J. Membrane Biol. 148, 185–191 (1995)

The Journal of
Membrane
Biology

Modulation of the Inwardly Rectifying K^+ Channel in Isolated Human Atrial Myocytes by α_1-Adrenergic Stimulation

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Received: 30 March 1995/Revised: 26 July 1995

Abstract. We have examined the α_1-adrenergic modulation of the inwardly-rectifying K^+ channel (I_{K1}) in isolated human atrial myocytes using the patch clamp technique. α_1-Adrenergic agonist methoxamine produced action potential prolongation and a depolarization of the resting membrane potential. Under whole-cell voltage-clamp conditions, bath application of methoxamine can inhibit macroscopic I_{K1}. The methoxamine-induced inhibition was reversible and concentration dependent, with the concentration for half-maximal inhibition being 18 μM. The methoxamine-induced inhibition of I_{K1} was prevented by bath application of α_1-adrenergic blocker prazosin. The current was similarly inhibited by phorbol ester (PMA), an activator of protein kinase C (PKC). In contrast, methoxamine failed to inhibit the current in the presence of a specific PKC inhibitor H-9, suggesting that PKC is involved in the methoxamine-induced inhibition of I_{K1}. In single channel recording from cell-attached patches, bath-applied methoxamine could suppress I_{K1} channels by decreasing the frequency and duration of bursting without affecting unitary amplitude. Direct application of purified PKC to excised inside-out patches inhibited channel activity similar to methoxamine in cell-attached patches. The PKC selective inhibitor, PKC19-36, prevented the PKC-induced inhibition of the channel. We conclude that human atrial I_{K1} can be inhibited by α_1-adrenergic stimulation via PKC-dependent pathways.

Key words: Inwardly rectifying K^+ channel — Patch-clamp technique — Human atrial myocytes — Methoxamine — Protein kinase C

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Introduction

Catecholamines can modulate heart rate and rhythm and can influence cardiac contractility (Noble, 1984; Corr, Yamada & Witkowski, 1986). Previous studies suggest that cardiac inwardly-rectifying K^+ channel (I_{K1}) is modulated by the α_1-adrenergic stimulation in rabbit atrial and ventricular myocytes (Fedida, Braun & Giles, 1991; Braun, Fedida & Giles, 1992), rat atrial (Jahnel et al., 1991) and ventricular muscle (Apkon & Nerbonne, 1988; Ravens, Wang & Wettwer, 1989) and canine Purkinje myocytes (Shah, Cohen & Rosen, 1988). Recently, α_1-adrenergic stimulation has been shown to cause a detectable positive inotropic effect in human atrial tissue under in vitro conditions (Jahnel, Jakob & Nawrath, 1992). However, it is still unclear about the effects of α_1-adrenergic agonists on human cardiac I_{K1} channels despite the fact that variety of human cardiac ion channels have been characterized (Heidbuchel, Vereecke & Carmeliet, 1990; Sato et al., 1990; Koumi & Wasserstrom, 1994; Koumi et al., 1994*b*).

The purpose of the present study was to determine if human atrial I_{K1} channels can be modulated by α_1-adrenergic stimulation.

Materials and Methods

Human Cardiac Specimens and Cell Isolation

Adult human atrial specimens were obtained from patients undergoing cardiac surgery. Institutional and National Institutes of Health guidelines for human experimentation were followed in obtaining surgical specimens and informed consent was obtained from all subjects. A total of 20 patients without atrial disease were studied (33–73 years; median age 47 years). Twelve patients were male and eight female.

Seventeen patients underwent surgery for ischemic heart disease and three for ischemic heart disease with valvular heart disease. None of the patients had symptomatic congestive heart failure, without elevation of mean right atrial pressure (3.6 ± 0.9 mmHg, n = 20, all patients < 5 mmHg) and without atrial enlargement as diagnosed by ultrasound cardiography. All patients were in sinus rhythm (SR). The administration of cardiac drugs was stopped 48 hr before surgery. Immediately after surgical explanation, the specimen was placed in a chilled transport solution and carried to the laboratory within one hr.

Human atrial myocytes were isolated by an enzymatic dissociation method identical to that described previously (Koumi et al., 1994*b*). Briefly, specimens were minced into small pieces using a fine razor and washed three times, 7 min each time, in oxygenated Ca^{2+}-free Tyrode's solution. The tissues were then incubated in oxygenated Ca^{2+}-free Tyrode's solution containing 300–350 U/ml collagenase (Sigma, Type V, St. Louis, MO), 0.5 U/ml protease (Sigma, Type XXIV) and 1 mg/ml of bovine serum albumin (Sigma) at 37°C, and gently stirred with a magnetic stirring bar until isolated myocytes appeared (~40 min). The tissue was then strained through a 200 μm nylon mesh to collect the individual myocytes. Myocytes were stored at room temperature in a modified Kraftbruhe (KB) medium (Isenberg & Klockner, 1982). The residual nondigested tissue was reincubated in enzyme-containing solution for an additional 10 min and isolated myocytes collected in the similar manner as above. This process was repeated until viable myocytes could no longer be isolated. Only Ca^{2+}-tolerant, clearly-striated, rod-shaped cells without any blebs were studied. All specimens were obtained from the right atrial appendage.

Solution

The external solution contained (in mM): NaCl 140.0, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 5.0, glucose 5.0 (pH = 7.4 with NaOH). The composition of internal solution used for the action potential and whole-cell recording was (in mM): K-aspartate 120, KCl 20, KH_2PO_4 1.0, $MgCl_2$ 1.0, Na_2-ATP 5.0, ethyleneglycol-bis(β-aminoethylether)-N,N′-tetraacetic acid (EGTA) 5.0, HEPES 5.0 (pH = 7.2). Pipette solution for both cell-attached and inside-out patch recordings and bath solution used for cell-attached patch recordings were (in mM): KCl 150, HEPES 5.0 (pH = 7.4). Bath solution used for the inside-out patch recordings (cytosolic side of the membrane) contained (in mM): K aspartate 129, KCl 20, KH_2PO_4 1.0, $MgCl_2$ 1.0, Na_2ATP 5.0, HEPES 5.0, EGTA 5.0 (pH = 7.2). Phorbol 12-myristate 13-acetate (PMA), the membrane permeable activator of PKC, was dissolved in DMSO. N-(2-aminoethyl)-5-isoquinolinesulfonamide (H-9) was applied to the external solution in whole-cell experiments. Purified PKC was prepared as described previously (Woodgett & Hunter, 1987). The selective PKC inhibitor, PKC 19–36, was prepared as described previously (House & Kemp, 1987; Alexander et al., 1989).

Electrical Recording

Membrane currents were recorded using the patch clamp technique (Hamill et al., 1981; Koumi, Sato & Aramaki, 1994*a*; Koumi et al., 1994*c*) and an Axopatch-1C amplifier (Axon Instruments, Foster City, CA). The pipettes were pulled in two stages from hard glass tubing (Narishige Scientific Instruments Laboratories, Tokyo, Japan) using a vertical microelectrode puller (Type PE-2, Narishige Scientific Instruments Laboratories, Tokyo, Japan) and then fire-polished using a microforge (Model MF-83, Narishige Scientific Instruments Laboratories, Tokyo, Japan). Electrodes had resistances of 2–3 MΩ for whole-cell recording and 5–10 MΩ for single-channel recording when filled with appropriate standard internal solutions. The action potential was measured by the whole-cell current-clamp mode. Ramp voltage-clamp pulses were applied from a holding potential of −120 to +40 mV at a rate of 100 mV/sec. The current-voltage (*I-V*) relationships were obtained during ramps. Single-channel currents were monitored with a storage oscilloscope (Type 5113, Tektronix, Beaverton, OR) and were stored continuously on digital audiotape (R-60DM, MAXELL, Tokyo, Japan) using a PCM data recording system (RD-100T, TEAC, Tokyo, Japan). The single-channel events were reproduced and filtered off-line with a cutoff frequency of 2 kHz through an eight-pole low-pass Bessel filter (48 dB/octave, Model 902-LPF, Frequency Devices, Haverhill, MA), digitized with 14-bit resolution at a sample rate of 10 kHz. The holding potential was set at −40 mV. The data were analyzed on a computer (PC-9801, NEC, Tokyo, Japan) using locally written analysis programs that are based on the half-amplitude threshold analysis method of Colquhoun & Sigworth (1983). Temperature of the bath was monitored and was maintained at 37 ± 1°C using a Peltier thermoelectrical device. The results are expressed as mean ± SD. Statistical analysis was performed using Student's *t*-test or ANOVA; the results are considered statistically significant when $P < 0.05$.

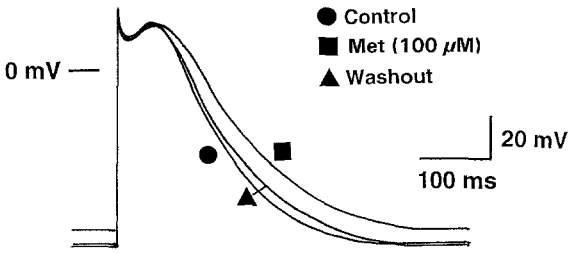

Fig. 1. Effect of methoxamine on the action potential in isolated human atrial myocytes. Representative action potential and the response to methoxamine in an isolated atrial myocyte recorded under the whole-cell current-clamp configuration at 37°C. Following application of methoxamine (100 μM) to the bath solution, prolongation of the action potential occurred (to 121.4% of control measured at 90% repolarization) with a small depolarization of the resting membrane potential (6 mV, square). The action potential recovered partially following washout of methoxamine (triangle).

Results

Action Potential Response to Methoxamine in Isolated Human Atrial Myocytes

Figure 1 shows representative action potentials recorded from an isolated human atrial myocyte obtained in the absence (circle) and presence (square) of the α_1-adrenergic agonist, methoxamine (100 μM), in the external solution and after washout of methoxamine (triangle) at the physiological temperature (37°C). Bath exposure to methoxamine caused prolongation of the action potential with a small depolarization of the resting membrane potential. In addition, the rate of repolarization (phase 3) during exposure to methoxamine became slower than in the control, so that the prolongation of the action potential duration at 90% of repolarization (APD_{90}) was greater than at 50% repolarization (APD_{50}).

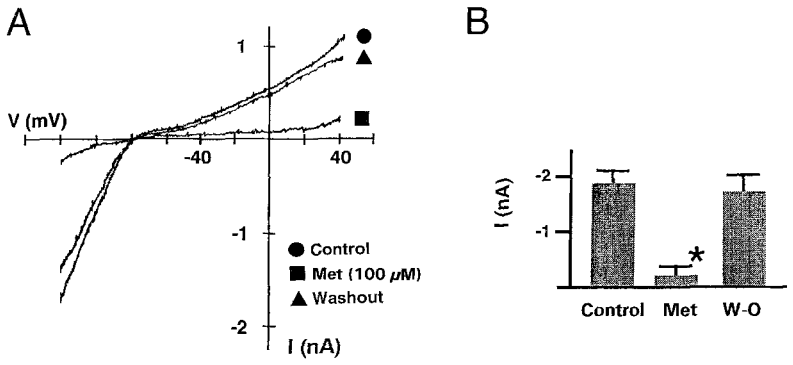

Fig. 2. Effect of methoxamine on the whole-cell I_{K1} measured by the ramp voltage clamp in isolated human atrial myocytes. (*A*) Representative current-voltage (*I-V*) curves of control whole-cell I_{K1} currents recorded in normal Tyrode's solution at 37°C. Tetrodotoxin (TTX, 5 μM), Co^{2+} (2 mM), 4-aminopyridine (4-AP, 2 mM) and nifedipine (5 μM) were added to the external solution. The *I-V* relationships for the membrane currents were measured during the control period (circle), during exposure to 100 μM methoxamine (square) and after wash out of methoxamine (triangle). (*B*) Averaged whole-cell current amplitude at −120 mV during control (1.89 ± 0.24 nA, n = 8), during exposure to 100 μM methoxamine (0.21 ± 0.16 nA, n = 8) and after washout of methoxamine (1.68 ± 0.30 nA, n = 8). *P < 0.001 different from other groups by ANOVA.

APD_{90} was 261.5 ± 22.1 msec (n = 14) in the control and 315.4 ± 24.9 msec (n = 14, P < 0.01) during exposure to methoxamine. After washout of methoxamine, APD_{90} recovered to almost the control level (279.0 ± 24.5 msec, n = 14). The action potential duration at 50% was 155.8 ± 13.0 msec (n = 14) in the control and 179.2 ± 14.1 msec (n = 14, P < 0.05) during exposure to methoxamine.

Methoxamine-induced Inhibition of Whole-Cell I_{K1}

Figure 2*A* illustrates the whole-cell current-voltage (*I-V*) relationships obtained in the absence (circle), during superfusion (square), and after washout of 100 μM methoxamine (triangle). Ramp voltage-clamp pulses were applied from a holding potential of −120 to +40 mV at the 37°C. Tetrodotoxin (TTX, 5 μM), Co^{2+} (2 mM), 4-aminopyridine (4-AP, 2 mM) and nifedipine (5 μM) were added to the external solution. In these conditions, bath-applied methoxamine could reversibly suppress the whole-cell I_{K1} conductance. Similar results were obtained in 14 of 15 cells. Figure 2*B* illustrates the averaged current magnitudes in the control, during superfusion and after washout of methoxamine. The current magnitude during superfusion with methoxamine (0.21 ± 0.16 nA, n = 8) was significantly smaller than control (1.89 ± 0.24 nA, n = 8) and after washout of methoxamine (1.68 ± 0.30 nA, n = 8, P < 0.001). Adding the Cl^- channel blocker, 9-anthracenecarboxylic acid (9-AC, 0.1 mM), to the bath solution did not alter the methoxamine-induced inhibition of I_{K1}.

Figure 3 characterized the concentration-dependence of the effect of methoxamine to inhibit I_{K1}. At −120 mV the methoxamine-induced inhibition could be detected at a methoxamine concentration as low as 3 μM and the inhibitory effect being nearly saturated at approximately 100 μM methoxamine. The concentration-response curve was fitted by the least-squares method to the Hall equation:

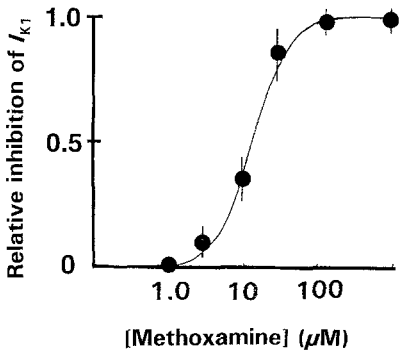

Fig. 3. Concentration-dependence of the inhibition of whole-cell I_{K1} by methoxamine. The normalized methoxamine-concentration dependence of the inhibition of the whole-cell I_{K1} at −120 mV. Inhibition by methoxamine was saturated at 100 μM. Analysis of the concentration dependence yielded an apparent dissociation constant (IC_{50}) of 18 μM and a Hill coefficient of ~2. Data plotted as mean ± SD (n = 5–10).

$$y = 1/\{1 + (IC_{50}/[M]^H\} \quad (1)$$

where IC_{50} is the concentration at the half-maximal effect and H is the Hill coefficient. The half-maximal inhibition (IC_{50}) occurred at 18 μM and the Hill coefficient value was ~2.

Figure 4 illustrates the effect of the α_1-blocker, prazosin, on the methoxamine-induced inhibition of I_{K1}. After prazosin (2 μM) had been present in the bath perfusate for 5 min, subsequent exposure to methoxamine (100 μM) did not inhibit I_{K1} (Fig. 4*A*). In the absence of prazosin (control), averaged I_{K1} current amplitude at −120 mV was inhibited to 11.9 ± 5.0% of control (P < 0.001, n = 8, Fig. 4*B* top panel). In contrast, in the presence of prazosin, averaged I_{K1} current amplitude at −120 mV was not significantly different from control level (n = 8, Fig. 4*B* bottom panel). β-Adrenergic blocker, propranolol (1 μM), did not prevent methoxamine-induced inhibition of I_{K1} in five of five cells. These results indicate that methoxamine can inhibit I_{K1} by a mechanism involving α_1-adrenergic receptors.

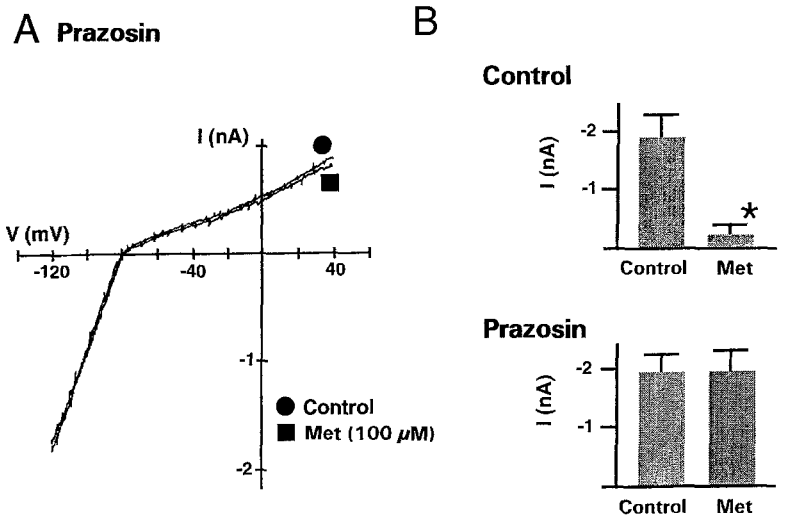

Fig. 4. Effect of methoxamine on I_{K1} in the presence of prazosin. (*A*) Representative current-voltage (*I-V*) curves for I_{K1} obtained during the control period (circle) and during exposure to methoxamine (100 μM, square) in the presence of prazosin (2 μM) in the bath. (*B*) (top panel): Averaged whole-cell current amplitude at −120 mV during control (1.89 ± 0.35 nA, n = 8) and during exposure to 100 μM methoxamine (0.22 ± 0.16 nA, n = 8, *P < 0.001 different from control) in the absence of prazosin. (bottom panel): Averaged whole-cell current amplitude (nA) at −120 mV during control (1.92 ± 0.29 nA, n = 8) and during exposure to 100 μM methoxamine (1.96 ± 0.36 nA, n = 8, not different statistically from control) in the presence of prazosin (2 μM) in the bath.

Effect of Protein Kinase C

To address the question of whether the inhibitory effect of methoxamine on I_{K1} involves activation of protein kinase C (PKC), the effect of an activator of PKC, phorbol ester (PMA), on macroscopic I_{K1} was determined. As illustrated in Fig. 5*A*, application of PMA (10 nM) to the bath solution inhibited the current. The current magnitude during superfusion with PMA (0.27 ± 0.14 nA, n = 11) was significantly smaller than control (1.99 ± 0.23 nA, n = 11) and after washout of PMA (1.85 ± 0.29, P < 0.001). Further support for PKC-induced inhibition of the current was provided by experiments with H-9, a specific blocker of PKC (Suzuki et al., 1991). As illustrated in Fig. 5*B*, methoxamine failed to inhibit I_{K1} in the presence of H-9 (10 μM). The current magnitude during superfusion with PMA (1.98 ± 0.25 nA, n = 7) was not significantly different from control (2.00 ± 0.23 nA, n = 7, NS) when H-9 was present. These results suggest that methoxamine inhibits I_{K1} by a mechanism involving α_1-adrenergic receptors-PKC pathways.

Effect of Methoxamine on Single I_{K1} Channel Activity

Figure 6 shows that bath application of methoxamine (100 μM) can reversibly inhibit I_{K1} channel activity recorded from a cell-attached patch. Single I_{K1} channel behavior in control (Fig. 6) exhibits slow bursting kinetics separated by long interburst period that are similar to typical human single channel I_{K1} described previously (Heidbuchel et al., 1990; Koumi, Backer & Arentzen, 1995). No basal activity of the muscarinic K^+ channel [$I_{K(ACh)}$] was detected which exhibited spike-like opening (Koumi et al., 1994*b*). In human cardiac tissues, basal $I_{K(ACh)}$ activity was hardly detected, similar to the report by Heidbuchel et al. (1990). When the myocyte was exposed to methoxamine (Fig. 6*A*), individual burst duration gradually decreased without affecting the unitary amplitude and resulted in the decrease of the channel open-state probability (P_o). The number of active channels in the patch did not change by methoxamine (0.4 ± 0.1/patch, n = 19, in the control and 0.4 ± 0.1/patch, n = 15, with methoxamine). After washout of methoxamine, P_o recovered to almost the control level (Fig. 6*B*). The averaged P_o was 0.77 ± 0.15 (n = 11) in the control and 0.20 ± 0.13 (n = 11, P < 0.001) during exposure to methoxamine (100 μM, Fig. 6*C*). P_o recovered to almost the control level after washout of methoxamine (0.71 ± 0.13, n = 11, not different statistically from control).

Effect of Purified PKC on I_{K1} in Inside-Out Patches

The nature of the methoxamine-induced inhibition of I_{K1} channel activity in cell-attached patches was further investigated using excised inside-out patches. When purified PKC (10 nM) was directly applied to the cytosolic side of an inside-out patch, channel activity was inhibited (Fig. 7*A*). The effect of purified PKC to mimic the effect of bath-applied methoxamine on cell-attached patch I_{K1} channel was confirmed in nine other inside-out patches. In contrast, subsequent application of the PKC selective inhibitor, PKC19-36 (1 μM), to the bath solution antagonized the PKC-induced channel inhibition (Fig. 7*B*) in seven of seven cells.

Discussion

The major findings in this study are as follows: (i) α_1-Adrenergic stimulation by methoxamine produced action potential prolongation and a depolarization of the resting membrane potential in isolated human atrial myocytes. (ii) Methoxamine inhibited whole-cell I_{K1} in a concentration dependent fashion. (iii) The current was inhibited similarly by PMA. In contrast, methoxamine failed to inhibit the current in the presence of a specific protein kinase C inhibitor H-9. (iv) Methoxamine could suppress unitary I_{K1} channel activity by decreasing the fre-

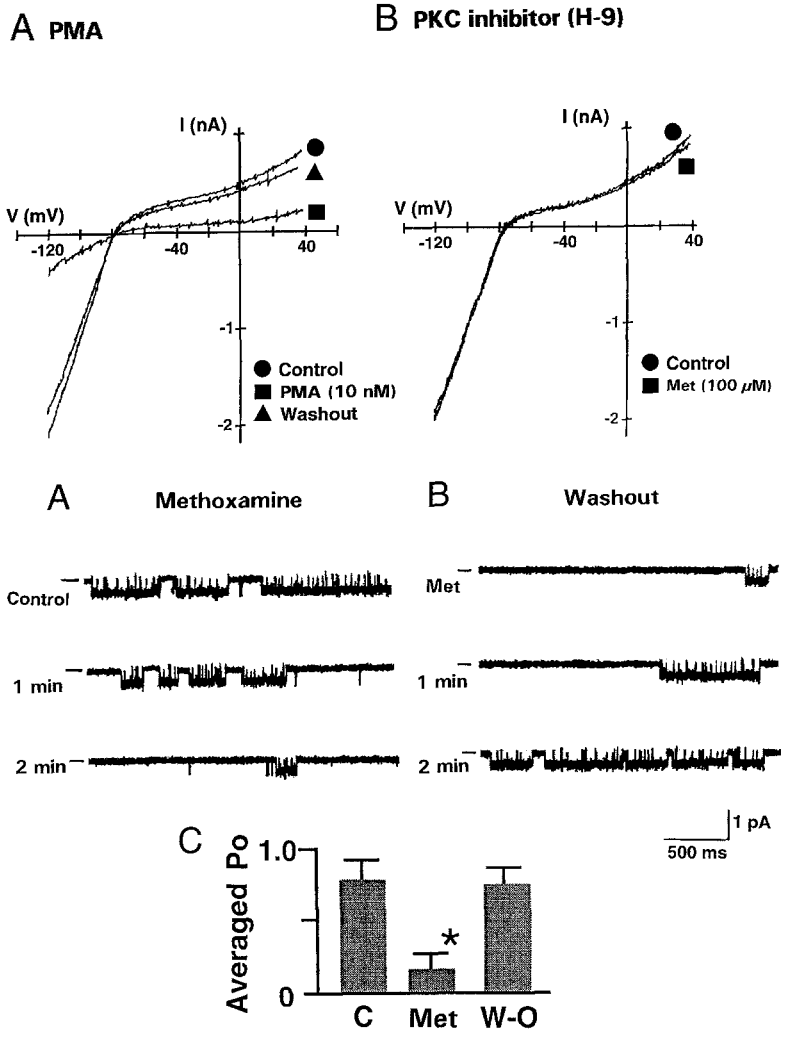

Fig. 5. Effect of Phorbol 12-myristate 13-acetate (PMA) and N-(2-aminoethyl)-5-isoquinolinesulfonamide (H-9) on I_{K1}. (*A*) Representative current-voltage (*I-V*) curves of whole-cell I_{K1} recorded during the control period (circle), during exposure to 10 nM PMA (square) and after washout of PMA (triangle). (*B*) Representative *I-V* curves for I_{K1} obtained during the control period (circle) and during exposure to 100 μM methoxamine (square) in the presence of H-9 (10 μM) in the bath.

Fig. 6. Effect of methoxamine on the single channel I_{K1} recorded in cell-attached patches in human atrial myocytes. (*A*) Representative example of current recorded from I_{K1} channels in a cell-attached patch that gradually decreased P_o after exposure to methoxamine (100 μM) applied via the bath. Prior to applying methoxamine, channel activity was continuously observed for more than 10 min. One min after introduction of methoxamine, the channels being open gradually decreased without affecting the unitary amplitude. The closed state (baseline level) was indicated as the bar at the left of each current trace. (*B*) After washout of methoxamine, channel activity recovered to almost the control level. (*C*) Averaged P_o in control, during exposure to methoxamine (100 μM), and after washout of methoxamine were measured in twelve different cells. The vertical bar through each bar represents the SD. $^*P < 0.001$ different from other groups by ANOVA.

quency and duration of bursting. (v) Purified PKC applied to excised inside-out patches inhibited channel activity similar to methoxamine in cell-attached patches. The PKC selective inhibitor, PKC19-36, prevented the PKC-induced inhibition of the channel. These results suggest that human atrial I_{K1} can be inhibited by α_1-adrenergic stimulation via PKC-dependent pathways.

The present results show that α_1-adrenergic stimulation can reversibly inhibit I_{K1} in isolated human atrial myocytes. Jahnel et al. (1992) demonstrated a positive inotropic response to α_1-adrenergic receptor stimulation in human atrial heart muscle without significant changes of the action potential. Although the exact reason of this difference is unknown, it may be caused by the different experimental conditions. α_1-Adrenergic inhibition of I_{K1} has been reported in rabbit atrial and ventricular myocytes (Braun et al., 1992; Fedida et al., 1991), rat atrial (Jahnel et al., 1991) and ventricular muscle (Apkon & Nerbonne, 1988; Ravens et al., 1989) and canine Purkinje myocytes (Shah et al., 1988). Braun et al. (1992) suggested that α_1-adrenergic stimulation does not involve the activation of PKC. Jahnel et al. (1991) demonstrated that phorbol 12,13-dibutyrate abolished the effects of α_1-adrenergic stimulation and suggested a negative feedback control of PKC to terminate the effects of α_1-adrenergic stimulation. In contrast, different from these reports, the methoxamine-induced inhibition appears to occur via PKC-dependent pathway in human atrial myocytes. The result suggests that the effects of α_1-adrenergic stimulation on I_{K1} are species and/or tissue-dependent. Radioligand binding studies have shown that the density of α_1-adrenergic receptors is species-dependent. Densities of α_1-adrenergic receptors in cat, dog, calf, primates and rabbit are virtually the similar levels (Mukherjee et al., 1983; Saffitz, 1989; Fedida, Braun & Giles, 1993 *for review*). In contrast, density in humans is below the range observed in other mammalian species (Ferry & Kaumann, 1987; Fedida, Braun & Giles, 1993 *for review*). In addition to these differences, the sensitivity of the channel itself to agonist stimulation may be different among species. Another possible reason for differences between present results and those of

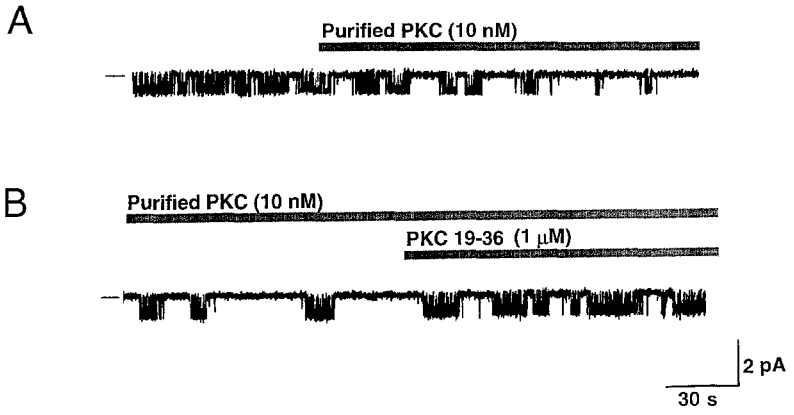

Fig. 7. Effect of purified PKC on the single channel I_{K1} recorded in inside-out patches in human atrial myocytes. (*A*) The effect of bath-applied purified PKC on the current. Channel activity was recorded during the period prior to exposure of the patch to purified PKC (10 nM) via the bath. After purified PKC was introduced into the bath, the channel was inhibited. The closed state (baseline level) was indicated as the bar at the left of each current trace. (*B*) The PKC-activated channel current recorded from another excised inside-out patch. The selective inhibitor of PKC, PKC19-36, treatment can antagonize the inhibition of the channel by purified PKC in an inside-out patch. Following inhibition of the channel that had been induced by exposing the cytosolic surface of an inside-out excised patch to purified PKC (10 nM), PKC19-36 (1 μM), applied to the cytosolic side of the membrane, resulted in reactivation of the channel. Currents were recorded with low-pass filtering at 2 kHz.

others may be caused by our use of relatively selective pharmacological tools.

From the recent cloning of an inwardly-rectifying K^+ (IRK) channel gene from mouse macrophage (IRK1 by Kubo et al., 1993), IRK1 has provided a tool with which to probe the composition of IRKs in a variety of mammalian hearts (Kubo et al., 1993; Dascal et al., 1993; Ashford et al., 1994). The IRK family includes GIRK1 (Kubo et al., 1993), KGA (Dascal et al., 1993) and rcKATP (Ashford et al., 1994). In addition, it has been demonstrated that the amino acid sequence of the cloned channel from human atrium is virtually identical to IRK1 (Wible et al., 1995). It is expected that α_1-adrenergic agonists can modulate this type of IRK1 in human atrium.

Inhibition of the outward current at positive potentials cannot simply be explained by an influence on I_{K1} (Jahnel et al., 1991). The transient outward current (I_{to}) (Apkon & Nerbonne, 1988; Fedida, Shimoni & Giles, 1989; Fedida, Shimoni & Giles, 1990) and a time-independent current in the plateau level (I_{Kp}) (Yue & Marban, 1988) may be affected by methoxamine. The experimental condition in the present study was selected to remove current systems other than I_{K1}. To eliminate sodium (I_{Na}) and L-type calcium current (I_{Ca}), tetrodotoxin (TTX, 5 μM), nifedipine (5 μM) and Co^{2+} (2 mM) were added to the external solution. The delayed-rectifier K^+ channel was also eliminated by the acute ramp voltage-clamp protocol (*see* Materials and Methods). 4-aminopyridine (4-AP, 2 mM) was added to the external solution to inhibit the KV 1.5 type delayed-rectifier K^+ current (HK2) that was recently cloned from human heart (Tamkun et al., 1991; Snyders et al., 1992; Coraboeuf & Nargeot, 1993). In addition, lack of effect of 9-AC on the methoxamine-induced inhibition of I_{K1} suggests that basal Cl^- conductance may not contaminate the background conductance. However, the *I-V* relationship of I_{K1} showed no negative slope nor does it show zero slope region in the present study. The leakage conductance and related overlapping conductances were not totally removed during the ramp-clamp recordings even after removal of other ionic current systems as described above. These nonselective overlapping currents could not be totally removed in the acute ramp voltage-clamp pulse protocol.

Activation of phospholipase C by α_1-adrenergic stimulation increases the production of inositol phosphates and diacylglycerol which stimulates PKC (Nishizuka, 1984; Exton, 1985). On the other hand, the breakdown of phosphatidyl inositol biphosphate (PIP_2) generates inositol triphosphate ($InsP_3$) which activates sarcoplasmic reticulum Ca^{2+}-release channel (Sterb et al., 1983; Berridge & Irvine, 1984; Nosek et al., 1986). Because intracellular Ca^{2+} can inhibit outward directed conductance of I_{K1} (Mazzanti & DiFrancesco, 1989), α_1-adrenergic stimulation can inhibit outward I_{K1} via phospholipase C-$InsP_3$ pathways. Further study is needed to determine the effect of the α_1-adrenergic stimulation-phospholipase C-$InsP_3$ pathways on I_{K1}.

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