Dietary sodium modulates the interaction between efferent and afferent renal nerve activity by altering activation of $\alpha_2$-adrenoceptors on renal sensory nerves

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Kopp UC, Cicha MZ, Smith LA, Ruohonena S, Scheinin M, Fritz N, Hökfelt T. Dietary sodium modulates the interaction between efferent and afferent renal nerve activity by altering activation of $\alpha_2$-adrenoceptors on renal sensory nerves. Am J Physiol Regul Integr Comp Physiol 300: R298–R310, 2011. First published November 24, 2010; doi:10.1152/ajpregu.00469.2010.—Activation of efferent renal sympathetic nerve activity (ERSNA) increases afferent renal nerve activity (ARNA), which then reflexively decreases ARNA via activation of the renorenal reflex to maintain low ERSNA. The ERSNA-ARNA interaction is mediated by norepinephrine (NE) that increases and decreases ARNA by activation of renal $\alpha_1$-and $\alpha_2$-adrenoceptors (AR), respectively. The ERSNA-induced increases in ARNA are suppressed during a low-sodium (2,470 ± 770% s) and enhanced during a high-sodium diet (5,670 ± 1,260% s). We examined the role of $\alpha_2$-AR in modulating the responsiveness of renal sensory nerves during low- and high-sodium diets. Immunohistochemical analysis suggested the presence of $\alpha_2A$-AR and $\alpha_2C$-AR subtypes on renal sensory nerves. During the low-sodium diet, renal pelvic administration of the $\alpha_2$-AR antagonist rauwolscine or the AT1 receptor antagonist losartan alone failed to alter the ARNA responses to reflex increases in ERSNA. Likewise, renal pelvic release of substance P produced by 250 pM NE (from 8.0 ± 1.3 to 8.5 ± 1.6 pg/min) was not affected by rauwolscine or losartan alone. However, rauwolscine+losartan enhanced the ARNA responses to reflex increases in ERSNA (4,680 ± 1,240%-s), and renal pelvic release of substance P by 250 pM NE, from 8.3 ± 0.6 to 14.2 ± 0.8 pg/min. During a high-sodium diet, rauwolscine had no effect on the ARNA response to reflex increases in ERSNA or renal pelvic release of substance P produced by NE. Losartan was not examined because of low endogenous ANG II levels in renal pelvic tissue during a high-sodium diet. Increased activation of $\alpha_2$-AR contributes to the reduced interaction between ERSNA and ARNA during low-sodium intake, whereas no/minimal activation of $\alpha_2$-AR contributes to the enhanced ERSNA-ARNA interaction under conditions of high sodium intake.

The kidney has a rich supply of sympathetic nerves, which innervate all parts of the kidney (5). The kidney also has abundant afferent innervation; the fibers proceed from the kidney to the neuraxis and contain substance P and calcitonin gene-related peptide (CGRP) as primary sensory neurotransmitters. The sensory nerve endings are located primarily in the renal pelvic wall (15, 21, 22, 24, 29). Sympathetic efferent nerve fibers and afferent sensory nerve fibers often run separately but intertwined in the same nerve bundles in the renal pelvic smooth muscle layer (22), providing anatomical support for a possible functional interaction between efferent renal sympathetic nerve activity (ERSNA) and afferent renal nerve activity (ARNA). Indeed, increasing ARNA by increasing renal pelvic pressure leads to a reflex decrease in ERSNA and increased natriuresis, i.e., a renorenal reflex response (26). The responsiveness of the renal sensory nerves is also modulated by ERSNA. Reflex increases and decreases in ERSNA increase and decrease ARNA, respectively (22, 24, 27). Taken together, there is strong evidence to support a negative feedback system, in which increases in ERSNA increase ARNA, which, in turn, decrease ERSNA via activation of the renorenal reflex. Changes in ERSNA modulate ARNA by the release of the neurotransmitter norepinephrine (NE), which activates $\alpha_1$-adrenoceptors (AR) and $\alpha_2$-AR on renal sensory nerves, leading to increases and decreases in ARNA, respectively (22). The physiological importance of the ERSNA-induced increases in ARNA is underlined by the interaction being modulated by dietary sodium. A high-sodium diet enhances and a low-sodium diet reduces the ERSNA-induced increases in ARNA (24). Under the high-sodium dietary condition, enhancement of the ERSNA-induced increases in ARNA would increase the inhibitory renorenal reflex control of ERSNA, resulting in reduction of ERSNA to prevent or limit sodium retention. Conversely, in low-sodium dietary conditions, suppression of the ERSNA-induced increases in ARNA would result in increased ERSNA by reducing the renorenal reflex inhibition of ERSNA, eventually leading to sodium retention. The importance of the renorenal reflex-induced inhibition of ERSNA in the control of body fluid and sodium homeostasis was demonstrated in our previous studies, which showed that rats lacking intact afferent renal innervation are characterized by increased ERSNA, increased responsiveness of ERSNA to various sympathetic stimuli, and increased arterial pressure when fed a high-sodium diet (17, 25).

The current studies were performed to examine the mechanisms involved in the altered responsiveness of the renal sensory nerves to increases in ERSNA produced by varying sodium dietary intake. The renin-angiotensin (ANG) system is one of the possible mechanisms involved in the diet-dependent modulation of the interaction between ERSNA and ARNA. Our previous studies examining mechanisms involved in the...
activation of the renal mechanosensory nerves showed that stretching the renal pelvic wall leads to induction of COX-2 and increased renal pelvic synthesis of PGE₂ (19, 20, 23). PGE₂ increases the release of substance P, which increases ARNA via activation of the cAMP-PKA signal transduction pathway. Under low-sodium dietary conditions, the reduced responsiveness of the renal mechanosensory nerves to increased renal pelvic pressure involves increased activation of ANG II type 1 (AT1) receptors. Activation of AT1 receptors inhibits the PGE₂-mediated activation of adenyl cyclase by a pertussis toxin (PTX)-sensitive mechanism (16). Because the ERSNA-induced increases in ARNA are dependent on intact PG synthesis (22), we reasoned that a similar mechanism(s), i.e., increased activation of AT1-receptors, would be involved in the reduced ARNA responses to increases in ERSNA in rats on low-sodium diets. However, in our initial studies, the AT1 receptor antagonist losartan failed to enhance the responsiveness of the renal sensory nerves to increases in ERSNA.

In view of the inhibitory effects of NE-mediated activation of α₂-AR on ARNA in rats on normal sodium diets (22), we then hypothesized that the reduced responsiveness of the renal sensory nerves to increases in ERSNA involves increased NE-mediated activation of renal pelvic α₂-AR in rats fed a low-sodium diet. If so, we reasoned that the enhanced responsiveness of the renal sensory nerves to increases in ERSNA in rats on a high-sodium diet would involve decreased NE-mediated activation of renal α₂-AR. We studied this hypothesis by examining the effects of renal pelvic administration of the α₂-AR antagonist rauwolscine (2) on the increases in ARNA produced by reflex increases in ERSNA by thermal cutaneous stimulation. Because the reflex-mediated increases in ERSNA were associated with increases in arterial pressure (AP) and heart rate (HR), these studies were complemented with studies in which we examined the ARNA responses to renal pelvic administration of NE at a concentration that did not alter systemic hemodynamics. To examine whether the effects produced by reflex increases in ARNA and NE on ARNA involved presynaptic or postsynaptic effects of NE, additional experiments were performed in an isolated renal pelvic wall preparation from rats on high- and low-sodium diets.

We also carried out immunohistochemical studies to determine whether α₂-ARs are located on or close to the sensory nerves and/or sympathetic nerves in the renal pelvic wall. We focused on the α₂A- and α₂C-AR subtypes because of their well-known expression on primary afferent neurons (7).

**METHODS**

The study was performed on male Sprague-Dawley rats weighing 202–464 g (mean 284 ± 5 g). Two weeks before the study, rats were placed on either sodium-deficient pellets (Na⁺ = 1.6 meq/kg; Dyets, Bethlehem, PA) and tap water as drinking fluid (low-sodium diet, n = 115) or normal-sodium pellets with 0.9% NaCl solution as drinking fluid (high-sodium diet, n = 16) (20).

The experimental protocols were approved by the Institutional Animal Care and Use Committee, and experiments were performed according to the “Guide for the Care and Use of Laboratory Animals” from the National Institutes of Health.

Anesthesia was induced with pentobarbital sodium (0.2 mmol/kg ip; Abbott Laboratories, Abbott Park, IL).

In Vivo Studies

After induction of anesthesia, an intravenous infusion of pentobarbital sodium (0.04 mmol·kg⁻¹·h⁻¹) at 50 μl/min into the femoral vein was started and maintained throughout the course of the experiment. Arterial pressure was recorded from a catheter in the femoral artery. The left renal pelvis was perfused with vehicle or various perfusates, described below (Groups I–VII), throughout the experiment at 20 μl/min via a PE-10 catheter placed inside a PE-60 catheter located in the ureter. ERSNA and ARNA were recorded from the central and peripheral portions, respectively, of the cut ends of two adjacent left renal nerve branches, which were placed on bipolar silver wire electrodes. ERSNA and ARNA were integrated over 1-s intervals, the unit of measure being microvolts per second per 1 second. All data were collected at 500 Hz and averaged over 2 s. Postmortem renal nerve activity, assessed by crushing the renal nerve bundles proximal or distal to the recording electrode, was subtracted from all values of ERSNA and ARNA, respectively. Renal nerve activity was expressed in percentage of its baseline value during the control period (15–27).

Stimulation of renal sensory nerves. Renal sensory nerves were stimulated by reflex-mediated increases in ERSNA, which were produced by placing the rat’s tail in 47°C water (22, 24) or renal pelvic administration of NE. In all experimental groups, a 10-min control and a 10-min recovery period bracketed the experimental period.

Group I, low-sodium diet: effects of an AT1 receptor antagonist on the ARNA responses to reflex increases in ERSNA. The experiments were divided into two parts. During each part, the rat’s tail was placed in 47°C water during two 3-min experimental periods. Following each experimental period, the rat’s tail was immediately placed in room-temperature water to quickly terminate the heat stimulation. Twenty minutes after the first recovery period, the renal pelvic perfusate was switched from vehicle to losartan (0.44 mM; n = 8) (16, 18). Ten minutes later, the control, experimental, and recovery periods were repeated.

Group II, low-sodium diet: effects of an α₂-AR antagonist on the ARNA responses to reflex increases in ERSNA. These experiments used a similar protocol as Group I, except rauwolscine, 0.1 μM (22), was administered into the renal pelvis (n = 14).

Group III, low-sodium diet: effects of an AT1 receptor antagonist plus an α₂-AR antagonist on the ARNA responses to reflex increases in ERSNA. These experiments used a similar protocol as Groups I and II, except losartan + rauwolscine were administered into the renal pelvis (n = 12).

Group IV, high-sodium diet: effects of an α₂-AR antagonist on the ARNA responses to reflex increases in ERSNA. The experiments performed in rats fed the high-sodium diet used a similar protocol as in Group II (n = 8).

Groups V–VII, low-sodium diet: effects of an AT1 receptor antagonist, an α₂-AR antagonist, and an AT1 receptor antagonist plus an α₂-AR antagonist on the ARNA responses to reflex increases in ERSNA. The experiments were divided into three parts. During each part, 10 pM of NE, subthreshold concentration of NE for activation of renal sensory nerves in low-sodium diet rats (24), was administered into the renal pelvis during three 5-min experimental periods. In Group V (n = 7), the renal pelvic perfusate was switched from vehicle to 0.44 μM losartan at the end of the first recovery period. Five minutes later, the control, experimental, and recovery periods were repeated. At the end of the second recovery period, the renal pelvic perfusate was switched from losartan to losartan + rauwolscine. Five minutes later, the control, experimental, and recovery periods were repeated once more. In Group VI (n = 8), the experimental protocol was similar, except rauwolscine was administered instead of losartan at the end of the first recovery period. In Group VII (n = 5), only two control, experimental, and recovery periods were performed, the first part in the presence of vehicle and the second part in the presence of losartan + rauwolscine.
In Vitro Studies

To study whether the mechanisms involved in the altered responsiveness of the afferent renal nerves to NE in low- and high-sodium diets involve presynaptic or postsynaptic mechanisms, we examined the mechanisms of the NE-mediated release of substance P in an isolated renal pelvic wall preparation (14–24). NE increases substance P release by a PG-dependent mechanism (22). Therefore, we also examined whether the altered responsiveness of the renal sensory nerves to NE in rats fed low- and high-sodium diets was associated with changes in NE-induced PGE2 release.

The isolated renal pelvic wall preparation has previously been described in detail (14–24). In brief, following anesthesia renal pelvises dissected from the kidneys were placed in wells containing 400 μl HEPES buffer maintained at 37°C. Each well contained the pelvic wall from one kidney. Throughout the experiment, the incubation medium was replaced with fresh HEPES every 5 min. The incubation medium, 1.25% (wt/vol) paraformaldehyde and 0.2% (wt/vol) picric acid. The kidneys were quickly dissected, postfixed in fixative for 90 min, and stored in −80°C for later analysis of substance P and PGE2. After a 2-h equilibration period, the experiment was started with four 5-min control periods followed by one 5-min experimental period and four 5-min recovery periods. NE was added to the incubation bath to both pelvises during the experimental periods.

Group VIII, low-sodium diet: effects of an AT1 receptor antagonist on the NE-induced increases in substance P and PGE2. One pelvis was incubated in HEPES buffer, and the other pelvis was incubated in HEPES buffer containing losartan (0.44 mM) throughout the control, experimental, and recovery periods. During the experimental period, both pelvises were exposed to NE at 6,250 pM (n = 4), 1,250 pM (n = 8), or 250 pM (n = 8).—threshold and subthreshold concentrations for substance P release in rats on a low-sodium diet (24). Renal pelvic release of substance P and PGE2 into the incubation bath was measured throughout the experiment.

Group IX, low-sodium diet: effects of an α2-AR antagonist on the NE-induced increases in substance P and PGE2. These experiments used a similar protocol as Group VIII, except one pelvis was incubated in HEPES buffer containing 0.1 μM rauwolscine instead of losartan. During the experimental period, both pelvises were exposed to NE at 6,250 pM (n = 10) or 1,250 pM (n = 6)—threshold and subthreshold concentrations for substance P release in rats on a low-sodium diet (24). Renal pelvic release of substance P and PGE2 into the incubation bath was measured throughout the experiment. Group X, high-sodium diet: effects of an AT1 receptor antagonist plus an α3-AR antagonist on the NE-induced increases in substance P and PGE2. One pelvis was incubated in HEPES buffer containing losartan (n = 8) or rauwolscine (n = 16), and the other pelvis was incubated in HEPES buffer containing losartan + rauwolscine throughout the control, experimental, and recovery periods. During the experimental period, both pelvises were exposed to NE at 250 pM.

Group XII, high-sodium diet: effects of an α2-AR antagonist on the NE-induced increases in substance P and PGE2. These experiments used a similar protocol as Group IX. During the experimental period, both pelvises were exposed to NE at 2 pM, a subthreshold concentration for substance P release in rats on a high-sodium diet (24).

Immunohistochemistry. The immunohistochemical procedures have been previously described in detail (15, 21, 22, 24). In brief, anesthetized male Sprague-Dawley rats fed a low- or high-sodium diet were transcardially perfused with calcium-free Tyrode solution followed by phosphate-buffered (0.1 M, pH 7.4) fixative containing 4% wt/vol paraformaldehyde and 0.2% wt/vol picric acid. The kidneys were quickly dissected, postfixed in fixative for 90 min, and stored in 10% sucrose at 4°C. Fourteen-micrometer-thick sections were cut on a cryostat and thaw-mounted onto gelatin-coated slides. All primary antibodies were incubated for 24 h at 4°C.

The sections were incubated with antisera against α2A-AR or α2C-AR [rabbit 1:2,000 (45)]. Immunoreactivity was visualized using the tyramide signal amplification system (TSA-Plus: PerkinElmer Life and Analytical Sciences, Waltham, MA). After completion of the protocol for TSA for detection of α2A-AR and α2C-AR, the tissue sections were incubated with antiserum for CGRP (mouse; 1:400; Drs. J. H. Walsh and H. C. Wong), a marker for sensory nerves, or the NE transporter (NE-1) (rabbit; 1:500; HA004063, www.proteinatlas.org) (22), a marker for sympathetic nerves, and processed by the indirect immunofluorescence technique.

The specificity of the antisera for α2A-AR and α2C-AR was tested by preincubation of the primary antisera with an excess amount (10−5 M) of the fusion protein used as immunogen and by labeling cultured Chinese hamster ovary (CHO) cells with antisera against α2A-AR or α2C-AR. These CHO cells had been transfected to express human α2A-AR or α2C-AR (36) or represented wild-type (WT) CHO cells devoid of α2-AR expression.

The tissue sections were examined using a Nikon Eclipse E600 fluorescence microscope (Tokyo, Japan) equipped with epifluorescence and the appropriate filter combinations. Photographs were taken with a Hamamatsu ORCA-ER C4762-80 digital camera (Hamamatsu City, Japan) using Hamamatsu photonics Wasabi software. For confocal analysis, a Radiance Plus confocal laser scanning system (Bio-Rad, Hemel Hempstead, UK) installed on a Nikon Eclipse E600 fluorescence microscope was used. Digital images from the microscopy were optimized for image resolution, brightness, and contrast, and color images were merged using Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA).

Drugs and Reagents

Substance P antibody (IHC 7,451) was acquired from Peninsula Laboratories (San Carlos, CA) and PGE2 from Cayman Chemicals (Ann Arbor, MI). All other reagents/chemicals were from Sigma Aldrich (St. Louis, MO) unless otherwise stated. NE was dissolved in 0.1% ascorbic acid in incubation buffer or 0.15 M NaCl. All other agents were dissolved in incubation buffer (in vitro studies) or 0.15 M NaCl (in vivo studies).

Analytical Procedures

Concentrations of Substance P and PGE2 in the incubation medium were measured by ELISA, as previously described in detail (14–24).

Statistical Analysis

In vivo, increases in ERSNA, ARNA, mean AP (MAP), and HR produced by placing the rat’s tail in warm water were evaluated by calculating the area under the curve for each parameter vs. time, with baseline being the average value of each control period. Likewise, the ARNA responses to NE were calculated as the area under the curve of ARNA vs. time. In vitro, the release of substance P and PGE2 during the experimental period was compared with the substance P and PGE2 release during the control and recovery periods. Because not all data were normally distributed (D’Agostino and Pearson, omnibus normality tests), nonparametric analysis methods were used. The data were analyzed by Wilcoxon signed rank test and Friedman one-way ANOVA with repeated measures followed by Dunn’s multiple-comparison test (GraphPad Prism 5.03, GraphPad Software, La Jolla, CA).

Table 1. Effects of thermal cutaneous stimulation on MAP, HR, ERSNA, and ARNA in rats fed a low- and high-sodium diet during vehicle administration

<table>
<thead>
<tr>
<th>n</th>
<th>Low-Sodium Diet</th>
<th>High-Sodium Diet</th>
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</thead>
<tbody>
<tr>
<td>ΔMAP, mmHg/s</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>ΔHR, beats/min</td>
<td>22,010 ± 2390*</td>
<td>35,090 ± 6650*</td>
</tr>
<tr>
<td>ΔERSNA, %/s</td>
<td>7920 ± 860*</td>
<td>12,910 ± 3780*</td>
</tr>
<tr>
<td>ΔARNa, %/s</td>
<td>2220 ± 450*</td>
<td>5670 ± 1260*</td>
</tr>
</tbody>
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Responses of mean arterial pressure (ΔMAP), heart rate (ΔHR), efferent renal sympathetic nerve activity (ERSNA) and afferent renal nerve activity (ARNA) are expressed as area under the curve of MAP, HR, ERSNA, and ARNA, respectively, vs. time. *P < 0.01 vs. baseline; †P < 0.05; ‡P < 0.01 vs. low-sodium diet.
A significance level of 5% was chosen. Data in the text and figures are expressed as means ± SE.

RESULTS

**In Vivo Studies**

Similar to our previous studies (24), placing the rat’s tail in warm water resulted in reversible increases in MAP, HR, ERSNA, and ARNA that were of a greater magnitude in rats fed a high-sodium diet than in rats fed a low-sodium diet, although the differences in the ERSNA responses to thermal cutaneous stimulation between the high- and low-sodium diet did not reach statistical significance (P > 0.05) in the current studies (Table 1).

**Groups I–III, low-sodium diet: effects of an AT1 receptor antagonist and an α2-AR antagonist alone and in combination on the ARNA responses to reflex increases in ERSNA.** Because endogenous ANG plays an inhibitory role in the activation of renal mechanosensory nerves by increased renal pelvic pressure (18) in rats fed a low-sodium diet, we reasoned that losartan would enhance the suppressed ARNA responses to increases in ERSNA in rats on a low-sodium diet. However, renal pelvic perfusion with losartan failed to enhance the ARNA responses to increases in ERSNA, as shown in Fig. 1A. In view of the inhibitory effects of the NE-mediated activation of α2-AR on ARNA in rats fed a normal-sodium diet (22), we then hypothesized that an α2-AR antagonist would enhance the ARNA responses to increases in ERSNA. In contrast to our previous findings in rats on a low-sodium diet that suggested important inhibitory roles for both α2-AR and AT1 receptors in the ERSNA-induced increases in ARNA, we next examined the role of α2-AR in the activation of renal sensory nerves in rats fed a high-sodium diet, a physiological condition of low

**Group IV, high-sodium diet: effects of an α2-AR antagonist on the ARNA responses to reflex increases in ERSNA.** Because our findings in rats on a low-sodium diet suggested important inhibitory roles for both α2-AR and AT1 receptors in the ERSNA-induced increases in ARNA, we next examined the role of α2-AR in the activation of renal sensory nerves in rats fed a high-sodium diet, a physiological condition of low

**Effects of losartan and rauwolscine on the ERSNA and ARNA responses to thermal cutaneous stimulation - Low sodium diet**

![Graph A](http://example.com/graph_a.png)

**Graph A:**

- Δ ERSNA % s (AUC) vs. Δ ARNA % s (AUC) for vehicle, losartan, and rauwolscine treatments.

**Graph B:**

- Δ ERSNA % s (AUC) vs. Δ ARNA % s (AUC) for vehicle, losartan, and rauwolscine treatments.

**Graph C:**

- Δ ERSNA % s (AUC) vs. Δ ARNA % s (AUC) for vehicle, losartan, and rauwolscine treatments.

**Effects of rauwolscine on the ERSNA and ARNA responses to thermal cutaneous stimulation - High sodium diet**

![Graph D](http://example.com/graph_d.png)

**Graph D:**

- Δ ERSNA % s (AUC) vs. Δ ARNA % s (AUC) for vehicle and rauwolscine treatments.

**Fig. 1. In vivo low-salt diet. Effects of renal pelvic administration of vehicle (open bar) and losartan, 0.44 mM, (hatched bar) (A), rauwolscine, 0.1 μM, (hatched bar) (B), or losartan + rauwolscine (cross-hatched bar) (C) on the increases in ERSNA and ARNA produced by placing the rat’s tail in 47°C water. *P < 0.05, **P < 0.01 vs. baseline. ‡P < 0.01, ARNA responses to thermal cutaneous stimulation in the absence and presence of renal pelvic perfusion with losartan + rauwolscine. ΔERSNA, efferent renal sympathetic nerve activity response; ΔARNA, afferent renal nerve activity response; AUC, area under the curve of RNA vs. time.

**Fig. 2. In vivo high-salt diet. Effects of renal pelvic administration of vehicle (open bar) and rauwolscine (hatched bar) on the increases in ERSNA and ARNA produced by placing the rat’s tail in 47°C water. **P < 0.01 vs. baseline.
Effects of losartan, rauwolscine and losartan+rauwolscine on the ARNA responses to NE - Low sodium diet

Fig. 3. In vivo low-sodium diet: Effects of renal pelvic administration of vehicle (open bar) and losartan (hatched bar), rauwolscine (hatched bar), or losartan+rauwolscine (cross-hatched bar) on the ARNA responses to renal pelvic administration of 10 pM NE. Losartan or rauwolscine (random order) preceded the administration of rauwolscine+losartan. *P < 0.05, **P < 0.01 vs. baseline, †P < 0.05, ‡P < 0.01, ARNA responses to NE during renal pelvic administration of rauwolscine+losartan vs. during vehicle administration. NE, norepinephrine.

endogenous renal ANG levels (5, 12, 16). In contrast to our findings in rats on a low-sodium diet treated with losartan, rauwolscine had no effect on the ERSNA-induced increases in ARNA (Fig. 2).

Groups V–VII, low-sodium diet: effects of an AT1 receptor antagonist and an α2-AR antagonist alone and in combination on the NE-induced increases in substance P and PGE2. Our in vivo studies suggested an important inhibitory role for NE-mediated activation of α2-AR in the reduced activation of renal sensory nerves in rats fed a low-sodium diet in the presence of AT1 receptor blockade. To examine whether this mechanism involved presynaptic or postsynaptic activation of α2-AR, we used the isolated renal pelvic wall preparation. Our previous in vitro studies showed that the threshold concentration of NE for ARNA responses to NE in the presence of vehicle and losartan+rauwolscine was observed in the experiments in which losartan or rauwolscine did not precede the losartan+rauwolscine perfusion (Group VII). In these rats, the ARNA responses to NE in the presence of vehicle and losartan+rauwolscine in the renal pelvis were 274 ± 137 and 1,859 ± 614%·s, respectively (P < 0.05 vs. vehicle, data not included in Fig. 3). MAP and HR were not affected by the renal pelvic administration of NE in any of the groups.

In Vitro Studies

Groups VIII–XI, low-sodium diet: effects of an AT1 receptor antagonist and an α2-AR antagonist alone and in combination on the NE-induced increases in substance P and PGE2. Our in vivo studies suggested an important inhibitory role for NE-mediated activation of α2-AR in the reduced activation of renal sensory nerves in rats fed a low-sodium diet in the presence of AT1 receptor blockade. To examine whether this mechanism involved presynaptic or postsynaptic activation of α2-AR, we used the isolated renal pelvic wall preparation. Our previous in vitro studies showed that the threshold concentration of NE for ARNA responses to NE in the presence of vehicle and losartan+rauwolscine was observed in the experiments in which losartan or rauwolscine did not precede the losartan+rauwolscine perfusion (Group VII). In these rats, the ARNA responses to NE in the presence of vehicle and losartan+rauwolscine in the renal pelvis were 274 ± 137 and 1,859 ± 614%·s, respectively (P < 0.05 vs. vehicle, data not included in Fig. 3). MAP and HR were not affected by the renal pelvic administration of NE in any of the groups.

Table 2. Effects of losartan, 0.44 mM, on the norepinephrine-mediated release of substance P and PGE2 from isolated renal pelvic wall preparations derived from low-sodium diet rats

<table>
<thead>
<tr>
<th>Substance P, pg/min</th>
<th>PGE2, pg/min</th>
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<tbody>
<tr>
<td>Control</td>
<td>NE 6250 pM</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td>Losartan</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5.3 ± 0.9</td>
</tr>
<tr>
<td>Losartan</td>
<td>5.6 ± 1.3</td>
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NE, norepinephrine. **P < 0.01, *P < 0.05 vs. average of control and recovery.
Table 4, 250 pM of NE resulted in significant increases in substance P and PGE$_2$ in the presence of rauwolscine plus losartan in the bath.

**Group XII, high-sodium diet:** effects of an $\alpha_2$-AR antagonist on the NE-induced increases in substance P and PGE$_2$. Adding rauwolscine to the incubation bath failed to enhance the increases in renal pelvic release of substance P and PGE$_2$ produced by 2 pM NE, subthreshold concentration of NE for activation of renal sensory nerves in rats fed a high-sodium diet (Fig. 5).

**Immunohistochemistry**

Localization of $\alpha_{2A}$- and $\alpha_{2C}$-AR in renal pelvis. Renal tissues were double labeled with antibodies against $\alpha_{2A}$-AR, $\alpha_{2C}$-AR, and CGRP or NE-t. As shown in Fig. 6, A–C and E–G, the antibodies against $\alpha_{2A}$-AR and $\alpha_{2C}$-AR labeled fibers that were on or close to CGRP-immunoreactive (ir) nerve fibers in the renal pelvic wall. Higher magnification suggested the presence of the $\alpha_{2A}$-AR being located on the renal sensory nerve fibers (Fig. 6, D and H). The $\alpha_{2C}$-AR antibody also labeled fibers on or close to NE-t-ir nerve fibers in the pelvic wall (Fig. 7, A–C), renal fat tissue (not shown), and blood vessels (Fig. 7D). No such labeling was observed with the $\alpha_{2A}$-AR antibody.

The $\alpha_{2A}$-AR and $\alpha_{2C}$-AR antibody labeling in the kidney was blocked by adsorption with the peptides used as immunogens for the generation of the $\alpha_{2A}$-AR and $\alpha_{2C}$-AR antibodies (Fig. 8A, a–d). $\alpha_{2A}$-AR-ir was only seen in CHO cells transfected to express $\alpha_{2A}$-AR (Fig. 8B, a–c). Likewise, $\alpha_{2C}$-AR-ir was only seen in CHO cells transfected to express $\alpha_{2C}$-AR (Fig. 8B, d–f). No labeling with either $\alpha_{2A}$-AR or $\alpha_{2C}$-AR antibodies was observed in nontransfected CHO cells.

**DISCUSSION**

The interaction between ERSNA and ARNA is modulated by dietary sodium (24). The present results show that reduced activation of ARNA by reflex increases in ERSNA of rats fed a low-sodium diet is not affected by renal pelvic perfusion with losartan or rauwolscine alone but is enhanced by the combined administration of these two drugs. Likewise, losartan or rauwolscine, but not either agent alone, enhanced the ARNA responses to renal pelvic administration of NE. In vitro studies in isolated renal pelvic wall preparations from rats fed a low-sodium diet showed that rauwolscine but not losartan, alone, enhanced the release of substance P and PGE$_2$ produced by 1,250 pM NE. At 250 pM, NE failed to increase substance P or PGE$_2$ release in the presence of either losartan or rauwolscine alone but increased substance P and PGE$_2$ significantly in the presence of both losartan and rauwolscine in the bath. In vivo and in vitro studies in rats fed a high-sodium diet showed that rauwolscine failed to enhance the responsiveness of the renal sensory nerves to reflex increases in ERSNA or NE. Immunohistochemical analysis suggested the presence of $\alpha_{2A}$-AR and $\alpha_{2C}$-AR on or close to the sensory nerves in the renal pelvic wall. Taken together, our studies support the notion that dietary sodium modulates the NE-mediated activation of $\alpha_2$-AR on renal pelvic sensory nerve endings, resulting in altered responsiveness of the renal sensory nerves to increases in ERSNA. Thus, in addition to endogenous ANG II inhibiting the responsiveness of the renal...
sensory nerves, increased activation of renal \(\alpha_2\)-AR appears to play an important inhibitory role in the interaction between ERSNA and ARNA in conditions of low sodium intake. Conversely, no or minimal activation of renal \(\alpha_2\)-AR facilitates the enhanced interaction between ERSNA and ARNA under high-sodium dietary conditions.

**Interaction Between ERSNA and ARNA**

Our previous studies in rats on a normal-sodium diet suggested that ERSNA increases ARNA by NE-mediated activation of \(\alpha_1\)-AR and decreases ARNA by NE-mediated activation of \(\alpha_2\)-AR (22). Our current functional studies showed that thermal cutaneous stimulation results in a general increase in sympathetic nerve activity, as evidenced by increases in MAP, HR, and ERSNA. The increases in these parameters were greater in the high- than in the low-sodium diet. The lack of statistically significant differences in the ERSNA responses to thermal cutaneous stimulation (\(P = 0.054\)) between rats fed high- and low-sodium diet in the current study is likely related to the magnitude of the ERSNA response being the result of both stimulatory reflexes (heating of the tail, per se) and inhibitory reflexes (activation of the renorenal and baroreceptor reflexes) (5). Nevertheless, the ERSNA-induced increases in ARNA differed in magnitude between the high- and low-sodium diet rats.

**Mechanisms involved in the reduced responsiveness of renal sensory nerves in the low-sodium diet.** Activation of endothelin A (ETA) receptors contributes to the reduced ERSNA-ARNA interaction (24). However, the ETA-receptor antagonist did not normalize the responsiveness of the renal sensory nerves to NE in the low-sodium diet, suggesting that additional mechanisms contribute to the reduced ERSNA-ARNA interaction. Because increased ANG II levels in the renal pelvic wall (16) reduced the ARNA responses to increased renal pelvic pressure in rats on the low-sodium diet (16, 24), we reasoned that increased activation of AT1 receptors would contribute to the reduced interaction between ERSNA and ARNA. AT1 receptors are located on renal vascular and tubular structures (32, 50) and, most importantly, in the renal pelvic wall (8, 10, 31, 50). However, our initial studies showed no effects of renal pelvic administration of losartan on the increases in ARNA produced by reflex increases in ERSNA or on the substance P release produced by 1,250 or 6,250 pM NE, supramaximal concentrations of NE for substance P release in rats on a normal-sodium diet. The threshold concentration of NE for substance P release is 250 pM in rats fed a normal-sodium diet (22). These studies suggested that additional mechanisms were involved in suppressing the responsiveness of renal sensory nerves to changes in ERSNA and NE.

In view of our studies in rats fed a normal-sodium diet, which showed that rauwolscine enhanced the interaction between ERSNA and ARNA (22), we reasoned that among other possible inhibitory mechanisms that may contribute to the reduced ERSNA-ARNA interaction during low-sodium intake would be increased activation of renal pelvic \(\alpha_2\)-AR. The involvement of \(\alpha_2\)-AR in the central nervous system cardiovascular regulation has long been known (35, 37), and activation of \(\alpha_2\)-AR on primary afferent neurons has anti nociceptive effects (34). Three subtypes of \(\alpha_2\)-AR have been cloned, \(\alpha_2A\), \(\alpha_2B\), and \(\alpha_2C\) (2). There is widespread distribution of \(\alpha_2A\)-AR and \(\alpha_2C\)-AR in the central nervous system (33). NE is suggested to have higher affinity for \(\alpha_2C\)-AR, which is activated at lower action potential frequencies (11, 35). Both \(\alpha_2A\)-AR and \(\alpha_2C\)-AR are expressed on primary afferent neurons in the spinal cord and dorsal root ganglia (DRG) (42, 43). In the central nervous system, the available evidence indicates a limited distribution of \(\alpha_2B\)-AR (7, 11, 42, 43). Studies examining the presence of \(\alpha_2B\)-AR on DRG have shown conflicting results, with one study showing a majority of the neurons expressing \(\alpha_2B\)-AR (9), and other studies showing only a few neurons expressing \(\alpha_2B\)-AR (3, 42). Whereas many neurons in lumbar DRG showed coexpression of \(\alpha_2A\)-AR and \(\alpha_2C\)-AR mRNA with CGRP mRNA, only a very few neurons showed coexpression of \(\alpha_2B\)-AR with CGRP (42). In the kidney, the \(\alpha_2A\)-AR transcript is present in the outer and inner medulla, and the renal pelvic wall (30). A similar distribution, albeit somewhat with lower intensity, was observed for \(\alpha_2C\)-AR mRNA. The distribution of mRNA for \(\alpha_2B\)-AR is quite different, with intense expression in the renal cortex and outer medulla but no expression in the inner medulla or renal pelvic cord and dorsal root ganglia (42, 43). In the central nervous system, the available evidence indicates a limited distribution of \(\alpha_2B\)-AR (7, 11, 42, 43). Studies examining the presence of \(\alpha_2B\)-AR on DRG have shown conflicting results, with one study showing a majority of the neurons expressing \(\alpha_2B\)-AR (9), and other studies showing only a few neurons expressing \(\alpha_2B\)-AR (3, 42). Whereas many neurons in lumbar DRG showed coexpression of \(\alpha_2A\)-AR and \(\alpha_2C\)-AR mRNA with CGRP mRNA, only a very few neurons showed coexpression of \(\alpha_2B\)-AR with CGRP (42). In the kidney, the \(\alpha_2A\)-AR transcript is present in the outer and inner medulla, and the renal pelvic wall (30). A similar distribution, albeit somewhat with lower intensity, was observed for \(\alpha_2C\)-AR mRNA. The distribution of mRNA for \(\alpha_2B\)-AR is quite different, with intense expression in the renal cortex and outer medulla but no expression in the inner medulla or renal pelvic

### Table 3. Effects of norepinephrine, 250 pM, on the release of substance P and PGE2 from isolated renal pelvic wall preparations derived from low-sodium diet rats

<table>
<thead>
<tr>
<th>Substance</th>
<th>Control</th>
<th>NE, 250 pM</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE2, pg/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>8.0 ± 1.2</td>
<td>8.5 ± 1.6</td>
<td>8.0 ± 1.4</td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>7.0 ± 1.0</td>
<td>9.4 ± 1.1</td>
<td>7.1 ± 1.0</td>
</tr>
</tbody>
</table>

NE, norepinephrine.

### Table 4. Effects of losartan alone and in combination with rauwolscine on the norepinephrine-mediated release of substance P and PGE2 from isolated renal pelvic wall preparations derived from low-sodium diet rats

<table>
<thead>
<tr>
<th>Substance</th>
<th>Control</th>
<th>NE, 250 pM</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE2, pg/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOS</td>
<td>5.9 ± 0.7</td>
<td>6.0 ± 0.7</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>LOS+RWC</td>
<td>4.5 ± 0.6</td>
<td>10.1 ± 1.4**</td>
<td>4.5 ± 0.7</td>
</tr>
</tbody>
</table>

NE, norepinephrine. LOS, losartan; RWC, rauwolscine. **P < 0.01 vs. average of control and recovery; †P < 0.01 vs. the NE-induced increase in substance P release during losartan alone.
There is limited immunohistochemical evidence for \( \alpha_2 \)-AR on peripheral sensory nerve endings. A recent study by Riedl et al. (40) demonstrated colocalization of \( \alpha_2 \)-AR-ir and substance P-ir in skin sensory nerves.

The results of the present studies showed that in contrast to losartan, rauwolscine enhanced the responsiveness of the renal sensory nerves to relatively high concentrations of NE, 1,250 and 6,250 pM in vitro. These findings demonstrated important suppressive effects of \( \alpha_2 \)-AR on renal sensory nerve activation in rats fed a low-sodium diet and suggested that activation of \( \alpha_2 \)-AR by pharmacological concentrations of NE can suppress the responsiveness of the renal sensory nerves to physiological activation of AT1 receptors produced by a low-sodium diet. The lack of an effect of rauwolscine alone on the responsiveness of the renal sensory nerves to stimuli of a more physiological nature, e.g., thermal cutaneous stimulation in vivo and/or 250 pM NE in vitro in rats on a low-sodium diet, would support this hypothesis.

It is unlikely that the lack of effects of renal pelvic administration of rauwolscine on the activation of the renal sensory nerves in vivo and in vitro were due to rauwolscine not producing significant blockade of renal pelvic \( \alpha_2 \)-AR. Rauwolscine was administered at a concentration, 0.1 \( \mu \)M, which is 25- and 500-fold higher than its \( K_i \) for \( \alpha_{2A}-\)AR and \( \alpha_{2C}-\)AR, respectively (2). Furthermore, the concentration of rauwolscine used in the current study is 10-fold higher than that shown to block renal vasoconstrictor responses to the \( \alpha_2 \)-AR agonists clonidine and guanabenz (6). In addition, our previous studies showed that 0.1 \( \mu \)M rauwolscine enhances the activation of renal sensory nerves by increases in ERSNA in vivo and NE in vitro in rats fed a normal-sodium diet (22), a dietary condition characterized by low renal tissue levels of ANG II levels (12). Rather, we reasoned that suppression of the ARNA responses to sympathetic stimuli of a magnitude within the physiological range may involve increased activation of both \( \alpha_2 \)-AR and AT1 receptors. If so, concurrent blockade of \( \alpha_2 \)-AR and AT1 receptors would enhance the responsiveness of the renal sen-

Fig. 5. In vitro isolated renal pelvises, high-sodium diet: effects of 2 pM NE on the release of substance P and PGE\(_2\) in the presence of vehicle (solid line) or 0.1 \( \mu \)M rauwolscine (dashed line) in the bath.

Fig. 6. Immunofluorescence double-labeling of renal tissue with antibodies against \( \alpha_{2A} \)-adrenoceptors (AR), \( \alpha_{2C} \)-AR (green) and calcitonin gene-related peptide (CGRP; red) shows \( \alpha_{2A} \)-AR-immunoreactive (ir) fibers (A) and \( \alpha_{2C} \)-AR-ir fibers (E) close or on CGRP-ir sensory nerve fibers (B, C, and F, G, respectively) (colocalization yellow, arrows) in the renal pelvic wall. Higher magnification showed \( \alpha_{2A} \)-AR-ir (green, D) and \( \alpha_{2C} \)-AR-ir (green, H) on CGRP-ir fibers (red), colocalization yellow (arrows).
sory nerves to increases in ERSNA and NE. The results confirm our hypothesis. Renal pelvic administration of losartan + rauwolscine enhanced the ARNA responses to increases in ERSNA in vivo and lowered the responsiveness of the renal sensory nerves to NE for substance P release to 250 pM in vitro. The similar findings in vivo and in vitro suggested a mechanism(s) at the sensory nerve endings, the isolated renal pelvic wall preparation being sympathetically denervated.

The mechanisms by which losartan + rauwolscine enhanced the interaction between ERSNA and ARNA are currently unknown. Activation of the renal sensory nerves by increases in ERSNA and NE is dependent on intact PG synthesis (22). Our previous studies showed that increased activation of AT1 receptors reduced the ARNA response to increased renal pelvic pressure by inhibiting the PGE2-mediated activation of adenylyl cyclase by a PTX-sensitive mechanism (16). These findings, together with the α2-AR being coupled to PTX-sensitive Gqi proteins (11, 48), suggest a similar mechanism being involved in the ANG II and NE-mediated suppression of the NE-mediated activation of renal sensory nerves in low-sodium dietary conditions. However, this reasoning does not explain the increases in NE-induced PGE2 release in the presence of rauwolscine and losartan. The increased PGE2 release may be related to cross-talk among the second messenger systems activated by Gqi, Gs, and/or Gz protein-coupled receptors. Activation of Gq-coupled receptors has been shown to affect PLC and/or PKC activity either directly or via inhibition of the AC/cAMP transduction pathway in neuronal and nonneuronal cells (1, 28, 38, 47). The relevance of these findings to the current studies relates to our previous studies, showing that activation of renal mechanosensory nerves involves activation of the PKC and cAMP/PKA transduction cascades (16, 23).

**Mechanisms involved in the enhanced responsiveness of renal sensory nerves under high-sodium dietary conditions.** A high-sodium diet enhances the responsiveness of the renal sensory nerves to NE, as evidenced by the greater ARNA responses to increases in ERSNA and the threshold concentration of NE for substance P release being 10 pM in high- vs. 6,250 pM in low-sodium dietary conditions (24). Our previous studies suggested an important role for ET-mediated activation of ETB receptors in the enhanced ARNA response to increases in ERSNA involving an interaction between α1-AR and ETB receptors (24). The present findings showed that rauwolscine has no effect on the ARNA responses to increases in ERSNA or the substance P release produced by 2 pM NE, a subthreshold concentration of NE for substance P release in rats on a high-sodium diet rats (24). It is unlikely that adding losartan to the renal pelvic perfusate containing rauwolscine would have resulted in increased responsiveness of the renal sensory nerves to increases in ERSNA and/or 2 pM NE in rats fed a high-sodium diet. Our previous studies showed that rats fed a high-sodium diet had markedly reduced endogenous ANG II concentrations in renal pelvic tissue compared with rats fed a low-sodium diet (16). It is highly likely that the responsiveness of the renal sensory nerves is modulated by renal pelvic tissue.

**Fig. 7.** Immunofluorescence double-labeling of renal tissue with antibodies against α2C-AR (green) and NE-t (red) shows α2C-AR-ir fibers (A) close or on NE-t-ir fibers (B, C) (colocalization yellow, arrows) in the renal pelvic wall. Higher magnification showed α2C-AR-ir (green, D) on NE-t-ir fibers (red), colocalization yellow (arrows) on a vessel in renal cortical tissue.
concentration and not urinary sodium concentration of ANG II because dietary sodium modulates the activation of the renal sensory nerves in a similar fashion in vivo and in vitro, the incubation bath being same for pelvises of rats on either a high or low-sodium diet. Furthermore, renal pelvic administration of losartan had no effect on the responsiveness of the renal sensory nerves to increases in renal pelvic pressure or PGE2 in rats fed a normal- or high-sodium diet (18). In view of the results from these previous studies, the findings from our current studies suggest that NE-mediated activation of $\alpha_2$-AR plays no or only a minimal role in the ERSNA-induced activation of the renal sensory nerves in rats on a high-sodium diet.

Taken together, our findings in rats fed a high-sodium or low-sodium diet suggest an important role for $\alpha_2$-AR in modulating the responsiveness of renal sensory nerves to increases in ERSNA during various dietary sodium intakes. The lack of effects of rauwolscine on the activation of the renal sensory nerves in rats fed a high-sodium diet is most likely not related to the low level of endogenous ANG II in renal pelvic tissue, since our previous studies showed that rauwolscine enhanced the responsiveness of the renal sensory nerves to similar stimuli in rats fed a normal-sodium diet (22), which is also characterized by low endogenous ANG II in renal tissue (12). Rather, the lack of effects of rauwolscine on the activation of the renal sensory nerves in rats fed a high-sodium diet is most likely related to the high-sodium diet, per se. Whether a high-sodium diet decreases the affinity of the $\alpha_2$-AR to NE and/or reduces the density of $\alpha_2$-AR on the renal sensory nerve fibers is currently unknown. Of interest in this context are studies in platelets and renal cortical tissue that showed decreased affinity of $\alpha_2$-AR with increasing Na$^+$ concentrations (46, 49). The current studies showed no apparent differences in the intensity of labeling or number of nerve fibers in kidneys

Fig. 8. A: immunofluorescence labeling of renal tissue shows $\alpha_2A$-AR-ir (a) and $\alpha_2C$-AR-ir (c) fibers in the renal pelvic wall (arrows). The $\alpha_2A$-AR and $\alpha_2C$-AR labeling is blocked by adsorption with the appropriate peptide (b and d). B: $\alpha_2A$-AR antibody labeled CHO cells transfected to express $\alpha_2A$-AR (a) but not cells transfected to express $\alpha_2C$-AR (b) or WT cells (c). The $\alpha_2C$-AR antibody labeled CHO cells transfected to express $\alpha_2C$-AR (d) but not cells transfected to express $\alpha_2A$-AR (e) or WT cells (f).
labeled with either antibody against the \( \alpha_2A\)-AR or \( \alpha_2C\)-AR between rats on high-sodium and low-sodium diets. It is well recognized that only large differences in antibody labeling can be detected by immunohistochemical analyses. Thus, these studies do not allow a firm conclusion about whether there is a difference in the labeling of the \( \alpha_2\)-AR antibodies of the renal sensory nerves among rats fed a high- or low-sodium diet.

**Immunohistochemical analysis of \( \alpha_2\)-AR in renal pelvic tissue.** Because of the lack of truly subtype-selective agonists or antagonists of the \( \alpha_2\)-AR subtypes, the subtype involved in the modulation of the responsiveness of the peripheral renal sensory nerves to changes in ERSNA could not be evaluated in our functional studies. Previous in situ hybridization studies suggested the presence of \( \alpha_2A\)-AR and \( \alpha_2C\)-AR in renal pelvic tissue, without any evidence for \( \alpha_2B\)-AR (30). Therefore, our immunohistochemical studies focused on the localization of the \( \alpha_2A\)-AR and \( \alpha_2C\)-AR subtypes on or close to the peripheral sensory nerves in the renal pelvic wall. We applied the very same antibodies against \( \alpha_2A\)-AR and \( \alpha_2C\)-AR that have been previously and consistently shown to recognize the receptors against which they were generated (e.g., 7, 40, 45). Our studies showed that the \( \alpha_2A\)-AR antibody and \( \alpha_2C\)-AR antibody-labeled fibers in the renal pelvic wall that were also labeled with the CGRP antibody. These data suggested the presence of both \( \alpha_2A\)-AR and \( \alpha_2C\)-AR on renal sensory nerve fibers. Our studies further showed \( \alpha_2C\)-AR-ir fibers on or close to NE-t-ir fibers throughout the kidney, suggesting the presence of \( \alpha_2C\)-AR on renal sympathetic nerve fibers. It is unlikely that activation of these presynaptic receptors played a major role in the inhibition of the activation of renal sensory nerves due to the similar effects produced by rauwolscine in the innervated in vivo and denervated in vitro preparations.

Similar to previous studies in the spinal cord (7, 40, 45), the \( \alpha_2A\)-AR and \( \alpha_2C\)-AR labeling of the nerve fibers in renal tissue was blocked by preadsorption with the antigens used to generate the antibodies against the two \( \alpha_2\)-AR subtypes. Further studies using CHO cells transfected to express either \( \alpha_2A\)-AR or \( \alpha_2C\)-AR or neither, i.e., WT cells, showed \( \alpha_2A\)-AR-ir only on cells transfected to express \( \alpha_2A\)-AR, and \( \alpha_2C\)-AR-ir was observed only on cells transfected to express \( \alpha_2C\)-AR, in agreement with previous studies using Madin-Darby canine kidney cells (45). Taken together, these studies suggest that the \( \alpha_2A\)-AR-ir and \( \alpha_2C\)-AR-ir observed on/close to the renal sensory nerves in the pelvic wall represented the \( \alpha_2A\)-AR and \( \alpha_2C\)-AR subtypes, respectively. However, applying the antibodies against \( \alpha_2A\)-AR and \( \alpha_2C\)-AR to renal tissue derived from mice deficient in \( \alpha_2A\)-AR or \( \alpha_2A\)-AR resulted in similar labeling as in WT mice (data not shown). The reasons for this apparent discrepancy in our studies examining the specificity of the two antibodies in labeling \( \alpha_2A\)-AR and \( \alpha_2C\)-AR are currently unclear. Although the \( \alpha_2A\)-AR and \( \alpha_2A\)-AR-deficient mice lack functional \( \alpha_2A\)-AR and \( \alpha_2C\)-AR, we can currently not discount the possibility that there are remnants of the \( \alpha_2\)-AR proteins that are recognized by the two antibodies.

**Perspectives and Significance**

An important role for the renal renorenal reflex mechanism in determining ERSNA is suggested by our studies showing that increases in ERSNA increase ARNA (22, 24), which, in turn, would lead to decreases in ERSNA via activation of the renorenal reflexes, i.e., a negative feedback mechanism (26). Importantly, a high-sodium diet enhances the ERSNA-induced increases in ARNA (24), which would lead to increased inhibitory renorenal reflex control of ERSNA to minimize any ERSNA-induced sodium retention in high-sodium dietary conditions. Conversely, low-sodium diet reduces the ERSNA-induced increases in ARNA, which would lead to decreased inhibitory renorenal reflex control of ERSNA. Because increases in ERSNA are essential to prevent sodium loss in low-sodium dietary conditions (5), the reduced renorenal reflex control of ERSNA would serve as an important physiological mechanism that will contribute to the required increases in ERSNA to prevent sodium loss.

The current studies suggest that the altered responsiveness of the renal sensory nerves to increases in ERSNA produced by changes in dietary sodium involves modulation of the NE-mediated activation of \( \alpha_2\)-AR on the renal pelvic sensory nerves. For rats on a high-sodium diet, NE-mediated activation of the renal sensory nerves is mediated by activation \( \alpha_2\)-AR with little or no inhibitory effects produced by activation of \( \alpha_2\)-AR. For rats on a low-sodium diet, increased NE-mediated activation of \( \alpha_2\)-AR together with increased endogenous ANG II suppresses the NE-mediated activation of \( \alpha_2\)-AR (Fig. 9). Interestingly, \( \alpha_2\)-AR density in renal tissue is greater in spontaneously hypertensive rats (SHR) than in Wistar-Kyoto rats (41) and increases further in conditions of high-sodium dietary intake in association with further increases in arterial pressure (41, 44). In this context, it is of interest that the responsiveness of the renal sensory nerves is impaired in SHR (14). In view of

![Activation of Afferent Renal Sensory Nerves by \( \uparrow \)ERSNA](image-url)
our current findings, we hypothesize that the impaired responsiveness of the renal mechanosensory nerves in SHR involves inappropriately increased activation of renal α2-AR, especially when rats are on a high-sodium diet. Thus, increased NE-mediated activation of α2-AR on the renal sensory nerves may play an important role in the development of salt-sensitive hypertension.

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