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INTERRELATION BETWEEN BARORECEPTOR AND MACULA DENSA MECHANISMS IN THE CONTROL OF RENIN SECRETION

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SUMMARY

1. It was the aim of this study to examine the interrelation between 'baroreceptor' function and the macula densa signal in the control of renin secretion from the kidneys. To this end we investigated the effects of frusemide and bumetanide, two different inhibitors of the macula densa Na^+–K^+–$2Cl^-$ cotransport, on pressure-dependent renin release from isolated perfused rat kidneys. In addition, pressure modulation of renin secretion from hydronephrotic kidneys devoid of macula densa structures was examined.

2. Basal flow rate through isolated kidneys was $13{\cdot}5 \pm 1{\cdot}0$ ml min^{-1} g^{-1} at a renal artery pressure of 100 mmHg and corresponding renin secretory rates were $5{\cdot}5 \pm 0{\cdot}5$ (ng angiotensin I (Ang I) h^{-1}) min^{-1} g^{-1} (mean ± S.E.M., $n = 15$).

3. Frusemide (10–100 μM) and bumetanide (5–50 μM) increased urine flow rates and stimulated urinary sodium excretion in a dose-dependent fashion from $13{\cdot}0 \pm 2{\cdot}5$ μmol min^{-1} g^{-1} ($n = 10$) under control conditions to maximal values of $38{\cdot}0 \pm 5{\cdot}0$ ($n = 5$) and $37{\cdot}0 \pm 2{\cdot}0$ μmol min^{-1} g^{-1} ($n = 5$), respectively. Both drugs also induced concentration-dependent decreases of the renal vascular resistance. The vasorelaxant effects of frusemide and bumetanide were paralleled by an increase of renin secretion to a maximum of 21 ± 4 (ng Ang I h^{-1}) min^{-1} g^{-1} ($n = 10$). On a molar basis bumetanide was twice as potent as frusemide in stimulating renin secretion.

4. Lowering of the perfusion pressure from 100 to 40 mmHg resulted in a prompt increase of renin release yielding secretion rates of 92 ± 10 (ng Ang I h^{-1}) min^{-1} g^{-1} ($n = 15$). In the presence of frusemide (100 μM) and bumetanide (50 μM) renin secretion rates at 40 mmHg were 97 ± 11 and 133 ± 24 (ng Ang I h^{-1}) min^{-1} g^{-1} ($n = 6$), respectively. Renin release stimulated by bumetanide was significantly reduced to $8{\cdot}0 \pm 1{\cdot}5$ (ng Ang I h^{-1}) min^{-1} g^{-1} ($n = 5$) by elevating the perfusion pressure from 100 to 140 mmHg.

5. Lowering the renal artery pressure from 100 to 40 mmHg in isolated perfused rat hydronephrotic kidneys devoid of tubular structures increased renin secretion rates from $4{\cdot}5 \pm 1{\cdot}0$ to $22{\cdot}5 \pm 2{\cdot}5$ (ng Ang I h^{-1}) min^{-1} ($n = 5$).

6. In conclusion, our results suggest that the pressure-dependent renin secretion system does not require the macula densa signal. At normal pressure values (around 100 mmHg), the macula densa mechanism mediates approximately 20 % of

pressure-related inhibition of renin release. Moreover, renin secretion which is susceptible to the macula densa signal is at the same time under the direct and predominant control of the renal artery pressure.

INTRODUCTION

At the organ level, renin secretion from renal juxtaglomerular (JG) cells is controlled by two inhibitory feedback loops: the sodium chloride concentration in the tubular fluid and the renal artery pressure (cf. Keeton & Campbell, 1981; Hackenthal, Paul, Ganten & Taugner, 1990).

In an elegant *in vitro* study Skott & Briggs (1987) have demonstrated that the amount of renin released from isolated perfused juxtaglomerular apparatus (JGA) was negatively correlated with the tubular sodium chloride concentration at the macula densa. The macula densa cells of the early distal tubule are equipped with a Na^+–K^+–$2Cl^-$ cotransport system in their luminal membranes and this cotransporter is pharmacologically inhibitable with, for instance, frusemide or bumetanide (Schlatter, Salomonsson, Persson & Greger, 1989). It has been found that renin secretion from isolated JGAs was directly stimulated by the luminal application of these transport blockers (Lorenz, Weihprecht, Schnermann, Skott & Briggs, 1991). On the other hand, increasing the Cl^- but not the Na^+ concentration at the macula densa significantly reduced renin release in the same preparation (Lorenz *et al.* 1991). In conclusion, the initiating signal for macula densa control of renin release most probably consists of an inverse change of Cl^- transport rate via the luminal Na^+–K^+–$2Cl^-$ cotransport system.

While the role of the macula densa in the control of renin secretion is well established, the cellular pathways along which the renal artery pressure regulates renin secretion are less clear. Different, in part contradictory, theories have been developed in order to explain the mechanism whereby a drop of the renal perfusion pressure enhances renin release. First, it appears possible that the pressure control of renin secretion is mediated via the macula densa mechanism (Vander & Miller, 1964). According to this concept, changes in the renal artery pressure would result in similar changes of the glomerular filtration rate (GFR) and in consequence of the NaCl delivery to the macula densa (Vander & Miller, 1964). The latter hypothesis is in keeping with findings made in rats, but it is at odds with results obtained from dogs. In conscious rats significant increases of renin secretion could only be produced by lowering the renal artery pressure to values below 90–100 mmHg (Conrad, Brinck-Johnson, Gellai & Valtin, 1984; Imagawa, Miyauchi & Satoh, 1984), i.e. in a range where GFR, and presumably the NaCl delivery to the macula densa, decreased in parallel with the renal perfusion pressure. In conscious dogs, however, pressure modulation of renin secretion was related to the autoregulatory plateau phase and reducing the renal artery pressure below the lower threshold for renal autoregulation did not further increase renin secretory rates (Eide, Loyning & Kiil, 1973; Kirchheim, Ehmke, Hackenthal, Löwe & Persson, 1987).

Since pressure dependence of renin release was preserved also in nominally non-filtering dog kidneys, the existence of an additional vascular 'baroreceptor' mechanism for the pressure control of renin secretion has been hypothesized (Blaine, Davis & Witty, 1970; Blaine, Davis & Prewitt, 1971).

The question arises, therefore, as to how this putative 'baroreceptor' and the macula densa work together in the control of renin secretion from the kidneys. Blaine, Davis & Harris (1972) suggested that both mechanisms would act more or less independently of each other with the vascular 'baroreceptor' being operative within the autoregulatory range only and the macula densa signal becoming effective at lower pressure values.

Alternatively, it also appears possible that the macula densa signal is part of the renal 'baroreceptor' mechanism. If the latter hypothesis is correct then one should expect that, even in the normal pressure range (around 100 mmHg), renin secretion from the kidneys is susceptible to the macula densa signal and at the same time can also be modulated by changes of the renal artery pressure.

In view of those controversial concepts it appeared reasonable to us to investigate systematically the interrelation between macula densa and 'baroreceptor' mechanisms in the control of renin release. To this end we used an experimental model that displays a pressure-dependent renin release and which also permits us to modulate the macula densa function. We have recently reported that isolated rat kidneys perfused with a medium containing human red cells released renin in a pressure-dependent fashion (Scholz & Kurtz, 1992). Moreover, the basic mechanisms of myogenic autoregulation were preserved under those experimental conditions (Scholz & Kurtz, 1992).

In our study we have therefore examined renin secretion from isolated perfused rat kidneys at varying renal artery pressures with and without alteration of the macula densa function. The efficacy of the drugs used to inhibit macula densa Na^+–K^+–$2Cl^-$ cotransport was assessed by determination of urine flow and urinary sodium excretion rates. In addition, we also examined pressure-related renin release from isolated perfused hydronephrotic rat kidneys which are devoid of macula densa structures.

METHODS

Male SIV strain rats (250–350 g body weight) having free access to commercial pellet chow and tap water were obtained from the local animal house and used throughout. Kidney perfusion was performed in a recycling system according to the technique of Schurek & Alt (1981) with minor modifications as described in detail previously (Scholz, Kaissling, Inagami & Kurtz, 1991). In brief, the animals were anaesthetized with 150 mg kg^{-1} of 5-ethyl-(1′-methyl-propyl)-2-thio-barbituric acid (Inactin®, Byk Gulden, Konstanz, FRG). Volume loss during the preparation was substituted by intermittent injections of physiological saline (about 2·5 ml in total) via a catheter inserted into the jugular vein. After opening of the abdominal cavity by a mid-line 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) which was connected to a larger polyethylene catheter (PE-50). After intravenous heparin injection (2 U g^{-1}, Liquemin®, Roche, Basel, Switzerland) the aorta was clamped distal to the right renal artery and the large vessels branching off the abdominal aorta were ligated. A double-barrelled cannula was inserted into the abdominal aorta and placed close 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^{-1}. 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 renal venous effluent via a metal cannula back into a reservoir (200–220 ml). The basic perfusion medium, which was taken from the thermostated (37 °C)

reservoir, consisted of a modified Krebs–Henseleit solution containing (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)$^{-1}$ bovine serum albumin, 1 mU (100 ml)$^{-1}$ vasopressin 8-lysine, and with freshly washed human red blood cells (10 ± 2 % haematocrit). Ampicillin (3 mg (100 ml)$^{-1}$) and flucloxacillin (3 mg (100 ml)$^{-1}$) were added to inhibit possible bacterial contamination of the medium. To improve the functional preservation of the preparation, the perfusate was continuously dialysed 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 95 % oxygen, 5 % carbon dioxide mixture. Perfusate flow rates were obtained from the revolutions of the peristaltic pump which was calibrated before and after each experiment. Renal flow rate and perfusion pressure were continuously monitored by a potentiometric recorder (Kipp & Zonen, Delft, Netherlands). Stock solutions of the drugs to be tested (see below) were dissolved in freshly prepared perfusate and infused into the arterial limb of the perfusion circuit directly before the kidneys (peristaltic pump 2132 Microperpex®, LKB, Bomma, Sweden) at 1 % of the rate of perfusate flow. For determination of perfusate renin activity (pRA) aliquots (about 0·1 ml) were drawn from the arterial limb of the circulation and the renal venous effluent, respectively. The samples were centrifuged (4 °C) at 1500 *g* for 15 min (Sorvall RT 6000) and the supernatants were stored at −20 °C until assayed for renin activity.

Determination of renin activity. Perfusate samples were incubated for 1·5 h at 37 °C with plasma from bilaterally nephrectomized male rats as renin substrate (Rightsel *et al.* 1982). The generated angiotensin I (Ang I) was determined by radioimmunoassay (Medipro AG, Teufen, Switzerland).

Renin release. In a previous study performed with the same experimental model we have found that renin is not inactivated during its passage through isolated perfused rat kidneys (Scholz *et al.* 1991). Therefore, renin secretory rates could be calculated from the arteriovenous differences of perfusate renin activity (AVD_{pRA}) and the corresponding renal flow rates.

Determination of sodium. Sodium concentrations were measured in perfusate and urine samples by means of a flame photometer equipped with an internal caesium standard (Instrumentation Laboratory 943).

Determination of urinary excreted frusemide and bumetanide. Until measurements, the collected urine samples were stored in the dark at −20 °C. Using a Beckman DU-62 spectrophotometer, wavelength scanning was performed in the range between 200 and 800 nm, and both frusemide and bumetanide were found to exhibit a characteristic absorption maximum at 330 nm. Therefore, this wavelength was taken for determination of urinary excreted frusemide and bumetanide. For generation of standard curves, various concentrations of both drugs were freshly dissolved in blank urine samples.

Hydronephrotic rat kidneys. Hydronephrotic kidneys were obtained according to the method of Steinhausen, Snoei, Parekh, Baker & Johnson (1983). Male SIV strain rats (190 ± 10 g body weight) were used. After anaesthetizing the rats as described above, a small lower abdominal incision was made and the right ureter was ligated near to its entrance into the urinary bladder. The abdomen was closed by surgery. Four weeks later hydronephrosis had developed in more than 90 % of operated rats. Hydronephrosis was identified by the typical balloon-like appearance of the kidneys being devoid of intact renal parenchyma. Perfusion of hydronephrotic kidneys was performed in the same way as described for intact kidneys, with the only exception that cannulation of the ureter was not done.

Agents. Pyruvate was obtained from Boehringer, Mannheim, FRG. Ampicillin and flucloxacillin were from Beecham, Bern, Switzerland. Glutamate, urea, and α-ketoglutarate were obtained from Merck, Darmstadt, FRG. L-malic acid and L-lactate as sodium salts were provided by Serva, Heidelberg, FRG. L-Amino acids were from Braun/Melsungen, FRG (Aminoplasmal 5 % paediatric, free of carbon hydrate). Vasopressin 8-lysine was provided by Sandoz, Basel, Switzerland. Frusemide, bumetanide, angiotensin II, isoproterenol and bovine serum albumin (fraction V powder) were purchased from Sigma International. Stock solutions of the drugs to be tested were 0·5 M in dimethyl sulphoxide (DMSO) and the final DMSO concentration in the perfusion medium was 0·1 %. Therefore, appropriate controls were performed by infusing the vehicle alone, and 0·1 % DMSO in the perfusate was found to have no significant effects on the functional parameters to be examined, in particular not on renal flow and renin secretion rates.

Presentation of results. Graphs showing renal perfusate flow and renin secretion rates are redrawings from original traces. As indicated by points, perfusate samples for determination of renin activity were collected at 2 min intervals.

Statistics. All experiments were performed in such a way that a single kidney preparation was first taken as a control and was then used for the experimental protocol. Statistical analysis was performed using Student's t test with Bonferroni's reduction for multiple comparisons. $P < 0{\cdot}05$ was considered significant.

RESULTS

The duration of the experiments was usually less than 2 h. Within this time period perfusate flow through the kidneys remained stable and was $13{\cdot}5 \pm 1{\cdot}0$ ml min^{-1} g^{-1} (mean ± S.E.M., $n = 15$) under control conditions at 100 mmHg (Fig. 1). Basal renin secretion rates were $5{\cdot}5 \pm 0{\cdot}5$ (ng Ang I h^{-1}) min^{-1} g^{-1} ($n = 15$, Fig. 1).

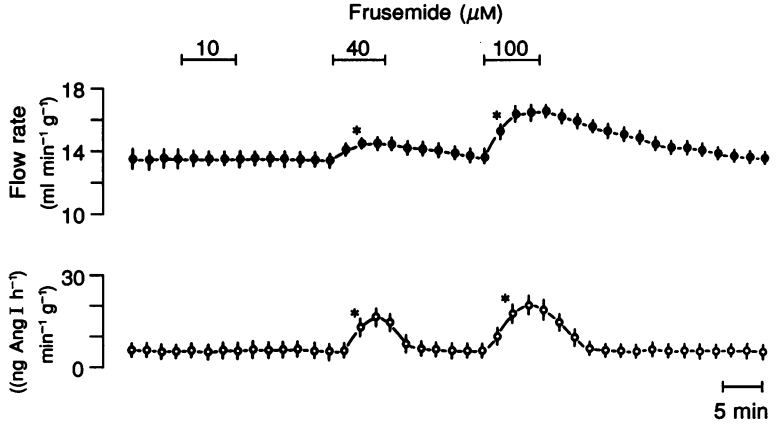

Fig. 1. Dose–response traces of added frusemide on perfusate flow (upper trace) and renin secretion rates (RSR; lower trace) from isolated rat kidneys perfused at a constant pressure of 100 mmHg. Frusemide was flow-proportionally (1 % of renal flow rate) infused into the arterial limb of the perfusion system and the concentrations indicated refer to the arterial perfusate. Values are means ± S.E.M., number of experiments, $n = 5$. *, indicate significant difference from control ($P < 0{\cdot}05$).

Frusemide, an inhibitor of the macula densa Na^+–K^+–$2Cl^-$ cotransport (Schlatter *et al.* 1989), used in the concentration range between 10 and 100 μM stimulated urine flow and urinary sodium excretion in a concentration-dependent fashion (Figs 2 and 3). Urine flow rates were 84 ± 15 μl min^{-1} g^{-1} ($n = 10$) under control conditions and 208 ± 25 μl min^{-1} g^{-1} ($n = 5$) with 100 μM frusemide (Fig. 2). During control periods, the mean urinary sodium excretion was $13{\cdot}0 \pm 2{\cdot}5$ μmol min^{-1} g^{-1} ($n = 10$) and this value was increased to a maximum of $38{\cdot}0 \pm 5{\cdot}0$ μmol min^{-1} g^{-1} ($n = 5$) in the presence of 100 μM frusemide (Fig. 3). Addition of frusemide to the perfusate led to a concentration-dependent increase of perfusate flow by up to 20 % of the control flow (Fig. 1). This vasorelaxant effect was paralleled by a dose-dependent increase in renin secretion reaching values of 21 ± 4 (ng Ang I h^{-1}) min^{-1} g^{-1} ($n = 5$) at a concentration of 100 μM frusemide and at a perfusion pressure of 100 mmHg (Fig. 1). Similarly, bumetanide, another specific

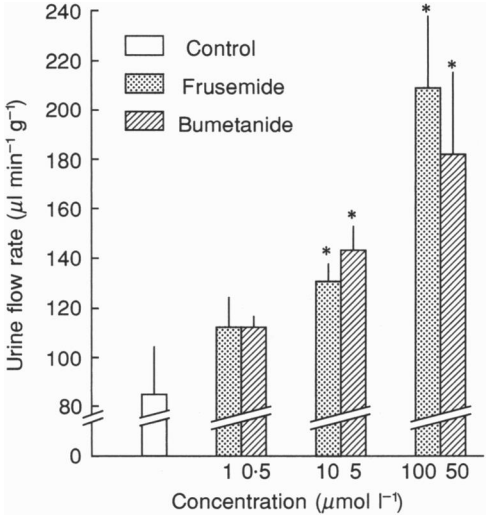

Fig. 2. Effects of frusemide and bumetanide on urine flow rates. The amount of urine produced by the kidneys was measured gravimetrically. The concentrations of both drugs were nominally adjusted in the arterial perfusate. Values are means ± S.E.M., $n = 5$. *, indicate significant difference from control ($P < 0.05$).

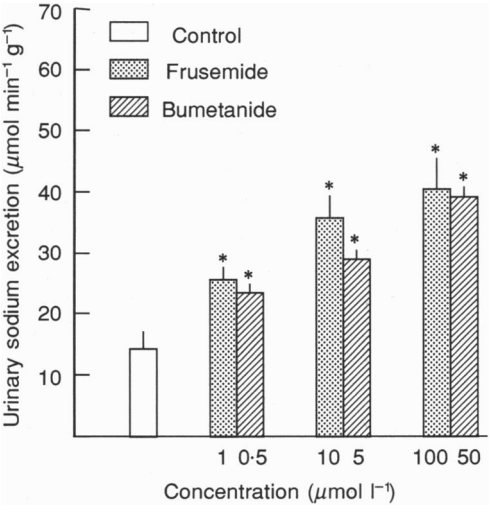

Fig. 3. Dose-dependent effects of frusemide and bumetanide on urinary sodium excretion. The concentrations indicated refer to the arterial perfusion medium. Values are means ± S.E.M., $n = 5$. *, indicate significant difference from control ($P < 0.05$).

Na^+–K^+–$2Cl^-$ cotransport inhibitor (Schlatter, Greger & Weidtke, 1983), also caused a dose-dependent increase of basal flow rate and a stimulation of renin release (Fig. 4). On a molar basis, bumetanide was around 2-fold more potent than frusemide in causing renal vascular relaxation and stimulation of renin secretion.

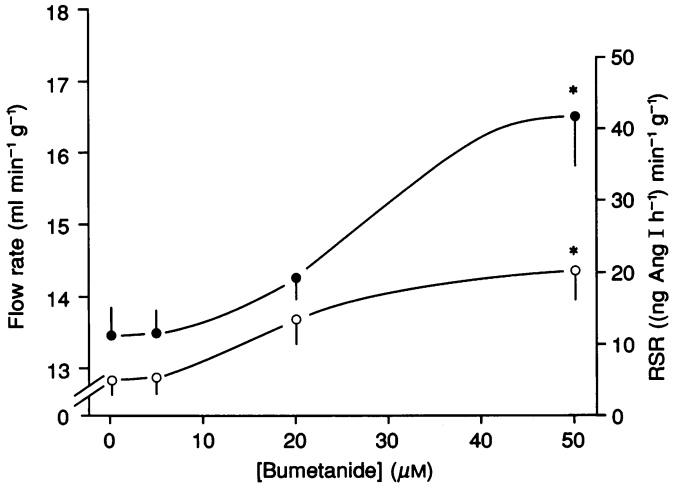

Fig. 4. Dose–response relationship for the effect of nominally added bumetanide on renal flow (●) and renin secretion rates (○) from isolated perfused rat kidneys. Values are means ± S.E.M., $n = 5$. *, indicate significant difference from control ($P < 0{\cdot}05$).

Like frusemide, bumetanide also increased urine flow rates and sodium excretion in a concentration-dependent fashion (Figs 2 and 3).

Since loop diuretics such as frusemide and bumetanide act on the luminal tubular membranes and since these diuretics are known to be not only filtered but also secreted (Friedman, 1988), it was of interest to know the concentration of the drugs in the tubular fluid facing the macula densa. As an approximate for this parameter we therefore measured the concentration of frusemide and bumetanide in the urine produced by the isolated kidneys. We found a positive correlation between the concentrations of both drugs in the perfusate and in the urine. Thus, administration of frusemide at perfusate concentrations of 100 and 10 μM resulted in urine concentrations of 47 ± 3 and 4 ± 2 μM frusemide, respectively ($n = 5$). When bumetanide was added to the perfusion medium at concentrations of 50 and 5 μM, the corresponding concentrations measured in the urine were 22 ± 2 and 2 ± 1 μM bumetanide, respectively ($n = 5$).

We next examined the effects of frusemide and bumetanide on pressure-related renin secretion from isolated perfused rat kidneys. As shown previously, stepwise reductions of the perfusion pressure by 20 mmHg steps from 100 to 40 mmHg in this preparation are associated with exponentially increasing renin secretion rates (Scholz & Kurtz, 1992). In this study a single step reduction of the perfusion pressure from 100 to 40 mmHg was therefore used as a standard protocol to stimulate pressure-related renin secretion. Lowering of the perfusion pressure from 100 to 40 mmHg yielded renin secretion rates of 92 ± 10 (ng Ang I h^{-1}) min^{-1} g^{-1} ($n = 15$, Fig. 5). The rise of renin release caused by a reduction of the renal artery pressure was of rapid onset and renin secretory rates started to increase within the first 2 min (Fig. 5). When the renal artery pressure was readjusted to 100 mmHg, renin secretion returned to control values within 4 min. Reducing the perfusion

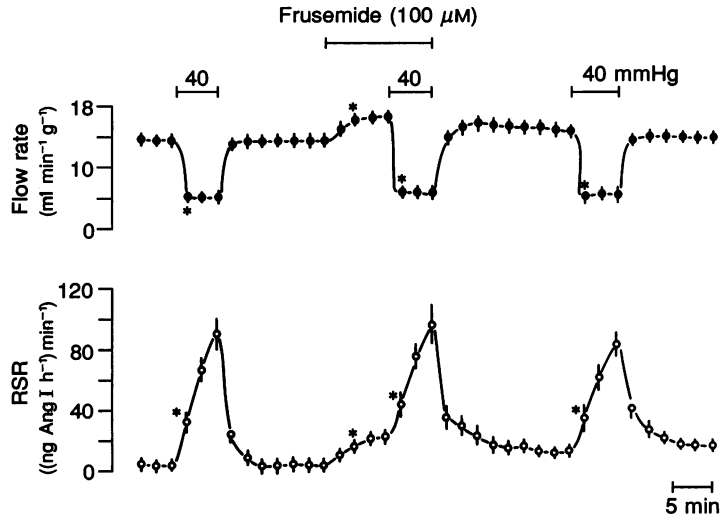

Fig. 5. Effect of frusemide on perfusate flow rate (upper trace) and renin secretion rate (lower trace) from isolated kidneys stimulated by a decrease of the renal artery pressure from 100 to 40 mmHg. Values are means ± S.E.M., $n = 6$. *, indicate significant difference from control ($P < 0.05$).

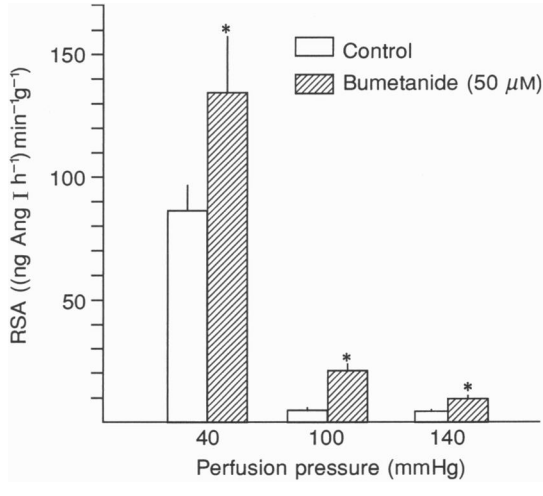

Fig. 6. Effect of bumetanide on pressure-related renin secretion from isolated kidneys. Values are means ± S.E.M., $n = 6$. *, indicate significant difference from control ($P < 0.05$).

pressure from 100 to 40 mmHg in the presence of frusemide (100 μM) and bumetanide (50 μM) increased renin secretion to maximal values of 97 ± 11 ($n = 6$) and 133 ± 24 (ng Ang I h^{-1}) min^{-1} g^{-1} ($n = 6$), respectively (Figs 5 and 6). Elevating the renal artery pressure from 100 to 140 mmHg did not significantly affect basal renin release under control conditions, but significantly reduced renin secretion rates in the presence of 50 μM bumetanide to 8·0 ± 1·5 (ng Ang I h^{-1}) min^{-1} g^{-1} ($n = 6$, Fig. 6).

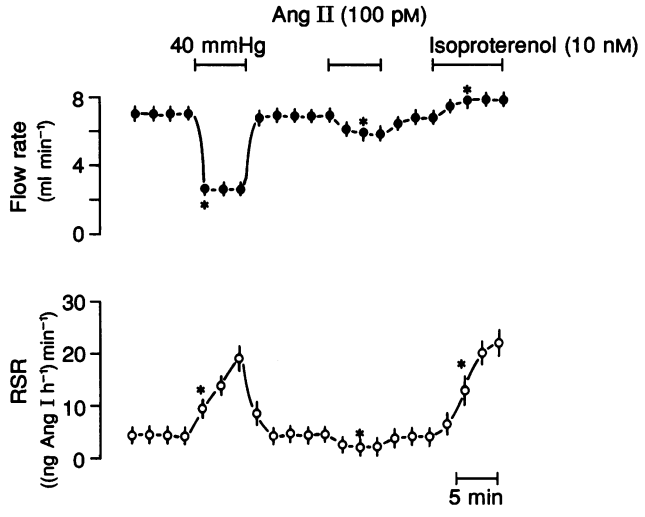

Fig. 7. Effect of decreasing the renal artery pressure from 100 to 40 mmHg on basal flow rate (upper trace) and renin release (lower trace) from isolated perfused hydronephrotic kidneys. For comparison the effects of angiotensin II (Ang II; 100 pM) and isoproterenol (10 nM) are also shown. Values are means ± S.E.M., $n = 5$. *, indicate significant difference from control ($P < 0{\cdot}05$).

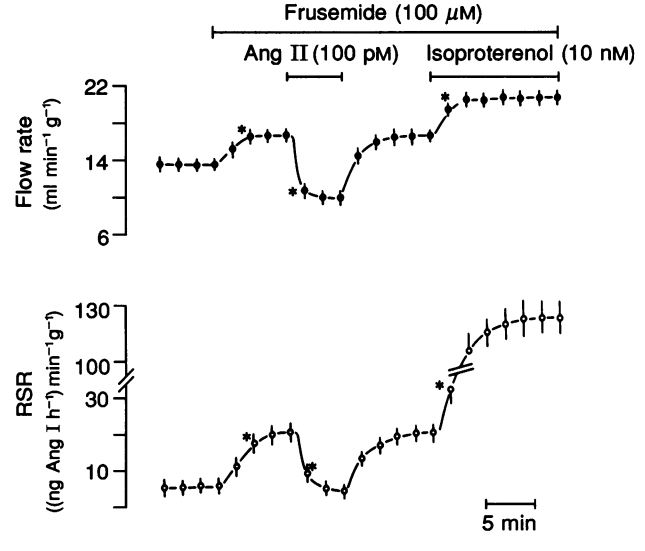

Fig. 8. Effects of angiotensin II (100 pM) and isoproterenol (10 nM) on flow rate (upper trace) and renin secretory rates (lower trace) in the presence of 100 μM frusemide. Values are means ± S.E.M., $n = 5$. *, indicate significant difference from control ($P < 0{\cdot}05$).

In order to find out to what extent pressure-related renin secretion was at all dependent on the existence of the macula densa, renin release from five isolated perfused hydronephrotic rat kidneys was examined. Hydronephrotic kidneys were prepared according to the method described by Steinhausen *et al.* (1983). Those kidneys are devoid of tubular structures including the macula densa. Basal flow

rates through these kidneys at 100 mmHg were $7{\cdot}0 \pm 0{\cdot}5$ ml min^{-1} ($n = 5$) and corresponding renin secretion rates were $4{\cdot}5 \pm 1{\cdot}0$ (ng Ang I h^{-1}) min^{-1} (Fig. 7). Lowering of the perfusion pressure from 100 to 40 mmHg caused a 5-fold increase of renin release (Fig. 7). A similar increase in renin secretion from this preparation was achieved with 10 nM isoproterenol, which stimulates renin release via cAMP formation (Fig. 7). Addition of angiotensin II (100 pM) to the perfusate led to a decrease of renin secretion rates to half of the controls (Fig. 7). For comparison in normal kidneys in which the macula densa was functionally blocked with frusemide, addition of isoproterenol (10 nM) resulted in renin secretion rates that were also comparable to those obtained by reducing the perfusion pressure to 40 mmHg (Fig. 8). Moreover, angiotensin II (100 pM) also had a similar inhibitory effect on renin secretion as in the hydronephrotic kidneys (Fig. 8).

DISCUSSION

Renin secretion from renal juxtaglomerular (JG) cells is under the inhibitory control of the renal artery pressure and the sodium chloride concentration in the tubular fluid (cf. Keeton & Campbell, 1981; Hackenthal *et al.* 1990). It is well documented that the macula densa cells of the early distal tubule modulate the secretory activity of JG cells in response to changes of the tubular NaCl concentration (Skott & Briggs, 1987; Lorenz, Weihprecht, Schnermann, Skott & Briggs, 1990). To this end, they are equipped with a luminal Na^+–K^+–$2Cl^-$ cotransport system that can be pharmacologically blocked with loop diuretics such as frusemide or bumetanide (Schlatter *et al.* 1989). Most probably, changes in the Cl^- transport rate via this cotransport system are the key signal for initiating inverse alterations in renin release (Lorenz *et al.* 1991).

In addition to the macula densa signal, the existence of a second, vascular mechanism that is directly sensitive to changes of the renal artery pressure has been hypothesized (Tobian, 1960; Skinner, McCubbin & Page, 1964). However, both the cellular localization of this putative vascular 'baroreceptor' as well as the signal transduction pathways for pressure-dependent regulation of renin secretion are rather unclear. In particular the interaction between the macula densa signal and the renal 'baroreceptor' in the control of renin release from JG cells is less understood.

In our study we have therefore systematically investigated the interrelationship between renal artery pressure and macula densa function in renin secretion. To this end we used the model of isolated rat kidneys perfused with a medium containing red cells, because under those conditions *in vitro* perfused kidneys have previously been found to release renin in a pressure-dependent fashion (Scholz & Kurtz, 1992). Several lines of evidence suggest that the macula densa control of renin secretion was also preserved in our preparations. Thus, frusemide and bumetanide, two different inhibitors of the macula densa Na^+–K^+–$2Cl^-$ transport (Schlatter *et al.* 1989), caused a dose-dependent increase of renal flow rates which was paralleled by a stimulation of renin release (Figs 1 and 4). Moreover, frusemide and bumetanide also increased urine flow rates and sodium excretion in a dose-dependent manner (Figs 2 and 3), suggesting that they effectively inhibited NaCl transport in the

thick ascending limb of Henle and presumably also at the macula densa. These effects are very similar to those obtained in studies on tubuloglomerular feedback and renin secretion performed with *in vitro* perfused isolated macula densas (cf. Briggs & Schnermann, 1986; Lorenz *et al.* 1991). Our findings are also in agreement with a previous study reporting that frusemide increased sodium excretion and stimulated renin release from isolated perfused rat kidneys 2- to 6-fold (Hofbauer, Zschiedrich, Hackenthal & Gross, 1974).

Since loop diuretics are actively secreted into the tubular lumen (Friedman, 1988) and their site of action is at the luminal membrane, their effective concentrations critically depend upon the activity of this transport process and may therefore differ in the tubular fluid and in the intravascular perfusate. For this reason, we also determined the amounts of frusemide and bumetanide in urine and we found that the concentrations of both drugs in the urine were approximately half of those administered in the perfusate. Assuming IC_{50} values for half-maximal inhibition of the cotransport system of 3 and 0·2 μM for frusemide and bumetanide, respectively (Schlatter *et al.* 1983), and assuming that the concentrations of the drugs in the tubular fluid at the macula densa site are close to the concentrations in the urine, then concentrations of 50 μM frusemide and 25 μM bumetanide should be sufficient to inhibit maximally macula densa Na^+–K^+–$2Cl^-$ transport activity. Such a concentration range for both diuretics is in keeping with the observation that 10 μM frusemide and 1 μM bumetanide already significantly increased afferent arteriolar diameter and stimulated renin release from the microperfused juxtaglomerular apparatus (Itoh & Carretero, 1990; Lorenz *et al.* 1991).

In addition to their inhibitory action on Na^+–K^+–$2Cl^-$ cotransport, loop diuretics may also decrease the activity of other cellular transport processes, including OH^-–Cl^- exchange (Aronson & Seifter, 1984), Na^+–K^+-ATPase (Kramer, 1976), and electroneutral KCl efflux (Larson & Spring, 1984). However, those effects were only seen at concentrations higher than 100 μM and it therefore appears less likely that frusemide and bumetanide stimulated renin release via inhibition of those transport pathways. Moreover, inhibition of the macula densa Na^+–K^+–$2Cl^-$ cotransporter obviously did not affect other characteristic signalling pathways in JG cells such as angiotensin II-induced inhibition and isoproterenol-produced stimulation of renin secretion (Fig. 8).

Lowering of the perfusion pressure from 100 to 40 mmHg in the presence of effective concentrations of frusemide and bumetanide led to a further increase of renin secretion (Figs 5 and 6), suggesting that additional mechanisms, which were not related to macula densa function, became activated by this manoeuvre. Recent studies reporting a pressure-modulated renin release from isolated perfused rabbit afferent arterioles devoid of macula densa raise the possibility that the cellular structures for renal 'baroreception' are located in this vascular segment (Bock, Hermle, Brunner & Thiel, 1992). One may therefore speculate whether the JG cells directly respond to changes of the afferent arteriolar wall stretch (Fray & Lush, 1984) or whether another cell type, for instance endothelial cells, is required that mediates pressure dependence of renin secretion. Evidence for a possible role of the local vascular endothelium in the control of renin release has recently been

provided by the demonstration that basal and stimulated renin secretion from isolated JG cells was significantly affected in the presence of co-cultured bovine endothelial cells (Kurtz, Kaissling, Busse & Baier, 1991).

A macula densa-independent component of pressure-related renin secretion is also strongly supported by the finding that the principles of pressure-controlled renin release were preserved in isolated perfused hydronephrotic rat kidneys being devoid of macula densa structures (Fig. 7). However, lowering the renal artery pressure in this preparation stimulated renin secretion to maximum values ranging only around 20 % of those obtained with normal control kidneys and with frusemide- or bumetanide-treated kidneys. We think that this discrepancy cannot be explained only by the lack of macula densa structures, because the effect of isoproterenol, which also directly stimulates renin release from JG cells by increasing cellular cyclic AMP levels (cf. Kurtz, 1986), was proportionally reduced in hydronephrotic compared to intact kidneys (Figs 7 and 8). A more general inhibition of renin secretion in hydronephrotic kidneys therefore becomes more likely and one may speculate whether this phenomenon is due to the relatively high vascular resistance of this preparation (Fray, 1976).

Interestingly, increasing the perfusion pressure to 140 mmHg in normal (non-hydronephrotic) kidneys significantly reduced the stimulatory effect of bumetanide on renin release from those preparations (Fig. 6). Thus, the inhibitory effect of a high renal artery pressure was predominant over stimulatory signals derived from the macula densa. In conclusion, it appears as if the role of the macula densa is not only to adjust renin secretion to the tubular sodium chloride concentration and to mediate the tubuloglomerular feedback but also to be part of the intrarenal 'baroreceptor' mechanism.

Taken together, our findings suggest that the principles of pressure-controlled renin release do not require macula densa signals. The macula densa function, however, appears to be part of the renal 'baroreceptor' mechanism that keeps renin secretion low at normal renal artery pressures (around 100 mmHg). Under those conditions probably 20 % of pressure-related inhibition of renin release is mediated by the macula densa mechanism.

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