Endothelin Blocks ATP-Sensitive K⁺ Channels and Depolarizes Smooth Muscle Cells of Porcine Coronary Artery

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ATP-sensitive K⁺ channels with a conductance of 30 pS in smooth muscle cells of porcine coronary artery were found to be highly active in the intact cell-attached patch configuration when the pipette contained a physiological concentration of Ca²⁺ (>10⁻⁴ M). In the inside-out configuration, these channels were activated by extracellular Ca²⁺ and blocked by cytosolic ATP and glibenclamide. Endothelin applied to the pipette specifically blocked these channels in a concentration-dependent manner in the cell-attached configuration (half-maximal inhibition, 1.3×10⁻⁹ M). A K⁺ channel opener, nicorandil, activated these channels even in the presence of 10⁻⁴ M endothelin. In the whole-cell current-clamp method, the cell membrane was depolarized by endothelin and then repolarized by nicorandil. The membrane depolarization is closely related to contraction of smooth muscle cells. These results suggest that the ATP-sensitive K⁺ channels are important in controlling the vascular tone of the coronary artery and that endothelin can increase vascular tone by blocking these channels. (Circulation Research 1992;70:612–616)

KEY WORDS • ATP • potassium channels • calcium • smooth muscle cells • endothelin

Endothelin is a potent vasoconstrictor with an amino acid sequence similar to those of peptide neurotoxins that bind directly to membrane ionic channels. Early studies showed that endothelin-induced vasoconstriction could be attenuated by a voltage-dependent Ca²⁺ channel antagonist, suggesting that endothelin might open Ca²⁺ channels. However, recent studies have indicated that endothelin might not be a Ca²⁺ channel opener and that its effect on Ca²⁺ channels may result from membrane depolarization.

We have shown that the ATP-sensitive K⁺ (Kₐtp) channels of smooth muscle cells of porcine coronary artery are active at a physiological concentration of extracellular Ca²⁺, even in the presence of cytosolic ATP. Because these channels are highly active at physiological concentrations of Ca²⁺, we postulate that they also contributed, at least in part, to the resting membrane potential of vascular smooth muscle cells. In addition, because their opening can be controlled by various endogenous and exogenous vasoactive substances, they are probably involved in the mechanism controlling vascular tone.

A number of peptide hormones and neurotransmitters are known to have effects on Kₐtp channels. In the present study, we examined the effect of endothelin on the Kₐtp channels and the contraction of smooth muscle cells of the porcine coronary artery.

Materials and Methods

Cell Preparation

We cultured smooth muscle cells according to the method reported by Ross. Large epicardial coronary arteries of either side were excised from fresh porcine heart obtained from a local slaughterhouse and cut into small pieces in normal Tyrode's solution after removing the endothelial tissue. The pieces were then explanted in culture dishes filled with medium 199 (Nissui Chemical, Japan) containing 10% fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.) and stored in a CO₂ incubator (5% CO₂ at 37°C). Three or four glass coverslips were placed on the bottom of each culture dish. For the experiments we used single smooth muscle cells that had migrated from the tissues during primary culture for 5–7 days and had adhered to the coverslips. The cultured cells formed hills and valleys after growing to confluence. The formation of hills and valleys is characteristic of smooth muscle cells.

Solutions and Chemicals

Tyrode's solution contained (mM) NaCl 137, KCl 2.7, sodium MOPS buffer 7.5 (pH 7.2), and glucose 5.5. High K⁺ solution contained (mM) KCl (or potassium aspartate) 140 and potassium MOPS buffer 10 (pH 7.2). Normal Tyrode's solution contained 1.4 mM CaCl₂, Ca²⁺ EGTA buffer was used for adjusting Ca²⁺ concentrations of <5×10⁻⁶ M. Free Ca²⁺ concentrations were

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determined with a $K_d$ of 87 nM. Endothelin-1 was purchased from the Peptide Institute Inc., Osaka, Japan, and NaATP and glibenclamide were from Sigma Chemical Co., St. Louis, Mo. Nicorandil was provided by Chugai Pharmaceutical Co., Japan.

**Electrophysiological Measurements**

Membrane currents were recorded in the cell-attached, excised inside-out, whole-cell, and outside-out configurations with a patch-clamp amplifier (model EPC-7, List Medical Electronics, Darmstadt, FRG). Membrane potential was recorded by the whole-cell current-clamp method. Soft glass patch pipettes with a heat-polished tip were used after Sylgard coating. The electric resistance of the patch pipettes was 5–7 MΩ for single-channel and whole-cell recording. Experiments were carried out at a temperature of 35–37°C. Data were stored in a PCM recorder (model PCM-501ES, Sony Co., Tokyo, Japan) with a high-cut filter (>3 kHz). The software for data analysis of single-channel currents was kindly provided by Dr. Y. Kurachi, Tokyo University.

**Results**

**Effect of Endothelin on the Membrane Potential**

The effect of endothelin on the membrane potential of primary cultured smooth muscle cells of porcine coronary artery was studied with the whole-cell configuration. Figure 1A shows an example of the effect of endothelin on the membrane potential of a single smooth muscle cell. The membrane potential was initially $-46.5$ mV and gradually depolarized to $-10.5$ mV after administration of $2 \times 10^{-8}$ M endothelin. The average resting membrane potential, calculated from the values for four cells in normal Tyrode’s solution, was $-48.1 \pm 2.6$ mV (mean$\pm$SEM). The average depolarization of the membrane by $2 \times 10^{-8}$ M endothelin was $25.8 \pm 4.4$ mV (mean$\pm$SEM, $n=4$). Figure 1B shows that the membrane could be repolarized by addition of $2 \times 10^{-4}$ M nicorandil to the bathing solution after depolarization by endothelin. The membrane potential was depolarized to $-12$ mV by $2 \times 10^{-4}$ M endothelin and then repolarized to $-42$ mV by $2 \times 10^{-4}$ M nicorandil.

**Single $K_{ATP}$ Channels in Vascular Smooth Muscle Cells**

The effects of cytosolic ATP and extracellular Ca$^{2+}$ are discussed in detail elsewhere. Figure 2A shows the $K_{ATP}$ channel currents recorded first from a cell-attached patch (tracing a) and then for the inside-out configuration (tracings b–d) with a pipette containing $150$ mM K$^+$ and $10^{-4}$ M Ca$^{2+}$ and bathing solution containing $150$ mM K$^+$ and $3 \times 10^{-8}$ M Ca$^{2+}$ at a pipette voltage of $+50$ mV. In the cell-attached patch configuration (tracing a) a K$^+$ channel of 30 pS was recorded. This channel activity was increased in the inside-out configuration (tracing b). This channel activity was only partially blocked by ATP (1 mM) applied to the cytosolic side (tracing c) but was significantly reduced by 20 µM glibenclamide (tracing d). Figure 2B shows that these channels were inactive in the presence of 1 mM ATP in the bathing solution when the Ca$^{2+}$ concentration in the pipette was reduced to $10^{-7}$ M. Thus, the channel activities in the presence of ATP on the cytosolic side became much higher when the extracellular Ca$^{2+}$ concentration was elevated. At $10^{-3}$ M Ca$^{2+}$, we could not record clear open–closed events, presumably because the channels were in a highly open state. Therefore, we studied the effect of endothelin using a solution containing $10^{-4}$ M Ca$^{2+}$ in subsequent experiments. We did not add Mg$^{2+}$ to the pipette solution, because it also affects the activity of these channels. Figure 3 shows that in the outside-out configuration the K$^+$ channel activity disappeared after $10^{-8}$ M endothelin was applied to the bathing Tyrode’s solution containing $10^{-4}$ M Ca$^{2+}$ at a pipette voltage of 0 mV. Therefore, we used endothelin in the concentration range of $10^{-10}$ to $10^{-7}$ M for the experiments.
**Effect of Endothelin on K\(_{ATP}\) Channels**

Figure 4A shows the K\(^+\) channel activities in the cell-attached patch configuration with pipette solution containing 10\(^{-4}\) M Ca\(^{2+}\). The mean value of the fraction of open channels at 10\(^{-4}\) M Ca\(^{2+}\) was 98.0±1.7\% (mean±SEM, n=5). When the pipette solution contained 10\(^{-8}\) M endothelin, the K\(^+\) channel activity decreased (Figure 4B). The fraction markedly decreased to 4.8±2.0\% (mean±SEM, n=5) when the pipette solution contained 10\(^{-8}\) M endothelin (Figure 4C). Addition of 50 \(\mu\)M nicorandil to the pipette solution blocked the effect of 10\(^{-8}\) M endothelin and increased the K\(^+\) channel activity (Figure 4D). Figure 4E shows the relation of endothelin concentration in the pipette solution to the fraction of open channels, calculated as 1 minus the fraction of closed channels. The channels that remained open after endothelin application had the same conductance (30 pS) as those before endothelin application (Figure 4F). Endothelin blocked the K\(_{ATP}\) channels in a concentration-dependent manner and did not alter their conductance but decreased their open probability.

**Discussion**

In cardiac muscle, K\(_{ATP}\) channels are thought not to be operational in physiological conditions but to be active only in conditions in which cytosolic ATP is depleted, such as in severe ischemia.\(^7\)\(^8\) In pancreatic B cells, however, K\(_{ATP}\) channels are known to control the resting membrane potential and to be related to insulin secretion, although even lower ATP concentration has been reported to abolish K\(^+\) channel opening in excised membrane patches.\(^9\)\(^10\) There has been no explanation for the activation of these channels in the presence of cytosolic ATP in intact cells. Our study showed that the K\(_{ATP}\) channels of porcine coronary artery smooth muscle cells are active in physiological conditions and also control the resting membrane potential in vascular smooth muscle cells. That is, these channels are still highly active in the presence of 1 mM ATP on the cytosolic side when the extracellular Ca\(^{2+}\) concentration is >10\(^{-4}\) M.

In our previous findings,\(^4\) these channels were shown to be activated by K\(^+\) channel openers cromakalim and nicorandil and blocked by glibenclamide. These channels were also sensitive to extracellular Ca\(^{2+}\) concentration.\(^11\) In our study we used the cultured smooth muscle cells, so we cannot deny that the cultured cells might be altered and have adopted new features. Standen et al\(^12\) found that the K\(_{ATP}\) channels in the smooth muscle cells of rabbit or rat mesenteric arteries had a higher conductance (135 pS) than that observed in our study and were activated by cromakalim applied to the cytosolic side; however, they did not discuss the Ca\(^{2+}\) dependency of these channels. Kajioka et al\(^13\) reported the presence of K\(_{ATP}\) channels with a conductance of 10 pS in the rat portal vein. These channels were sensitive to intracellular Ca\(^{2+}\) concentration and were activated by nicorandil. Recently, it has been suggested that K\(_{ATP}\) channels play an important role in vasodilation by controlling the membrane potential.\(^14\)

Endothelin is a potent vasoconstrictive peptide produced by endothelial cells of blood vessels.\(^1\) It is known to cause a rapid, but transient, increase in the cytosolic...
Ca\(^{2+}\) concentration, followed by a sustained increase of the cytosolic Ca\(^{2+}\).\(^{15,16}\) The increase in cytosolic Ca\(^{2+}\) induced by endothelin was considered to be due to activation of the voltage-dependent Ca\(^{2+}\) channels.\(^{15,16}\) Contraction of the artery induced by various agonists (such as serotonin, prostaglandin F\(_2\), norepinephrine, and vasopressin) has also been found to depend largely on extracellular Ca\(^{2+}\) and to be very sensitive to Ca\(^{2+}\) antagonist inhibition. However, the initial increase in cytosolic Ca\(^{2+}\) induced by endothelin was recently shown not to be blocked by Ca\(^{2+}\) antagonists,\(^{2}\) suggesting that endothelin must have other effects in addition to its stimulation of Ca\(^{2+}\) influx or that even Ca\(^{2+}\) influx may be a secondary effect of endothelin. Inoue et al\(^{17}\) practically suggested that endothelin does not act directly on the voltage-dependent Ca\(^{2+}\) channels but through intrinsic products, such as second messengers.

Kauser et al\(^{18}\) found that endothelin induces concentration-dependent contraction of arteries and depolarizes arterial smooth muscle cells. They concluded that the depolarization was due to an endothelium-dependent factor, because the constriction by endothelin was augmented by removal of the endothelium or by superfusion of the intact vessel with indomethacin. In our study, however, endothelin directly depolarized the membrane of cultured smooth muscle cells without endothelium. Endothelin specifically blocked the K\(_{ATP}\) channels in a concentration-dependent manner. These effects induced by endothelin could be reversed by nicorandil. The blockade of these channels might result in membrane depolarization and an increase in intracellular Ca\(^{2+}\). An increase of intracellular Ca\(^{2+}\) contributes to an increase in vascular tone.\(^{19}\)

We consider that induction of cell contraction by endothelin is related, at least partially, to depolarization by decreased conductance of K\(^{+}\) channels. Thus, we hypothesized that endothelin acts as a blocker of K\(^{+}\) channels in primary cultures of smooth muscle cells from porcine coronary artery and induces cell contraction by depolarizing the membrane.

In a previous study, we found that application of tetraethylammonium, a K\(^{+}\) channel blocker, to the left coronary artery of the isolated perfused rabbit heart induced a contraction of the coronary artery similar to that observed in vasospastic angina in humans.\(^{20}\) In the present study, we found that endothelin is a blocker of the K\(^{+}\) channels that may contribute to the resting membrane potential generation. These results suggest that endothelin might also play a role in vasospasm as a K\(^{+}\) channel blocker.

![Figure 4](http://circres.ahajournals.org/)
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