Kidney International, Vol. 46 (1994), pp. 1577–1579

Endothelium-mediated regulation of renin secretion

BERNHARD K. KRÄMER, THERESIA RITTHALER, MICHAEL ACKERMANN, STEPHAN HOLMER, KARIN SCHRICKER, GÜNTER A.J. RIEGGER, and ARMIN KURTZ

Klinik und Poliklinik für Innere Medizin II; and Physiologisches Institut I, University of Regensburg, Regensburg, Germany

Endothelium-mediated regulation of renin secretion. The aim of the present study was to investigate the endothelial influence on renin secretion of isolated juxtaglomerular cells. Specifically the role of nitric oxide (NO) and of endothelin was studied. Coculture of primary cultures of juxtaglomerular cells with aortic and microvascular endothelial cells decreased renin secretion. Inhibition of NO formation by absence of l-arginine or presence of N^{ω}-nitro-l-arginine caused a marked decrease in cGMP accumulation and a reduction in renin secretion in cocultures. Exogenous NO (NO liberators sodium nitroprusside/SIN 1) stimulated the 20-hour renin secretion from juxtaglomerular cells markedly, too. The effect of NO on renin secretion was biphasic: short-time inhibition and long-time stimulation of renin release. NO's stimulatory effect on renin secretion is dependent on extracellular calcium, but independent of cAMP or cGMP accumulation. Endothelin 1, 2, and 3 did not affect basal renin secretion, but inhibited cAMP stimulated renin release to a similar extent. Endothelin's action is not mediated via the subtype A endothelin receptor, but seems to involve calcium mobilization in juxtaglomerular cells that is dependent on extracellular calcium and associated with prominent calcium activated chloride channels. Taken together, coculture of juxtaglomerular cells with endothelial cells inhibits renin secretion despite the stimulatory effect of native NO released from endothelial cells. cAMP stimulated renin secretion is inhibited by all three endothelin isoforms thus contributing to the inhibition of renin secretion in coculture.

Since juxtaglomerular cells are located in direct contact to vascular endothelial cells, it is of obvious interest to study the effects of endothelial mediators endothelin and nitric oxide (NO) as well as of endothelial cells *per se* on renin release in isolated juxtaglomerular cells. In addition, it had been shown in preliminary studies that endothelial cells modulate renin secretion in coculture with juxtaglomerular cells and that the endothelial mediator endothelin inhibits renin secretion [1, 2]. The role of NO for renin release with regard to results obtained in isolated perfused kidneys and kidney slices is controversial [3–6].

Therefore, the aim of our ongoing research is to elucidate the role of the endothelium in renin secretion from juxtaglomerular cells [1, 2, 4, 7–9].

Methods

Primary cultures of mouse juxtaglomerular cells (C57B16 mice; Charles River; isolation of cells using 0.1% collagenase and 0.25% trypsin) were done as previously described [1, 8]. After enzymatic dissociation the tissue was sieved (22 μm screen) and the cell suspension was separated using Percoll density gradients. Juxtaglomerular cells were cultured using RPMI 1640 medium (Amimed) supplemented with insulin, penicillin, streptomycin and 2% FCS in a humidified atmosphere containing 5% CO_2. For cocultures endothelial cells were cultured for three days and then juxtaglomerular cells were added. After 24 hours the culture medium (and nonattached cells) was removed and the experiments were started.

Bovine aortic endothelial cells were isolated after incubation with collagenase (1 mg/ml) and plated in α-MEM with 10% FCS as described [8]. Cloned capillary endothelial cells from bovine adrenals were handled as described [1, 8].

Renin release was measured after three and 20 hours of incubation. Renin activity was measured by its ability to generate angiotensin I measured by radioimmunoassay (Sorin). Renin release is given as fractional release of renin (renin activity in supernatant/total renin activity after lysis of cells with Triton X-100) [1, 8]. Accumulation of cGMP was measured after extraction with ice cold 95% ethanol + 20 mM HCl after prior inhibition of phosphodiesterase activity with 0.5 mM IBMX using a radioimmunoassay (Amersham). Measurement of free, intracellular calcium concentrations in juxtaglomerular cells was done using Fura-2/AM and whole cell patch clamp experiments were done as previously described [1, 10]. NO formation was inhibited by the absence of l-arginine or the presence of N^{ω}-nitro-l-arginine.

Results and Discussion

Role of coculture of juxtaglomerular cells with endothelial cells for renin secretion

Employing bovine microvascular endothelial cells and mouse renal juxtaglomerular cells for coculture experiments, we recently reported a decrease of renin secretion in comparison with primary cultures of renal juxtaglomerular cells [1]. These results have been confirmed and extended by recent studies using bovine aortic endothelial as well as bovine microvascular endothelial cells [8, 9]. Basal 20-hour renin secretion rates are reduced by about 30 to 40% in cocultures compared with primary cultures (Fig. 1) [1, 8, 9]. The attenuation of basal renin release by endothelial cells in coculture was not related to their capability of NO formation, since bovine aortic endothelial cells of different passages as well as bovine microvascular endothelial cells were equally effective with regard to reduction of renin secretion despite large differences in cGMP accumulation [8, 9]. In addition to basal renin secretion, cAMP-stimulated renin secretion (10 μM of forskolin or isoproterenol) was also inhibited by coculture with endothelial cells. Lipoxygenase products seem not to be involved in the effects of coculture on renin release, whereas cyclooxygenase products are

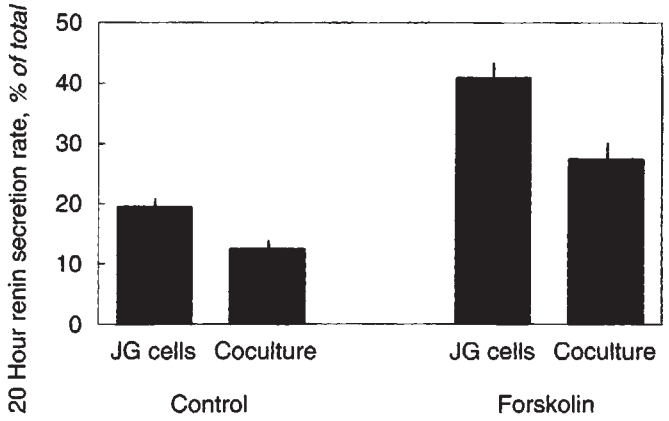

Fig. 1. *Renin secretion during 20 hours from cultured mouse juxtaglomerular cells in coculture with bovine microvascular endothelial cells during control conditions and during stimulation with 10 μM forskolin.* Data are means ± SEM of 4 to 8 individual experiments, each representing the mean of four replicate cultures.

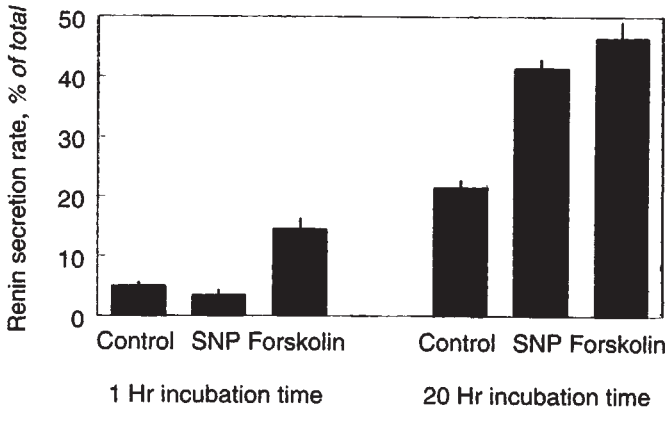

Fig. 2. *Renin secretion during 1 and 20 hours from cultured mouse juxtaglomerular cells incubated with 100 μM SNP (sodium nitroprusside) or 10 μM forskolin in comparison to control conditions.* Data are means ± SEM of 4 individual experiments, each representing the mean of four replicate cultures.

supposed to have a stimulatory role on renin secretion in coculture, since indomethacin decreased spontaneous but not cAMP-stimulated renin release in cocultures [1]. Prostacyclin is known to stimulate renin secretion via cAMP formation, thus explaining the effect of indomethacin on renin secretion [11].

Role of endothelial mediators for renin release from isolated juxtaglomerular cells

In our work we concentrated on the endothelial mediators NO and endothelins 1-3, since both the effects and the mechanisms of action of these mediators with regard to renin secretion have not been well characterized. Bovine aortic endothelial cells released significant amounts of endogenous NO as assayed by guanylate cyclase activity measured separately in endothelial cells, juxtaglomerular cells, and in cocultures [8, 9]. cGMP accumulation was <10% in juxtaglomerular cells compared with endothelial cells, but was overadditively high in cocultures (about 180% of endothelial cells alone). The production of NO was markedly attenuated in the presence of 200 μM of N^{ω}-nitro-l-arginine or in the absence of l-arginine.

Inhibition of endothelial NO formation was associated with a significant decrease of renin release (30 to 40% of baseline) from cocultures, whereas N^{ω}-nitro-l-arginine and the absence of l-arginine were without effect in primary cultures of juxtaglomerular cells. The high cGMP activity in endothelial cells was thought to be due to NO formation for several reasons; marked inhibition of cGMP activity by lack of l-arginine and presence of N^{ω}-nitro-l-arginine, and action as a diffusible agent in cocultures with synergistic effects on cGMP activity were demonstrable [8, 9]. Taken together, we demonstrated that endothelial cells in coculture release relevant amounts of endogenous NO causing a significant stimulation of renin secretion.

Exogenous NO (100 μM of sodium nitroprusside) stimulated renin secretion by about 45 to 110% in primary cultures of juxtaglomerular cells (Fig. 2) as well as in the cocultures both at normal and inhibited NO formation [2, 8, 9]. In dose response curves a near doubling of 20-hour renin secretion was achieved by 100 μM sodium nitroprusside or 5 to 10 mM SIN-1 [7].

In contrast to some investigators who suggest an inhibitory effect of NO on renin release in kidney slices, we found a stimulatory effect of endogenous as well as exogenous NO on renin secretion, confirming the results of previous studies done in isolated kidney as well as when using primary cultures of juxtaglomerular cells [3–9]. The mode of action of NO on renin release of juxtaglomerular cells remains unclear, since stimulation of cGMP is an inhibitory signal for renin release [7]. The stimulatory action of NO on renin release has been shown to be slow-acting, calcium-dependent, and independent of cGMP (ANF despite causing a similar cGMP accumulation than NO was associated with a significant inhibition of renin secretion) or cAMP activity [7]. In fact the effect of NO on renin secretion is biphasic: A short (probably cGMP mediated) inhibition is followed by a long-term stimulation of renin secretion (Fig. 2) [7].

NO-induced stimulation of renin secretion from juxtaglomerular cells appears to be of physiological importance during low renal perfusion pressure where the renin secretion is dependent on stimulation by NO [4, 12]. During high renal perfusion pressure the stimulatory role of NO on renin secretion is overridden by inhibitory signals. In addition to low renal perfusion pressure, renin secretion in response to low tubular sodium chloride appears also to be dependent on the action of NO [13].

In addition to studying the effects of the endothelium-derived relaxing factor NO, we have concentrated our interest on the effects of the endothelium-derived constricting factors endothelins 1-3 on renin secretion. Basal renin secretion was basically unchanged by endothelins 1, 2, and 3, whereas endothelins 1, 2, and 3 blocked the stimulatory effect of cAMP (3 or 10 μM Forskolin; Fig. 3) [2, 9]. The endothelin isoforms 1-3 (1 nM to 1 mM) blocked the cAMP mediated stimulation of renin secretion in a dose-dependent manner, whereas they were without significant effects on renin secretion stimulated by NO, low or high extracellular calcium (manuscript in preparation). Endothelin-induced inhibition of cAMP-stimulated renin secretion was achieved whether 3 μM forskolin, 10 μM isoproterenol, 3 mM 8-br-cAMP or 500 μM IBMX were used, and all three endothelin isoforms were similar in their extent of inhibition of cAMP mediated stimulation of renin secretion (manuscript in preparation). Thus endothelin

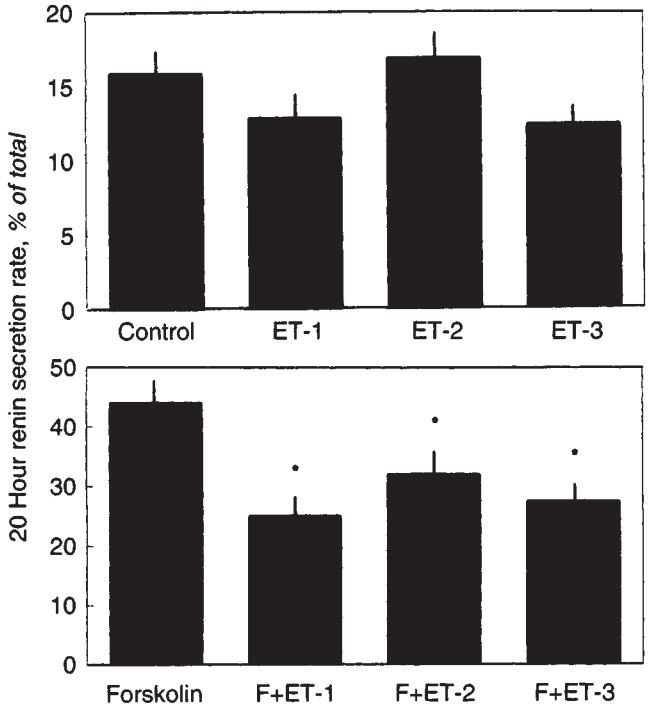

Fig. 3. *Basal (upper panel) and forskolin-stimulated (lower panel; 3 μM of forskolin) renin secretion during 20 hours from cultured mouse juxtaglomerular cells incubated with 100 nM endothelin 1, endothelin 2, and endothelin 3.* Data are means ±SEM of 3 to 5 individual experiments, each representing the mean of four replicate cultures. $^*P < 0.05$ in comparison with forskolin alone.

seems to interfere with cAMP-stimulated renin secretion at a point of the intracellular signal transduction downstream to the site of cAMP formation. The well established inhibitor of the endothelin subtype A receptor, BQ123, was without effect on endothelin's inhibition of cAMP induced renin secretion (manuscript in preparation), suggesting that this effect is not mediated by the endothelin subtype A receptor. Endothelins induced calcium oscillations in juxtaglomerular cells that were dependent on extracellular calcium and that were associated with prominent calcium-activated chloride currents (manuscript in preparation). Calcium mobilization is thought to be essentially involved in the regulation of renin secretion from juxtaglomerular cells [14].

Endothelin's effects on stimulated renin secretion may be one factor responsible for inhibition of renin release in coculture with endothelial cells [1, 8, 9].

NO and endothelin are only two of several endothelium-derived factors that interfere with renin secretion from juxtaglomerular cells [15]. Prostacyclin has been shown to stimulate renin secretion from juxtaglomerular cells, whereas lipoxygenase products (12- or 15-HETE, 12- or 15-HPETE) and 14,15-EET inhibit renin secretion [15]. The interplay between these factors (and factors that are not yet known) and effects that are mediated by direct cell-to-cell contact between juxtaglomerular and endothelial but also, for example, macula densa cells, is complex and more studies are needed to further clarify the basic principles of endothelium-mediated regulation of renin secretion.

Acknowledgments

This study was financially supported by a grant from the Deutsche Forschungsgemeinschaft (Ku 859/2-1) (to AK) and by a grant from the Doktor Robert Pfleger-Stiftung (to BKK).

Reprint requests to Dr. Bernhard K. Krämer, Klinik und Poliklinik für Innere Medizin II, Klinikum der Universität, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany.

References

1. KURTZ A, KAISSLING B, BUSSE R, BAIER W: Endothelial cells modulate renin secretion from isolated mouse juxtaglomerular cells. *J Clin Invest* 88:1147–1154, 1991
2. KRÄMER BK, ACKERMANN M, RITTHALER T, RIEGGER GAJ, KURTZ A: Role of endothelium-derived mediators on renin release in isolated mouse juxtaglomerular cells. (abstract) *J Am Soc Nephrol* 4:557, 1993
3. GARDES J, POUX JM, GONZALES MF, ALHENC-GELAS F, MENARD J: Decreased renin release and constant kallikrein secretion after injection of L-NAME in isolated perfused rat kidney. *Life Sci* 50:987–993, 1992
4. SCHOLZ H, KURTZ A: Involvement of endothelium-derived relaxing factor in the pressure control of renin secretion from isolated perfused kidneys. *J Clin Invest* 91:1088–1091, 1993
5. VIDAL MJ, ROMERO JC, VANHOUTTE PM: Endothelium derived relaxing factor regulates renin release in vivo. *Eur J Pharmacol* 149:401–402, 1988
6. BEIERWALTES WH, CARRETERO OA: Non-prostanoid endothelium-derived factors inhibit renin release. *Hypertension* 19 (Suppl II):68–73, 1992
7. SCHRICKER K, KURTZ A: Liberators of NO exert a dual effect on renin secretion from isolated mouse renal juxtaglomerular cells. *Am J Physiol* 265:F180–F186, 1993
8. SCHRICKER K, RITTHALER T, KRÄMER BK, KURTZ A: Effect of endothelium-derived relaxing factor on renin secretion from isolated mouse juxtaglomerular cells. *Acta Physiol Scand* 149:347–354, 1993
9. KRÄMER BK, RITTHALER T, ACKERMANN M, SCHRICKER K, RIEGGER GAJ, KURTZ A: Effect of EDRF and endothelin on renin secretion from isolated juxtaglomerular cells, in *Biology of Nitric Oxide,* edited by MONCADA S, FEELISCH M, BUSSE R, HIGGS EA, London, Chapel Hill, Portland Press, 1994 (in press)
10. KURTZ A, PENNER R: Angiotensin II induces oscillations of intracellular calcium and inhibits anomalous inward rectifying potassium current in renal juxtaglomerular cells. *Proc Natl Acad Sci USA* 86:3423–3427, 1989
11. KURTZ A: Cellular control of renin secretion. *Rev Physiol Biochem Pharmacol* 113:1–40, 1989
12. PERSSON PB, BAUMANN JE, EHMKE H, HACKENTHAL E, KIRCHHEIM HR, NAFZ B: Endothelium-derived NO stimulates pressure-dependent renin release in conscious dogs. *Am J Physiol* 264:F943–F947, 1993
13. HE X-R, GREENBERG SG, BRIGGS JP, SCHNERMANN JB: Macula densa (MD)- and baroreceptor-mediated renin secretion during inhibition of nitric oxide (NO) synthesis. (abstract) *Nieren-Hochdruckkr* 22:492, 1993
14. SCHRICKER K, DELLA BRUNA R, KURTZ A: Extracellular calcium exerts a dual effect on renin secretion from isolated mouse juxtaglomerular cells. *Pflügers Arch* 423:14–20, 1993
15. CAMPBELL WB, HENRICH WL: Endothelial factors in the regulation of renin release. *Kidney Int* 38:612–617, 1990