\( \alpha_1 \)-Adrenoceptor Stimulation Enhances the Delayed Rectifier \( K^+ \) Current of Guinea Pig Ventricular Cells Through the Activation of Protein Kinase C

Noritsugu Tohse, Haruaki Nakaya, and Morio Kanno

The effect of \( \alpha_1 \)-adrenoceptor stimulation on the delayed rectifier \( K^+ \) current (\( I_K \)) was examined in isolated guinea pig ventricular cells by use of the patch-clamp method. \( I_K \) was evoked by a 3-second depolarizing pulse from a holding potential of \(-30 \) mV in a \( Na^+ \)- and \( K^+ \)-free solution containing 3 \( \mu M \) nifedipine. Phenylephrine (30 \( \mu M \)) in the presence of propranolol (1 \( \mu M \)) produced an increase in \( I_K \). In five cells, phenylephrine increased the tail current of \( I_K \) by 23 \( \pm \) 5\%. This effect of phenylephrine was blocked by prazosin (0.3 \( \mu M \)), a selective \( \alpha_1 \)-blocker. Phenylephrine produced only a small effect on the voltage and time dependence of \( I_K \). Pretreatment with 1-(5-isouquinolinesulfonyl)-2-methylpiperazine (H-7, 10 \( \mu M \)) abolished the phenylephrine-induced increase in \( I_K \). In addition, pretreatment with a maximally effective concentration of 12-\( O \)-tetradecanoylphorbol 13-acetate (100 \( nM \)) abolished the phenylephrine-induced increase in \( I_K \). In conclusion, \( \alpha_1 \)-adrenoceptor stimulation increases \( I_K \) in guinea pig cardiomyocytes. This \( \alpha_1 \)-adrenoceptor-mediated response may be related to an activation of protein kinase C. The increase in \( I_K \) may explain a shortening of action potential duration observed after \( \alpha_1 \)-adrenoceptor stimulation in guinea pig cells. (Circulation Research 1992;71:1441-1446)

**KEY WORDS** *\( \alpha_1 \)-adrenoceptors* • *delayed rectifier \( K^+ \) current* • *protein kinase C* • *cardiomyocytes* • *phenylephrine* • *H-7* • *patch clamp*

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ecently, many reports\(^1\-^5\) have indicated that \( \alpha_1 \)-adrenoceptor stimulation evoked the phosphatidylinositol turnover in cardiomyocytes. Otani et al\(^2\) and Scholz et al\(^3\) proposed that an increase in intracellular inositol trisphosphate produced by \( \alpha_1 \)-adrenoceptor stimulation may cause \( Ca^{2+} \) mobilization, resulting in a positive inotropic response. However, it is still controversial whether or not inositol trisphosphate can mobilize \( Ca^{2+} \) from the sarcoplasmic reticulum in cardiac myocytes.\(^5\)-\(^8\) In addition, Endoh et al\(^4\) have suggested that activation of protein kinase C may not be involved in the \( \alpha_1 \)-adrenoceptor-mediated positive inotropic response, because phorbol esters (activators of protein kinase C) could not mimic and 1-(5-isouquinolinesulfonyl)-2-methylpiperazine (H-7, an inhibitor of protein kinase C) could not inhibit the positive inotropic response. On the other hand, Capogrossi et al\(^5\) have recently shown that activation of protein kinase C may be responsible for the \( \alpha_1 \)-receptor-mediated negative inotropic response in \( Ca^{2+} \)-loaded rat cardiomyocytes. Thus, the causal relation between increases in the phosphatidylinositol turnover (inositol trisphosphate and diacylglycerol) and physiological (e.g., inotropic and chronotropic) responses evoked by \( \alpha_1 \)-adrenoceptor stimulation remains unclear.

\( \alpha_1 \)-Adrenoceptor stimulation is known to produce various electrophysiological changes in several mammalian cardiac muscles.\(^5\) It was demonstrated that \( \alpha_1 \)-adrenoceptor stimulation produces a prolongation of action potential duration (APD) in rat\(^10\) and rabbit\(^5\) cardiac muscles. The \( \alpha_1 \)-adrenoceptor-mediated APD prolongation might be ascribed to an inhibition of the transient outward current (\( I_{to} \))\(^11,12\) in rat and rabbit cardiomyocytes. However, the inhibition of \( I_{to} \) was not mimicked by the extracellular application of phorbol esters\(^11,12\) in these cells.

On the other hand, in guinea pig cardiac muscles, \( \alpha_1 \)-adrenoceptor stimulation is known to shorten the APD.\(^13\) Previously, we demonstrated that 12-\( O \)-tetradecanoylphorbol 13-acetate (TPA) increased the delayed rectifier potassium current (\( I_K \)) in isolated guinea pig cardiomyocytes and that the increase in \( I_K \) was blocked by H-7.\(^14\) Furthermore, we showed that intracellular perfusion with purified protein kinase C could mimic the effect of TPA.\(^15\) Therefore, \( \alpha_1 \)-adrenoceptor stimulation may produce an increase in \( I_K \) through the activation of protein kinase C, resulting in shortening of the APD. In the present study, we tested this hypothesis in guinea pig cardiomyocytes by use of patch-clamp techniques.

**Materials and Methods**

Single ventricular cells were enzymatically isolated by a previously described method.\(^15\) Membrane currents were recorded in the whole-cell voltage-clamp mode.
The pipette solution contained (mM) KOH 110, KCl 20, MgCl₂ 1, K₃ATP 5, K₇ creatine phosphate 5, aspartic acid 90–100, and EGTA 10. The pH was adjusted to 7.4 by 5 mM HEPES. Total K⁺ concentration was 150 mM. The concentration of free Ca²⁺ in the pipette solution (pCa 10) was buffered by use of 10 mM EGTA. Composition of Na⁺⁻ and K⁺⁻ free external solution was (mM) choline chloride 149, CaCl₂ 1.8, MgCl₂ 0.5, glucose 5.5, and HEPES-LiOH buffer 5.5; the pH was adjusted to 7.4. The temperature of the perfusate was kept constant at 34–36°C. Phenylephrine (Sigma Chemical Co., St. Louis, Mo.), propranolol (Sigma), prazosin (Pfizer Laboratories, New York), and H-7 (Seikagaku Corp., Tokyo) were dissolved in water to prepare a 10-mM stock solution. TPA (Sigma) was dissolved in dimethyl sulfoxide as a stock solution (1 mM). The stock solutions were diluted to a desired concentration by the perfusate. In all experiments, 3 μM nifedipine (Bayer) was used to block the calcium current. Nifedipine was dissolved with ethanol for a 10-mM stock solution.

All experiments were carried out in cells superfused with Na⁺⁻ and K⁺⁻ free external solution containing 3 μM nifedipine. In this condition, Iₓ was isolated from other membrane currents, as previously described.¹⁴,¹⁶ To eliminate a weak β-adrenergic action of phenylephrine, all experiments were carried out in the presence of the β-blocker propranolol (1 μM).

All values are presented as mean±SEM. Statistical analyses were performed using Student’s paired t test.

Results

Figure 1 shows the effects of phenylephrine (30 μM) in the presence of propranolol (1 μM) on the isolated Iₓ. Depolarizing test pulses of 3 seconds were applied from a holding potential of −30 mV to 10 different potentials by 10-mV steps between −20 and 70 mV. Outward membrane currents were evoked by the test pulses, and outward tail currents were observed after repolarization to the holding potential (Figure 1A). These time-dependent currents were designated as Iₓ. After an attainment of a current–voltage relation in the control condition (with 1 μM propranolol), 30 μM phenylephrine was applied to the single cell. Phenylephrine increased the amplitudes of the current during the test pulse and the tail current at every test pulse. Figure 1B shows the activation curve of Iₓ obtained from the same cell as in panel A. In five different cells, 30 μM phenylephrine increased the tail current of Iₓ in response to a depolarizing pulse to 50 mV by 23.3±5.3% over the control value (p<0.05). Because propranolol blocks the β-adrenergic action of phenylephrine, the effect of phenylephrine may be mediated by α-adrenoceptors. In addition, it was confirmed that the selective α₁-adrenoceptor antagonist prazosin (0.3 μM) could abolish the increase in tail current by phenylephrine (n=3). Concentration-related effects of phenylephrine are indicated in Table 1. Although phenylephrine at a concentration of 3 μM produced little effect on Iₓ, a significant increase in Iₓ was observed at 10 and 30 μM. Therefore, 30 μM appeared to be the maximally effective concentration of phenylephrine for an α₁-adrenoceptor–mediated increase in Iₓ.

To examine the effects of phenylephrine on the voltage dependence of Iₓ, we constructed normalized activation curves for the Iₓ tail current. As shown in Figure 2, phenylephrine very slightly shifted the activation curve to the hyperpolarized direction. The half-activation potential was 24.1 and 21.6 mV for control and phenylephrine, respectively. The slope factor was hardly affected by phenylephrine (14.1 for control cells versus 14.8 for phenylephrine-treated cells).

<table>
<thead>
<tr>
<th>Phenylephrine</th>
<th>Increase in delayed rectifier K⁺ tail current (%)</th>
<th>n</th>
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<tbody>
<tr>
<td>3 μM</td>
<td>−4.7±2.4</td>
<td>4</td>
</tr>
<tr>
<td>10 μM</td>
<td>29.9±9.1*</td>
<td>5</td>
</tr>
<tr>
<td>30 μM</td>
<td>23.3±5.3*</td>
<td>5</td>
</tr>
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</table>

n, Number of experiments.
* p<0.05 vs. control.
The tail current, in response to a depolarizing pulse to 50 mV, was not increased but even slightly decreased by 10.5±7.2% by 30 μM phenylephrine in the presence of H-7. As shown in Table 2, phenylephrine produced little effect on the time constants of the I\textsubscript{K} tail current in the presence of H-7. Therefore, these findings indicate that activation of protein kinase C is involved in the α\textsubscript{1}-adrenoceptor–mediated increase in I\textsubscript{K}.

The hypothesis that α\textsubscript{1}-adrenoceptor stimulation enhances I\textsubscript{K} through activation of protein kinase C was also supported by experiments using a combination of phenylephrine and TPA. Figure 4 shows a representative experiment in which an effect of 30 μM phenylephrine was examined in the presence of 100 nM TPA. Although TPA alone markedly increased I\textsubscript{K}, phenylephrine failed to produce a further increase in I\textsubscript{K} in the presence of TPA. In four cells, phenylephrine produced a 4.6±7.4% decrease in I\textsubscript{K} in the presence of TPA. Because the concentration of 100 nM was assumed to be the maximal concentration for the TPA-induced increase in I\textsubscript{K}, as shown in the previous report, the α\textsubscript{1}-adrenoceptors may possess the same pathway as TPA for the increase in I\textsubscript{K}, namely, the activation of protein kinase C.

We previously reported that the I\textsubscript{K} in rat ventricular cells was decreased by α\textsubscript{1}-adrenoceptor stimulation, resulting in the prolongation of the APD. In the present study, we examined whether I\textsubscript{K} exists in guinea pig ventricular cells. Figure 5A shows a family of membrane currents elicited by a 300-msec depolarizing test pulse from -20 to 50 mV from a holding potential of -70 mV in guinea pig ventricular cells. To observe all types of K\textsuperscript{+} currents, Na\textsuperscript{+}-free external solution containing 5.4 mM K\textsuperscript{+} and 3 μM nifedipine was used. At potentials from -20 to 0 mV, a negative slope of the inward rectifier K\textsuperscript{+} current (I\textsubscript{K1}) was observed. At a more positive potential range, slowly activating I\textsubscript{K} was prominent, but any transient surge of outward current in the early phase of test pulses was not observed. After an application of 3 mM 4-aminopyridine (4-AP), a blocker of I\textsubscript{K1}, I\textsubscript{K} and I\textsubscript{K1} were reduced. The right tracings in Figure 5 (subtracted) are current tracings produced by subtraction of the tracings with 4-AP from control tracings. Transient inward deflections observed in the early phase may be artifacts because of subtraction. The subtracted tracings indicate that only I\textsubscript{K} and I\textsubscript{K1} were partially blocked by 4-AP. Therefore, it is evident that there is no 4-AP–sensitive I\textsubscript{Ko} in guinea pig ventricular cells. Similar results were obtained in three other cells. In contrast with guinea pig cells, the 4-AP–sensitive I\textsubscript{Ko} was prominent in rat ventricular cells when the same voltage protocol was used (Figure 5B), which is consistent with our previous report.

**Discussion**

We reported that TPA, an activator of protein kinase C, increased I\textsubscript{K} in guinea pig ventricular cells. The effect of TPA was blocked by 10 μM H-7 and mimicked by 1-oleoyl-2-acetylglycerol, a synthetic diacylglycerol. A similar effect of another phorbol ester, phorbol 12,13-dibutyrate (PDBu), was also reported by other investigators. Furthermore, we showed that protein kinase C (type III [α]) purified from bovine brain re-

**Table 2. Effects of 30 μM Phenylephrine on the Time Constants of the Delayed Rectifier K\textsuperscript{+} Tail Current in the Absence and Presence of 10 μM Protein Kinase C Inhibitor H-7**

<table>
<thead>
<tr>
<th>Without H-7 (n=6)</th>
<th>Fast time constant (msec)</th>
<th>Slow time constant (msec)</th>
</tr>
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<tbody>
<tr>
<td>Before PHE</td>
<td>164±35</td>
<td>735±190</td>
</tr>
<tr>
<td>After PHE</td>
<td>227±36*</td>
<td>1,090±207*</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>With H-7 (n=4)</th>
<th>Fast time constant (msec)</th>
<th>Slow time constant (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before PHE</td>
<td>187±67</td>
<td>849±398</td>
</tr>
<tr>
<td>After PHE</td>
<td>157±45*</td>
<td>784±306*</td>
</tr>
</tbody>
</table>

\(n\), Number of experiments; H-7, protein kinase C inhibitor; PHE, phenylephrine. Values are mean±SEM. *p=NS vs. corresponding value before PHE.
markedly increased $I_k$ when the enzyme was internally applied by the cell dialysis method\textsuperscript{15}. Therefore, activation of protein kinase C definitely enhances $I_k$ in guinea pig cardiomyocytes. Recently, the cloned cardiac K$^+$ channel expressed in *Xenopus* oocytes was reported by Honore et al\textsuperscript{18} to be decreased by activation of protein kinase C. This discrepancy may come from differences of experimental conditions, e.g., our "native" channels versus their expressed and cloned channels.

Many reports indicate that $\alpha_2$-adrenoceptor stimulation can increase phosphatidylinositol turnover and then activate protein kinase C in cardiac preparations of various species.\textsuperscript{1-5} In the present study, $\alpha_2$-adrenoceptor stimulation produced an increase in $I_k$. The $\alpha_2$-adrenoceptor–mediated effect was abolished by 10 $\mu$M H-7. As mentioned above, H-7 blocked the $I_k$ increase produced by protein kinase C in our previous study.\textsuperscript{14} Therefore, $\alpha_2$-adrenoceptor stimulation appears to activate protein kinase C and then increase $I_k$. This hypothesis is also supported by the observation that phenylephrine failed to increase $I_k$ in the presence of the maximally effective concentration of TPA. This finding implies that protein kinase C activator and $\alpha_2$-adrenoceptor stimulation share a common pathway for the increase in $I_k$. Another $\alpha_2$-adrenergic modulation of cardiac ion channels through the activation of protein kinase C was recently reported in the Cl$^-$ channel of guinea pig heart cells.\textsuperscript{19}

The voltage dependence of $I_K$ was hardly affected by $\alpha_2$-adrenoceptor stimulation. However, Walsh and Kass\textsuperscript{20} showed that PDBu produced steepened voltage dependence of $I_k$ and a slight shift of the half-activation potential. This discrepancy might stem from differences in experimental conditions. We used phenylephrine and data during the deactivation time course (tail current), whereas they used PDBu and data during the activation time course.

Recently, it was reported that $I_k$ in guinea pig cardiomyocytes was the composite of two currents, rapidly activating $I_k$ ($I_{Ks}$) and slowly activating $I_k$ ($I_{Ko}$).\textsuperscript{21} In that report, $I_{Ks}$ was almost completely suppressed in K$^+$-free external solution. Therefore, $I_k$ in the present study is the same current as $I_{Ko}$ in that report.

There are species differences in electrophysiological responses to $\alpha_2$-adrenoceptor stimulation. In rat\textsuperscript{10} and rabbit\textsuperscript{5} cardiac muscles, $\alpha_2$-adrenoceptor stimulation prolonged APD. However, phorbol esters failed to prolong the APD in rat cardiac muscles.\textsuperscript{4} Recent reports from our laboratory\textsuperscript{11} and other laboratories\textsuperscript{12,22,23} showed that the $\alpha_2$-adrenoceptor–mediated prolongation of the APD can be ascribed to the inhibition of $I_{no}$ in rat\textsuperscript{11,22} and rabbit\textsuperscript{12,23} cardiac cells. These reports also showed that the activation of protein kinase C did not inhibit $I_{no}$\textsuperscript{11,12}. On the other hand, Dirksen and Shue\textsuperscript{13} showed that $\alpha_2$-adrenoceptor stimulation and phorbol ester shortened the APD in guinea pig papillary muscles. Because $I_{no}$ channels do not exist in guinea pig ventricular cells, as shown in this study, $\alpha_2$-adrenoceptor stimulation might fail to prolong the APD. The $\alpha_2$-adrenoceptor–mediated shortening of the APD may be

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**Figure 3.** Effect of pretreatment with protein kinase C inhibitor H-7 (10 $\mu$M) on the $\alpha_2$-adrenoceptor–mediated increase in the delayed rectifier K$^+$ current ($I_k$). Panel A: Tracings showing $I_{Ks}$ elicited by 3-second depolarizing test pulses to 10 different potentials by 10-mV steps between −20 and 70 mV. The holding potential was −30 mV. Dashed lines indicate the zero current level. Note that phenylephrine (30 $\mu$M) failed to increase $I_k$ in the presence of H-7. Panel B: Activation curves of the tail current before and after phenylephrine in the H-7–pretreated cell. Note that both curves almost overlap.

**Figure 4.** Time course of changes in the delayed rectifier K$^+$ tail current produced by 100 nM 12-O-tetradecanoylphorbol 13-acetate (TPA) and 30 $\mu$M phenylephrine. The amplitude of the delayed rectifier K$^+$ tail current was elicited by a 3-second test pulse to 50 mV from a holding potential of −30 mV every 15 seconds.
produced by the increase in $I_K$ through the activation of protein kinase C. On the other hand, the delayed rectification was not observed in rat ventricular cells (see Figure 5B). Therefore, $\alpha_1$-adrenoceptor-mediated inhibition of $I_{so}$ is dominant in rat cells, resulting in APD prolongation. Thus, the species differences of the $\alpha_1$-adrenoceptor-mediated response may come from the species differences in the composition of the membrane current, especially the presence of $I_K$ and $I_{so}$.

Previously, we showed that TPA (10 nM) and protein kinase C (10 $\mu$g/ml) increased $I_K$ by 60% and 150%, respectively. However, in the present study, phenylephrine (30 $\mu$M) increased $I_K$ by only 23%. This discrepancy in $I_K$-increasing ratios may imply that $\alpha_1$-adrenoceptor stimulation in cardiac cells could not produce a full activation of protein kinase C. In the present study, we demonstrated that phenylephrine failed to further increase $I_K$ in cells pretreated with the maximally effective concentration of TPA (100 nM).

Inversely, TPA at the same concentration produced an increase in $I_K$ in a cell pretreated with 30 $\mu$M phenylephrine (authors’ unpublished observation). These findings support the idea that $\alpha_1$-adrenoceptor stimulation produces incomplete activation of protein kinase C. This low efficiency could explain that the phosphatidylinositol turnover only plays a minor role in $\alpha_1$-adrenoceptor-mediated inotropic responses.

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**References**


**FIGURE 5.** Membrane currents of guinea pig and rat ventricular cells in Na$^+$-free external solution. Panel A: Membrane currents of guinea pig ventricular cells in the Na$^+$-free external solution currents were elicited by 300-msec depolarizing test pulses from –20 to –50 mV, from a holding potential of –70 mV. After the application of 3 mM 4-aminopyridine (4-AP), the delayed rectifier and inward rectifier K$^+$ currents were reduced. The subtracted tracings are shown at the right. These tracings were obtained by subtracting the tracings with 4-AP from the control tracings for each test pulse. Panel B: Membrane currents of rat ventricular cells in Na$^+$-free external solution. Voltage protocol was the same as in guinea pig ventricular cells. The transient outward current was prominent and abolished by 3 mM 4-AP. In subtracted tracings, only the transient outward current was observed.
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