

DIFFERENTIAL RESPONSE OF RENIN SECRETION TO VASOCONSTRICTORS IN THE ISOLATED PERFUSED RAT KIDNEY

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(Received 26 November 1990)

SUMMARY

1. We have examined whether an increase of renal vascular resistance is generally accompanied by an inhibition of renin secretion. The effects of vasoconstriction produced by angiotensin II (Ang II), arginine-vasopressin (AVP), and potassium (KCl) depolarization on vascular resistance and on renin release from isolated rat kidneys perfused at constant pressure of 100 mmHg were investigated.

2. Histological examination performed on some representative kidneys revealed that the tubular lumina of all segments within the cortex were patent and the brush borders of the proximal tubules were well preserved. The renal vasculature and the juxtaglomerular region appeared to be morphologically intact. By immunocytochemistry, renin-positive cells were found exclusively in the wall of the afferent arterioles.

3. Basal flow rate through isolated kidneys was 14.5 ± 2.0 ml min⁻¹ (g kidney weight (gkw))⁻¹ (mean \pm s.e.m., $n = 10$). Under control conditions renin secretory rates were in the range of 30–40 (ng Ang I h⁻¹) min⁻¹ gkw⁻¹.

4. Ang II (100 pM) caused a decrease of renal flow rate to $42 \pm 2\%$ of control which was accompanied by a reduction of renin secretion rates by a factor of 4.

5. AVP (10 pM to 1 nM) reduced renal perfusate flow in a dose-dependent fashion to a minimum of $25 \pm 3\%$ of control. The vasoconstrictor effect of AVP was paralleled by a concentration-dependent increase of renin secretory rates reaching a factor of maximally 5 when AVP was used at a concentration of 1 nM. The stimulatory effect of AVP on renin release could be mimicked by [deamino-Cys¹, D-Arg⁸]-vasopressin (dDAVP), a vasopressin analogue with prevalent V₂ receptor agonistic properties. In the presence of dDAVP (100 nM, 1 μ M) renal flow rate reversibly increased by 8 and 12% of control values, respectively.

6. Depolarizing concentrations of KCl (30 mM) decreased perfusate flow to $20 \pm 4\%$ of control. The vasoconstrictor effect of KCl was paralleled by an increase of the arterio-venous difference of perfusate renin activity to such an extent that the rate of renin release remained unaltered.

7. Our findings suggest that there exists no general inverse relationship between renal arteriolar resistance and renin secretion. Our study, moreover, does not support

a functional role of potential operated calcium channels in the control of renin secretion. Finally, we conclude that V_2 receptors are present on juxtaglomerular epithelioid cell membranes and mediate the stimulatory effect of AVP on renin release from isolated rat kidneys.

INTRODUCTION

Renin secretion from renal juxtaglomerular epithelioid (JGE) cells is believed to be under the inhibitory control of calcium (Churchill, 1988; for review see Kurtz, 1989). This phenomenon, termed the calcium paradox of renin secretion, might seem surprising at first sight because calcium normally initiates, facilitates or maintains secretory activity in a variety of glands (Rasmussen, 1986*a, b*). From a teleological point of view, however, the inhibitory effect of intracellular calcium on renin secretion appears meaningful: by generating angiotensin II (Ang II), renin indirectly induces calcium mobilization in vascular smooth muscle cells and thereby causes vasoconstriction (Churchill & Churchill, 1982; Rasmussen, 1986*a, b*; Karaki & Weiss, 1988). If renin secretion from JGE cells, which are metaplastically transformed smooth muscle cells (Barajas, 1979; Taugner, Bührle, Hackenthal, Mannek & Nobling, 1984), was stimulated by a rise of cytosolic free calcium, the enhanced formation of Ang II would lead via a positive feedback loop to unrestricted vasoconstriction. An inhibitory effect of intracellular calcium on renin release would, however, represent a very direct negative feedback control of renin secretion and of vascular tone.

Under the assumption that both cell types, arteriolar vascular smooth muscle cells and JGE cells, are equipped with the same membrane signal transduction systems (Fray, Lush & Valentine, 1983; Hackenthal & Taugner, 1986) one could speculate therefore that the rate of renin secretion and renal arteriolar resistance are generally related in an inverse fashion. In fact, a number of observations exist that would support such a relationship. In addition to Ang II, both the macula densa signal and an increase of perfusion pressure cause renal arteriolar constriction and suppression of renin secretion (cf. Kurtz, 1989). Conversely, a fall of perfusion pressure, hormones activating adenylate cyclase, and calcium antagonists lead to renal arteriolar dilatation and enhancement of renin secretion (cf. Kurtz, 1989).

Such a unifying concept linking the state of contraction of renal vascular smooth muscle cells and renin secretion has not been systematically tested. It seemed reasonable therefore to examine the relationship between renal arteriolar resistance, reflecting state of contraction of vascular smooth muscle cells and renin secretion in a preparation that allows both parameters to be monitored simultaneously. To this end we have used the model of the isolated rat kidney perfused at constant pressure (Schurek & Alt, 1981; cf. Scholz & Kurtz, 1990). To improve the functional conservation of the preparation we have chosen a recirculatory system with a perfusate containing red cells.

With this model we have examined the effects of three different vasoconstrictors, namely Ang II, AVP, and potassium depolarization on renal vascular resistance and renin secretion. Our results indicate that at the whole kidney level there exists no general inverse relationship between vascular tone and renin secretion. The

experimental variables chosen to produce renal vasoconstriction can inhibit, have no effect, or stimulate renin secretion.

METHODS

Male SIV strain rats (250–350 g body weight) having free access to commercial pellet chow and tap water were used throughout. Kidney perfusion was performed according to the technique developed by Schurek & Alt (1981). In brief: at the day of experiment the animals were anaesthetized by intraperitoneal injection of 150 mg (kg body weight (Kgbw))⁻¹ of 5-ethyl-1'-methyl-propyl-2-thio-barbituric acid (Inactin[®]; Byk Gulden, Konstanz, Germany). The trachea was cannulated with a polyethylene tube for artificial respiration with 25% O₂ (Harvard respirator 75 strokes per minute, stroke volume 2 ml). Substitution for volume loss was performed by intermittent injections of small volumes of physiological saline (about 0.5 ml) via a jugular vein catheter. Following opening of the abdominal cavity the right kidney was exposed and placed in a thermostatically controlled metal chamber which was fixed to a micropuncture table. After intravenous heparin injection (2 U (g body weight (gbw))⁻¹; Liquemin[®], Roche, Basel, Switzerland) and ligation of the large arteries branching off the abdominal aorta, a double-barrelled cannula was inserted into the aorta and placed at the origin of the right renal artery. To avoid ischemia, perfusion was started *in situ* with an initial flow rate of 8 ml min⁻¹. Thereafter, the kidney was isolated from the animal and perfused at constant pressure of 100 mmHg by means of a feedback regulated peristaltic pump. Perfusion pressure was monitored through the inner part of the perfusion cannula (Statham Transducer P 10 EZ). The basic perfusion medium, which was taken from a thermostated (37 °C) reservoir (200–250 ml), consisted of a Krebs–Henseleit solution containing (in mM) all physiologic amino acids in concentrations between 0.2 and 2.0, and 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 2 g (100 ml)⁻¹ bovine serum albumin. Ampicillin (3 mg (100 ml)⁻¹) and flucloxacilline (3 mg (100 ml)⁻¹) were used as antibiotics. Freshly washed human red blood cells were added to the perfusion medium to a hematocrit of 10 \pm 2%. Kidney perfusion was performed in a recycling perfusion system by draining the renal venous effluent via a metal cannula back into the reservoir. To improve the perfusion conditions the perfusate was continuously dialysed against a 25-fold volume of the same composition but without erythrocytes and albumin. Oxygenation of the dialysate was performed with a gas mixture containing 95% oxygen and 5% carbon dioxide. Perfusate flow rate was 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 drawn perfusate and infused into the arterial limb of the perfusion circuit directly before the kidney (peristaltic pump 2132 Microperpex[®], LKB, Bomma, Sweden) at 1% of the rate of perfusate flow. For determination of perfusate renin activity (pRA) aliquots (about 100 μ l) were drawn from the arterial limb of the circulation and the renal venous effluent, respectively. The samples were cooled on ice and stored at -20 °C until assayed for renin activity.

Determination of renin activity

Aliquots of perfusate were incubated for 1.5 h at 37 °C with plasma of bilaterally nephrectomized male rats as renin substrate (Rightsel, Okamura, Inagami, Pitcock, Taki, Brooks, Brown & Muirhead, 1982). The generated angiotensin I was determined by radioimmunoassay (Medipro AG, Teufen, Switzerland).

Renin release

Renin secretion from isolated perfused kidneys was calculated from differences between the arterio-venous perfusate renin activities (AVD_{pRA}) and the perfusion flow rate through the kidneys.

Histology and immunocytochemistry

Fixation of the renal tissue. At the end of the experiments (after about 3.0 h) several kidneys were perfused for 5 min with 2.5% paraformaldehyde, 0.1% glutaraldehyde, 3 mM-MgCl₂, and 0.5%

picric acid, dissolved in 0.1 M-cacodylate buffer, pH 7.4, adjusted to 300 mosmol with sucrose. Thereafter, the kidneys were flushed for 10 min by perfusion with iso-osmotic cacodylate buffer.

Histology and immunocytochemistry. One part of the kidneys was cut into small tissue blocks, which were dehydrated in graded series of alcohols and embedded in LR-White resin (London Resin, Basingstoke, UK) according to routine procedures. Semithin sections (1 μM) were made with an ultramicrotome (Ultracut E, Reichert, Jung), stained with AzurII/Methylene Blue and studied by light microscopy (Polyvar, Reichert-Jung). The other part of the kidneys was used for immunocytochemistry. To this end, tissue slices (about 1 mm thick) across the kidneys were frozen in liquefied propane, cooled by liquid nitrogen and 5 μm sections were cut with a cryostat at temperatures of about -20°C . Small tissue blocks (side length about 1 mm) were immersed for at least 1 h in 2.3 M-sucrose, mounted in 2.3 M-sucrose on specimen holders and frozen as described above. From these blocks 1 μm thin sections were prepared at temperatures of about -90°C with an ultramicrotome, equipped with a cryo-chamber (Ultracut E, FC 4D, Reichert-Jung, Vienna, Austria). All sections were transferred to chrome-alum gelatin coated slides, which were then placed for at least 1 h in 0.05 M- NH_4Cl in order to quench residual aldehyde function of the fixative. Subsequently, the sections were incubated in normal swine serum, diluted 1:50 in phosphate buffered saline (PBS) for 30 min, then in rabbit anti-rat renin serum (Takii, Figueiredo & Inagami, 1985), diluted 1:1000 in PBS for 1 h. After washing in PBS the binding sites of the anti-renin serum were visualized by incubation in a swine anti-rabbit serum, conjugated with fluorescein-isothiocyanate (FITC; Dakopatts, Denmark). All incubations were made at room temperature. Following a wash in PBS the sections were counterstained with a 0.1% solution of Evans Blue (Merck, Darmstadt, FRG) and mounted in glycerine gelatin (Glycergel DAKO, Dakopatts, Denmark), containing 2.5 g (100 ml)⁻¹ of 1,4-diazabicyclo[2,2,2]octane (Sigma International) as fading retardant. The sections were studied by epifluorescence (Polyvar, Reichert-Jung). Photographs were taken on Kodak Technical Pan (light microscopy) and Kodak T-Max 400 ASA (fluorescence).

Agents

Pyruvate was from Boehringer, Mannheim, Germany. Ampicillin and flucloxacillin were from Beecham, Bern, Switzerland. Glutamate, urea, and α -ketoglutarate were obtained from Merck, Darmstadt, Germany. L-Malic acid and L-lactate as sodium salts were provided by Serva, Heidelberg, Germany. L-Amino acids were from Braun/Melsungen, Germany (Aminoplasmal paediatric free of carbon hydrate). Bovine serum albumin, angiotensin II, arginine-vasopressin, and [deamino-Cys¹, D)Arg⁸]-vasopressin were purchased from Sigma International. D-Sucrose was from Fluka, Buchs, Switzerland. The RIA kit for determination of Ang I was obtained from Medipro AG, Teufen, Switzerland.

Statistics

Levels of significance were calculated by using Student's *t* test. $P < 0.05$ was considered significant.

RESULTS

The kidneys were usually perfused *in vitro* for 3 h. Since, to our knowledge, the isolated rat kidney (IPRK) perfused with a medium containing red cells has not yet been used as an experimental model to study renin secretion, we have characterized this preparation also by histological examination. To perceive possible morphological alterations, in particular, of the vasculature and the juxtaglomerular regions, some kidneys were fixed after 3 h of perfusion. They were processed for histological examination and for renin immunohistochemistry.

General appearance. The tubular lumina of all segments within the cortex (Fig. 1A) and the medulla were patent. Occasionally, some cell debris and shedded necrotic cells were present in the lumina. Epithelial cells were not swollen. The basal infoldings in most segments were readily discernible, due to a regular, slight widening

of the intercellular spaces. This might indicate a small degree of cellular shrinkage. The interstitial spaces in all zones contained largely open capillaries.

Renal corpuscles. The glomerular tuft appeared to be unaltered by the *in vitro* conditions. The capillaries were patent. Occasionally, some cell debris was present in their lumina but did not obstruct them. The structure of podocytes and Bowman's capsule revealed the same aspect as in the kidneys fixed by vascular perfusion *in vivo* (Fig. 1A).

Tubules. The brush border was well preserved in all three segments of the proximal tubule (Fig. 1A). In S1 segments vacuoles of rather uniform size, belonging to the vacuolar apparatus, were well evident at the base of the brush border. The cytoplasm of S2 segments appeared to be rather dense. In the apical cytoplasm of S2 segments great accumulations of endocytotic vesicles were present, protruding the apical cell pole into the lumen (Fig. 1A). These vesicles most probably indicate uptake of proteins from the tubular fluid. All segments situated in the medullary zones (not shown) appeared to be well preserved. In particular, no cellular swelling was apparent. The same applies to the distal segments in the cortex. The apical cytoplasm of distal convoluted cells showed some small cytoplasmic protrusions. Occasionally, some cells in the connecting tubule and collecting duct were vacuolated and shed into the lumen.

Vasculature. Endothelial cells in peritubular capillaries (Fig. 1A), in arteries and arterioles appeared to be intact (Fig. 1C). The smooth muscle cells of the arterial walls were discernible and revealed no vacuoles.

Juxtaglomerular apparatus (JGA). The intercellular spaces between the macula densa cells were dilated, as usually found in rat kidneys, in which the tubuloglomerular feedback had not been inhibited by, for instance, furosemide. In proximity of the vascular pole and among the smooth muscle cells along the afferent arteriole, granular cells were discernible (Fig. 1C). By immunocytochemistry, using a polyclonal antibody against rat kidney renin (Takii *et al.* 1985), the presence of renin within these cells is evident by the bright fluorescence. Other structures of the kidney than the granular cells did not bind to this antibody (Fig. 1B).

The time courses of perfusion flow rate, arterial and venous perfusate renin activity (pRA) and renin release from IPRKs run under control conditions are shown in Fig. 2. Perfusate flow through isolated kidneys was 14.5 ± 2.0 ml min⁻¹ gkw⁻¹ (mean \pm s.e.m., $n = 10$) under control conditions. Renal flow rate remained constant during the first 2 h of perfusion. From then on, renal perfusate flow continuously decreased reaching a value of 12.5 ± 2.5 ml min⁻¹ gkw⁻¹ at 3 h after the onset of perfusion. Arterial perfusate renin activity (A_{pRA}) as measured in aliquots taken from the arterial limb of the perfusion circuit, steadily increased from 3 ± 1 to 18 ± 3 ng Ang I h⁻¹ ml⁻¹ gkw⁻¹ ($n = 6$) during the 3 h perfusion periods (Fig. 2). The increase of A_{pRA} was accompanied by a parallel rise of the renin activity determined in the renal venous effluent (V_{pRA}) which increased from 5 ± 1 at the beginning to 25 ± 3 ng Ang I h⁻¹ ml⁻¹ gkw⁻¹ at the end of perfusion ($n = 6$). Consequently, the arterio-venous difference of perfusate renin activity (AVD_{pRA}) remained almost constant during the course of the experiments (Fig. 2). Only towards the end of the perfusion periods was an increase of AVD_{pRA} usually observed. The time courses of renin secretion rates (RSR) which were calculated from the AVD_{pRA} and the renal

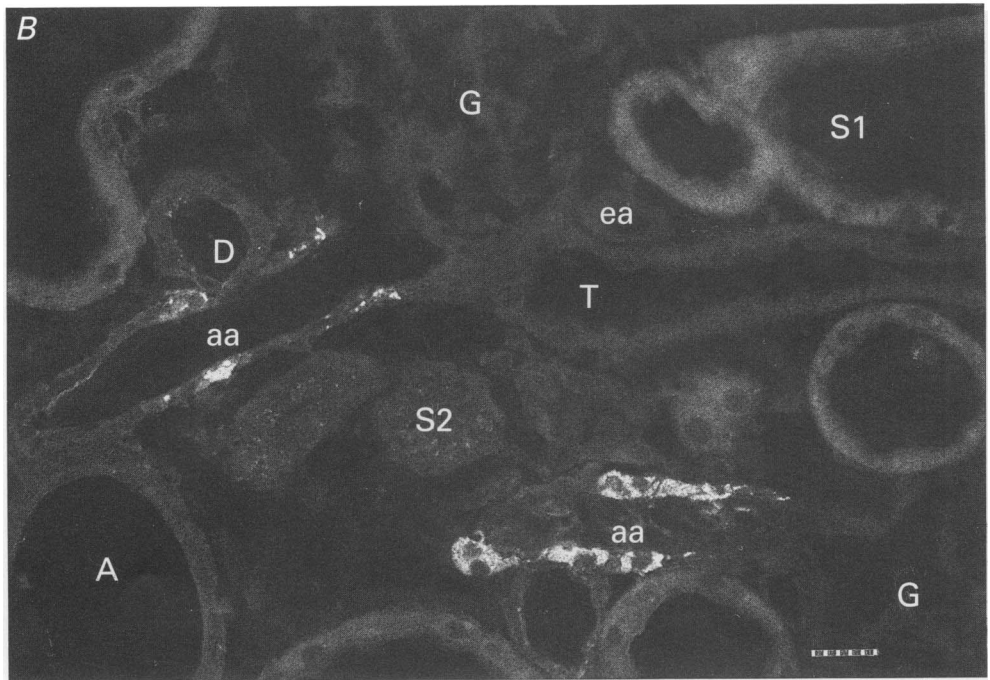
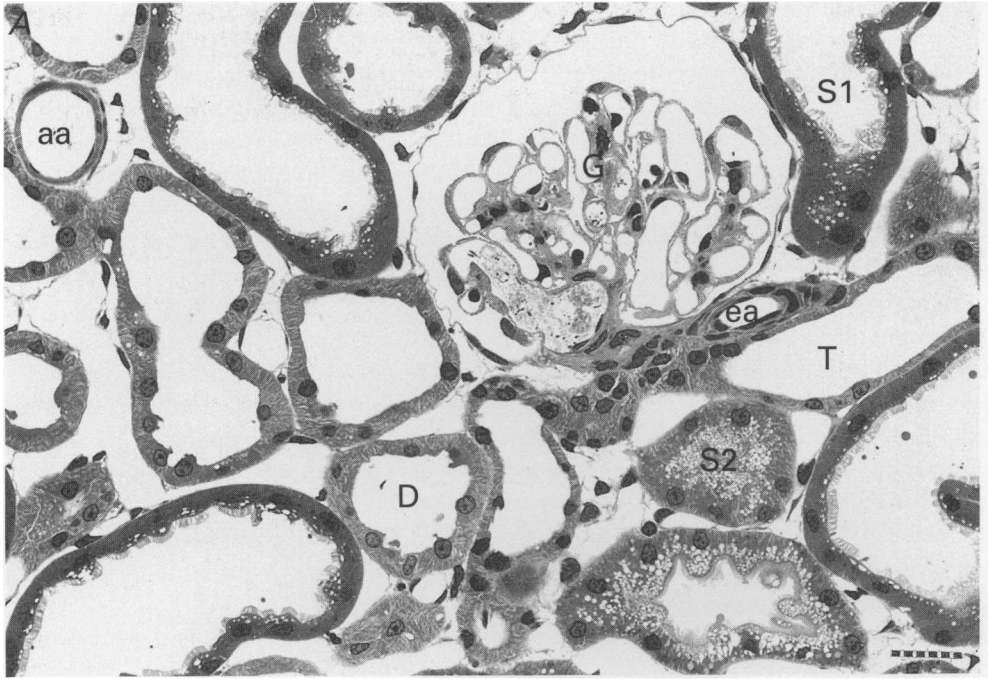


Fig. 1. For legende see facing page.

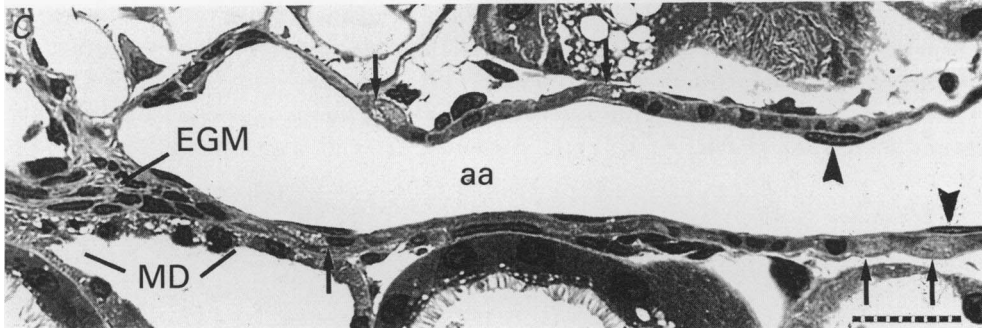


Fig. 1. *A*, semithin section of LR-White embedded renal cortex from a kidney perfused *in vitro* for 3 h. The tubular lumina as well as the peritubular capillaries are open. The brush border in S1 and S2 segments of the proximal tubule is rather well preserved. Note the accumulation of vesicles in the apical pole of S2 cells. Some cell debris is visible in a glomerular capillary. In some cells of the distal tubule (D) small cytoplasmic protrusions are present. G, glomerulus; ea, efferent arteriole; aa, afferent arteriole; T, thick ascending limb of Henle's loop. Scale bar, 20 μm . *B*, cryostat section; FITC-immunofluorescence, revealing binding sites in the tissue to antibodies against renin. Only granular cells in the afferent arterioles (aa) display a bright fluorescence. The granules in the cells are readily visible. A, cortical radial artery; D, distal tubule; G, glomerulus; ea, efferent arteriole; S1 and S2, segments of the proximal tubule; T, thick ascending limb of Henle's loop. Scale bar, 20 μm . *C*, semithin section of LR-White embedded tissue; juxtaglomerular apparatus. The cell plaque of the macula densa (MD) is obvious within the TAL by the dilated intercellular spaces. The endothelial cells (arrow heads) in the afferent arteriole (aa) are flat; granular cells (arrows) among the smooth muscle cells are evident by their granular content; EGM, extraglomerular mesangium. Scale bar, 20 μm .

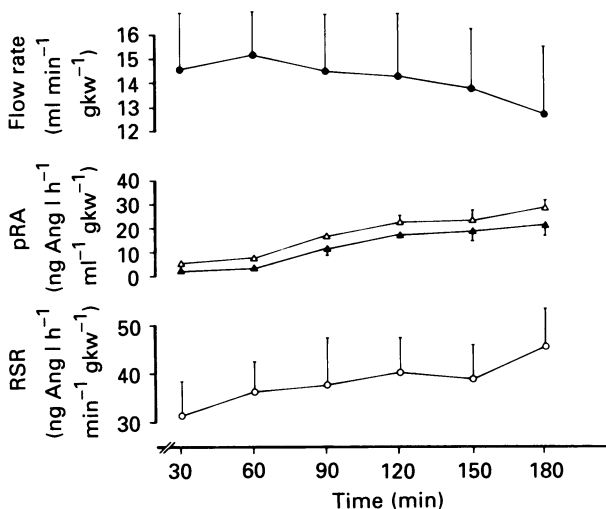


Fig. 2. Time courses of perfusate flow rates, arterial (▲) and venous (△) perfusate renin activities (pRA), and renin secretion rates (RSR) of isolated rat kidneys perfused for 3 h under control conditions. As can be seen, basal flow rates were in the range of 14.5 ml min⁻¹ gkw⁻¹ during the first 2 h and decreased to values of 12.5 ml min⁻¹ gkw⁻¹ towards the end of perfusion. Arterio-venous differences of pRA also remained nearly constant for a 2.5 h perfusion period. Renin secretion rates displayed a slow and almost linear increase over the courses of the experiments. Values are means \pm s.e.m. ($n = 10$).

perfusate flow rates displayed a slight and almost linear increase ranging from 31 ± 7 at 30 min to 45 ± 8 ($\text{ng Ang I h}^{-1} \text{ ml}^{-1} \text{ gkw}^{-1}$) at 180 min after perfusion start (Fig. 2). Since the arterio-venous difference of pRA is the sum of intrarenal renin inactivation and intrarenal renin release, we have examined a possible inactivation of renin within the IPRK. To this end systemic (arterial) and effluent (venous) pRA

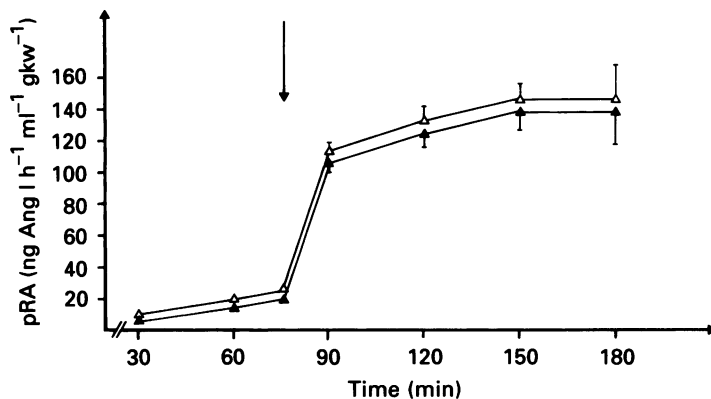


Fig. 3. Effect of the addition of exogenous rat renin to the perfusion medium on arterial (▲) and venous (△) perfusate renin activity (pRA). A bolus of exogenous rat renin given at about 80 min after the onset of perfusion (arrow) increased arterial and venous pRA to the same extent thus leaving the arterio-venous difference of pRA unchanged ($n = 5$). This suggests that under our experimental conditions no significant inactivation of renin occurred during the passage through the isolated perfused rat kidney. Values are means \pm s.e.m. ($n = 5$).

were monitored before and after the addition of exogenous rat renin to the perfusate. A pool of perfusate with high renin activity was produced by pre-incubating 1 ml of perfusate with isolated rat JGE cells (Kurtz, Pfeilschifter, Hutter, Bührle, Nobiling Taugner, Hackenthal & Bauer, 1986). As can be seen from Fig. 3, venous pRA rose to the same extent after the addition of renin as arterial pRA did. This suggests that under our experimental conditions no significant inactivation of renin occurred during the passage through the isolated kidney.

We then examined the effects of angiotensin II (Ang II), arginine-vasopressin (AVP), and of high extracellular potassium (KCl; 29 ± 2 mM) on perfusate flow rates, arterio-venous differences of pRA and on the resulting renin secretion rates. Ang II (100 μM) decreased renal perfusate flow rates to $42 \pm 2\%$ of the control values (mean \pm s.e.m., $n = 5$; Fig. 4). The delay of the vasoconstrictor effect of Ang II was less than 15 s. After removal of Ang II flow rates returned to control values within 6 min. Ang II-induced renal vasoconstriction was accompanied by a significant reduction of the arterio-venous differences of pRA (Fig. 4). In presence of Ang II AVD_{pRA} was decreased to 1.4 ± 0.3 as compared to 2.2 ± 0.3 $\text{ng Ang I h}^{-1} \text{ ml}^{-1} \text{ gkw}^{-1}$ during the control periods (Fig. 4). In consequence, the fall of flow rates caused by Ang II was paralleled by a reduction of renin secretory rates by a factor of 4 (Fig. 4).

AVP (1 nM) also reversibly decreased renal perfusate flow to $25 \pm 3\%$ of control ($n = 5$; Fig. 5). After removal of AVP perfusate flow reached control values with a delay of approximately 13 min. The vasoconstrictor effect of AVP, moreover, was dependent upon the concentration used. AVP at a concentration of 10 pM decreased renal flow rate to $95 \pm 0.5\%$ and in presence of 100 pM-AVP perfusate flow was

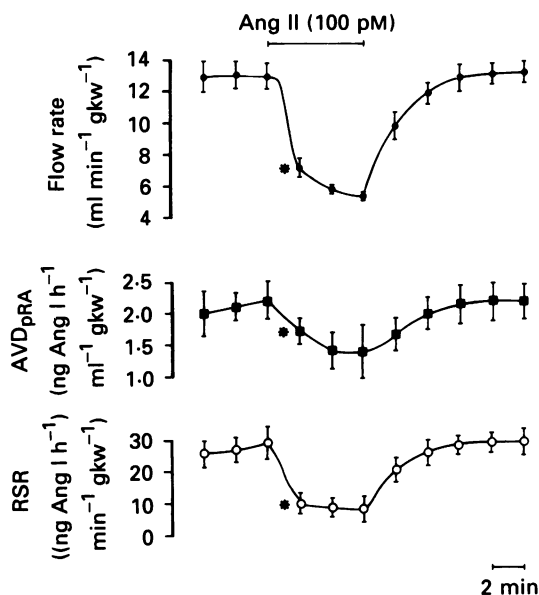


Fig. 4. Effect of angiotensin II (Ang II) on perfusate flow rates, arterio-venous differences of perfusate renin activity (AVD_{pRA}) and renin secretory rates (RSR) of isolated rat kidneys. Values are means \pm s.e.m., $n = 5$. * Significantly different from control. Student's t test ($P < 0.05$).

reduced to $80 \pm 1\%$ of control values ($n = 3$; Fig. 6). AVP (1 nM)-induced increase of renal vascular tone was accompanied by an approximately 15-fold increase of the AVD_{pRA} (Fig. 5). As a result renin secretion from isolated kidneys increased from 20 ± 5 (ng Ang I h⁻¹) min⁻¹ gkw⁻¹ during control periods to maximally 100 ± 5 (ng Ang I h⁻¹) min⁻¹ gkw⁻¹ during AVP (1 nM; Fig. 5). As illustrated in Fig. 6, the stimulatory effect of AVP on renin release was concentration dependent. When used at 10 pM, AVP caused an approximately 50% increase of renin secretory rates; at a concentration of 100 pM, AVP stimulated renin release from isolated rat kidneys by a factor of nearly 2.5. Renin secretion rates returned to control values in parallel with the recovery of renal vascular resistance. [deamino-Cys¹,D-Arg⁸]-Vasopressin (dDAVP), a vasopressin analogue with prevalent V₂ receptor agonistic properties (Weingartner, Gold, Ballenger, Smallberg, Summers, Rubinow, Post & Goodwin, 1981), increased renal flow rate by $8 \pm 0.5\%$ and $12 \pm 1\%$ of control, when used at concentrations of 100 nM and 1 μ M, respectively ($n = 3$; Fig. 7). In addition, dDAVP stimulated renin secretion from isolated rat kidneys in a dose-dependent fashion. When used at 100 nM, dDAVP caused an approximately 2.5-fold rise of renin

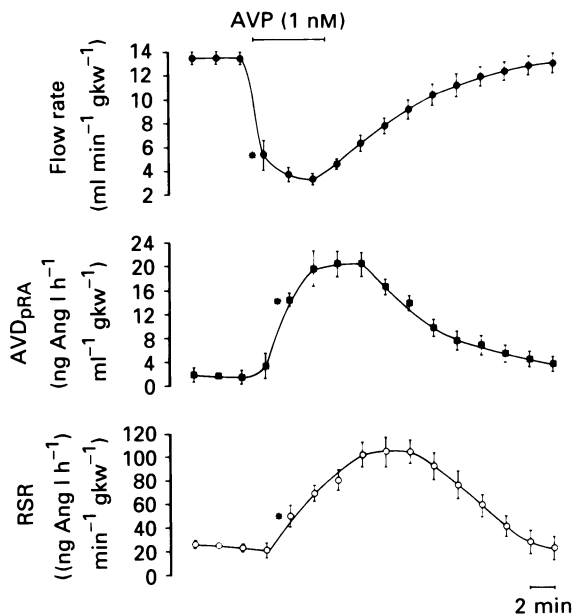


Fig. 5. Effect of arginine vasopressin (AVP) on renal flow rates and renin secretion from isolated perfused rat kidneys. AVP (1 nM) decreased perfusate flow rates to $25 \pm 3\%$ of control ($n = 5$). At the same time, a 15-fold increase of the arterio-venous difference of perfusate renin activity (AVD_{pRA}) was observed. As a result, AVP at a concentration of 1 nM stimulated renin release (RSR) from isolated kidneys by a factor of about 5. Values are means \pm s.e.m., $n = 5$. *Significantly different from control. Student's t test ($P < 0.05$).

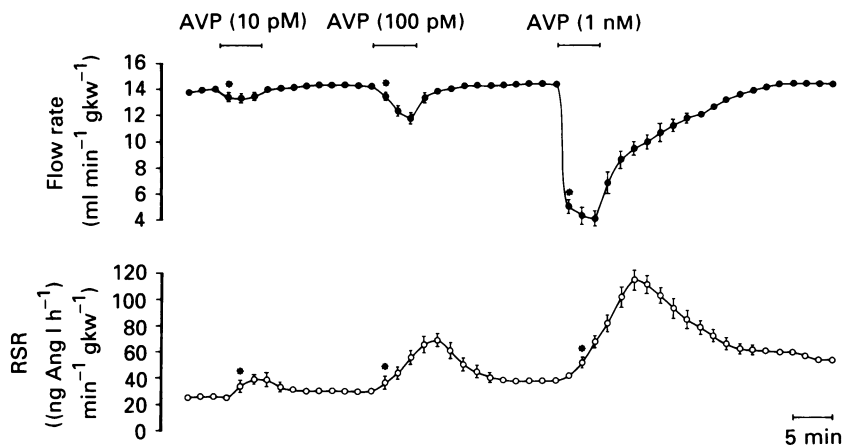


Fig. 6. Dose-response traces of arginine vasopressin (AVP). AVP used at concentrations of 10 pM, 100 pM, and 1 nM decreased renal flow rates to 95 ± 0.5 , 80 ± 1 and $25 \pm 3\%$ of control, respectively. AVP-induced renal vasoconstriction was paralleled by a concentration-dependent rise of renin secretory rates (RSR). Values are means \pm s.e.m., $n = 3$. *Significantly different from control. Student's t test. ($P < 0.05$).

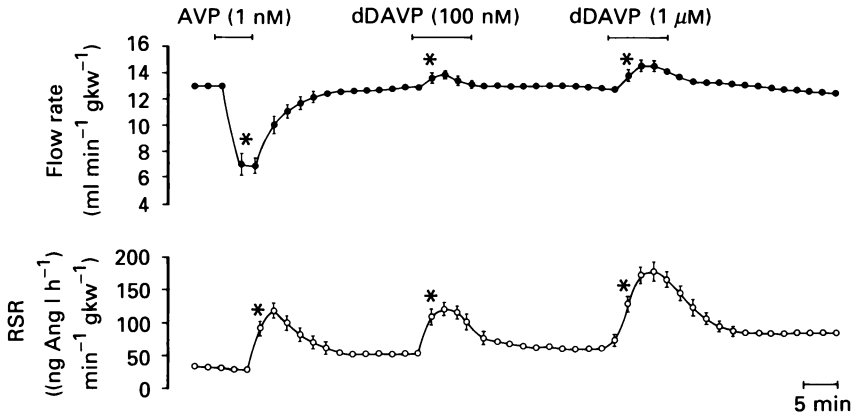


Fig. 7. Effect of [deamino-Cys¹,D-Arg⁸]-vasopressin (dDAVP), a vasopressin analogue with prevalent V₂ receptor agonistic properties, on renal flow rate and renin secretion. dDAVP used at concentrations of 100 nM and 1 μM increased renal flow rate by 8 ± 0.5 and 12 ± 1%, respectively. Renal vasodilatation caused by dDAVP was associated with a dose-dependent stimulation of renin release (RSR) from isolated rat kidneys. AVP (1 nM) was taken as control. Values are means ± s.e.m., *n* = 3. *Significantly different from control. Student's *t* test (*P* < 0.05).

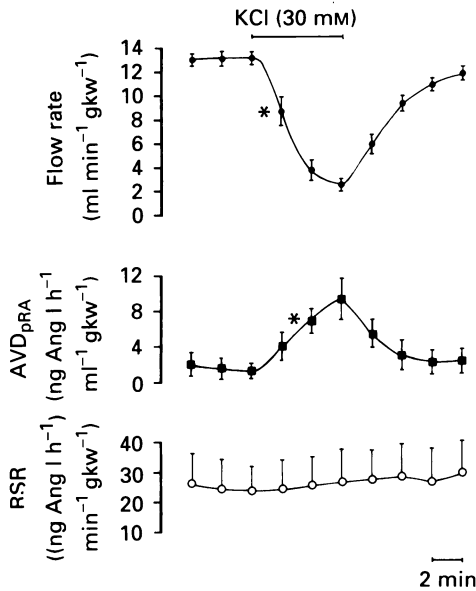


Fig. 8. Effect of the addition of depolarizing concentrations of potassium chloride (KCl) to the perfusate. KCl led to a fall of flow rate to 20 ± 4% of control (*n* = 5). KCl-induced increase of renal vasotone was accompanied by a uniform rise of the arterio-venous difference of perfusate renin activity (AVD_{pRA}). In consequence, renin secretion rates (RSR) remained unchanged in presence of depolarizing concentrations of KCl. Values are means ± s.e.m., *n* = 5. *Significantly different from control. Student's *t* test (*P* < 0.05).

secretory rates; at a concentration of $1 \mu\text{M}$, dDAVP stimulated renin release by a factor of about 3 (Fig. 7).

Depolarizing concentrations of KCl (30 mM, final concentration) led to a reduction of perfusate flow rates to $20 \pm 4\%$ of the control values ($n = 5$; Fig. 8). The delay of the vasoconstrictor effect of KCl was usually less than 15 s. Arterio-venous differences of pRA increased in the presence of KCl (30 mM) from $2.5 \pm 1.0 \text{ ng Ang I h}^{-1} \text{ ml}^{-1} \text{ gkw}^{-1}$ during control periods to maximally $9.5 \pm 2.5 \text{ ng Ang I h}^{-1} \text{ ml}^{-1} \text{ gkw}^{-1}$ (Fig. 8). This increase was inversely correlated to the drop of flow rate induced by KCl. As a consequence renin secretion rates did not significantly change in the presence of depolarizing concentrations of potassium (Fig. 8). Since osmolality of the perfusate was increased during administration of KCl appropriate controls were made by the addition of 25 mM-NaCl or 50 mM-sucrose to the perfusate. Addition of both, NaCl or sucrose, to the perfusion medium did not alter renin secretion rates (not shown).

DISCUSSION

Our study suggests that the isolated rat kidney perfused at constant pressure in a recirculatory system containing erythrocytes is a suitable model to study the regulation of renal vascular resistance and of renin secretion in parallel. The morphology of the renal vasculature and of the juxtaglomerular region is well preserved in this preparation at least during 4 h of perfusion (Fig. 1). We think that the low basal secretion rates of renin also suggest a preserved function of the juxtaglomerular region. For comparison, basal renin secretion rates from IPRK in single pass experiments were reported to be in the range of 500–1000 ($\text{ng Ang I h}^{-1} \text{ min}^{-1}$) (Konrad, Hofbauer, Werner & Gross, 1978; Nobiling, Münter, Bührle & Hackenthal, 1990). These values are higher by a factor of about 10–30 than those found in this study.

Since there is no appreciable degradation of renin activity during passage through the IPRK (Fig. 3), renin secretion rates can be calculated directly from the values of the arterio-venous differences of pRA and the renal flow rates.

Our findings that Ang II, AVP, and potassium reproducibly and reversibly increased renal vascular resistance fits well with the results produced by others (Cooper, Shaffer & Malik, 1985; Loutzenhiser, Hayashi & Epstein, 1989). They confirm the concept that a rise of cytosolic calcium brought about by mobilization of calcium from intracellular stores or by enhancement of calcium entry due to the activation of receptor and potential operated calcium channels is a key signal also for the contraction of renal vascular smooth muscle cells (Cooper *et al.* 1985; Carmines & Navar, 1989). The vasoconstrictor effect of Ang II was paralleled by a decrease of the arterio-venous difference of perfusate renin activity (pRA; Fig. 4). This observation is in harmony with previous studies showing that Ang II inhibits renin secretion *in vivo* and *in vitro* (Keeton & Campbell, 1981). In the presence of high potassium the arterio-venous differences of pRA increased in relation to the increase of vascular resistance (Fig. 8). In consequence, the renin secretion rate was not influenced by potassium. The increase of perfusate osmolality as a possible explanation for the lacking effect of K on renin secretion appears unlikely, because the addition of NaCl or sucrose in equiosmolar concentrations did not affect renin

secretion. Our finding that potassium did not alter renin secretion contrasts with studies reporting that depolarizing concentrations of potassium inhibit renin secretion from renal slices (Churchill & Churchill, 1982) and also from isolated rat kidneys but only if extracellular calcium concentrations are in the supraphysiological range (Fray, 1978). High concentrations of potassium (50 mM) have been reported to stimulate renin secretion from IPRK if extracellular calcium is low (Fray, 1980).

There are two possible explanations for our findings with respect to the existence of potential operated calcium channels (POCC) in JGE cells. First, if we assume that JGE cells have in fact POCC, then we have to infer from our results, that a rise of calcium in JGE cells through POCC itself is not sufficient to block renin secretion. The second possible explanation for our findings is that renal JGE cells do not possess POCC. This conclusion would be in concordance with our previous *in vitro* studies providing no evidence for the existence of those channels in JGE cells (Kurtz, Skott, Chegini & Penner, 1990).

Another unexpected finding in our study was that AVP in a concentration range of 10 pM to 1 nM stimulated renin release from isolated perfused rat kidneys in a dose-dependent fashion (Figs 5 and 6). *In vivo* effects of AVP on renin release are controversial. In several studies vasopressin has been found to decrease renin secretion *in vivo* (cf. Keeton & Campbell, 1981; Schwartz & Reid, 1986). However, it has also been shown that the non-osmotic release of AVP is associated with a concomitant activation of the renin-angiotensin system (Ishikawa & Schrier, 1984; Riegger, Liebau, Bauer & Kochsiek, 1985). From *in vitro* studies, using isolated kidneys perfused without red cells, it has been reported that vasopressin did not affect basal renin secretion but attenuated the stimulatory effect of β -adrenergic agonists on renin release (Vandongen, 1975; Konrads *et al.* 1978). At the cellular level, vasopressin is considered to act primarily via two distinct receptor types classified by their different second messengers (Michell, Kirk & Billah, 1979). The V_1 receptor acts via phosphatidylinositol hydrolysis thus increasing cellular inositol-triphosphate (IP_3) and diacylglycerol (DAG) content (Michell *et al.* 1979). The rise of cellular IP_3 levels leads to calcium mobilization from intracellular stores whereas DAG is a potent activator of protein kinase C (PKC) (Castagna, Takai, Kaibuchi, Sano, Kikkawa & Nishizuka, 1982; Berridge, 1984). Both events, cellular calcium mobilization and activation of PKC, are thought to act in concert in mediating the vasoconstrictor effect of vasopressin (Caramelo, Okada, Tsai & Schrier, 1989). Furthermore, the so-called V_2 receptor which is mainly located in the kidneys (late distal tubule, collecting duct) is coupled to adenylate cyclase and acts by increasing cellular cyclic AMP levels (cf. Morel, Imbert-Teboul & Chabardès, 1987). The antidiuretic effect of AVP is mediated by the V_2 receptor (cf. Morel *et al.* 1987). At the cellular level, the concentration-dependent increase of renin secretion observed in this study could have been due to either direct action of AVP on the JGE cell membrane or due to some indirect e.g. tubular effect of AVP. In fact, it has been reported that AVP at micromolar concentrations inhibits renin release from cultures of isolated JGE cells (Kurtz *et al.* 1986). Moreover, inhibition of renin secretion from isolated JGE cells by AVP was associated with increased phosphatidylinositol breakdown indicating that the inhibitory effect of AVP in cultured JGE cells was most likely mediated by the V_1 receptor (Kurtz *et al.* 1986). This conclusion is in

harmony with results obtained by microelectrode studies on JGE cells. There it has been found that AVP at concentrations of $1 \mu\text{M}$ caused a marked depolarization of JGE cells as it was seen also with other calcium mobilizing hormones (Bührle, Scholz, Hackenthal, Nobiling & Taugner, 1986). Since the signal transduction mechanism of the V_1 receptor is probably similar to that of the Ang II receptor (Kurtz *et al.* 1986) it appears rather unlikely that the stimulatory effect of AVP on renin release from the IPRK was mediated via V_1 receptors. A much better candidate for stimulation of renin secretion would be V_2 receptors coupled to adenylate cyclase, because a rise of the cellular cyclic AMP levels is a stimulatory signal for renin release from JGE cells (cf. Kurtz, 1989). Our finding that dDAVP, a vasopressin analogue acting predominantly on V_2 receptors (Weingartner *et al.* 1981), significantly decreased renal arteriolar resistance and at the same time stimulated renin secretion from isolated rat kidneys (Fig. 7) suggests that V_2 receptors are not only located on renal tubular cells, but they also occur in the renal vasculature. This conclusion is in concordance with recent studies showing that both, selective V_2 receptor agonists and AVP during V_1 receptor blockade, elicit V_2 -like haemodynamic effects (peripheral vasodilatation, increase of heart rate and cardiac output) in conscious dogs (Liard, 1989, 1990) and also in rats (Walker, 1986). Moreover, it has been shown recently that vasopressin-induced forearm vasodilatation in humans is mediated by vascular V_2 receptors (Hirsch, Dzau, Majzoub & Creager, 1989). These findings suggest that beside their location on renal tubular cells, V_2 -like receptors may also occur in the vasculature, e.g. on vascular smooth muscle cells. Since JGE cells are metaplastically transformed smooth muscle cells (Barajas, 1979; Taugner *et al.* 1984), one may infer that V_2 receptors could also occur on JGE cells and thus mediate the stimulatory effect of AVP on renin secretion from isolated perfused rat kidneys. Possible reasons why vasopressin has been found in previous studies to decrease rather than to stimulate renin secretion could be that AVP in those studies has mostly been used in the micromolar concentration range (Schwartz & Reid, 1986). At micromolar concentrations which are higher by a factor of 1000 than we have used in our study, however, AVP has been shown to inhibit renin release from isolated JGE cells via the V_1 receptor (Kurtz *et al.* 1986). A further possibility could be that V_2 receptor degradation usually occurs very rapidly under *in vitro* conditions. Therefore, our finding that AVP increased renin secretion by a V_2 receptor dependent signal transduction mechanism suggests that V_2 receptors are well preserved in the isolated rat kidney perfused with a medium containing red cells.

Taken together, our study suggests that there is no general link between renal arteriolar resistance and inhibition of renin secretion.

The expert technical assistance of W. Baier, C. Champion, B. Gnos, and U. Vogel is gratefully acknowledged. The authors also wish to thank C. Gasser and M. Müller for doing the artwork. This study was supported by a grant of the Swiss National Foundation (grant no. 31-26381.89 and 31-25354.88).

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