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Activation of α_1-adrenoceptors modulates the inwardly rectifying potassium currents of mammalian atrial myocytes

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Abstract. The selective α_1-adrenergic agonist methoxamine ($10^{-4}-10^{-3}$ M), in the presence of propranolol (10^{-6} M), can reduce both the inwardly rectifying K^+ background current (I_{K1}) and the muscarinic cholinergic receptor-activated K^+ current ($I_{K,ACh}$) in rabbit atrial myocytes resulting in action potential prolongation during the final phase of repolarization and a depolarization of the resting membrane potential. The reduction of these K^+ current(s) by α_1-adrenoceptor stimulation was insensitive to pre-treatment of artial myocytes with pertussis toxin (0.15–0.5 μg/ml) and was irreversible following intracellular dialysis with the non-hydrolysable guanosine triphosphate (GTP) analogue, Gpp(NH)p ($1-5\times10^{-3}$ M). Neither the protein kinase C (PKC) inhibitors, 1-(5-isoquinolinesulphonyl)-2-methylpiperoxine (H-7) (5×10^{-5} M) and staurosporine (1×10^{-7} M), nor "downregulation" of PKC by prolonged phorbol ester exposure (5×10^{-7} M, for 7–8 h) had an effect on the α_1-adrenergic modulation of this K^+ current. Under cell-attached patch-clamp conditions, bath application of methoxamine reversibly decreased acetylcholine-induced single-channel activity, thus confirming the observed reduction of the ACh-induced current under whole-cell voltage clamp. These results demonstrate that the α_1-adrenoceptor, once activated, can reduce current through two different inwardly rectifying K^+ channels in rabbit atrial myocytes. These current changes are mediated via a pertussis toxin-insensitive GTP-binding protein, and do not appear to involve the activation of PKC.

Key words: Rabbit heart – Alpha-adrenoceptors – Potassium currents – Protein kinase C

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Introduction

In the heart, α_1-adrenoceptor activation can lead to changes in cell resting membrane potential and underlying diastolic membrane currents. In Purkinje fibres, α_1-agonists increase the maximum diastolic potential [27, 29] and activate the electrogenic Na/K pump [35]. In contrast, α_1-agonists also reduce the background K^+ conductance in Purkinje fibres [35], the inwardly rectifying K^+ current in rabbit ventricle [12] and the steady-state outward current in rat ventricle [28] and atrium [21]. Either positive [38] or negative [32] chronotropic effects can be observed; these may arise from differences in the membrane currents which are affected by α_1-agonists in the sinus node.

In view of these complex electrophysiological effects, we investigated the effects of α_1-adrenergic stimulation on two K^+ currents that can affect electrical activity at diastolic potentials in rabbit atrial myocytes. Two prominent inwardly rectifying K^+ currents in the heart are I_{K1} and $I_{K,ACh}$, a K^+ current activated by muscarinic cholinergic agonists. These two types of K^+ current have somewhat similar steady-state current/voltage ($I-V$) relations but $I_{K,ACh}$ has not been observed in ventricular myocytes. In single-channel recordings $I_{K,ACh}$ has much shorter single-channel open times than I_{K1} [31] and, since it exhibits less inward rectification, $I_{K,ACh}$ may carry more outward current than I_{K1} [5, 36] (for review, see [18]). Another important difference between these two K^+ currents is that $I_{K,ACh}$ can be activated by acetylcholine (ACh) or adenosine and is directly modulated via a heterotrimeric guanosine triphosphate (GTP)-binding regulatory protein (G-protein) (for review, see [3]). Recently it has been shown by Kurachi et al. [23] that phenylephrine, a mixed α_1- and β-agonist in the heart [7], can increase $I_{K,ACh}$ single-channel activity in cell-attached patches, an effect which appears to be mediated by arachidonic acid metabolites. In the present experiments, we used the whole-cell voltage-clamp and cell-attached patch-clamp methods to study the effect of the selective α_1-adrenoceptor agonist methoxamine, in the

presence of the β-adrenoceptor antagonist propranolol, on the action potential and inwardly rectifying K^+ currents of rabbit atrial myocytes. In the majority of our experiments, we find that both of these inwardly rectifying K^+ currents are reduced by α_1-agonists, and that the signal transduction mechanism does not appear to involve either a pertussis toxin-sensitive G-protein or intracellular protein kinase C (PKC).

Materials and methods

Cell dissociation and storage were carried out using standard methods [11]. The experimental solution was the modified Tyrode's solution we have used in our previous experiments. In consisted of (in mM): NaCl, 121; KCl, 5.0; sodium acetate, 2.8; $NaHCO_3$, 24; $MgCl_2$, 1.0; Na_2HPO_4, 1.0; glucose, 5.49; pH 7.4 after equilibration with 95% O_2, 5% CO_2. The $CaCl_2$ concentration was 2.2 mM, 0.3 mM $CdCl_2$ was present continuously to block the Ca current (except for experiments shown in Fig. 1) and 10^{-6} M propranolol was used to prevent activation of β-adrenoceptors. Experiments were carried out at 21–23 °C using whole-cell voltage-clamp recording techniques previously described. The micropipette filling solution had the following composition (in mM): potassium aspartate, 120; KCl, 20; adenosine 5′-triphosphate, sodium salt, 4; 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) 5; $MgCl_2$, 1. The pH was edjusted to, 7.2 with KOH. In some experiments, 10^{-3} M ethylglycol-bis-(β′aminoethyl)*N,N,N′N′*-tetraacetic acid (EGTA) was added to this solution to give a calculated pCa in the range 9.1–10.1 (assuming a contaminant Ca concentration of 10^{-6}–10^{-5} M [8]. A liquid junction potential of approximately 10 mV arose from the use of potassium aspartate in microelectrodes; all results have therefore been corrected by this amount.

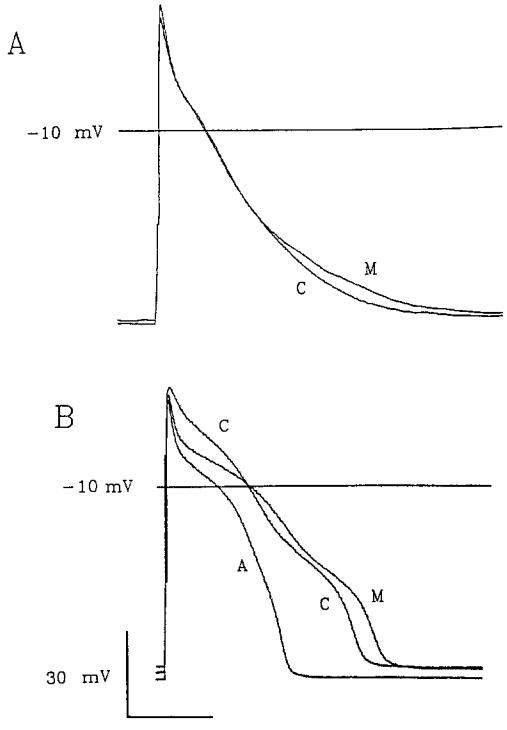

Fig. 1A, B. Effects of α_1-adrenergic stimulation on the rabbit atrial action potential in the presence of (10^{-3} M) 4-aminopyridine (4-AP). Action potentials were elicited by 2 ms suprathreshold current pulses applied at 0.2 Hz. The *horizontal time bar* corresponds to 100 ms in **A** and 200 ms in **B**. **A** Atrial cell action potentials; trace *C* control, trace *M* recorded during exposure to 4×10^{-4} M methoxamine. **B** Effects of methoxamine on atrial action potential during superfusion with 10^{-7} M acetylcholine (ACh). Trace *C*, control action potential; trace *A*, during exposure to 10^{-7} M ACh; *M*, recorded in presence of 10^{-7} M ACh and 2×10^{-4} M methoxamine. Experimental temperature 21–23 °C in this and subsequent figures.

A voltage-clamp protocol consisting of a sawtooth voltage cycle from −125 mV to +50 mV at 0.16 Hz was used to measure changes in K^+ current. This is illustrated in the lower panel of the chart recording in Fig. 3A. The depolarizing phase of the voltage cycle lasted 5 s and was used to inactivate time-dependent currents. Membrane *I/V* relations were recorded during the subsequent repolarizing phase which lasted 1 s. Current changes during voltage ramp experiments (Fig. 2–6) were continuously monitored on a chart recorder (Gould 2400 series). Digital averages of membrane current over five cycles were stored online on computer. All *I/V* relations shown in the Results are therefore averaged data. Voltage ramps recorded during repolarization measure mainly current through K^+ channels (See Discussion). At positive membrane potentials, though, a steady-state component of current due to the incomplete inactivation of transient outward K^+ current channels (I_t) can sometimes be observed. We used 4-aminopyridine (4-AP) to block I_t (unless otherwise stated), but concentrations of $1-2\times10^{-3}$ M were required for complete abolition of I_t and these sometimes reduced other K^+ currents (e.g. Fig. 6). The component of current attributable to I_t could also be removed by subtraction of data obtained after exposure to 0.4 mM $BaCl_2$ since I_t is resistant to $BaCl_2$, but both of the inwardly rectifying K^+ currents are blocked completely (see Fig. 4). No quantitative difference between results obtained in the presence or absence of 4-AP was observed.

$I_{K,ACh}$ single-channel events were recorded at 21–23 °C in the Tyrode's bath solution described above using the cell-attached patch-clamp configuration. The tips of the patch pipettes were first coated with Sylgard and then heat-polished. Pipettes were filled with a solution containing 140 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10–100 nM acetylcholine and 5 mM HEPES. The pH was adjusted to 7.4 with KOH. Tip resistances were typically 5–5 MΩ. Single-channel events were low-pass filtered at 2 kHz using a 4-pole Bessel filter, digitized at 10 kHz and stored both on computer and video tape. Analyses of single-channel activity were performed using IPROC-2 software (Axon Instruments).

Results

α_1-Adrenergic effects on the action potential

Fedida et al. [11] have shown that, in rabbit artial cells, α_1-adrenergic stimulation can prolong the action potential by causing a reduction in I_t. In the present experiments, we prevented α_1-induced changes in I_t from affecting the action potential configuration by using 4-AP (10^{-3} M) to block this time- and voltage-dependent transient outward current. In Fig. 1A action potentials are shown from a single rabbit atrial myocyte during constant stimulation at 0.2 Hz. Due to the presence of 4-AP and the resulting block of I_t, these action potentials have only a brief phase of rapid repolarization followed by a well-defined plateau phase. Very little difference can be seen between control action potentials and those recorded in the presence of the selective α_1-adrenoceptor agonist methoxamine (2×10^{-4} M), except for a modest lengthening of the final phase of action potential repolarization. There is also a small depolarization of the membrane in the presence of the α_1-agonist (see also [11], Fig. 1). These rather small effects of α_1-adrenergic stimulation may be contrasted with the marked lengthening of the action potential that occurs when I_t has not been blocked by 4-AP [11].

Prolongation of action potential duration and depolarization of the resting potential are suggestive either of a reduction in outward K^+ current or an increase in inward current [21]. Two outward K^+ currents which have been identified in rabbit myocytes over this potential range are I_{K1}, an inward rectifier identified as a 30–50 pS channel in cell attached recordings and, in atrial cells, $I_{K,ACh}$, a K^+ conductance of similar unitary magnitude, but distinct kinetics (for review see [18]). We have investigated the possibility that the action potential prolongation induced by α_1-agonists during late repolarization may involve α_1-adrenoceptor modulation of I_{K1} and $I_{K,ACh}$ in these myocytes. In Fig. 1 B, results from another atrial myocyte are shown. Again, the control action potential in the presence of 4-AP has a triangular waveform (denoted C). Addition of 10^{-7} M ACh to the bathing solution produces a marked shortening of the action potential and a hyperpolarization of the resting membrane potential, as expected from the known effects of ACh on transmembrane ionic currents in heart. At plateau potentials, ACh reduces I_{Ca} [15] and during repolarization increases $I_{K,ACh}$, an inwardly rectifying, selective K^+ current [18]. These changes will shorten the plateau phase, accelerate repolarization and hyperpolarize the resting potential toward E_K. All these effects are exhibited by the action potentials in Fig. 1 B (trace A). Subsequent addition of the α_1-adrenoceptor agonist methoxamine reverses both the action potential shortening negative to −10 mV and hyperpolarization of the resting potential. (Fig. 1 B, trace M), suggesting that α_1-adrenoceptor activation can reverse the effects of ACh.

α_1-Agonists can reduced $I_{K,ACh}$ in atrial cells

To dertemine whether α_1-agonists can independently reduce $I_{K,ACh}$ in atrial cells, the effects of α_1-agonists on ACh-activated background K^+ current were examined. Typical results are illustrated in Fig. 2. In this cell, 10^{-7} M ACh was present as indicated by the continuous bar in Fig. 2 A. The trace labelled C was recorded first (Fig. 2 B); thereafter exposure to methoxamine resulted in a large reduction in ACh-activated K^+ current (trace M in Fig. 2 B). The effect of the α_1-agonist was readily reversible upon washout from the bath (Fig. 2 A) confirming that there was a large reduction of ACh-activated K^+ current by methoxamine. When ACh was then removed from the superfusate, $I_{K,ACh}$ slowly declined (Fig. 2 A, right-hand end). The residual current in the absence of ACh (Fig. 2 B, trace C_2) is very small, as expected in mammalian atrial cells. Additional measurements demonstrated that methoxamine dose dependently reduced the ACh-induced current in these cells; further information describing this dose dependence is given in Table 1. These observations confirm that the methoxamine effect is mediated via stimulation of α_1-adrenoceptors.

The change in membrane current induced by exposure to the α_1-agonist, that is, the difference current, corresponds to a reduction of outward current, as is shown in Fig. 2 C. Note that the reversal potential suggests K^+ selectivity and that this difference current exhibits strong

Table 1. Dose-dependence of the α_1-adrenergic-induced decrease in inwardly rectifying K^+ currents in rabbit atrial myocytes

Methoxamine concentration	Percentage of control current
5×10^{-5} M	110.5 ± 6.6%
2.5×10^{-4} M	35.8 ± 3.7%
8×10^{-4} M	24.5 ± 8.5%
Washout	75.1 ± 9.8%

Each cell was dialysed via the pipette with 4×10^{-4} M Gpp(NH)p to activate $I_{K,ACh}$, and control measurements were taken under steady-state conditions. Thereafter, cells were exposed consecutively to increasing concentrations of methoxamine, followed by washout after the highest concentration. The α_1-adrenergic effect is expressed as a percentage of the inward current measured at −125 mV under control conditions (taken as 100%). Absolute levels of inward current at −125 mV under control conditions ranged from −180 to −450 pA. Results are expressed as means ± SD; $n = 4-5$

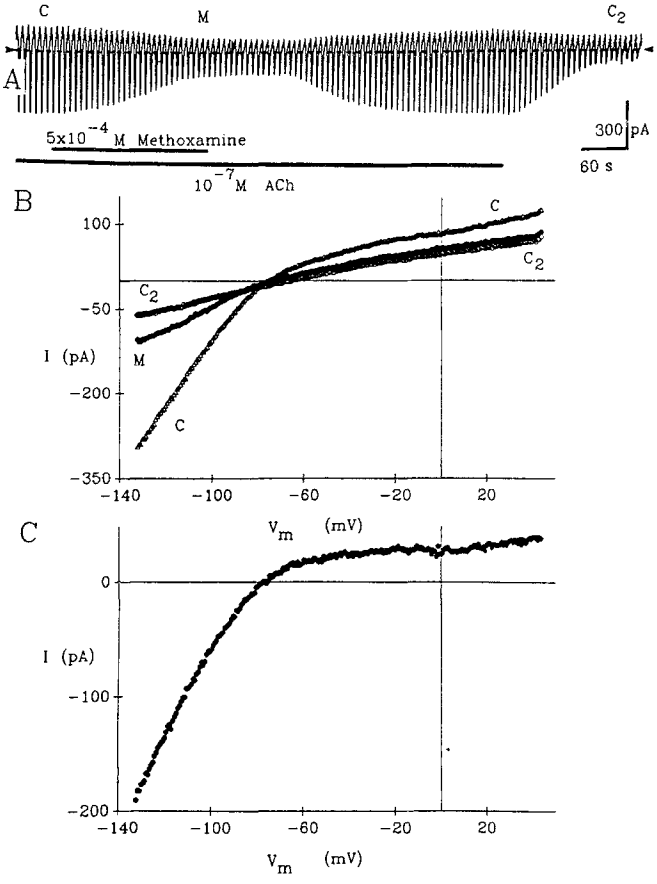

Fig. 2A–C. Reduction of $I_{K,ACh}$ by methoxamine in atrial cells. **A** ACh-activated inwardly rectifying current in an atrial myocyte. Continuous recording of membrane current from chart recorder during application of voltage ramp protocols. Exposure to 10^{-7} M ACh and 5×10^{-4} M methoxamine is indicated by *bars* below the records. Note that the final part of the chart record illustrates the reduction of current that occurred when acetylcholine was removed from the bathing solution. **B** Control *I/V* relation (*C*, in the presence of ACh), after exposure to methoxamine (trace *M*), and finally after washout of ACh (trace C_2). *I/V* relations were obtained at the times indicated by the *labels* above the chart record in **A**. **C** *I/V* difference current illustrating the ACh-activated current which is sensitive to 5×10^{-4} M methoxamine, obtained by subtraction of *M* from *C* in **B**

inward rectification, although significant outward current is present at positive potentials. This pattern of difference current was obtained consistently in rabbit atrial cells. Although this current is somewhat similar to I_{K1} in ventricle, there are important differences: (1) it is smaller in magnitude and (2) it lacks a region of negative slope conductance and therefore passes significant outward current at positive potentials. These characteristics fit well with the known properties of $I_{K,ACh}$ in atrial cells [18].

Two additional characteristics of K^+ current in rabbit atrial cells require further description and this is shown in the next two Figures. First, in some atrial cells, a component of K^+ current was present, even in the absence of ACh, that was sensitive to α_1-agonists. An example of this is shown in Fig. 3. In the chart record in Fig. 3A, exposure to 2×10^{-4} M methoxamine results in a reduction of membrane current which is subsequently blocked almost completely by $BaCl_2$. In this example, no attempt was made to wash out the α_1-agonist to reverse its effect; however, in many other experiments (e.g. data in Fig. 6),

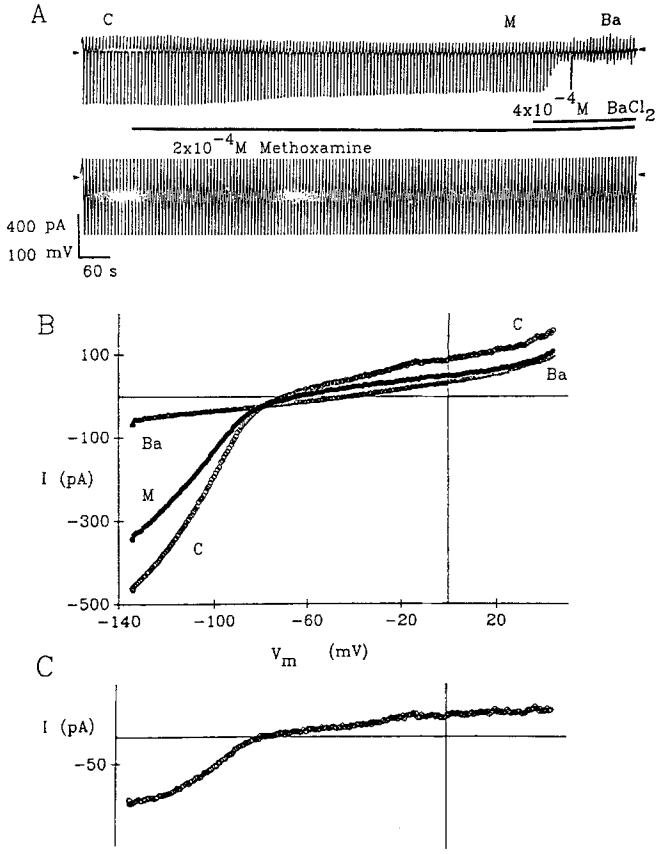

Fig. 3A–C. Currents recorded in a rabbit atrial cell during ramp voltage clamps and effect of α_1-agonist stimulation in the absence of ACh. **A** Chart recording of membrane current (*upper panel*) and voltage (*below*) during ramp voltage cycles. Cell was exposed to 2×10^{-4} M methoxamine and later 4×10^{-4} M $BaCl_2$ as indicated by the *horizontal bars*. *Letters* above record refer to labelled tracees in **B**. **B** Current/voltage relations from **A**. Trace *C* is the control prior to methoxamine exposure. Trace *M* recorded after methoxamine effect had reached a steady state. Trace *Ba* denotes current in presence of $BaCl_2$. **C** Difference *I/V* relation between traces *M* and *C* in **B**. Note that this represents a reduction in current caused by the α_1-agonist

the effects of α_1-agonists could be quickly and completely reversed. The labelled data from Fig. 3A are plotted in Fig. 3B. The methoxamine-sensitive difference current is plotted in Fig. 3C; although it is very small it has many similarities to the difference current in Fig. 2C. There is inward rectification, a reversal potential near E_K and significant outward current at positive potentials.

In 74 cells out of the 100 studied, exposure to α_1-agonists caused this type of reduction of the inwardly rectifying whole-cell K^+ current. In 11 cells there was no apparent effect on this current. In 15 cells studied with the pipette containing 10^{-3} M EGTA to buffer internal Ca at a very low level, the inwardly rectifying K^+ current initially increased on exposure to α_1-agonists and then decreased again. An example of this kind of response is shown in Fig. 4. The section of chart record in Fig. 4A illustrates the changes in this current that occurred over a 25-min period. In Fig. 4B, data recorded at the times indicated on the chart in Fig. 4A are shown as $I-V$ relations. The initial control record (labelled C) in the absence of any drugs shows some inward rectification but also significant outward current (positive to -30 mV) that may be attributable to a steady-state component of transient outward current. Soon after, the cell was exposed to 2×10^{-4} M methoxamine and there was an increase in peak to peak current. The inward component reached a maximum in about 1 min. At this time, the trace labelled M_1 was recorded demonstrating a measurable increase in inwardly rectifying K^+ current which reversed near -80 mV. With continued exposure to the α_1-agonist two additional changes were observed. First, the increase in inward current at negative potentials declined, so that after 10 min exposure to the α_1-agonist (trace M_2) the inward current was only slightly greater than the control level (see Fig. 4B). Second, there was a significant reduction in the outward current at potentials positive to -20 mV due to an inhibition of the transient outward current. This is seen in Fig. 4B as the divergence of traces M_2 and C at positive potentials, and closely resembles previous reports of methoxamine-induced reduction in I_t in rabbit atrial myocytes [10].

When the α_1-agonist was removed from the superfusate in the experiment shown in Fig. 4, the inward current quickly recovered to the control level; thereafter a slow recovery of outward current occurred. The cell was then exposed to 4×10^{-4} M $BaCl_2$ to block the inwardly rectifying K^+ currents (but *not* I_t) and the trace labelled Ba was recorded. Under these conditions, almost no inward current was present but the outward current was very similar to the control trace positive to -20 mV. This outward current is I_t and is insensitive to this concentration of $BaCl_2$, but can be abolished by 4-AP (last record in Fig. 4B).

From these interventions we obtained the methoxamine-sensitive background K^+ current by subtraction of trace M_1 from trace C. The resulting difference current is shown in Fig. 4C. This I/V relation thus reflects the *increase* in background current that sometimes occurred during exposure to methoxamine. This difference current is similar to that shown in Figs. 2C and 3C in its voltage dependence and reversal potential – however, it

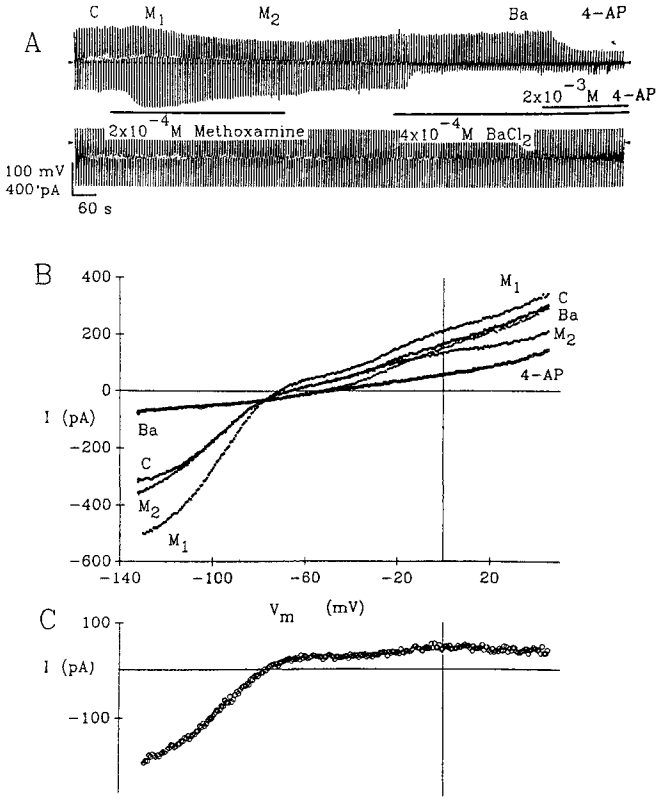

Fig. 4A–C. *I/V* relations demonstrating an increase in inwardly rectifying current caused by methoxamine in a rabbit atrial myocyte. **A** Continuous chart record of experiment lasting 30 min. Current in *upper panel*, voltage the *lower panel*. Cell was exposed to 2×10^{-4} M methoxamine, 4×10^{-4} M $BaCl_2$ and 2×10^{-3} M 4-AP as indicated by the *horizontal bars*. *Labels* above the chart record refer to plotted *I/V* relations in part *B*. **B** Trace *C* was recorded soon after whole-cell recording was started. Trace M_1 was recorded soon after α_1-agonist was introduced into bath and current magnitude had increased. Trace M_2, was obtained later during exposure to methoxamine, and trace *Ba* was recorded after washout of methoxamine and after block of I_{K1} by $BaCl_2$. Finally, trace *4-AP* was recorded in presence of both $BaCl_2$ and 4-AP to obtain the "leak" *I/V* relation when all I_{K1} and I_t channels were blocked. **C** Difference *I/V* relation for data in **B**. Note that in this case the difference current (traces M_1-C) represents an increase in K^+ current, as opposed to the decrease in Figs. 2 and 3. Micropipette filling solution contained 10^{-3} M ethylglycol-bis-(β'aminoethyl)*N,N,N',N'*-tetraacetic acid (EGTA) (see Discussion)

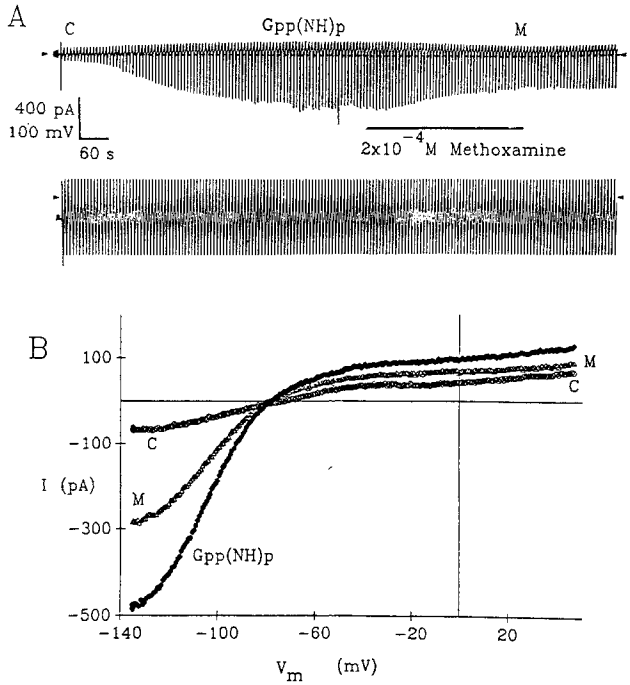

Fig. 5A,B. Effect of a non-hydrolysable guanosine triphosphate (GTP) analogue on inwardly rectifying current and effect of α_1-agonist. **A** Continuous chart record of experiment, with membrane current in the *upper panel* above, voltage in the *lower panel*. Microelectrode filling solution contained 10^{-3} M Gpp(NH)p in addition to normal constituents (see Materials and methods). Immediately on establishment of whole-cell recording (transient at left of record), voltage ramps were started. Methoxamine (2×10^{-4} M) was added to the bath solution as indicated by *bar* on record. *Labels* above record correspond to current recordings in **B** and indicate time at which they were made. **B** *I/V* relations recorded as shown in **A**. Trace *C* recorded soon after whole-cell recording started and before Gpp(NH)p had activated $I_{K,ACh}$. Trace *Gpp(NH)p* recorded when inward current had increased to a steady level. Trace *M* recorded during period of maximum effect of 2×10^{-4} M methoxamine. All traces shown in **B** represent Ba^{2+}-sensitive currents

represents an increase in K^+ current rather than the decrease described in Fig. 2C.

Modulation of α_1-adrenergic effects on inwardly rectifying K^+ current

We have shown previously that the reduction in the transient outward current in rabbit atrial myocytes by α_1-agonists is mediated via a pertussis toxin (PTX) insensitive G-protein [2]. In these experiments PTX pretreatment of atrial myocytes caused the adenosine diphosphate (ADP) ribosylation of some 90% of the available PTX-sensitive G-proteins in atrial cells [2]. In the present study, we incubated cells for periods of 7–9 h at 31 °C in the absence or presence of 0.5 μg/ml PTX to block the hormonal activation of the two sub-groups of PTX-sensitive G-proteins, G_o and G_i, which are present in cardiac tissue [24]. The α_1-adrenergic effects on $I_{K,ACh}$ were then studied in both control and PTX-treated myocytes using dialysis of the recording micropipette with the non-hydrolysable GTP analogue, Gpp(NH)p (2.5×10^{-4} M) to activate $I_{K,ACh}$ directly via its G-protein (see Fig. 5). In myocytes incubated in the absence of PTX, 2×10^{-4} M methoxamine decreased the inward current at −125 mV to 71.1±10.5% (Mean±SD, $n=3$] of control levels; in myocytes pre-treated with PTX, the methoxamine-induced reduction was to 67.8±10.6% ($n=3$) of control. Subsequent exposure to a high dose of ACh (10^{-6} M) in two atrial cells failed to activate $I_{K,ACh}$ via the muscarinic cholinergic receptor (mAChR), demonstrating that the mAChR-coupled, PTX-sensitive G-protein pathway was disrupted by this procedure. We conclude from these findings that the α_1-adrenergic mediated reduction of inwardly rectifying K^+ currents is mediated via a PTX insensitive G-protein, as is the reduction in I_t [2].

We have also studied the effects of including the non-hydrolysable GTP analogues, guanosine 5'-O-(3'-thiotriphosphate) (GTPγS) and Gpp(NH)p in the pipette filling

solution on the response to α_1-agonists. The effect of 10^{-3} M Gpp(NH)p is shown in Fig. 5. Voltage ramps were imposed on the cell as soon as the membrane patch was ruptured and whole-cell recording was started. At the time indicated in Fig. 5 A, the control data (the trace labelled C) was recorded. Soon after this, a large increase in current was observed that we attributed to the Gpp(NH)p-dependent activation of $I_{K,ACh}$, as previously described in atrial cells [18]. After some 5 min of recording, a steady-state was reached (trace labelled Gpp(NH)p), at which point a large increase had occurred in both outward and inward components of $I_{K,ACh}$. Subsequent exposure to 2×10^{-4} M methoxamine resulted in a 30%–40% reduction in $I_{K,ACh}$ (trace labelled M). This reduction was not reversible on prolonged washout of the α_1-agonist. It is interesting to note that the reduction in current by methoxamine was larger than the current present before $I_{K,ACh}$ channels were activated by intracellular Gpp(NH)p. This observation is similar to that illustrated in Fig. 2, in which the α_1-mediated reduction of ACh-activated K^+ current was larger than the current in the absence of agonist.

In many tissues α_1-adrenergic receptors are coupled to phosphoinositide metabolism [25]. The hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) leads to the formation of inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (1,2-DAG), which is known to stimulate PKC [26]. Previously we have shown that the α_1-adrenergic modulation of I_t in rabbit atrial myocytes is not mediated via activation of PKC [2, 9]. In the experiment shown in Fig. 6 the hypothesis that PKC may be involved in the second messenger modulation of background K^+ current by α_1-agonists was tested. The data are from a cell that was superfused continually with the PKC inhibitor 1-(5-isoquinolinesulphonyl)-2-methylpiperazine (H-7) (5×10^{-5} M). The initial section of the chart record in Fig. 6 A shows the effect of 2×10^{-3} M 4-AP on the membrane current during voltage ramp cycles. Note that as well as removing the outward current (due to I_t), 4-AP causes an approximately 20% reduction in inward current in this experiment. After stabilization of the current in the presence of 4-AP, the trace labelled C was recorded. The cell was then exposed to 2×10^{-4} M methoxamine, and a large, reversible, reduction of inwardly rectifying K^+ current was observed even in the presence of 5×10^{-5} M H-7. The difference I/V relation, indicative of a reduction in current is shown in Fig. 6 C. Similar results were obtained in five other cells. Furthermore, methoxamine caused a reduction in K^+ current in three cells exposed to another PKC inhibitor, staurosporine (10^{-7} M, included in the pipette filling solution). Finally, in four cells pretreated with 5×10^{-7} M phorbol-12-myristate-13-acetate (PMA) for 8 h to downregulate PKC [2], a clear reduction in K^+ current was still observed following exposure to the α_1-agonist (data not shown).

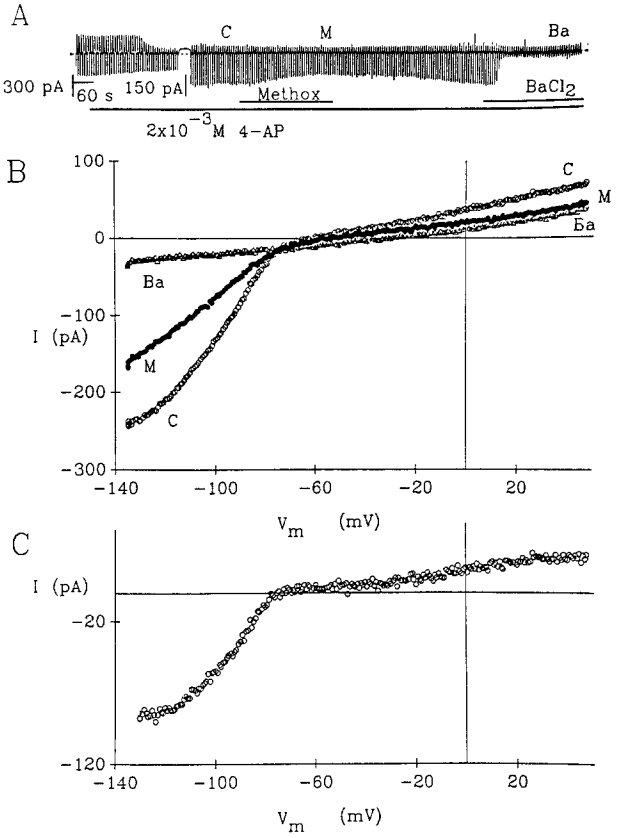

Fig. 6 A–C. Effect of PKC inhibitor 1-(5-isoquinolinesulphonyl)-2-methylpiperazine (H-7) on changes in inwardly rectifying current following application of α_1-agonist. **A** Continuous chart record of membrane current during experiment. The inhibitor H-7 (5×10^{-5} M) was continuously present in the superfusate throughout. As denoted by the *horizontal bar*, 2×10^{-3} M 4-AP was present in bath. At the break in the chart record the gain was increased 2-fold. Drugs (2×10^{-4} M methoxamine and 10^{-3} M $BaCl_2$ were included in the bathing solution, as indicated by the *horizontal bars*. Labels above the record refer to the times at which the labelled I/V relations in **B** were recorded. **B** I/V relations showing trace *C*, a control relation in the presence of 4-AP and H-7. Trace *M* shows reduction in current following exposure to α_1-agonist. **C** Difference I/V relation from data in **B**, corresponding to a reduction in current that occurs on exposure to methoxamine, even in the presence of H-7 (residual current in presence of $BaCl_2$ subtracted from records)

α_1-Agonist effects on ACh-induced single-channel activity

$I_{K,ACh}$ single-channel events were recorded using the cell-attached patch configuration to confirm that the observed effect of methoxamine on the ACh-induced current under whole-cell voltage-clamp conditions reflected changes in $I_{K,ACh}$ single-channel activity. With 100 nM ACh in the pipette, single-channel openings with a conductance of about 40 pS and a mean open time of approx 0.5 ms were observed (see Fig. 7). These findings are in agreement with previous reports from our laboratory [22], and others (for review, see [18]) describing $I_{K,ACh}$ single-channel events. The brief open times of these single-channel events much more closely resemble those for $I_{K,ACh}$ than those for I_{K1}, which are typically much longer (approximately 50 ms) [18, 22]. Addition of 2×10^{-4} M methoxamine to the bath led to a significant reduction in the ACh-induced single-channel acitivity over the entire voltage range studied, in agreement with whole-cell data (see Fig. 2). Methoxamine did not alter either the single-channel conductance or open time, rather

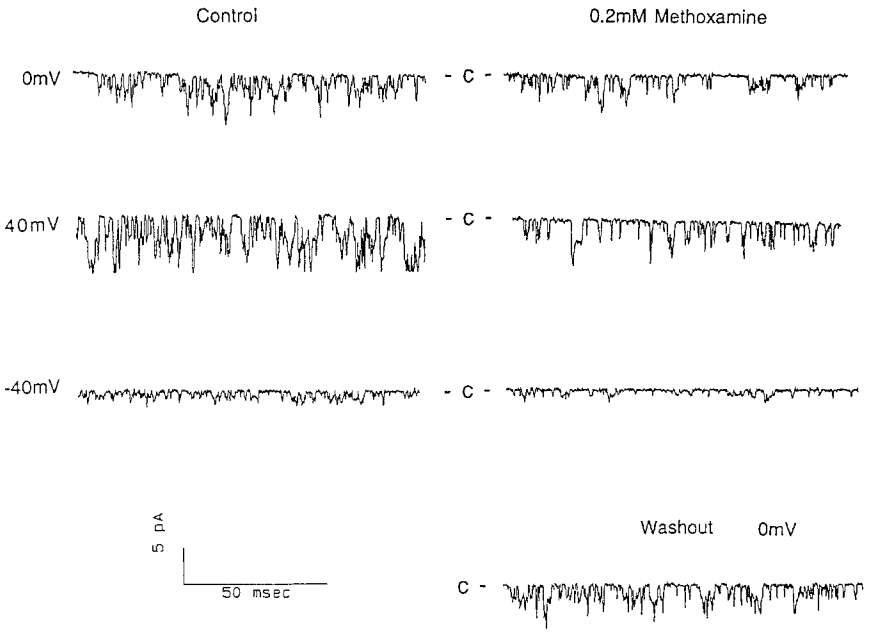

Fig. 7. Effect of α_1-adrenergic agonist on ACh-induced single-channel activity in a rabbit atrial myocyte under cell-attached, patch-clamp conditions. Cells were superfused with normal Ca^{2+}-containing Tyrode's solution, and the pipette contained 140 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM 4-(2-hydroxyethyl)-1-piperozineethanesulphonic acid (HEPES) pH 7.4, and 10^{-7} M ACh. Inward currents are shown as downward deflections, and the closed level of the channels is indicated by the -*c*- between the two panels. The pipette potentials denoted on the left-hand side are relative to the resting membrane potential (typically −70 mV). Currents were low-pass filtered at 2 kHz (−3 dB) and sampled at 10 kHz. After control recordings (*left panel*), the cell was superfused with 2×10^{-4} M methoxamine for 2 min and single-channel events were again recorded at the indicated pipette potentials (*right panel*). Recovery of the ACh-induced channel acitvity was recorded at 0 mV following a 3 min washout of methoxamine from the bath. Results shown are representative of four similar experiments

it reduced the probability of channel opening. In additional patch-clamp recordings pre-exposure of atrial cells to the α_1-antagonist phentolamine (2×10^{-5} M) blocked the methoxamine-induced (2×10^{-4} M) reduction in $I_{K,ACh}$, although this inhibition was not studied in detail.

Previously, we have reported that the α_1-agonists decrease the burst open probability of transient outward K^+ current single-channel events [2]; the data shown in Fig. 7 suggest that a similar phenomenon may also occur in the case of ACh-activated single-channel events. Additional details of this α_1-mediated decrease in single-channel activity are the subject of an ongoing study and thus will not be further elaborated here.

Discussion

α_1-Agonist effects on I_{K1} and $I_{K,ACh}$

In mammalian atrial cells, the inwardly rectifying K^+ current is usually very small (Figs. 5, 6) amounting to only some 10% of that in ventricular myocytes (Figs. 2–4; [12, 14]). There are also other important differences between the *I/V* diagrams of the background K^+ currents in atrial and ventricular cells [12]. In ventricular cells I_{K1} shows more pronounced rectification, as well as region of negative slope conductance. In atrial myocytes, both the K^+ current recorded under basal conditions and that activated by ACh show less inward rectification and very little negative slope; the latter therefore can carry outward current positive to −20 mV (Figs. 2, 3, 5). Previously, Simmons and Hartzell [36], Hartzell [18] and Clark et al. [5] have shown that $I_{K,ACh}$, may pass outward current. Under our standard experimental conditions, I_{K1} was very small in atrial cells and $I_{K,ACh}$ was activated to a variable degree. Recent observations from our laboratory [22] and others [20] suggest that $I_{K,ACh}$ may undergo spontaneous activation in the absence of an extracellular agonist. This activity appears to result from the actions of membrane-associated nucleoside diphosphate kinase, which causes G-protein activation through the formation of GTP, leading to a transient, G-protein-dependent, receptor-independent activation of $I_{K,ACh}$. Indeed, under cell-attached patch-clamp conditions in the absence of ACh, we observed single-channel events very similar to those recorded with ACh in the patch pipette (data not shown). Thus, some atrial cells exhibit very little or no inwardly rectifying K^+ current when ACh is absent, but in others a current with very similar characteristics to $I_{K,ACh}$ can be identified. These recent findings, coupled with our present observations, suggest that the K^+ current recorded under basal conditions in rabbit atrial myocytes may represent a combination of I_{K1} and spontaneous openings of $I_{K,ACh}$ channels. The effect of methoxamine on the ACh-induced single-channel activity (see Fig. 7) provides clear evidence for the α_1-adrenergic modulation of $I_{K,ACh}$ in rabbit atrial myocytes. Similar direct evidence for an α_1-adrenergic effect on I_{K1} will require further studies at the single-channel level, so that I_{K1} and $I_{K,ACh}$ events can be more clearly separated.

In three-quarters of the atrial cells studied, exposure to the α_1-adrenergic agonist methoxamine resulted in a reduction of I_{K1} and/or $I_{K,ACh}$. The observed dose dependence of the methoxamine-induced effects at 23 °C in rabbit atrial myocytes (see Table 1) is comparable with the known concentration dependence for mediation of the α_1-dependent, positive inotropic effects in rabbit [6] and cat [17] papillary muscles, the decrease of I_{K1} in rabbit ventricular myocytes [12] and the reduction of the transient outward current, I_t, in rabbit atrial and rat ventricular myocytes [10, 11, 28].

The "difference currents" that we have recorded from atrial myocytes (Figs. 2C, 3C, 4C, 6C) have properties which suggest that K^+ channel activity is reduced by α_1-agonists. These include inward rectification, K^+ selectivity as determined by the reversal potential, and sensi-

tivity to low concentrations of $BaCl_2$.In combination, these characteristics exclude other currents, such as N/K pump current and Na/Ca exchange current from being primarily responsible for these α_1-adrenergic effects. There is no apparent shift of the voltage dependence of either I_{K1} or $I_{K,ACh}$ under the influence of α_1-agonists as shown by the common crossing points of all control and drug-treated I/V relations (Figs. 2–6). The α_1-agonist appeared to reduce inward and outward K^+ currents equally, although in atrial cells this was difficult to assess since the current changes, especially in the outward direction, are small. However, the difference currents in Figs. 2C, 3C, 4C, 6C are similar to the control currents recorded in each case, which suggests that α_1-agonists can mediate an equivalent reduction of both inward and outward I_{K1} and $I_{K,ACh}$.

It has been suggested previously that $I_{K,ACh}$ can be modulated by α_1-agonists. However, in this report single-channel activity was increased by the α_1-action of phenylephrine in guinea-pig atrial cells [23]. In our experiments using a similar cell-attached configuration, bath application of 2×10^{-4} M methoxamine produced a significant reduction in the level of ACh-induced $I_{K,ACh}$ single-channel activity. It is possible that species-dependent effects are responsible for these differences. Alternatively, the increase in background K^+ current we observed in some of our cells (Fig. 4) may correspond to the increase seen in cell-attached patches [23]. In our experiments though, the small increases in the current always declined to the control level during maintained exposure to the agonist.

Other investigators have found that α_1-adrenergic stimulation can affect potassium conductances (g_K) present in quiescent cardiac muscle preparations. Cardiac Purkinje fibres/myocytes exhibit a reduction in background g_K when exposed to α_1-agonists [13, 19, 35]. In rat ventricular myocytes, Ravens et al. [28] have suggested that noradrenaline, acting via α_1-adrenoceptors, can reduce the outward background K^+ current withou affecting the current negative to the K^+ reversal potential.

Modulation of α_1-effects on background K^+ currents

Our previous biochemical measurements have demonstrated that pre-treatment of atrial myocytes with PTX inactivates a significant proportion (~90%) of the PTX-sensitive G-proteins (i.e. G_i and G_o) without affecting the α_1-mediated decrease in transient outward current [2]. In the present experiments, a similar pre-treatment protocol prevented hormonal activation of $I_{K,ACh}$ in atrial cells, but did not prevent reduction of inwardly rectifying K^+ currents by methoxamine. This observation is consistent with recent reports in cardiac tissue describing the PTX insensitivity of α_1-adrenoceptor agonist binding [4], of α_1-mediated PIP_2 metabolism [33, 37], and of positive inotropy [1]. However, others have reported that the α_1-modulation of automaticity [30], of K^+ conductance in Purkinje myocytes [35], and of cytosolic Ca^{2+} transients in hamster cardiac myocytes [34] is sensitive to blockade by PTX treatment. The reason for this difference in unclear, but may be related to differing types and/or amounts of G-proteins in these tissues.

The non-hydrolysable analogues of GTP, Gpp(NH)p (Fig. 5) or GTPγS when included in the pipette filling solution led to an irreversible decrease in background K^+ current following α_1-adrenoceptor stimulation. These observations, in light of the known actions of non-hydrolysable guanine nucleotide analogues [16], provide strong evidence for the involvement of a G-protein in the modulation of I_{K1} and $I_{K,ACh}$ by α_1-adrenergic agonists. Our observations that the PKC inhibitors H-7 (Fig. 6) and staurosporine, as well as down-regulation of myocyte PKC by prolonged exposure to phorbol ester, do not interfere with the α_1-mediated reduction of K^+ currents agree with our previous data, showing that this pathway is not directly responsible for the reduction in I_t in rabbit atrial cells following α_1-adrenergic stimulation [2, 9]. The intracellular cascade underlying the α_1-induced reduction of K^+ current(s) therefore remains unexplained.

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