RESEARCH PAPER

Regulation of renal sympathetic neurotransmission by renal α2A-adrenoceptors is impaired in chronic renal failure

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BACKGROUND AND PURPOSE

The mechanisms underlying increased renal noradrenaline in renal failure are still unclear. In this study, the role of α2A-adrenoceptors in controlling sympathetic neurotransmission in chronic renal failure was evaluated in a subtotal nephrectomy model. Also, the influence of this receptor subtype on angiotensin II (Ang II)-mediated noradrenaline release was evaluated.

EXPERIMENTAL APPROACH

α2A-Adrenoceptor-knockout (KO) and wild-type (WT) mice underwent subtotal (5/6) nephrectomy (SNx) or SHAM-operation (SHAM). Kidneys of WT and KO mice were isolated and perfused. Renal nerves were stimulated with platinum electrodes and noradrenaline release was measured by HPLC.

KEY RESULTS

Noradrenaline release induced by renal nerve stimulation (RNS) was significantly increased in WT mice after SNx. RNS-induced noradrenaline release was significantly higher in SHAM-KO compared with SHAM-WT, but no further increase in noradrenaline release could be observed in SNx-KO. α-Adrenoceptor antagonists increased RNS-induced noradrenaline release in SHAM-WT but not in SHAM-KO. After SNx, the effect of α2-adrenoceptor blockade on renal noradrenaline release was attenuated in WT mice. The mRNA expression of α2A-adrenoceptors was not altered, but the inhibitory effect of α2-adrenoceptor agonists on cAMP formation was abolished after SNx. Ang II facilitated RNS-induced noradrenaline release in SHAM-WT but not in SHAM-KO and SNx-WT.

CONCLUSION AND IMPLICATIONS

In our model of renal failure autoregulation of renal sympathetic neurotransmission was impaired. Presynaptic inhibition of noradrenaline release was diminished and the facilitatory effect of presynaptic angiotensin AT1 receptors on noradrenaline release was markedly decreased in renal failure and depended on functioning α2A-adrenoceptors.

Abbreviations

Ang II, angiotensin II; CRF, chronic renal failure; HPLC, high performance liquid chromatography; IBMX, 3-isobutyl-1-methylxanthine; KO, knockout; RNS, renal nerve stimulations; SHAM, SHAM-operation; SNx, subtotal (5/6) nephrectomy; WT, wild-type

Introduction

Chronic renal failure (CRF) and end stage renal disease (ESRD) are major health issues in modern populations. Patients suffering from CRF or ESRD are at higher risk for cardiovascular events leading to increased morbidity and mortality (London, 2000; Guérin *et al.*, 2008). It is well known that CRF is associated with an activation of the sympathetic nervous system which contributes to the progression of CRF and cardiovascular complications, such as hypertension (Converse *et al.*, 1992; Rump *et al.*, 2000; Hausberg *et al.*, 2002; Vonend *et al.*, 2004; 2008; Bakris *et al.*, 2006; Schlaich *et al.*, 2009b). Moreover, elevated catecholamine plasma levels correlate with cardiovascular events (Zoccali *et al.*, 2002). However, the mechanisms that lead to the impaired regulation of sympathetic neurotransmitter release in CRF are poorly understood. Insights into the underlying mechanism(s) might help to improve treatment of hypertension and CRF.

Only recently has it been recognized that even a minor injury to the kidney (Ye *et al.*, 2006) triggers afferent nerve traffic to the hypothalamus and thereby increases efferent sympathetic nerve activity and blood pressure (Rump *et al.*, 2003). It is not entirely clear which signals in the damaged kidney trigger afferent signals [e.g. hypoxia, adenosine, angiotensin II (Ang II) or others], but sympathetic overactivity has been observed in a variety of experimental models of renal damage including subtotal nephrectomy. Campese has shown that selective renal de-afferentiation (dorsal rhizotomy) prevents the development of hypertension in rats after 5/6-nephrectomy (Campese, 2000). In patients with resistant hypertension, renal denervation reduced sympathetic nerve activity and blood pressure (Krum *et al.*, 2009; Schlaich *et al.*, 2009a).

α_2-Adrenoceptors play a major role in regulation of sympathetic neurotransmitter release (Starke *et al.*, 1989). Three subtypes (α_{2A}, α_{2B}, α_{2C}) have been described (Bylund *et al.*, 1994; Hein, 2006; receptor nomenclature follows Alexander *et al.*, 2009). The α_{2A}-adrenoceptor subtype represents the major autoinhibitory receptor modulating neurotransmitter release from sympathetic nerve endings (Altman *et al.*, 1999; Vonend *et al.*, 2007). Besides affecting noradrenaline release, the α_{2A}-receptor acts as a heteroreceptor and also mediates release of ATP (Vonend *et al.*, 2007).

In addition, the sympathetic nervous system interacts with vasoactive hormone systems such as nitric oxid (Sartoria *et al.*, 2005) or the renin-angiotensin system (DiBona, 2001). Local Ang II facilitates presynaptic noradrenaline release in the human or murine heart and kidney. There is strong evidence that this interaction between the renin-angiotensin system and the sympathetic nervous system plays a role in the genesis of hypertension in CRF (Rump *et al.*, 1995; Stegbauer *et al.*, 2005).

The aim of the present study was to investigate local mechanisms that regulate sympathetic neurotransmitter release in the kidney in a model of CRF. In this respect, the roles of presynaptic α_2-adrenoceptors and angiotensin AT_1 receptors were evaluated using a mouse model with CRF.

Methods

All animal care and experimental investigations were in accordance with the current European Communities regulations (Office Journal of the European Communities L358/1 18 December 1986) and approved by a University-independent governmental Ethics Committee. α_{2A}-Adrenoceptor-knockout (KO) and wild-type (WT) mice, both on FVB-background, were obtained from Experimental Animal Centre of the Ruhr University of Bochum. Primarily, α_{2A}-adrenoceptor-KO mice were generated on C57Bl/6-background by Lutz Hein, Department of Pharmacology, University of Freiburg, Germany (Hein *et al.*, 1999) and later backcrossed on FVB-background for seven generations. Backcrossing the C57Bl/6-α_{2A}-adrenoceptor-KO mice on FVB-background was necessary because mice on C57Bl/6-background are not susceptible to glomerulosclerosis after subtotal nephrectomy (Ma and Fogo, 2003). Adult (60–75 days) male mice with a body weight of 21–26 g were used for the experiments. All animals were housed under standard conditions and had free access to water and food.

Isolated perfused kidneys

Mice were anaesthetized by intraperitoneal injection of sodium pentobarbital (0.270 mg·g^{-1} body weight). Kidneys were isolated microscopically (Olympus CO11) and perfused with Krebs–Henseleit solution [composition (mM): NaCl 118, KCl 4.7, CaCl$_2$ 2.5, MgSO$_4$ 0.45, NaHCO$_3$ 25, KH$_2$PO$_4$ 1.03, D-(+)-glucose 11.1, Na$_2$EDTA 0.067 and ascorbic acid 0.07; all from Fluka, Switzerland] at a constant rate (7.25 mL·min^{-1}·g^{-1} kidney) as described previously (Vonend *et al.*, 2005). The perfusion medium was gassed continuously with a mixture of 95% O$_2$ and 5% CO$_2$ and passed through a 0.45 μm filter before reaching the kidney. The kidneys were transferred into a jacketed glass chamber maintained at a temperature of 37°C. Bipolar platinum electrodes were placed around the renal arteries to stimulate the renal sympathetic nerves. The perfusate was collected from the renal vein and ureter. Five minutes before nerve stimulation, drugs were infused into the perfusion line by a perfusion apparatus (Harvard Pump 11 Plus Dual Syringe) at a constant flow rate of 0.158 mL·min^{-1}. To test the viability of the preparation, the renal nerve was stimulated (RNS) at a frequency of 5 Hz (3 s, 1 ms pulse width, 40 mA) followed by the administration of 60 mM KCl 15 min later.

Effect of renal nerve stimulation on renal noradrenaline release. After a stabilization period of 30 min, cocaine (10 μM) and corticosterone (20 μM) were added to the perfusion solution in order to prevent neuronal and extraneuronal uptake of released noradrenaline respectively. After another 20 min, 3 min fractions of the perfusate were collected by a fraction collector (LKB, Bromma, Sweden) into vials containing 125 μL HCl (1 M), 13.3 μL of EDTA (0.067 M) and 2.5 μL Na$_2$SO$_3$ (1 M). Kidneys were stimulated at frequencies of 5, 7.5 and 10 Hz (3 s, 1 ms pulse width, 40 mA) as described above.

Effect of α-adrenoceptor blockade on renal noradrenaline release. The experiment started after a stabilization period of 30 min. Then, six RNS (S$_0$–S$_5$) at a frequency of 5 Hz (3 s, 1 ms pulse width, 40 mA) were applied. Using a perfusion appara-

tus (Harvard Pump 11 Plus Dual Syringe) the non-selective α-adrenoceptor antagonist phentolamine was applied in a cumulative manner at 0.01, 0.03, 0.1, 0.3 and 1 μM, the selective α2-adrenoceptor antagonist rauwolscine at 0.01, 0.1 and 1 μM and the selective α1-adrenoceptor antagonist prazosin at 0.01, 0.1, 1 and 10 μM at a constant rate of 0.158 mL·min^{-1} accompanying the stimulations. The interval of perfusion started 5 min before RNS and lasted until 1 min after. The perfusate of the kidneys was collected in two fractions of 3 min after each RNS. Noradrenaline release was measured by HPLC.

Effect of angiotensin II on renal noradrenaline release. Five RNS (S_0–S_4), each at 5 Hz (3 s, 1 ms pulse width, 40 mA), were applied at 6, 22, 38, 54 and 72 min after the start of fraction collection. Ang II was added directly to the perfusion system via perfusion apparatus (Harvard Pump 11 Plus Dual Syringe) in a cumulative manner (0.01, 0.1, 1 and 10 nM), starting 5 min before and stopping 1 min after S_1, S_2, S_3 and S_4. In the set of experiments evaluating the effect of α2-adrenoceptor blockade on Ang II-mediated facilitation of noradrenaline release the selective α2-adrenoceptor antagonist rauwolscine was added 10 min before the first stimulation period (S_0) at a concentration of 1 μM to the perfusion solution for the total time of experiment.

Quantitative determination of release of endogenous noradrenaline. Noradrenaline in the collected samples was extracted (adsorption onto alumina, elution with HClO$_4$). The quantity of noradrenaline in each sample was determined by reversed-phase HPLC detection (Stegbauer *et al.*, 2005) and corrected for recovery using an internal standard (3,4-dihydroxybenzylamine 12 pg·mL^{-1}, Chromsystems, Germany). RNS-induced outflow of noradrenaline was determined as the difference between the content of noradrenaline present in two 3-min samples collected immediately after onset of stimulation and spontaneous noradrenaline content present in the 3-min sample collected immediately before RNS (Stegbauer *et al.*, 2005).

Calculation of data. In some experiments (Figures 1, 2 and 3A',B') the absolute amounts of noradrenaline release were calculated and expressed in pg·g^{-1} kidney wet weight. S_0 served as a reference stimulation, and the RNS-induced noradrenaline outflow in S_1–S_3 in experiments with rauwolscine and S_1–S_4 in those with application of Ang II and prazosin was expressed as a percentage of the outflow in S_0 (S_n as % of S_0). For further evaluations of the effects of rauwolscine, prazosin and Ang II, the S_n/S_0 ratios were calculated as a percentage of values, which were determined in the corresponding control experiments presented in Figure 4 (S_n/S_0 as % of control).

Subtotal (5/6) nephrectomy

The animals were placed on a heating pad to maintain a constant body temperature (37°C). Under anaesthesia with sodium pentobarbital (0.270 mg·g^{-1} body weight), right flank incisions were made, and the right kidney was decapsulated and removed. To achieve a subtotal (5/6) nephrectomy (SNx), upper and lower pole of the left kidney were removed. To control for the effect of the surgery, WT and KO mice were
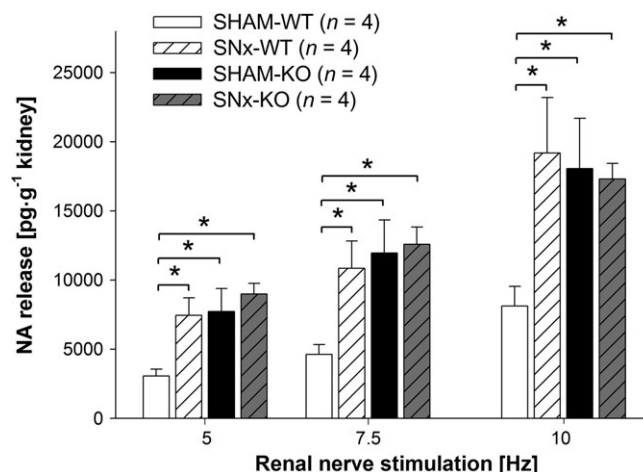

Figure 1

Renal nerve stimulation (RNS)-induced endogenous noradrenaline (NA) release in isolated kidneys of SHAM-operated (SHAM) and subtotal nephrectomized (SNx) wild-type (WT) and knockout (KO) mice at frequencies of 5, 7.5 and 10 Hz (each for 3 s, with 1 ms pulse width at 40 mA). RNS-induced NA release was measured and expressed in pg·g^{-1} kidney wet weight (data given are mean and vertical lines show SEM). Noradrenaline release was significantly increased in WT mice after SNx. In SHAM-KO mice, NA release was significantly greater than in SHAM-WT mice. There was no increase in NA release in KO mice after SNx. Experiments in SNx-animals were performed 60 days postoperatively. *$P < 0.05$ indicates significant differences.

treated with a SHAM-operation (SHAM), in which the right and left flank incisions were made in anaesthetized mice and sutured without any tissue removal. Thirty days after the operation, the serum urea (mean ± SEM; in SNx-WT mice, 1781 ± 758 mg·L^{-1}; in SHAM-WT mice, 553 ± 106 mg·L^{-1}) and the albumin to creatinine ratio in urine (mean ± SEM; in SNx-WT mice, 16715 ± 1237 mg·g^{-1}; in SHAM-WT mice, 73 ± 31 mg·g^{-1}) were significantly increased, indicating CRF.

Quantification of mRNA expression

The α2A-, α2B- and α2C-adrenoceptor mRNA expression was estimated by real-time-quantitative PCR analyses. RNA was isolated from tissue of α2A-adrenoceptor-KO and -WT mice using RNeasy Plus Mini Kit (Qiagen). The extracted RNA was transcribed into cDNA by QuantiTect Reverse Transcription Kit (Qiagen). Quantitative mRNA analysis was performed by 7300 real-time PCR system (Applied Biosystems) using TaqMan Gene Expression assays (Applied Biosystems). Changes in expression level were calculated using Rest 2008 Software (Corbett Research, USA) according to Pfaffl *et al.* (Pfaffl *et al.*, 2002).

Quantification of cAMP accumulation

The SHAM-operated and SNx-WT mice were anaesthetized by intraperitoneal injection of sodium pentobarbital (0.270 mg·g^{-1} body weight) and perfusion was performed through the abdominal aorta with Dulbecco's modified Eagle's medium (DMEM) in order to remove blood from the kidneys. The kidneys were removed and cut by a sharp blade
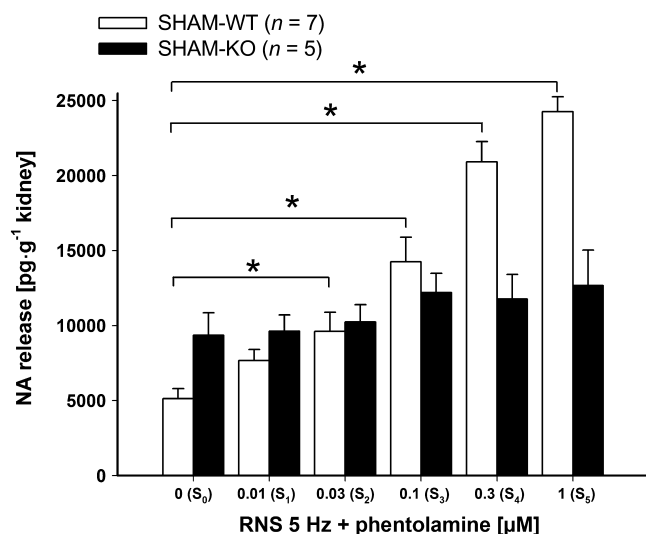
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Figure 2

Effect of the non-selective α-adrenoceptor antagonist phentolamine on renal nerve stimulation (RNS)-induced endogenous noradrenaline (NA) release in isolated kidneys. There were six stimulation periods (S_0–S_5, each at 5 Hz for 3 s, 1 ms pulse width, 40 mA). Phentolamine was applied in increasing concentrations (0.01, 0.03, 0.1, 0.3 and 1 μM) and was added to the perfusion solution to S_1–S_5. RNS-induced NA release was measured and expressed in pg·g^{-1} kidney wet weight (data given are mean and vertical lines show SEM). A significant increase in RNS-induced NA release after α-adrenoceptor blockade by phentolamine was found in wild-type (WT) but not in knockout (KO) mice. $*P < 0.05$ indicates a significant difference in NA release by increasing concentrations phentolamine in WT mice.

(Apollo, Germany) into thin tissue slices. The tissue slices were transferred into 2 mL of prewarmed (37°C) DMEM, gassed with 5% CO_2 and 95% O_2 and incubated for 90 min. After the incubation period, 3-isobutyl-1-methylxanthine (IBMX) was added to the medium culture. After 20 min IBMX treatment, forskolin was added in increasing concentration (0, 1 and 5 μM) in the presence or absence of the selective α_2-adrenoceptor agonist UK14.304 (25 μM). Tissue slices were collected after 30 min of incubation with forskolin and cAMP accumulation was measured by cAMP EIA kit (Cayman Chemical, USA). The effect of forskolin and the combination of forskolin and UK14.304 on cAMP formation was expressed as a percentage of basal cAMP formation in the absence of other drugs except IBMX.

Statistics

All data were expressed as mean ± SEM. Differences were analysed by one-way ANOVA for repeated measurement combined with a *post hoc* test (Bonferroni). Probability levels of $P < 0.05$ were considered statistically significant. The number of experiments indicates the number of individual kidneys.

Materials

The following drugs were used: corticosterone, phentolamine HCL, rauwolscine, prazosin, Ang II, IBMX, forskolin, UK14.304 (Sigma-Aldrich, Germany); cocaine HCl (Merck, Germany). Drugs were dissolved in distilled water before

being diluted with the Krebs–Henseleit solution, except corticosterone (absolute ethanol).

Results

Effect of subtotal nephrectomy on renal noradrenaline release

The WT and KO mice were treated with SNx or a SHAM. RNS was performed to induce noradrenaline release from renal sympathetic nerve endings. RNS-induced noradrenaline release at all frequencies (5, 7.5 and 10 Hz) was significantly higher in SNx-WT than in kidneys of SHAM-WT mice (Figure 1). In SHAM-KO mice, RNS-induced noradrenaline release was already at same level as in WT mice after SNx and significantly higher at all frequencies compared with SHAM-WT mice (Figure 1). Experimental CRF had no effect on renal noradrenaline release in KO mice. There was no significant difference in noradrenaline release at all frequencies in KO mice after SNx- compared with SHAM-KO mice (Figure 1).

Renal nerve stimulation-induced noradrenaline release in the presence of α-adrenoceptor blockade

The non-selective α-adrenoceptor antagonist phentolamine increased RNS (5 Hz)-induced noradrenaline release in a concentration-dependent manner (0.01, 0.03, 0.1, 0.3 and 1 μM) in kidneys of SHAM-WT mice (Figure 2). In WT mice the increase in noradrenaline release by phentolamine was significant at concentrations of 0.03, 0.1, 0.3 and 1 μM. Only a marginal increase in noradrenaline release by phentolamine was observed in SHAM-KO mice. In the absence of α-adrenoceptor antagonists, noradrenaline release, induced by RNS (S_0–S_5 at 5 Hz), was similar in kidneys of WT and KO mice (Figure 4A).

Quantitative expression of α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors

The expression of mRNA for α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors was determined by quantitative PCR analysis in renal tissue of SHAM and SNx α_{2A}-adrenoceptor-KO and -WT mice. The expression of α_{2B}- and α_{2C}-adrenoceptors revealed no significant difference between SHAM-KO and SHAM-WT mice (Figure 5A). SNx did not change the mRNA expression level of α_{2A}-adrenoceptors (Figure 5B). There was also no difference between expression of mRNA for α_{2B}- and α_{2C}-adrenoceptors in SNx-KO and SHAM-KO mice as well as SNx-KO and SNx-WT mice (data not shown).

Renal nerve stimulation-induced noradrenaline release in the presence of selective α_1- and α_2-adrenoceptor blockade

The selective α_2-adrenoceptor antagonist rauwolscine facilitated RNS (5 Hz)-induced noradrenaline release in a concentration-dependent manner (0.01, 0.1 and 1 μM) in kidneys of SHAM-WT and SNx-WT mice (Figure 3A/A'). Application of rauwolscine induced an increase in noradrenaline release in SHAM-WT up to 331.5%, and in SNx-WT up to
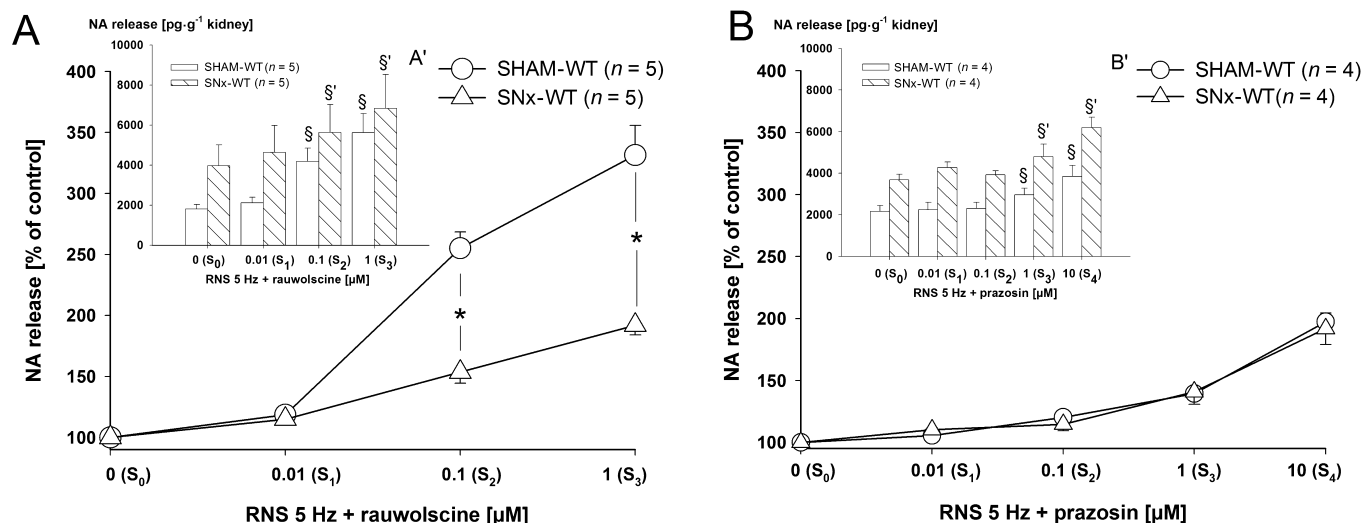
Figure 3

(A,B) Effects of the selective α_2-adrenoceptor antagonist rauwolscine (A) and the selective α_1-adrenoceptor antagonist prazosin (B) on renal nerve stimulation (RNS)-induced endogenous noradrenaline (NA) release in isolated kidneys of SHAM-operated (SHAM) and subtotal nephrectomized (SNx) wild-type (WT) mice. There were four stimulation periods in experiments with rauwolscine (S_0–S_3) and five stimulation periods in experiments with prazosin (S_0–S_4). Each stimulation period was performed at 5 Hz for 3 s, 1 ms width, 40 mA. Rauwolscine was applied in increasing concentrations of 0.01, 0.1 and 1 μM to the perfusion solution to S_1–S_3 and prazosin in increasing concentrations of 0.01, 0.1, 1 and 10 μM to S_1–S_4. Noradrenaline release was measured and expressed as a percentage of control S_0 (S_n as % of S_0) (A, B). In (A') and (B') the same data are given but expressed in pg·g^{-1} kidney wet weight for SHAM and SNx mice. Data given are mean and vertical lines show SEM. The increase in NA release after α_2-adrenoceptor blockade by rauwolscine was significantly reduced in SNx-WT, compared with SHAM-WT mice (A; *$P < 0.05$ indicates a significant difference). The increase in NA release after α_1-adrenoceptor blockade was similar in kidneys of SHAM and SNx-WT mice (B). Graphs indicating absolute NA release demonstrate the higher level of stimulation-induced transmitter release in SNx compared with SHAM animals (A',B'). Rauwolscine and prazosin lead to a concentration-dependent increase in NA release in SHAM and SNx animals (A',B') (§$P < 0.05$ indicates significant differences compared with S_0, §P within SHAM group and §'P within SNx group).
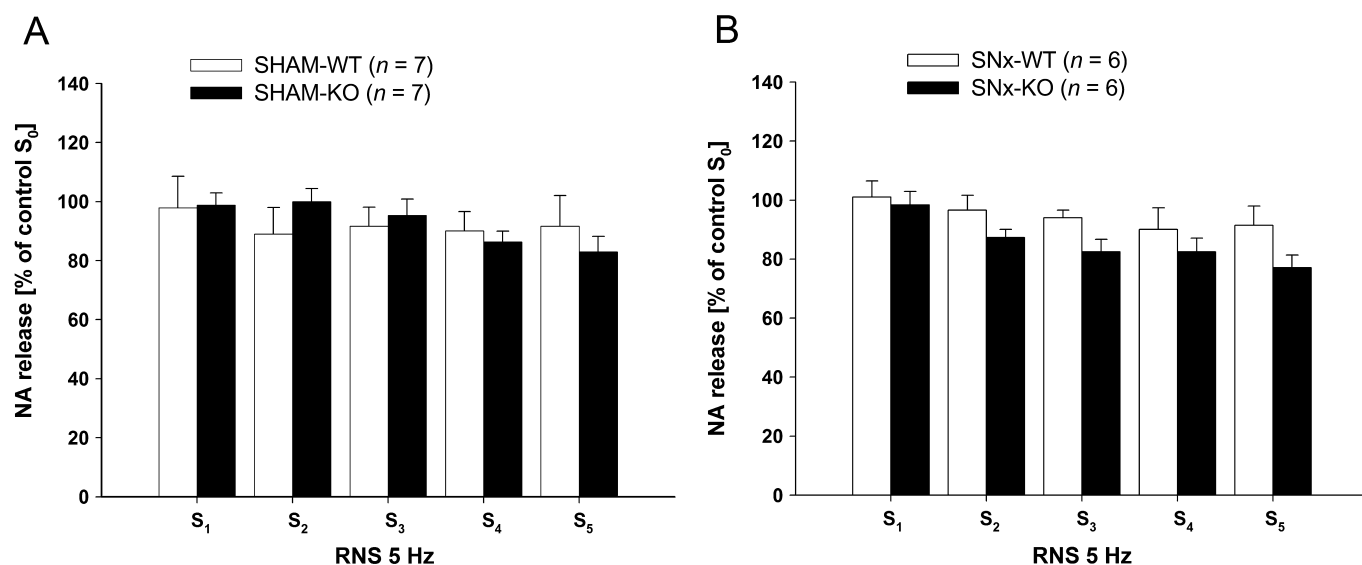

Figure 4

(A,B) Renal nerve stimulation (RNS)-induced endogenous noradrenaline (NA) release in isolated kidneys of SHAM-operated (SHAM) (A) and subtotal nephrectomized (SNx) (B) wild-type (WT) and knockout (KO) mice, six times consecutively (S_0–S_5) at a frequency of 5 Hz (each for 3 s, with 1 ms pulse width at 40 mA) without application of any drugs. The stimulation-induced NA release was measured and expressed in percentage of S_0 (S_1/S_0, to S_5/S_0) (data given are mean and vertical lines show SEM). Noradrenaline release after RNS was stable over time in all four groups. Experiments in SNx animals were performed 30 days postoperatively.
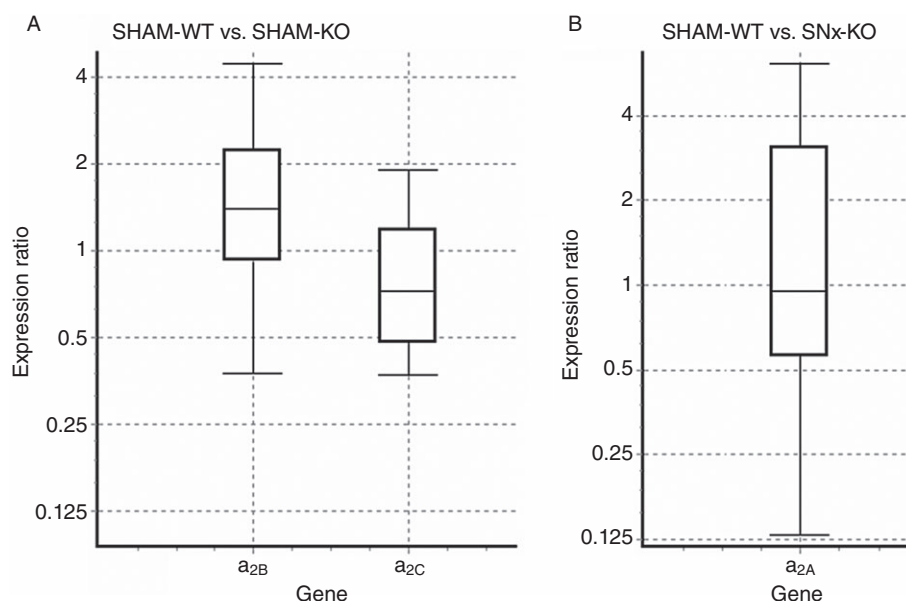
Figure 5

(A,B) Effect of knockout (KO) compared with WT (A) and of subtotal nephrectomized (SNx)-WT compared with SHAM-operated (SHAM)-WT (B) on mRNA expression of α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor subtypes in the kidneys. The mRNA expression was quantified by real-time-quantitative PCR. Results indicated no significant differences in mRNA expression (data are shown as medians (black lines in boxes), with box (interquartile range) and whiskers (maximum and minimum); $n = 4$ for each group).

192%. This increase was significantly lower in SNx-WT compared with SHAM-WT mice (Figure 3A). The selective α_1-adrenoceptor antagonist prazosin facilitated RNS (5 Hz)-induced noradrenaline release at 1 and 10 µM in kidneys of SHAM-WT and SNx-WT mice (Figure 3B/B'). Application of prazosin induced an increase in noradrenaline release in SHAM-WT up to 197.3% similar to that seen in SNx-WT (Figure 3B).

Inhibition of forskolin-induced cAMP accumulation

Kidney tissue slices of SHAM- and SNx-WT mice were stimulated by the adenylyl cyclase activator forskolin (0, 1 and 5 µM) in the presence and in the absence of the α_2-adrenoceptor agonist UK14.304 (25 µM). Forskolin 1 µM and 5 µM increased cAMP accumulation in SHAM-WT to 457% and 773% respectively (Figure 6). Application of UK14.304 inhibited forskolin-induced cAMP accumulation significantly in SHAM- WT mice. In contrast, the inhibiting effect of UK14.304 on forskolin-induced cAMP accumulation was abolished in SNx-WT mice (Figure 6).

Effect of angiotensin II on renal noradrenaline release

Ang II application facilitated noradrenaline release, induced by RNS (5 Hz), in a concentration-dependent manner (0.01, 0.1, 1 and 10 nM) in kidneys of SHAM-WT mice up to 197% (Figure 7). The increase in noradrenaline release by 1 nM Ang II was significantly attenuated in SNx-WT mice. The facilitatory effect of 1 nM Ang II on RNS-induced noradrenaline release was significantly smaller in SHAM-KO compared with
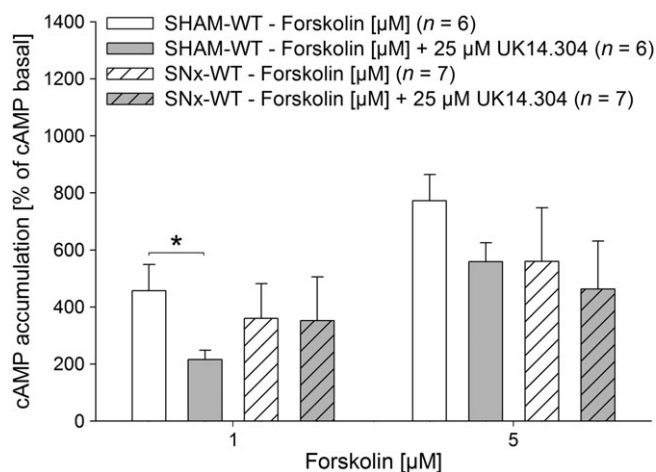

Figure 6

Effect of UK14.304 (25 µM) on forskolin-induced (0, 1 and 5 µM) cAMP accumulation in kidney slices of SHAM-operated (SHAM) and subtotal nephrectomized (SNx) mice. At 1 µM forskolin, a significant inhibition of cAMP accumulation by UK14.304 was observed in SHAM-wild-type (WT) but not in SNx-WT mice. Experiments in SNx animals were performed 30 days after subtotal nephrectomy. cAMP accumulation is presented as percentage of cAMP basal level (data given are mean and vertical lines show SEM.). *$P < 0.05$ indicates a significant difference.

SHAM-WT mice. In addition, no difference in the facilitatory effect of noradrenaline release could be observed between SHAM-KO and SNx-KO (Figure 7). As demonstrated in α_{2A}-adrenoceptor KO mice, the pharmacological blockade of
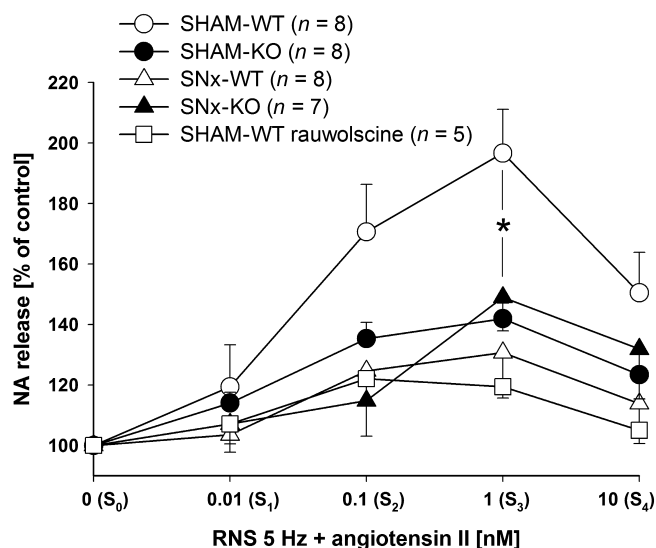
Figure 7

Effect of angiotensin II (Ang II) on renal nerve stimulation (RNS)-induced endogenous noradrenaline (NA) release in isolated kidneys of SHAM-operated (SHAM) and subtotal nephrectomized (SNx) knockout (KO) and wild-type (WT) mice. There were five stimulations periods (S_0–S_4, each at 5 Hz for 3 s, 1 ms pulse width, 40 mA). Ang II was applied in increasing concentrations (0.01, 0.1, 1 and 10 nM) to the perfusion solution to S_1–S_4. The RNS-induced NA release was measured and calculated as a percentage of control S_0 (S_n as % of S_0) (data given are mean and vertical lines show SEM.). Experiments in SNx mice were performed 30 days postoperatively. The enhancement of NA release by Ang II was reduced in mice lacking the α_{2A}-adrenoceptor (KO) and in mice after SNx, compared with SHAM-WT mice. The antagonism of α_{2A}-adrenoceptors by rauwolscine (1 μM) reduced the enhancing effect in a similar manner. *$P <$ 0.05 indicates significant differences in the facilitatory effect of Ang II on NA release between SHAM-WT and all other groups.

α_{2A}-adrenoceptors using rauwolscine attenuated the facilitatory effect of Ang II in SHAM-WT in a similar manner. Noradrenaline release induced by consecutive RNS at 5 Hz (S_0–S_4) decreased slightly over time in SHAM and SNx mice.

Discussion

Impaired function of presynaptic α_{2A}-adrenoceptors in chronic renal failure

In the present study, we showed that noradrenaline release induced by RNS was significantly increased in SNx mice, our model of CRF. There is evidence for an impaired autofeedback regulation in uraemia in the CNS (Klein *et al.*, 2005). In the present set of experiments, we tested whether the function of presynaptic α_2-adrenoceptors was impaired in CRF. Autoinhibition by presynaptic α_2-adrenoceptors is one of the most potent mechanisms regulating sympathetic neurotransmitter release. Indeed, the increase in noradrenaline release after SNx, as observed in WT mice, could not be seen in α_{2A}-adrenoceptor SNx-KO mice. However, SHAM-KO mice were characterized by a higher RNS-induced renal noradrenaline release compared with SHAM-WT mice.

In order to prove that in FVB-WT mice, similar to previous observations in C57Bl/6 mice (Vonend *et al.*, 2007), the presynaptic autoinhibition of noradrenaline release was mediated by the α_{2A}-adrenoceptor subtype, a non-specific α-adrenoceptor antagonist phentolamine was applied. In WT mice, phentolamine uncoupled RNS-induced noradrenaline release in a concentration-dependent manner. In contrast, the application of phentolamine led only to a marginal increase in noradrenaline release in KO mice. It is possible that other α_2-adrenoceptor subtypes (α_{2B}-and α_{2C}-adrenoceptors) could participate in renal sympathetic neurotransmission (Hein, 2001; Starke, 2001). However, in our experimental setup, a compensatory overexpression of α_{2B}- and α_{2C}-adrenoceptors on mRNA level was not observed in mice lacking the α_{2A}-adrenoceptor.

The marginal increase in renal noradrenaline release in KO mice by phentolamine could be due to inhibition of α_1-adrenoceptors. Previously, facilitatory effects of α_1-adrenoceptor blocking drugs on noradrenaline release have been described in rats (Rump and Majewski, 1987; Bohmann *et al.*, 1993a,b). It was proposed that prazosin increased noradrenaline release induced by RNS, by preventing a transjunctional inhibitiory feedback loop which is mediated through release of prostaglandins and adenosine, following stimulation of postjunctional α_1-adrenoceptors. Furthermore, these authors discussed a blockade of α_2-adrenoceptors at higher prazosin concentrations. Indeed, in the present study, prazosin facilitated RNS-induced noradrenaline release in SHAM and SNx mice. It remains unclear which of the above mentioned mechanisms may be responsible for the observed effect of phentolamine in KO mice. In any case, the facilitatory effect of prazosin on noradrenaline release was unaffected in mice with CRF.

The α_2-adrenoceptor antagonist rauwolscine increased the RNS-induced noradrenaline release to a significantly greater extent in SHAM than in SNx mice, indicating impaired α_2-adrenoceptor function in CRF. These observations are in contrast to previous results in rat kidneys in which an α_2-adrenoceptor antagonist had a greater facilitatory effect on RNS-induced noradrenaline release after SNx, compared with control animals (Amann *et al.*, 2000). We do not have a definite explanation, but species differences may account for the contrasting findings. The present data suggest that the inhibitory α_{2A}-adrenoceptor function was impaired in kidneys of SNx mice and this might be one reason for the observed increase in presynaptic release of noradrenaline.

To further elucidate the underlying mechanism for this impairment we analysed α_{2A}-adrenoceptor mRNA expression level. The SNx *per se* did not alter the mRNA expression of α_{2A}-adrenoceptors, compared to SHAM-WT mice. We carried out additional experiments to test α_2-adrenoceptor signalling. As α_{2A}-adrenoceptors are G_i coupled receptors, their activation leads to a reduction in cAMP accumulation (Bylund *et al.*, 1994). Incubation of kidney slices with the selective α_2-adrenoceptor agonist UK14.304 inhibited cAMP accumulation in SHAM-WT mice. This effect was attenuated in kidneys of SNx-WT mice indicating α_2-adrenoceptor desensitization in CRF. The reduced activity of α_{2A}-adrenoceptors might be caused by desensitization after receptor phosphorylation or internalization by the binding of arrestins to phosphorylated receptors as suggested previously (Olli-Lahdesmaki *et al.*,

2004). However, as most tissues, including the kidney, contain both pre- and postsynaptic α_2-adrenoceptors, it is difficult to distinguish between these two sites by binding or signalling studies. A transgenic mouse model expressing the dopamine β-hydroxylase promoter combined with general deletion of the endogenous α_{2A}-adrenoceptor gene could be used to unequivocally distinguish between pre- and postsynaptic α_2-adrenoceptors (Gilsbach *et al.*, 2009; 2010).

Angiotensin AT$_1$ receptor-mediated noradrenaline release depends on presynaptic α_2-adrenoceptors

Apart from α_{2A}-adrenoceptor function, other presynaptic mechanisms might be affected in this model of CRF. And it is well known that the renin-angiotensin system is activated in CRF. In this respect, Ang II has been described as a potent modulator of sympathetic neurotransmitter release (Stegbauer *et al.*, 2005) and it was possible that the facilitatory effect of Ang II on noradrenaline release was also altered in CRF. There is evidence that angiotensin AT$_1$ receptors interact with α_2-adrenoceptors. Cox and colleagues showed that a marked presynaptic facilitatory effect of Ang II in atria isolated from mice required an ongoing presynaptic α_2-autoinhibition (Cox *et al.*, 2000). Trendelenburg *et al.* confirmed these findings and pointed out that the α_{2C}-adrenoceptor subtype in particular was responsible for this receptor interaction (Trendelenburg *et al.*, 2003).

Indeed, exogenously applied Ang II caused a significant facilitation of renal noradrenaline release in SHAM-WT mice. According to Stegbauer *et al.*, the effect is mediated by presynaptic angiotensin AT$_1$ receptors (Stegbauer *et al.*, 2003). This facilitatory effect of Ang II was significantly diminished in WT mice with CRF. As described above, the α_{2A}-adrenoceptor-mediated autoinhibition was impaired in our model of CRF and, in case of a functional cross talk with presynaptic angiotensin AT$_1$ receptors, might disrupt the facilitatory effect of Ang II on noradrenaline release. To test this hypothesis, we repeated the experiments using α_{2A}-adrenoceptor-KO mice. As expected, the facilitatory effect of Ang II on noradrenaline release was significantly diminished in SHAM-KO mice. In addition, pharmacological α_2-adrenoceptor blockade by rauwolscine attenuated the facilitatory effect of Ang II on noradrenaline release in a similar way.

In this set of experiments, it was shown for the first time that α_{2A}-adrenoceptors are involved in the receptor interaction between α-adrenoceptors and angiotensin AT$_1$ receptors. This interaction probably represents a necessary mechanism of fine regulation of sympathetic neurotransmission under physiological conditions and may also have important pathophysiological implications.

In summary, we conclude that the α_{2A}-adrenoceptor plays a key role in regulating sympathetic activity in the kidney and its function is impaired in CRF. Moreover, there is an interaction between α_{2A}-adrenoceptors and angiotensin AT$_1$ receptors that represents an interface between the sympathetic nervous system and the renin-angiotensin system.

Acknowledgements

Conflict of interest

None.

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