Impaired Function of Endothelial Pressure-Activated Cation Channel in Salt-Sensitive Genetic Hypertension

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Abstract. Mechanosensitive ion channels have been suggested to act as endothelial mechanosensors for hemodynamic forces. The present study tested the hypothesis that the pressure-activated cation channel (PAC), a novel type of endothelial mechanosensitive ion channel, is involved in salt sensitivity in the Sabra rat model of hypertension. Groups of Sabra salt-sensitive (SBH/y) and salt-resistant (SBN/y) rats were loaded with deoxycorticosterone-acetate (DOCA)-salt for 8 wk or were fed a regular diet. Single channel function of PAC in SBH/y and SBN/y rats was investigated in intact endothelium of mesenteric artery using the patch-clamp technique. After DOCA-salt treatment, the SBH/y rats showed a full hypertensive response, whereas SBN/y rats were normotensive. Rats of both strains that received a regular diet were normotensive. In endothelium of both Sabra rats, Ca^{2+} permeable PAC that was activated by positive pipette pressures was identified. Apparent PAC density (percentage of patches with PAC activity) was reduced in hypertensive SBH/y rats that were loaded with DOCA-salt compared with salt-loaded normotensive SBN/y rats ($6 \pm 2\%$ *versus* $24 \pm 8\%$, respectively; $P < 0.05$). In normotensive SBH/y and SBN/y rats that received a regular diet, PAC density was not altered. Mechanosensitivity and unitary conductance of endothelial PAC were similar in both strains under a regular diet as well as salt loading with DOCA-salt. In conclusion, the decreased density of PAC in mesenteric endothelium from hypertensive SBH/y rats indicates an impaired ion channel regulation. The defective PAC function presumably leads to an impaired mechanosensitive Ca^{2+} entry and might contribute to endothelial dysfunction and high BP in this type of salt-sensitive genetic hypertension.

The endothelium plays a pivotal role in controlling vascular tone in response to hemodynamic stimulation by the release of vasoactive factors (1,2). Mechanosensitive ion channels (MSC) might act as endothelial mechanotransducers, which convert hemodynamic stimuli into Ca^{2+} fluxes and thus stimulate endothelial function by increasing the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (3,4). In patch-clamp experiments in intact endothelium of rat vessel preparations, we recently identified a novel type of Ca^{2+}-permeable MSC that is regulated by membrane pressure (5). This pressure-activated channel (PAC) in intact endothelium represents a class of MSC with unique mechanosensitive properties. Unlike stretch-activated cation channels, the PAC is inhibited by membrane stretch and activated solely by applying positive pressure to the cell membrane. The PAC is a nonselective cation channel with a considerable high Ca^{2+} conductance and is sensitive to gadolinium, a blocker of MSC (5,6).

After induction of hypertension in the two-kidney-one-clip (2K1C) rat model of secondary renovascular hypertension without genetic predisposition, apparent channel density of the PAC was found to be increased in endothelium of aorta and mesenteric resistance arteries (5). This PAC upregulation was interpreted as a counter-regulatory mechanism of the endothelium, because an enhanced Ca^{2+} entry through the PAC could increase Ca^{2+}-dependent formation of vasodilating factors in response to hemodynamic stimulation. A comparable upregulation of PAC also was observed in endothelium of aorta from genetically spontaneously hypertensive rats (SHR) (5) with a genetically determined hypertension. However, PAC function in resistance arteries from genetically hypertensive rats has not been investigated.

It has been hypothesized that an inherited defect of transmembrane sodium transport might be one of the underlying causes of salt-sensitive hypertension (7). The Sabra salt-sensitive (SBH/y) and the Sabra salt-resistant (SBN/y) rats are a useful experimental model to study this hypothesis. The unique feature of this experimental model is that salt susceptibility is genetically determined and expressed only after salt-loading, without the development of spontaneous hypertension (8). In support for a role of endothelial dysfunction in the Sabra model, a diminished acetylcholine-induced endothelium-dependent relaxation has been described in Sabra salt-sensitive

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rats (9). Because endothelial PAC might act as an important membranous regulator of endothelial function in response to hemodynamic stimulation by modulating Ca^{2+} and Na^{+} influx, we studied PAC properties in mesenteric endothelium of Sabra salt-sensitive hypertension.

Materials and Methods

Animals

Male SBH/y and SBN/y rats were obtained from our colony at the Barzilai Medical Center (8). SBH/y and SBN/y rats were salt loaded starting at the age of 10 wk by administration of 1% NaCl in drinking water after subcutaneous implantation of a 75-mg sustained-release deoxycorticosterone-acetate (DOCA) pellet (Innovative Research, Tampa, FL; $n = 7$ each). Untreated SBN/y and SBH/y rats were sham operated and had free access to tap water ($n = 7$ each). After 8 wk, systolic BP was monitored in the awake and undisturbed animals by the tail-cuff method using an automated detection device (TSE, Bad Homburg, Germany).

Patch-Clamp Experiments

Rats were killed at the age of 18 wk during ether anesthesia by excising the heart. Small tissue slices of mesenteric arteries of approximately 2 mm in length (150 to 200 μm outer diameter) were dissected carefully. Patch-clamp experiments in intact endothelium and data analysis were carried out as described previously (5,10). Membrane currents were recorded with a EPC-9 patch-clamp amplifier (HEKA Electronics, Lambrecht, Germany). Data were low-pass-filtered (−3 dB, 800 Hz) at a sample frequency of 2 kHz. Membrane potentials were recorded in the current-clamp mode of the EPC-9. The standard pipette solution contained 140 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH 7.4). The "high" Ca^{2+} pipette solution contained 90 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH 7.4). In current-clamp experiments, the pipette solution contained 140 mM KCl, 1 mM $MgCl_2$, and 10 mM HEPES (pH 7.2). Bath solution contained 140 mM NaCl, 4.3 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH 7.4). Experiments were performed at 37°C.

Mechanical stimulation of the cell membrane was performed by applying negative or positive hydrostatic pressures to the rear of the patch pipette (5). The hydrostatic pressure was adjusted and controlled with a water manometer and monitored with a differential pressure transducer. The pipette pressures used for mechanical manipulation of cell membrane are not equivalent to BP amplitudes that occur *in vivo*.

PAC function in intact endothelium of Sabra rats was determined as described previously (5). The source of the vessels with respect to normotensive or hypertensive Sabra rats was blinded to the investigator who performed the patch-clamp experiments. Only tight-seal patch-clamp experiments with a seal resistance of more than 4 GΩ were included in the statistical analysis.

As a quantitative measure of PAC function in the cell membrane of each animal, we determined the percentage of patches in which PAC activity could be detected after applying positive pressure to the cell membrane (5,6). In addition, we determined the number of PAC in each of 10 different patches by counting the number of current amplitudes. In multichannel patches, we assessed the maximum number of superimposed openings of PAC during maximal mechanical stimulation (40.8 cm of H_20 pipette pressure). The probability of a single PAC being open (P_o) was calculated by integration using the formula $P_o = F_{open}/F_{total}$, where F_{open} is the current face of PAC in the open state and F_{total} is the total face. As a measure of pressure sensitivity, single-channel activity induced by 13.6, 27.2, and 40.8 cm of H_2O pipette pressure, respectively, was determined in each group.

Statistical Analysis

Differences between groups were calculated by use of the Mann-Whitney *U*/Wilcoxon rank sum test. Data are given as mean ± SEM.

Results

Endothelial PAC in Sabra Strains

In intact endothelium of mesenteric arteries from SBN/y and SBH/y rats, we identified a PAC whose mechanosensitive and electrophysiologic properties resemble the characteristics of the PAC previously identified by us in intact aortic and endocardial endothelium from WKY rats (5,6). The PAC in Sabra strains exhibited low spontaneous channel activity with a probability of a channel being open (P_o) of <0.1. Channel activity was strongly increased by applying positive pipette pressure to the membrane patch (Figure 1A). In intact endothelium of mesenteric artery, PAC activation highly depended on the degree of the applied pressure (Figure 1B). In some cell-attached patches, PAC exhibited low spontaneous channel activity that was almost completely abolished by applying negative pipette pressure (−27.2 cm of H_20), *i.e.*, membrane stretch (not shown).

At negative membrane potentials and with a KCl pipette solution, single-channel conductance was 25 ± 2 ps ($n = 14$) in SBN/y rats. In experiments with a 90-mM $CaCl_2$ pipette solution, single-channel conductance was 5 ps ± 1 SD ($n = 4$). Cation selectivity of the PAC was described in detail previously (see reference 5). In a series of cell-attached patch-clamp experiments ($n = 12$), activation of PAC was followed by the opening of a Ca^{2+}-activated nonselective cation channel (5,6) as illustrated in Figure 1C. Such a co-activation was never observed when a pipette solution with 0 mM Ca^{2+} and 1 mM ethyleneglycotetraacetic acid was used ($n = 25$). On the basis of these observations, we concluded that at physiologic $[Ca^{2+}]$ gradients, the Ca^{2+} influx through the PAC in Sabra strains is sufficient to raise $[Ca^{2+}]_i$. Therefore, Ca^{2+} influx through PAC also could stimulate Ca^{2+}-dependent formation of vasodilating factors.

Comparative Study

Systolic BP was increased significantly in salt-loaded SBH/y rats compared with salt-loaded SBN/y rats (Figure 2; $P < 0.01$). In animals that were fed a normal diet, systolic BP was similar in both strains (Figure 2).

In salt-loaded hypertensive SBH/y rats ($n = 7$), apparent density of PAC was 6 ± 2%, which was significantly lower than in salt-loaded normotensive SBN/y rats (24 ± 8% [$n = 7$]; $P < 0.05$; Figure 2). Accordingly, in salt-loaded hypertensive SBH/ rats, the absolute number of PAC detected in 10 patches was 1.3 ± 0.6, which was significantly lower than in salt-loaded normotensive SBN/y rats (7.3 ± 0.3; $P < 0.05$). In animals that were provided regular chow, PAC density in normotensive SBH/y rats ($n = 7$) was 20 ± 5%, which was not different from SBN/y rats (26 ± 6% [$n = 7$]; $P = 0.61$; Figure

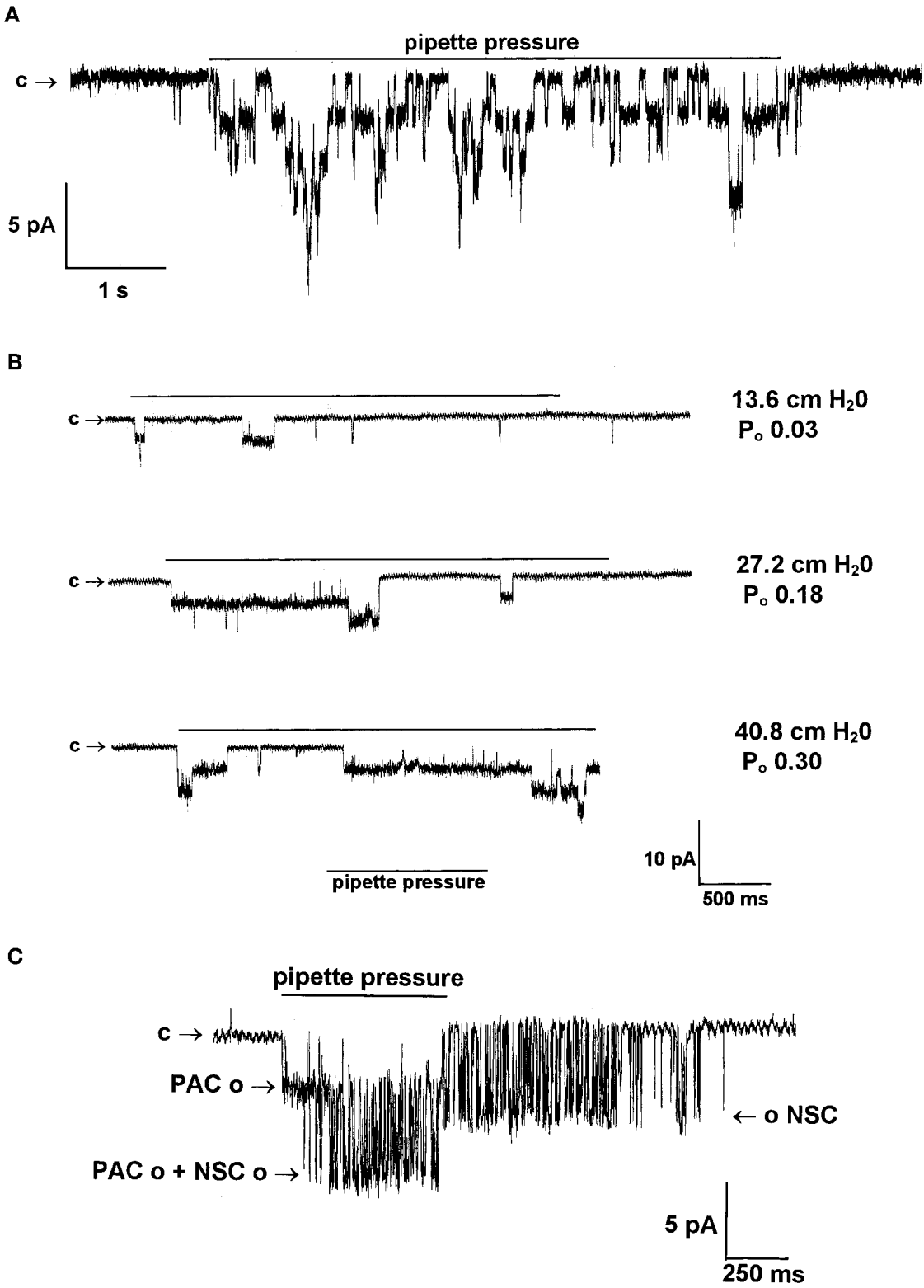

Figure 1. Pressure-activated cation channel (PAC) in intact endothelium of mesenteric arteries from Sabra rats. (A) Activation of PAC by positive pipette pressure (10 mmHg) recorded from a cell-attached patch at a membrane potential of −80 mV; 4 to 5 channels were activated. (B) Gradual increase of channel activity in response to increasing pipette pressures. Channel openings in the downward direction indicate K^+ currents moving from the pipette into the cell. (C) Co-activation of Ca^{2+}-dependent nonselective cation channel (NSC) in cell-attached patches with PAC activity. Channel activity of a single NSC can be differentiated from the activity of the PAC by the larger current amplitude and shorter open states of the NSC. Channel activity was recorded at a holding potential of −80 mV in cell-attached patches. c→, closed state of channels.

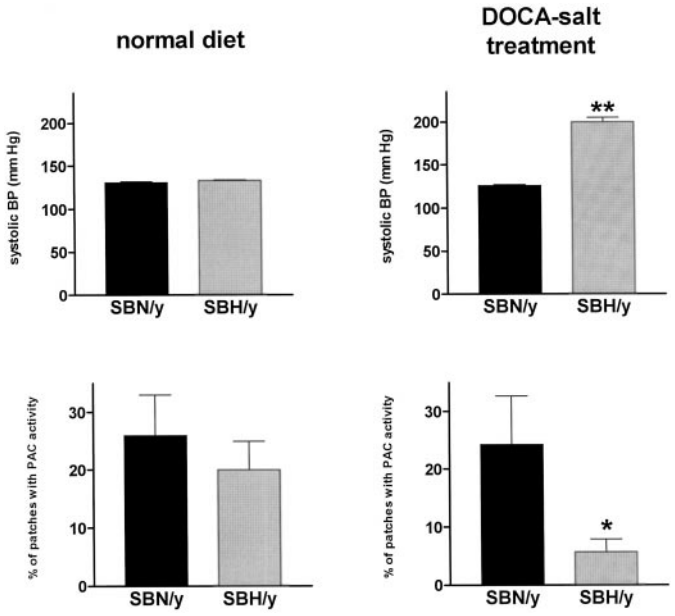

Figure 2. (A) Systolic BP (top) and apparent density of PAC (bottom) in intact endothelium of mesenteric arteries from Sabra salt-sensitive (SBH/y; $n = 7$) and salt-resistant (SBN/y; $n = 7$) rats that were fed a normal diet. (B) Systolic BP (top) and apparent density of PAC (percentage of patches with PAC activity, bottom) in endothelium of mesenteric arteries from SBH/y ($n = 7$) and SBN/y ($n = 7$) rats that were loaded with deoxycorticosterone-acetate (DOCA)-salt for 8 wk. Channel density was determined in each rat as a percentage of patches with PAC activity. **, $P < 0.01$; *, $P < 0.05$.

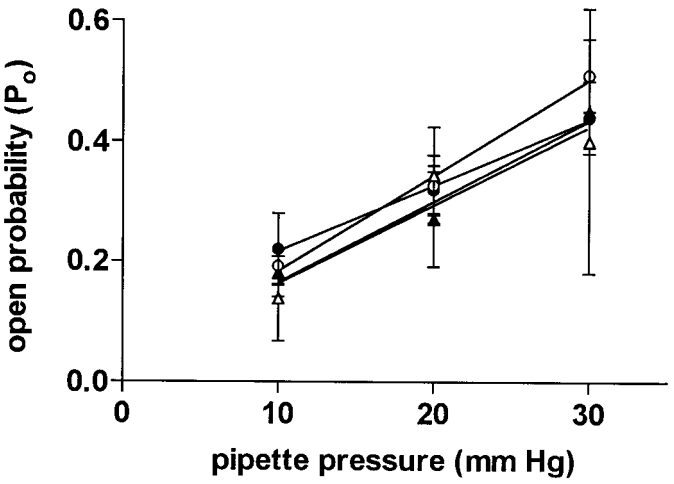

Figure 3. Pressure sensitivity of PAC in intact endothelium of SBH/y ($n = 4$; ○) and SBN/y ($n = 9$; △) rats that were loaded with DOCA-salt and SBH/y ($n = 4$; ●) and SBN/y ($n = 6$; ▲) rats that were fed a normal diet. Open probability of PAC (P_o) was determined at a holding potential of −80 mV.

2). The absolute number of PAC detected in 10 patches was not significantly different in normotensive SBH/y rats (5.3 ± 1.4) compared with SBN/y rats (6.1 ± 1.9; $P = 0.65$).

In endothelium of aorta from salt-loaded hypertensive SBH/y and SBN/y rats, apparent density of PAC was higher than in endothelium of the mesenteric artery. However, in salt-loaded hypertensive SBH/y rats, aortic density of PAC was 34 ± 4%, which was significantly lower than aortic density of PAC in SBN/y rats (58 ± 4; $P = 0.003$). Corresponding, in salt-loaded hypertensive SBH/y rats, the absolute number of PAC detected in 10 patches was 7.6 ± 1.6, which was significantly lower than in salt-loaded normotensive SBN/y rats (13.1 ± 2.0; $P = 0.048$).

A difference in successful seal formation and in seal resistance was not noticed between rats from all groups.

Pressure sensitivity of PAC was similar in both strains that were fed a normal diet or loaded with DOCA-salt (Figure 3). Channel conductance of PAC was not altered significantly in either strain that was fed a normal diet (SBH/y, 26 ± 1 ps; SBN/y, 25 ± 2 ps) or loaded with DOCA-salt (SBH/y, 23 ± 4 ps; SBN/y, 24 ± 1 ps).

In a series of current-clamp experiments, we measured resting potentials in salt-loaded SBH/y and SBN/y rats. In SBH/y rats, resting potential (−24 ± 2 mV [$n = 7$]) was significantly lower compared with SBN/y rats (−33 ± 3 mV [$n = 5$]; $P = 0.018$). In normotensive rats that were provided regular chow, resting potential was statistically not different (SBH/y, −31 ± 3 mV [$n = 8$]; SBN/y, −36 ± 4 mV [$n = 6$]; $P = 0.33$).

Discussion

Endothelial function has been reported to be impaired in human and experimental hypertension (11–14). Particularly, the flow-dependent vasodilation, an endothelial mechanism to protect the vessel wall from damage by increased shear stress, is defective in experimental genetic hypertension (15,16). We showed previously that an upregulation of PAC occurred in 2K1C rats and SHR (5). Upregulation of PAC density was interpreted as a counter-regulatory mechanism that increases Ca^{2+} entry and subsequent formation of vasodilating factors. An increased ability of nitric oxide formation has been described in SHR (16,17). A protective and counter-regulatory ion channel function also has been suggested for Ca^{2+}-dependent potassium channels in vascular smooth muscle cells of aorta and cerebral arteries from SHR (18) and in endothelial potassium-selective MSC (19). These findings indicate that adaptive changes in vascular ion channel densities presumably are important in the maintenance of blood flow during hypertension (18).

One major goal of the present study was to explore whether adaptive changes of PAC function, as had been observed in endothelium of mesenteric resistance arteries from 2K1C rats (5), occur also in the Sabra model of salt-sensitive genetic hypertension. We observed a pronounced decrease of the apparent PAC density in the mesenteric endothelium of hypertensive SBH/y rats compared with normotensive SBN/y rats. This finding is in sharp contrast to our previous observation made in 2K1C hypertensive rats in which, compared with controls, a steep increase of PAC density was detected (5). In the present study, the observation of decreased PAC density in mesenteric endothelium of SBH/y rats suggests that the presumed protective upregulation of PAC observed in secondary

renovascular hypertension is missing in the genetic Sabra model of salt-sensitive hypertension. The decreased PAC density in mesenteric endothelium from salt-loaded SBH/y rats presumably leads to a reduced Ca^{2+} entry in response to hemodynamic stimulation and subsequently to a diminished Ca^{2+}-dependent formation of vasodilating factors. An impaired nitric oxide formation has been suggested to be present in the Sabra (9) as well as in the Dahl model of salt-sensitive genetic hypertension (20,21). Therefore, a decreased PAC function might be indicative of a disturbed endothelial function and diminished flow-induced vasodilation in the Sabra model of salt-sensitive hypertension.

To determine whether this decreased density of PAC is a primary phenomenon in SBH/y rats or is a consequence of hypertension, we made use of the unique specificity of this model and determined PAC densities in normotensive SBH/y and SBN/y rats that were fed a normal diet. It is interesting that PAC densities tended to be decreased, although not significantly so, in mesenteric endothelium of normotensive SBH/y rats compared with SBN/y rats. However, compared with normotensive SBH/y rats, PAC density was significantly lower in hypertensive SBH/y rats. This indicates that the pronounced decrease in PAC density observed in hypertensive SBH/y rats occurred only after induction of hypertension by DOCA-salt treatment.

Alternatively, one could argue that the decrease in PAC was a consequence of high salt intake and/or DOCA treatment alone rather than of hypertension. However, the finding that PAC density was unchanged in SBN/y rats that received a standard diet compared with normotensive SBN/y rats that were treated with DOCA-salt does not support the idea of a direct effect of high salt intake and DOCA on PAC regulation.

As reported previously, chronic Na^+ overload leads to increased intraerythrocytic Na^+ content in SBH/y but not in SBN/y rats (22). Because the PAC is a nonselective cation channel with a fourfold higher permeability for Na^+ than for Ca^{2+}, another possible interpretation of decreased PAC densities in SBH/y rats that are loaded with DOCA-salt is that PAC function and consequently Na^+ influx are downregulated, thus protecting the endothelium from further intracellular Na^+ overload. Therefore, it is tempting to speculate that the protective PAC upregulation as observed in secondary renovascular hypertension might be effectively overruled by a compensatory downregulation of PAC densities in SBH/y rats under DOCA-salt treatment as a result of intracellular Na^+ overload and increased passive Na^+ permeability. Our finding of a lowered resting potential in endothelium of SBH/y rats that were loaded with DOCA-salt could support this interpretation. However, the molecular basis that leads to a lower PAC density has not yet been defined. Possible mechanisms might be a decreased channel expression or decreased integration of the channel protein into the cell membrane. In this regard, such mechanisms remain to be defined by molecular-biologic and immunohistologic studies after molecular-biologic characterization of PAC. Moreover, measurements of vasodilation in response to hemodynamic stimulation in the presence of selective inhibitors of PAC will help to elucidate further the functional consequences of decreased PAC function in salt-loaded hypertensive SBH/y rats.

Mechanosensitivity and unitary conductance of the PAC were similar either in hypertensive SBH/y rats that were loaded with DOCA-salt or in SBH/y rats that were fed a normal diet compared with their respective SBN/y controls. This suggests that the underlying membranous mechanisms of PAC mechanosensitivity or the pore-forming region of the channel protein is not disturbed in Sabra salt-sensitive hypertension.

In conclusion, the decreased PAC densities observed in endothelium of mesenteric arteries might indicate endothelial dysfunction and contribute to impaired flow-induced vasodilation and high BP in Sabra salt-sensitive hypertension. Our findings of an impaired PAC function in salt-sensitive hypertension provide the first evidence of diminished endothelial ion channel function in hypertension and a novel mechanism for salt-sensitive hypertension.

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