Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 8756–8760, November 1988
Physiological Sciences

α_1-Adrenergic agonists selectively suppress voltage-dependent K^+ currents in rat ventricular myocytes

(cardiac muscle/voltage-clamp/protein kinase C)

MICHAEL APKON AND JEANNE M. NERBONNE*

Department of Pharmacology, Washington University Medical School, 660 South Euclid Avenue, St. Louis, MO 63110

Communicated by Philip Needleman, July 1, 1988

ABSTRACT **The effects of α_1-adrenergic agonists on the waveforms of action potentials and voltage-gated ionic currents were examined in isolated adult rat ventricular myocytes by the whole-cell patch-clamp recording technique. After "puffer" applications of either of two α_1 agonists, phenylephrine and methoxamine, action-potential durations were increased. In voltage-clamped cells, phenylephrine (5–20 μM) or methoxamine (5–10 μM) reduced the amplitudes of Ca^{2+}-independent voltage-activated outward K^+ currents (I_{out}); neither the kinetics nor the voltage-dependent properties of I_{out} were significantly affected. The effects of phenylephrine or methoxamine on I_{out} were larger and longer-lasting at higher concentrations and after prolonged or repeated exposures; in all experiments, however, I_{out} recovered completely when puffer applications were discontinued. The suppression of I_{out} is attributed to the activation of α_1-adrenergic receptors, as neither β- nor α_2-adrenergic agonists had measurable effects on I_{out}; in addition, the effect of phenylephrine was attenuated in the presence of the α antagonist phentolamine (10 μM), but not in the presence of the β antagonist propranolol (10 μM). Voltage-gated Ca^{2+} currents, in contrast, were not altered measurably by phenylephrine or methoxamine and no currents were activated directly by these agents. Suppression of I_{out} was also observed during puffer applications of either of two protein kinase C activators, phorbol 12-myristate 13-acetate (10 nM–1 μM) and 1-oleoyl-2-acetylglycerol (60 μM). We conclude that the activation of α_1-adrenergic receptors in adult rat ventricular myocytes leads to action-potential prolongation as a result of the specific suppression of I_{out} and that this effect may be mediated by activation of protein kinase C.**

Although it seems certain that the major effects of sympathomimetic agents on the mammalian heart are mediated by activation of β-adrenergic receptors, the presence of α-adrenergic receptors, which appear to be predominantly of the α_1 subtype (1, 2), has also been demonstrated in many preparations, including human atrial (3) and ventricular (4) muscle. In addition to variations among species (2), both the absolute and relative numbers of α_1- and β-adrenergic receptors are altered under some pathological conditions (5, 6). Although stimulation of α receptors results in action-potential (AP) prolongation and inotropic responses similar (although not identical) to those seen on β-receptor activation (1, 2, 6), the mechanisms involved in mediating these effects are not well understood (1, 6). This contrasts markedly with β-receptor stimulation, which results in increased cAMP, activation of cAMP-dependent protein kinase, and, presumably, protein phosphorylation (7); at the membrane level, β-receptor agonists increase the amplitude (7) of voltage-gated inward Ca^{2+} currents (I_{Ca}), by increasing the number and/or the opening probability of functional Ca^{2+} channels (8, 9). The effects of α_1 agonists are apparently not mediated by a similar mechanism, as these agents increase the hydrolysis (10), not the production (1), of cAMP. Activation of α_1 receptors also increases membrane inositolphospholipid turnover and the production of the inositol tris-, bis-, and monophosphates (11–13), as well (presumably) as diacylglycerol, an endogenous activator of protein kinase C (PKC) (14). Recently, it was suggested that inositol trisphosphate and/or diacylglycerol might function as second messengers in mediating the effects of α_1 agonists (13). Biochemical studies also suggest a role for PKC, as α_1 agonists increase the phosphorylation of a 15-kDa sarcolemmal protein (15) that appears identical to the protein phosphorylated by PKC in isolated sarcolemmal vesicles (16). Although myocardial effects of PKC activators have been demonstrated (17–19), the relationship between the observed effects and α_1 receptors has not been explored.

The underlying conductance mechanism(s) involved in mediating the effects of α_1-receptor stimulation has similarly not been clarified (1, 6). Although it has been proposed that α_1 agonists increase I_{Ca} (20–23), others have suggested that the predominant effect is not an increase in I_{Ca} (24, 25) but, rather, a reduction in time-independent (26) or time-dependent (27, 28) outward K^+ currents. Although these differences may reflect distinct mechanisms in different preparations (23), most studies have been conducted on multicellular preparations and effects on individual conductance pathways were not evaluated directly. To probe the mechanisms mediating the effects of α_1 agonists and to examine the relationships between α_1-receptor activation, changes in AP waveforms, inositolphospholipid turnover, and protein phosphorylation, we examined the effects of α_1 agonists and PKC activation on excitable membrane properties in isolated myocytes. A preliminary account of some of this work has been published (29).

MATERIALS AND METHODS

Materials. Clonidine, creatine, insulin, isoproterenol, 1-oleoyl-2-acetylglycerol (OAG), Percoll, phentolamine, phenylephrine, phorbol 12,13-diacetate (PDA), phorbol 12-myristate 13-acetate (PMA), and tetrodotoxin (TTX) were obtained from Sigma; methoxamine was obtained from Burroughs Wellcome (Research Triangle Park, NC). The (type II) collagenase used in myocyte dissociations was from Cooper-Worthington (Freehold, NJ); laminin was from Collaborative Research (Waltham, MA). Medium 199 was ob-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AP, action potential; RP, resting membrane potential; HP, holding potential; I_{out}, Ca^{2+}-independent, voltage-activated outward K^+ current(s); I_{Ca}, voltage-gated inward Ca^{2+} current(s); I_{Na}, voltage-gated inward Na^+ current(s); TTX, tetrodotoxin; PMA, phorbol 12-myristate 13-acetate; PDA, phorbol 12,13-diacetate; OAG, 1-oleoyl-2-acetylglycerol; PKC, protein kinase C.

*To whom reprint requests should be addressed.

tained from Irvine Scientific and other tissue culture reagents from GIBCO.

Cell Preparations. Single ventricular myocytes were isolated from adult Long–Evans rat heart by described procedures (30, 31). Intact myocytes were separated by density gradient sedimentation over isotonic Percoll, suspended in serum-free medium 199 (with penicillin and streptomycin), and plated on laminin (5 μg/ml)-coated glass coverslips. As laminin promotes the preferential attachment of viable, rod-shaped myocytes (32), replacement of the medium a few hours after plating facilitated the removal of "rounded-up" cells, thereby enriching the cultures to ≥80% rod-shaped cells; thereafter, the medium was exchanged daily. Dissociated myocytes could be maintained *in vitro* for 3–5 days with little change in morphological or electrophysiological characteristics (33); as changes were evident in cells in culture for ≥1 week (33, 34), the experiments were conducted on rod-shaped cells within 3–5 days of plating.

Electrophysiological Recordings. The whole-cell recording method (35) was employed to measure resting membrane potentials (RPs), APs, and ionic currents. The voltage/current-clamp circuit was provided by either a Dagan (model 8900; 1-GΩ feedback resistor) or an Axon (Axopatch; $\beta = 1$) whole-cell patch-clamp amplifier. Recording pipettes were fabricated from flint glass and coated with Sylgard; after fire-polishing, pipettes had tip diameters of 0.5–1.0 μm and resistances of 2–4 MΩ. Series resistance compensation was employed in all voltage-clamp experiments and was checked at regular intervals; data were discarded if series resistance increases were evident. Experimental parameters [e.g., holding potentials (HPs)] were controlled with an IBM PC equipped with a Labmaster (Scientific Solutions, Solon, OH) analog interface. After linear leakage subtraction, current signals were filtered (2–3 kHz), digitized, and stored. Data acquisition was performed with PClamp (Axon); data analyses employed PClamp and Asystant (Macmillan). All data were obtained at 20–22°C.

For AP measurements, bath solutions contained (in mM) NaCl, 136; KCl, 4; $CaCl_2$, 1; $MgCl_2$, 2; Hepes, 10; and glucose, 10, at pH 7.4, and recording pipettes contained (in mM) KCl, 135; EGTA, 10; Hepes, 10; glucose, 5; Mg·ATP, 3; and Tris·GTP, 0.5, at pH 7.3. Outward K^+ currents were measured in bath solutions containing 20 μM TTX to block inward Na^+ currents (I_{Na}) and either $CoCl_2$ (5 mM) or $CdCl_2$ (200 μM) to block I_{Ca}; pipettes contained the KCl solution above. Although it may have facilitated suppression of I_{Ca}, extracellular Ca^{2+} could not be removed completely as the cells became leaky. Since I_{Ca} is suppressed ≥95% by 5 mM $CoCl_2$ or 200 μM $CdCl_2$ (ref. 36 and unpublished data), the measured currents are assumed to reflect Ca^{2+}-independent, voltage-gated K^+ currents (I_{out}). From HPs of −40 to −90 mV, I_{out} was evoked during depolarizations positive to −30 mV; I_{out} was abolished when the K^+ in the pipettes was replaced by Cs^+. As I_{Na} in these cells is relatively resistant to TTX (37), most experiments were conducted at depolarized HPs to inactivate the residual I_{Na}. In the absence of $CoCl_2$, I_{Ca} was measured with isotonic CsCl in the pipettes to block I_{out} (≥95%), and I_{Ca} was evoked during depolarizations positive to −30 mV from HPs of −50 to −90 mV.

Pharmacological Manipulations. Pharmacologic agents were applied by pressure ejection from 1- to 3-μm "puffer" pipettes placed within ≈25 μm of the cell surface. A PMA stock solution was prepared at 1 mM in dimethyl sulfoxide, aliquoted, and stored under N_2 at −70°C; the PDA stock was 0.1 mM in dimethyl sulfoxide. OAG was prepared at 2.5 mM in chloroform, aliquoted, and stored under N_2 at −70°C (38). For experiments, the PMA or PDA stock was thawed, diluted 1:100 in phosphate-buffered saline, and further diluted in bath solution to the desired concentration (0.01–1.0 μM); the dimethyl sulfoxide in the final solutions never exceeded 1% (vol/vol), a concentration that, by itself, had no effects on APs or ionic currents. In experiments with OAG, the chloroform was evaporated before dilution in bath solution (38). The concentrations of all agents refer to the concentrations in the puffer pipettes.

RESULTS

Whole-cell recordings (35) from isolated adult rat ventricular myocytes (Fig. 1) revealed AP waveforms indistinguishable from those measured (31, 34) with intracellular microelectrodes: the absence of a prominent plateau phase and rapid repolarization, which appears to comprise two kinetically distinct phases, are characteristic features of APs in these cells. Within 2 sec of initiating puffer application of the α_1 agonist methoxamine (5 μM), the AP was prolonged (Fig. 1, episode 2); the RP was not significantly affected. As the application of methoxamine was continued, further increases in the AP duration were evident at longer times (Fig. 1, episodes 3 and 4). Puffer applications of bath solution, in contrast, were without effects. Although prolonged or repeated applications of methoxamine led to larger and longer-lasting effects on APs and, in some experiments, prevented AP repolarization, when methoxamine applications were discontinued, AP durations returned to control values. The time course of recovery paralleled the duration of the exposure to methoxamine and was accelerated by puffer applications of bath solution. As the effects of α_1 agonists on AP waveforms are similar to those reported for rat ventricular (20) and papillary (28) muscle strips, it appears that isolated myocytes can be considered a reliable system in which to examine the mechanistic details of α_1-receptor-mediated changes in electrical excitability.

Effects of α_1 Agonists on Voltage-Gated Currents. In subsequent experiments, the effects of α_1 agonists on voltage-activated inward and outward currents were examined directly. With I_{Na} and I_{Ca} blocked, I_{out}, evoked during 125-msec depolarizations, was observed to activate rapidly and

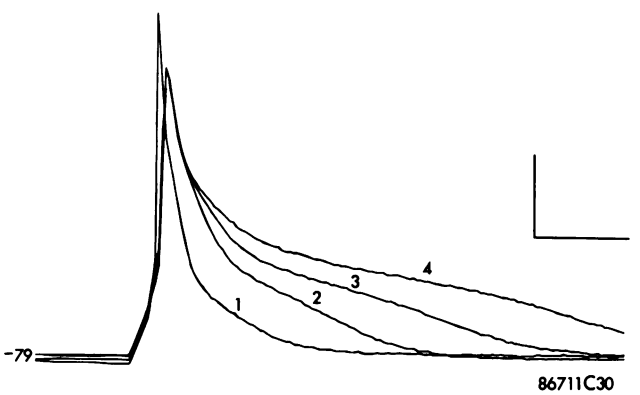

FIG. 1. Effect on the AP of continuous application of methoxamine. APs were evoked at 10-sec intervals by 2.5-msec superthreshold depolarizing current pulses delivered via the recording pipette; four successive APs are shown. After the control AP (episode 1) was recorded, puffer application of methoxamine (5 μM) was initiated; within ≈2 sec, the AP was prolonged (episode 2). If the puffer was turned off after episode 2, the AP returned to its control waveform in ≈1 min. As the application of methoxamine was continued here, further increases in AP duration were observed in episodes 3 and 4; similar results were seen for four cells. Note that the velocity of the AP upstroke, as well as the peak AP amplitude, in episode 1 appears reduced in episode 2. As the time to reach threshold has increased slightly, this effect may reflect a decrease in the stimulus artifact, although we cannot rule out a methoxamine-mediated reduction in I_{Na}; no further changes in the rising phase or the amplitude of the AP are evident in episodes 3 and 4. Calibration bars = 30 mV and 25 msec; RP was −79 mV.

then decay to a plateau level (Fig. 2A, episode 1). Puffer application of phenylephrine (5 μM) reduced I_{out} amplitude by ≈30% (Fig. 2A, episode 2); partial recovery was evident in subsequent records (Fig. 2A, episode 3). After a single 300-msec puff, I_{out} was maximally suppressed within 5 sec and returned to its original level within 2 min; recovery was accelerated by puffing bath solution. No irreversible inhibition of I_{out} was observed, even under conditions leading to ≥50% attenuation of I_{out} amplitudes. Similar results were obtained with methoxamine. Although puffer applications of bath solution produced no suppression of I_{out}, in ≈20% of the cells, small increases in the time to peak I_{out} were observed; to date, however, we have not investigated this effect.

Although phenylephrine is generally considered an α_1 agonist (39), receptor specificity was evaluated directly by examining the effects of α_2- and β-adrenergic agonists on I_{out}: neither the α_2 agonist clonidine (10 μM) nor the β agonist isoproterenol (25 μM) measurably altered the waveforms of I_{out} when applied continuously for up to 5 sec; similar applications of phenylephrine (10 μM) resulted in ≥30% suppression of I_{out}. In addition, the effect of phenylephrine (5 μM) on I_{out} was attenuated when both phenylephrine and the α-adrenergic antagonist phentolamine (10 μM) were present in the same puffer pipette (Fig. 2B); in the presence of the β antagonist propranolol (10 μM), however, the effects of phenylephrine were not altered measurably. These observations suggest that the phenylephrine- or methoxamine-induced suppression of I_{out} is mediated through activation of α_1-adrenergic receptors.

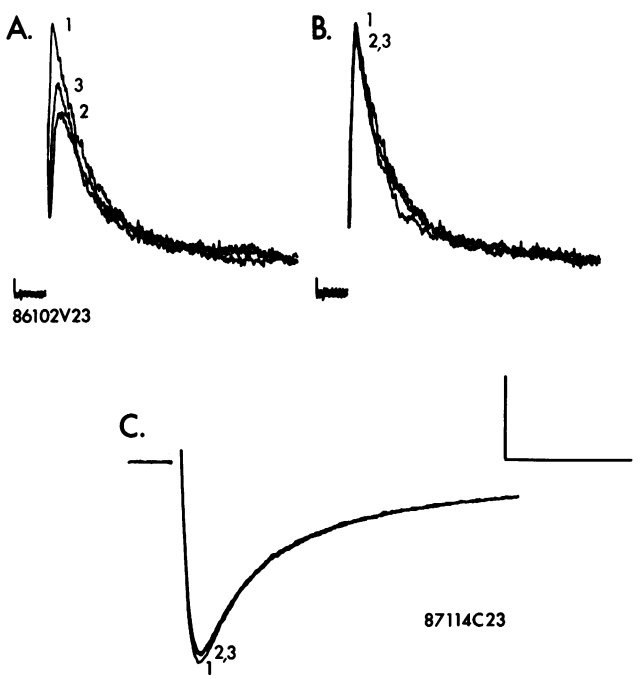

FIG. 2. Effects of phenylephrine on I_{out} (A and B) and I_{Ca} (C). (A and B) I_{out} was evoked at 5-sec intervals during depolarizations to +30 mV from a HP of −60 mV; the bath contained 20 μM TTX and 5 mM Co^{2+} to block I_{Na} and I_{Ca}. Three episodes (numbered 1–3, in the order recorded) are superimposed. (A) The control I_{out} waveform (episode 1) was recorded prior to the application of phenylephrine (5 μM). After a 2-sec puffer application, peak I_{out} amplitude was reduced (episode 2); partial recovery is evident in episode 3, recorded 20 sec after cessation of the phenylephrine application. (B) Voltage-clamp data obtained from the same cell by using an experimental paradigm identical to that in A, except that the cell was exposed to a solution containing 5 μM phenylephrine and 10 μM phentolamine for 2 sec; the suppression of I_{out} produced by phenylephrine alone (A) was markedly attenuated in the presence of phentolamine (B). Similar results were obtained with nine cells. (C) In the absence of Co^{2+}, I_{Ca} was measured in the presence of 5 mM Ca^{2+} during depolarizations to 0 mV from a HP of −60 mV; 20 μM TTX was present to block I_{Na} and the recording pipette contained 140 mM CsCl to block I_{out}. After episode 1 (control), the cell was exposed to methoxamine (20 μM) and episodes 2 and 3 were recorded 10 (episode 2) and 60 (episode 3) sec later. Similar results were obtained with four cells. Calibration bars = 150 pA and 100 msec (A and B) or 150 pA and 40 msec (C).

The waveforms of I_{out} in isolated adult rat ventricular myocytes appear to comprise two distinct components: a fast, transient K^+ current [similar to that described previously (40)] and a delayed, slowly activating, K^+ current (ref. 36 and unpublished data). In the composite I_{out} waveforms, the peak and plateau currents reflect primarily the transient and the delayed components, respectively. Although both components of I_{out} are evident in all cells, their relative amplitudes vary markedly among cells. The effects of α_1 agonists on the two components of I_{out} were examined separately, therefore, by measuring the percent suppression of peak and plateau I_{out} after puffer applications of phenylephrine (10 μM): both components were attenuated to approximately the same extent (Table 1), although, in most cases, there appeared to be a greater reduction in the peak. Similar results were obtained with methoxamine (Table 1).

As α-adrenergic agonists reportedly increase I_{Ca} in bovine ventricular strips (23), the effects of phenylephrine and methoxamine on I_{Ca} in isolated rat ventricular myocytes were also examined. In contrast to the effects on I_{out}, however, prolonged puffer applications of methoxamine (20 μM) did not alter the waveforms of I_{Ca} (Fig. 2C); phenylephrine (10 μM) was similarly without effect. With KCl in the electrodes, both inward and outward currents were evoked during depolarizations to potentials positive to −30 mV from HPs of −50 to −90 mV. Although it is difficult to distinguish inward and outward currents during the depolarizations, Ca^{2+} tail currents can be measured in isolation during repolarizations to E_K (−70 to −80 mV). Since puffer applications of methoxamine (20 μM), which led to 25–50% reductions in the net outward currents, did not affect Ca^{2+} tail currents (data not shown), α_1 agonists do not appear to have any direct effects on I_{Ca} in this preparation.

It also seems unlikely that the phenylephrine- or methoxamine-induced suppression of I_{out} reflects the activation of an inward current not blocked by Co^{2+} or TTX, as no effects of prolonged puffer applications of methoxamine (20 μM) were observed on holding (or "leak") currents measured (in the absence of depolarizations) at HPs of −60 and −20 mV. In addition, with outward K^+ currents blocked (by Cs^+), no inward or outward currents were evoked during depolarizations in the presence or absence of phenylephrine.

As reductions in I_{out} amplitudes (Table 1) might be accounted for by changes in the voltage dependences of outward-current activation and/or inactivation, peak and plateau I_{out} amplitudes were measured in the absence or presence of phenylephrine during depolarizations from various HPs. When phenylephrine (10 μM) was applied, no changes in either the normalized current–voltage relations

Table 1. Attenuation of I_{out} by α_1-adrenergic agonists and activators of PKC

Test agent*	Duration, sec	Mean % suppression (range)		$n^\dagger$
		Peak I_{out}	Plateau I_{out}	
PE (10 μM)	>30	30 (23–45)	23 (18–32)	9
ME (10 μM)	3–5	31 (26–37)	14 (3–22)	5
PMA (1 μM)	1–3	34 (12–63)	17 (7–36)	7
OAG (60 μM)	>10	31 (15–50)	32 (17–53)	13

*PE, phenylephrine; ME, methoxamine.
†No. of cells.

(Fig. 3A) or the voltage dependences of steady-state inactivation (Fig. 3B) were observed for either the peak or the plateau components of I_{out}. It seems unlikely, therefore, that changes in voltage-dependence can account for the phenylephrine-induced attenuation of I_{out}. Reductions in amplitude, in the absence of effects on the time- and voltage-dependent properties of I_{out}, suggest a reduction in either the number of functional channels or the average single-channel conductance. It is not possible, however, to distinguish between these possibilities from the macroscopic recordings; single-channel analyses would be required.

Activation of PKC and K^+-Current Suppression. Since inositolphospholipid turnover (11–13) is accompanied by the production of diacylglycerol (14), the possibility that PKC might be involved in mediating the effects of α_1 agonists was explored. Puffer applications of the phorbol ester (41) PMA (100 nM) resulted in the rapid and reversible suppression of I_{out} (Fig. 4A); applications of the diacylglycerol analogue (42) OAG (60 μM) also suppressed I_{out} (Table 1). In contrast, PDA (100 nM), an analogue of PMA with 1/1000th the affinity for purified brain PKC (41), had little or no effect on I_{out} (Fig. 4B). After 1- to 3-sec exposures to PMA (1 μM), both the peak and plateau components of I_{out} were attenuated (Table 1); as with α_1 agonists, the peak appeared to be suppressed more than the plateau. In addition, brief (200-msec) applications of PMA resulted in the dose-dependent attenuation of I_{out} (Table 2). Interestingly, although the percent reductions in the peak and plateau components were nearly identical (Table 2) at 10 nM PMA, at 1 μM, peak currents were attenuated to a larger extent. Neither PMA (10 nM–1 μM) nor OAG (60 μM) measurably affected holding currents or I_{Ca} in these cells, and no changes in the kinetics or voltage-dependent properties of I_{out} were evident during prolonged applications of these agents. These results, taken together, suggest that phenylephrine, methoxamine, PMA, and OAG suppress I_{out} specifically. As PMA and OAG activate PKC (41, 42), it seems likely that I_{out} suppression is mediated through a PKC-dependent pathway.

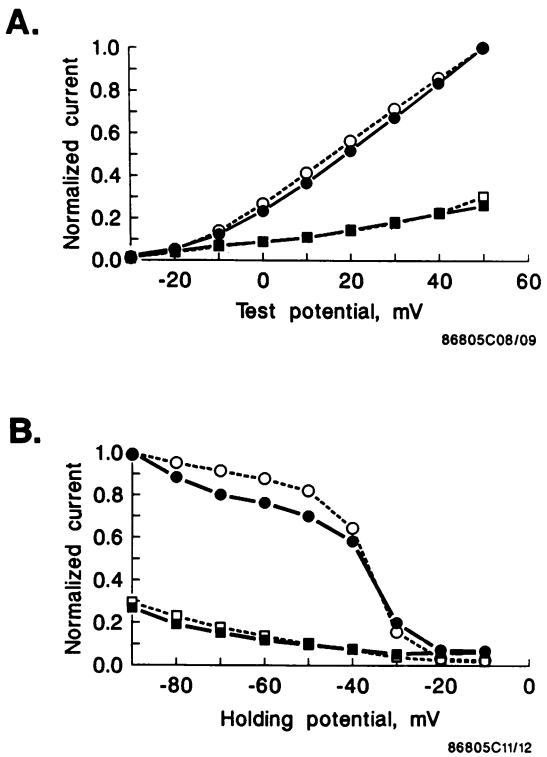

FIG. 3. Lack of effect of phenylephrine on the voltage dependence of activation (A) and steady-state inactivation (B) of the peak (○, ●) and plateau (□, ■) components of I_{out}. (A) The voltage-dependence of I_{out} activation was examined during depolarizations to −30 mV to +50 mV from a HP of −60 mV. In the absence of phenylephrine, the peak (○) and plateau (□) I_{out} amplitudes were measured and normalized to the peak current observed during depolarization to +50 mV. Peak (●) and plateau (■) current amplitudes were then remeasured during continuous exposure to phenylephrine (10 μM) and similarly normalized. No measurable effects of phenylephrine on the current–voltage relations were evident; similar results were obtained with six cells. (B) The voltage dependence of steady-state inactivation of I_{out} was examined during depolarizations to +30 mV from HPs between −10 and −90 mV. Control peak (○) and plateau (□) current amplitudes, recorded in the absence of phenylephrine, were normalized to the peak current observed during depolarization from −90 mV. Peak (●) and plateau (■) currents were then remeasured during continuous application of phenylephrine (10 μM) and similarly normalized. No significant changes in the voltage dependences of steady-state inactivation were evident in the presence of phenylephrine; similar results were obtained with two cells.

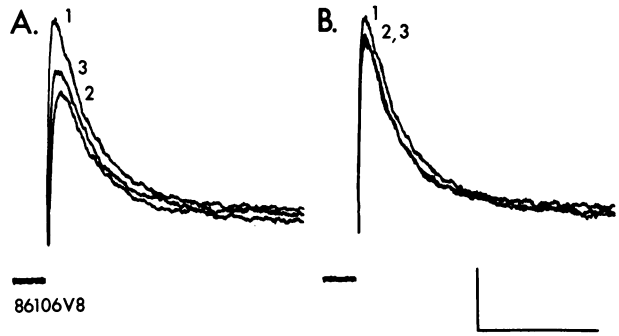

FIG. 4. Effects of phorbol esters on I_{out}. Outward currents were recorded as described in the legend of Fig. 2. (A) After the measurement of the control current (episode 1), the cell was exposed for 2 sec to PMA (100 nM) and episode 2 was recorded. Recovery from the PMA-induced suppression of I_{out} is evident in episode 3, recorded 30 sec after cessation of the PMA application. (B) The experimental paradigm was identical, except that prior to episode 2, the cells was exposed to PDA (100 nM) for 2 sec. It is evident that PDA was relatively ineffective at reducing the amplitude of I_{out}. Calibration bars = 150 pA and 100 msec.

Following brief exposures, the effects of phenylephrine or methoxamine developed rapidly; I_{out} suppression after a single 200-msec application was maximal when puffs preceded voltage steps by 100–200 msec. As the effects of PMA were also observed rapidly (Table 2) and over a time course similar to that seen with phenylephrine, it might be suggested that second-messenger generation is likely not the rate-limiting step in the pathway leading to modulation of I_{out} after α_1-receptor stimulation. In contrast to the rapid onset, recovery of I_{out} following phenylephrine- or PMA-induced suppression was slow; as recovery was accelerated by puffing bath solution, the slow time course likely reflects diffusion of phenylephrine or PMA away from binding sites.

DISCUSSION

The results presented here reveal that α-adrenergic agonists suppress I_{out} in adult rat ventricular myocytes; this suppression is attributed to the specific activation of α_1 receptors because the effects of phenylephrine and methoxamine are attenuated by α-adrenergic, but not β-adrenergic, antagonists

Table 2. Brief (200-msec) applications of PMA suppress I_{out}

PMA, μM	Mean % suppression (range) Peak I_{out}	Plateau I_{out}	n
0.01	12 (7–18)	15 (12–22)	5
0.1	18 (3–45)	17 (4–25)	8
1.0	27 (8–48)	16 (11–26)	5

and, in addition, neither α_2- nor β-adrenergic agonists alter I_{out}. Although β-adrenergic-induced increases in voltage-gated K^+ currents might be expected (43), the effects of β agonists on mammalian ventricular myocytes appear to be evident only at temperatures ≥28°C (44). As no direct effects on I_{Ca} were observed here, these results conflict with a previous report (23) that α_1 agonists increase I_{Ca} in bovine ventricular trabeculae. Although the reason for this discrepancy is not clear, it may be that different mechanisms predominate in different species (23). Alternatively, as it is difficult in multicellular preparations (23) to separate I_{Ca} completely from overlapping outward K^+ currents, it may also be difficult to determine whether a net increase in inward current resulted from an increase in I_{Ca} directly or from a decrease in I_{out}. It is not possible to distinguish between these possibilities from our data alone; further studies of α_1-adrenergic-mediated effects on isolated myocytes from different species will be necessary to resolve this issue.

It has become increasingly apparent that membrane ion channels can be regulated through a variety of phosphorylation-dependent mechanisms. Modulation of cardiac Ca^{2+} channels by a mechanism involving cAMP-dependent protein kinase in response to β-adrenergic stimulation, for example, is well documented (7–9). More recently, a role for PKC in the regulation of ion channels in neurons (38, 45, 46) and in cardiac cells (17–19) has also been demonstrated. In adult guinea pig ventricular myocytes, for example, PMA increases delayed K^+ currents (17). Although it was suggested (17) that this effect might be related to the positive inotropic effects of α_1 agonists, it is not clear how an increase in outward current would lead to AP prolongation and increases in contractile force. In neonatal rat ventricular myocytes, PMA has negative inotropic effects and increases I_{Ca} (19); the physiological significance of these observations is similarly not evident. As the results of both of these studies are quite different from those described here, it may be that different cell types respond differently to PKC activators. Alternatively, it is possible that PMA has effects that are not mimicked by either diacylglycerol analogues or endogenous physiological stimuli (47). In agreement with the latter suggestion, phorbol esters and diacylglycerol analogues have different effects on isolated guinea pig atria and only the diacylglycerol analogues mimic the positive inotropic effects of α_1 agonists (18). Nevertheless, the similarities in the effects of α_1 agonists and activators of PKC on I_{out} observed here suggest that these agents act through the same (or a very similar) mechanism in adult rat ventricular myocytes. These observations, together with previous biochemical evidence demonstrating α_1-receptor-stimulated turnover of inositol-phospholipids (11–13), also suggest the possibility that the positive inotropic effects of α_1 agonists are mediated by a mechanism involving PKC-dependent protein phosphorylation. It would be interesting to examine the effects of this modulation on the properties of single K^+ channels. In addition, since the inositol trisphosphate produced by inositolphospholipid hydrolysis (11–13) has been suggested to mediate some of the effects of α_1 agonists (13), it would be of interest to explore the possibility that inositol trisphosphate affects the membrane properties of isolated myocytes.

We thank Joel Solomon, Joe Henry Steinbach, and Jay Yang for discussion and critical reading of the manuscript. This work was supported by an American Heart Association Established Investigator Award (J.M.N.) and Grant-in-Aid, with partial support provided by the Missouri Affiliate, and by the National Institutes of Health (HL34161 and T32-GM07805).

1. Brückner, R., Mügge, A. & Scholz, H. (1985) *J. Mol. Cell. Cardiol.* **17**, 639–645.
2. Mukherjee, A., Haghani, Z., Brady, J., Bush, L., McBride, W., Buja, L. M. & Willerson, J. T. (1983) *Am. J. Physiol.* **245**, H957–H961.
3. Schümann, H. J., Wagner, J., Knorr, A., Reidemeister, J. Ch., Sadony, V. & Schramm, G. (1987) *Naunyn-Schmiedebergs Arch. Pharmacol.* **302**, 333–336.
4. Brückner, R., Meyer, W., Mügge, A., Schmitz, W. & Scholz, H. (1984) *Eur. J. Pharmacol.* **99**, 345–347.
5. Corr, P. B., Shayman, J. A., Kramer, J. B. & Kipnis, R. J. (1981) *J. Clin. Invest.* **67**, 1232–1236.
6. Maisel, A. S., Motulsky, H. J. & Insel, P. A. (1987) *Biochem. Pharmacol.* **36**, 1–6.
7. Reuter, H. (1983) *Nature (London)* **301**, 569–574.
8. Brum, G., Flockerzi, V., Hofmann, F., Osterrieder, W. & Trautwein, W. (1983) *Pflügers Arch.* **398**, 147–154.
9. Bean, B. P., Nowycky, M. C. & Tsien, R. W. (1984) *Nature (London)* **307**, 371–375.
10. Buxton, I. L. & Brunton, L. L. (1985) *J. Biol. Chem.* **260**, 6733–6737.
11. Brown, J. H., Buxton, I. L. & Brunton, L. L. (1985) *Circ. Res.* **57**, 532–537.
12. Poggioli, J., Sulpice, J. C. & Vassort, G. (1986) *FEBS Lett.* **206**, 292–298.
13. Otani, H., Otani, H. & Das, D. K. (1988) *Circ. Res.* **62**, 8–17.
14. Nishizuka, Y. (1984) *Nature (London)* **308**, 693–698.
15. Lindemann, J. P. (1986) *J. Biol. Chem.* **261**, 4860–4867.
16. Presti, C. F., Scott, B. T. & Jones, L. R. (1985) *J. Biol. Chem.* **260**, 13879–13889.
17. Tohse, N., Kameyama, M. & Irisawa, H. (1987) *Am. J. Physiol.* **22**, H1321–H1324.
18. Teutsch, I., Weible, A. & Seiss, M. (1987) *Eur. J. Pharmacol.* **144**, 363–367.
19. Dösemeci, A., Dhallan, R. S., Cohen, N. M., Lederer, W. J. & Rogers, T. B. (1988) *Circ. Res.* **62**, 347–357.
20. Wenzel, D. G. & Su, J. L. (1966) *Arch. Int. Pharmacodyn.* **160**, 379–389.
21. Wagner, J. & Schümann, H.-J. (1979) *Life Sci.* **24**, 2045–2052.
22. Miura, Y., Inui, J. & Imamura, H. (1978) *Naunyn-Schmiedebergs Arch. Pharmacol.* **301**, 201–205.
23. Brückner, R. & Scholz, H. (1984) *Br. J. Pharmacol.* **82**, 223–232.
24. Ledda, F., Marchetti, P. & Mugelli, A. (1975) *Br. J. Pharmacol.* **54**, 83–90.
25. Ledda, F., Mantelli, L. & Mugelli, A. (1980) *Br. J. Pharmacol.* **69**, 565–571.
26. Hauswirth, O., Wehner, H. D. & Ziskoven, R. (1976) *Nature (London)* **263**, 155–156.
27. Handa, Y., Wagner, J., Inui, J., Averesch, H. & Schümann, H. J. (1982) *Naunyn-Schmiedebergs Arch. Pharmacol.* **318**, 330–335.
28. Skomedal, T., Osnes, J.-B. & Oye, I. (1982) *Acta Pharmacol. Toxicol.* **50**, 1–12.
29. Apkon, M. & Nerbonne, J. M. (1986) *Soc. Neurosci. Abstr.* **12**, 15.
30. Wittenberg, B. A. & Robinson, T. F. (1981) *Cell Tissue Res.* **216**, 231–251.
31. Powell, T., Terrar, D. A. & Twist, V. W. (1980) *J. Physiol. (London)* **302**, 131–153.
32. Borg, T. K., Rubin, K., Lundgren, E., Borg, K. & Obrink, B. (1984) *Dev. Biol.* **104**, 86–96.
33. Schwartz, P., Piper, H. M., Spahr, R. & Spieckermann, P. G. (1985) in *Isolated Adult Cardiac Myocytes: Structure, Function, and Metabolism*, eds. Spieckermann, P. G. & Piper, H. M. (Springer, New York), pp. 181–185.
34. Meier, C. F., Jr., Briggs, G. M. & Claycomb, W. C. (1986) *Am. J. Physiol.* **250**, H731–H735.
35. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391**, 85–100.
36. Apkon, M. & Nerbonne, J. M. (1986) *Biophys. J.* **49**, 55a (abstr.).
37. Reuter, H. (1984) *Annu. Rev. Physiol.* **46**, 473–484.
38. Rane, S. G. & Dunlap, K. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 184–188.
39. Berthelson, S. & Pettinger, W. A. (1977) *Life Sci.* **21**, 595–606.
40. Josephson, I. R., Sanchez-Chapula, J. & Brown, A. M. (1984) *Circ. Res.* **54**, 157–162.
41. Leach, K. L., James, M. L. & Blumberg, P. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4208–4212.
42. Kaibuchi, K., Takai, Y., Sawamura, M., Hoshijima, M., Fujikura, T. & Nishizuka, Y. (1983) *J. Biol. Chem.* **258**, 6701–6704.
43. Nakayama, T. & Fozzard, H. A. (1988) *Circ. Res.* **62**, 162–172.
44. Walsh, K. B., Begenisich, T. & Kass, R. S. (1988) *Pflügers Arch.* **411**, 232–234.
45. DeRiemer, S. A., Strong, J. A., Albert, K. A., Greengard, P. & Kaczmarek, L. K. (1985) *Nature (London)* **313**, 313–316.
46. Malenka, B. C., Madison, D. V., Andrade, R. & Nicoll, R. A. (1986) *J. Neurosci.* **6**, 475–480.
47. Cochet, C., Keramidas, M., Souvignet, C. & Chambaz, E. M. (1986) *Biochem. Biophys. Res. Commun.* **138**, 1283–1290.