β_3 adrenergic receptor in the kidney may be a new player in sympathetic regulation of renal function

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see commentary on page 471

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To date, the study of the sympathetic regulation of renal function has been restricted to the important contribution of β_1- and β_2-adrenergic receptors (ARs). Here we investigate the expression and the possible physiologic role of β_3-adrenergic receptor (β_3-AR) in mouse kidney. The β_3-AR is expressed in most of the nephron segments that also express the type 2 vasopressin receptor (AVPR2), including the thick ascending limb and the cortical and outer medullary collecting duct. *Ex vivo* experiments in mouse kidney tubules showed that β_3-AR stimulation with the selective agonist BRL37344 increased intracellular cAMP levels and promoted 2 key processes in the urine concentrating mechanism. These are accumulation of the water channel aquaporin 2 at the apical plasma membrane in the collecting duct and activation of the Na-K-2Cl symporter in the thick ascending limb. Both effects were prevented by the β_3-AR antagonist L748,337 or by the protein kinase A inhibitor H89. Interestingly, genetic inactivation of β3-AR in mice was associated with significantly increased urine excretion of water, sodium, potassium, and chloride. Stimulation of β3-AR significantly reduced urine excretion of water and the same electrolytes. Moreover, BRL37344 promoted a potent antidiuretic effect in AVPR2-null mice. Thus, our findings are of potential physiologic importance as they uncover the antidiuretic effect of β_3-AR stimulation in the kidney. Hence, β_3-AR agonism might be useful to bypass AVPR2-inactivating mutations.

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In the kidney, the antidiuretic hormone arginine vasopressin (AVP) is a critical regulator of water and electrolyte homeostasis. AVP is released from the pituitary gland into the bloodstream and binds to the type 2 vasopressin receptor (AVPR2),[1] a G protein–coupled receptor localized in the thick ascending limb of Henle, the distal convolute tubule, and the collecting duct, acting mainly through the cAMP–protein kinase A pathway.

In the thick ascending limb of Henle, AVP stimulates NaCl reabsorption across the Na-K-Cl cotransporter (NKCC2), increasing its phosphorylation,[2] thus generating the cortico-medullary osmotic gradient providing the driving force for water reabsorption in the kidney tubules.

In the CD, AVP stimulates the exocytosis of the water channel aquaporin 2 (AQP2)[3] at the apical membrane of the principal cells, dramatically increasing water reabsorption (for a review, see ref. 4). Inactivating mutations of the *AVPR2* gene cause X-linked nephrogenic diabetes insipidus (XNDI), characterized by constant diuresis and the risk of severe dehydration.[5] Many studies have shown that hormones other than AVP also exhibit antidiuretic effect,[6–10] suggesting novel strategies to manage XNDI.

The β-adrenergic system controls several renal functions. In particular, types 1 and 2 β-adrenoreceptors ($\beta_{1\text{-}2}$-AR)[11] regulate renal blood flow, glomerular filtration rate (GFR), sodium and water reabsorption, acid-base balance, and secretion of renin (for a review, see Johns *et al.*[12]).

Among β-ARs, the β_3-AR is the last identified member of this family. At first, it was shown to regulate lipolysis and thermogenesis in adipose tissue,[13] whereas subsequently it was shown to play important roles in the pathophysiology of the cardiovascular[14] and urinary[15] systems. However, its expression and possible physiologic role in the kidney remains to be fully clarified. There are indications in mice that β_3-AR mRNA is expressed by renal arteries.[16] In addition, in the rat kidney, a cDNA microarray screening showed that β_3-AR is expressed in the kidney medulla.[17] Moreover, in humans, β_3-AR polymorphisms seem to be associated with the effect of thiazide diuretics,[16,18] suggesting a role for β_3-AR in regulating renal water reabsorption. In this respect, demonstrating this novel role of β_3-AR in renal physiology is particularly intriguing in light of potential therapeutic applications of β_3-AR–acting drugs in diseases characterized by

altered diuresis. Moreover, β_3-AR is relatively resistant to agonist-induced desensitization,[19] which would ensure prolonged pharmacologic stimulation *in vivo*. In addition, due to the limited number of tissues expressing β_3-AR, compared with $\beta_{1\text{-}2}$-AR, β_3-AR agonists are supposed to show a low systemic off-target effect.[14]

RESULTS

β_3-AR expression in the mouse kidney

Reverse transcriptase polymerase chain reaction revealed that β_3-AR mRNA was clearly detectable in the RNA samples from the mouse kidney, brown adipose tissue, and bladder (Figure 1a). In particular, the intron-spanning primers amplified 2 bands of 234 bp and 337 bp, representing β_{3a}-AR and β_{3b}-AR transcripts, respectively.[20] Sequencing confirmed the specificity of the obtained bands (data not shown).

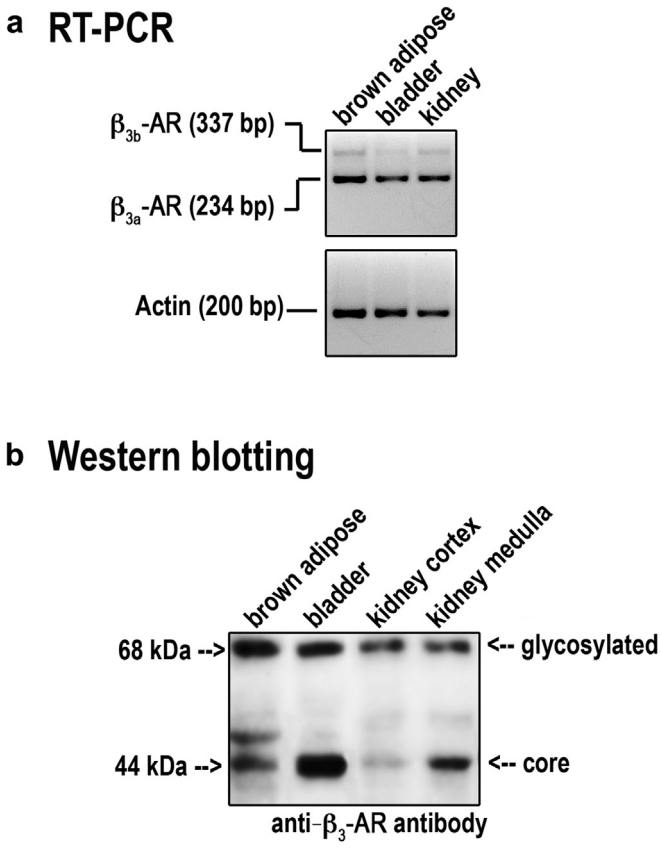

Figure 1 | Expression of β_3-ARs mRNA and protein in mouse kidney. (**a**) Total RNA from mouse kidney was probed for the presence of mRNA coding β_3-adrenergic receptors (β_3-ARs). Brown adipose tissue and bladder were used as positive controls. Two amplicons corresponding to β_{3b}-AR (337 bp) and β_{3a}-AR (234 bp) were visualized in all samples. Control reverse transcriptase polymerase chain reaction was performed using primers amplifying mouse β-actin. (**b**) Total protein extracts from mouse kidney cortex and total medulla were analyzed by Western blotting using anti–β_3-AR antibodies. Two bands, corresponding to the core and the glycosylated protein, were detected in the kidney fractions at the same molecular size as those revealed in brown adipose and urinary bladder. Experiments were repeated 3 times with comparable results. RT-PCR, reverse transcriptase polymerase chain reaction.

Immunoblotting analysis revealed that mouse kidney cortex and total medulla expressed a band of 44 kDa for the core protein and 1 at 68 kDa for the glycosylated form in all samples (Figure 1b). Both bands were also revealed in β_3-AR–expressing control tissues.

Immunolocalization of β_3-ARs in the mouse kidney

As shown in Figure 2, β_3-AR was expressed at the apical and basolateral membrane of the epithelial cells of the thin ascending limb, identified by the presence of the kidney-specific chloride channel ClC-K1.[21,22] β_3-AR was also localized at the basolateral membrane of the epithelial cells of (i) the thick ascending limb of Henle, expressing the apical NKCC2 cotransporter[23]; (ii) the distal convolute tubule, expressing the apical thiazide-sensitive NaCl symporter (NCC)[24]; and (iii) the cortical CD and the outer medullary CD (the latter not shown), expressing AQP2 at the apical membrane.[25] The staining for β_3-AR completely disappeared when the anti–β_3-AR antibody used for immunofluorescence was preadsorbed on its immunizing peptide (Supplementary Figure S1).

We also demonstrated that β_3-AR was neither expressed in the proximal convolute tubule nor in the thin descending limb of Henle's loop, the inner medullary CD, and the *vasa recta* (Supplementary Figure S2). Overall, the current data show that β_3-AR is localized in those nephron tracts also expressing AVPR2.

Effect of β_3-AR activation on cAMP production, AQP2 trafficking, and NKCC2 phosphorylation: *ex vivo* experiments

Our finding that β_3-AR is expressed in the AVPR2-positive kidney segments prompted us to investigate whether β_3-AR activation may mimic the effect of AVP on cAMP production, AQP2 intracellular trafficking, and NKCC2 activation. Using an *ex vivo* model consisting of freshly isolated mouse kidney tubule suspensions, we measured changes in intracellular cAMP concentrations in response to either the specific β_3-AR agonist BRL37344 (1, 10, 100 μM) or the AVP analog 1-deamino-8-D-arginine-vasopressin (dDAVP), 10^{-7} M, used as positive control for cAMP production (Figure 3a). Results are reported as the percentage of the cAMP concentration measured in resting tubules. Treatment with BRL37344 led to a concentration-dependent increase in intracellular cAMP levels, with the maximal effect observed at 10 μM (+173%, $P < 0.0001$).

Accordingly, we used 10 μM BRL37344 for all the following experiments performed in freshly isolated live mouse kidney slices, untreated (resting) or incubated with either dDAVP or BRL37344 (Figure 3b). Confocal microscopy showed that both BRL37344 and dDAVP promoted AQP2 accumulation at the luminal plasma membrane of cortical collecting duct cells (Figure 3b, white arrows) compared with the cytoplasmic localization of AQP2 observed in control slices (Figure 3b, white arrowheads). In line with the absence of β_3-AR in the inner medullary CD, BRL37344 failed to induce AQP2 apical accumulation in this portion of the CD (not shown). Importantly, the effect of BRL37344 was prevented by