Tonic Postganglionic Sympathetic Inhibition Induced by Afferent Renal Nerves?

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Abstract—Other than efferent sympathetic innervation, the kidney has peptidergic afferent fibers expressing TRPV1 receptors and releasing substance P. We tested the hypothesis that stimulation of afferent renal nerve activity with the TRPV1 agonist capsaicin inhibits efferent renal sympathetic nerve activity tonically by a neurokinin 1 receptor–dependent mechanism. Anesthetized Sprague-Dawley rats were instrumented as follows: (1) arterial and venous catheters for recording of blood pressure and heart rate and drug administration; (2) left-sided renal arterial catheter for selective intrarenal administration of the TRPV1 agonist capsaicin (3.3, 6.6, 10, 33*10^{-7} M; 10 μL; after 15, 30, 45, and 60 minutes, respectively) to stimulate afferent renal nerve activity; (3) right-sided bipolar electrode for continuous renal sympathetic nerve recording; and (4) specialized renal pelvic and renal artery catheters to separate pelvic from intrarenal afferent activity. Before and after intrarenal capsaicin application, increasing intravenous doses of the neurokinin 1 receptor blocker RP67580 were given. Intrarenal capsaicin decreased integrated renal sympathetic activity from 65.4±13.0 mV*s (baseline) to 12.8±3.2 mV*s (minimum; P<0.01). This sustained renal sympathetic inhibition reached its minimum within 70 minutes and was not directly linked to the transient electric afferent response to be expected with intrarenal capsaicin. Suppressed renal sympathetic activity transiently but completely recovered after intravenous administration of the neurokinin 1 blocker (maximum: 120.3±19.4 mV*s; P<0.01). Intrarenal afferent activity could be unequivocally separated from pelvic afferent activity. For the first time we provide direct evidence that afferent intrarenal nerves provide a tonically acting sympathoinhibitory system, which seems to be rather mediated by neurokinin release acting via neurokinin 1 receptor pathways rather than by electric afferent effects on central sympathetic outflow. (Hypertension. 2012;59[part 2]:467-476.)

Key Words: renal nerve • afferent • efferent • TRPV1 • NK_{1}-receptor • tonic inhibition

The kidney has a very complex sympathetic efferent and peptidergic afferent innervation¹ that recently became of increased interest as renal nerve ablation was introduced into the treatment of severely hypertensive patients.² However, especially the role of the afferent renal innervation in hypertension is still far from being fully understood.³ We know that afferent renal nerve traffic is able to suppress the contralateral renal nerve activity by a sympathodepressory renorenal reflex that is altered in hypertension.⁴ So far afferent nerve fibers involved in this reflex were said to be mainly projecting from the renal pelvis to the first neuron in the dorsal root ganglion,⁵ although afferent nerve fibers are also found intrarenally in close vicinity to efferent sympathetic nerve fibers.⁶

Furthermore, it is very likely that afferent nerve fibers are able to secrete transmitters, specifically substance P (SP) and calcitonin gene-related peptide (CGRP), as described for other organ systems where these substances are released from afferent peptidergic nerve fibers, which might even be aggregated in inflammation.⁷,⁸ Astonishingly, these peptides may be secreted along the whole surface of the axon⁹ and not only at the nerve endings as described very recently for vagal afferent sensory nerve fibers.¹⁰

Afferent and efferent nerve fibers lie in close proximity to each other within the kidney.⁶ Therefore, the question arises in how far the close contact between afferent peptidergic and efferent sympathetic nerve fibers at other sites, such as the peripheral ganglia, will enable the afferent nervous system to interact with efferent nerve fibers by secreting peptidergic transmitters. Because afferent nerve fibers electrophysiologically subserve a sympathodepressory renorenal reflex,⁴ it is possible that neuronally secreted peptides would enhance this effect during stimulation of afferent peptidergic axons from the kidney.

One may argue that there is not much evidence for such an assumption, especially because nearly the only evidence of the effect of a peptide like SP on sympathetic nerve structures

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elicited an excitatory response.\textsuperscript{11} However, these responses were seen in the spinal cord on presympathetic fibers on the neurokinin 1 (NK\textsubscript{1})–mediated release of \(\gamma\)-aminobutyric acid. We rather thought of an interaction taking place more peripherally, outside the spinal cord on postganglionic sympathetic nerve structures, where afferent and efferent portions of renal innervation are in even closer contact. The possibility of a tonic sympatho-depression seemed to be even more likely for us when we observed that the intravenous application of a tachykinin NK\textsubscript{1} receptor antagonist,\textsuperscript{12} presumably inhibiting the effects of SP, was able to induce a sympatho-excitatory response with increases in blood pressure, heart rate, and renal sympathetic nerve activity in preliminary experiments.

Hence, we wanted to test the hypothesis that renal afferent nerve fibers are able to inhibit renal afferent sympathetic nerve activity via an NK\textsubscript{1} receptor–dependent mechanism. We developed a catheter, allowing the direct injection of substances into the kidney via the renal artery, to be able to separate systemic responses from effects mediated via the kidneys. We used capsaicin to stimulate intrarenal TRPV1 receptors\textsuperscript{13} putatively involved in the release of tachykinins like SP.\textsuperscript{14} We searched for colocalization of afferent renal nerve fibers and sympathetic neurons within the aortorenal ganglion associated with renal innervation. Eventually we performed experiments to separate responses from renal afferents in the pelvis from afferents within the renal interstitium because we wanted to clearly demonstrate that the kidney itself (not only the pelvis) has a functional, relevant peptidergic afferent innervation.

**Methods**

**Animals**

Male Sprague-Dawley rats (Charles River, Kislegg, Germany) weighing 250 to 300 g were maintained in cages at 24±2°C and fed a standard rat diet (No. C-1000, Altromin, Lage, Germany) containing 0.2% sodium by weight and were allowed free access to tap water. All of the procedures performed on animals were done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the local government agency (Regierung von Mittelfranken, Ansbach, Germany).

**Anesthesia**

For all of the surgical procedures animals were anesthetized with a mixture of \(\text{O}_2\), 50% \(\text{N}_2\text{O}\), and ~1.5% isoflurane. When surgical procedures were finished, anesthesia was switched to methohexital (800 \(\mu\)g/kg per minute, IV) and animals were allowed 90 minutes to stabilize.

**Arterial and Venous Lines**

A right femoral artery catheter was connected to a transducer (Stratham P23Db) to record arterial blood pressure and heart rate (pressure processor type 13-4615-52; Gould Instrument Systems, Valley View, OH). Two right-sided femoral venous lines were inserted for infusion of saline (1.3 mL/kg per hour) and for administration of substances. The other catheter was used for methohexital maintenance infusion. A catheter was placed in the jugular vein to monitor the right atrial pressure and the respiratory rate.

**Renal Artery Catheter**

The tip of a PE-10 catheter (OD: 0.61 mm; ID: 0.28 mm) was stretched by heat to an OD of 120 \(\mu\)m over 10-mm length. This heat-stretched tip was coiled around a glass rod with a diameter of 1.2 mm, immersed in hot water (80°C) for 5 seconds, and afterward cooled in ice water. The coil was cut so that a 90° curve with a radius of 0.6 mm was left. It was inserted into a vascular introducer sheath made from an intravascular infusion catheter (22G, Insys-e-W, Becton Dickinson, Germany) of which the Luer cone was assembled with a silastic valve to prevent bleeding during catheter manipulation. The system was inserted into the left femoral artery and advanced up the aorta to position the tip just 1 to 2 mm cranial to the ostium of the left renal artery. The left kidney was exposed via a left lateral incision and carefully retracted ventrally while the rat was lying on its right side. The curved microtip catheter was manipulated to advance the tip 1.0 to 1.5 mm into the left renal artery to then be used for the injection of substances.

**Renal Pelvic Catheter**

The left ureter was cannulated with a triple lumen catheter. The intraluminal part consisted of the 10-mm tip of a 22G intravascular infusion catheter. Two heat-stretched lines (OD: 150 \(\mu\)m) made from PE-10 tubing, running through a silastic tubing (ID: 1.0 mm; OD: 1.4 mm), were inserted into the intraluminal catheter and advanced into the renal pelvis. The silastic tubing was then connected to the intraluminal catheter tip and served to drain urine and perfusion fluids delivered via the small catheters. One line delivered normal saline at a constant flow rate of 20 \(\mu\)L/min, whereas the other was used to pharmacologically stimulate or inhibit renal pelvic afferent nerves. By rising or lowering the end of the drainage line, renal pelvic pressure could be set to defined pressure values between ~5 and 20 cm H\(_2\)O.

**Recording of Afferent Renal Nerve Activity and Renal Sympathetic Nerve Activity**

Recordings of left-sided afferent renal nerve activity (ARNA) or right-sided and renal sympathetic nerve activity (RSNA) were performed in separate experiments (except for the first basic experiments, see below), as described previously.\textsuperscript{15-17} Through a right-sided dorsolateral or left lateral approach, a renal nerve bundle was dissected free from connective tissue and placed on a bipolar electrode (0.2-mm stainless steel wire; Science Products, Frankfurt, Germany). ARNA was recorded from a proximal right-sided renal nerve branch and ARNA from the distal cut end of a left sided renal nerve. ARNA and RSNA signals were amplified 50 000 times and filtered (1-kHz low pass; 100-Hz high pass) using a band pass amplifier (CyberAmp 320 with AI402x50 Ultra Low Noise Differential Amplifier; Axon Instruments, Foster City, CA). The signal was channeled to an A/D oscilloscope (RM 305-3; Hameg, Frankfurt, Germany) and an audio amplifier (AM8 audio monitor; Grass-Telefactor, West Warwick, RI) for visual and auditory evaluation. RSNA signal quality was assessed by its pulse synchronous rhythmicity and by the decrease attributed to ganglionic blockade by trimetaphan-camsylate (10 mg/kg of IV; Arfonad, Roche, Basel, Switzerland). As soon as an optimal signal could be observed, the right-sided nerve bundle was fixed to the electrode using silicone adhesive (Bisico 54i; Bielefelder Dentalsilicone, Bielefeld, Germany). After the left sided nerve bundle (ARNA) was cut proximal to the electrode, bursting activity disappeared but single action potentials were visible. The electrode was tunneled to the neck, and the situs was closed in layers. All of the nerve signals were full-wave rectified and integrated over 1-second intervals using a commercially available data acquisition and analysis software (SciWorks 7.2, DataWave Technologies, Loveland, CO). At the end of nerve recording protocol, Arfonad and potassium chloride were given to evaluate baseline noise, as well as maximum depolarized nerve bundle activity.

**Functional Discrimination of Renal Pelvic and Intrarenal ARNA**

Eight rats were instrumented for ARNA recordings as follows: (1) arterial and venous catheters for recording of blood pressure, right atrial pressure, and for fluid and drug administration; (2) left-sided electrode for ARNA recording; (3) renal pelvic (RP) catheter for
urine drainage and mechanical ARNA stimulation by RP pressure changes, as well as for RP drug perfusion to selectively stimulate or block renal pelvic ARNA via TRPV1 receptors; and (4) renal artery catheter for intrarenal chemical ARNA stimulation. In first basic experiments (n=5), a change in renal pelvic pressure (Δrpp) of +15 cm H2O over 30 seconds (3 times within 3 minutes) turned out to induce a reproducible mechanosensory ARNA peak response, which was defined as a reference for the adjustment of the capsaicin dosage that was used for chemical TRPV1 stimulation. For chemical ARNA stimulation, the renal pelvis was perfused with 10 μL of a capsaicin solution for 30 seconds (3.3×10⁻⁷ M in ethanol, dissolved in saline). During the capsaicin perfusion, the background saline perfusion was halted and restarted afterward. The perfusate was drained via the renal pelvic catheter. This induced a similar ARNA peak response as the mechanical stimulus. After a 3-minute recovery interval, 4 different doses of capsaicin (3.3×10⁻⁷ M, 6.6×10⁻⁷ M, 1.0×10⁻⁶ M, 3.3×10⁻⁶ M, 10 μL each, randomized order, 1 dose per 5 minutes) were injected into the renal artery (IRA) via the renal artery catheter.

After a 10-minute recovery period, the renal pelvis was perfused with capsaicine (3.3×10⁻⁴ M, 90 μL per 9 minutes) instead of normal saline to block renal pelvic TRPV1 receptors. During the capsaicine perfusion the protocol was repeated with shorter intervals.

RSNA Response to Intrarenal ARNA/TRPV1 Stimulation
First basic experiments were done to elucidate the effect of repetitive ARNA stimulation on RSNA and to screen the effects of the NK1 receptor antagonist RP67580, the NK2 receptor antagonist MEN10376, and the calcitonin gene-related peptide (CGRP) antagonist CGRP8-37. Five rats were instrumented as above, with an additional left-sided RSNA electrode. Only in these first basic experiments were ARNA and RSNA recorded simultaneously, whereas renal afferents were stimulated as above and special attention was turned to RSNA.

In 20 other rats, the renal pelvic catheter and the ARNA electrode were omitted, otherwise the instrumentation was the same as above. These RSNA recording experiments commenced with baroreceptor loading and unloading, by IV bolus administration of the α1-agonist methoxamine (10 μg) and the vasodilator Na⁺-nitroprusside (1 μg), respectively, in randomized order with a recovery period of 15 minutes.

Different doses of the NK1 receptor antagonist RP67580 (10⁻² M; 2, 5, 10, 15, and 20 μL) were given IV before and after administration of increasing doses of capsaicin (3.3×10⁻⁷ M, 6.6×10⁻⁷ M, 1.0×10⁻⁶ M, 3.3×10⁻⁶ M, 10 μL each, 1 dose per 15 minutes) either into the renal artery (IRA Cap; n=10 rats) or intravenously (IV capsaicin; n=10 rats).

Histological Investigation
Sections of aortorenal ganglia were investigated to substantiate the putative existence of afferent peptidergic nerve fibers in these ganglia, which might account for an afferent-to-effenter interaction at this site. The main renal nerve branch was followed cranially until its fusiform aorticorenal ganglion was clearly visible. The ganglion at this site. The main renal nerve branch was followed cranially until its fusiform aorticorenal ganglion was clearly visible. The ganglion

Data Analysis
Integrated ARNA and RSNA were recorded as microvolts × seconds (μV·s). To compare baseline values between groups, individual values were corrected for background noise, that is, the minimum activity after ganglionic blockade with trimetaphan-camyslute (10 mg/kg, IV; Arfonad, Roche) and/or postmortem activity (30-minute average). Baseline values of ARNA and RSNA (μV·s), mean arterial pressure (millimeters of mercury [mm Hg]), and heart rate (beats per minute [bpm]) were averaged from 10-minute periods before intervention. Baseline parameters and differences between groups at different points in time were analyzed using 1-way ANOVA with Student-Newman-Keuls post hoc “multiple comparisons versus control” (ie, baseline). Statistical significance was defined as P<0.05. Data are given as group mean±SEM. SigmaStat 3.5 (Systat Software) was used for statistical analysis.

Results
Baseline Parameters
Analysis of mean arterial pressure, heart rate, ARNA, and RSNA did not show differences between groups; therefore, pooled data are given. Averaged over all of the groups, mean arterial pressure was 99±4 mm Hg and heart rate was 377±21 bpm. Amplified, filtered, and noise-corrected mean baseline RSNA was 63234±5120 μV·s, and baseline ARNA was 7770±989 μV·s.

Functional Discrimination of Renal Pelvic and Intrarenal ARNA
The 3 different methods of ARNA stimulation showed specific response patterns (see Figure 1). Mechanical RP ARNA stimulation (Δrpp +15 cm H2O) induced a differential receptor response peaking with increase and decrease of RP pressure. Chemical renal pelvic stimulation by RP capsaicin perfusion induced a steep increase in ARNA, which returned toward baseline already during stimulus, indicating rapid tachyphylaxis as expected in TRPV1 receptors. When intrarenal TRPV1 receptors were stimulated (IRA Cap) by capsaicin injection into the renal artery, ARNA responded with a short peak of a few seconds. Furthermore, renal pelvic TRPV1 blockade (RP capsazepine perfusion) abolished the renal pelvic but not the intrarenal stimulus response.

For data analysis, the maximum peak responses from 8 rats were evaluated. Different stimuli (RP capsaicin [3.3×10⁻⁷ M; 10 μL/30 seconds], RP pressure [Δrpp +15 cm H2O; 30 seconds], IRA Cap [1.0×10⁻⁶ M; 10 μL]) induced similar peak values. The noise-corrected peak ARNA was ~750% above baseline. It could be shown (see Figure 2) that renal pelvic perfusion with the TRPV1-antagonist capsazepine (RP capsazepine perfusion) completely abolished the responses to
Figure 1. A through C, Original data traces of blood pressure (BP) and integrated afferent renal nerve activity (ARN; int_ARNA) from a representative experiment illustrating the response patterns of different ARNA stimuli and the blocking effect of renal pelvic (RP) TRPV1 antagonism (RP capsazepine [CPZ] perfusion). A, Mechanical RP stimulation induced a 2-peak, differential receptor-like response (abolished by RP CPZ perfusion). B, Chemical RP stimulation indicating rapid tachyphylaxis (abolished by RP CPZ perfusion). C, Short-lasting peak response attributed to intrarenal TRPV1 stimulation, which was unaffected by RP TRPV1 antagonism (RP CPZ perfusion).
renal pelvic stimuli, whereas ARNA responses to intrarenal TRPV1 stimulation were unaffected. These findings indicate that renal pelvic and intrarenal ARNA can be functionally discriminated and that intrarenal ARNA seems to be independent from renal pelvic ARNA.

RSNA Response to Intrarenal ARNA/TRPV1 Stimulation

The first basic experiments (n=5) indicated a long-lasting RSNA suppression because of repetitive ARNA stimulation. It turned out that neither the NK2 antagonist MEN10376 nor CGRP8-37 affected RSNA suppression when given intravenously. Only the NK1 antagonist RP67580 seemed to unmask RSNA after TRPV1-dependant suppression. Hence, further experiments focused on intrarenal TRPV1 stimulation (IRA Cap) and systemic NK1 antagonism. The main data from the first basic experiments are shown in Figure 3.

Intrarenal TRPV1 agonism by 4 consecutive bolus doses of capsaicin into the renal artery (IRA Cap, n=10 rats) induced a sustained and long-lasting, contralateral RSNA suppression (Δrpp +15 cm H2O) induced a similar ARNA peak response as RP capsaicin perfusion (RP Cap). Intrarenal TRPV1 stimulation (IRA Cap) was unaffected by RP TRPV1 blockade (RP CPZ perfusion), whereas renal pelvic stimuli where abolished. *P<0.01; RP saline vs RP CPZ perfusion.

When RSNA was suppressed, the NK1 antagonist RP67580 was able to unmask RSNA when given systemically (IV). There seemed to be no relevant change in blood pressure, heart rate, and respiratory rate because of either intrarenal TRPV1 agonism or systemic NK1 antagonism (Figure 5).

Systemic (IV) NK1 antagonism increased RSNA already under baseline conditions, that is, before any ARNA stimulation (IRA Cap). A dose-dependent saturating effect was found. With doses by which RSNA response was already maximally increased (RP67580, 10^{-2} M; >5 µL IV), very small but statistically significant increases in blood pressure and heart rate were found. This suggests a very high RSNA specificity of the observed NK1-dependant mechanism. All of these effects were nonsustained, lasting only for a few seconds (see Figure 6).

The effect of systemic NK1 antagonism after TRPV1 stimulation by either intrarenal capsaicin application (IRA Cap) or by systemic administration of capsaicin (IV Cap) is shown in Figure 7. RSNA, which was suppressed because of IRA capsaicin, could be significantly increased by systemic NK1 antagonism. Furthermore, RSNA, which was not altered after IV Cap, could be stimulated to the same extend as under
control conditions (as shown in Figure 6). There was no statistical difference in the RSNA peak responses when IRA capsaicin-treated animals (n=10) were compared with those treated with IV capsaicin (n=10). Small increases in blood pressure and heart rate were observed as under control conditions (Figure 6).

In sections of aorticorenal ganglia, we could clearly stain for sympathetic neurons with the help of tyrosin hydroxylase antibodies. The bulk of sympathetic neurons within this ganglion is part of the renal sympathetic innervation. A major peripheral input to this ganglion comes from the kidney, as could be demonstrated when we followed the renal nerve from the hilus of the kidney cranially to the fusiform aorticorenal ganglion. As shown in Figure 8, we found a dense meshwork of CGRP- and SP-containing afferent nerve fibers in close vicinity to sympathetic neurons. This colocalization of afferent and efferent components of the autonomic nervous system in a ganglion strongly connected to renal innervation is in accordance with the idea of an afferent-to-efferent interaction at this site.

Discussion

Our study provides the first direct evidence that the renal afferent innervation is involved in a tonically acting sympathoinhibitory system via an NK₁ receptor–dependant mechanism. This sympatho-inhibitory neurogenic effect seems to be mediated rather by neurokinin release from afferent renal nerves via NK₁ receptors than merely by electric ARNA that acts on central sites generating sympathetic outflow to the periphery. We could only observe a very short increase in afferent nerve activity after repeated bolus injections of the TRPV1 receptor agonist capsaicin into the renal artery (refer to Figure 1C and Results section for details). *P<0.05; IRA Cap vs IV Cap.

Concerning the site of this NK₁-mediated effect on RSNA, we can provide the first hints of the putative anatomic
Figure 5. A through C, Original data traces of blood pressure (BP), ECG, respiratory rate (indicated by right atrial pressure dips; RAP), and renal sympathetic nerve activity (RSNA) from a representative experiment. A, Baseline condition; B, TRPV1 induced RSNA suppression because of intrarenal capsaicin (IRA Cap); C, unmasking of RSNA by systemic neurokinin 1 (NK1) antagonism. There was no change in other parameters than RSNA.
location by the demonstration of afferent peptidergic afferent within the aorticorenal ganglion in close vicinity to sympathetic neurons of which the axons project to the kidney. Because peptidergic afferent nerve fibers are said to release their transmitters not only at the nerve endings but along the surface of the whole axon, sympato-afferent interaction is putatively also possible within peripheral ganglia. It is known that cutaneous afferent nerves already release CGRP and probably SP if they are merely spontaneously active. In our experiment, the afferent renal nerves always showed spontaneous activity. Hence, putative afferent peptidergic nerve fibers traveling from the kidney through the ganglion aortico renal could release neuropeptides that modulate renal sympathetic activity.

The influence of afferent renal nerve fibers via an NK₁-dependant mechanism on sympathetic drive to other sites than the kidneys seems to be limited: intrarenal stimulation of TRPV1 receptors with capsaicin produced a very long-lasting highly significant renal sympathodepression but had no significant influence on blood pressure or heart rate, although the very same doses of capsaicin had no significant effect on blood pressure, heart rate, or RSNA at all when administered intravenously. Because TRPV1 receptors are only localized on peptidergic afferent nerves and also on nerve fibers within the kidney, the effect must be initiated by these very nerve fibers.

Our study provides direct neurophysiological evidence for the existence of a functionally relevant intrarenal peptidergic innervation that is independent from renal pelvic innervation. In this respect renal arterial application of substances provides a tool for the investigation of intrarenal afferent nerve function without the need for renal pelvic manipulation. Other than its intrarenal role, TRPV1 proved also to be an important component of the renal pelvic mechanosensory and chemosensory systems. A putative intrarenal mechanosensory system would need further elucidation beyond the scope of this article.

A certain independence of the innervation of the renal pelvis from intrarenal neuronal pathways is not completely unexpected given the ontogenetical development of the 2 sites. The renal pelvis develops out of the Wolffian duct, whereas the kidney derives from the metanephrogenic mesenchyme that eventually gets into contact with the Wolffian duct via the ureter bud. In how far these differences in developmental anatomy would also suggest that afferent
nerves with axons from these sites exert different functions within the renal autonomous nervous system is not known. So far, we are not aware of any report suggesting this assumption, nor do our own data support such a hypothesis.

The question arises as to how the tonic inhibition of RSNA by an SP-dependent mechanism related to afferent renal innervation can be explained. First, is it exerted via central downregulation of renal sympathetic outflow because of increased afferent nerve traffic? Second, will the release of SP from afferent axons along their putative pathway through sympathetic ganglia modulate RSNA peripherally? It is well documented that stimulation of TRPV1 receptors on renal pelvic afferents will increase ARNA, which is followed by a decrease in renal sympathetic efferent activity traditionally recorded on the contralateral side.

Afferent tonic inhibition of efferent sympathetic activity dependent on tachykinin mechanisms as described by us could potentially occur everywhere on the afferent branch of this reflex arch. However, a central interaction would be rather related to increased afferent electric input that did not occur. We only saw short afferent activity increases during capsaicin administration but a long-lasting RSNA suppression. Concerning spinal interactions between afferent and efferent pathways, only a γ-aminobutyric acid–related sympathoexcitatory mechanism has been described so far. With respect to TRPV1 receptor stimulation of renal pelvic afferents, it is known that SP release from these nerve fibers is induced.

Eventually ARNA may even be dependent on SP and its receptors. Because TRPV1 receptors can regulate cation influx, it was argued that activation of TRPV1 itself might directly generate ARNA. The published evidence on renal pelvic afferent fibers rather supports only a direct effect of TRPV1 on SP release. By means of NK₁ receptor antagonism it could be proved that TRPV1-induced sensory activation was dependent on NK₁ receptor function, which is downstream of TRPV1.

However, neither the TRPV1 receptor–dependent intrarenal release of SP nor the NK₁ receptor dependency of electric ARNA is able to explain the long-lasting NK₁-dependent renal sympathodepression after intrarenal TRPV1 receptor stimulation with capsaicin that we saw in our experiments. This sympathodepression was characterized by a completely different time constant when compared with the short increases of ARNA attributed to intrarenal capsaicin injection. Traditionally, a subsequent short-lasting dip in RSNA would be expected. However, such a response was never observed in our experiments. Obviously additional tonic sympathoinhibition dependent on NK₁ receptors could totally override such a classic neurogenic afferent-efferent reflex arch. Hence, with respect to the 2 questions outlined above, the tonic inhibitory effect of afferent renal innervation that we observed is not likely attributed to increased afferent electric renal nerve traffic but rather to release of tachykinins from the afferent branch of the very renal innervation downregulation efferent sympathetic nerve fiber activity to the kidney.

**Perspective**

RSNA is increased, whereas its control is impaired in arterial hypertension. A decreased efficacy of the newly described tonically acting sympatho-inhibitory system via an NK₁ receptor–dependent mechanism may contribute to renal sympatho-regulation under these circumstances. The mechanism leading to a decline in sympathoinhibition by this NK₁-dependent mechanism could be an increased release of SP, a potent proinflammatory substance, from intrarenal nerve fibers during inflammatory processes in hypertensive nephropathy at the expense of renal sympathoinhibition. Release of neurogenic SP from peptidergic afferent nerve fibers in inflammation has been described for other organ systems than the kidney.

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**Disclosures**

None.

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