reached a steady state, 50 μM 293B was added to the bath solution, as indicated by the horizontal bar. Inset in (A) and (B) shows superimposed original current traces recorded at time points indicated by numerals on the graph. (C) Superimposed current traces in response to 2 s voltage-clamp steps to membrane potentials of -40 to $+50$ mV in 10 mV steps applied from a holding potential of -50 mV, recorded $\sim$1 min (left-hand panel) and $\sim$15 min (right-hand panel) after a rupture of the patch membrane. Current records shown in (B) and (C) were obtained from distinct cells. (D) $I-V$ relationships for I_{Ks} tail currents, from the data shown in (C). (E) $I-V$ relationships for normalized I_{Ks} tail currents. Amplitude of I_{Ks} tail current at each test potential was normalized with reference to the maximum value at $+50$ mV and was plotted against test potential. Continuous curves through the data points show the least-squares fit of a Boltzmann equation ($\sim$1 min after whole-cell, $V_{1/2}$, 12.8 mV; k, 11.5 mV; $\sim$15 min after whole-cell, $V_{1/2}$ 4.8 mV; k, 12.5 mV).

view that stimulatory effect of cGMP was entirely due to modification of I_{Ks}.

Figure 2C shows the superimposed current traces during 2 s depolarizing steps to membrane potentials of -40 to $+50$ mV applied in 10 mV steps from a holding potential of -50 mV, recorded $\sim$1 min and $\sim$15 min after internal dialysis with cGMP. Figure 2D illustrates $I-V$ relationships for I_{Ks} tail current recorded under these two conditions (open circles, $\sim$1 min after internal dialysis; filled circles, $\sim$15 min after internal dialysis). In a total of four cells, dialysis of the cell interior with a pipette solution containing 100 μM cGMP increased I_{Ks} by a factor of 2.37 ± 0.39 on average, as judged by comparing the amplitudes of tail currents elicited upon return to the -50 mV holding potential following 2 s depolarization to $+30$ mV $\sim$1 min and $\sim$15 min after internal dialysis within the same cells.

In order to assess the effect of internal cGMP on the voltage-dependent activation of I_{Ks}, the amplitude of the tail current at each test potential was normalized with reference to its maximal value at $+50$ mV and was fitted by a Boltzmann equation:

$$I_{K,tail} = 1/(1+\exp((V_{1/2}-V_m)/k)) \quad (1)$$

where $V_{1/2}$ is the voltage at which the activation is half-maximal, V_m is the test potential and k is the slope factor. In a total of four cells, $V_{1/2}$ and k values respectively averaged 9.5 ± 2.4 mV and 11.5 ± 1.4 mV for the data recorded $\sim$1 min after internal dialysis, and 3.3 ± 1.0 mV and 11.6 ± 1.8 mV for the data recorded $\sim$15 min after internal dialysis ($n=4$), thus showing that the voltage-dependence of I_{Ks} activation was significantly shifted in a negative direction (9.5 ± 2.4 mV *vs* 3.3 ± 1.0 mV, $n=4$; $P<0.05$) while the slope factor was not appreciably affected (11.5 ± 1.4 mV *vs* 11.6 ± 1.8 mV, $n=4$) when I_{Ks} was enhanced by internal dialysis with 100 μM cGMP.

Enhancement of I_{Ks} by atrial natriuretic peptide

Since atrial natriuretic peptide (ANP) has been demonstrated to elevate intracellular levels of cGMP by activating pGC-coupled receptors in a variety of cell types including mammalian cardiac myocytes (Cramb *et al*., 1987; Lin *et al*., 1995), we tested whether this peptide which is expected to elevate intracellular levels of endogeneous cGMP can potentiate I_{Ks} in guinea-pig SA node cells. As demonstrated in Figure 3, a bath application of 100 nM ANP evoked an increase in I_{Ks} in a guinea-pig SA node cell. In a total of five cells, bath application of ANP produced an increase in I_{Ks} tail current, measured upon return to the -50 mV holding potential following depolarization to $+30$ mV for 2 s, by $47.7\pm4.8\%$. This observation is consistent with intracellular cGMP producing an increase in I_{Ks} in guinea-pig SA node cells (Figure 2B–E).

Potentiation of I_{Ks} by inhibition of phosphodiesterases

We then examined the intracellular mechanisms mediating the stimulatory effect of cGMP on I_{Ks} in guinea-pig SA node cells. In cardiac cells I_{Ks} is potentiated by an elevation in intracellular cAMP levels and resultant activation of PKA (Walsh & Kass, 1988; 1991; Yazawa & Kameyama, 1990). To test the possibility that a blockade of the cGMP-inhibited phosphodiesterase (PDE3) mediates the stimulatory action of cGMP on I_{Ks}, the effect of a selective PDE3 inhibitor milrinone (Harrison *et al*., 1986) on I_{Ks} was examined (Figure

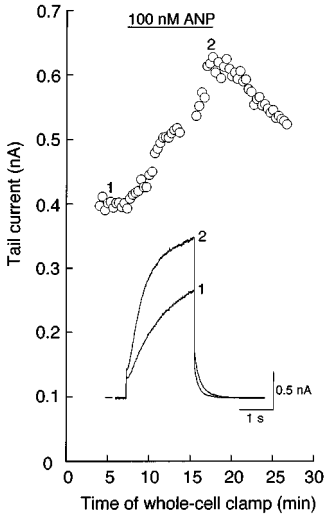

Figure 3 Time course of I_{Ks} response to 100 nM ANP. Amplitude of I_{Ks} tail currents elicited upon repolarization to a holding potential of −50 mV following depolarization to +30 mV for 2 s is plotted as a function of time after a rupture of the patch membrane. ANP (100 nM) was added to the bath solution during the period indicated by the horizontal bar. Initial rundown not shown. The inset shows superimposed original current traces recorded at the time points indicated by numerals on the graph.

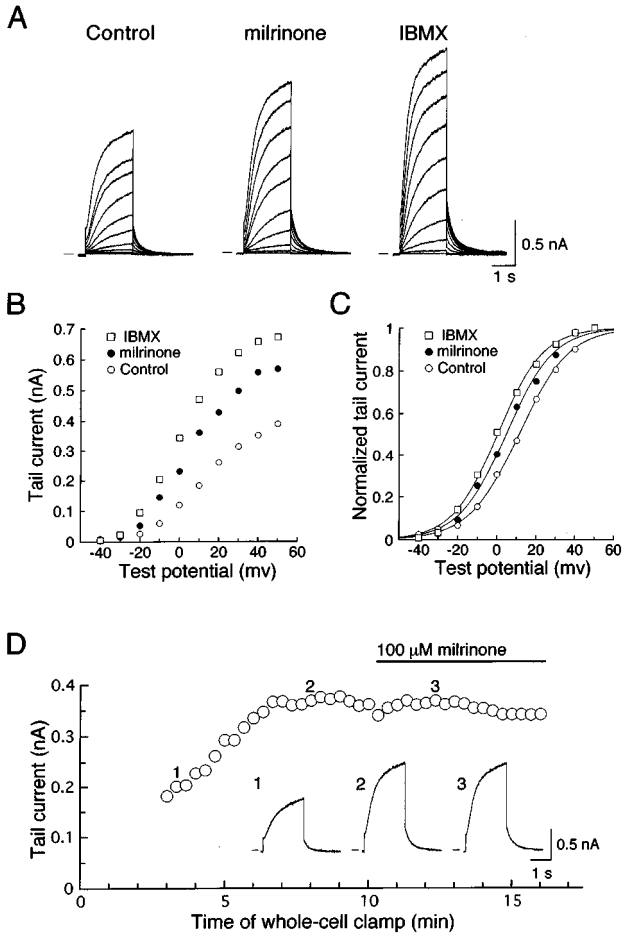

Figure 4 Potentiation of I_{Ks} by milrinone and IBMX. (A) Superimposed current traces during 2 s voltage-clamp pulses to membrane potentials of −40 to +50 mV in 10 mV steps applied from a holding potential of −50 mV, recorded under control conditions (left-hand panel), 5 min after application of 100 μM milrinone (middle panel), and 3 min after subsequent addition of 100 μM IBMX with milrinone (right-hand panel). (B) $I-V$ relationships for I_{Ks} tail currents obtained from the records in (A), under control conditions, during exposure to milrinone, and after a further addition of IBMX. (C) $I-V$ relationships for normalized I_{Ks} tail currents. The amplitude of I_{Ks} tail current measured at each test potential was normalized with respect to its maximum value at +50 mV. The least-squares fit of the data points to a Boltzmann equation provides $V_{1/2}$ and k (Control: $V_{1/2}=12.1$ mV, $k=13.5$ mV; milrinone: $V_{1/2}=5.3$ mV, $k=12.3$ mV; IBMX: $V_{1/2}=0.3$ mV, $k=11.4$ mV). (D) Time course of response of I_{Ks} tail current to 100 μM milrinone, recorded from a cell dialyzed with a pipette solution containing 100 μM cGMP. Depolarizing voltage steps (to +30 mV for 2 s) were applied from a holding potential of −50 mV every 30 s and amplitudes of tail currents upon return to the holding potential were measured. Inset shows examples of original current traces recorded at the times indicated by numerals on the graph.

4). Bath application of 100 μM milrinone increased the amplitude of I_{Ks}, evoked by 2 s depolarizing pulses applied from a holding potential of −50 mV to test potentials of −40 to +50 mV in 10 mV steps, indicating that a blockade of PDE3 results in an enhancement of I_{Ks}. A subsequent application of a potent but nonspecific PDE inhibitor IBMX (Strada *et al*., 1984) at 100 μM in the continued presence of milrinone produced an additional increase in I_{Ks}. Figure 4B illustrates $I-V$ relationships for the tail currents recorded under control conditions (open circles), during exposure to 100 μM milrinone (closed circles) and further addition of 100 μM IBMX (open squares). On average, the application of milrinone at 100 μM increased the amplitude of I_{Ks} tail current, elicited upon return to a holding potential of −50 mV after a 2 s pulse to +50 mV, by 50.4±8.5% and subsequent addition of 100 μM IBMX further increased I_{Ks} tail current by 26.5±6.5% of its control amplitude ($n=4$). Thus the stimulatory effect of IBMX on I_{Ks} was additive with that of mil-rinone, suggesting that PDE subtypes other than PDE3 also coexist in guinea-pig SA node cells. Bath application of mil-rinone at a lower concentration (10 μM) also increased the amplitude of I_{Ks} tail current, measured at −50 mV following 2 s depolarization to +50 mV, by 42.1±6.8% ($n=3$, data not shown).

The smooth curves through the normalized tail current amplitude (Figure 4C) represent fit of the data to a Boltzmann equation (eqn. 1). In a total of four cells, $V_{1/2}$ and k values respectively averaged 13.0±3.3 and 11.4±0.7 mV for control, 8.1±2.7 and 11.8±0.2 mV for milrinone, and 4.0±2.6 and 12.3±0.7 mV for IBMX. Milrinone shifted the I_{Ks} activation in a negative direction by 4.8±1.1 mV and IBMX further shifted by 4.1±1.2 mV. The slope factor was not appreciably affected by these agents (control, 11.4±0.7 mV; milrinone, 11.8±0.2 mV; IBMX, 12.3±0.7 mV). A similar shift in the I_{Ks} activation has been reported for the potentiation of I_{Ks} by cAMP-PKA activating agents in guinea-pig ventricular myocytes (Harvey & Hume, 1989b; Yazawa & Kameyama, 1990; Walsh & Kass, 1991).

Blockade of PDE3 activity by milrinone in guinea-pig SA node cells was thus found to produce a significant increase in I_{Ks}. In order to assess whether PDE3 inhibition mediates the stimulatory action of cGMP on I_{Ks}, we tested whether the stimulatory effect of milrinone is additive with that of intracellular cGMP. In the experiment shown in Figure 4D, an SA node cell was dialyzed with a pipette solution containing 100 μM cGMP, which gradually increased the

amplitude of I_{Ks} tail current, measured upon repolarization to −50 mV following depolarization to +30 mV. Bath application of milrinone at 100 μM could not produce any further increase in I_{Ks} tail current after potentiation by internal dialysis with 100 μM cGMP, suggesting that the stimulatory action of cGMP on I_{Ks} involves the cGMP-induced inhibition of PDE3 activity and a resultant elevation of intracellular cAMP (and activation of PKA).

Potentiation of I_{Ks} by activation of PKG

In order to assess the role of PKG in the enhancement of I_{Ks}, the effect of 8-Br-cGMP on I_{Ks} was investigated in guinea-pig SA node cells. 8-Br-cGMP is a nonhydrolyzable cGMP analogue which penetrates the cell membrane and selectively activates PKG in the cytoplasm but has little action on the cGMP-regulated PDEs, namely, PDE2 and PDE3 (Corbin *et al*., 1986; Butt *et al*., 1992; Beltman *et al*., 1995). Figure 5A shows the membrane currents in response to 2 s voltage pulses to potentials between −40 and +50 mV in 10 mV steps applied from a holding potential of −50 mV, recorded under control conditions (left-hand panel) and 5 min after superfusion of 100 μM 8-Br-cGMP (right-hand panel). Figure 5B illustrates $I-V$ relationships for tail currents, from the experiment shown in Figure 5A. In a total of five SA node cells, application of 8-Br-cGMP at 100 μM increased the amplitude of I_{Ks} tail current, measured upon return to the holding potential following depolarization to +50 mV, by 47.7±10.5%, thus suggesting that activation of PKG produces an increase in I_{Ks} in guinea-pig SA node cells. 8-Br-cGMP at 100 μM significantly shifted the voltage-dependence of I_{Ks} activation in a negative direction ($V_{1/2}$; control, 8.4±1.5 mV; 8-Br-cGMP, 4.6±2.3 mV; $n=5$, $P<0.05$) without appreciably affecting the slope factor (k; control, 11.0±0.3 mV; 8-Br-cGMP, 10.8±0.6 mV; N.S.).

Further experiments were performed to clarify whether potentiation of I_{Ks} by 8-Br-cGMP (Figure 5) is indeed evoked by activation of PKG. For this purpose we used KT-5823, which has been demonstrated to inhibit the activity of PKG in a highly selective manner (Kase *et al*., 1987; Hidaka & Kobayashi, 1992). As demonstrated in Figure 6, extracellular application of 100 μM 8-Br-cGMP increased the amplitude of I_{Ks} tail current from 0.120 to 0.188 nA, representing a 56.7% increase, a value similar to results shown in Figure 5, and this increase was completely reversed by the subsequent application of 500 nM KT-5823. This observation further supports the view that activation of PKG results in the enhancement of I_{Ks} in guinea-pig SA node cells.

Additive enhancement of I_{Ks} by inhibition of phosphodiesterases and activation of PKG

The results so far represented are consistent with the hypothesis that intracellular cGMP potentiates I_{Ks} not only by blocking PDE3 but also by activating PKG. To further assess whether intracellular cGMP potentiates I_{Ks} by affecting these two distinct intracellular target proteins, we checked whether the stimulatory effect of PKG activation on I_{Ks} is additive with that produced by PDEs inhibition. Bath application of 100 μM IBMX evoked an increase in I_{Ks} activated by 2 s depolarizing steps to membrane potentials between −40 and +50 mV (Figure 7A,B). Subsequent addition of 100 μM 8-Br-cGMP in the continued presence of IBMX produced an additional increase in I_{Ks} by 52.1±8.1% ($n=4$) of the control amplitude, when evaluated by the amplitudes of the tail currents evoked following depolarization to +50 mV. This value is not significantly different from the increase evoked by 8-Br-cGMP

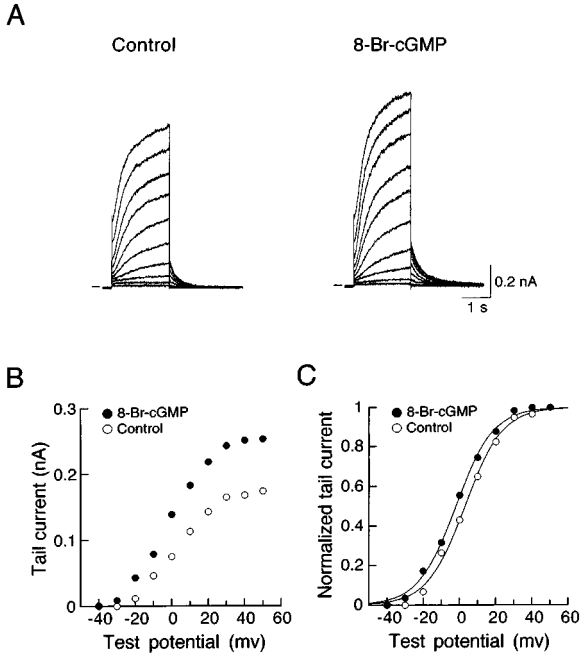

Figure 5 Potentiation of I_{Ks} by 8-Br-cGMP. (A) Superimposed current traces during 2 s voltage-clamp pulses to membrane potentials from −40 to +50 mV applied in 10 mV steps from a holding potential of −50 mV under control condition (left-hand panel) and after 5 min exposure to 100 μM 8-Br-cGMP (right-hand panel). (B) $I-V$ relationships for I_{Ks} tail currents obtained from the records in (A), before and during application of 8-Br-cGMP. (C) $I-V$ relationships for normalized I_{Ks} tail currents. The amplitude of I_{Ks} tail current measured at each test potential was normalized with reference to its maximum value at +50 mV. Continuous curves through the data points show the least-squares fit of a Boltzmann equation (Control: $V_{1/2}=2.9$ mV, $k=10.4$ mV; 8-Br-cGMP: $V_{1/2}=-1.1$ mV, $k=10.9$ mV).

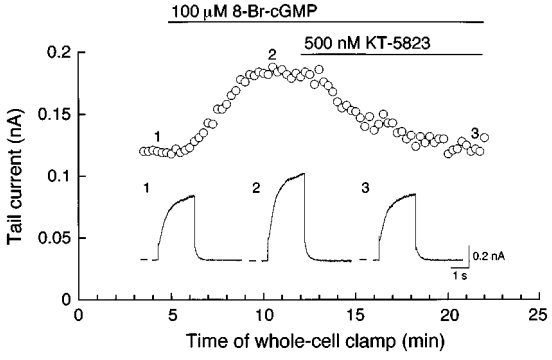

Figure 6 Time course of I_{Ks} response to 8-Br-cGMP and KT-5823. The amplitude of I_{Ks} tail current was measured upon return to a holding potential of −50 mV after 2 s voltage step to +30 mV every 20 s. Periods of exposure to 100 μM 8-Br-cGMP and 500 nM KT-5823 are denoted by horizontal bars. Initial rundown not shown. The inset shows examples of the original current traces recorded at the time points indicated on the graph.

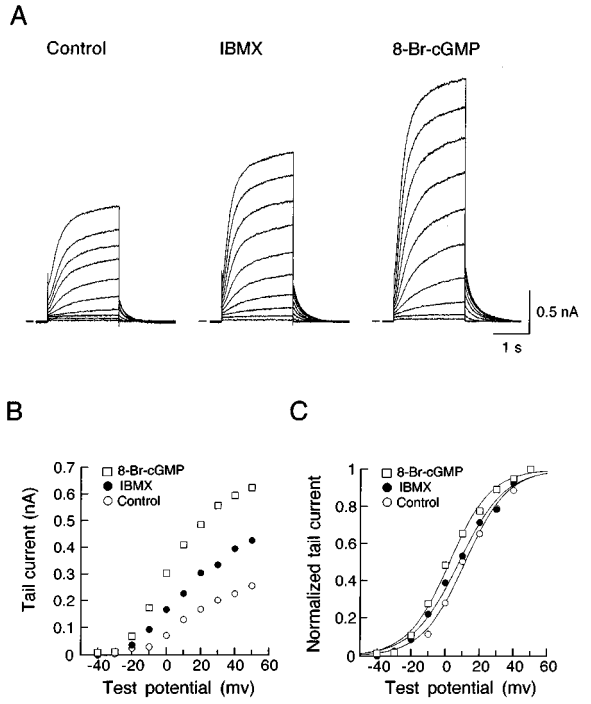

Figure 7 Enhancement of I_{Ks} by 8-Br-cGMP in the presence of IBMX. (A) Superimposed current traces in response to 2 s voltage-clamp pulses to membrane potentials from −40 to +50 mV applied in 10 mV steps from a holding potential of −50 mV under control condition (left-hand panel), during exposure to 100 μM IBMX (middle panel), and after addition of 100 μM 8-Br-cGMP in the presence of IBMX (right-hand panel). (B) $I-V$ relationships for I_{Ks} tail currents obtained from the records in (A) under control conditions, during application of IBMX, and after addition of 8-Br-cGMP. (C) $I-V$ relationships for normalized I_{Ks} tail currents. The amplitude of I_{Ks} tail current at each test potential was normalized with reference to its peak amplitude at +50 mV and was fitted with a Boltzmann equation (Control: $V_{1/2}=12.3$ mV, $k=12.8$ mV; IBMX: $V_{1/2}=8.9$ mV, $k=13.9$ mV; IBMX+8-Br-cGMP: $V_{1/2}=2.1$ mV, $k=11.8$ mV).

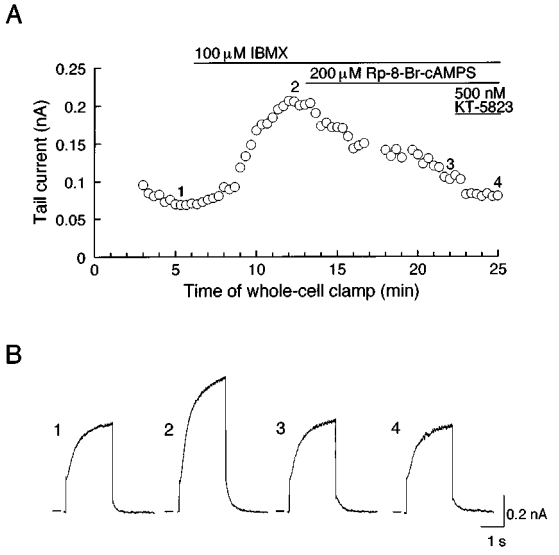

Figure 8 Effect of *R*p-8-Br-cAMPS and KT-5823 on I_{Ks} in the presence of IBMX. (A) Time course of response of I_{Ks} tail current to 100 μM IBMX, 200 μM *R*p-8-Br-cAMPS and 500 nM KT-5823. A cell was repetitively depolarized every 15 s from a holding potential of −50 mV to +30 mV for 2 s and the tail current was measured upon return to the holding potential. Initial rundown not shown. (B) Original current traces recorded at the time points indicated by numerals on the graph in (A).

(47.7±10.5%, $n=5$; Figure 5) in the absence of IBMX, thus showing that potentiation of I_{Ks} by the activation of PKG (by 8-Br-cGMP) was not appreciably influenced by the presence of non-specific inhibition of PDEs (by IBMX). In this example, IBMX shifted the I_{Ks} activation to more negative potentials by 3.4 mV and further application of 8-Br-cGMP produced additional shift to a negative direction by 6.8 mV but the effect of both agents on the slope of the activation curve was considerably small (see k values in legend). These results suggest that the inhibition of PDEs and the activation of PKG independently potentiate I_{Ks} in guinea-pig SA node cells.

Involvement of PKA and PKG in IBMX-induced I_{Ks} enhancement

It has been suggested that exposure to IBMX at higher concentrations such as 1 mM results in an intracellular accumulation of cGMP as well as cAMP through a nonspecific inhibition of PDEs in guinea-pig ventricular cells (Shirayama & Pappano, 1996). We checked the relative contribution of PKA and PKG activity in the enhancement of I_{Ks} during exposure to IBMX. In the experiment shown in Figure 8, a bath application of 100 μM IBMX induced a marked (~2.9 fold) increase in the amplitude of I_{Ks} tail currents, measured upon repolarization to a holding potential of −50 mV following 2 s depolarization to +30 mV. After I_{Ks} response to IBMX reached a steady state, the cell was exposed to a membrane permeable PKA inhibitor *R*p-8-Br-cAMPS (De Wit *et al*., 1984) at 200 μM, which reversed the IBMX-evoked I_{Ks} potentiation by 77%. Further addition of a selective PKG inhibitor KT-5823 at 500 nM fully abolished the IBMX-induced potentiation of I_{Ks} in guinea-pig SA node cells. These results indicate that potentiation of I_{Ks} by IBMX is largely (but not totally) mediated by activation of PKA while PKG activity, probably due to cGMP accumulation, also contributes partly to the enhancement of I_{Ks} by IBMX.

Discussion

The major findings of the present investigation are: (a) I_{Ks} is potentiated not only by internal dialysis with cGMP (Figure 2) but also by external application of ANP (Figure 3) in guinea-pig SA node cells; (b) the signal transduction pathways mediating this stimulatory action of intracellular cGMP appear to involve activation of PKG (Figures 5 and 6) as well as inhibition of PDE3 (Figure 4) leading to activation of cAMP-PKA system.

In cardiac muscle, $I_{Ca,L}$, I_{Ks} and the CFTR (cystic fibrosis transmembrane conductance regulator) Cl^- channels ($I_{Cl,CFTR}$) are stimulated through an elevation of intracellular cAMP levels and subsequent activation of PKA (Reuter, 1983; Kameyama *et al*., 1985; Harvey & Hume, 1989a, b; Bahinski *et al*., 1989; Yazawa & Kameyama, 1990). Intracellular cAMP levels are determined by the balance between the production from ATP by AC and the hydrolytic degradation into 5′-AMP by PDEs. β-Adrenergic agonist

isoprenaline, acting through the stimulatory G protein (G_s), stimulates AC, whereas muscarinic agonist acetylcholine (ACh) exerts an inhibitory action on AC *via* the inhibitory G protein (G_i). The present finding that an inhibition of PDE3 by milrinone results in a significant increase in basal (not stimulated through the cAMP-PKA pathway) I_{Ks} (Figure 4) indicates that intracellular cAMP levels are sufficiently elevated by a blockade of PDE3 alone. It is therefore reasonable to assume that AC is substantially active under basal conditions in guinea-pig SA node cells and that PDE3 activity is also equivalently high to keep the steady state levels of cAMP at a subthreshold level to affect I_{Ks}. In human atrial cells, the inhibition of either PDE2 or PDE3 has been demonstrated to greatly increase $I_{Ca,L}$ in the absence of any prestimulation with cAMP-elevating agents, suggesting a substantially high activity of basal AC as well as PDE2 or PDE3 in this tissue (Kirstein *et al*., 1995; Rivet-Bastide *et al*., 1997; Vandecasteele *et al*., 2001).

It has been demonstrated in guinea-pig ventricular cells that application of milrinone little affects the basal $I_{Ca,L}$ but further potentiates the β-adrenergically stimulated $I_{Ca,L}$ (Ono & Trautwein, 1991), which appears to reflect lower basal activity of AC in ventricular cells, compared with that in SA node cells. In these ventricular cells, ACh antagonizes the β-adrenergically stimulated I_{Ks} but produces little, if any, effect on the basal I_{Ks} (an accentuated antagonism; Harvey & Hume, 1989b; Yazawa & Kameyama, 1990). On the other hand, the muscarinic agonist carbachol (CCh) was shown to greatly depress I_{Ks} in guinea-pig SA node cells even under basal conditions (Freeman & Kass, 1993). These observations may also be accounted for by assuming the substantial difference in the basal activity of AC in SA node and ventricular cells of guinea-pig heart.

Cardiac muscle was previously shown to possess four distinct PDE subtypes (PDE1, PDE2, PDE3 and PDE4), including both of the cGMP-regulated PDEs (PDE2 and PDE3; for a review see Beavo, 1995). As judged from the observation that the increase in amplitude of I_{Ks} evoked by milrinone was larger than the additional increase in I_{Ks} evoked by IBMX (Figure 4), PDE3 appears to be a functionally dominant PDE subtype involved in the regulation of basal I_{Ks} while other PDE subtypes may also exist in guinea-pig SA node cells. In rabbit SA node cells, however, PDE2 was demonstrated to be a dominant PDE subtype, which mediates the nitric oxide (NO)-induced decrease in intracellular cAMP levels and resultant attenuation of $I_{Ca,L}$ (Han *et al*., 1995; 1998). In guinea-pig ventricular cells the cGMP-induced inhibition of PDE3 was shown to play an important role in potentiating the stimulatory effect of intracellular cAMP on $I_{Ca,L}$ and $I_{Cl,CFTR}$, thus cGMP exerts a synergistic action on the β-adrenergic stimulation through inhibition of PDE3 (Ono & Trautwein, 1991; Ono *et al*., 1992). In contrast, cGMP has been shown to attenuate the isoprenaline-induced potentiation of $I_{Ca,L}$ by activating PDE2 in frog ventricular cells (antagonistic action, Fischmeister & Hartzell, 1987). Thus the cGMP-regulated PDEs (PDE2 and PDE3) mediate either stimulatory or inhibitory effect of cGMP on either the basal or β-adrenoceptor-stimulated $I_{Ca,L}$ and $I_{Cl,CFTR}$ in various cardiac cell types.

In the present study, I_{Ks} was found to be regulated by PKG in guinea-pig SA node cells (Figures 5 and 6). This process was accompanied by the negative shift of the voltage dependence of I_{Ks} activation (Figure 5). It has been demonstrated in guinea-pig ventricular cells that PKA and PKC produce an increase in I_{Ks} but differentially affect the voltage-dependent activation curves; PKA shifts the I_{Ks} activation in a negative direction without appreciably altering the slope factor, whereas PKC affects the slope factor with a small effect on the half-activation voltage (Harvey & Hume, 1989b; Yazawa & Kameyama, 1990; Walsh & Kass, 1991). Addition of fixed negative charge to channel proteins by a phosphorylation process has been ascribed to these changes in the voltage-dependence of I_{Ks} activation evoked by PKA and PKC (Walsh & Kass, 1991). Although it is presently unknown as for the site and mechanism of PKG actions underlying the stimulatory effect on I_{Ks}, it is probable that phosphorylation of channel proteins by PKG may affect the sensing of the transmembrane potentials by channel proteins and thereby alters the voltage-dependence of activation curve. In recent years, phosphorylation of serine 533 in the α_{1C} subunit was suggested to be involved in the inhibitory action of PKG on $I_{Ca,L}$ (Jiang *et al*., 2000). Further experiments are called for to explore the molecular basis underlying the stimulatory action of PKG on I_{Ks}.

The pacemaker activity of the SA node cells in the mammalian heart has been shown to be generated by the interaction of multiple ionic currents, including I_f, $I_{Ca,L}$, I_{Ks} and I_{Kr} (for a review see Irisawa *et al*., 1993). It has been demonstrated in SA node cells of pig (Ono *et al*., 2000) and guinea-pig (Anumonwo *et al*., 1992; Matsuura *et al*., 2002) that I_{Ks} is a dominant component of I_K and plays an essential role in the spontaneous activity under control conditions. On the other hand, Lei *et al*. (2002) have recently shown that in rabbit SA node cells, I_{Ks} contributes minimally to the spontaneous electrical activity under control conditions but can play a substantial role during β-adrenergic stimulation. Thus, I_{Ks} can be regarded as one of the most relevant current systems for electrical activity of SA node cells in many species. It is probable that such cGMP-elevating hormones or neurotransmitters as ANP, NO or ACh can affect the electrical activity of SA node cells through regulation of I_{Ks} as well as $I_{Ca,L}$ (Han *et al*., 1995; 1998) and I_f (Musialek *et al*., 1997; Yoo *et al*., 1998). Furthermore, ANP- or NO-induced decrease of action potential duration in cardiac cells (Kecskemeti *et al*., 1996) might be ascribed, at least partly, to the potentiation of I_{Ks}. It will be interesting to examine whether the cGMP-dependent potentiation of I_{Ks} either through activation of PKG or *via* inhibition of PDE3 is present in other cardiac cell types, which will further elucidate the physiological and pathophysiological role of cGMP-elevating compounds in the regulation of cardiac electrical activity.

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