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RESEARCH PAPER

α_{2A}-Adrenoceptors Regulate Sympathetic Transmitter Release in Mice Kidneys

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Background and purpose: In the present study, a rodent model was used to investigate whether the α_{2A}-adrenoceptor (α_{2A}) represents the presynaptic autoinhibitory receptor regulating sympathetic transmitter release in the kidney. Moreover, the potential role of α_{2A} as a heteroceptor regulating adenosine triphosphate (ATP) release was tested.
Experimental approach: Kidneys from wild-type (WT) and α_{2A}-knockout (KO) mice were isolated and perfused. Renal nerves were stimulated with platinum-electrodes. Endogenously released noradrenaline (NA) was measured by HPLC. The perfusion pressure was monitored continuously.
Key results: Renal nerve stimulation (RNS) induced a frequency (1,2,5,7.5,10,15 Hz)-dependent release of NA in WT mice (994 ± 373, 2355 ± 541, 6375 ± 950, 11626 ± 1818, 19138 ± 2001 pg NA g^{-1} kidney (means $\pm$ s.e.m.)). There was a 2.7-fold (5 Hz) increase of NA release in α_{2A}-KO mice. In WT animals α-adrenoceptor blockade by phentolamine increased RNS-induced NA release in a concentration-dependent manner up to 350% of control. No facilitation by phentolamine was observed in α_{2A}-KO mice. Pressor responses to 1 Hz and 2 Hz were resistant to α_1-adrenoceptor blockade (0.03 μM prazosin) but abolished by P_2 receptor blockade (5 μM PPADS). Blockade of α_2-adrenoceptors (1 μM rauwolscine) increased these purinergic pressor responses to 296 ± 112% (1 Hz) in WT but not in α_{2A}-KO mice. Exogenous ATP (100 μM) increased basal but not RNS-induced NA release.
Conclusions and Implications: α_{2A}-Adrenoceptor-activation inhibits NA and ATP release from renal sympathetic nerves. Pressor responses to RNS at higher stimulation frequencies (>2 Hz) are mediated by NA. At lower frequencies neuronally released ATP seems to be the predominant transmitter mediating renovascular resistance.
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Keywords: α_{2A}-adrenoceptor; sympathetic neurotransmission; noradrenaline; ATP; P2 receptor; receptor-deficient mouse

Abbreviations: ATP, adenosine triphosphate; HPLC, high-performance liquid chromatography; KO, knockout; NA, noradrenaline; RNS, renal nerve stimulation; WT, wild type

Introduction

Sympathetic overactivity has been linked to elevated cardiovascular morbidity and mortality in chronic renal failure (Rump *et al*., 2000). Besides central mechanisms, the kidney appears to be a major modulator of sympathetic drive. Kidneys are densely innervated by afferent and efferent sympathetic nerve fibres. In animal models and patients with chronic renal failure it was shown that diseased kidneys are the origin of an overactive sympathetic nervous system and trigger hypertension. Accordingly, cutting the afferent nerve fibres by dorsal rhizotomy or removing the affected kidneys reduces sympathetic nerve activity and blood pressure (Campese, 1997; Ritz *et al*., 1998; Campese and Krol, 2002).

Presynaptic α_2-adrenoceptors are known to play a predominant role in the regulation of central and peripheral sympathetic nerve activity (Trendelenburg *et al*., 2001, 2003). Sympatholytic drugs, such as the non-selective α_2-adrenoceptor agonist clonidine, have been used to control hypertension for more than 30 years. They are most effective in patients with chronic renal failure (Garrett and Kaplan, 1980; Schohn *et al*., 1985; Vonend *et al*., 2003b). Three different α_2-adrenceptor subtypes have been cloned (α_{2A}, α_{2B} and α_{2C}). However, only a limited number of selective ligands are available to study the physiological and pathophysiological significance of each receptor subtype

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(Lorenz *et al*., 1990). By using subtype-specific knockout (KO) mice, it is now possible to determine the function of each α_2-adrenoceptor subtype (Hein *et al*., 1999). Experiments in vasa deferentia, isolated brain and atrial tissue of α_{2A}-, α_{2B}- and α_{2C}-KO mice revealed a predominant role for α_{2A}- and α_{2C}-adrenoceptor subtypes regulating synaptic noradrenaline (NA) release (Trendelenburg *et al*., 2001, 2003). Deletion of α_{2A}- and α_{2C}-adrenoceptor subtypes increased the susceptibility to develop heart failure following chronic pressure overload *in vivo* (Brede *et al*., 2002). There is still a lack of studies focusing on the kidney, despite its key role as the origin and target of increased sympathetic activity. Therefore, experiments in isolated perfused kidneys of wild-type (WT) and α_{2A}-adrenoceptor KO mice were conducted to analyse whether the α_{2A}-adrenoceptor subtype represents the autoinhibitory receptor that modulates renal NA release. Furthermore, its function as a heteroceptor modulating neuronal release of the sympathetic cotransmitter adenosine triphosphate (ATP) was investigated. This is of particular interest as NA and ATP appear to mediate renovascular and mitogenic effects in rodent and human kidneys via specific G-protein-coupled P2-purinoceptors (Amann *et al*., 2001; Vonend *et al*., 2002, 2003a, 2005a, b).

Methods

α_{2A}-Adrenoceptor KO (C57/Bl6 $\alpha_{2A^{-/-}}$) and WT (C57/Bl6 $\alpha_{2A^{+/+}}$) mice were obtained from L Hein, Department of Pharmacology, University of Würzburg, Germany (Hein *et al*., 1999). Adult (60–75 days) male mice with a body weight of between 21 and 26 g were used for the experiments. The investigations were performed in accordance with the current EC regulations (OJ of EC L358/1 12/18/1986). A University independent governmental Ethics Committee approved the study protocol.

Isolated perfused kidney

Mice were anaesthetized by intraperitoneal injection of sodium pentobarbitone (0.270 mg g^{-1} body weight). Kidneys were isolated microscopically (Olympus CO11) and perfused with Krebs–Henseleit solution at a constant rate (7.25 ml min^{-1} g^{-1} kidney equals 1.12 ± 0.02 ml min^{-1}) as described previously (Vonend *et al*., 2005b). 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 it reached 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 allowed to drip out of the cut end of the renal vein and ureter and was then collected. Perfusion pressure was monitored continuously with a Statham P23 Db pressure transducer (Gould, Oxnard, CA, USA) coupled to a Watanabe pen recorder (Graphtec Corp., Tokyo, Japan). The basal perfusion pressure was 46.0 ± 17.0 mm Hg in kidneys of wild-type mice and 47.4 ± 21.5 in α_{2A}-KO mice.

When agonists or antagonists were used, the drugs were infused into the perfusion line 5 min before stimulation by a perfusion apparatus (Braun, Melsungen, Germany) at a constant flow rate of 0.158 ml min^{-1}. To eliminate potential hydraulic pressure differences by drug-infusion a second vehicle-filled, perfusion apparatus was stopped during drug infusion leading to a constant perfusion volume (7.25 ml min^{-1} g kidney). To test the viability of the preparation the renal nerve was stimulated (RNS) with 5 Hz followed by the administration of 60 mM KCl 10 min later.

Effect of renal nerve stimulation on renal perfusion pressure

After a stabilization period of 30 min kidneys were stimulated with 1, 2, 5, 7.5, 10 and 15 Hz (30 s duration, 1 ms pulse width, 40 mA amplitude) with a time interval of 9 min between each stimulus. Pressor responses to renal nerve stimulation (RNS) were measured as the maximum increase of perfusion pressure above basal perfusion pressure ($\Delta P_{max} = P_{max} - P_{basal}$). This increase was expressed in mm Hg and as a percentage of the pressor response to 60 mM KCl. In some experiments, kidneys were stimulated only with 1 and 2 Hz in the absence of drugs (time interval 6 min). After a time interval of 20 min a second 1 Hz and 2 Hz stimulation (time interval 6 min) was performed in the presence of the α_1-adrenoceptor blocker prazosin (0.03 μM), added 5 min before RNS. A final 1 and 2 Hz stimulation was delivered after another 20 min time interval in the presence of α_1- and α_2-adrenoceptor blockade, induced by a combination of prazosin (0.03 μM) and rauwolscine (5 μM).

Effect of renal nerve stimulation on 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 NA, respectively. After another 20 min, 3-min fractions of the effluent were collected by a fraction collector (LKB, Bromma, Sweden) into vials containing 167 μl of 1 M HCl, 13.3 μl of 0.067 M ethylene diamine tetraacetic acid (EDTA) and 3.3 μl of 1 M Na_2SO_3. Kidneys were stimulated with frequencies of 1–15 Hz as described above. In selected experiments six RNS at 5 Hz (S_1–S_6) were applied 3, 18, 33, 48, 63 and 78 min after the start of fraction collection. When antagonists were used, the drug was infused into the perfusion line by a perfusion apparatus (Braun, Melsungen, Germany) at a constant flow rate of 0.158 μl min^{-1} starting 5 min before S_1, S_2, S_3, S_4 and S_5. In experiments in which the influence of ATP and analogues on 5 Hz RNS was analysed, three RNS (S_1–S_3) with 5 Hz were applied 6, 30 and 56 min after the start of fraction collection. In this experimental setup, three additional 3-min fraction samples were collected before RNS to evaluate the effect of ATP and analogues on NA release. ATP and analogues were applied 9 min before RNS.

Noradrenaline in the collected samples was extracted (adsorption onto alumina, elution with $HClO_4$). The quantity of NA in each sample was determined by reversed-phase high-performance liquid chromatography (HPLC) detection (Stegbauer *et al*., 2005) and corrected for recovery (average recovery of noradrenaline-HCl was $62.1 \pm 4.0\%$; $n = 45$) using internal standard (3,4-dihydoxybenzylamine 12 pg μl^{-1},

Chromsystems, Munich, Germany). RNS-induced outflow of NA was determined as the difference between the content of NA present in two 3-min samples collected immediately after onset of stimulation and spontaneous NA content present in the 3-min sample collected immediately before RNS (Stegbauer *et al*., 2005). RNS-induced NA release was expressed in pg NA g^{-1} kidney wet weight.

Effect of agonists and antagonists on renal perfusion pressure
After a stabilization period of 30 min, the non-selective α-adrenoceptor agonist NA was added to the perfusion solution, in a cumulative manner, at a constant rate of 0.158 ml min^{-1} using a perfusion apparatus (Braun, Melsungen, Germany). The concentration was changed when the perfusion pressure had reached a maximum, or when no effects were observed, respectively. When antagonists were used, a 10-min wash-in period was performed before the first agonist application.

Statistical analysis
All data are expressed as mean $\pm$ s.e.m. Differences were analysed by two-factorial analysis of variance (ANOVA) (SPSS12.0G) for repeated measurements followed by a *post hoc* test according to Bonferroni. Probability levels of $P<0.05$ were considered statistically significant. The number of experiments indicates the number of individual kidneys.

Drugs and vehicles
The Krebs–Henseleit solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NAHCO_3$ 25, KH_2PO_4 1.03, D-(+)-glucose 11.1, Na_2EDTA 0.067 and ascorbic acid 0.07 (all Fluka, Buchs, Switzerland). The following drugs were used: noradrenaline-HCl, corticosterone, phentolamine-HCL, methoxamine, UK14304 (5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline), PPADS (pyridoxalphosphate-6-azophenyl-2′,4′-disulphonate) tetrasodium salt, ATP, uridine triphosphate (UTP), uridine diphosphate (UDP), α,β-meATP, ATP-γS (Sigma, Buchs, Switzerland); cocaine-HCl (Merck, Darmstadt, Germany). Drugs were dissolved in distilled water before being diluted with the Krebs–Henseleit solution, except corticosterone (absolute ethanol).

Results

Effect of exogenous α-adrenoceptor agonists on renal perfusion pressure
Exogenous NA and the selective α_1-adrenoceptor agonist methoxamine induced a concentration-dependent rise in perfusion pressure (Figure 1a and b). The concentration–response curves were similar in α_{2A}-receptor KO and WT mice (EC_{50} NA: α_{2A}-KO: 0.25 μM; WT: 0.24 μM, EC_{50} methoxamine: α_{2A}- KO: 1.80 μM; WT: 2.01 μM). The selective α_2-adrenoceptor agonist UK14304 failed to increase perfusion pressure in wild-type and α_{2A}-receptor KO mice up to 1 mM (data not shown).

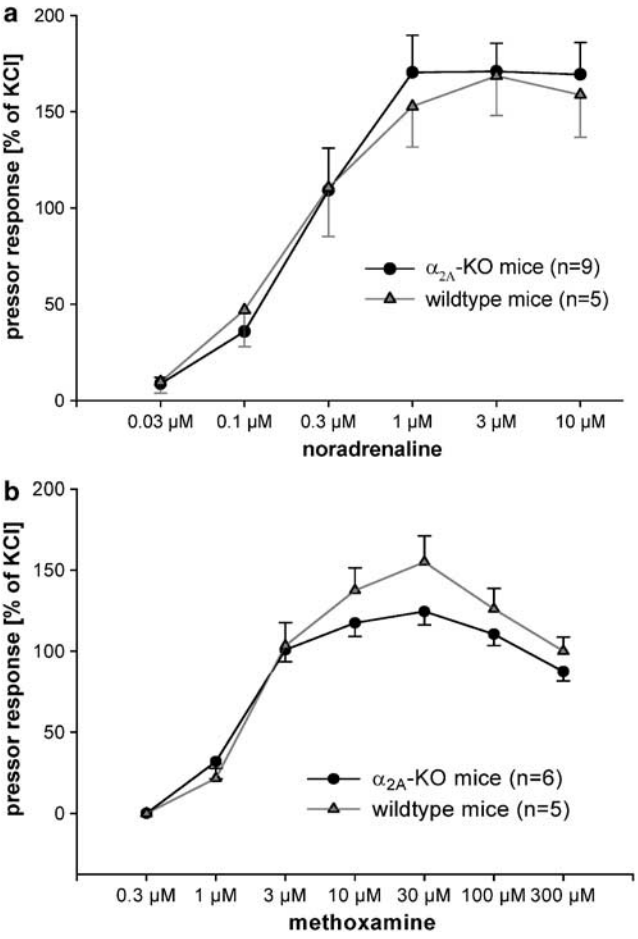

Figure 1 Concentration–response curves for noradrenaline (**a**) and methoxamine (**b**) in wild-type and α_{2A}-adrenoceptor knockout (KO) mice. Pressor responses are expressed as % of KCl (60 mm)-induced responses (data given are mean and vertical lines show s.e.m.).

Renal nerve stimulation induced pressor responses and noradrenaline release
RNS was performed to induce sympathetic neurotransmitter release. RNS-induced pressor responses at frequencies of 1, 2, 5, 7.5, 10 and 15 Hz were significantly greater in α_{2A}-adrenoceptor KO than in WT mice kidneys (Figure 2). Correspondingly, NA release (ng g^{-1} kidney weight) was also markedly higher in kidneys of α_{2A}-adrenoceptor KO than in age-matched WT mice (Figure 3).

Renal nerve stimulation-induced noradrenaline release in the presence of α_2-adrenoceptor blockade
The non-selective α-adrenoceptor blocker phentolamine (0.01, 0.03, 0.1, 0.3 and 1 μM) facilitated RNS (5 Hz)-induced NA release in a concentration-dependent manner in the kidneys of WT mice (Figure 4a). No increase in NA release by phentolamine was observed in α_{2A}-receptor KO mice (Figure 4a). In the presence of the highest concentration of phentolamine RNS-induced NA release in the kidneys of WT mice was comparable to that observed in the kidneys of α_{2A}-receptor KO mice in the absence of phentolamine (Figure 4a). When the renal nerves of WT ($n=8$) and α_{2A}-

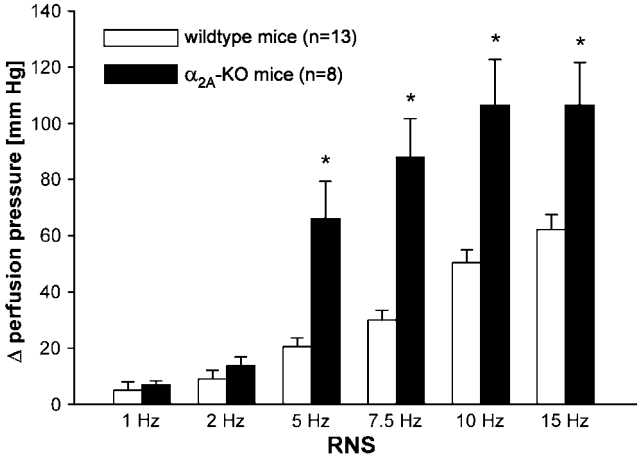

Figure 2 The increase in perfusion pressure induced by renal nerve stimulation (RNS) in wild-type and α_{2A}-knockout (KO) mice (data given are mean and vertical lines show s.e.m.). *$P<0.05$ indicates significant differences between wild-type and KO mice.

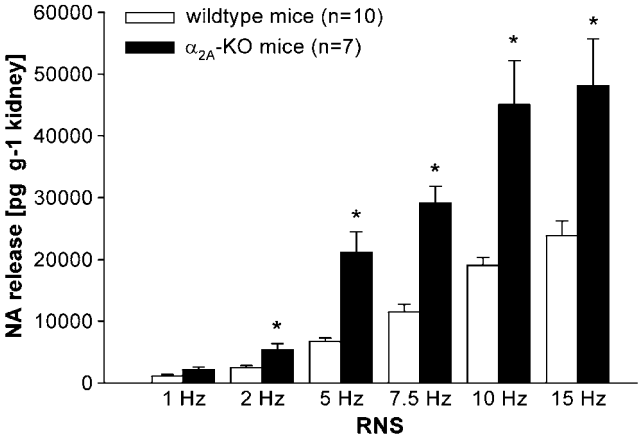

Figure 3 Renal nerve stimulation (RNS)-induced NA release in wild-type and α_{2A}-adrenoceptor knockout (KO) mice. Noradrenaline (NA) release was measured by HPLC and expressed in pg g^{-1} kidney (data given are mean and vertical lines show s.e.m.). *$P<0.05$ indicates significant differences between wild-type and KO mice.

adrenoceptor KO ($n=5$) mice kidneys were stimulated at 5 Hz for six consecutive times in the absence of any drug, RNS-induced NA release was stable in both strains (Figure 4b).

Renal nerve stimulation-induced pressor responses resistant to α-adrenoceptor blockade

RNS induced frequency-dependent pressor responses in the WT mice kidneys (1, 2, 5, 7.5, 10 and 15 Hz) (Figure 5). In the presence of the non-selective α-adrenoceptor blocker phentolamine (1 μM), RNS-induced pressor responses were significantly reduced only at 7.5, 10 and 15 Hz. Phentolamine failed to reduce pressor responses to RNS at 1 and 2 Hz (Figure 5). RNS induced pressor responses resistant to α-adrenoceptor blockade were totally blocked by the non-selective P_{2X}-receptor blocker PPADS (1 μM) (Figure 5).

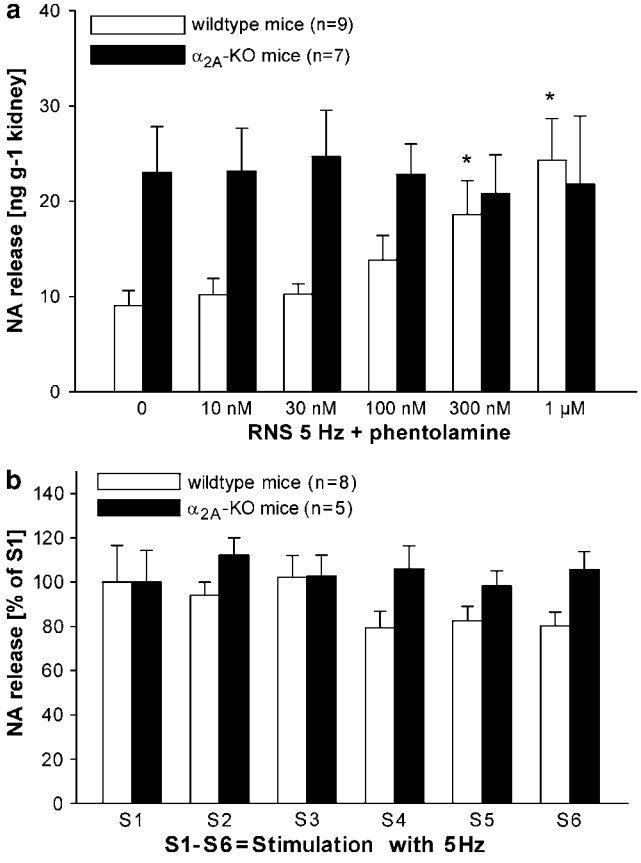

Figure 4 Kidneys of α_{2A}-adrenoceptor knockout (KO) and wild-type mice were electrically stimulated (RNS) six times in a row with 5 Hz in the presence of phentolamine (**a**) or in the absence of any other drug (**b**). The non-selective α-adrenoceptor blocker phentolamine was added in increasing concentrations (0–1 μM) (**a**). NA release was measured and expressed in ng g^{-1} kidney. Throughout six consecutive stimulations, RNS-induced NA release was stable over time (**b**). A significant increase in RNS-induced NA release by phentolamine was found in wild-type but not in α_{2A}-adrenoceptor KO mice. (*$P<0.05$ indicates a significant increase in NA release by phentolamine compared to control – 0 nM phentolamine.)

Presynaptic α_2-adrenoceptor modulation of postsynaptic pressor responses resistant to α-adrenoceptor blockade

Renal nerves of mice kidneys were stimulated at 1 and 2 Hz for three consecutive times with 20-min intervals. Prazosin (0.03 μM) was added before the second stimulation period to block α_1-adrenoceptors. Rauwolscine (1 μM) was added, in addition to prazosin, before the third stimulation period to block α_2-adrenoceptors (Figure 6a and b). In WT and in α_{2A}-adrenoceptor KO mice, the blockade of α_1- (and $\alpha_{2B/C}$) adrenoceptors by prazosin reduced pressor responses to RNS at 1 and 2 Hz significantly (Figure 6a and b). In WT (Figure 6a) but not α_{2A}-adrenoceptor KO (Figure 6b) mice, the addition of rauwolscine to the perfusion solution markedly potentiated RNS-induced pressor responses despite α_1- and α_2-adrenoceptor blockade. These α_{2A}-adrenoceptor blockade-resistant, non-adrenergic pressor responses were blocked by the non-selective P_{2X}-receptor antagonist PPADS (5 μM) (Figure 6a).

Control experiments were performed in WT mice to ensure that the prazosin concentration used was sufficient

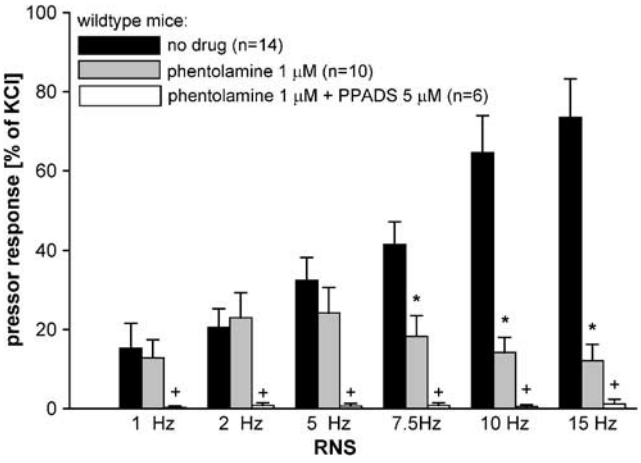

Figure 5 Renal nerve stimulation (RNS, 1–15 Hz)-induced pressor responses in wild-type mice in the absence of drugs, after α-adrenoceptor blockade by phentolamine and after addition of the non-selective P_2 receptor blocker PPADS. The data are expressed as a % of the response to 60 mM KCl and the means and s.e.m. (vertical lines) are shown. $^*P<0.05$ indicates a significant reduction of RNS-induced pressor responses by phentolamine compared to no drug. $^+P<0.05$ indicates a significant difference between phentolamine alone and phentolamine plus PPADS.

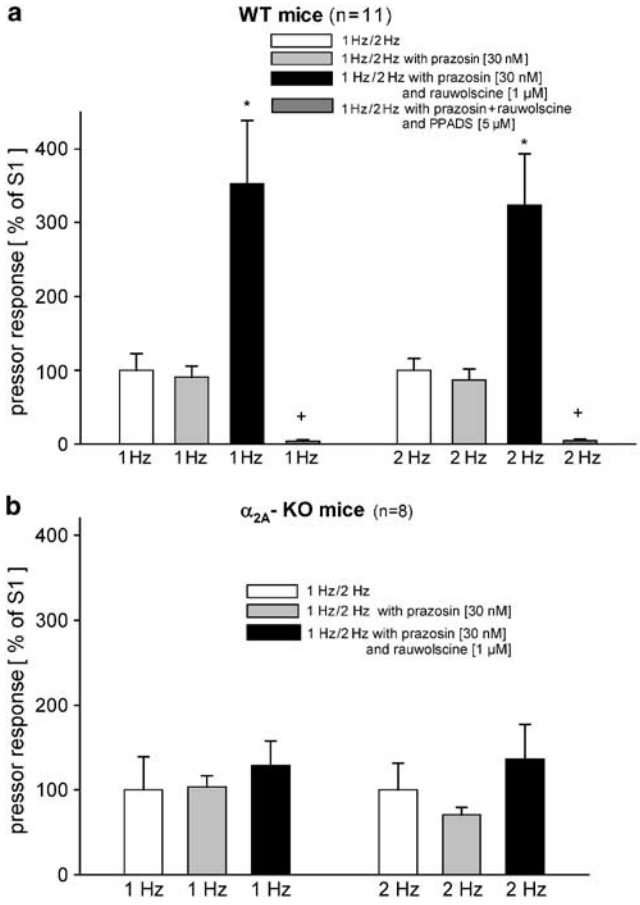

Figure 6 The effect of α_2-adrenoceptor blockade (rauwolscine 1 μM) on α_1-adrenoceptor-resistant (prazosin 30 μM) renal nerve stimulation (RNS, 1 Hz and 2 Hz)-induced pressor responses in wild-type (**a**) and α_{2A}-adrenoceptor knockout (KO) mice (**b**). RNS-induced pressor responses were obtained in the absence of drugs, in the presence of prazosin, prazosin plus rauwolscine and prazosin plus rauwolscine plus PPADS. $^*P<0.05$ indicates significant differences in RNS-induced pressor responses by adding rauwolscine to prazosin. $^+P<0.05$ indicates significant differences in RNS-induced pressor responses by adding PPADS to the combination of rauwolscine and prazosin.

to block the effect of NA on postsynaptic α-adrenoceptors, but failed to facilitate RNS-induced NA release. A rauwolscine concentration had to be selected to assure the opposite effect.

Noradrenaline (0.03, 0.1, 0.3, 1, 3 and 10 μM) increased renal perfusion pressure in a concentration-dependent manner. Prazosin (0.03 μM) reduced these pressor responses to exogenous NA (1, 3 and 10 μM) to 0±0, 7.4±3.1 and 29.0±10.1% ($n=5$), respectively. Furthermore, prazosin (0.03 μM) failed to alter RNS (5 Hz)-induced NA release (109.1±2.7% of control; $n=6$). In contrast, rauwolscine (1 μM) enhanced RNS (5 Hz)-induced NA release significantly to 316.7±10.5% of control ($n=6$) but did not significantly inhibit pressor responses to NA (1 μM) (91.2±2.3% of control; $n=4$).

The effect of adenosine triphosphate and its analogues on NA release

Adenosine triphosphate (100 μM) did not significantly alter RNS (5 Hz)-induced NA release (Figure 7), but increased basal NA release. This effect was blocked by the non-selective P_{2X}-receptor antagonist PPADS (5 μM) (Figure 7). The analogues ADP, UTP, ATP-γS, 2-methyl-thioADP, α,β-mATP (10–100 μM) were without any effect on RNS-induced and basal NA release (data not shown).

Discussion

The present study was conducted to elucidate the influence of α_{2A}-adrenoceptors on renal sympathetic neurotransmission. The kidney plays a major role in regulating blood pressure and hypertensive patients with chronic renal failure are characterized by a dramatic increase in cardiovascular mortality (Rump *et al*., 2000; Koomans *et al*., 2004). There is unswerving evidence that sympathetic overactivity is a main factor that contributes to this unfavourable outcome in patients with kidney diseases. Understanding the local release mechanisms of sympathetic neurotransmitters might help to develop a strategy that slows down progression of renal disease and decreases cardiovascular risk.

Presynaptic autoinhibition by the α_{2A}-adrenoceptor subtype

First, WT and α_{2A}-adrenoceptor KO mice kidneys were stimulated with increasing concentrations of NA to find out whether α_{2A}-adrenoceptors contribute to postsynaptic renovascular effects. There is evidence that in vas deferens α_{2A}-adrenoceptors partly mediate contractions to exogenous NA (Bultmann *et al*., 1991; Cleary *et al*., 2003). However, in

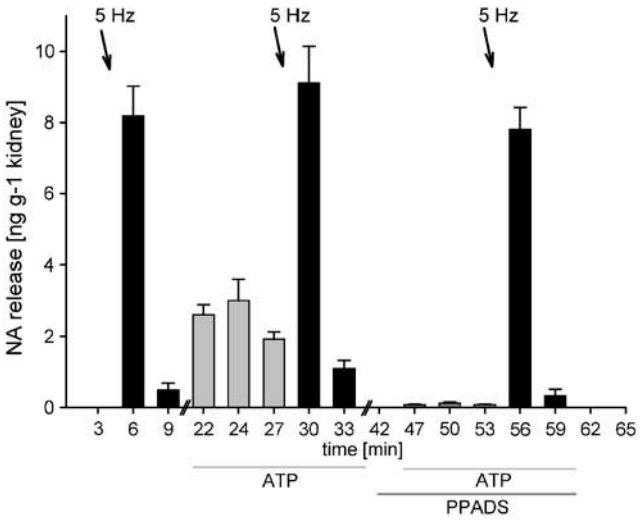

Figure 7 Kidneys of wild-type mice were electrically stimulated (RNS) three times with 5 Hz and the superfusate was collected in 3 min samples for NA analysis by HPLC. The columns represent the NA content in each sample. ATP (100 μM) was added 9 min before the second RNS. ATP was added before the third RNS in the presence of the non-selective P_2 receptor blocker PPADS (5 μM). The NA release was measured and expressed in ng g^{-1} kidney.

mice isolated perfused kidneys, we found no difference between WT and α_{2A}-adrenoceptor KO mice, suggesting that α_{2A}-adrenoceptors are not involved in postsynaptic effects of NA. In line with this finding the α_2-adrenoceptor agonist UK14304 failed to increase renal perfusion pressure in both strains, whereas the α_1-adrenoceptor agonist methoxamine increased renovascular resistance to a similar extent in both strains.

When the renal nerves were stimulated at 1–15 Hz to induce endogenous neurotransmitter release, there was a significant difference between WT and KO mice. Despite a comparable response to KCl 60 mM, pressor responses to RNS were significantly greater in KO than in WT mice. Measuring endogenous NA release by HPLC revealed that RNS (1–15 Hz)-induced NA release in KO mice was significantly enhanced compared to that in WT mice. When renal nerves were stimulated repeatedly with 5 Hz in WT mice, a concentration-dependent facilitation of RNS-induced NA release was observed by increasing concentrations of the α-adrenoceptor blocker phentolamine. No facilitation by phentolamine was observed in KO mice. Also, the RNS-induced NA release in KO mice in the absence of phentolamine was quantitatively almost the same as the NA release in WT mice under conditions of maximum blockade of presynaptic autoreceptors. As phentolamine is a non-selective adrenoceptor blocker this observation indicates that in mice kidneys the α_{2A}-adrenoceptor represents the only α_2-adrenoceptor subtype that acts as an inhibitory presynaptic α-adrenoceptor. This is in contrast to observations in vas deferens, atrial and brain tissue where α_{2C}-adrenoceptors and possibly also α_{2B}-adrenoceptors at least partly contribute to presynaptic control of NA release at the sympathetic nerve terminal (Hein *et al*., 1999; Trendelenburg *et al*., 2001; Brede *et al*., 2002; Trendelenburg *et al*., 2003).

α_{2A}-Adrenoceptors act as presynaptic inhibitory 'heteroceptors'

In WT mice RNS induced a frequency-dependent increase in renal perfusion pressure. At frequencies of 1–5 Hz, blockade of postsynaptic α-adrenoceptors by phentolamine failed to reduce RNS-induced pressor responses significantly. Adding the non-selective P_2-purinoceptor inhibitor PPADS abolished the RNS- induced α-adrenoceptor-resistant, non-adrenergic pressor responses. This suggests that at lower stimulation frequencies neuronally released ATP is the predominant neurotransmitter in mouse kidney. The important role of purinergic neurotransmission in rat and mice kidney has been described previously (Schwartz and Malik, 1989; Inscho, 2001; Vonend *et al*., 2005a, b).

RNS-induced pressor responses are mediated by a 'cocktail' of neuronally released NA and ATP. In the presence of the non-selective α-adrenoceptor blocker phentolamine, RNS-induced pressor responses were not diminished but rather increased. Thus, one can speculate that neuronal ATP release is facilitated by blocking the presynaptic inhibitory α-adrenoceptors. Cleary and co-workers observed an increase in purinergic contractions in the presence of high concentrations of an α_1-adrenoceptor blocker in mouse vas deferens. It was assumed that the high antagonist concentrations used had blocked presynaptic inhibitory α_2-adrenoceptors to enhance ATP release from sympathetic nerve endings (Cleary *et al*., 2003). The amount of ATP in the renal effluent is not a reliable indicator of neuronal ATP release since large amounts of ATP are also released non-neuronally (Vonend *et al*., 2002). Therefore pressor responses to RNS in the presence of complete α_1- and $\alpha_{2B/C}$-adrenoceptor blockade by prazosin were analysed. These α-adrenoceptor blockade-resistant, purinergic pressor responses better reflect neuronal ATP release. Further addition of the α_2-adrenoceptor antagonist rauwolscine caused a significant increase in purinergic pressor responses in WT mice. As this effect was absent in α_{2A}-adrenoceptor knockout mice the hypothesis that α_{2A}-adrenoceptors are presynaptic heteroceptors mediating neuronal ATP release is feasible.

The assumption that ATP in turn modulates neuronal NA release was not confirmed in mice kidneys. Previously, it has been observed that ATP and analogues inhibit sympathetic nerve stimulation-induced NA release by the activation of presynaptic P_{2Y} receptors (von Kugelgen *et al*., 1999; Queiroz *et al*., 2003). In addition, excitatory P_{2X}-receptors have also been shown to mediate an increase in stimulation-induced NA release by ATP (Bohmann *et al*., 1997; Queiroz *et al*., 2003; Sesti *et al*., 2003). Although exogenous ATP had no effect on RNS-induced NA release in the present study, ATP strongly enhanced basal NA release. Other ATP analogues that were tested, in order to characterize the P_2-receptor subtype involved, were without any effect. As PPADS blocked the increase in basal NA release by ATP, the observed effect is likely to be mediated by a P_{2X}-receptor (Lambrecht, 2000).

In conclusion, the α_{2A}-receptor represents the presynaptic α-adrenoceptor subtype that inhibits RNS-induced NA release in mice kidney. Moreover, the α_{2A}-receptor acts as a heteroceptor and also mediates ATP release. However, ATP had no influence on RNS-induced NA release but increased basal NA release by a yet uncharacterized mechanism.

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Conflict of interest

The authors state no conflict of interest.

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