Sensory renal innervation: a kidney-specific firing activity due to a unique expression pattern of voltage-gated sodium channels?

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Freisinger W, Schatz J, Ditting T, Lampert A, Heinlein S, Lale N, Schmieder R, Veelken R. Sensory renal innervation: a kidney-specific firing activity due to a unique expression pattern of voltage-gated sodium channels? Am J Physiol Renal Physiol 304: F491–F497, 2013. First published January 2, 2013; doi:10.1152/ajprenal.00011.2012.—Sensory neurons with afferent axons from the kidney are extraordinary in their response to electrical stimulation. More than 50% exhibit a tonic firing pattern, i.e., sustained action potential firing throughout depolarization, pointing to an increased excitability, whereas nonrenal neurons show mainly a phasic response, i.e., less than five action potentials. Here we investigated whether these peculiar firing characteristics of renal afferent neurons are due to differences in the expression of voltage-gated sodium channels (Navs). Dorsal root ganglion (DRG) neurons from rats (Th11-L2) were recorded by the current-clamp technique and distinguished as “tonic” or “phasic.” In voltage-clamp recordings, Navs were characterized by their tetrodotoxoxin (TTX) sensitivity, and their molecular identity was revealed by RT-PCR. The firing pattern of 66 DRG neurons (41 renal and 25 nonrenal) was investigated. Renal neurons exhibited more often a tonic firing pattern (56.1 vs. 12%). Tonic neurons showed a more positive threshold (−21.75 ± 1.43 vs. −29.33 ± 1.63 mV; P < 0.05), a higher overshoot (55.74 [53.6–60.96] vs. 46.79 mV [38.63–54.75]; P < 0.05) and longer action potential duration (4.61 [4.15–5.85] vs. 3.35 ms [2.12–5.67]; P < 0.05). These findings point to an increased presence of the TTX-resistant Na1.8 and 1.9. Furthermore, tonic neurons exhibited a relatively higher portion of TTX-resistant sodium currents. Interestingly, mRNA expression of TTX-resistant sodium channels was significantly increased in renal, predominantly tonic, DRG neurons. Hence, under physiological conditions, renal sensory neurons exhibit predominantly a firing pattern associated with higher excitability. Our findings support that this is due to an increased expression and activation of TTX-resistant Na1.s.

renal afferent nerve; DRG; tonic; phasic; excitability

THE RENAL INNERVATION PLAYS an important role in the regulation of physiological processes such as renal perfusion or blood pressure regulation. In the last years, catheter-based thermal ablation of renal nerves has been shown to be an elegant and effective tool in the treatment of resistant hypertension (32, 33), thus indicating that numerous reports with experimental data (18) are of substantial clinical relevance. Clinical reports on patients after thermal ablation point to an independent role of afferent renal innervation on blood pressure (33). Experimental data suggest that renal afferent denervation may be protective against the development of high blood pressure (26, 42) as well as the progression of renal structural damage in renal disease (6, 43). Hence, nonselective thermal ablation of afferent renal nerves in resistant human hypertension may not be always beneficial since the exact pathophysiological situation is normally not well defined (27).

Unfortunately, the efferent sympathetic innervation of the kidney is far better understood than the afferent peptidergic renal innervation, the latter mainly being investigated with respect to the renal pelvis (18). One possible explanation for this fact is the difficult access to the afferent sensory nerve endings situated in the kidney as well as the demanding task to record afferent activity in multifiber preparations of renal nerves containing a high number of actually firing sympathetic units. On the other hand, the cell body of the first neuron of the afferent innervation is situated in the dorsal root ganglion (DRG) and therefore accessible for cell culture and electrophysiological investigation (28).

We described earlier that renal afferent neurons, situated in the DRG Th11-L2, exhibited a specific response to acidification compared with nonrenal neurons with projections mostly from the lower limb (19, 20, 28). Interestingly, neurons with projections to the kidney seemed to show a significant different reaction upon changes in membrane potential. Whereas nonrenal cells exhibit predominantly a phasic response, e.g., one to four action potential spikes after depolarizing current injection, renal cells showed mostly a tonic response, e.g., continuous firing after depolarization (19). This response to depolarization is indicative of renal neuronal cells, which could be a mechanism to regulate the central sympathetic output and efferent renal innervation. Since action potential generation is dependent on voltage-gated sodium channels (Na1.s), we tested the hypothesis that distinct firing characteristics of renal DRG neurons are due to the expression of specific sets of Na1.s on the DRG cell surface.

With whole cell patch-clamp experiments, we characterized renal and nonrenal cells according to their firing properties as tonic or phasic (34, 35). To assess voltage-gated sodium currents in each cell, we switched to voltage-clamp mode. The sodium channel blocker tetrodotoxin (TTX) was applied to identify the TTX-resistant Na1 current component. To further characterize the Na1 subtypes expressed in renal or nonrenal DRGs, we separated labeled renal neurons from unlabeled neurons with the help of flowcytometry and determined the expression of channel-mRNA.

METHODS

Male Sprague-Dawley rats (Charles River, Kisslegg, Germany) weighing 180–250 g (9 to 12 wk of age) were maintained in cages at 24 ± 2°C. They were 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 procedures performed in animals were done in accordance with the guidelines of the American Physiological Society and approved by the local government agency. 

Labeling of renal afferent neurons. To identify neurons with renal afferents, these cells were labeled using the dicarboxylic acid dye Dil (1,1’dioleoyl-3,3’,3’-tetramethyl-indocarbocyanine methanesulfonate, Ψ9-Dil, 50 g/ml in EtOH; Molecular Probes, Darmstadt, Germany) by subcapsular application (5 μl; 10 g/ml in both kidneys. This established technique (13) was adapted for renal purposes. The subcapsular renal Dil application could be brought about with minor surgery under isoflurane anesthesia (2–3% Forene; Abbott, Baar, Switzerland; N2O, O2, Linde Gas Therapeutics, Unterschleifheim, Germany). For analysis, rats were given buprenorphine before surgery (0.05 mg/kg, Temgesic; RB Pharmaceuticals, Berkshire, UK) and, if necessary, after surgery. Small flank incisions through skin and muscles were necessary to expose renal poles. The lipophilic tracer was transported retrogradely along the nerves to the cell bodies; 6 days later animals were euthanized and the neurons were harvested from DRG T11-L2 (19, 28).

Neuronal cell culture. Male Sprague-Dawley rats were anesthetized with isoflurane and euthanized by decapitation, and DRGs from T11-L2 were dissected. Primary cultures of DRG neurons were obtained by mechanical and enzymatic dissociation as described previously (28). The ganglia were incubated with collagenase IA [2 mg/ml C9891 (Sigma-Aldrich, Munich, Germany) in DMEM (PAA Laboratories, Linz, Austria)] for 1 h in a 5% CO2 incubator at 37°C. After 1 h of enzymatic dissociation, enzyme activity was terminated by removing collagenase containing DMEM with a sucking pipette and by adding fresh DMEM + culture medium (DMEM plus 10% FCS 1% penicillin/streptomycin, and 0.1% insulin). Tissue digestion was stopped by FCS.

Ganglia were triturated using sterile Pasteur pipettes (Sigmacot; Sigma-Aldrich) to dissociate individual cells. After centrifugation at 705 rpm, cells were resuspended again in 10 ml DMEM + to repeat centrifugation. The pellet was resuspended in 1.8 ml DMEM +, and cells were plated on six glass coverslips coated with poly-L-lysine. The cells were plated on coverslips in DMEM + for 1 day before the electrophysiological experiments.

To demonstrate that labeled cells were neurons, they were tested for fast sodium currents by stepwise depolarization. A laser beam (532 nm) was mounted to the patch-clamp setup to detect DiI-positive, renal DRG cells by fluorescence. From previous experiments, we knew that the nonrenal neurons in the DRGs mostly have axonal input from the hindlimbs (28).

Patch clamp. Patch-clamp recordings were obtained from respective DRG neurons using a pipette solution (intracellular solution) containing the following (in mM) 140 KCl, 5 NaCl, 2 MgCl2, 1 CaCl2, 2 Mg-ATP, 0.3 Na-GTP, 10 EGTA, and 10 HEPES, pH adjusted with KOH at pH 7.4. To achieve whole-cell-mode, pipette resistances from 1 to 3 MΩ were accepted, gigaseal resistance was required, and input resistance was 6 MΩ at most. Patch-clamp recordings were obtained with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Data were sampled at 20 kHz for voltage and current-clamp and stored on a computer using a software package (pClamp 10.2; Axon Instruments). For current-clamp measurements, the extracellular solution contained the following (in mM): 140 NaCl, 5 KCl, 1 MgCl2, 10 HEPES, and 10 glucose.

After current-clamp recordings, the bath solution (extracellular solution) was changed to minimize leak currents and contained the following (in mM): 30 NaCl, 100 NaHCO3, 1.6 CaCl2, 2 MgCl2, 10 HEPES, 0.2 CdCl2, and 15 glucose; pH 7.4 adjusted with tetramethylammonium-OH. Neurons were only accepted for analysis if their resting membrane potentials were below –40 mV. Only cells that stained brightly for Dil at laser excitation were considered as renal neurons. The experimental protocol was also performed in neurons with nonrenal afferents likewise located in DRG of vertebrae T11-L2 to use the results from a neuron population with afferents of a different anatomical site for comparison, i.e., mainly the hindlimbs (28). All recordings were done at room temperature, i.e., 22 ± 2°C.

Current-clamp protocols. To classify DRG neurons with renal vs. nonrenal afferent projections in terms of firing patterns, we used an adaptation of a current-clamp approach recently described (19). PCsamp 10.2 software (Axon Instruments, Foster City, CA) was used to control current-pulse generation to record membrane potentials as well as offline data analysis. Action potentials were induced by rectangular current-pulse injections as follows: a 5-ms prepulse, followed by a 600-ms pulse with an interpulse delay of 100 ms was delivered in three consecutive steps of stepwise increased intensity; 40–400, 400–4,000, and 4,000–12,000 pA, in 10 consecutive steps, with each step lasting 5.16 s. This protocol allowed categorization of each DRG neuron as tonic or phasic (19, 34, 35). After a recovery time of at least 5 min, when the resting membrane potential returned to its individual baseline value, the patch basin was perfused with a sodium-reduced, potassium free solution as mentioned above to measure inward currents mediated by voltage-gated sodium channels on the same cell.

Voltage-clamp protocols. Cell capacitance was compensated manually, and cell parameters (size, capacitance, and resistance) were documented. Induced currents by voltage changes were adjusted to cell capacitance to express current densities. Inward currents were induced by using a voltage-ramp protocol (−100 to 40 mV in 10 ms), 10 times with a 20-s delay. To further analyze the subgroups of voltage-gated sodium channels, the measurements were repeated after the channel blocker TTX (250 nmol/l) was added to the bath solution. The concentration of TTX has been chosen since it is known to securely block all TTX-sensitive channels and does not influence TTX-resistant currents (7). This blocker allows the distinction between the amount of current mediated by TTX-sensitive channels (Na1.1, Na1.2, Na1.6, and Na1.7) and TTX-resistant channels (Na1.8 and Na1.9).

Neuronal cell sorting. To investigate mRNA levels of voltage-gated sodium channels in renal and nonrenal DRGs, we separated the renal from the nonrenal neuron population in the DRG by flow cytometry.

Therefore, the above-mentioned tracing protocol was modified. The dicarboxylic acid DiD [1,1’dioctadecyl-3,3’,3’-tetramethyl-indocarbocyanine perchlorate, DiIC18(5) iod, Molecular Probes] features the same properties as Dil at the wavelength of the far red laser (excitation 647 nm/emission 670 nm) for further analysis. After dissection, the ganglia were triturated mechanically and enzymatically [collagenase IA, 4 mg/ml C9891 (Sigma-Aldrich) in DMEM (PAA Laboratories) and 100 μM DNase (RNase-free DNase, Nr. 79254; Qiagen, Hilden, Germany)]. The cells were incubated for 30 min with an AlexaFluor (ex 488 nm)-coupled anti-CD-90 antibody serving as neuronal cell marker situated in the neuronal membrane [10 μl anti-CD90, MCA47A488, Morphosys AbD (Serotec, Düsseldorf, Germany) in 200 μl DMEM], and then 1 μl DAPI (4’,6-diamidino-2-phenylindole, D 21490, ex 358 nm, em 461 nm; Invitrogen, Darmstadt, Germany) in 800 μl DMEM, a vitality marker, was added to the solution. After 5 min of incubation, cells were filtered (70-μm filter, Falcon; BD Bioscience, Heidelberg, Germany), washed, centrifuged, and resuspended for cell sorting.

With the help of these fluorescent dyes, vital peripheral neurons could be identified. With the use of a MoFlo High Speed cell sorter (BeckmannCoulter, Krefeld, Germany), renal neurons, staining positive for DiD, could be separated from vital neurons from nonrenal sites.

Isolation of mRNA and RT-PCR. The sorted cell population was captured with 350 μl RLT-buffer, containing β-mercaptoethanol (RNeasy Mini Kit, 74106; Qiagen, Hilden, Germany). The following steps were performed according to RNeasy Mini Handbook “Purification of Total RNA from Animal Cells Using Spin Technology” (Qiagen). For reverse transcription, we used the TaqMan reverse transcriptase...
transcription reagents (N808234; Applied Biosystems, Darmstadt, Germany). cDNA samples were amplified in a Trio-Thermoblock (Biometra, Göttingen, Germany). Relative mRNA expression of the channel was determined by quantitative PCR using a Step One Plus Real-Time PCR System (Applied Biosystems). The reaction volume (10 μl) contained the following: 100 nM each of forward and reverse primers (Eurofins MWG Operon, Ebersberg, Germany) and fast SYBR green master mix (Applied Biosystems). Final RNA concentration in the reaction mixture was adjusted to 5 ng/μl, and fluorescence was normalized with the reference dye ROX (6-carboxy-X-rhodamin, component of SYBR green master mix, Applied Biosystems). The relative amount of the specific mRNA was normalized to the housekeeping gene hypoxanthine ribosyltransferase (14). The primers used for the identification of voltage-gated sodium channels were designed as described previously in the literature (Table 1) (40).

Care was also taken to avoid regions of homology with other Na channels. PCR products were verified by melting point analysis at the end of each experiment.

Data analysis. The data were statistically analyzed with a two-tailed t-test (where appropriate) or a Mann-Whitney rank sum test using a SigmaStat software package (SYStat, Erkrath, Germany). Furthermore, a z-test was used to test for significant differences in the frequency distribution of characteristics of the DRG neurons. Only a priori fixed comparisons were tested. Statistical significance was defined as P < 0.05. Data are given as means ± SE or as median [interquartile range].

RESULTS

Labeled cells in situ and in culture. One day after dissection and adhesion on coverslips, neurons could be easily distinguished from fibroblasts and other cells by their typical size and soma. A fraction of these neurons (between 15–25%) was brightly labeled with DiI, and renal afferent neurons (n = 41) could be distinguished from nonrenal ones (n = 25) by fluorescence of DiI in the laser beam.

Renal DRGs display mainly tonic firing patterns. Current-clamp experiments were done to classify the neurons into tonic (n = 26; i.e., >4 action potentials; Fig. 1B) and phasic (n = 40; i.e., 1–4 action potentials; Fig. 1A) according to their firing pattern upon suprathreshold current injection. Among renal neurons, tonic neurons could be found significantly more likely than among nonrenal ones (n = 23 of 41 in renal vs. n = 3 of 25 in nonrenal; P < 0.001, Fig. 2), which means tonic neurons were almost exclusively seen in renal neurons.

Electrophysiological properties of tonic and phasic neurons. Current-clamp results reveal that current-threshold was significantly lower (593.3 ± 131 vs. 2,234.3 ± 511 pA; P < 0.05) in tonic neurons (n = 26) compared with phasic neurons (n = 40). Tonic neurons were characterized by significantly more depolarized voltage thresholds (−21.75 ± 1.43 vs. −29.33 ± 1.63 mV; P < 0.05), higher overshoots (56.74 ± 3.63 vs. 46.79 ± 4.69 mV; P < 0.05) and longer duration of action potentials at threshold level (4.61 ± 0.45–5.85 vs. 3.35 ± 0.26 ms; P < 0.05; Fig. 3).

Tonic DRG neurons exhibit relatively higher TTX-resistant sodium currents. After current-clamp measurements, we switched to voltage-clamp measurements and investigated the currents mediated by Na_v, in these DRG neurons: potassium and calcium channels were blocked pharmacologically to exclude interferences. TTX was added to the perfusion solution, which blocks TTX-sensitive currents and reveals the TTX-resistant component. Cells with phasic behavior (n = 40) had significantly larger ramp current densities than tonic (n = 26) cells (156.96 ±

Table 1. Primers for RT-PCR of Na-channel mRNA

<table>
<thead>
<tr>
<th>Name</th>
<th>Rat Gene Symbol</th>
<th>Forward Primers</th>
<th>Reverse Primers</th>
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<tbody>
<tr>
<td>Na_1.1</td>
<td>Scn1a</td>
<td>5'-ATC CCA GTC CAA AGA TAG CA-3'</td>
<td>5'-GTC TCG GGG AAA ACA GTC AG-3'</td>
</tr>
<tr>
<td>Na_1.2</td>
<td>Scn2a</td>
<td>5'-GGG AGG GTG CTT CAG TAG CA-3'</td>
<td>5'-AAG AGA GAG TGG TGG GGA GA-3'</td>
</tr>
<tr>
<td>Na_1.7</td>
<td>Scn9a</td>
<td>5'-GAC AGC TTC TTC GAG AGG GAA TA-3'</td>
<td>5'-CCA TGG TGG AGA TTA TTG TCT-3'</td>
</tr>
<tr>
<td>Na_1.8</td>
<td>Scn10a</td>
<td>5'-GAC CCA TGA CAA CAG GTC AC-3'</td>
<td>5'-GAT CCC GTC AGG AAA TGA GA-3'</td>
</tr>
<tr>
<td>Na_1.9</td>
<td>Scn11a</td>
<td>5'-GGC AGC GCA GTC AAT CTT TC-3'</td>
<td>5'-GCG CCA CAG TTG TGG TTA AT-3'</td>
</tr>
<tr>
<td>HPRT</td>
<td>Hprt</td>
<td>5'-GCA GAC TTT GCT TCC GGT-3'</td>
<td>5'-TAC TGG CCA CAT CAA CAG GAC-3'</td>
</tr>
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HPRT, hypoxanthine ribosyltransferase.
29.76 phasic vs. 75.43 ± 22.79 tonic pA/pF; P < 0.05). With respect to TTX-resistant and TTX-sensitive current densities, we found a significantly higher proportion of currents that were mediated by TTX-resistant channels in tonic than phasic neurons (Fig. 4).

Renal DRGs have higher mRNA-levels of Na\(_v\)1.7, Na\(_v\)1.8, and Na\(_v\)1.9. Real-time PCR was performed to investigate the mRNA expression of Na\(_v\)s in renal and nonrenal neurons. The primers were used as described in the literature (40). DiD labeling identified renal neurons. Cell vitality was tested with DAPI, and anti-CD 90 antibodies were used as general neuronal marker. Thus voltage-gated sodium channel mRNA expression in vital renal neurons could be compared with nonrenal, vital neurons (Fig. 5).

We found that renal neurons expressed a significantly greater amount of mRNA of the TTX-sensitive channel Na\(_v\)1.7 (7.65 ± 2.80-fold increase; P < 0.05), the TTX-resistant channels Na\(_v\)1.8 (21.98 ± 4.80-fold increase; P < 0.05) and Na\(_v\)1.9 (8.61 ± 1.68-fold increase; P < 0.05) in relation to nonrenal neurons (Fig. 6).

Unfortunately, neurons cannot be sorted according to their firing pattern into tonic or phasic. Since nonrenal DRG neurons are mostly phasic, whereas renal neurons exhibit mostly a tonic firing pattern, these changes in mRNA expression point to a different channel protein expression in renal neurons.

DISCUSSION

We demonstrated for the first time that physiological characteristics of the renal afferent innervation, including a high number of neurons with a tonic firing pattern, may be linked to the presence of distinctive voltage-gated sodium channels. Given the influence of afferent renal innervation on e.g., blood pressure regulation (18, 33), this finding will improve our understanding of the role that renal afferent innervation is playing in health and disease.

Repetitive firing in DRG-neurons has been described before (23, 25) and is linked to hyperexcitability of neurons, particu-
larily concerning various pain disorders, e.g., inflammatory pain (16). In these states, hyperexcitability is defined by increased (tonic) firing frequency in response to suprathreshold stimulation and decreased current threshold for action potentials (12). We found a significantly decreased threshold in tonic compared with phasic neurons. Recent studies on hyperexcitability of neurons, particularly investigating various pain disorders (e.g., painful neuropathy) have pointed out the importance of specific voltage-gated sodium channels (NaVs) in these states (22).

These channels, of which nine pore-forming α-subunits (Na\(_{\text{v}}\)1.1–1.9) have been identified, show heterogeneous functional properties and specialized functions of each subunit. In DRG-neurons, Na\(_{\text{v}}\)1.1-Nav1.7 [except Na\(_{\text{v}}\)1.4 and Na\(_{\text{v}}\)1.5, which occur in muscle (39) and cardiac tissue (15), respectively] are relatively sensitive to the neurotoxin TTX whereas Na\(_{\text{v}}\)1.8 and Na\(_{\text{v}}\)1.9 are resistant to TTX. Research of electrogenesis in DRG-neurons indicates that repetitive firing is dependent on sodium currents especially mediated by Na\(_{\text{v}}\)1.7, Na\(_{\text{v}}\)1.8, and Na\(_{\text{v}}\)1.9, which are in a complex cooperation with each other and the other sodium channels in DRG neurons (41).

Particularly Na\(_{\text{v}}\)1.8 plays an important role in the concept of repetitive firing. It uniquely shows slow activation and a fast recovery at depolarized membrane potentials after inactivation, so that solely these channels are available for activation in subsequent action potentials (21, 30, 31). Furthermore, Na\(_{\text{v}}\)1.8 is activated at a relatively depolarized potential in DRG neurons and contributes the majority of the inward current underlying the action potential in DRG-neurons (5, 30).

In accordance with these reports, we found typical impacts of Na\(_{\text{v}}\)1.8 in the shapes of our tonic action potentials: Tonic neurons, highly influenced by Na\(_{\text{v}}\)1.8 in their electrogenesis, are characterized by significantly more depolarized voltage thresholds than phasic neurons. Overshoots are significantly higher as TTX-resistant channels seem to play a predominant role only in tonic neurons, which has been similarly described before (30). With reference to the distinguishing long duration of tonic action potentials at threshold level, Na\(_{\text{v}}\)1.8 has been identified to cause broader action potentials in neurons due to their kinetics (9).

These observations made from current-clamp data go in line with the voltage-clamp findings. We found a relatively higher proportion of TTX-resistant currents, mediated by Na\(_{\text{v}}\)1.8 and Na\(_{\text{v}}\)1.9 in tonically firing neurons.

While differences in current-clamp mode are strikingly evident, our measurements in voltage-clamp mode need to be discussed more closely as they raise far-reaching questions. Although measurement of overshoots in current-clamp mode reveals greater values for tonic cells, a result of comparatively high influence of Na\(_{\text{v}}\)1.8 - phasic neurons show significantly higher total sodium current densities. The greater total sodium densities in phasic neurons depend on greater TTX-sensitive currents as TTX-resistant currents show no significant differences in tonic and phasic neurons. These findings become allowable by comparing membrane potentials before our measurements. In current-clamp mode, cells were exposed to membrane potentials from ~55 to ~40 mV so that TTX-sensitive channels are predominantly inactivated and subsequently large parts are not available for firing at all (8). In contrast, all cells were clamped to ~100 mV before ramp measurements, which means a much larger fraction of TTX-sensitive sodium channels are capable of ion passage. We conclude from these observations that under physiological conditions TTX-sensitive channels mediate only small parts of the inward current during action potentials.

To further quantify the finding that Na\(_{\text{v}}\)1.8-mediated currents dominate in tonic firing, we measured mRNA-levels for this channel in renal and nonrenal neurons. As tonic neurons were barely found in nonrenal neurons (only 3 in 28) and repetitive firing is dependent on occurrence of Na\(_{\text{v}}\)1.8, we assume a significantly higher expression of Na\(_{\text{v}}\)1.8 (similarly Na\(_{\text{v}}\)1.9 and Na\(_{\text{v}}\)1.7) not only in renal but also in tonic neurons.

Interestingly, we found not only higher expression levels of Na\(_{\text{v}}\)1.8 mRNA in renal DRG neurons but also a higher amount of Na\(_{\text{v}}\)1.9 mRNA. This TTX-resistant channel has very slow activation and inactivation kinetics with an overlap of both curves near resting potential resulting in a permanent current near resting potential (10). Accordingly, the current plays an important role by setting the electrogenic properties by upregulating their resting potentials and responses to subthreshold stimulation leading to hyperexcitability (2, 24). Significantly depolarized resting membrane potentials were seen in tonic compared with phasic neurons apart from hyperexcitability possibly indicating a higher activity of Na\(_{\text{v}}\)1.9.

We furthermore showed that also Na\(_{\text{v}}\)1.7 mRNA was up-regulated in renal neurons. Na\(_{\text{v}}\)1.7 is important in early phases of electrogenesis in DRG neurons, producing graded depolarizations that may boost subthreshold inputs (11) to bring DRG neurons to voltages at which Na\(_{\text{v}}\)1.8, activating at more depolarized thresholds (1), opens to produce all-or-none action potentials (17). By this mechanism, a higher expression of Na\(_{\text{v}}\)1.7 probably leads to easier activation of Na\(_{\text{v}}\)1.8 and therefore jointly causes hyperexcitability in tonic neurons.

Taken together the results of mRNA expression and electrophysiology support the assumption that especially in renal neurons, expression of Na\(_{\text{v}}\)1.8 is widespread and most likely the key condition for tonic firing.

It was demonstrated lately that 75% of all DRG neurons from L4–L5 express Na\(_{\text{v}}\)1.8, including >90% of neurons expressing markers of nociceptors like calcitonin gene-related peptide (CGRP), substance P (SP), and transient receptor potential vanilloid 1 (TRPV1). Ninety-seven percent of CGRP-positive neurons, 92% of SP-positive neurons, and 86% of TRPV1-positive neurons were found to have a coexpression of Na\(_{\text{v}}\)1.8 (37). In our previous work, we found that renal afferent neurons innervating the pelvic wall as well as the renal cortex in course next to sympathtic fibers almost throughout stained positive for CGRP and in parts SP. We also reported that almost all of the CGRP-positive primary afferent nerve fibers costained for TRPV1 and that we could also observe that tonic afferent nerve fibers were extraordinarily sensitive to protons (19). This is of physiological and clinical interest, as it had been shown that in rat models of hypertension as well as in salt-sensitive humans, metabolic acidosis increased renal acid excretion as well as metabolic production of acid (38). Plasma pH and bicarbonate were significantly lower in spontaneously hypertensive rats compared with their normotensive controls (29). Since metabolic acidosis preceded the development of hypertension in hypertensive rats, it is unlikely that the acid-base changes were the consequence of elevated blood pressure or associated renal insufficiency (29). Rats on high- and low-
salt diets have demonstrated a greater urinary acid excretion in salt-sensitive than in salt-resistant rats (4). Furthermore, it appeared that salt-sensitive rats exhibited a greater acid excretion because they were likely to produce more acid (3). Comparable results could be obtained from salt sensitive humans (36). As mentioned earlier, intact afferent renal innervation protects animals from hypertension when they are put on a high-salt diet (18).

Taking these findings together, we believe that Na1.8 plays a pivotal role in the transmission of information from the kidneys to central neuronal structures. Under physiological conditions, they might be involved in acid-base and salt metabolism but also respond to inflammatory alterations in the kidney as in hypertension or renal disease.

**Perspective**

Our study demonstrates that the renal afferent innervation exhibits an organ-specific firing characteristic that distinguishes renal afferent neurons from neurons related to other sites. It points to an increased excitability of these neurons under physiological conditions that can be associated with TTX-resistant voltage-gated sodium channels. As this mechanism is likely to be involved in sodium and acid base homeostasis, the role of afferent renal neurons and their firing activity has to be urgently elucidated in pathological models such as renal inflammation and hypertension. Prospectively, the selective modulation of, e.g., Na1.8 locally in the kidney could provide an even more elegant tool than unselective thermal ablation.

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**AUTHOR CONTRIBUTIONS**


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