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# Role of eicosanoids in alteration of membrane electrical properties in isolated mesenteric arteries of salt-loaded, Dahl salt-sensitive rats

[1]Koji Fujii, Uran Onaka, Yusuke Ohya, Susumu Ohmori, Mitsuhiro Tominaga, Isao Abe, Yutaka Takata & Masatoshi Fujishima

Second Department of Internal Medicine, Faculty of Medicine, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-82, Japan

**1** The role of eicosanoids in altered membrane electrical properties of Dahl salt-sensitive (DS) rats was investigated, by use of conventional microelectrodes technique, in isolated superior mesenteric arteries of DS rats and Dahl salt-resistant (DR) rats fed either a high or low salt diet.

**2** The membrane was significantly depolarized in salt-loaded DS rats compared with the other three groups. In addition, the arteries of salt-loaded DS rats exhibited spontaneous electrical activity.

**3** Spontaneous electrical activity in salt-loaded DS rats was inhibited by the following: indomethacin, a cyclo-oxygenase inhibitor; ONO-3708, a prostaglandin $H_2$/thromboxane $A_2$ receptor antagonist; OKY-046, a thromboxane $A_2$ synthase inhibitor; nicardipine, a $Ca^{2+}$-channel antagonist and by $Ca^{2+}$-free solution. In addition, spontaneous electrical activity was enhanced by a thromboxane $A_2$ analogue and by prostaglandin $H_2$. Spontaneous electrical activity was unaffected by phentolamine, atropine and tetrodotoxin.

**4** Membrane potential in arteries of salt-loaded DS rats was not affected by either indomethacin or ONO-3708.

**5** Spontaneous contraction, sensitive to indomethacin, was present, and contractile sensitivity to high potassium solution was enhanced in arteries of salt-loaded DS rats.

**6** These findings suggest that eicosanoid action, together with membrane depolarization, may lead to the activation of voltage-dependent $Ca^{2+}$-channels, thereby causing spontaneous electrical activity in mesenteric arteries of salt-loaded DS rats. In addition, tension data suggest that these changes in membrane properties are related to enhanced contractile activities in salt-loaded DS rats. Mechanisms of depolarization remain to be determined.

**Keywords:** Dahl salt-sensitive rat; hypertension; salt; vascular smooth muscle cell; membrane potential; spontaneous electrical activity; thromboxane $A_2$

## Introduction

Although a positive relationship between a high salt intake and blood pressure has been demonstrated (Intersalt, 1988; MacGregor *et al*., 1989), the precise mechanism is unclear. Dahl salt-sensitive (DS) rats become hypertensive when fed a high salt diet, whereas Dahl salt-resistant (DR) rats remain normotensive, despite salt loading (Dahl *et al*., 1962; Rapp, 1982). The DS rat thus serves as a useful model for exploring the mechanism of salt-induced hypertension.

Salt-induced hypertension in DS rats is associated with an increase in total peripheral resistance (Boegehold *et al*., 1991; Simchon *et al*., 1991). The isolated mesenteric vascular bed of salt-loaded DS rats exhibits an increased response to noradrenaline (Takeda *et al*., 1994). Although it is known that the membrane properties of vascular smooth muscle cells greatly influence vascular reactivity (Hermsmeyer *et al*., 1981; Mulvany *et al*., 1982), data on the membrane properties of arterial smooth muscle cells of DS rats are limited (Abel *et al*., 1981).

The generation of thromboxane $A_2$ ($TXA_2$) is increased in the vascular walls of DS rats (Ishimitsu *et al*., 1991). U46619, a thromboxane $A_2$ agonist, inhibits calcium-activated potassium channels in porcine coronary arteries (Scornik & Toro, 1992). Because potassium conductance contributes to the membrane potential of smooth muscle cells (Hermsmeyer, 1982), it is conceivable that alterations in the eicosanoid production in DS rats may lead to a depolarization of the resting membrane potential, spontaneous electrical activity or both in arterial smooth muscle cells.

We thus hypothesized that the membrane is depolarized and/or other electrical activity might be brought about by eicosanoids in arterial smooth muscle cells of salt-loaded DS rats. To test this hypothesis, we recorded the membrane potential of superior mesenteric arteries of DS and DR rats fed either a high or low salt diet. In addition, contractile responses were examined to see whether any change in membrane properties is related to altered contractile activities.

## Methods

### *Preparation of arteries*

Six-week-old male Dahl-Iwai salt-sensitive (DIS/Eis) rats (DS rats) and Dahl-Iwai salt-resistant (DIR/Eis) rats (DR rats) (Yamazaki *et al*., 1994) were fed a high salt diet (8% NaCl) or a low salt diet (0.3% NaCl) for 7 weeks. They had free access to tap water. The procedures were in accordance with our institutional guidelines. Systolic blood pressure was measured by the tail-cuff method. Rats were killed by decapitation and exsanguinated, and the main branches of the superior mesenteric artery were excised and placed on a plate containing cold Krebs solution. The arteries were then cleaned of adherent connective tissues, and cut into rings of 3 mm and 1 mm for the electrophysiological and tension experiments, respectively. In some rings, the endothelium was removed by a gentle rubbing of the intimal surface with polyethylene tubing. The absence of the endothelium was confirmed by the lack of hyperpolarization or relaxation to acetylcholine (10 $\mu$M).

[1] Author for correspondence.

### Recording of membrane potentials

Transverse strips cut along the longitudinal axis of the rings were placed with the endothelial layer uppermost in an experimental chamber (capacity 2 ml). Tissues were carefully pinned to a rubber bed fixed at the bottom of the chamber and then superfused with Krebs solution (36°C) bubbled with 95% $O_2$ and 5% $CO_2$ (pH 7.3–7.4) at a rate of 3 ml $min^{-1}$. Strips were allowed to equilibrate for at least 60 min before recordings were started. Membrane potentials were recorded with glass capillary microelectrodes filled with 3 mol $l^{-1}$ KCl and with tip resistances of 50–80 Mohm (Fujii, 1988). Microelectrodes were impaled into the smooth muscle cell from the endothelial side (Bény, 1990; Fujii *et al*., 1992). Membrane potential recordings obtained without any mechanical resistance upon impalement of the electrodes were discarded to exclude possible recordings from the endothelial cells. Following this step, the electrode was farther advanced until it met a mechanical resistance; then a fine vibration was applied to the electrode by gently tapping the manipulator, allowing the electrode to penetrate the membrane. Only the recordings obtained through these procedures were accepted as membrane potentials of smooth muscle cells (Bény, 1990). Other criteria for successful impalement were an abrupt drop in voltage upon entry of the microelectrode into the cell, a stable membrane potential for at least 2 min and a sharp return of the membrane potential to zero upon withdrawal of the electrode (Fujii *et al*., 1992). Electrical responses were monitored on an oscilloscope (VC-11, Nihon Kohden Co. Ltd., Tokyo, Japan) and recorded with a pen writing recorder (RJG-4002, Nihon Kohden Co. Ltd., Tokyo, Japan).

### Force measurements

Rings were placed in organ chambers (capacity 5 ml) containing Krebs solution (36°C, pH 7.4). Two fine stainless wires were placed through the lumen of the ring, one was anchored and the other was connected to the mechano-transducer (UM-203, Kishimoto, Kyoto, Japan). An optimal resting tension of 1.0 g was applied to the rings (Fujii *et al*., 1992). The rings were allowed to equilibrate for at least 60 min before the start of recordings. In some preparations, indomethacin (10 $\mu$M) was applied 60 min before the start of the experiments and was present throughout the experiments. The rings were first challenged by 39.2 mM KCl until the response became steady. Then the rings were exposed to increasing concentrations of KCl (10.7, 20.2, 39.2, 77 and 118 mM). Responses were displayed on a pen writing recorder (Recti-Horiz-8K, NEC Sanei, Tokyo, Japan).

### Solutions and drugs

The ionic composition of the Krebs solution was as follows (mM): $Na^+$ 137, $K^+$ 5.9, $Mg^{2+}$ 1.2, $Ca^{2+}$ 2.5, $HCO_3^-$ 15.5, $H_2PO_4^-$ 1.2, $Cl^-$ 134 and glucose 11.5. Drugs used were: acetylcholine, chloride, indomethacin, phentolamine hydrochloride, atropine sulphate, tetrodotoxin, ouabain (Sigma Chemical Co., St Louis, U.S.A.), ONO-3708 ((9,11),(11,12)-Dideoxa-9α,11α-dimetylmethano m-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanor-15-epi-thromboxane $A_2$, OKY-046 ((E)-3[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate), 9,11-epithio-11,12-methano-$TXA_2$ ($STA_2$), prostaglandin $H_2$ ($PGH_2$) (Ono Pharmaceuticals, Osaka, Japan), and nicardipine (Yamanouchi Pharmaceuticals, Tokyo, Japan). In solutions containing high $K^+$ (10.7 to 118 mM), NaCl was replaced with KCl on an equimolar basis. Indomethacin was dissolved in 10 mM $Na_2CO_3$ and $STA_2$ was dissolved in ethanol. Tetrodotoxin was dissolved in 0.1 M acetic acid. Other drugs used were dissolved in distilled water. All drugs were further diluted 1000 times or more in Krebs solution to produce final bath concentrations. The solvents used to dissolve the drugs did not affect electrical responses in the final bath concentrations. Preliminary experiments showed that the depolarizing response to ouabain was not affected by phentolamine (1 $\mu$M), thus suggesting that the contribution of neurotransmitter release from the residual nerve terminals to ouabain-induced depolarization might be small, if any, in this preparation.

### Statistics

Data are given as mean ± s.e.mean. The number of animals is indicated by *N*. The number of cells is indicated by *n*. The average values of membrane potentials were first calculated for each animal. These values were compared statistically between groups. Variables were compared by one-way or two-way

**Table 1** Body weight and systolic blood pressue of Dahl rats

| *Strain/diet* | *Number of animals* | *Body weight* (g) | *Systolic blood pressure* (mmHg) |
|---|---|---|---|
| DS high salt diet | 22 | 331 ± 10*·† | 236 ± 6**·†† |
| low salt diet | 14 | 385 ± 12 | 160 ± 5 |
| DR high salt diet | 18 | 388 ± 8 | 149 ± 4 |
| low salt diet | 5 | 426 ± 36 | 143 ± 8 |

Values are mean ± s.e.mean. *$P<0.05$, **$P<0.001$ vs DS low salt diet, †$P<0.05$, ††$P<0.001$ vs DR high salt diet. DS, Dahl salt-sensitive; DR, Dahl salt-resistant..

**able 2** Membrane potential in superior mesenteric artery of Dahl rats

| *Strain/diet* | *Control* (mV) | *ONO-3708* (10 $\mu$M) | *Indomethacin* (10 $\mu$M) | *Without ouabain* | *With ouabain* (1 mM) | *Changes induced by ouabain* |
|---|---|---|---|---|---|---|
| DS high salt diet | −41.4 ± 0.5**·†† (N = 22, n = 120) | −42.4 ± 0.4* (6,42) | −41.3 ± 0.3** (8,33) | −40.1 ± 0.7**·†† (4,21) | −31.5 ± 2.9*·†·‡ (4,31) | 8.4 ± 2.3 (*N* = 4) |
| low salt diet | −47.3 ± 0.6 (14,84) | −47.6 ± 0.4 (5,32) | −47.6 ± 0.7 (5,27) | −47.3 ± 0.7 (5,27) | −40.6 ± 1.5‡ (5,26) | 6.8 ± 1.2 (*N* = 5) |
| DR high salt diet | −47.0 ± 0.7 (18,45) | ND | ND | −47.3 ± 0.8 (4,20) | −38.8 ± 1.9‡ (4,20) | 8.5 ± 2.5 (*N* = 4) |
| low salt diet | −47.8 ± 0.4 (5,14) | ND | ND | ND | ND | ND |

Values are mean ± s.e.mean. *N*, number of animals; *n*, number of cells. *$P<0.05$, **$P<0.001$ vs DS low salt diet, †$P<0.05$, ††$P<0.001$ vs DR high salt diet. ‡$P<0.01$ vs without ouabain of the same strain and diet. DS, Dahl salt-sensitive; DR, Dahl salt resistant. ND, not determined.

analysis of variance, followed by Scheffe's test for multiple comparison, or by unpaired Student's $t$ test. $P$ values less than 0.05 were considered statistically significant.

## Results

### *Body weight and systolic blood pressure of Dahl rats*

Mean systolic blood pressure was significantly higher in DS rats fed a high salt diet than in DS rats fed a low salt diet or in DR rats fed a high salt diet (Table 1). Blood pressure did not differ significantly between DR rats fed a high or low salt diet. Body weight was lower in DS rats fed a high salt diet than in DS rats fed a low salt diet or in DR rats fed a high salt diet (Table 1).

### *Membrane potential in mesenteric artery of Dahl rats*

The resting membrane potential was significantly more positive in the mesenteric arteries of DS rats fed a high salt diet compared with DS rats fed a low salt diet or DR rats fed a high salt diet (Table 2). The resting membrane potential was similar in the arteries of DR rats fed a high or low salt diet. Neither ONO-3708 (10 $\mu$M), a specific antagonist of $TXA_2/PGH_2$ receptors (Katsura *et al*., 1983), nor indomethacin (10 $\mu$M), a cyclo-oxygenase inhibitor, affected the membrane potential in DS rats fed a high or low salt diet (Table 2, Figure 1). The electrogenic component of the membrane potential was assessed by the addition of ouabain (1 mM), an inhibitor of $Na^+$, $K^+$-ATPase. Ouabain produced a comparable depolarization in DS rats fed a high and low salt diet, and DR rats fed a high salt diet (Table 2). Accordingly, in the presence of ouabain, the membrane potential remained significantly more positive in DS rats fed a high salt diet than in the other two groups (Table 2).

### *Spontaneous electrical activity in mesenteric artery of salt-loaded DS rats*

Most arteries of DS rats fed a high salt diet exhibited spontaneous electrical activity (5 to 20 mV in amplitude, 1 to 3 $min^{-1}$ in frequency) (Figures 1–3, 5). Minor spontaneous electrical activity was observed in only 1 of 18 preparations from DR rats fed a high salt diet. Spontaneous electrical activity was not affected by phentolamine (1 $\mu$M), atropine (1 $\mu$M) or tetrodotoxin (0.3 $\mu$M) (data not shown, $N=3-5$, respsectively). Indomethacin (10 $\mu$M) gradually reduced the amplitude of spontaneous electrical activity, then abolished it ($N=6$) (Figure 1). ONO-3708 (10 $\mu$M) abolished spontaneous electrical activity immediately after its application ($N=6$) (Figure 1). OKY-046 (10 $\mu$M), a $TXA_2$ synthetase inhibitor (Iizuka *et al*., 1981), slowed the frequency and decreased the amplitude of spontaneous electrical activity ($N=4$) (Figure 2). At a concentration of 10 pM, $STA_2$, a stable $TXA_2$ analogue, increased the frequency of spontaneous electrical activity with no apparent effect on resting membrane potential (Figure 3a). Higher concentrations of $STA_2$ depolarized the membrane and accelerated the activity ($N=5$) (Figure 3a). $PGH_2$ also enhanced spontaneous electrical activity ($N=4$) (Figure 3b). Higher concentrations of $STA_2$ (1 and 3 nM) depolarized the membrane, but did not evoke spontaneous electrical activity in the arteries of DS rats fed a low salt diet ($N=8$) (Figure 4). Similarly, in 5 out of 6 arteries from DR rats fed a high salt diet, $STA_2$ produced only depolarization. However, in one preparation from DR rats, small oscillatory activities were superimposed on $STA_2$-induced depolarization (Figure 4). In a small number of DS rats fed a high salt diet, spontaneous electrical activity was absent. Interestingly, in such preparations ($N=3$), $STA_2$ elicited large oscillatory electrical responses which were similar in shape to spontaneous electrical activity (Figure 4).

Spontaneous electrical activity was abolished by nicardipine (5 to 10 nM), a $Ca^{2+}$-channel antagonist ($N=5$) (Figure 5a). Spontaneous electrical activity also disappeared in $Ca^{2+}$-free solution, and reappeared after the addition of $Ca^{2+}$ ($N=4$)

**Figure 1** Effects of (a) indomethacin, a cyclo-oxygenase inhibitor, and (b) ONO-3708, a specific antagonist of prostaglandin $H_2$ ($PGH_2$)/thromboxane $A_2$ ($TXA_2$) receptors, on spontaneous electrical activity in the mesenteric arteries of Dahl salt-sensitive rats fed a high salt diet. Indomethacin or ONO-3708 was applied at the point indicated by (●) and was present throughout the rest of the experiment.

**Figure 2** Effects of OKY-046, a specific blocker of $TXA_2$ synthetase, on spontaneous electrical activity in the mesenteric arteries of Dahl salt-sensitive rats fed a high salt diet. OKY-046 was applied at the point indicated by (●) and was present throughout the rest of the experiment. The traces above represent parts of a continuous recording.

**Figure 3** Effects of (a) $STA_2$ and (b) $PGH_2$ on spontaneous electrical activity in the mesenteric arteries of Dahl salt-sensitive rats fed a high salt diet. Traces in (a) and (b) were obtained from different rats. $STA_2$ is a stable analogue of $TXA_2$.

**Figure 4** Effects of $STA_2$ on membrane potential in the mesenteric arteries of Dahl rats. DS high salt, Dahl salt-sensitive rats fed a high salt diet; DS low salt, Dahl salt-sensitive rats fed a low salt diet; DR high salt, Dahl salt-resistant rats fed a high salt diet. $STA_2$ (1 nM), a stable analogue of $TXA_2$ was applied during the period indicated by the horizontal bar.

(Figure 5b). Spontaneous electrical activity was also transiently suppressed by the hyperpolarization produced by acetylcholine in endothelium-intact preparations (Figure 5c upper trace) ($N=7$). Spontaneous electrical activity was present in preparations without the endothelium (Figure 5c lower trace) ($N=4$), which was also abolished by indomethacin and ONO-3708.

### *Contractile responses in mesenteric artery of Dahl rats*

Contractile responses were recorded in mesenteric arteries of DS rats fed either a high or low salt diet and DR rats fed a high salt diet. Contractile responses to 20.2 mM KCl were significantly greater in endothelium-intact preparations of salt-loaded DS rats than in DS rats fed a low salt diet and DR rats fed a high salt diet (Figures 6 and 7). Such differences were not observed in endothelium-intact preparations pretreated with indomethacin. In endothelium-rubbed preparations of salt-loaded DS rats, spontaneous contractions were frequently observed (5 out of 7 rats) (Figure 6). Spontaneous contractions were abolished by indomethacin (10 $\mu$M) and by nicardipine (100 nM) ($N=5$, respectively; note that spontaneous contraction was absent in preparations treated with indomethacin; Figure 6). In endothelium-rubbed preparations, contractile responses to 10.7 and 20.2 mM KCl were markedly greater in salt-loaded DS rats compared with DS rats fed a low salt diet and DR rats fed a high salt-diet (Figures 6 and 7).

**Figure 5** (a) Effects of nicardipine on spontaneous electrical activity in the mesenteric arteries of Dahl salt-sensitive rats fed a high salt diet. Nicardipine (5 nM) was applied during the period indicated by the horizontal bar. (b) Effects of $Ca^{2+}$-free solution and re-addition of $Ca^{2+}$ on spontaneous electrical activity. $Ca^{2+}$-free solution was applied during the period indicated by the horizontal bar. Upper and lower traces were taken from the same preparation but from the different cells. (c) Effects of acetylcholine (ACh) on spontaneous electrical activity in preparations with and without the endothelium. ACh (10 $\mu$M) was applied during the period indicated by the horizontal bar. Note that a hyperpolarizing response to ACh was absent in preparations without the endothelium.

## Discussion

The present study demonstrated that in the mesenteric arteries of salt-loaded DS rats: (a) the membrane was depolarized compared with the other groups; (b) spontaneous electrical activity was present; (c) the $TXA_2/PGH_2$ receptor antagonist, ONO-3708, and the cyclo-oxygenase inhibitor, indomethacin, abolished spontaneous electrical activity without altering the membrane potential; (d) spontaneous electrical activity was dependent on voltage-dependent $Ca^{2+}$-influx; (e) spontaneous contraction was present, and contractile sensitivity to high KCl solution was increased. These findings indicate that the action of $TXA_2/PGH_2$, together with membrane depolarization, may lead to the activation of voltage-dependent $Ca^{2+}$-channels, thereby causing spontaneous electrical activity in the mesenteric arteries of salt-loaded DS rats. In addition, these changes in membrane properties appeared to be related to enhanced

**Figure 6** Representative tracings of KCl-induced contractions in mesenteric arteries of Dahl rats. The number in the figure represents concentration of KCl (mM). Sudden vertical deflections are artifacts caused by changing the solution. Endothelium (+), endothelium-intact preparation; Endothelium (−), endothelium-rubbed preparation; Indomethacin (−), without indomethacin; Indomethacin (+), with indomethacin (10 $\mu$M). DS high salt, DS rats fed a high salt diet; DS low salt diet, DS rats fed a low salt diet; DR high salt, DR rats fed a high salt diet.

contractile activities. The mechanisms that account for membrane depolarization in salt-loaded DS rats remain unclear.

### *Membrane depolarization in mesenteric artery of salt-loaded DS rats*

The depolarization of the resting membrane potential of arterial cells has been demonstrated in some models of experimental hypertension (Pamnani *et al*., 1982; Shoemaker & Overbeck, 1986), but not in others (Harder *et al*., 1983; 1985; Lamb & Webb, 1989). There is only one study of the membrane potential of arterial cells in DS rats. Abel *et al*. (1981) found no significant difference in the membrane potential of caudal arteries in female DS and DR rats fed either a 8% or a 0.4% NaCl diet. In the present study, significant depolarization was observed in mesenteric arteries of salt-loaded DS rats in comparison to that observed in other groups.

Membrane potential is largely determined by ionic conductance and the electrogenic $Na^+$, $K^+$ pump, i.e. $Na^+$, $K^+$-ATPase activity (Hermsmeyer, 1982). An imbalance of eicosanoid production has been demonstrated in the aorta of DS rats, i.e., an increase in the synthesis of $TXA_2$ and a decrease in the synthesis of vasodilator prostaglandins, such as $PGI_2$ and $PGE_2$ (Ishimitsu *et al*., 1991). In the present experiments, high concentrations of $STA_2$, a $TXA_2$ analogue, and $PGH_2$ produced membrane depolarization in the arteries of DS rats. Although the ionic mechanism that accounts for this depolarization was not evaluated in the present study, a recent study showed that U46619, a $TXA_2$ mimetic, inhibited calcium-activated potassium channels from the pig coronary artery that had been reconstituted in a lipid layer (Scornik & Toro, 1992). We thus tested the hypothesis that $TXA_2/PGH_2$ may be involved in the membrane depolarization in the arteries of salt-loaded DS rats. However, membrane potential was not significantly affected by ONO-3708 and indomethacin, although spontaneous electrical activity was abolished by these agents. These findings exclude the involvement of $TXA_2/PGH_2$ in the membrane depolarization in salt-loaded DS rats.

It has been suggested that ouabain, an inhibitor of $Na^+$, $K^+$-ATPase, is identical to the endogenous digitalis like factor (Hamlyn *et al*., 1991). If membrane depolarization in salt-loaded DS rat arteries is due to diminished $Na^+$, $K^+$-ATPase activity, ouabain would minimize the difference between membrane potentials of salt-loaded DS rats and control rats. However, the membrane was still significantly depolarized in DS rats fed a high salt diet as compared with control rats after treatment with ouabain (1 mM), indicating that membrane depolarization in salt-loaded DS rats could not be explained by a decrease in $Na^+$, $K^+$-ATPase activity. Our findings are consistent with previous findings that $Na^+$, $K^+$-ATPase activity, as assessed by ouabain-sensitive $^{86}$rubidium uptake, is not decreased in the isolated arteries of salt-loaded DS rats (Pamnami *et al*., 1980; Overbeck *et al*., 1981). However, the present findings should be interpreted with caution, since ouabain at higher concentrations has been shown to inhibit $K^+$-channels in certain smooth muscle cells (Barajas-López *et al*., 1989).

Other possibilities that could account for membrane depolarization may include changes in the ionic permeability and a greater contribution of adrenergic neurotransmitters to membrane potential (Harder *et al*., 1981; Stekiel *et al*., 1986). In the

**Figure 7** Line graphs show concentration-response curves to KCl in mesenteric arteries of Dahl rats. (a) Endothelium-intact preparation; (b) endothelium-intact preparation with indomethacin; (c) endothelium-rubbed preparation; (d) endothelium-rubbed preparation with indomethacin. DS rats fed a high salt diet (○); DS rats fed a low salt diet (▲); DR rats fed a high salt diet (□). Means are shown with vertical lines indicating s.e.mean. $N=6-7$ in each group. $*P<0.001$ vs DS fed a low salt diet, $\dagger P<0.001$ vs DR fed a high salt diet by two-way analysis of variance, followed by Scheffe's test.

study by Abel *et al*. (1981) no difference was found in membrane potential between DS and DR rats; adrenergically denervated preparations by a prior *in vitro* exposure to 6-hydroxydopamine (Aprigliano & Hermsmeyer, 1976) were used, thereby eliminating a neurogenic contribution to membrane potential. Whether the presence or absence of residual nerve varicosities can explain the different results between our study and theirs remains to be determined. However, there are a number of other differences between these two studies; e.g., in the present study, rats received a high-salt diet for 7 weeks beginning at 6 weeks of age, while in the study by Abel *et al*. (1981) rats were fed a high salt diet for 5 weeks beginning at 12 weeks of age. It is conceivable that the effects of salt loading may have been affected by the time of initiation and the duration of salt loading. Differences in the experimental conditions, the vascular bed studied, and the source and gender of the Dahl rats used may also have contributed to the differing results. An additional study is needed to clarify the mechanisms responsible for membrane depolarization in the arteries of salt-loaded DS rats.

### *Mechanisms of spontaneous electrical activity in mesenteric artery of salt-loaded DS rats*

Spontaneous electrical activity was present in most of the arteries of salt-loaded DS rats, but not in those of the other groups of rats. Except for the portal vein, vascular smooth muscle cells rarely exhibit spontaneous electrical activity. We previously observed spontaneous electrical activity in the mesenteric arteries of spontaneously hypertensive rats (SHRs) older than 20 months of age, but not in younger SHRs (Fujii *et al*., 1992; 1993). Oscillations in the resting membrane potential were previously observed only in the middle cerebral arteries of SHRs (Harder *et al*., 1983). The precise mechanism responsible for spontaneous electrical activity in the present study remains to be established. However, our findings suggest several possibilities.

Spontaneous electrical activity in the arteries of salt-loaded DS rats was abolished by ONO-3708, a specific $TXA_2/PGH_2$ antagonist (Katsura *et al*., 1983), and by indomethacin, a cyclooxygenase inhibitor, and was reduced by OKY-046, a $TXA_2$ synthesis inhibitor (Iizuka *et al*., 1981). In contrast, spontaneous electrical activity was enhanced by a thromboxane analogue and by $PGH_2$. Spontaneous electrical activity was also abolished by nicardipine, a $Ca^{2+}$-antagonist, by $Ca^{2+}$-free solution, and was transiently suppressed by the hyperpolarization produced by acetylcholine. Adrenergic neurotransmitter release is unlikely to be involved in spontaneous electrical activity as this activity was unaffected by tetrodotoxin and phentolamine. In fact, these findings suggest that spontaneous electrical activity in the arteries of salt-loaded DS rats is mediated in part by $TXA_2/PGH_2$ and is related to the activation of voltage-dependent $Ca^{2+}$-channels. As far as we know, this is the first study that has demonstrated the possible relationship between eicosanoids and active electrical activity in vascular smooth muscle cells.

It is not known how the stimulation of $TXA_2/PGH_2$ receptors is coupled to the activation of voltage-dependent $Ca^{2+}$-channels. Even though $STA_2$, a $TXA_2$ mimetic, depolarized the membrane, $STA_2$ failed to evoke oscillatory electrical activity in arteries of DS rats fed a low salt diet and in most arteries of DR rats fed a high salt diet. On the other hand, in preparations of salt-loaded DS rats, which did not exhibit spontaneous electrical activity, $STA_2$ evoked oscillatory electrical responses similar in shape to spontaneous electrical activity. Thus, a depolarization of the resting membrane potential, as observed in salt-loaded DS rats, may be one of the prerequisites for the generation of spontaneous electrical activity. Based on these observations, it seems more likely that $TXA_2/PGH_2$ activated voltage-dependent $Ca^{2+}$-channels indirectly via a subtle depolarization, thereby leading to spontaneous electrical activity. Alternatively, the characteristics of voltage-dependent $Ca^{2+}$-channels *per se* may be altered in arteries of hypertensive rats (Rusch & Hermsmeyer, 1988; Ohya *et al*., 1993). Studies with the voltage-clamp technique would clarify these issues.

It is also important to determine the exact source of prostaglandins involved in spontaneous electrical activity. Peripheral nerve terminals can be excluded, since spontaneous electrical activity was not inhibited by tetrodotoxin. $PGH_2$ or $TXA_2$ has been shown to be a major endothelium-derived contracting factor that is released in response to acetylcholine in the aorta of SHR and aged rats (Koga *et al*., 1989; Kato *et al*., 1990; Lüscher *et al*., 1992). However, the messenger RNA of cyclo-oxygenase is also expressed in non-endothelial components of vascular walls (Ge *et al*., 1995). Furthermore, in the present study, similar spontaneous electrical activity was also present in preparations without the endothelium. Although additional studies are required to clarify this issue, it seems that the endothelial cells do not play an obligatory role in the generation of spontaneous electrical activity.

### *Contractile properties in mesenteric arteries of salt-loaded DS rats*

Spontaneous contractions were frequently observed in arteries of salt-loaded DS rats, especially in endothelium-rubbed preparations. These contractions had similar characteristics to those of spontaneous electrical activity; both were abolished by indomethacin and nicardipine, indicating that prostaglandins and voltage-dependent $Ca^{2+}$-influx are involved in both activities. It thus appears that spontaneous contractions are closely related to spontaneous electrical activity.

Contractile sensitivity to high KCl solution was increased in arteries of salt-loaded DS rats. We evaluated KCl-induced contraction, because it is in most part mediated by voltage-dependent $Ca^{2+}$-influx, and is, therefore, dependent on membrane potential. Accordingly, the most likely explanation for the enhanced sensitivity to KCl in arteries of salt-loaded DS rats may be the depolarization of the resting membrane potential. Prostaglandins might also be partially involved in the increased sensitivity, as treatment with indomethacin tended to decrease the difference between salt-loaded DS rats and the other two groups. The increased KCl-induced contraction in salt-loaded DS rats was more pronounced in endothelium-rubbed preparations than in endothelium-intact preparations. The reasons for this remain to be elucidated; one possibility is that the inhibitory influence of the endothelium on contraction may partly counteract the enhanced contractile activity in salt-loaded DS rats.

### *Pathophysiological implications*

The present study demonstrated membrane depolarization and spontaneous electrical activity in the mesenteric arteries of salt-loaded DS rats; the latter appears to be related to eicosanoids and to the activation of voltage-dependent $Ca^{2+}$-channels. Theoretically, any of these changes could lead to an increase in vascular reactivity, and indeed results obtained from tension experiments have demonstrated enhanced contractile activity in salt-loaded DS rats. However, the physiological significance of our findings cannot be determined on the basis of this study alone, partially because the observations were limited to *in vitro* conditions and only the conduit artery was used. Nevertheless, there are several experimental and clinical studies, suggesting that eicosanoids might be involved in the pathogenesis of genetic, salt-induced, or pregnancy-induced hypertension. For instance, in SHRs and Lyon hypertensive rats, chronic administration of $TXA_2$ synthase inhibitors ameliorated the elevation of blood pressure (Shibouta *et al*., 1985; Geoffroy *et al*., 1990). An imbalance of eicosanoid production, i.e., increased production of $TXA_2$, has been demonstrated in pregnancy-induced hypertension in man (Fitzgerald *et al*., 1990). In salt-sensitive hypertensive patients, blood pressure after salt loading has been found to be closely associated with the urinary excretion of thromboxane $B_2$, a metabolite of $TXA_2$ (Gomi *et al*., 1992). There is also evidence that the enhancement of cellular $Ca^{2+}$-dependent vasoconstriction may be associated with salt sensitivity in man (Oshima *et al*., 1988; Alexiewicz *et al*., 1992). Oshima *et al*. (1988) showed that the increase in mean blood pressure after salt-loading was positively correlated with changes in the hypotensive response to nifedipine, a $Ca^{2+}$-antagonist, as well as in the intracellular $Ca^{2+}$ content in lymphocyte in patients with essential hypertension. It is conceivable that our findings might thus provide some experimental clues to elucidate eventually the underlying mechanisms of these observations.

DS rats rapidly develop severe target organ damage on exposure to a high salt diet (Yamazaki *et al*., 1994). Activation of the $TXA_2/PGH_2$ receptors exerts other biological actions such as platelet aggregation and the proliferation of vascular smooth muscle cells (Nagata *et al*., 1992). It is, therefore, possible that the enhanced manifestation of eicosanoid action together with membrane depolarization might contribute to the target organ damage seen early in the process of salt-induced hypertension in DS rats. These questions should be addressed in future studies on the effects of chronic administration of $TXA_2$ synthase inhibitors or $TXA_2/PGH_2$ receptor antagonists to DS rats.

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