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Characterization of the Acetylcholine-sensitive Muscarinic K^+ Channel in Isolated Feline Atrial and Ventricular Myocytes

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Abstract. M_2-cholinergic receptor activation by acetylcholine (ACh) is known to cause a negative inotropic and chronotropic action in atrial tissues. This effect is still controversial in ventricular tissues. The ACh-sensitive muscarinic K^+ channel ($I_{K(ACh)}$) activity was characterized in isolated feline atrial and ventricular myocytes using the patch-clamp technique. Bath application of ACh (1 μM) caused shortening of action potential duration without prior stimulation with catecholamines in atrial and ventricular myocytes. Resting membrane potential was slightly hyperpolarized in both tissues. These effects of ACh were greater in atrium than in ventricle. ACh increased whole-cell membrane current in atrial and ventricular myocytes. The current-voltage (*I-V*) relationship of the ACh-induced current in ventricle exhibited inward-rectification whose slope conductance was smaller than that in atrium. In single channel recording from cell-attached patches, $I_{K(ACh)}$ activity was observed when ACh was induced in the pipette solution in both tissues. The channel exhibited a slope conductance of 47 ± 1 pS (mean ± SD, n = 14) in atrium and 47 ± 2 pS (n = 10) in ventricle (not different statistically; NS). The open times were distributed according to a single exponential function with mean open lifetime of 2.0 ± 0.3 msec (n = 14) in atrium and 1.9 ± 0.3 msec (n = 10) in ventricle (NS); these conductance and kinetic properties were similar between the two tissues. However, the relationship between the concentration of ACh and single channel activity showed a higher sensitivity to ACh in atrium (IC_{50} = 0.03 μM) than in ventricle (IC_{50} = 0.15 μM). In excised inside-out patches, ventricular $I_{K(ACh)}$ required higher concentrations of GTP to activate the channel compared to atrial channels. These results suggest a reduced $I_{K(ACh)}$ channel sensitivity to M_2-cholinergic receptor-linked G protein (G_i) in ventricle compared to atrium in feline heart.

Key words: Patch clamp technique — Acetylcholine — Muscarinic K^+ channel — GTP — G protein

Introduction

M_2-cholinergic receptor stimulation by acetylcholine (ACh) is known to cause a negative inotropic and chronotropic action in atrial tissues (Loffelholz & Pappano, 1985, for review). This effect is associated with a reduction of inward Ca^{2+} current (I_{Ca}) and an increase of an outward K^+ conductance (Loffelholz & Pappano, 1985, for review). These muscarinic actions do not require prior stimulation by catecholamines (Inoue, Hachisu & Pappano, 1983). Previous reports have confirmed the direct activation of the muscarinic K^+ channel ($I_{K(ACh)}$) by G protein in amphibian (Breitwieser & Szabo, 1985), embryonic chick (Pfaffinger et al., 1985) and mammalian atrial myocytes (Kurachi, Nakajima & Sugimoto, 1986; Koumi et al., 1994*a*).

In contrast to atrial and pacemaker tissues, previous studies showed that muscarinic activation of the ventricular tissue involves a reduction of I_{Ca} alone (Giles & Noble, 1976; Hino & Ochi, 1980; Josephson & Sperelakis, 1982; Hescheler, Kameyama & Trautwein, 1986; Antzelevitch et al., 1991). According to these studies, ACh is thought to have only an indirect effect on ventricular tissue that had not received prior stimulation by catecholamines. Recently, Hartzell & Simmons (1987)

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reported that the ACh-induced K^+ conductance is responsible for negative chronotropic effect of muscarinic stimulation in frog ventricular tissues using the whole-cell patch-clamp technique. Boyett et al. (1988) showed the shortening of action potential duration by ACh in ferret ventricular myocytes which is induced by increased background K^+ conductance. However, the direct action of ACh on ventricular tissues is still undetermined.

In this study, we have focused on $I_{K(ACh)}$ activity in ventricular tissue and have compared their properties to those in atrial tissues in feline heart.

Materials and Methods

Cell Preparation

Feline atrial and ventricular myocytes were isolated in a manner similar to that described previously (Silver et al., 1983; Harvey & Ten Eick, 1988; Martin, Koumi & Ten Eick, 1995). Cats were anaesthetized with either sodium pentobarbital (42.0 mg/kg body weight, i.p.) or ketamine HCl (11.0 mg/kg body weight, IM) followed by sodium pentobarbital (24.3 mg/kg body weight, i.v.). Hearts were excised and coronary arteries were retrogradely perfused following cannulation of the aorta. After 2–3 min of perfusion with a Ca^{2+}-free Krebs-Henseliet buffer solution (KHB), the heart was perfused with KHB containing 0.15% collagenase (Worthington, Type II). After 30–40 min, perfusion was stopped. Atrial and ventricular tissues were then minced, and incubated in a shaker bath for 5–10 min in collagenase-containing solution. The remaining tissue pieces were removed by filtering, and cells were washed free of collagenase and stored in KHB containing 1% albumin and 1mM Ca^{2+}. Both isolated atrial and ventricular cells were quiescent and had a resting membrane potential less than −80 mV.

Solutions

The control Tyrode's solution contained (in mM): NaCl 140.0, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 5.0, glucose 5 (pH = 7.4). Ca^{2+}-free Tyrode's solution was made by omitting $CaCl_2$ from the normal Tyrode's solution. The composition of KHB solution was (in mM): NaCl 130, KCl 4.8, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 12.5 at pH 7.4. For whole-cell voltage-clamp recordings, the external solution was normal Tyrode's. To block L-type Ca^{2+} current (I_{Ca}) and Na^+ current (I_{Na}), nifedipine (5 μM) and tetrodotoxin (TTX, 10 μM) were added to the normal Tyrode's solution. The composition of internal pipette solution for whole-cell recording was (in mM): potassium aspartate 110, KCl 20, KH_2PO_4 1.0, $MgCl_2$ 1.0, Na_2-ATP 5.0, HEPES 5.0 and EGTA 5.0 (pH = 7.2). Internal solution used for both cell-attached and inside-out patch recordings contained (in mM): KCl 150, HEPES 5.0 (pH = 7.4). Normal Tyrode's solution was used for the external solution in cell-attached patch recordings. In excised inside-out patch recordings, the cytosolic surface of the membrane was perfused with a bath solution containing (in mM): K aspartate 120, KCl 30, Na_2-ATP 5, $MgCl_2$ 1, HEPES 5 and EGTA 5 (pH = 7.2). ACh (1–20 μM) and atropine (1 μM) were added to the pipette solution. Guanine nucleotide (GTP, 100 μM) was added to the bath solution in inside-out patch recordings. Pertussis toxin (PTX) was dissolved in KB solution at a final concentration of 5 μg/ml with albumin (3 mg/ml) during myocyte incubations for up to 90 min at 35°C.

The experimental chamber (0.08 ml) was continuously perfused with solution at a rate of 6–7 ml/min, and complete solution exchange were achieved within 1.3 ± 0.2 sec (mean ± SD, n = 25). To facilitate the rapid exchange of test solutions, they were delivered to the patch from the mouth of a fine bore polyethylene tube positioned within one mm from the patch. Temperature of the bath was monitored and was maintained at 37 ± 0.5°C using a Peltier thermoelectrical device. All agents were purchased from Sigma Chemical Co. (St. Louis, MO).

Whole Cell and Single Channel Recording

Patch clamp recordings were made of whole-cell currents and single channel unitary currents using cell-attached and excised inside-out patch configurations (Hamill et al., 1981; Koumi, Sato & Aramaki, 1994; Koumi et al., 1994*b*) with an Axopatch-1C amplifier (Axon Instruments, Inc., 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). The feedback resistance of the head stage was 500 MΩ for recording whole-cell current and 50 GΩ for recording single channel current. Electrodes with tip resistances of 1.5 to 2.5 MΩ were used to record whole-cell currents and those with 5.0 to 10.0 MΩ for single channel currents. Seal resistances were 50–100 GΩ for recording single channel currents.

For whole-cell recording, the series resistance attributed to the pipette tip and the cell interior was compensated by summing a fraction of the converted current signal to the command potential and feeding it to the positive input of the operational amplifier. Series resistance compensation was done to minimize the time course of the capacitative surge. Cell membrane capacitance (C_m) was calculated using the equation: $C_m = Q/V$, where Q is total charge movement determined by integrating the area defined by the capacitative transient, and V is the voltage step. Mean cell capacitance (C_m) was calculated to be 96 ± 11 pF (mean ± SD, n = 45) in atrial cells and 224 ± 26 pF (n = 46) in ventricular cells. The capacitative transient remaining after series resistance compensation was constant throughout the experiments. The output of the voltage-clamp amplifier was adjusted to give zero current when the tip of the patch pipette (filled with internal solution) was immersed in the bath containing Tyrode's solution. 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. Current obtained within the first 5 mV after the onset of the ramp was not analyzed because of the contamination of the capacitative transient of the membrane. Action potentials were measured in the whole-cell current-clamp mode.

Single-channel currents were monitored with a storage oscilloscope (Type 5113, Tektronix, Inc., Beaverton, OR) and were stored continuously on digital audio tape (R-60DM, MAXELL, Tokyo, Japan) using a PCM data recording system (RD-100T, TEAC, Tokyo, Japan). The recorded single channel events were reproduced and filtered off-line with a cutoff frequency of 5 kHz through an eight-pole low-pass Bessel filter (48 dB/octave, Model 902-LPF, Frequency Devices, Inc., Haverhill, MA), digitized with 14-bit resolution at a sample rate of 10 kHz. 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). When multiple open channel events were observed in a single patch, the total currents were calculated using an averaging technique for determining channel amplitude and channel open probability was estimated from the total amplitude, unitary current amplitude and the functional channel number in the patch. The measurements derived from the channel transitions were collected into histograms to allow an analysis of single channel kinetics. Mean dwell times were determined

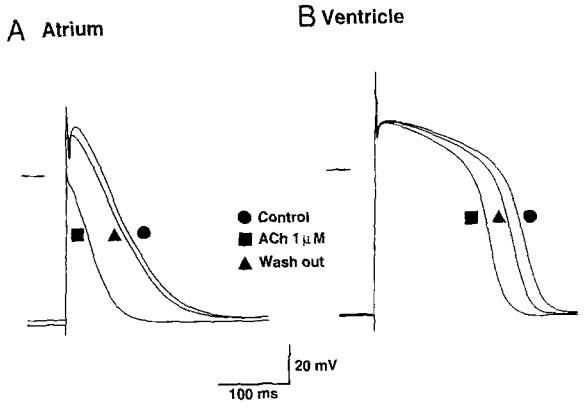

Fig. 1. Effect of ACh on the action potentials in feline atrial and ventricular myocytes. (*A*) Representative action potential and the response to ACh in an atrial myocyte recorded under the whole-cell current-clamp configuration. Following application of ACh (1 μM) to the bath solution, shortening of the action potential occurred with a small hyperpolarization of the resting membrane potential (square). The action potential partially recovered after washout of ACh (triangle). (*B*) Representative action potential and the response to ACh in a ventricular myocyte. The action potential shortened and the resting membrane potential was hyperpolarized in the presence of ACh (square). These effects were partially reversed after washout of ACh (triangle).

from the sum of exponential fits to the distributions of open and closed times recorded from patches with only one channel.

Data Analysis and Statistics

The results are expressed as mean ± SD. Statistical analysis was done using Student's *t*-test. Results were considered to be significant when the $P < 0.05$.

Results

Action Potential Response to ACh in Atrial and Ventricular Myocytes

Figure 1 illustrates typical action potential response to ACh in atrial (Fig. 1*A*) and ventricular (Fig. 1*B*) myocytes. Bath application of ACh (1 μM) caused a shortening of the action potential duration, shifted the plateau level to more negative potentials and increased the resting membrane potentials in both tissues. However, these responses to ACh in ventricular myocytes were much less than in atrium. The shortening of the action potential was partially reversed after washout of ACh in both cells. Table 1 summarizes the action potential parameters and their response to ACh in both cells. The hyperpolarizing shift of the resting membrane potential and shortening of action potential durations at 50% and 90% repolarization by ACh were significantly reduced in ventricular myocytes compared to atrium ($P < 0.001$). These results suggest that the sensitivity of transmembrane potential characteristics to ACh is reduced in ventricle compared to atrium.

Whole-Cell Membrane Current Response to ACh in Atrial and Ventricular Myocytes

Figure 2 shows the whole-cell current-voltage (*I-V*) relationships obtained in control, during superfusion with ACh (1 μM) and after washout of ACh in atrial (Fig. 2A) and ventricular (Fig. 2B) myocytes. Voltage-clamp ramps were applied from a holding potential of −120 to +40 mV at a rate of 100 mV/sec. ACh (1 μM) increased both the inward and outward currents and shifted the reversal potential to more negative potentials in both cells, but both the original current magnitude and the response to ACh were different in the two cells. Table 2 summarizes the whole-cell current parameters and their response to ACh in both tissues. The increase in whole-cell currents by ACh in ventricle was less than in atrium: the normalized slope conductance (pS/pF) and the normalized current density (pA/pF) in the ACh-sensitive current in ventricle were significantly smaller than those in atrium ($P < 0.001$). These results are consistent with those obtained from the action potential measurements.

Muscarinic K^+ Channel Activity in Atrial and Ventricular Myocytes

Figure 3 shows the unitary $I_{K(ACh)}$ currents in isolated atrial and ventricular myocytes recorded in cell-attached patches with 1 μM ACh in the pipette solution bathed in the normal Tyrode's. The original current recordings in Fig. 3*A* show channel activity typical of the fast open-close kinetics of $I_{K(ACh)}$. The channel exhibited brief openings during individual bursts. When atropine (1 μM) was included in the pipette solution or myocytes were preincubated with pertussis toxin (PTX, 5 μg/ml), ACh failed to activate the channel even at a concentration of 20 μM in the pipette in both cells (n = 9 in atrium, n = 8 in ventricle, data not shown). Channel activity was observed in 81.8% (36/44) of patches in atrium compared to 32.3% (21/65) of patches in ventricle. The inward *I-V* relationships for both cells were almost linear (Fig. 3*B*). Outward currents were not detected at holding voltages more positive than zero current potential. The single channel slope conductance was 47 ± 1 pS (n = 14) in atrium and 47 ± 2 pS (n = 10) in ventricle (not different statistically; NS).

Figure 4 shows representative histograms of single channel open time distributions in atrium (Fig. 4*A*) and in ventricle (Fig. 4*B*). Open time distributions could be best fitted by a single exponential function in both cells. The open times were distributed according to a single exponential function in both cells with averaged mean

Table 1. Action potential parameters in atrial and ventricular myocytes

	RP (mV)	APD_{50} (msec)	APD_{90} (msec)
Atrium (n = 8)			
Control	−81.0 ± 4.5	93.7 ± 8.8	151.4 ± 14.2
ACh	−83.4 ± 4.9	32.8 ± 4.9[b]	65.3 ± 7.0[b]
Difference	2.4 ± 0.4	34.9 ± 3.7 (%)	43.0 ± 4.5 (%)
Ventricle (n = 8)			
Control	−81.2 ± 4.7	206.5 ± 19.3	230.7 ± 25.1
ACh	−82.0 ± 5.0	166.7 ± 15.0[a]	176.5 ± 18.2[a]
Difference	0.8 ± 0.3[c]	80.8 ± 9.3 (%)[c]	78.2 ± 9.6 (%)[c]

RP, resting membrane potential; APD_{50} and APD_{90}, action potential duration measured at 50% and 90% repolarization, respectively; and n, number of cells. Values are means ± SD.
[a] $P < 0.01$ different from control, [b] $P < 0.001$ different from control and [c] $P < 0.001$ different from atrium using t test.

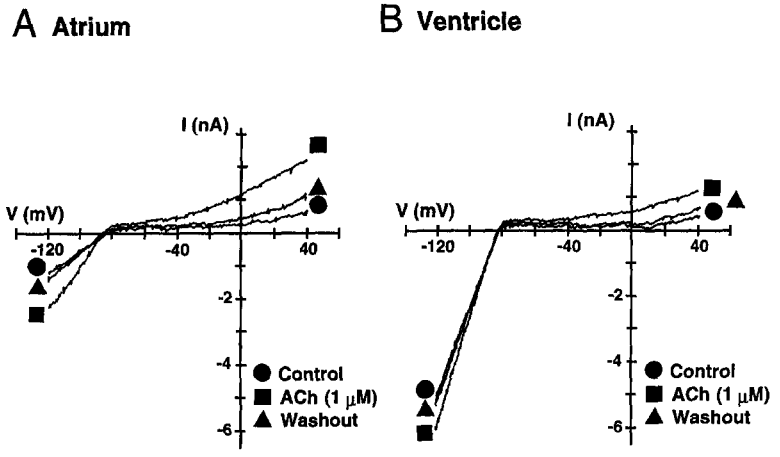

Fig. 2. The whole-cell current-voltage (I-V) relationships and the responses to ACh in feline atrial and ventricular myocytes measured by the ramp voltage clamp. (*A*) Representative whole-cell membrane current I-V relationship and the response to ACh (1 μM) in an isolated atrial myocyte. The I-V relationships for the membrane currents were measured during the control period (circle), during exposure to ACh (square) and after wash out of ACh (triangle). (*B*) Representative whole-cell membrane current I-V relationship and the response to ACh (1 μM) in an isolated ventricular myocyte. The I-V relationships for the membrane currents were measured during the control period (circle), during exposure to ACh (square) and after wash out of ACh (triangle).

Table 2. Whole-cell current parameters in atrial and ventricular myocytes

	E_K (mV)	γ (pS/pF)	D (pA/pF)
Atrium (n = 9)			
Control	−80.4 ± 4.2	38.2 ± 3.3	13.5 ± 2.0
ACh	−82.9 ± 5.0	80.7 ± 5.9[b]	26.4 ± 2.5[b]
$I_{K(ACh)}$	−83.3 ± 3.8	42.7 ± 4.4	12.9 ± 2.1
Ventricle (n = 7)			
Control	−80.6 ± 4.4	120.5 ± 16.1	23.8 ± 2.1
ACh	−81.7 ± 5.1	132.9 ± 17.0[a]	27.2 ± 2.5[a]
$I_{K(ACh)}$	−82.9 ± 4.0	12.5 ± 1.3[c]	3.4 ± 0.3[c]

E_K, reversal potential; γ, normalized slope conductance at E_K; D, normalized current density measured at −120 mV; $I_{K(ACh)}$, ACh-sensitive current obtained by subtracting the current in the presence of ACh from control; and n, number of cells. Values are means ± SD.
[a] $P < 0.05$ different from control, [b] $P < 0.001$ different from control and [c] $P < 0.001$ different from atrium using t test.

open lifetime of 2.0 ± 0.3 msec (n = 14) in atrium and 1.9 ± 0.3 msec (n = 10) in ventricle (NS). These values are consistent with other mammalian species (Sakmann, Noma & Trautwein, 1983; Soejima & Noma, 1984; Kurachi et al., 1986; Kirsch et al., 1988; Heidbuchel, Vereecke & Carmeliet, 1990; Sato et al., 1990). These results suggest that feline ventricular myocytes share the same population of $I_{K(ACh)}$ as in atrium. However, the estimated functional channel number in atrial myocytes was greater than that in ventricular myocytes.

Figure 5 illustrates the dependence of single $I_{K(ACh)}$ channel activity on ACh concentration in atrial and ventricular myocytes in cell-attached patches. Channel activity was estimated by NP_o, where N is the number of

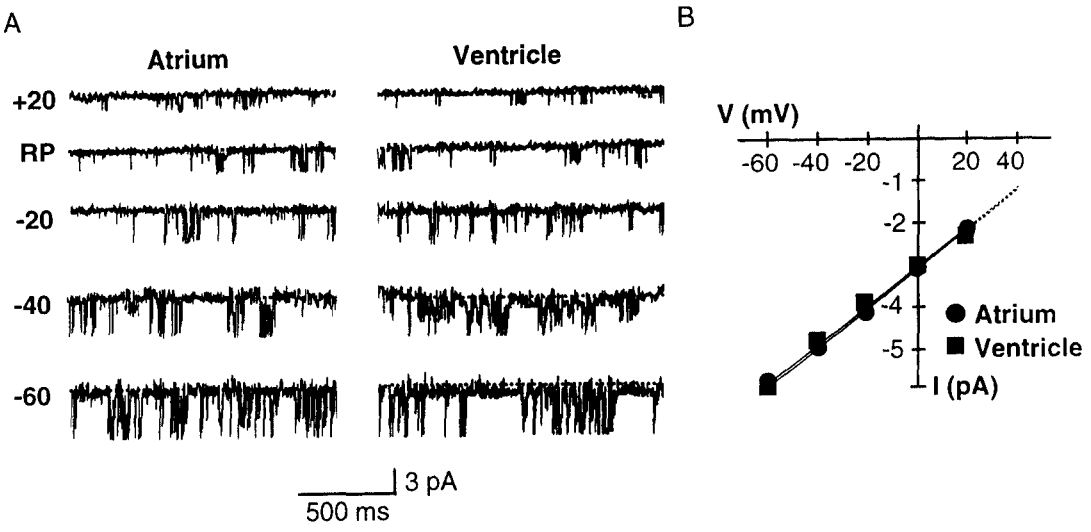

Fig. 3. Characteristics of Muscarinic K^+ channel activity in atrial and ventricular myocytes. (*A*) Representative original $I_{K(ACh)}$ channel recordings in isolated atrial and ventricular myocytes recorded under cell-attached patch conditions with 150 mM K^+ in the pipette solution bathed in the normal Tyrode's (5.4 K^+). The holding potential (HP) is expressed as the voltage deviation from the resting membrane potential (RP) and is indicated to the left of each current trace. The dotted line running above parts of the trace indicates the closed state (baseline level). Currents were low pass filtered at 5 kHz. (*B*) The *I-V* relationships obtained from the same patches as *A*. Inward current showed a slope conductance of 47 pS in atrial (circles) and ventricular (squares) myocytes.

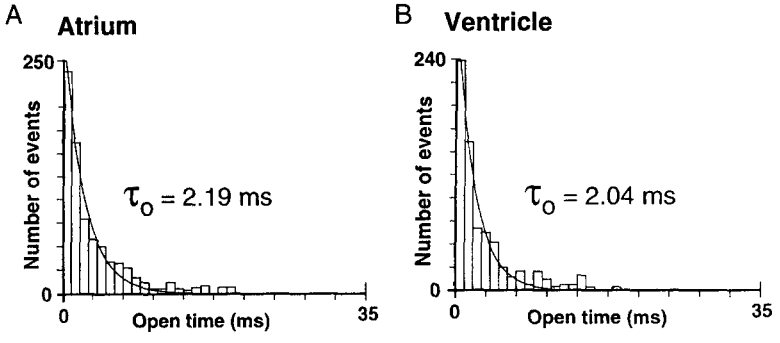

Fig. 4. Dwell time histograms in feline atrial and ventricular muscarinic K^+ channels. (*A*) Histogram of open times in cell-attached patch recordings at HP = –60 mV in an isolated atrial myocyte with low pass filtering at 5 kHz. The measurements were made from a patch containing only one channel. The lifetimes of openings were distributed according to a single exponential function with a time constant (τ_o) of 2.19 msec. (*B*) Histogram of open times in an isolated ventricular myocyte determined under the same conditions as that in *A*. The open times were distributed according to a single exponential function with τ_o of 2.04 msec.

channels in the patch and P_o is the channel open probability. The concentration-dependent activation of $I_{K(ACh)}$ was fitted by a least-squares method to the following form of the Hill equation:

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

where IC_{50} is the concentration of ACh at half-maximal channel activation and H is the Hill coefficient. The relationship between the concentration of ACh and single channel activity fit with values for the Hill coefficient of 1.8 for both cells. However, the graph showed a higher sensitivity to ACh in atrium (IC_{50} = 0.03 μM) than in ventricle (IC_{50} = 0.15 μM). These results suggest that the reduced whole-cell membrane current response to ACh in ventricle may be caused by reduced muscarinic receptors, a reduced total number of functional channels (density) and/or reduced activity of individual $I_{K(ACh)}$ channels in ventricle.

Coupling of G Proteins to Muscarinic K^+ Channels in Atrial and Ventricular Myocytes

The role of G proteins in the activation of the $I_{K(ACh)}$ in atrial and ventricular myocytes was tested using the excised inside-out patch configurations (Fig. 6). In the presence of ACh (1 μM) in the pipette solution, $I_{K(ACh)}$ activity was observed in cell-attached patches in atrial and ventricular myocytes. Channel activity disappeared following the formation of excised inside-out patch configuration in both cells. Application of GTP (100 μM) to the bath solution (cytosolic side of membrane) restored channel activity in both patches (Fig. 6*A* and *B*). After

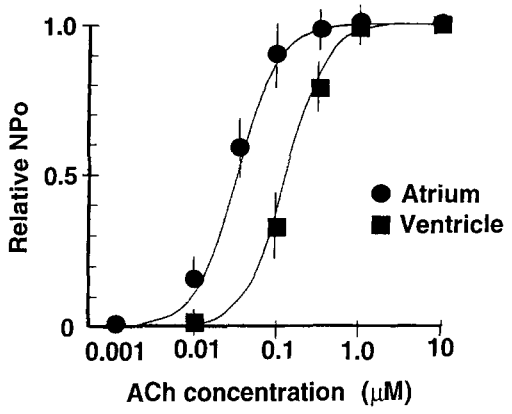

Fig. 5. Concentration-dependent activation of muscarinic K^+ channels by ACh in atrial and ventricular myocytes in cell-attached patch recordings. The relationship between the concentration of ACh in the pipette solution and channel relative NP_o (%) in atrial (circles) and ventricular (squares) myocytes. The ACh concentration at half-maximal channel activation (IC_{50} was 0.03 μM in atrial and 0.15 μM in ventricular $I_{K(ACh)}$. Vertical bars through each point represent standard deviation from 8–14 cells in atrium and from 7–12 cells in ventricle.

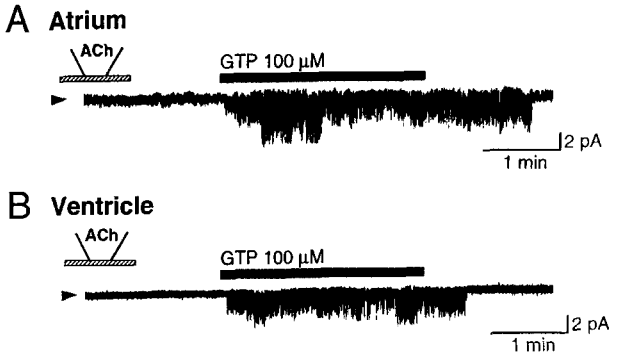

Fig. 6. GTP-dependent activation of muscarinic K^+ channels in atrial and ventricular myocytes in excised inside-out patch recordings. (*A*) Original current recordings in atrial myocytes at HP = −60 mV. Application of GTP (100 μM) to the bath solution caused activation of the channel. The channel activated without a significant delay following application of GTP. The channel remained active for over 1 min after washout of GTP. The arrowhead indicates the baseline level (zero current, channel closed). (*B*) Original current recordings in ventricular myocytes at HP = −60 mV. The channel activated without a significant delay following application of GTP similar to that in atrium, but channel activity disappeared faster than in atrium during washout of GTP.

washout of GTP from the bath, channel activity disappeared in both patches. However, the time to channel closure in ventricle (a half time of 19.3 ± 4.6 sec, n = 14) was significantly faster than that in atrium (82.9 ± 20.5 sec, n = 10, $P < 0.001$).

Figure 7 illustrates the dependence of single $I_{K(ACh)}$ channel activity on GTP concentration in atrial and ventricular myocytes. Channel activity was estimated by

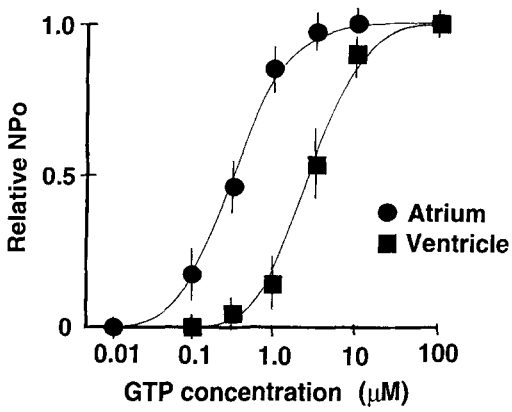

Fig. 7. Concentration-dependent activation of muscarinic K^+ channels by GTP in atrial and ventricular myocytes in excised inside-out patch recordings. The relationship between the concentration of GTP and channel relative NP_o (%) in atrium (circles) and ventricle (squares). The GTP concentration at half-maximal channel activation was 0.32 μM in atrium and 2.9 μM in ventricle. Vertical bars through each point represent standard deviation from 9–13 cells in atrium and from 9–12 cells in ventricle.

NP_o. The concentration-dependent activation of $I_{K(ACh)}$ was fitted by the equation (1). The relationship between the concentration of GTP and single channel activity fit with values for the Hill coefficient of 1.9 for both groups but showed a higher sensitivity to GTP in atrium (IC_{50} = 0.32 μM) than in ventricle (IC_{50} = 2.9 μM).

Discussion

The major findings in this study are as follows: (i) ACh hyperpolarized and shortened the action potential in cat ventricular myocytes, but these effects were diminished compared to atrium. (ii) ACh increased the whole-cell membrane current in ventricle, but the normalized ACh-sensitive current slope conductance and the current density were significantly smaller in ventricle than in atrium. (iii) In cell-attached patches, single $I_{K(ACh)}$ channel activity was present in ventricular myocytes. (iv) Single $I_{K(ACh)}$ channel characteristics in ventricle were similar to those in atrium with the exception of a lower number of functional channels. (v) Single $I_{K(ACh)}$ channel sensitivity to ACh and GTP in ventricle was less than in atrium. These results suggest that reduced electrophysiological response to ACh in ventricular myocytes may be caused by reduced muscarinic receptors, reduced density of $I_{K(ACh)}$ channels and/or reduced channel sensitivity to ACh and G proteins.

There are several in vivo studies of a direct effect of ACh on ventricle in cat (Blair, Shimizu & Bishop, 1980), dog (Martins & Zipes, 1980; Martins et al., 1989) and human (Prystowsky et al., 1981) as well as in vitro studies in ferret (Boyett et al., 1988) and dog (Litovsky &

Antzelevitch, 1990) hearts. Several reports have shown that ACh can directly abbreviate ventricular action potential duration (Boyett et al., 1988; Litovsky & Antzelevitch, 1990). However, ACh effects on action potential and the heart beat are still unclear. Even in sino-atrial (SA) node, the contribution of $I_{K(ACh)}$ to the total membrane conductance is relatively small (Noma et al., 1984). Although $I_{K(ACh)}$ channels open even in the absence of ACh (Sakmann, Noma & Trautwein, 1983), the resting conductance is almost unchanged by removing K^+ ions from both the internal and the external solutions in rabbit SA node cells (Noma et al., 1984). The effects of ACh vary with different species and regions of the heart and are influenced by other factors including ACh concentrations. Contamination of effects on Ca^{2+} current also makes net changes in action potential unpredictable. Furthermore, both sympathetic and parasympathetic stimulation modulates cardiac action potential and the balance of ACh effects on each will determine the net effect on the action potential in vivo.

Previous studies demonstrated that the inwardly-rectifying K^+ current is enhanced by M_2-cholinergic receptor stimulation by ACh in atrial tissues (Ten Eick et al., 1976; Garnier et al., 1978; Kurachi et al., 1986), sino-atrial and atrio-ventricular nodes (Noma & Trautwein, 1978), and cardiac Purkinje fibers (Carmeliet & Mubagwa, 1986*a,b*). In contrast to the increasing action of ACh on these tissues, it has been unclear whether ACh has a negative inotropic and chronotropic effect on ventricular tissues. Previous studies suggested that ACh has no direct action on the inwardly rectifying background K^+ current in guinea-pig papillary muscle (Hino & Ochi, 1980) and chick ventricle (Josephson & Sperelakis, 1982; Inoue et al., 1983). However, Hartzell & Simmons (1987) documented that ACh can increase K^+ conductance in frog ventricular myocytes. Boyett et al., (1988) demonstrated that the ACh-induced K^+ conductance is responsible for negative inotropic and chronotropic action of muscarinic stimulation in ferret ventricular tissues. Ventricular tissue generally has a lower concentration of $I_{K(ACh)}$ channels than does atrium or pacemaker tissue (Hartzell, 1988 for review). In the present study, macroscopic background current response to ACh was prominent in ventricular myocytes after removal of the contamination of I_{Na} and I_{Ca}. This increase in macroscopic current conductances were caused by the activation of the specific K^+ channel ($I_{K(ACh)}$).

Although both the inward and outward currents were increased by ACh in ventricle, the increase in whole-cell currents by ACh in ventricle was less than in atrium. One of the major reason of this observation is that the channel density of macroscopic $I_{K(ACh)}$ in ventricle was lower than in atrium. The lower channel incidence in single channel recordings was consistent with a reduced whole-cell current density in ventricle. The reduced M_2-cholinergic receptor density in ventricle may be, in part, responsible for the reduced apparent channel density. Fields et al. (1978) examined the density of muscarinic receptors in rabbit heart and showed that the density of muscarinic receptors in rabbit ventricle was approximately 20% or less than that in atrium. Similar results were reported in frog, rat, rabbit, guinea-pig and dog hearts (Wei & Sulakhe, 1978; Hartzell, 1980). Possibly another reason for the reduced whole-cell current density in ventricle is that the channel sensitivity of $I_{K(ACh)}$ to G protein in ventricle is reduced compared to atrium. Excised patch experiments with GTP allowed the direct comparisons of G protein (G_i) coupling of the channel between the two cell types under the identical condition. The higher apparent IC_{50} value for channel activation by GTP in ventricle may be caused by a diminished sensitivity to G protein stimulation. On the other hand, Galper & Smith (1983) and Sorota, Adam & Pappano (1986) reported that the atrium has a higher apparent affinity for a number of agonists that the ventricle in chick heart, indicating the M_2-receptor affinity for ACh may be decreased in ventricle compared to atrium. These results suggest that the relationship between G protein and the channel may be reduced in ventricle. Therefore, reduced $I_{K(ACh)}$ channel activation in ventricle may be caused by reduced number of M_2-cholinergic receptors and the reduced signal transduction pathway between G protein and the channel.

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