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Effect of Prostaglandins D_2, E_2 and I_2 on the Regulation of K_{ATP} Channel Activity in Rat Cardiac Myocytes

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Contribution of prostaglandins D_2, E_2 and I_2 (PGD_2, PGE_2 and PGI_2) on the regulation of ATP-sensitive K^+ channel (K_{ATP} channel) was investigated in isolated single rat ventricular cardiac myocytes using the patch clamp technique. PGD_2, PGE_2 and PGI_2 did not affect K_{ATP} channel activity in the inside-out patch, but increased channel activity in a dose-dependent manner when the channel activities were attenuated by the administration of 100 μM ATP to the internal solution in the inside-out patch. Channel activations by the prostaglandins were abolished by 50 μM glibenclamide, a K_{ATP} channel blocker. Dose-response curves of relative channel activity against the ATP concentrations of internal solution in the inside-out patch were shifted to the right in the presence of those three prostaglandins. The rank order of the channel stimulatory potencies (as IC_{50} for ATP) calculated from the dose-response curves were $PGI_2 > PGD_2 > PGE_2$. Conductance of the channel was not changed by those three prostaglandins. In conclusion, we suggest that prostaglandins D_2, E_2 and I_2 are involved in the regulation of K_{ATP} channel activity in certain circumstances, and that those three prostaglandins may cause myocardial relaxation by opening K_{ATP} channels, thus protecting the heart from ischema.

Key Words: Prostaglandins, K_{ATP} channel, Cardiac myocytes, Patch clamp, Rat

INTRODUCTION

Since ATP-sensitive potassium channels (K_{ATP} channels) were first identified in cardiac muscle of adult guinea pig in 1983 (Noma), it has been well known that the channel play a major role in the control of blood vessel tone in certain circumstances, particularly in hypoxic or ischemic vascular beds (Nichols & Lederer, 1991; Noma, 1991; Quast, 1993). This channel activity, which is inactive under normal cellular conditions, is regulated by the level of intracellular ATP or the ATP/ADP ratio (Pfruender et al, 1993; Ripoll et al, 1993). The channels, however, open in response to metabolic inhibition or cellular ischemia which lowers the cytosolic ATP concentration, resulting in action potential shortening due to an increase in K^+ efflux. Therefore, K_{ATP} channels may play a crucial role in protecting tissues from ischemia through physiological functions such as vasodilation and reduction in heart contractility in the cardiovascular system (Ashcroft, 1988; Nichols & Lederer, 1991; Noma & Takano, 1991; Lynch et al, 1992; Escande & Standen, 1993; Mcpherson, 1993). Recently, some studies have reported that arachidonic acid inhibits K_{ATP} channels and activates a new type of arachidonate-induced K^+ current in rat cardiac myocytes (Kim & Duff, 1990; Wallert et al, 1991). Prostaglandins are cyclooxygenase products derived from arachidonic acid, and their actions are diverse, particularly in the cardiovascular system, such as vasoactive and cardiac stimulation (Burton et al, 1986; Needleman et al, 1986; Campbell, 1991). More recently, it has also been demonstrated that prostaglandin I_2, E_2, and D_2 may activate K_{ATP} channels in Langendorff rat hearts (Bouchard et al, 1994) and vasodilation induced by prostaglandin E_2 can be significantly reduced by glibenclamide, a K_{ATP} channel

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blocker (Jackson et al, 1993). However, little is actually known about the relationship between K_{ATP} channels and prostaglandins in regard to cellular electrophysiology. In addition, its action site also remained unclear. The aim of this study was, therefore, to examine the effects and characteristics of various prostaglandins on K_{ATP} channels using the excised inside-out patch clamp technique in isolated single rat cardiac myocytes.

METHODS

Single cell isolation

Single cardiac myocytes were isolated from male Sprague Dawley rats (250~300 g) stunned by a blow on the neck. The heart was immediately removed and mounted on a Langendorff apparatus, and then retrogradely perfused through the aorta with Krebs-Henseleit solution (Composition; 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 10 mM HEPES, 25 mM $NaHCO_3$, 10 mM Pyruvate, 11 mM dextrose and 1 mM $CaCl_2$) warmed to 37°C, 4 ml/min for 5 min. The heart was then perfused with calcium-free Krebs-Henseleit solution until cessation of the heartbeat, then perfused for approximately 30 min with the same Ca^{2+}-free Krebs-Henseleit solution containing 0.7 mg/ml collagenase (Type II, Worthington Biochemical Co.). Finally, the softened ventricular tissues were cut into small pieces and mechanically dissociated into single cells. Isolated cells were stored in Ca^{2+}-free Krebs-Henseleit solution supplemented with 1% albumin. All cells used in the experiments were clear and rod-shaped as viewed under an inverted micoscope (Biostar, American Optical Co.).

Electrophysiological recordings and data analysis

Patch clamp micropipettes (4~5 MΩ) were pulled from borosilicate glass (#7052, World Precision Instruments) with a two-stage pipette puller (PP-83, Narishige Scientific Instruments). The shank of each pipette was covered with sylgard (Corning Co.) under stereozoom microscope (SMZ-2B, Nikon), then dried using a coiled heater (Lab-made). The electrode tip was fire-polished under Microforge (MF-83, Narishige Scientific Instruments) and pipettes having 5 - 10 MΩ tip resistances were used. Single channel currents were recorded from excised inside-out patches in a method described by Hamil et al (1981). Electrical signals were recorded with a patch clamp amplifier (Axopatch 200 A, Axon Instruments Inc.) and stored on a videotape through a digital data recorder (VR-10B, Instrutech Co.). The cut-off frequency of the filter was set at 2 kHz before display. Single channel currents were analyzed by computer software (pClamp 6.0, Axon Instruments Inc.) with an A/D converter (Digidata 1200, Axon Instruments Inc.). To detect events, the half-maximum single-unit amplitude threshold method was used. As a measure of channel activity, open probability (Po) was calculated by the equation described by Spruce et al (1985).

$$Po = \left(\sum_{j=1}^{n} tj\right) / (Td \cdot n)$$

where tj was the time spent at each level, j corresponded to 1, 2, 3, · · n channel opens, Td was the total recorded time. n was the maximum number of channels seen in ATP-free solutions. Po was calculated via continuous single channel recordings over a 30 sec. period, and relative Po was illustrated as a percentage change, compared of pre- and post-drug exposure.

Solutions and drugs used

The external and internal solution used throughout the experiment had the following composition: 140 mM KCl, 2 mM $MgCl_2$, 5 mM EGTA, 10 mM HEPES (K-5 solution), and pH was adjusted to 7.2. The drugs used in this study were prostaglandins (PGD_2, PGE_2 and PGI_2), glibenclamide and adenosine triphosphate (ATP). All drugs were purchased from the Sigma Chemical Co., and the stock solutions were prepared in ethanol then diluted with the K-5 experimental solution prior to administration. All experiments were carried out at room temperature ranging from 22±2°C.

RESULTS

Characteristics of K_{ATP} channel activity

The channel activity employed in the present study appeared immediately after the excised inside-out patch, and ATP (1 mM) in the internal solution in-

hibited channel activity almost completely. Channels were reactivated without ATP, and glibenclamide, a K_{ATP} channel blocker, inactivated the channel.

The current-voltage (I-V) relationship of K_{ATP} channel activity showed a trend of inward rectification and the channel conductance calculated from the slope of the I-V relation curve was approximately 62 pS. The mean amplitude at -60 mV holding potential was 3.7 ± 0.02 pA, and channel activity was inversely proportional to the dwell time.

Effects of prostaglandins D_2, E_2 and I_2 on K_{ATP} channel activity

PGD_2, E_2 and I_2 did not affect full-activated channel activity, immediately following making the inside-out patch where the ATP concentration in the bath solution was almost zero. However when channel activity was reduced by the addition of 100 μM ATP to the bath solution, PGD_2 increased channel activity and augmented channel activity subsided with the addition of 50 μM glibenclamide (Fig. 1). 50 μM PGE_2 and PGI_2 also activate K_{ATP} channels in the presence of internal 100 μM ATP.

The relationship between channel activation by the prostaglandins and internal ATP concentration

We examined the effects of prostaglandins D_2, E_2 and I_2 on K_{ATP} channel activity in the presence of ATP at various concentrations (ranging from 1 to 1000 μM) in the bath solution. 50 μM PGI_2 increased all channel activities which were recorded in the presence of ATP at various concentrations in the bath solution (Fig. 2). The change of relative channel activity by those prostaglandins (50 μM PGD_2, PGE_2 and PGI_2) was plotted against a dose of ATP which was added to the bath solution. Each prostaglandin-

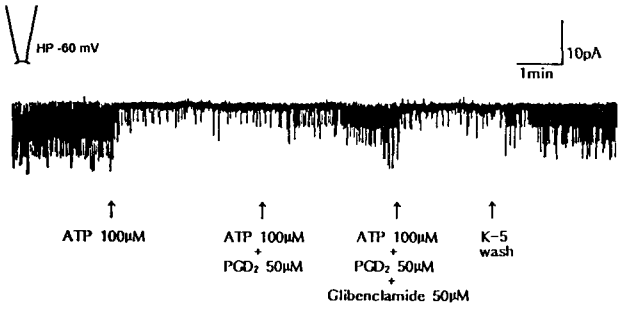

Fig. 1. Typical recording showing the prostaglandin D_2 (PGD_2) effects on the single K_{ATP} channel currents in excised inside-out patch membrane of isolated rat single cardiac ventricular myocytes at -60 mV holding potential.

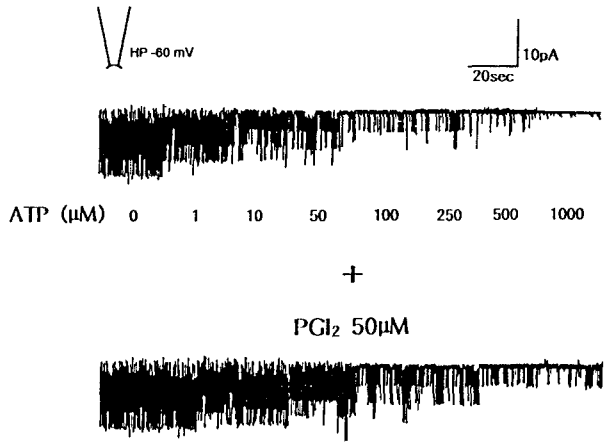

Fig. 2. Typical recording showing ATP dependency of the spontaneous and prostaglandin I_2 (PGI_2)-induced K_{ATP} currents in excised inside-out patch membrane of isolated rat single cardiac ventricular myocytes. K_{ATP} channel currents were inhibited by ATP in a concentration-dependent manner (top row). Prostaglandin I_2 (PGI_2) increased the K_{ATP} channel currents in the presence of each ATP concentration (bottom row).

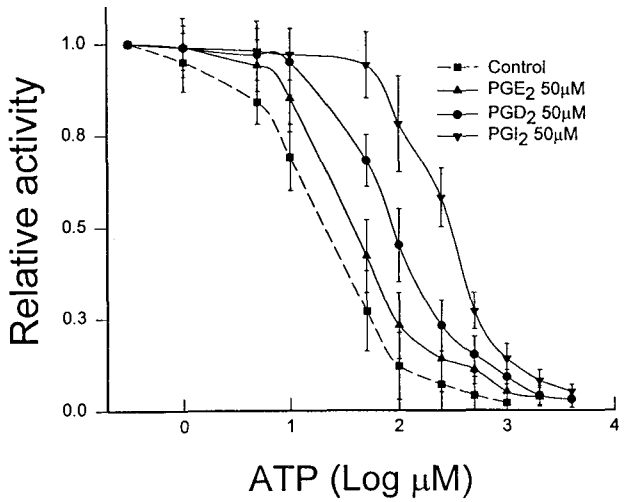

Fig. 3. Effects of 50 μM prostaglandins D_2, E_2 and I_2 (PGD_2, PGE_2, PGI_2) on the K_{ATP} channel activity in the presence of each dose of ATP. Relative activity represents the channel acitivity at immediate after making excised inside-out patch preparation. Control means dose-response curve of ATP alone. Each point represents mean $\pm$SEM of 4~5 experiments.

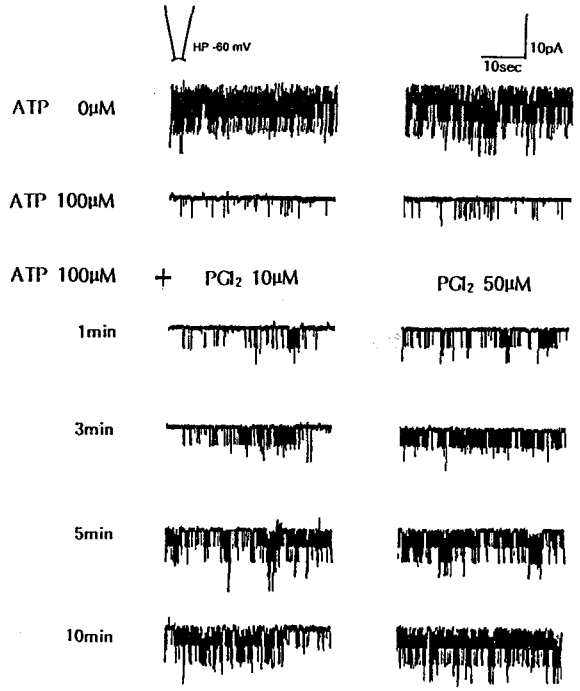

Fig. 4. Effects of prostaglandin I_2 (PGI_2) on the K_{ATP} channel currents of excised inside-out patch membrane of isolated single rat cardiac ventricular myocytes at −60 mV holding potential in the presence of 100 μM internal ATP. Time indicates the minutes after administration of each PGI_2.

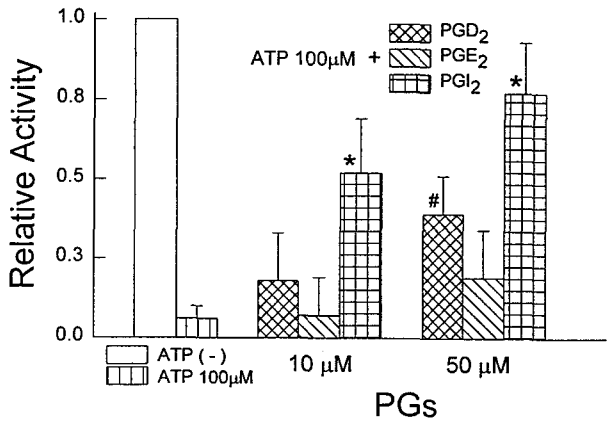

Fig. 5. Effects of prostaglandins (PGs; PGD_2, PGE_2, PGI_2) on the K_{ATP} channel activity of excised inside-out patch membrane at −60 mV holding potential of isolated single rat cardiac ventricular myocytes in the presence of 100 μM internal ATP. Relative activity represents the channel acitivity at immediate after making excised inside-out patch preparation. Each bar represents the mean ± SEM of 4~5 experiments. * and # indicate significance differences at $p<0.01$ and $p<0.05$, compared with the 100 μM internal ATP group.

present dose-response curve shifted the dose-response curve for ATP alone to the right, and the IC_{50} for ATP was 79±5.2, 40±3.4 and 316±16.4 μM in the presence of PGD_2, PGE_2 and PGI_2, respectively (Fig. 3).

Influence of dose and time on channel activation by prostaglandins D_2, E_2 and I_2

In the presence of 100 μM ATP in the bath solution, PGI_2 gradually increased channel activity over time, with a maximum effect 5 or 10 min after administration. Futhermore, the higher the admini stered dose of PGI_2, the greater the increase in channel activity (Fig. 4). 10 and 50 μM of PGI_2 and 50 μM PGD_2 significantly increased channel activity in the presence of 100 μM ATP compared to that of 100 μM ATP alone (Fig. 5).

DISCUSSION

In the present study, we evaluate the contribution of prostaglandins D_2, E_2 and I_2 in the regulation of K_{ATP} channel activity. Buchard et al (1994) already reported that infusion of PGE_2, PGD_2 and iloprost (a PGI_2 analogue) in isolated hearts induced marked vasodilatation and that glibenclamide significantly reduced the vasodilatation induced by those three prostaglandins suggesting a relationship between those prostaglandins and the regulation of K_{ATP} channel activity. However, evidence that prostaglandins I_2, E_2 and D_2 activate ATP sensitive potassium channels was not found in the study via channel activity analysis, but rather demonstrated indirectly in isolated Langendorff hearts. We, therefore, directly examined the contribution of prostaglandins D_2, E_2 and I_2 in the regulation of K_{ATP} channel activity using the patch clamp technique in isolated single cardiac myocytes.

The channel activities in the present study were almost maximally activated after making excised inside-out patch preparations, and were attenuated by internal ATP or glibenclamide. Although the channel conductance (62 pS) calculated from the slope of the

current-voltage relation curve was slightly lower than those in previous reports (Bechem et al, 1983; Trube & Hescheler, 1983; Kakei et al, 1985), this profile of channel activity proved that it was from the K_{ATP} channel.

Prostaglandins D_2, E_2 and I_2 had no effects on channel activity just after making inside-out patch preparations. K_{ATP} channel activity in inside-out patch should be very high, because the ATP concentration of internal solution in the inside-out patch is theoretically zero. Agents which are capable of activating the channel were not expected to increase channel activity any more in these situations. On the other hand, 50 μM PGD_2 slowly increased channel activity which was subsequently reduced by the addition of 100 μM ATP to the internal solution. Moreover, an increase in channel activity caused by the PGD_2 was also inhibited by glibenclamide. PGE_2 and PGI_2 also facilitated channel activity reduced by ATP in nearly the same fashion. That is, prostaglandins D_2, E_2 and I_2 seem to activate K_{ATP} channels. In reality, intracellular ATP concentration cannot be exactly zero even during inhibition of metabolism by ischemia or hypoxia and, consequently, there may be an opportunity to futher increase K_{ATP} channel activity in this situation. The rank order of potency to activate K_{ATP} channels (as obtained from the dose-response curves for ATP in the presence of each of the prostaglandins) was $PGI_2 > PGD_2 > PGE_2$, and results which coincide with the findings of Bouchard et al (1994). Bouchard, as you may recall, measured coronary perfusion pressure in isolated Langendorff hearts.

In conclusion, it was inferred that prostaglandins D_2, E_2 and I_2 participate in the regulation of K_{ATP} channel activity and that channel activation by the prostaglandins is mediated in part by K_{ATP} channels, thus protecting the myocardium from the ischemia during myocardial relaxation. However, no plausible explanations for this phenomenon were found distinguish whether the cause was due to direct action on the channel itself (Cook & Hales, 1984) or if it was coupled with the intracellular signal transduction system, like a secondary messenger. In fact, there have been reports that prostaglandin indirectly regulates channel activity via a secondary messenger system, such as, cAMP (Needleman et al, 1986; Campbell, 1990), IP3 or DAG (Kurachi et al, 1989; Scherer et al, 1990; Ling, 1991; Minami et al, 1993; Miyoshi et al, 1993; Nakhostine et al, 1994). Further experiments are necessary to pinpoint the exact mechanisms.

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