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 (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.
Electrophysiology of juxtaglomerular cells

U.G. Friis, F. Jorgensen, B.L. Jensen and O. Skott

Physiology and Pharmacology, University of Southern Denmark, Odense, Denmark

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 calcisepine 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 calcisepine, indicating that the activated L-type Cav were responsible for the inhibition of cAMP-stimulated renin release. Thus, 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.


Where applicable, the experiments described here conform with Physiological Society ethical requirements.
Multiphoton imaging of juxtaglomerular cell functions
J. Peti-Peterdi
Physiology & Biophysics and Medicine, University of Southern California, Los Angeles, CA, USA

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 of 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.

This work was supported by NIH RO1 DK64324, AHA 00230074N, and an ASN Gottschalk Grant.

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