Enhanced Electrical Activity in Mesenteric Arteries From Salt-Loaded Dahl Salt-Sensitive Rats

Actions of Prostaglandin H$_2$ on Membrane Channels

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Salt loading increases blood pressure in Dahl salt-sensitive (Dahl S) rats. We have previously shown that the mesenteric artery of salt-loaded Dahl S rats exhibits enhanced electrical activity that is corrected by a cyclooxygenase inhibitor, indomethacin. Prostaglandin H$_2$ (PGH$_2$) is a product of cyclooxygenase that is known as an intrinsic vasoconstricting factor in spontaneously hypertensive rats. Our hypothesis is that tissue production of PGH$_2$ would be involved in the enhanced electrical activity of arteries from salt-loaded Dahl S rats. In the present study, to clarify this possibility, we evaluated the actions of PGH$_2$ on membrane channels in arterial cells from Dahl S rats. Membrane currents were recorded by whole-cell voltage clamp technique in single smooth muscle cells from the mesenteric artery. Application of PGH$_2$ evoked an inward current that was mainly dependent on extracellular Na$^+$ in the physiological extracellular solution. When high Ba$^{2+}$ solution was used for the extracellular solution, PGH$_2$ also evoked the inward current, suggesting that a divalent cation, such as Ba$^{2+}$ or Ca$^{2+}$, could permeate the PGH$_2$-activated channels. In contrast, the L-type Ca$^{2+}$ channel currents were not enhanced by the application of PGH$_2$. The present results suggest that production of PGH$_2$ contributes to the enhanced electrical activity by activating cation-permeable channels and depolarizing the membrane potential. PGH$_2$ also directly stimulates the Ca$^{2+}$ influx by activating Ca$^{2+}$ permeable channels. Am J Hypertens 1997;10:112S–115S © 1997 American Journal of Hypertension, Ltd.

KEY WORDS: Salt, Dahl salt-sensitive rat, calcium channel, prostaglandin, electrophysiology, receptor operated channels.

Dahl salt-sensitive (Dahl S) rats develop hypertension after dietary salt loading.$^{1-3}$ Neural, humoral, and renal factors have been reported to be important for the development of this salt-induced hypertension.$^{1,2}$ In contrast, changes in the characteristics of vascular tissues in this model have not been fully clarified, although vascular resistance is known to be increased after salt loading.$^{4,5}$

We have previously reported that membrane electrical activity in the mesenteric arteries was enhanced in Dahl S rats fed a high salt diet; the resting membrane potential was depolarized by several millivolts and spontaneous electrical activity was observed in most of the tissues examined.$^6$ These alterations were corrected by pretreatment with either indomethacin or a Ca$^{2+}$ antagonist, suggesting that products of cyclooxygenase or Ca$^{2+}$ influx through L-type Ca$^{2+}$ channels might be involved. We have also reported,
using the patch clamp technique, that activation of L-type Ca$^{2+}$ channels was enhanced in arterial cells from Dahl S rats fed a high salt diet compared with those fed low salt diet. However, the precise mechanism for these membrane alterations has not been clarified. We hypothesized that the products of cyclooxygenase may affect membrane channels and thus enhance the membrane electrical activity of arterial cells. Prostaglandin H$_2$ (PGH$_2$) is one of products of cyclooxygenase and has been shown to be an intrinsic vasoconstricting factor in spontaneously hypertensive rats (SHR). In the present study, we evaluated the actions of PGH$_2$ on the membrane channels in arterial cells from Dahl S rats using a voltage clamp technique.

**METHODS**

**Animals** The Dahl S rats used in the present study had been originally obtained from Brookhaven National Laboratories (Upton, NY) and were inbred at the Laboratory Animal Research Center, Eisai, Tokyo, Japan. The rats were fed a 0.3% NaCl diet from 6 weeks of age at the Animal Center in Kyushu University, and used for experiments at 10 to 12 weeks of age (n = 5 rats). The study protocol was approved by the Committee on Ethics of Animal Experimentation of the Faculty of Medicine, Kyushu University.

**Single Cells and Electrical Recording** Single smooth muscle cells were obtained from the resistance mesenteric arterial branch (diameter < 300 µm) by collagenase treatment. Whole-cell voltage-clamp experiments were performed with a patch pipette through a voltage-clamp amplifier (Axopatch 1-D, Axon Instruments, Foster City, CA). Conditions and procedures were basically the same as those previously described. Currents were recorded at room temperature (22°C to 24°C). The liquid junction potential of 10 mV was corrected.

The conductance of the current was calculated at a membrane potential of −80 to 0 mV in the current-voltage relationship and expressed as a normalized value by the cell capacitance. The cell capacitance was estimated by the cancellation network in the voltage clamp amplifier.

**Solutions and Chemicals** To eliminate K$^+$ channel currents, the pipette was filled with a high Cs$^+$ solution that consisted of (in mmol/L): 120 Cs aspartate, 30 CsCl, 3 ATPNa$_2$, 3 MgCl$_2$, 10 EGTA, 10 HEPES, pH 7.3 titrated with CsOH. The bath solution contained (in mmol/L) 150 NaCl, 2 CaCl$_2$, 2 MgCl$_2$, 10 glucose, 10 HEPES; or 50 BaCl$_2$, 75 TrisCl, 10 glucose, 10 HEPES, at pH 7.3, titrated with CsOH. In some experiments, 150 mmol/L TrisCl was used instead of 150 mmol/L NaCl.

PGH$_2$ (Funakoshi Co., Tokyo, Japan) was stored at −80°C and diluted to make the test solutions 1 to 2 min before the application to the cell.

**RESULTS**

**Action of PGH$_2$ on the Basal Current** Membrane currents were recorded with a whole-cell voltage clamp technique. With a high Cs$^+$ solution in the pipette and physiological salt solution in the bath, the ramp voltage from −80 to 40 mV evoked a small linear basal current. The inward Ca$^{2+}$ channel current was usually small with low Ca$^{2+}$ concentration. The application of PGH$_2$ (0.3 nmol/L and higher concentrations) amplified the conductance of the basal current. Figure 1 demonstrates the currents before and after the application of 3 nmol/L PGH$_2$. The slope conductance induced by 0.3, 3, and 30 nmol/L PGH$_2$ were 4.1 ± 0.3, 21.3 ± 1.8, and 52.3 ± 4.1 pS/pF, respectively (mean ± SEM, n = 4 to 6). PGH$_2$-activated current exhibited a straight current-voltage relationship, suggesting that channels for this current had a voltage-insensitive nature. When extracellular Na$^+$ was replaced with equimolar Tris$^+$, the current disappeared (the evoked conductance with 3 nmol/L PGH$_2$ was 0.9 ± 0.1 pS/pF, mean ± SEM, n = 4). In addition, the reversal potential of the evoked current was nearly 0 mV, which was far from the reversal potential for Na$^+$, Ca$^{2+}$, K$^+$, or Cl$^-$. These results suggest that the amplified conductance is due to the opening of nonselective cation channels.

**Actions of PGH$_2$ on the Ca$^{2+}$ Channel Currents** L-Type Ca$^{2+}$ channel currents were recorded with a high Ba$^{2+}$ solution in the bath (Ba$^{2+}$ is usually used as a charge carrier instead of Ca$^{2+}$ for Ca$^{2+}$ permeating channels in the voltage clamp study). A voltage ramp from −80 to 40 mV evoked an inward Ba$^{2+}$ current (via voltage operated L-type Ca$^{2+}$ channels) and a small

![Figure 1](image-url)
background current (Figure 2). Application of 3 nmol/L PGH$_2$ did not increase, but rather slightly decreased, the amplitude of L-type Ca$^{2+}$ channel current. However, 3 nmol/L PGH$_2$ amplified the background current also in this condition (Figure 2; 4.8 ± 0.3 pS/pF, mean ± SEM, n = 4). Since Tris$^+$ did not permeate the PGH$_2$-activated channel, Ba$^{2+}$ was considered to permeate PGH$_2$-activated channels. This result suggests that divalent cations, including Ba$^{2+}$ and Ca$^{2+}$, also permeate the PGH$_2$-activated channels.

**DISCUSSION**

In the present study, we demonstrated, using the voltage clamp method, that PGH$_2$ activated nonselective cation channels but did not directly stimulate the activation of L-type Ca$^{2+}$ channels in mesenteric arterial cells from Dahl S rats. Activation of nonselective cation channels would depolarize the cell membrane by Na$^+$ influx and secondarily activate L-type Ca$^{2+}$ channels. In addition, Ca$^{2+}$ would directly permeate the PGH$_2$-activated channels. These actions of PGH$_2$ on membrane channels may explain at least in part the enhanced electrical activity of arteries, which would contribute to the increase in peripheral resistance.

**Is Membrane Electrical Activity Enhanced by Salt Loading in Dahl S Rats?** Only a few studies have examined the electrical characteristics of arterial tissues from Dahl S rats. Abel et al reported that the membrane potential of the tail artery was not altered by salt loading. We have previously reported that the membrane potential was depolarized and showed spontaneous electrical activity in the mesenteric artery from salt-loaded Dahl S rats. Differences in these results may be explained by the differences in the tissues or in the experimental conditions. The enhanced electrical activity in salt-loaded rats was inhibited by the application of Ca$^{2+}$ antagonist, suggesting that Ca$^{2+}$ influx via L-type Ca$^{2+}$ channels was increased. The increased Ca$^{2+}$ influx may enhance the arterial tone and contribute to the maintenance of hypertension.

**Is Production of PGH$_2$ Associated With the Enhanced Electrical Activity?** The spontaneous electrical activity observed in the mesenteric artery from salt-loaded rats disappeared with the application of indomethacin. This result suggests that products of cyclooxygenase may be involved in this alteration. PGH$_2$ is one of these cyclooxygenase products, which is considered as an intrinsic vasoconstricting factor in SHR artery. We hypothesize that PGH$_2$ enhances membrane electrical properties in arteries from Dahl S rats after salt loading; however, it is not known whether production of PGH$_2$ is increased with salt-loading or with hypertension.

**PGH$_2$ Activated Nonselective Cation Channels in Arterial Cells** We demonstrate in the present study that the vasoconstrictive eicosanoid, PGH$_2$, activated nonselective cation channels in vascular smooth muscle cells. Other agonists, such as ATP, acetylcholine, arginine vasopressin, and endothelin, have also been shown to activate nonselective cation channels in smooth muscle cells. The properties of agonist-activated channels in the present study for PGH$_2$ and those for other agonists were basically the same: cation channels that were permeable for Na$^+$ and Ca$^{2+}$. Since an influx of cations would cause membrane depolarization, the result that PGH$_2$ activated the nonselective cation channels supports our hypothesis that the tissue production of PGH$_2$ is involved in the depolarized membrane potential in arterial tissue from salt-loaded Dahl S rats.

**PGH$_2$ Did Not Alter the L-Type Ca$^{2+}$ Channels** Since spontaneous electrical activity was abolished by application of Ca$^{2+}$ antagonists, Ca$^{2+}$ influx through L-type Ca$^{2+}$ channels was increased in arterial tissue from salt-loaded Dahl S rats. However, in the present study, activation of L-type Ca$^{2+}$ channels was not enhanced by PGH$_2$ in the voltage clamp condition. Thus, the depolarized membrane potential would explain the enhanced activation of L-type Ca$^{2+}$ channels. In addition, we have previously shown that the threshold po-
potential for activation of L-type $\text{Ca}^{2+}$ channels was shifted to the negative direction in arterial cells from salt-loaded Dahl S rats. This alteration also contributes to the enhanced activation of L-type $\text{Ca}^{2+}$ channels.

In conclusion, we have demonstrated that PGH$_2$ activates nonselective cation channels in arterial cells from Dahl S rats. Since PGH$_2$ is considered as an intrinsic vasoconstricting factor in hypertension, the present study suggests that the membrane electrical activity could be enhanced by the tissue production of PGH$_2$. Further study is required to examine whether the potency of PGH$_2$ for activating nonselective cation channels in arterial cells is altered or whether production of PGH$_2$ in arterial tissues is increased in the Dahl S rat fed a high salt diet.

**REFERENCES**