Functional role of sodium-calcium exchange in the regulation of renal vascular resistance

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Schweda, Frank, Helga Seebauer, Bernhard K. Krämer, and Armin Kurtz. Functional role of sodium-calcium exchange in the regulation of renal vascular resistance. Am J Physiol Renal Physiol 280: F155–F161, 2001.—Our study aimed to assess a possible functional role of the Na+/Ca2+ exchanger in the regulation of renal vascular resistance (RVR). Therefore, we investigated the effects of pharmacological blockade of the Na+ channel on RVR, by using isolated perfused rat kidneys. Graded decreases in [Na+]e led to dose-dependent increases in RVR to 4.3-fold (35 mM Na+). This vasoconstriction was markedly attenuated by lowering the extracellular calcium concentration, by the L-type calcium channel blocker amlo-dipine or by the chloride channel blocker niflumic acid. Further lowering of [Na+]e to 7 mM led to an increase in RVR to 7.5-fold. In this setting, amloidipine did not influence the magnitude but did influence the velocity of vasoconstriction. Pharmacological blockade of the Na+/Ca2+ exchanger either by lowering the extracellular sodium concentration ([Na+]e) or, pharmacologically on RVR, by using isolated perfused rat kidneys. Several studies provided evidence that under physiological conditions the Na+/Ca2+ exchanger acts as a calcium extrusion mechanism, transferring calcium from the intracellular to the extracellular space, the so-called “forward mode” (2, 21). The activity of this calcium extrusion mechanism has been shown to be dependent on extracellular sodium concentration ([Na+]e) and [Ca2+]i. Lowering of [Na+]e results in an increase in [Ca2+]i (2, 12, 13).

Recently, the presence of the Na+/Ca2+ exchanger in isolated renal afferent arterioles has been demonstrated as lowering extracellular sodium, led to a marked increase in [Ca2+]i independently of L-type calcium channels (4). Also, a possible role of this exchanger in the pathogenesis of arterial hypertension in spontaneously hypertensive and salt-sensitive rats has been suggested (15, 16). Despite these evidences for a functional role of the Na+/Ca2+ exchanger in the regulation of intracellular calcium levels in VSMC, an impact of the exchanger on vascular contractility has not been demonstrated as yet. As the Na+/Ca2+ exchanger might be a regulator of renal blood flow besides hormonal regulation of vascular resistance, we examined the influence of the Na+/Ca2+ exchanger on renal vascular tone by using the isolated perfused rat kidney model. This experimental model provides the opportunity to examine renal vascular resistance (RVR) without hormonal or neural influence. Especially, the systemic renin-angiotensin system, which regulates the sodium balance of the body, is not effective in the isolated perfused rat kidney model as the perfusion medium lacks angiotensinogen, the substrate of renin.

MATERIALS AND METHODS

Isolated perfused rat kidney. Male Sprague-Dawley rats (280–330 g body wt), having free access to commercial pellet chow and tap water were obtained from the local animal...
Kidney perfusion was performed in a recycling system (19). In brief, the animals were anesthetized with 100 mg/kg of 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid (Trapanal, Byk Gulden, Germany). Volume loss during the preparation was substituted by intermittent injections of physiological saline via a catheter inserted into the jugular vein. After opening of the abdominal cavity by a midline incision, the right kidney was exposed and placed in a thermoregulated metal chamber. The right ureter was cannulated with a small polypropylene tube (PP-10) that was connected to a larger polyethylene catheter (PE-50). After intravenous heparin injection (2 U/g), the aorta was clamped distal to the right renal artery so that the perfusion of the right kidney was not disturbed during the following insertion of the perfusion cannula in the aorta distal to the clamp. After ligation of the large vessels branching off the abdominal aorta, a double-barreled perfusion cannula was inserted into the abdominal aorta and placed close to the aortic clamp distal to the origin of the right renal artery. After ligation of the aorta proximal to the right renal artery the aortic clamp was quickly removed and perfusion was started in situ with an initial flow rate of 8 ml/min. By using this technique, a significant ischemic period of the right kidney was avoided. The right kidney was excised, and perfusion at constant pressure (100 mmHg) was established. To this end, the renal artery pressure was monitored through the inner part of the perfusion cannula (Statham transducer P 10 EZ), and the pressure signal was used for feedback control of a peristaltic pump. The perfusion circuit was closed by draining the vein.

Fig. 1. Original tracing of perfusate flow at constant perfusion pressure of 100 mmHg. Stepwise reduction of extracellular sodium concentration ([Na\(^+\)\(_{e}\)]\(_{e}\)) results in a graded reduction of perfusate flow.

Fig. 2. Effects of lowering [Na\(^+\)\(_{e}\)] to 130, 121, 87, 35, or 7 mM on renal vascular resistance (RVR). Inset: RVR (mmHg·min·ml\(^{-1}\)) in dependency of [Na\(^+\)\(_{e}\)].
nous effluent via a metal cannula back into a reservoir (200–220 ml). The basic perfusion medium, which was taken from a thermostated (37°C) reservoir, consisted of a modified Krebs-Henseleit solution containing (in mM) all physiological amino acids in concentrations between 0.2 and 2.0 mM, 8.7 glucose, 0.3 pyruvate, 2.0 L-lactate, 1.0 α-ketoglutarate, 1.0 l-malate, and 6.0 urea. The perfusate was supplemented with 6 g/100 ml bovine serum albumin, 1 mU/100 ml vaso- pressin 8-lysine, and with freshly washed human red blood cells (10% hematocrit). Ampicillin (5 mg/100 ml) and flucloxicillin (3 mg/100 ml) were added to inhibit possible bacterial growth in the medium. To improve the functional preservation of the preparation, the perfusate was continuously dialyzed against a 25-fold volume of the same composition but lacking erythrocytes and albumin. For oxygenation of the perfusion medium the dialysate was gassed with a 94% O2-6% CO2 mixture. Under these conditions both glomerular significance within individual kidneys. P values. Student’s paired average values of an adjoining experimental period or control These RVR values were averaged and compared with the least four time points within this experimental period (2, 5, 10, 15 min after change of perfusate/drug-administration). For evaluation of significance of a certain experimental maneuver on RVR, RVR values were calculated for at least a 3% of the rate of perfusate flow.

RVR was calculated as RVR = perfusion pressure/ perfusate flow.

Experimental maneuvers and chemicals. Different [Na+]e in the perfusate were achieved by replacing NaCl in the dialysate by choline-chloride (Sigma). A nearly complete exchange of sodium was achieved by additional replacement of Na-bicarbonate by choline-bicarbonate (Sigma). Resulting [Na+]e in the perfusate was determined by using a flame-photometer (Jenway PP7).

For lowering the calcium concentration into the submicromolar range, we added the calcium-chelator EGTA (3.12 mM) to the perfusate.

Benzamil, nickel chloride, amlodipine, EGTA, and niflumic acid were obtained from Sigma. KB-R7943 was obtained from Tocris Cookson.

Statistics. Five kidneys were used for each experimental protocol. For evaluation of significance of a certain experimental maneuver on RVR, RVR values were calculated for at least four time points within this experimental period (2, 5, 10, 15 min after change of perfusate/drug-administration). These RVR values were averaged and compared with the average values of an adjoining experimental period or control values. Student’s paired t-test was used to calculate levels of significance within individual kidneys. P < 0.05 was considered significant.

RESULTS

To asess the influence of [Na+]e on RVR, we lowered [Na+]e by replacing increasing amounts of sodium by choline. As shown in the original tracing (Fig. 1) and schematically in Fig. 2, this procedure led to a progressive vasoconstriction, with a significant increase in RVR already after replacement of 10 mM Na+ (1.25-fold of control, P < 0.05) (Fig. 2). Further reduction of [Na+]e to 35 mM led to a 4.3-fold increase in RVR (P < 0.001) (Fig. 2). Lowering of [Na+]e to 7 mM, and therefore below the intracellular sodium concentration, resulted in a further significant vasoconstriction. Time controls at constant [Na+]e did not change vascular resistance over a period of at least 60 min (Fig. 2).

To test whether the observed vasoconstriction was dependent on the availability of extracellular calcium, we added the calcium chelator EGTA (3.12 mM) to the perfusate and subsequently lowered [Na+]e to 35 mM (Fig. 3). Administration of EGTA resulted in a significant vasodilation (RVR 0.92-fold of control). Lowering of [Na+]e in this situation tended to increase RVR (1.1-fold of EGTA); however, this change was not statistically significant (P = 0.09). However, after withdrawal of EGTA, vascular resistance almost instantaneously increased to 7.8-fold. After 15 min, a new plateau of RVR was reached at 3.9-fold of control (Fig. 3).

As calcium influx via L-type calcium channels is involved in the induction of the vasoconstrictive effects of a variety of vasoconstrictors (i.e., angiotensin II, endothelin-1, KCl), we were interested in the role of these channels in the mediation of vasoconstriction by lowering [Na+]e. Therefore, we administered the L-type calcium channel blocker amlodipine (5 μM) before or after switching to a low-sodium perfusate ([Na+]e 35 mM). Addition of amlodipine led to a slight but significant decrease in RVR from 6.2 ± 0.1 to 5.7 ± 0.1 mmHg·min·ml−1 (P < 0.05) (Fig. 4, top). Subsequent lowering of the [Na+]e mediated a significant vasoconstriction (1.26-fold vs. amlodipine, 1.16-fold vs. control), which was marginal, however, compared with the vasoconstriction observed without blockade of calcium channels (4.3-fold vs. control) (Fig. 4, top). Moreover, amlodipine reversed the existing vasoconstriction when added after a switch to low extracellular sodium from 4.3- to 1.6-fold of control (P < 0.05) (Fig. 4, bottom). In the control group without administration of amlodipine, RVR remained constantly on an elevated level (P < 0.05 vs. control) (Fig. 4, bottom).
The next experiments were performed to examine a possible involvement of chloride channels in the mediation of the vasoconstriction by lowering $[Na^+]_e$. To this end, we added the chloride channel blocker niflumic acid (300 μM) to the perfusate and lowered $[Na^+]_e$ to 35 mM subsequently. As shown in Fig. 5, top, administration of niflumic acid resulted in a vasodilation (RVR 5.7 ± 0.1 vs. 6.2 ± 0.10 mmHg·min·ml$^{-1}$ for control) and markedly attenuated vasoconstriction after the switch to the low-sodium perfusate (1.3- to 4.4-fold of control without niflumic acid), indicating an important role of chloride channels in this mechanism. Similar to the results obtained with amlodipine, niflumic acid was able to reverse a preexisting vasoconstriction induced by low sodium from 3.7- to 1.6-fold of control in additional experiments (Fig. 5, bottom).

In the next set of experiments, we lowered $[Na^+]_e$ below the intracellular concentration, thereby turning the direction of calcium transport to an inward direction (Fig. 6). A stepwise reduction of $[Na^+]_e$ to 35 and 7 mM resulted in significant increases in RVR, as shown in Figs. 1 and 2. Performing this maneuver in the presence of amlodipine attenuated the increase in RVR at 35 mM $[Na^+]_e$ (1.3- vs. 4.5-fold of control). However, lowering $[Na^+]_e$ to 7 mM resulted in an increase in RVR to 6.6-fold of control, even under a blockade of L-type calcium channels (7.7-fold of control without amlodipine). Although the magnitude of these increases in RVR was not statistically different, the velocity of the increase was lowered in the kidneys exposed to amlodipine as the maximum vasoconstriction...
occurred 10–15 min later in this group (Fig. 6). These results were confirmed by additional experiments in which [Na\(^+\)]\(_e\) was lowered from 140 to 7 mM with or without amlodipine (5 \(\mu\)M; Fig. 6, inset). Again, the magnitude of vasoconstriction 20 min after lowering of [Na\(^+\)]\(_e\) was similar in both groups, whereas the velocity of vasoconstriction was different.

To strengthen our hypothesis that the vasoconstriction induced by the lowering of [Na\(^+\)]\(_e\) was attributable to an inhibition of the Na\(^+\)/Ca\(^{2+}\) exchanger, we performed a pharmacological blockade of this transporter with the selective inhibitor KB-R7943. As maximum vasoconstriction was achieved at a concentration of 50 \(\mu\)M in preceding experiments, this dosage was used in the further experiments. As shown in Fig. 7, administration of KB-R7943 resulted in a significant increase in RVR to 2.5-fold of control. Subsequent addition of amlodipine (5 \(\mu\)M) reversed this vasoconstriction significantly (RVR 1.14-fold of control). Moreover, benzamil (100 \(\mu\)M) or nickel (3 mM), both unspecific blockers of the Na\(^+\)/Ca\(^{2+}\) exchanger, induced significant vasoconstrictions (RVR 1.8- or 4.2-fold of control) (data not shown).

**DISCUSSION**

Our data demonstrate a functional role of the Na\(^+\)/Ca\(^{2+}\) exchanger in the renal vasculature in mediating vasoconstriction due to decreases in extracellular sodium. As shown in Figs. 1 and 2, this effect already occurs in a physiological concentration range, as lowering of [Na\(^+\)]\(_e\) from 140 to 130 mM resulted in an increase in RVR to 1.25-fold. This marked effect at rather small changes in [Na\(^+\)]\(_e\) seems to be somewhat contradictory to cell culture studies, as in those investigations where an increase in [Ca\(^{2+}\)]\(_i\) was observed only at rather low levels of [Na\(^+\)]\(_e\) (2). However, as the Na\(^+\)/Ca\(^{2+}\) exchanger acts as a calcium extrusion pathway, its activity depends, beside other regulators, on [Ca\(^{2+}\)]\(_i\) (20). As in our experimental model, in contrast to cell culture studies, the VSMC of the renal vasculature are exposed to a physiological perfusion pressure of 100 mmHg. L-type calcium channels are activated under control conditions to maintain vascular resistance. Therefore as shown in Fig. 3, elimination of extracellular calcium or blockade of L-type calcium channels (Fig. 4) results in a small but significant...
inward calcium pump in conditions with intracellular sodium concentrations exceeding \([\text{Na}^+]_e\) (2, 4, 15). Lowering \([\text{Na}^+]_e\) to 7 mM might therefore result in an active calcium transport into the cell. If this calcium influx exceeds calcium extrusion by the remaining extrusion mechanisms, an intracellular calcium accumulation leading to vasoconstriction will occur.

As replacing extracellular sodium not only effects the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger but also other sodium-driven exchange mechanisms, we performed a selective pharmacological blockade of the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger using KB-R7943. This novel compound has been shown to block the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger without affecting sodium channels or other sodium-driven transport systems (10). As shown in Fig. 7, pharmacological blockade of the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger resulted in a marked vasoconstriction, which was reversible by blockade of L-type calcium channels; behavior similar to this was observed after inhibition of the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger by partial replacement of sodium. Moreover, nickel and benzamil, both unspecific inhibitors of the exchanger, exerted marked vasoconstrictive effects. However, because benzamil, similar to lowering \([\text{Na}^+]_e\), attenuates the exchange capacity of the \(\text{Na}^+/{\text{H}}^+\) exchanger (5), the vasoconstriction might be related in part to this inhibition. However, the inhibition of the \(\text{Na}^+/{\text{H}}^+\) exchanger with ethylisopropylamiloride resulted in a decline of intracellular pH but did not change the \([\text{Ca}^{2+}]_i\), in aortic VSMC (2). Moreover, several studies provided evidence for a vasodilating action of \(\text{Na}^+/{\text{H}}^+\) exchange inhibition (1, 17, 18).

The comparison of the different maneuvers to block sodium-calcium exchange capacity shows that lowering of \([\text{Na}^+]_e\) and nickel are more potent vasoconstrictors than KB-R7943 and benzamil. As we used the most effective dose of each drug in our experiments, we should expect a similar extent of vasoconstriction in all groups if vasoconstriction was related exclusively to the inhibition of the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger. We have to conclude from this discrepancy either that choline and nickel exert additional vasoconstrictory effects or that KB-R7943 and benzamil were not capable of blocking the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger completely. As demonstrated for the different isoforms (9, 11) even the different splice variants found in the kidney may confer different sensitivity on the blockers used.

Another possible problem of our experimental design lies in the replacement of sodium by choline. As, for example, the permeability of the cytoplasmic membrane is smaller for choline than for sodium, choline exerts a higher osmotic action and might thereby influence contractility. However, we did not examine an increase in vascular resistance after raising extracellular osmolarity with sucrose or after adding choline to the perfusate in additional experiments. Moreover, an inhibitory effect of choline itself on the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger has been described (5). As this was the desired effect of our procedures, this "side effect" did not influence the interpretation of our results.

In summary, our data are highly compatible with a functional role of the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger in the renal
vasculature, mediating vasoconstriction due to a lowering of the [Na\(^+\)]\(_e\). In the physiological concentration range, sodium mediation of vasoconstriction is dependent on L-type calcium channels and chloride channels, whereas the reverse-mode action of the Na\(^+\)/Ca\(^2+\) exchanger mediates vasoconstriction only at [Na\(^+\)]\(_e\), far below the physiological range.

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REFERENCES