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Macula densa independent stimulatory effect of furosemide on renin secretion in mice

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Acute administration of loop diuretics like furosemide leads to a stimulation of renin secretion, an effect thought to result from inhibition of NKCC2-mediated salt transport at the macula densa. However, furosemide also inhibits NKCC1 with similar potency. In the present study we examined the influence of furosemide on renin secretion in NKCC1 knock-out and wild type mice and in isolated juxtaglomerular (JG) cells (all humanely killed animals) in order to distinguish between macula densa dependent and independent effects of furosemide.

Baseline plasma renin concentration (PRC) was 259 ± 61 ng Ang I/ml h in NKCC1+/+ (n = 9) and 917 ± 144 ng Ang I/ml h in NKCC1-/- mice (n = 11). Acute administration of furosemide (50 mg/kg i.p.) increased PRC significantly ($p=0.0001$) to 1794 ±229 ng Ang I/ml h in NKCC1+/+, whereas it reached a value of 2130 ± 286 ng Ang I/ml h in NKCC1-/- mice ($p<0.001$). Wild type mouse JG cells were isolated by enzymatic digestion of the renal cortex, and the effect of increasing concentrations of furosemide (10-5 M-10-3 M) on renin secretion was measured by RIA. Forskolin (10-5 M) was used as control. Forskolin and furosemide (10-3 M) significantly increased renin release (renin in medium in % of renin in cell lysate + medium)from 20.5 ±1.6% to 35.9 ±2.4% and 31.7 ±3.4%, respectively ($p<0.05$, n = 12). Furthermore, we determined membrane capacitance as a measure of exocytosis and renin release by using patch clamp methods in isolated JG cells (n = 5). Furosemide (10-4 M) increased membrane capacitance significantly by 8.1 ± 0.9% compared with basal capacitance over a period of 1200 s. Finally, we determined the expression of NKCC1 mRNA in single JG cells by RT-PCR. Using mRNA from 2.5 JG cells as template and 36 cycles we found that NKCC1 is expressed in JG cells while no band was seen in the control minus RT.

Our data suggest that besides modulating the macula densa pathway, furosemide also stimulates renin secretion directly on juxtaglomerular granular cells, and we suggest that this effect is mediated by inhibition of NKCC1.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Role of nitric oxide in neural control of intra-renal homodynamic in anaesthetised normotensive and hypertensive rats

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Nitric oxide (NO) produced in the renal cortex and medulla by nitric oxide synthase (NOS) regulates basal levels of renal haemodynamics and may be involved in neurally mediated vasoconstriction. This study investigated whether NO modulated renal sympathetic nerve (RSN) mediated reductions in renal cortical and medullary blood flow normally and in hypertension.

Four groups (n= 6-10) of male Wistar and stroke-prone spontaneously hypertensive rats (SHRSP), 250-300g, were anaesthetised with 1 ml chloralose/urethane, 16.5/250 mg/ml, i.p. The right femoral vein and artery were cannulated for saline infusion (154mM NaCl, at 3ml/h) and measurement of blood pressure (BP), respectively. The left kidney was exposed via the flank and a cannula inserted 4.5mm for intramedullary (i.m.) saline or drug infusion at 0.6-1.0 ml/h. Laser-Doppler microprobes were inserted 1.5 and 4.0 mm into the kidney to measure cortical and medullary blood perfusion (CP and MP), respectively (100 perfusion units (PU) = 1 V). Bipolar stainless steel electrodes were applied to the the renal nerves. After 90 min, baseline CP and MP were taken, and the RSN stimulated, 15V, 2 ms for 1 min, at 0.5, 1, 2 and 4 Hz, then either vehicle or L-NAME, 10 µg/kg/min, was infused i.m. for 90 min and the stimulation protocol was repeated. Data±SEM were subjected to Student's t test and significance taken at $P<0.05$. The animals were killed with an anaesthetic overdose.

In the Wistar rats, baseline levels of BP, MP and CP were 109±5 mmHg, 125±17 PU and 61±13 PU. respectively and for the SHRSP rats, BP was 123±3 mmHg, CP was 108±18 PU and MP was 60±9 PU. L-NAME infusion i.m. increased BP in both Wistar and SHRSP rats by 15±4% and 7±2% mmHg, respectively (both $P<0.05$), and reduced MP in the Wistar rats by 15±5% ($P<0.05$), with no significant change in the SHRSP; L-NAME had no effect on CP in either Wistar or SHRSP. In the Wistar rats, RSN stimulation reduced the MP and CP by 18 and 32%, respectively (both $P<0.05$), at 4 Hz while in the presence of L-NAME, the reductions in MP and CP were similar reaching 16 and 32% at 4 Hz, respectively ($P<0.05$). In the SHRSP, the RSN-mediated reductions in MP and CP were 7 and 8% at 4 Hz, respectively (both $P<0.05$). Following L-NAME infusion i.m., both MP and CP were reduced by 14 and 29% at 4 Hz, respectively (both $P<0.05$).

These results indicate that over the low frequency range of stimulation NO plays little role in buffering the renal medulla from the RSN induced vasoconstrictions. However, NO does appear to have modulatory action in the hypertensive state.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Electrophysiology of juxtaglomerular cells

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Renin is produced, stored and released from juxtaglomerular (JG) cells, which are modified smooth muscle cells located in the lamina media of the afferent arteriole at the site of entrance to the glomerulus. Using whole-cell patch clamp on JG-cells in isolated mouse afferent arterioles Kurtz & Penner (5) showed that JG-cells were nearly electrically silent at holding potentials between -50 and -10 mV, while there were outward K-currents at positive potentials and inward K-currents at negative potentials. They further identified a high density of calcium-activated chloride channels. We have isolated mouse and rat JG-cells ad modum (1) and studied them with whole-cell patch clamp technique in order to delineate the ion channels responsible for their electrical behaviour (2, 3, 4).

Single JG cells displayed marked outward current at positive membrane potentials. Tetraethylammonium inhibited 4/5 of the outward current, suggesting that K+ channels carry most of the current. Inhibition of Kv channels with 4-AP blocked 1/5 of the current. Inhibition of BKCa channels with iberiotoxin blocked 4/5 of the outward current. Furthermore, chelation of intracellular calcium with EGTA abolished the outward current. Thus, the outward potassium current is mainly carried through BKCa channels, the presence of which was confirmed with immunocytochemistry. Cyclic AMP increases outward currents in JG-cells (2, 3), and these currents were blocked by BKCa-specific inhibitors, suggesting that the BKCa splice variant in JG cells is the cAMP-stimulated ZERO variant (KCa1.1, ZERO variant). This was confirmed by RT-PCR. Activation of BKCa with cAMP led to a 16 mV hyperpolarisation of membrane potential while inhibition of the channels caused a 16 mV depolarisation. Thus, the BKCa channels influence the resting membrane potential of JG cells. In spite of this, inhibition of the BKCa channels had no effect on cAMP-induced renin secretion, showing that hyperpolarisation is not a prerequisite for renin secretion.

Mouse JG-cells display inward rectification of current at negative potentials, and the current has functional characteristics as the KIR (2, 5). By contrast, inward rectification was not observed in any of 326 rat JG-cells (4).

At variance with a number of functional studies, we found that JG-cells are endowed with high-voltage activated Ca channels (Cav) that are activated at a membrane potential of -20 mV and display maximal activation at +10- +20 mV (4). The current was blocked by the L-type channel blocker calciseptine and its expression of Cav 1.2 was confirmed by RT-PCR analysis. Immunostaining of kidney cryosections and of JG-cells showed colocalisation of renin and Cav. To examine the functional role of Cav we measured renin secretion (change in membrane capacitance) at different holding potentials. In unstimulated JG-cells the membrane capacitance was unaffected by holding potentials from -30 mV to +10 mV. Cyclic AMP increased membrane capacitance about 10% at -30 mV, but had no effect at +10 mV where Cav are activated. The inhibition of cAMP effects at +10 mV was abolished by calciseptine, indicating that the activated L-type Cav were responsible for the inhibition of cAMP-stimulated renin release. Thus, at depolarised potentials calcium influx through Cav inhibits renin release.

In conclusion, cAMP-activated BKCa. are involved in setting the resting membrane potential of juxtaglomerular cells. They are responsible for the major part of the outward current observed at depolarised membrane potentials, and they are responsible for the hyperpolarisation observed after cAMP stimulation. Rat juxtaglomerular cells express L-type voltage-dependent calcium channels (Cav 1.2) and activation of these channels inhibits cAMP-induced renin secretion. Cyclic AMP-induced stimulation of renin secretion may be protected against activation of L-type Cav by the hyperpolarisation induced by opening of BKCa.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Control of renin secretion

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Renin is stored in juxtaglomerular cells within granules and is thought to be released by exocytosis. The release of renin is precisely regulated by intracellular signal cascades, which either stimulate or inhibit renin secretion. The cyclic AMP pathway involving activation of protein kinase A potently stimulates renin secretion. The efficacy of the cAMP pathway is firstly determined by the balance of factors stimulating (example: ?-adrenoreceptor) or inhibiting adenylate cyclase (example: adenosine A1 receptor) as well as by the activity of cAMP-phosphodiesterases (PDE-3, PDE-4). Secondly, the efficacy of the stimulatory cAMP pathway is controlled by an inhibitory calcium dependent signaling route, which is powerful enough to virtually blunt any stimulation of renin secretion by cAMP. This inhibitory pathway is for example initiated by angiotensin II or by endothelins and their effects require both intracellular calcium release as well as transmembrane calcium influx. Further downstream elements of the inhibitory calcium dependent pathway are still subject of speculation. The same holds for the mechanism of the inhibitory effect of protein kinase G (type II) on renin secretion from juxtaglomerular cells. The final mechanisms that control the fusion of renin containing vesicles with the plasma membrane during the event of exocytosis are yet also only poorly understood.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Multiphoton imaging of juxtaglomerular cell functions

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Multiphoton fluorescence microscopy is an exciting new optical sectioning technique which has great potential for numerous future developments and is ideal for applications that require deep optical sectioning of living tissue samples. In combination with microperfusion techniques, the major functions of the juxtaglomerular apparatus (JGA), the tubuloglomerular feedback (TGF) and renin release, can be studied with high spatial and temporal resolution. Salt-dependent changes in macula densa (MD) cell volume, vasoconstriction of the afferent arteriole (AA), and activity of an intraglomerular precapillary sphincter composed of renin granular cells are visualized in real-time. Imaging cytosolic calcium levels of the microperfused JGA dissected from kidneys from humanely killed rabbits, we observed a fast calcium wave using ratiometric real-time imaging with Fluo-4 and Fura-Red. This calcium wave initiated from the extraglomerular mesangium and renin granular cells underneath the MD cells and was spreading towards both proximal AA smooth muscle cells and intraglomerular elements (mesangial cells and podocytes) with a time delay of 5 and 10 s, respectively. The terminal, intraglomerular part of the AA, a precapillary sphincter that includes renin granular cells, produced an almost complete closure of the AA during activation of TGF. This renin-positive sphincter acted as the first-response element of TGF activation and appeared to be the most significant vascular resistance to flow.

In addition, release and tissue activity of renin can be studied on the individual granule level. Renin release from JG cells represents a unique form of exocytosis: even large granules can release their content very rapidly (within 300ms) and without any significant movement relative to the JG cell membrane. Using a novel, FRET-based fluorogenic renin substrate, we demonstrated interstitial renin activity around renin granular cells in response to the beta-mimetic isoproterenol, simultaneously with renin exocytosis.

Imaging methods including the newest innovations in confocal fluorescence microscopy provide direct, visual information on JGA function with exceptional time and spatial resolution on the level of individual cells and organelles.

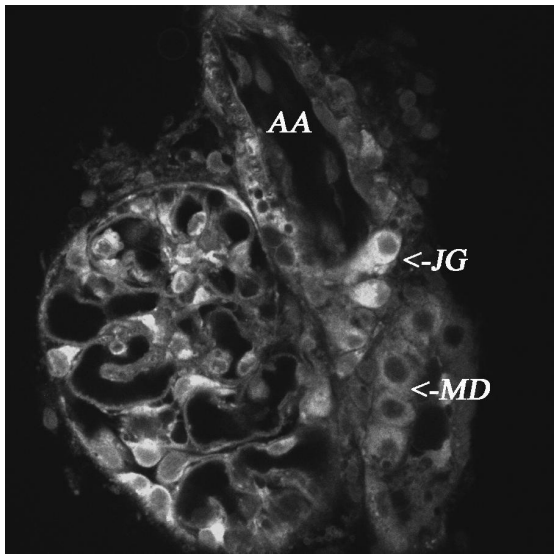

Figure 1. Two-photon image of a microperfused juxtaglomerular apparatus labelled with quinacrine. Diffuse labelling of the JGA structure, including macula densa (MD) cells, and the terminal part of the afferent arteriole (AA) containing renin granular cells or juxtaglomerular (JG) cells. JG cells contain a number of renin granules labelled with quinacrine.

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