Pflügers Arch (1991) 419: 32–37
003167689100132W

Pflügers Archiv
European Journal of Physiology
© Springer-Verlag 1991

Amiloride enhances the secretion but not the synthesis of renin in renal juxtaglomerular cells

Armin Kurtz[1], Roberto Della Bruna[2], Holger Scholz[2], and Wolfgang Baier[2]

[1] Physiologisches Institut der Universität Regensburg, Universitätsstrasse 31, W-8400 Regensburg, Federal Republic of Germany
[2] Physiologisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Received January 14, 1991/Received after revision April 11, 1991/Accepted May 25, 1991

Abstract. In this study we have examined a potential role of the sodium/proton exchange system in the regulation of renin secretion. We found that the inhibitors of the Na^+/H^+ antiport, amiloride (1 mM) and ethylisopropylamiloride (EIPA, 50 μM), led to a 125% increase of renin secretion from cultured mouse juxtaglomerular cells. The stimulatory effect of EIPA on renin secretion was dependent on the extracellular concentrations of sodium and hydrogen ions. While lowering the extracellular pH from 7.3 to 7.0, and lowering $[Na^+]_e$ from 130 mM to 5 mM had no effect on basal renin release, it markedly attenuated or even blunted the effect of EIPA on renin secretion. The stimulatory effect of forskolin on renin secretion, however, was not altered by decreases of extracellular pH and of sodium. Inhibition of basal renin release was achieved with angiotensin II (1 μM). In the presence of EIPA the inhibitory effect angiotensin II was markedly attenuated. Although effective on renin secretion, neither amiloride nor EIPA exerted a significant effect on the de novo synthesis of renin in cultured mouse JG cells. These findings are compatible with the idea that an amiloride-sensitive transport process, presumably the Na^+/H^+ exchanger, acts indirectly as an inhibitory signal transduction system for renin secretion from renal juxtaglomerular cells.

Key words: Juxtaglomerular cells – Renin secretion – Renin synthesis – Na/H exchange – Amiloride

Introduction

Membrane-bound signal transduction systems, such as nucleotide cyclases and phospholipases, are thought to play a major role in the control of renin secretion from renal juxtaglomerular (JG) cells (Kurtz 1989). Less attention has so far been paid to a possible role of plasmalemmal ion-exchange systems in the control of renin secretion. Such systems include the sodium/proton exchange influencing intracellular pH, which in turn has been found to be important for the function of vascular smooth muscle (VSM) cells (Wray 1988) to which juxtaglomerular cells belong (Barajas 1979). Although, its functional relevance is less clear for small arteries (Mulvany and Aalkjaer 1990), the Na^+/H^+ exchange is a major acid extruder from VSM cells and plays therefore an important role for the cytosolic pH homeostasis (Boyarsky et al. 1988; Kahn et al. 1990; Weissberg et al. 1987). The Na^+/H^+ exchange is also a major route for sodium entry into VSM cells and thus a major determinant for $[Na^+]_i$ (Little et al. 1986). Moreover, the Na^+/H^+ exchanger has been found to be hormonally regulated in VSM cells, thus providing the possibility that this exchange system could serve as signal transduction system for the hormone control of renin secretion. Angiotensin II and other vasoactive hormones, for instance, have been found to activate Na^+/H^+ exchange in VSM cells (Berk et al. 1987a, b; Ganz et al. 1988). Since angiotensin II and vasoactive hormones in general are known to exert marked effects on renin secretion from JG cells (cf. Kurtz 1989), it appeared reasonable to investigate if Na^+/H^+ exchange could serve as a signal transduction system relevant for the function of JG cells, in particular for the synthesis and the secretion of renin. The Na^+/H^+ exchanger can be rather specifically blocked by amiloride and its derivatives (cf. Rothstein 1989). Recent studies with short-term incubations have shown that amiloride does not alter basal renin release from rat renal cortical slices (Martinez-Maldonado et al. 1990) and does not change the response of renin secretion to moderate increases of extracellular NaCl in superfused rat glomeruli (Skott and Jensen 1989).

In order to assess possible longer-lasting roles for the Na^+/H^+ exchanger in the control of renin secretion and renin synthesis it seemed reasonable to examine the effects of amiloride and its more potent derivative ethylisopropylamiloride (EIPA) on the secretion and on the de novo synthesis of renin in cell cultures. Utilizing a

Offprint requests to: A. Kurtz

recently established cell-culture model of mouse JG cells (Della Bruna et al. 1991), we found that amiloride and EIPA markedly enhanced renin secretion without altering the synthesis rate of renin.

Materials and methods

Isolation of mouse juxtaglomerular cells. Mouse juxtaglomerular (JG) cells were isolated according to the method described previously (Della Bruna et al. 1991). For one preparation the kidneys of eight male C57B16 mice (age 4–6 weeks) were used. The animals were sacrificed by cervical dislocation, the kidneys were extirpated, decapsulated and minced with a scalpel blade. The minced tissue was incubated under gentle stirring in buffer 1 (130 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 10 mM glucose, 20 mM sucrose, 10 mM TRIS/HCl, pH 7.4) supplemented with 0.25% trypsin and 0.1% collagenase at 37 °C for 70 min. The material was then sifted over a 22 μm screen and single cells passing the screen were collected and washed with buffer 1 (at 500 *g* for 10 min). The washed single cells (final volume 6 ml) were mixed with 120 ml 30% isoosmotic Percoll solution, equally distributed into four centrifugation cups and centrifuged at 27000 *g* in a SS34 rotor and a Sorvall RC5C centrifuge for 25 min.

Four apparent bands were obtained with the gradients. Specific renin activity of band III cells (1.075 g/ml) was 2-fold and 64-fold higher than that of band II and band I cells, respectively. Band III cells were used for primary culture.

Primary culture of isolated mouse juxtaglomerular cells. Band III cells were freed from Percoll by washing twice with 50 ml buffer 1. The cells were then suspended in 12 ml culture medium (RPMI-1640 medium supplemented with 0.66 U/ml insulin, 100 U/ml penicillin, 100 μg/ml streptomycin and 2% dialysed fetal bovine serum) and distributed in 500-μl portions into 24-well plates. The cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 in air.

Preparation of cellular extracts. For determination of intracellular renin activity and radiolabelled renin immunoreactivity, cellular extracts were prepared. At the end of the incubation experiments, the culture medium as thoroughly removed. Then 0.5 ml phosphate-buffered saline (PBS) supplemented with 0.1% Triton X-100 was added to the cells, which were kept on a shaker for 20 min at room temperature. Afterwards the solutions were centrifuged at 2000 *g* for 10 min and the supernatants were saved at −20 °C for further processing.

Experiments. Experiments on renin secretion and renin synthesis were performed between the 20th and 40th h of primary culture. After 20 h of primary culture the culture medium was removed and the cultures were washed once with culture medium. Then fresh and prewarmed culture medium containg [^{35}S]-methionine {RPMI-1640 medium without methionine (Amimed, Basel, Switzerland) plus 0.66 U/ml insulin, 100 U/ml penicillin, 100 μg/ml streptomycin, 2% dialysed fetal bovine serum and 40 μCi/ml [^{35}S]-methionine (New England Nuclear)} together with the drugs to be tested or their respective solvent controls was added.

Renin secretion rates were estimated from the appearance rate of extracellular renin activity. At the end of the incubations the cells were harvested and their renin activity was determined. If not otherwise indicated, the incubation experiments were usually performed for 20 h.

For certain experimental conditions 6 wells were used per preparation.

Determination of renin synthesis. Rates of renin synthesis were estimated by measuring [^{35}S]-methionine immunoreactivity present in the culture medium and cellular extracts that was specifically bound to a rabbit antiserum against mouse renin (Della Bruna et al. 1991). Prior to immunobinding the cellular extracts (0.5 ml) or culture media (0.5 ml) were concentrated to a volume of 10–20 μl and then diaysed against 2×2 ml PBS by ultrafiltration on Centricon 30 membranes (Amicon) (molecular mass cut-off 30 kDa) at 2000 *g* in swing-out buckets in a Sorvall RT6000 centrifuge at 4 °C. The dialysed cellular extracts and culture media were then brought to a volume of 0.5 ml with PBS supplemented with 1% bovine serum albumin and protease inhibitors [leupeptin 0.1 mg/ml; antipain 0.1 mg/ml; chymostatin 0.1 mg/ml; aprotinin 0.2 mg/ml (all from Sigma Int.)]. The samples were then preincubated with PBS supplemented with 1% bovine serum albumin and 0.1% normal rabbit serum at 37 °C for 2 h. Next they were incubated with Omnisorb cells (Calbiochem, Luzern, Switzerland) (50 μl/0.5 ml) for 1 h. Omnisorb was removed by centrifugation at 2000 *g* for 15 min at 4 °C. The supernatants were split into duplicates, which in turn were incubated either with rabbit-(anti mouse renin) serum or with normal rabbit serum at final dilutions of 1 : 1000 at 4 °C overnight. After an incubation with Omnisorb cells for 60 min at room temperature, Omnisorb was removed by centrifugation as described before. The pellets were washed with ice-cold PBS until the radioactivity in the wash solution was less than 1 cpm/μl (usually after three or four washes). After the last wash the supernatants were thoroughly removed. The pellets were dissolved in a liquid scintillator and counted in a β-counter. Specific binding was considered to be the difference of radioactivity bound by the renin antiserum and the normal rabbit serum (i.e. nonspecific binding).

Measurement of renin activity. Renin activity was determined by its ability to generate angiotensin I from the plasma of bilaterally nephrectomized rats (Kurtz et al. 1986). Angiotensin I was measured by radioimmunoassay (Medipro, Teufen, Switzerland).

Measurement of protein. Protein concentrations in cellular extracts were determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Statistics. Levels of significance were calculated utilizing Student's *t*-test, $P<0.05$ was considered significant.

Materials. Angiotensin II, α-methylglucamine and [Sar^1, Ala^8]-angiotensin II were purchased from Sigma International. D-Sucrose came from Fluka, Buchs, Switzerland.

Results

The putative inhibitors of Na^+/H^+ exchange, amiloride and ethylisopropylamiloride (EIPA), significantly increased the 20-h renin release from cultured mouse JG cells by 75% and 125% respectively (Fig. 1). The threshold concentration to evoke this effect was between 0.1 mM and 1 mM for amiloride and around 10 μM for EIPA. The stimulatory effect of EIPA was a temporally monophasic process with a rapid onset of action within the first hour after addition of EIPA to the cells (Fig. 2). As shown in Fig. 2 the kinetics of action of EIPA was markedly different from that of forskolin, which stimulates renin secretion by increasing cyclic AMP formation (cf. Kurtz 1989).

Sodium/proton exchange rates via the Na^+/H^+ antiport are known to be dependent on the extracellular concentrations of sodium and protons (Rothstein 1989). We therefore examined how far changes of extracellular pH and of $[Na^+]_e$ influence basal renin release and the effectiveness of EIPA on renin release. Since these experiments required precise definition of extracellular pH as well as the omission of sodium bicarbonate from the culture medium, the following experiments were performed with a bicarbonate/CO_2-free and HEPES-buffered RPMI-1640 medium. Switching from CO_2/bicarbonate medium to HEPES-buffered medium led to a significant increase of basal renin release by 6% of total renin activi-

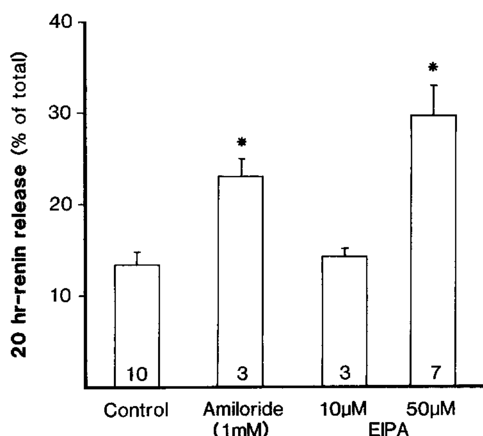

Fig. 1. Effects of amiloride and ethylisopropylamiloride (*EIPA*) on the rate of renin release from cultured mouse juxtaglomerular cells between the 20th and 40th h of primary culture. Release rates are given as percentages of total, i.e. sum of renin activity released + renin activity remaining in the cells. Absolute renin release rates under control conditions were 291 ± 27 ng angiotensin I h^{-1} 100 µg cellular $protein^{-1}$ in 20 h (mean ± SEM; $n = 10$). *Figures at the bottom* indicate the numbers of different experiments. Each experiment represents the mean of 6 replicate wells. The coefficient of variation within a single experiment was less than 20% under all conditions. * $P < 0.05$ vs control

ty. When the extracellular pH was then lowered from 7.3 to 7.0 basal renin release was not altered but the stimulatory effect of EIPA was blunted (Fig. 3, upper panel). For comparison the stimulatory effect of forskolin on renin secretion was not changed by extracellular acidification (Fig. 3, upper panel). Similar results were obtained when the extracellular sodium concentration was lowered isoosmotically (by substitution of sodium with α-methylglucamine) from 130 mM to 5 mM. Basal renin release was slightly reduced by this manoeuvre but the stimulatory effect of EIPA was markedly attenuated (Fig. 3, lower). The stimulatory effect of forskolin, on the other hand, was not affected when extracellular sodium was reduced.

When amiloride (1 mM, not shown) and EIPA (50 µM) were examined for their effects on the de novo synthesis rate of renin in cultured mouse JG cells, it turned out that neither drug altered the synthesis rate of renin within 20 h after addition (Fig. 4). For comparison, forskolin (10 µM), which stimulates renin secretion via cAMP formation, also significantly enhanced the de novo synthesis of renin in the cell cultures.

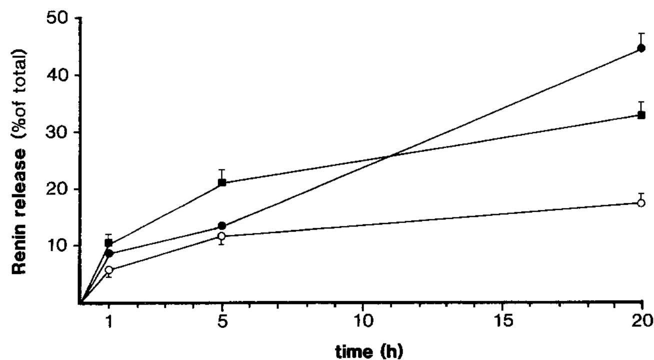

Fig. 2. Kinetics on renin release from cultured mouse juxtaglomerular cells in the absence and the presence of EIPA (50 µM) and forskolin (10 µM). The experiments were started after the 20th h of primary culture. Data are means ± SEM of three experiments each. Data are presented as explained in the legend to Fig. 1, ○, control; ■, EIPA (50 µM); ●, forskolin (10 µM)

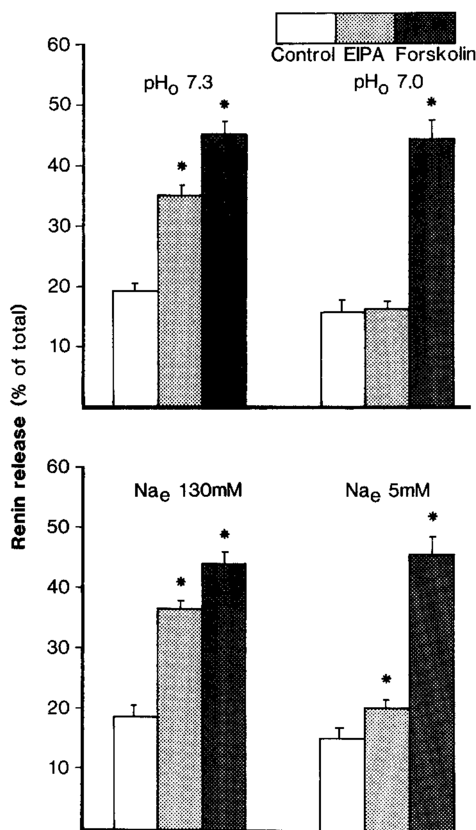

Fig. 3. Influence of extracellular pH (*upper*) and extracellular concentration of sodium (*lower*) on the effects of EIPA (50 µM) and forskolin (10 µM) on 20-h renin release from cultured mouse juxtaglomerular cells. Experiments shown in this figure were done with a bicarbonate/CO_2-free HEPES (25 mM)-buffered culture medium. Isoosmotic replacement of sodium was achieved with α-methylglucamine. Data are means ± SEM of 6 culture wells each. Data are presented as explained in the legend to Fig. 1

In order to obtain some further information about the intracellular cellular mechanisms by which EIPA led to a stimulation of renin secretion, we examined how this drug interfered with cAMP-induced renin secretion stimulated by forskolin (10 µM). As shown in Fig. 6 (lower panel) the effects of EIPA and of forskolin on renin secretion were not additive.

Renin secretion from JG cells in known to be sensitive to changes of cell volume, in the way that cell swelling is associated with increased renin secretion and vice versa (Skott 1988). To examine whether the stimulatory effect of EIPA on renin secretion required cell swelling we also tested its effectiveness in culture media, in which osmolality was increased by the addition of 100 mM sucrose. Basal and EIPA-stimulated renin secretion (Fig. 5), but not forskolin-stimulated renin secretion (not shown) were slightly decreased in the presence of sucrose.

Finally, we examined how EIPA interfered with the action of angiotensin II, which presumably also stimulat-

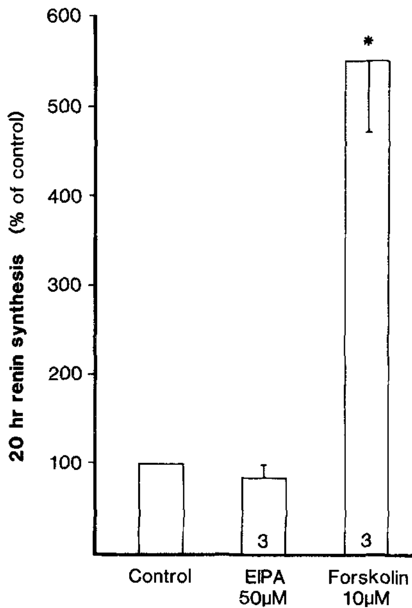

Fig. 4. Effects of EIPA (50 µM) and forskolin (10 µM) on 20-h renin synthesis in cultured mouse juxtaglomerular cells. Data are given as percentage of respective controls. *Figures at the bottom of the bars* indicate numbers of different experiments. Each experiment is the mean of 6 replicate wells. The coefficient of variation within a single experiment was less than 35% under all conditions. Absolute renin synthesis rates under control conditions were 3140±580 cpm mg cellular protein^{-1} 20 h^{-1} (mean±SEM; $n = 4$). * $P<0.05$ vs control

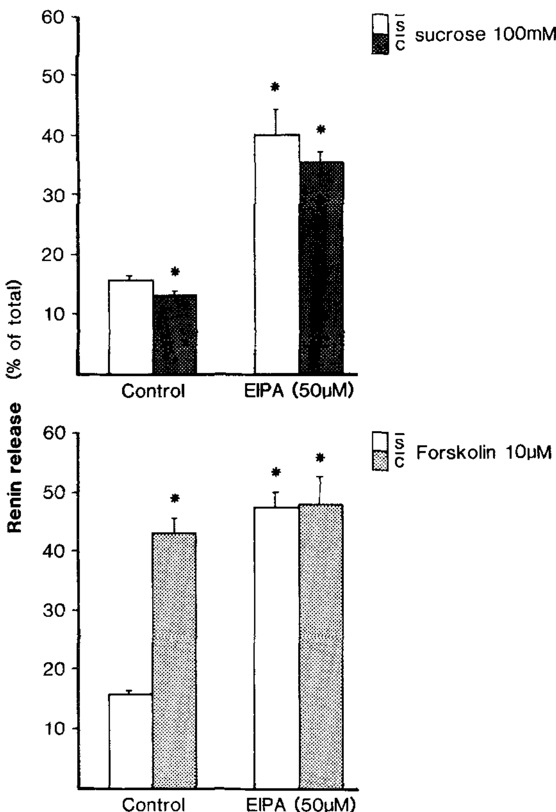

Fig. 5. Influence of sucrose (*upper*) and forskolin (*lower*) on the effect of EIPA on 20-h renin release from cultured mouse juxtaglomerular cells. Data are means±SEM of 6 replicate wells and they are presented as described in the legend to Fig. 1. * $P<0.05$ vs control

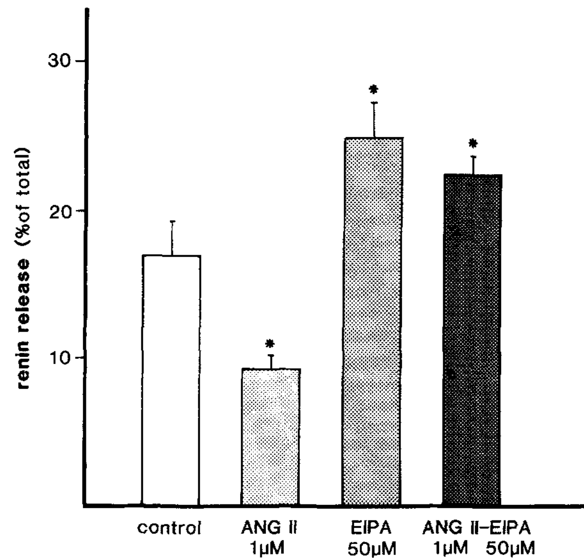

Fig. 6. Effect of [Sar^1, Ala^8]angiotensin II (1 µM) on 20-h renin release in the absence and the presence of EIPA (50 µM). Data are means±SEM of 6 replicate wells each and they are presented as described in the legend to Fig. 1. * $P<0.05$ vs control

es Na^+/H^+ exchange in JG cells. Considering the relatively long times of incubation and the weak stability of native angiotensin II even in vitro (Keppens et al. 1982), we used the more stable angiotensin II analogue [Sar^1, Ala^8] angiotensin II (1 µM) (Hall et al. 1974) for our experiments. As shown in Fig. 6 this compound caused a significant inhibition of basal renin release. The stimulatory effect of EIPA, however, was only slightly attenuated by the angiotensin II analogue.

Discussion

With this study we provide evidence that amiloride and its derivatives have influence on the secretion of renin from mouse renal juxtaglomerular cells in vitro, a finding that would fit with the observations that amiloride leads to a rise of plasma renin activity in humans (Kremer et al. 1977; Nicholls et al. 1976). A recent study utilizing renal cortical slices failed to detect such a stimulatory effect of amiloride within 75 min of incubation (Martinez-Maldonado et al. 1990). Possible explanations for these different findings may be given by the different experimental models used, by the relatively weak effect of amiloride also seen in our system and by the complex mode of action of amiloride on the juxtaglomerular cells as discussed in the following.

Several lines of evidence suggest that the stimulatory effect of amiloride and its derivative EIPA, as seen in this study, could be mediated via the sodium/proton exchange. Thus, amiloride and its more potent derivative EIPA have been found to block Na^+/H^+ antiport in a variety of tissues in a concentration range of 0.1 – 1 mM and 10 – 50 µM, respectively (Rothstein 1989). This is also the concentration range in which they stimulated renin secretion from cultured mouse JG cells (Fig. 1). Moreover, the effect of EIPA on renin secretion was dependent on

the extracellular concentrations or protons and sodium ions, in such a way that that an increase of proton concentration and a fall of sodium concentration inhibited the effect of EIPA on renin secretion (Fig. 4). On the other hand the basal renin secretion and the stimulatory effect of forskolin were not attenuated by these manoeuvres, indicating that drops of pH_0 and Na_e are not generally inhibitory for renin release and do not generally prevent stimulation of renin secretion. Na^+/H^+ exchange activity can, moreover, be attenuated by external ammonium, which competes for the external sodium-binding site (Aronson et al. 1983). As with EIPA, isoosmotic addition of 20 mM NH_4Cl to the culture medium also led to an increase of 20-h renin secretion from $17.5 \pm 1.2\%$ to $28.4 \pm 1.9\%$ (mean ±SEM; three experiments).

The question arises, however, how the finding that drops of extracellular pH and sodium, which are known to decrease net Na^+/H^+ exchange activity (Rothstein 1989), did not stimulate basal renin release is compatible with the aforementioned results. Since Na^+/H^+ antiport is considered as a major acid extruder in vascular smooth muscle cells (Boyarsky et al. 1988; Kahn et al. 1990; Weissberg et al. 1987), one could imagine that inhibition of Na^+/H^+ exchange leads to intracellular acidification of JG cells. However, it appears not very likely that a cytosolic acidification is the stimulatory trigger for renin secretion, because several manoeuvres expected to cause intracellular acidification, such as the addition of fix acids (Fig. 4, upper), increase of CO_2 from 5% to 10% at constant (25 mM) bicarbonate or decrease of bicarbonate to 12.5 mM at constant CO_2 (5%) (not shown), did not enhance renin release.

The Na^+/H^+ exchange is not only a major way for protons to exit from vascular smooth muscle cells but also a major way for sodium entry into these cells (Little et al. 1986; Rothstein 1989). Inhibition of Na^+/H^+ exchange could therefore lead to a sink of $[Na^+]_i$, which consequently could lead to changes of the cell volume (Rothstein 1989) and probably to more important changes in the activity of systems dependent on the transmembrane sodium gradient. For instance one should expect that inhibition of Na^+/H^+ exchange could increase the activity of other sodium-driven transport systems relevant for smooth muscle cells such as, for instance, sodium/bicarbonate exchange (Kahn et al. 1990), sodium/calcium exchange (cf. Carafoli 1987), sodium/potassium/chloride 2 cotransport (Owen 1984) and sodium/potassium ATPase. In fact, the involvement of a second sodium-dependent system is strongly suggested by the findings that a decrease of extracellular sodium markedly reduces the effectiveness of EIPA on renin secretion and slightly decreases basal renin release (Fig. 3). Although this second sodium-driven system still requires identification, it should be mentioned that a possible role of the sodium/calcium exchange in the control of renin secretion has recently been discussed (Churchill 1985). Enhancement of calcium exit via this system should lower resting cytosolic calcium levels or at least attenuate rises of this particular parameter. Since the intracellular calcium concentration is considered as an inhibitory signal for renin secretion, lowering of cytosolic calcium levels via an increased Na^+/Ca^{2+} exchange could lead to an enhancement of renin secretion (Churchill 1985). The observations that acidification blunted the stimulatory effect of EIPA but did not alter basal renin secretion would also fit with such a key role of intracellular sodium. Since it has been found that the affinity of amiloride for the Na^+/H^+ exchanger is markedly reduced at lower extracellular pH (Aaronson et al. 1983), it appears reasonable to assume that the lack of effect of EIPA on renin secretion at low extracellular pH was due to a diminished binding of the drug. The finding that basal renin release was not altered during acidosis would be expected if $[Na^+]_i$ had not changed during this condition, a situation which in fact is not unlikely. Although the efficiency of the Na^+/H^+ exchange for sodium entry is decreased during low extracellular pH, its turnover rate is markedly increased by intracellular acidification (Rothstein 1989). As a result, net sodium influx rate contributing to $[Na^+]_i$ may be unchanged in states of intra- and extracellular acidosis.

Although at present we can not clearly define how Na^+/H^+ exchange could influence renin secretion, we have ascribed some initial characteristics of stimulation of renin secretion by inhibition of Na^+/H^+ exchange. Our findings suggest that the pool of stored renin that is released by EIPA is the same as that activated by cAMP-induced renin secretion (Fig. 5), but it is unlikely that EIPA increases renin secretion via cAMP, because the influence of EIPA and of forskolin on renin secretion and on renin synthesis shows a number of marked differences (Figs. 2–4). It also appears not very likely that inhibition of Na^+/H^+ exchange acts on renin secretion solely via changes of the cell volume, in particular by causing swelling of JG cells, because increase of extracellular osmolality did not abolish the effect of EIPA (Fig. 5). The finding that the inhibitory effect of angiotensin II on renin secretion was markedly attenuated in the presence of EIPA (Fig. 6) may be of physiological relevance, and could indicate that activation of NA^+/H^+ exchange is involved in the action of angiotensin II on renin secretion.

Finally, the result that EIPA enhanced renin secretion but did not increase renin synthesis supports our previous notion that renin secretion and renin synthesis are not obligatorily linked in renal juxtaglomerular cells (Della Bruna et al. 1991).

Acknowledgements. We wish to thank Heini Murer for the generous gift of EIPA and for providing many helpful discussions. We are, moreover, indebted to Pierre Corvol for providing us with antiserum directed against mouse renin. This work was financially supported by a grant from the Swiss National Science Foundation (grant 31-26381.89).

References

Aronson PS, Suhm MA, Nee J (1983) Interaction of external H^+ with the Na^+-H^+ exchanger in renal microvillus membrane vesicles. J Biol Chem 258:6767–6771

Barajas L (1979) Anatomy of the juxtaglomerular apparatus. Am J Physiol 236:F333–F343

Berk BC, Aronow MS, Brock TA, Cragoe E, Gimbrone MA, Alexander RW (1987a) Angiotensin II-stimulated Na^+/H^+ exchange in cultured vascular smooth muscle cells. J Biol Chem 262:5057–5064

Berk BC, Brock TA, Gimbrone MA, Alexander RW (1987b) Early agonist-mediated ionic events incultured vascular smooth muscle cells. J Biol Chem 262:5065–5072

Boyarsky G, Ganz MB, Sterzel RB, Boron WF (1988) pH regulation in single glomerular mesangial cells I. Acid extrusion in absence and presence of HCO_3^-. Am J Physiol 255:C844–C856

Carafoli E (1987) Intracellular calcium homeostasis. Annu Rev Biochem 56:395–433

Churchill PC (1985) Second messengers in renin secretion. Am J Physiol 249:F175–F184

Davis JO, Freeman RH (1976) Mechanisms regulating renin release. Physiol Rev 56:1–56

Della Bruna R, Pinet F, Corvol P, Kurtz A (1991) Regulation of renin secretion and renin synthesis in isolated mouse juxtaglomerular cells. Cell Physiol Biochem 1:98–110

Ganz MB, Boyarski G, Boron WF, Sterzel RB (1988) Effects of angiotensin II and vasopressin on intracellular pH of glomerular mesangial cells. Am J Physiol 254:F787–F794

Hall MM, Khosla MC, Khairallah PA, Bumpus FM (1974) Angiotensin analogs: the influence of sarcosine substituted in position 1. J Pharmacol Exp Ther 88:222–228

Kahn AM, Cragoe EJ, Allen JC, Halligan RD, Shelat H (1990) Na^+-H^+ and Na^+-dependent Cl^--HCO_3^- exchange control pH_i in vascular smooth muscle. Am J Physiol 259:C134–C143

Keppens S, De Wulf H, Clauser P, Jard S, Morgat JL (1982) The liver angiotensin receptor involved in the activation of glycogen phosphorylase. Biochem J 208:809–817

Kremer D, Boddy K, Brown JJ, Davies DC, Fraser R, Lever AF, Morton JJ, Robertson JIS (1977) Amiloride in the treatment of primary hyperaldosteronism and essential hypertension Clin Endocrinol 7:151–157

Kurtz A (1989) Cellular control of renin secretion. Rev Physiol Biochem Pharmacol 113:1–40

Kurtz A, Pfeilschifter J, Hutter A, Bührle CP, Nobiling R, Taugner R, Hackenthal E, Bauer C (1986) Role of protein kinase C in vasoconstrictor caused inhibition of renin release from isolated juxtaglomerular cells. Am J Physiol 250:C563–C571

Little PJ, Cragoe EJ, Bobik A (1986) Na-H exchange is a major pathway for Na influx in rat vascular smooth muscle. Am J Physiol 251:C707–C712

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Martinez-Maldonado M, Gely R, Tapia E, Benabe JE (1990) Role of macula densa in diuretics-induced renin release. Hypertension 16:261–268

Mulvany MJ, Aalkjaer C (1990) Structure and function of small arteries. Physiol Rev 70:921–962

Nicholls MG, Espiner EA, Hughes H, Rogerd T (1976) Effect of potassium sparing diuretics on the renin-angiotensin-aldosterone system and potassium retention in heart failure. Br Heart J 38:1025–1030

Owen N (1984) Regulation of Na/K/Cl cotransport in vascular smooth muscle cells. Biochem Biophys Res Commun 125:500–508

Rothstein A (1989) The Na^+/H^+ exchange system in cell pH and volume control. Rev Physiol Biochem Pharmacol 112:235–257

Skott O (1988) Do osmotic forces play a role in renin secretion? Am J Physiol 255:F1–F10

Skott O, Jensen BL (1989) Influence of bicarbonate on the sensitivity of renin release to sodium chloride. Pflügers Arch 414:651–655

Weissberg PL, Little PJ, Cragoe EJ, Bobik A (1987) Na-H antiport in cultured rat aortic smooth muscle: its role on cytoplasmic pH regulation. Am J Physiol 251:C707–C712

Wray S (1988) Smooth muscle intracellular pH: measurement, regulation, and function. Am J Physiol 254:C213–C225