Activation of Na\textsuperscript{+},H\textsuperscript{+} Exchanger Produces Vasoconstriction of Renal Resistance Vessels
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To evaluate the influence of the sodium/proton exchanger (Na\textsuperscript{+},H\textsuperscript{+} exchanger) on the constriction of rat resistance vessels and on the iliac artery, the isometric vasoconstrictions of renal resistance vessels and strips from iliac artery derived from Wistar-Kyoto rats were measured using a vessel myograph. The Na\textsuperscript{+},H\textsuperscript{+} exchanger was activated by intracellular acidification using propionic acid. Cytosolic pH (pHi) and cytosolic free sodium concentration ([Na\textsuperscript{i}]) in vascular smooth muscle cells were measured using the fluorescent dye technique. The activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger increased the [Na\textsuperscript{i}] by 12.4 ± 1.3 mmol/L (n = 8). The activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger caused a contractile response of the renal resistance vessels (increase of tension, 1.5 ± 0.1 × 10\textsuperscript{-3} N; n = 13) and of the rat iliac artery (increase of tension, 7.5 ± 0.8 × 10\textsuperscript{-3} N; n = 5). The contractile response after activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger was significantly inhibited in the absence of external sodium or in the presence of amiloride, confirming the involvement of the Na\textsuperscript{+},H\textsuperscript{+} exchanger. The contractile response after activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger was significantly reduced in the absence of external calcium, after inhibition of calcium channels by nifedipine, and in the presence of an intracellular calcium antagonist 8-(diethylamino)-octyl-3,4,5-trimethoxybenzoate (TMB-8), indicating that the activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger consecutively caused transplasma membrane calcium influx. On the other hand, the inhibition of the Na\textsuperscript{+},Ca\textsuperscript{2+} exchanger by NiCl\textsubscript{2} significantly increased the vasoconstriction of renal resistance vessels after activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger. The activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger produces vasoconstriction by an increased cytosolic sodium concentration, inhibition of the Na\textsuperscript{+},Ca\textsuperscript{2+} exchanger, and activation of transplasma membrane calcium influx through potential dependent calcium channels. Am J Hypertens 1998;11:1214–1221 © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: Na\textsuperscript{+},H\textsuperscript{+} exchanger, vasoconstriction, resistance vessels, Na\textsuperscript{+},Ca\textsuperscript{2+} exchanger, fluorescent dye technique.

The sodium/proton exchanger (Na\textsuperscript{+},H\textsuperscript{+} exchanger) is an integral membrane protein found in all eukaryotic cells. It catalyzes the electroneutral and reversible exchange of Na\textsuperscript{+} for H\textsuperscript{+}. Therefore, the activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger causes intracellular alkalinization and increases intracellular sodium concentration. The Na\textsuperscript{+},H\textsuperscript{+} exchanger is involved in intracellular pH regulation in unstimulated cells, cell volume control, stimulus response coupling, and cell proliferation.\textsuperscript{1} There are several diseases that may be associated with or induced by altered Na\textsuperscript{+},H\textsuperscript{+} exchange, including hypertension,\textsuperscript{2} acid-base disorders, diabetic nephropathy,\textsuperscript{3} and cardiac ischemia and reperfusion stunning.\textsuperscript{4} Studies in a variety of tissues from hypertensive animals have shown enhanced sodium flux and cell growth compared to control. Cultured vascular smooth muscle cells derived from spontaneously hy-
pertensive rats show a higher rate of \( \text{Na}^+,\text{H}^+ \) exchange activity.\(^5\) Mesenteric arteries from spontaneously hypertensive rats show an increased activity of the \( \text{Na}^+,\text{H}^+ \) exchanger when compared to normotensive Wistar-Kyoto rats.\(^6,7\) Altered intracellular pH\(_i\) regulation may be responsible for both vasoconstriction and vascular hypertrophy in primary hypertension. However, the relation between the \( \text{Na}^+,\text{H}^+ \) exchanger and contractility is a point of considerable discussion.\(^8\) Changes of intracellular pH\(_i\) may produce marked effects on contraction in smooth muscle cells.\(^9\)

A broad number of contractile functions is potentially regulated by intracellular pH\(_i\).\(^9\) An increased \( \text{Na}^+,\text{H}^+ \) exchanger activity, for example, in response to vasoconstrictor hormones, leading to increased intracellular sodium concentration will probably activate the \( \text{Na}^+,\text{K}^+\)-ATPase, and changes of the intracellular sodium concentration might not appear. However, an increase of intracellular sodium might produce vasoconstriction attributable to the interrelations of the \( \text{Na}^+,\text{Ca}^{2+} \) exchanger, membrane potential and calcium channels, and numerous \( \text{Ca}^{2+} \)-ATPases.

In the present study the relationship between activation of the \( \text{Na}^+,\text{H}^+ \) exchanger and the vasoconstrictive response was evaluated. It appeared that the activation of the \( \text{Na}^+,\text{H}^+ \) exchanger causes vasoconstriction in renal resistance vessels and iliac artery, increases cytosolic free sodium concentration ([\( \text{Na}^+ \)]\(_i\)), and leads to transplasma membrane calcium influx through calcium channels.

**METHODS**

**Measurements of Tension of Renal Resistance Vessels and Strips of Iliac Artery** Six-month-old male Wistar-Kyoto rats (WKY) were used as previously described.\(^10\) The rats were fed a standard pellet diet and water ad libitum. The active force and wall tension of the vasculature was measured in renal resistance vessels under isometric conditions using the established methodology.\(^11–16\) Briefly, the vessel was mounted by wires between the two vessel supports of the small vessel myograph that were facing each other. The right vessel support was movable and connected to a force transducer (Swema, Stockholm, Sweden; maximum amplitude \( \pm 25 \times 10^{-3} \) N). The left vessel support was linked to a linear slide that was attached to a micrometer to adjust the resting wall tension of the vessel.

After removal of the kidney it was incised from cortex to medulla, washed, and kept unfolded at a temperature of 4°C in a medium containing (in mmol/L): NaCl, 115; KCl, 4.6; MgSO\(_4\), 1.2; NaH\(_2\)PO\(_4\), 1.2; NaHCO\(_3\), 22; CaCl\(_2\), 1; d-glucose, 5.5; equilibrated with 95% O\(_2\)/5% CO\(_2\); pH 7.4 at 4°C. By microdissection, renal proximal resistance vessels with diameters of approximately 200 \( \mu \)m were prepared from the medulla under a dissecting microscope. An isolated vessel segment was threaded onto two parallel 40-\( \mu \)m steel wires that were attached to the two vessel supports of the vessel myograph. The vasoconstriction was measured in the medium at 37°C. The resistance vessels were equilibrated for 20 min. The resting tension was set to \( 1 \times 10^{-3} \) N.

The iliac artery was excised and transferred into the medium. The vessels were freed of connective tissue under a dissecting microscope. The 10 \( \times \) 1.5-mm strips were suspended in an organ bath containing 5 mL of the medium kept at 37°C. The strips were equilibrated for 20 min. By stretching the strips the resting tension was set to \( 1 \times 10^{-3} \) N. Stimulation of the strips with 60 mmol/L potassium was repeated every 15 min until a reproducible contractile response was obtained.\(^11–16\) Additional experiments were carried out using strips after gentle removal of the endothelium.

After a period of equilibration with potassium stimulations, the activation of the \( \text{Na}^+,\text{H}^+ \) exchanger of the vasculature was performed using 100 mmol/L propionic acid. Separate experiments were undertaken in sodium-free medium where sodium had been isoosmotically replaced by choline or by \( \text{N}-\text{methyl}-\text{D}-\text{glucamine.} \) In some experiments the \( \text{Na}^+,\text{H}^+ \) exchanger was inhibited by the addition of 100 \( \mu \)mol/L amiloride. For calcium-free medium, calcium was omitted and 50 \( \mu \)mol/L ethyleneglycol-bis-aminoethyl-eether-\( \text{N}^{\prime},\text{N}^\prime\)-tetraacetic acid was added. The calcium channel blocker nifedipine was used at a concentration of \( 1 \mu \)mol/L. In additional experiments the intracellular calcium antagonist 8-(diethylaminoc)-octyl-3,4,5-trimethoxybenzoate (TMB-8) was added at a concentration of \( 1 \mu \)mol/L. All substances were purchased from Sigma (Deisenhofen, Germany) if not indicated otherwise.

**Measurements of Cytosolic-Free Sodium Concentration in Vascular Smooth Muscle Cells** Vascular smooth muscle cells (VSMC) were obtained from male WKY and cultured by tissue explant method according to published procedures.\(^17–19\) Briefly, VSMC were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Eggenstein, Germany) containing 10% (vol/vol) fetal calf serum (Boehringer, Mannheim, Germany), 100 U/mL penicillin G, and 100 \( \mu \)g/mL streptomycin, pH 7.4. The medium was changed initially after 24 h and then every 2 to 3 days. Cell cultures were incubated at 37°C in a humidified atmosphere of 95% air/5% CO\(_2\). The VSMC became confluent in 7 to 12 days and were then subcultured using 0.05% trypsin. After the first subculture, cells were cultured every week at a seeding density of 0.5 to 1.0 \( \times \) 10\(^4\) cells/cm\(^2\). Cultured cells up to the eighth passage were used.\(^17\) Separate experiments showed that resting [\( \text{Na}^+ \)] in cultured VSMC from rats were...
not significantly different in these passages, as it has been confirmed for intracellular calcium in recent literature.²⁰,²¹ Cells were made quiescent by incubation in serum-free medium containing 0.1% bovine serum albumin, 100 U/mL penicillin, and 100 µg/mL streptomycin. To ascertain that cultured cells were VSMC, immunocytochemical localization of smooth muscle-specific alpha-actin was carried out using monoclonal antibodies ASM-1 (Progen, Heidelberg, Germany) raised against smooth muscle alpha-actin and labeled with a fluorescence marker.¹⁷ Staining of cultured VSMC with this antibody revealed that all cells in the preparation were labeled, and actin stress fibers were seen throughout the cytosol. It was confirmed that cultured vascular smooth muscle cells were free from contamination with endothelial cells or fibroblasts by immunocytochemical staining of cells with antibodies against von Willebrand factor coupled to a fluorescence marker.

Fluorescence measurements of [Na⁺] in VSMC were performed using sodium-binding benzofuran isophthalate acetoxymethylester (SBFI-AM; Calbiochem, Bad Soden, Germany) according to recently published methods.²²–²⁸ A stock solution of SBFI-AM (1 mmol/L final concentration) was prepared in dimethyl sulfoxide. The VSMC were suspended in Hanks’ balanced salt solution containing (in mmol/L) NaCl, 136; KCl, 5.4; KH₂PO₄, 0.44; Na₂HPO₄, 0.34; CaCl₂, 1; d-glucose, 5.6; N-2-hydroxyethylpiperazine-N’-2-hydroxyethylpiperazinediethanesulfonic acid (HEPES) 10, pH 7.4. To 5 mL of VSMC (1 × 10⁶ cells/mL), 30 µL of the membrane-permanent SBFI-AM at a final concentration of 6 µmol/L and 5 µL of the nonionic detergent Pluronic F-127 (Molecular Probes, Eugene, OR) at a final concentration of 0.1% wt/vol were added. VSMC were incubated with the dye for 120 min at 37°C. After centrifugation at 11,000 g for 3 min, the supernatant was measured and subtracted from the observed values of SBFI-loaded VSMC. The amount of external dye was 11 ± 2% of the fluorescence intensity of the whole VSMC suspension. The calibration of the F340 nm/F385 nm excitation ratio in terms of [Na⁺], was performed in situ on each cell preparation.²³–²⁹ SBFI-loaded VSMC were added to solutions of known extracellular sodium concentration that were made by appropriate mixtures of high-sodium and high-potassium solutions in the presence of 5 µmol/L monensin and 5 µmol/L nigericin. The high sodium solution contained (in mmol/L) sodium gluconate, 110; NaCl, 30; CaCl₂ 1.2; MgCl₂ 0.6; and Na-HEPES, 10. The high potassium solution was identical except for complete replacement of sodium by potassium. The calibration curve is shown in Figure 1. The addition of sodium propionate did not change extracellular pH or autofluorescence.

**Measurements of Cytosolic pH in Vascular Smooth Muscle Cells** Fluorimetric determinations of cytosolic pH (pHᵢ) were performed according to published methods.³⁰–³³ VSMC were incubated with 10 µmol/L of the pH-sensitive fluorescent dye 2′-7′-bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxyethyl-ester (BCECF-AM; Calbiochem) at 37°C for 120 min. Fluorescence measurements were undertaken at 530 nm emission with an excitation wavelength of 495 nm. At each experiment the fluorescence intensities were converted to estimates of pH using the nigericin, high K⁺ protocol of Thomas et al.³³ It is known that exact characterization of the antiport’s kinetic parameters cannot be achieved by the use of propionic acid. Determination of true maximum exchange activity
(V\textsubscript{max}) requires acidification to pH 6.0, whereas propionate acidification beyond pH 6.4 cannot be achieved. Furthermore pH\textsubscript{r} recovery rates are dampened and the original baseline pH\textsubscript{r} is not reestablished due to the continuous influx of propionic acid. Therefore, comparisons of the V\textsubscript{max} of the Na\textsuperscript{+},H\textsuperscript{+} exchanger are not attempted in the present study.

Statistical Analysis

Data are presented as mean ± SEM. Where error bars do not appear on figures, errors are within the symbol size. Results were tested for statistical significance using Wilcoxon’s test. Two-tailed \( P < .05 \) were considered to be significant.

RESULTS

Effect of Intracellular Acidification on pH\textsubscript{r} and [Na\textsuperscript{+}], in Vascular Smooth Muscle Cells

Cytosolic pH\textsubscript{r} and cytosolic [Na\textsuperscript{+}], were measured using the fluorescent dye technique. The pH\textsubscript{r} in resting VSMC was 7.22 ± 0.13 (n = 6). After the addition of 100 mmol/L propionic acid there was a rapid decrease of pH\textsubscript{r} as shown in Figure 2. The pH\textsubscript{r} recovery after intracellular acidification due to the stimulation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger increased the pH\textsubscript{r} by 0.13 ± 0.04. The [Na\textsuperscript{+}], in resting VSMC was 19.3 ± 2.5 mmol/L (n = 20). After addition of 100 mmol/L propionic acid there was a rapid increase of [Na\textsuperscript{+}], by 12.4 ± 1.3 mmol/L (n = 8; Figure 3). Measurements of pH\textsubscript{r} and [Na\textsuperscript{+}], support the view that intracellular acidification produces an increase of [Na\textsuperscript{+}], after activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger.

Activation of the Na\textsuperscript{+},H\textsuperscript{+} Exchanger Causes Vasoconstriction of Renal Resistance Vessels

Figure 4 shows the typical vasoconstriction of the renal resistance vessel obtained from WKY rats after intracellular acidification with increasing concentrations of propionic acid. The administration of propionic acid produced a rapid vasoconstriction. After activation of the Na\textsuperscript{+},H\textsuperscript{+} exchanger with 100 mmol/L propionic acid the increase of the tension was 1.5 ± 0.1 × 10\textsuperscript{-3} N (n = 13). Control experiments showed that the addition of 100 mmol/L NaCl had no effect on the tension of renal resistance vessels. Additional experiments

![Figure 2](image2.png)

**Figure 2.** Original tracing showing the effect of propionic acid on cytosolic pH (pH\textsubscript{r}) in vascular smooth muscle cells (VSMC). pH\textsubscript{r} was measured in cultured VSMC from Wistar-Kyoto rats using the fluorescent dye 2\textsuperscript{-}9\textsuperscript{-}7\textsuperscript{-}bis-carboxyethyl-5(6)-carboxyfluorescein. The pH\textsubscript{r} in resting VSMC was 7.22 ± 0.13 (n = 6). As indicated by the bar 100 mmol/L propionic acid were added to VSMC. The tracing is representative of six similar experiments.

![Figure 3](image3.png)

**Figure 3.** Original tracing showing the effect of propionic acid on cytosolic-free sodium concentration ([Na\textsuperscript{+}]) in vascular smooth muscle cells (VSMC). [Na\textsuperscript{+}], was measured in cultured VSMC from Wistar-Kyoto rats using the fluorescent dye sodium-binding benzofuran isophthalate. The [Na\textsuperscript{+}], in resting VSMC was 19.3 ± 2.5 mmol/L (n = 20). As indicated by the bar 100 mmol/L propionic acid were added. The tracing is representative of eight similar experiments.

![Figure 4](image4.png)

**Figure 4.** Original tracings showing the vasoconstriction of rat renal resistance vessels after activation of Na\textsuperscript{+},H\textsuperscript{+} exchange by intracellular acidification. Isometric vasoconstrictions of renal resistance vessels from Wistar-Kyoto rats were measured in a vessel myograph. As indicated by the bars increasing concentrations of propionic acid were added at concentrations of 10, 50, or 100 mmol/L, respectively. The tracings are representative of a minimum of three similar experiments.
were done using strips after gentle removal of the endothelium. Under these conditions in the absence of endothelium, the vasoconstriction was 47.2 ± 4.2% of the potassium response. In the presence of endothelium the propionic acid-induced vasoconstriction was 44.6 ± 5.4% of the potassium response. These data indicate that propionic acid acts directly on the vascular smooth muscle cells and not by way of the endothelium.

To test whether the vasoconstriction subsequent to intracellular acidification may be due to activation of the Na⁺,H⁺ exchanger, experiments were conducted in the absence of extracellular sodium or in the presence of amiloride, an inhibitor of the Na⁺,H⁺ exchanger (Figure 5). In the absence of extracellular sodium, where sodium had been replaced isoosmotically by choline, the increase of tension after activation of the Na⁺,H⁺ exchanger with 100 mmol/L propionic acid was significantly reduced compared to control conditions (replacement by choline, 0.6 ± 0.3 × 10⁻³ N; n = 3; P < .05 compared to control). The increase of tension after activation of the Na⁺,H⁺ exchanger with 100 mmol/L propionic acid was significantly reduced in the presence of 100 μmol/L amiloride (0.6 ± 0.2 × 10⁻³ N; n = 3; P < .05 compared to control). From these experiments it was deduced that the vasoconstriction of renal resistance vessels after intracellular acidification is mediated by the Na⁺,H⁺ exchange.

Next, it was investigated whether the activation of the Na⁺,H⁺ exchange leads to transplasma membrane calcium influx (Figures 5 and 6). In the absence of extracellular calcium the contractile response of renal resistance vessels after activation of the Na⁺,H⁺ exchanger was significantly reduced (0.4 ± 0.1 × 10⁻³ N; n = 3; P < .05 compared to control), indicating that transplasma membrane calcium influx is a prerequisite for induction of vasoconstriction after activation of the Na⁺,H⁺ exchanger. After blockade of the potential dependent calcium channels by 1 μmol/L nifedipine the increase of tension subsequent to activation of the Na⁺,H⁺ exchanger was 0.3 ± 0.1 × 10⁻³ N (n = 3; P < .05 compared to control). The contractile response after administration of propionic acid was significantly reduced compared to control conditions in the presence of NiCl₂.
ence of an intracellular calcium antagonist, TMB-8 (final concentration 1 \( \mu \text{mol/L} \); increase of tension, \( 0.4 \pm 0.1 \times 10^{-3} \text{ N} \); \( n = 3; P < .05 \) compared to control).

Then, the role of the \( \text{Na}^+,\text{Ca}^{2+} \) exchange on the vasoconstriction after activation of the \( \text{Na}^+,\text{H}^+ \) exchanger was evaluated. The administration of 6 \( \text{mmol/L} \) NiCl\(_2\) significantly elevated the increase of tension after activation of the \( \text{Na}^+,\text{H}^+ \) exchanger from \( 1.5 \pm 0.1 \times 10^{-3} \text{ N} \) (\( n = 13 \)) to \( 5.8 \pm 0.4 \times 10^{-3} \text{ N} \) (\( n = 4; P < .05 \); Figures 5 and 6).

**Activation of the \( \text{Na}^+,\text{H}^+ \) Exchanger Causes Vasoconstriction of the Rat Iliac Artery**

The activation of the \( \text{Na}^+,\text{H}^+ \) exchanger caused a contractile response of the rat iliac artery by \( 7.5 \pm 0.8 \times 10^{-3} \text{ N} \) (\( n = 5; \) Figure 5). In the absence of extracellular sodium, where sodium had been isosmotically replaced by choline, the increase of tension after activation of the \( \text{Na}^+,\text{H}^+ \) exchanger with 100 \( \text{mmol/L} \) propionic acid was also significantly reduced compared to control conditions (\( 3.5 \pm 0.6 \times 10^{-3} \text{ N} \); \( n = 3; P < .05 \) compared to control). The increase of tension after activation of the \( \text{Na}^+,\text{H}^+ \) exchanger with 100 \( \text{mmol/L} \) propionic acid was also significantly reduced in the presence of 100 \( \text{mmol/L} \) amiloride (\( 3.0 \pm 0.8 \times 10^{-3} \text{ N} \); \( n = 3; P < .05 \) compared to control).

The vasoconstriction of rat iliac artery was also significantly reduced in the absence of extracellular calcium (\( 1.2 \pm 0.1 \times 10^{-3} \text{ N} \); \( n = 3; P < .05 \) compared to control), after inhibition of calcium channels by 1 \( \mu \text{mol/L} \) nifedipine (\( 1.2 \pm 0.4 \times 10^{-3} \text{ N} \); \( n = 3; P < .05 \) compared to control), or in the presence of TMB-8 (\( 3.8 \pm 0.4 \times 10^{-3} \text{ N} \); \( P < .05 \)). After administration of 6 \( \text{mmol/L} \) NiCl\(_2\) the increase of tension following activation of the \( \text{Na}^+,\text{H}^+ \) exchanger was \( 8.8 \pm 1.6 \times 10^{-3} \text{ N} \) (\( n = 3 \)). Therefore, the enhancing effect of inhibition of \( \text{Na}^+,\text{Ca}^{2+} \) exchange on the vasoconstriction subsequent to activation of the \( \text{Na}^+,\text{H}^+ \) exchanger was less pronounced in rat iliac arteries compared to rat renal resistance vessels.

**DISCUSSION**

The activation of the \( \text{Na}^+,\text{H}^+ \) exchanger produces vasoconstriction in both resistance vessels and large vessels from rats. Using the fluorescent dye technique it was confirmed that intracellular acidification caused an increase of cytosolic [Na\(^+\)] in vascular smooth muscle cells through activation of the \( \text{Na}^+,\text{H}^+ \) exchanger. The undissociated propionic acid permeates the cell membrane and acidifies the cytosol. The decrease in intracellular pH activates the \( \text{Na}^+,\text{H}^+ \) exchanger that extrudes intracellular H\(^+\) for extracellular Na\(^+\). In the presence of amiloride, an inhibitor of the \( \text{Na}^+,\text{H}^+ \) exchanger, the intracellular acidification did not cause vasoconstriction, indicating that indeed the activation of the \( \text{Na}^+,\text{H}^+ \) exchanger is a prerequisite for vasoconstriction under these conditions.

The addition of acetate or propionate caused a relaxation in arginine vasopressin precontracted rat tail artery strips.\(^{34}\) That effect was not dependent on endothelium. On the other hand, Miyahara et al\(^{35}\) showed that propionate markedly potentiated the potassium-induced contractions of internal mammary arteries. In addition, inhibition of the \( \text{Na}^+,\text{H}^+ \) exchange by amiloride blocked the phenylephrine-induced contraction of rat aorta.\(^{36}\) In the present study it was confirmed that the propionate-induced vasoconstriction could be blocked by amiloride and by inhibition of voltage-dependent calcium channels.

An increased \( \text{Na}^+,\text{H}^+ \) exchanger activity is associated with primary hypertension and diabetic nephropathy.\(^1\) However, it is not clear how disturbances of the \( \text{Na}^+,\text{H}^+ \) exchanger activity are directly linked to vasoconstriction. In particular, it is uncertain whether or not resting pH\(_1\) is reduced in primary hypertension, as some investigators showed marked differences, whereas others did not observe any differences.\(^{37–41}\) On the other hand it is well-known that potent vasoconstrictors (eg, angiotensin II) stimulate the \( \text{Na}^+,\text{H}^+ \) exchanger in vascular smooth muscle cells.\(^{42,43}\) It has been reported that the \( \text{Na}^+,\text{H}^+ \) exchange is the major sodium influx pathway in vascular smooth muscle cells.\(^{44}\) It has been recently shown that the \( \text{Na}^+,\text{Ca}^{2+} \) exchanger is working in the Ca\(^{2+}\) influx mode after the stimulation of cells.\(^{45–50}\) Therefore, an increase of [Na\(^+\)]\(_i\) may block the \( \text{Na}^+,\text{Ca}^{2+} \) exchanger. As confirmed in the present study the activation of the \( \text{Na}^+,\text{H}^+ \) exchanger causes an increase of [Na\(^+\)]. The [Na\(^+\)]\(_i\) measurements were undertaken in suspensions of cultured cells due to methodologic reasons; however, it may be speculated that similar effects could be observed in the whole vessel. An elevation of [Na\(^+\)]\(_i\) in vascular smooth muscle cells leads to the elevation of cytosolic calcium, probably attributable to the inhibition of the \( \text{Na}^+,\text{Ca}^{2+} \) exchanger working in the Ca\(^{2+}\) efflux mode.\(^{45–50}\) It should be noted that the vasoconstriction subsequent to activation of the \( \text{Na}^+,\text{H}^+ \) exchanger and \( \text{Na}^+,\text{Ca}^{2+} \) exchanger was less pronounced in rat iliac arteries compared to rat renal resistance vessels.

In the absence of extracellular sodium, where sodium had been isosmotically replaced by choline, the increase of tension after intracellular acidification was significantly reduced. That effect is probably attributable to the activation of cholinergic receptors. The replacement of extracellular sodium by N-methyl-D-glucamine produced a rapid vasoconstriction probably due to the activation of the \( \text{Na}^+,\text{Ca}^{2+} \) exchanger working in the Ca\(^{2+}\) influx mode.\(^{45}\) As confirmed by Miyahara et al\(^{35}\) the intracellular acidification by pro-
pionic acid still caused vasoconstriction in the presence of N-methyl-d-glucamine.

In conclusion, the activation of the \( \text{Na}^+,\text{H}^+ \) exchanger causes vasoconstriction in renal resistance vessels and iliac artery by an increase of [\( \text{Na}^+ \)]. That [\( \text{Na}^+ \)] increase blocks the \( \text{Na}^+\text{Ca}^{2+} \) exchanger and may also, in concert with low pH, activate transplasma membrane calcium influx through voltage-dependent calcium channels.

REFERENCES


