Effects of a High-Salt Diet on TRPV-1-Dependent Renal Nerve Activity in Dahl Salt-Sensitive Rats

Chaoqin Xie Donna H. Wang

Department of Medicine, the Neuroscience Program, and the Cell and Molecular Biology Program, Michigan State University, East Lansing, Mich., USA

Key Words

Transient receptor potential vanilloid type 1 • Afferent renal nerve activity • Dahl rats • Salt sensitivity • Substance P • Calcitonin gene-related peptide

Abstract

Objective: To test the hypothesis that transient receptor potential vanilloid type 1 channel (TRPV1)-mediated increases in afferent renal nerve activity (ARNA) and release of substance P (SP) and calcitonin gene-related peptide (CGRP) from the renal pelvis are suppressed in Dahl salt-sensitive (DS), but not -resistant (DR), rats fed a high-salt (HS) diet. **Methods and Results:** Male DS and DR rats were given a HS or low-salt (LS) diet for 3 weeks. Perfusion of capsaicin (CAP, 10^{-6} M), a selective TRPV1 agonist, into the left renal pelvis increased ipsilateral ARNA in all groups, but with a smaller magnitude in DS-HS compared to other groups. CAP increased contralateral urine flow in all groups except DS-HS rats. CAP-induced release of SP and CGRP from the renal pelvis was less in DS-HS compared to other groups. Western blot showed that TRPV1 expression in the kidney decreased while expression of neurokinin 1 receptors increased in DS-HS compared to other groups. **Conclusion:** TRPV1-mediated

increases in ARNA and release of SP and CGRP in the renal pelvis are impaired in DS rats fed a HS diet, which can likely be attributed to suppressed TRPV1 expression in the kidney and contributes to increased salt sensitivity.

Copyright © 2010 S. Karger AG, Basel

Introduction

The transient receptor potential vanilloid type 1 (TRPV1) is a nonselective cation channel, consisting of 6 transmembrane domains with the pore-forming region located between S5 and S6 and the N- and C-terminals positioned intracellularly [1]. The sequence for TRPV1 selectivity is $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Na}^+$ [2, 3]. TRPV1 may be activated by multiple chemical and physical stimuli including vanilloid compounds, noxious heat, lipid metabolites, NaCl and protons [4, 5]. It has been shown that activation of TRPV1 expressed in the renal pelvis leads to increases in afferent renal nerve activity (ARNA), renal sodium and water excretion, and the release of substance P (SP) and calcitonin gene-related peptide (CGRP) [6]. While TRPV1-induced increases in ARNA and renal excretory function are governed by activation of the neuro-

kinin receptor 1 (NK1) by SP released from renal afferent nerves, CGRP-induced enhancement in ARNA and renal excretory function appears to be attributed to TRPV1 activation [6]. Results from single nerve fiber recording confirm that enhancement in ARNA induced by capsaicin (CAP), a selective TRPV1 agonist, perfused into the renal pelvis can be abolished not only by capsazepine, a selective TRPV1 antagonist, but also by L-703,606, a selective NK1 receptor antagonist, indicating that NK1 receptor activation plays a key role in mediating TRPV1-induced increases in ARNA [7].

TRPV1 channels are primarily expressed in sensory neurons located in dorsal root ganglia and sensory nerve terminals including unmyelinated C-fibers and thinly myelinated A δ -fibers [8, 9] that innervate various tissues, including the lung, heart, kidney and blood vessels [10–15]. In the kidney, TRPV1-positive sensory nerves heavily innervate the tubules of the renal cortex and medulla, renal pelvis, pelvi-ureteric junction and ureter, with these fibers being found between the layers of smooth muscle and epithelia in the renal pelvis [7, 16]. Activation of TRPV1 expressed in these sensory nerves leads to the release of sensory neuropeptides including SP and CGRP that may possess profound effects on the kidney and cardiovascular tissues in regulating sodium and water homeostasis and blood pressure [6, 16–20].

In the genetically predisposed salt-sensitive model, i.e. Dahl salt-sensitive (DS) rats, TRPV1 expression and function have been found to be suppressed in response to salt load [21], which may contribute to decreased glomerular filtration rate (GFR) and renal excretory function in DS rats fed a high-salt (HS) diet [22]. While these studies in DS rats are indicative of a role of TRPV1 in the regulation of salt sensitivity, no direct evidence of functional impairment of renal TRPV1 or TRPV1-positive sensory nerves innervating the kidney is available as these previous studies were conducted using either systemic activation/blockade of TRPV1 or isolated perfused kidneys [21, 22]. Thus, the purpose of the current study was to test the hypotheses that: (1) TRPV1-induced increases in ipsilateral ARNA and contralateral renal excretory function are impaired in DS, but not Dahl salt-resistant (DR), rats in response to HS intake; (2) TRPV1-triggered release of SP and CGRP from the renal pelvis is suppressed in DS, but not DR, rats fed a HS diet; and (3) impaired ARNA and neuropeptide release are accompanied with suppressed TRPV1, but not NK1, expression in the kidney in DS rats fed a HS diet.

Methods

All experiments were approved by the Institutional Animal Care and Use Committee of Michigan State University. Five-week-old male DR and DS rats (Charles River Laboratories; Wilmington, Mass., USA) were housed in the animal facility 1 week before the experiments and were randomly assigned to a low-salt (LS, 0.3% NaCl, Harlan Teklad) or HS (8% NaCl, Harlan Teklad) diet for 3 weeks and grouped as DR-LS, DR-HS, DS-LS or DS-HS. All rats drank water ad libitum throughout the experiment. To serve as a hypertensive control group, deoxycorticosterone acetate (DOCA) and salt were given to Sprague-Dawley rats (Charles River Laboratories) for 3 weeks as described previously [21]. Briefly, rats underwent uninephrectomy via a flank incision on the left hand side and a silicone rubber DOCA implant (200 mg/kg) was placed subcutaneously between the shoulder blades. After surgery, rats received 1.0% NaCl and 0.2% KCl in water to drink.

Surgical Procedures and Nerve Activity Recording

As described previously [6, 23], 50 mg·kg⁻¹ pentobarbital sodium was administered intraperitoneally for anesthesia for the recording of mean arterial pressure (MAP), ARNA with or without drug perfusion into the renal pelvis, and urine collection from the ureters (see detail in supplement). Renal nerves were isolated via a left flank incision and placed on the stainless steel electrode for the recording of multifiber nerve activity (see detail in supplement). ARNA is expressed as the percent of its basal value [6, 23].

The experiment started 1.5 h after the surgery when the rats were stabilized. After recording the basal value, 10⁻⁶ M CAP was perfused into the left renal pelvis for 3 min, and the ipsilateral ARNA was recorded during the perfusion and recovery periods. Urine from the contralateral kidney was collected for three 10-min periods for determination of the urine flow rate (Uflow), i.e. 10 min before the beginning of drug perfusion as baseline; 10 min starting at the beginning of drug perfusion, and 10 min following the second 10-min collection as the recovery period. Uflow was expressed per microliter per minute of kidney weight (μ l/min/g) [6, 23].

Radioimmunoassay for SP and CGRP Release from the Renal Pelvis

The renal pelvis from both kidneys was dissected and incubated at 37°C for 30 min as described [6, 23]. The incubation solution was collected, purified and analyzed by radioimmunoassay (rat RIA kits; Peninsula Laboratories Inc., San Carlos, Calif., USA) for quantification of SP and CGRP release. The concentrations of SP and CGRP were normalized by the kidney weight [23].

Immunofluorescence Assay

Frozen kidney sections obtained from DR and DS rats were fixed with formalin for 15 min and washed with PBS-0.01% Tween 20 for 5 min. After blocking nonspecific binding sites, the sections were incubated with goat anti-TRPV1 receptor antiserum (1:50, Santa Cruz) and/or rabbit anti-NK1 receptor antiserum (1:50, Sigma) overnight. The negative controls were incubated with 5% bovine serum albumin overnight only. After washing, the sections were incubated with donkey-anti-goat FLIC-labeled IgG (1:200, Jackson ImmunoResearch) or donkey-anti-rabbit CY2-labeled IgG (1:200, Jackson ImmunoResearch). The sections were rinsed and covered with anti-fade mounting medium and coverslips before viewing under the microscope [6, 7].

Western Blot Analysis

Membrane proteins were extracted and 50- μ g proteins were loaded to SDS gel lanes and electroblotted onto the PVDF polyvinylidene difluoride membrane (Bio-Rad). After blocking with 5% non-fat dry milk, the membranes were incubated with goat anti-TRPV1 receptor antiserum (1:400, Santa Cruz) or rabbit anti-NK1 receptor antiserum (1:800, Sigma) overnight. After washing, the membranes were incubated with secondary antibody conjugated with horseradish peroxidase (1:1,000, Santa Cruz). The membranes were developed using an ECL kit (Amersham Pharmacia Biotech) and exposed to films (Hyperfilm-ECL, Amersham Pharmacia Biotech). The films were scanned and analyzed with the use of the Image Quantity Program (Scion) to obtain integrated densitometric values. β -Actin was used to normalize protein loading on membranes.

Statistical Analysis

All values were expressed as means $\pm$ SE. The differences among groups were analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparison tests. Comparisons of MAP before and after administration of drugs were performed with the use of a paired *t* test. Differences were considered statistically significant at $p < 0.05$.

Results

There was no significant difference in MAP between DR-LS, DR-HS and DS-LS, but MAP was elevated in DS-HS and DOCA-salt rats, albeit the magnitude of the elevation was slightly but significantly smaller in the latter (fig. 1). MAP in all groups was maintained at these levels before, during and after CAP perfusion into the renal pelvis.

To assess the function of TRPV1-positive renal afferent nerves, ARNA at the basal, during and after CAP perfusion was examined in DR, DS and DOCA-salt rats. Ipsilateral ARNA was increased in all groups when 10^{-6} M CAP was perfused into the left renal pelvis (fig. 2). While the magnitude of the increases in ARNA was not different between DR-LS, DR-HS, DS-LS and DOCA-salt rats, the increase in ARNA was significantly smaller in DS-HS compared to all the other groups (fig. 2 and 3).

To examine the role of TRPV1-positive renal afferent nerves in the regulation of Uflow, urine from the contralateral kidneys was collected at the basal, during and after CAP perfusion in DR and DS rats only, given that DOCA-salt rats had only one kidney due to prior uninephrectomy. Uflow at the basal level was not different among groups, except that it was higher in DR-HS compared to DS-HS rats (fig. 4). 10^{-6} M CAP perfused into the left renal pelvis increased contralateral Uflow in DR-LS, DR-HS and DS-LS, but not DS-HS, rats (fig. 4).

Fig. 1. MAP from DR-LS, DR-HS, DS-LS, DS-HS and DOCA-salt rats ($n = 7-8$ in each group). * $p < 0.05$ compared with DR-LS, DS-LS and DR-HS groups; # $p < 0.05$ compared with DS-HS group.

To examine TRPV1-dependent SP release from the renal pelvis, radioimmunoassay was used for measurement of the levels of SP released from the renal pelvis incubated in vitro with or without CAP (fig. 5). The SP levels were not different among groups at the basal levels, but were increased in all the groups in response to 10^{-6} M CAP administration. Moreover, CAP-triggered SP release was markedly less in DS-HS compared to DR-LS, DR-HS, DS-LS and DOCA-salt rats.

To examine TRPV1-dependent CGRP release from the renal pelvis, the levels of CGRP release from the incubated renal pelvis with or without CAP was studied (fig. 6). The CGRP levels were not different among groups before CAP treatment, but were increased in all the groups in response to 10^{-6} M CAP administration. Furthermore, CAP-induced CGRP release was significantly less in DS-HS compared to DR-LS, DR-HS, DS-LS and DOCA-salt rats.

Immunofluorescence staining was used to examine the anatomical expression of TRPV1 and NK1 in the renal cortex (see online supplementary figure 7A, www.karger.com/doi/10.1159/000316528), medulla (see online suppl. fig. 7C) and pelvis (see online suppl. fig. 7E). TRPV1, but not NK1-positive, staining was seen in the tubules of the renal cortex of DR and DS rats (see online suppl. fig. 7A), whereas TRPV1 as well as NK1-positive staining was found in the renal medulla and co-localized in tubules in DR and DS rats (see online suppl. fig. 7C). Furthermore, TRPV1 and NK1-positive nerve fibers were seen between the epithelial layer and smooth muscle layer in the renal pelvis and co-localized in a subpopulation of these fibers in DR and DS rats (see online suppl. fig. 7E).

Fig. 2. ARNA activated by CAP perfused into the left renal pelvis in DR-LS, DR-HS, DS-LS, DS-HS and DOCA-salt groups (n = 5–6 in each group). ** p < 0.01 compared with basal value of each group.

Fig. 3. CAP-induced ipsilateral ARNA in DR-LS, DR-HS, DS-LS, DS-HS and DOCA-salt rats (n = 5–6 in each group). ** p < 0.01 compared with DR-LS, DR-HS, DS-LS and DOCA-salt groups.

Fig. 4. CAP-induced contralateral urine flow in DR-LS, DR-HS, DS-LS and DS-HS rats (n = 5–6 in each group). * p < 0.05 compared with DR-LS, DR-HS and DS-LS groups; # p < 0.05 compared with the DS-HS group.

Fig. 5. CAP-triggered SP release from incubated renal pelvis in DR-LS, DR-HS, DS-LS, DS-HS and DOCA-salt rats (n = 5–6 in each group). * p < 0.05 compared with respective basal value; ** p < 0.01 compared with respective basal value; # p < 0.05 compared with DR-LS, DR-HS, DS-LS and DOCA-salt groups.

Fig. 6. CAP-triggered CGRP release from incubated renal pelvis in DR-LS, DR-HS, DS-LS, DS-HS and DOCA-salt rats (n = 5–6 in each group). * p < 0.05 compared with respective basal value; ** p < 0.01 compared with respective basal value; # p < 0.05 compared with DR-LS, DR-HS, DS-LS and DOCA-salt groups.

To quantify the levels of TRPV1 and NK1 protein expression in the renal cortex (see online suppl. fig. 7B), medulla (see online suppl. fig. 7D) and pelvis (see online suppl. fig. 7F), Western blot analysis was used. In the renal cortex, medulla and pelvis, TRPV1 expression was at similar levels between DR-LS, DR-HS, DS-LS and DOCA-salt rats, but was significantly decreased in DS-HS rats compared to all other groups (see online suppl. fig. 7B, D and F). In contrast, NK1 expression was elevated in the renal medulla in DS-HS rats compared to DR-LS, DR-HS, DS-LS and DOCA-salt rats, while its expression was at similar levels among all groups in the renal pelvis and not detectable in the renal cortex (see online suppl. fig. 7B, D and F).

Discussion

The results of the present studies showed the following. First, the activation of TRPV1 by CAP perfusion into the left renal pelvis increased ipsilateral ARNA in all groups, but the increase in ARNA was smaller in DS rats fed a HS diet compared to DS rats fed a LS diet or DR rats fed a HS diet. This indicates that TRPV1-induced increases in ARNA are impaired in DS rats in the face of HS intake. In contrast, a CAP-induced increment of ARNA was not attenuated in DOCA-salt rats that had elevated blood pressure similar to that of DS-HS rats, indicating that impaired increment of ARNA induced by TRPV1 activation in DS-HS rats is model-specific and may not be the result of elevated blood pressure. Furthermore, CAP perfused into the unilateral renal pelvis increased contralateral urine flow in DR rats fed a LS or HS diet or in DS rats fed a LS diet, but not in DS rats fed a HS diet. These results indicate that TRPV1-dependent increases in renal excretory function are impaired in DS rats in response to salt loading, which may contribute to increased salt sensitivity in this strain. Additionally, CAP-triggered release of SP and CGRP from the renal pelvis was suppressed in DS, but not in DR or DOCA, rats given HS, indicating that not only afferent function (i.e. ARNA) but also effector function of TRPV1-positive nerves are impaired in DS rats when loaded with salt. Finally, impaired ARNA and neuropeptide release were accompanied with suppressed TRPV1, but not NK1, expression in the kidney in DS, but not DR or DOCA, rats given HS, indicating that HS intake impairs TRPV1 expression in the kidney of DS rats that may underlie salt-induced development of hypertension in this strain.

TRPV1-positive sensory nerves have been shown to play a key role in the regulation of cardiovascular function and blood pressure [15–20], evidenced by the facts that activation of TRPV1 expressed in the heart protects the heart from ischemia/reperfusion injury via the release of CGRP and SP [15, 19] and that activation of TRPV1 systemically results in a decrease in blood pressure due to, at least in part, vasodilatation caused by CGRP release upon TRPV1 activation [18]. In the kidney, activation of TRPV1 *in vivo* or in isolated perfused kidneys increases the GFR that results in enhanced renal sodium and water excretion [24, 25]. Moreover, activation of TRPV1 expressed in the unilateral renal pelvis leads to increases in ipsilateral ARNA, contralateral diuresis and natriuresis, and the release of SP and CGRP from the renal pelvis [6, 20, 23]. This indicates that TRPV1 plays a vital role in mediating renal function and sodium and water homeostasis. Thus, it follows that TRPV1 dysfunction would lead to impaired renal excretory function and disturbed hemodynamic homeostasis [22, 26]. The results from the present study support this notion, given that TRPV1-induced increases in ARNA and renal excretory function are impaired in DS, but not DR, rats in response to HS intake. Furthermore, while DOCA-salt-hypertensive rats have similar blood pressure as that of DS rats on a HS diet, TRPV1-dependent increases in ARNA are not diminished by DOCA-salt treatment. These results indicate that salt-induced impairment of TRPV1 expression and function in DS rats may lie intrinsically with this strain. Given that TRPV1 expression and function in the kidneys of Wistar rats could be altered as early as 3 days after HS treatment [27], failure of such changes early on or predisposed dysfunctional regulation and function of TRPV1 in DS rats in response to HS intake may contribute to increased blood pressure and associated end-organ damage. Ascertaining this would require future additional functional and genetic/genomic studies in DS rats.

Both TRPV1 and NK1 receptors have been shown to express in sensory nerves, with NK1 receptors governing the action of TRPV1 once TRPV1 is activated, leading to SP release [6, 7]. It has been shown that NK1 receptor antagonists perfused into the renal pelvis abolish TRPV1-induced increases in ARNA and diuresis and natriuresis [6, 7]. Moreover, SP-induced activation of NK1 receptors contributes to bradykinin- or mechano-mediated increases in ARNA [28, 29]. Thus, TRPV1-triggered release of SP may mediate a number of TRPV1-dependent or -independent processes. The data in the present study show that TRPV1-induced SP release from the renal pelvis is suppressed in DS, but not in DR or DOCA, rats given HS,

indicating that effector function, in addition to afferent function, of TRPV1-positive nerves is impaired in DS rats when loaded with salt and that such impairment in diminished SP release upon TRPV1 activation may underlie observed weakened TRPV1 action in DS rats fed a HS diet. Furthermore, TRPV1-triggered release of CGRP from the renal pelvis is also suppressed in DS rats given a HS diet, indicating that TRPV1-mediated protection against salt-induced hypertension via CGRP release may be disturbed in DS rats challenged with salt loading [30].

TRPV1-positive sensory nerves innervate the renal pelvis, pelvi-ureteric junction, and ureter [16, 31, 32]. In the renal pelvis, TRPV1-positive sensory nerve fibers are found between the layers of smooth muscle and epithelia [7]. In addition, TRPV1 is expressed in the tubules of the renal cortex and medulla [7, 21]. The data from the present study show that TRPV1 expression is suppressed in the renal cortex, medulla and pelvis in DS, but not DR or DOCA, rats given HS. This indicates that HS intake impairs TRPV1 expression in the kidney of DS rats leading to impaired TRPV1-dependent enhancement of ARNA and neuropeptide release. Moreover, NK1 receptors have been shown to be expressed in sensory neurons in dorsal root ganglia as well as in the renal pelvis [6]. We found that NK1 receptors are distributed in the tubules of the renal medulla in addition to the renal pelvis, but not in the renal cortex in DS and DR rats. In contrast to the change of TRPV1 expression in the kidney, NK1 expression is enhanced in the renal medulla in DS, but not DR or DOCA, rats given HS. These results indicate that altered TRPV1 expression in the kidney in DS rats fed a HS diet is molecule-specific and that suppressed TRPV1, but not NK1, receptor expression may be the cause of impaired afferent renal nerve function and renal excretory function in DS rats fed a HS diet. A cautionary note: TRPV1 is a polymodal receptor, i.e. it can be activated by various stimuli including chemical and physical factors, leading to distinct effects that are tissue-, dose- and pathophysiological condition-specific. For example, while the peripheral effect of TRPV1 is antihypertensive, TRPV1 expressed in the CNS has an antidiuresis effect by triggering the release of arginine-vasopressin in response to serum hyperosmolality [33]. As a result, it has been suggested that the difficulty to induce hypertension by merely giving salt in TRPV1 knockout mice is possibly due to, at least in part, deletion of TRPV1 in both central and peripheral sites that mediate opposed effects. Future development of conditional knockout strains may shed some light on the functional significance of TRPV1 in the kidney as well as other organs/tissues.

Prospective

The molecular mechanisms underlying salt-dependent regulation of blood pressure remain to be defined in light of the facts that over half of hypertensives are salt-sensitive and that strategies targeting reducing salt sensitivity, particularly in high-risk individuals, are urgently needed [34–36]. Impairment in several endocrine/paracrine/autocrine systems or factors occurs in DS rats, a model which closely mimics human salt-sensitive hypertension. These systems/factors include (but are not limited to) the renin-angiotensin system, the endothelin system, transforming growth factor- β , nuclear factor- κ B and 20-hydroxyeicosatetraenoic acid (20 HETE) [37–40]. It has been shown that TRPV1 mediates ETB (endothelin receptor subtype B)-dependent increases in ARNA, diuresis and natriuresis as well as 20 HETE-induced depolarization of sensory nerves and consequent vasoactive neuropeptide release [41, 42]. Thus, it is conceivable that salt-induced suppression of renal TRPV1 expression and function in DS rats could cause further functional and structural deterioration of the kidney due to its inability to mediate the beneficial effects of ETB activation and 20 HETE production, leading to withdrawal of the protective mechanisms in the face of salt challenge in DS rats. It follows that improvement of function of TRPV1 or sensory nerves may confer a therapeutic potential in the treatment of salt-sensitive hypertension and its associated end-organ damage.

Sources of Funding

This work was supported in part by National Institutes of Health (grants HL-57853, HL-73287 and DK67620) and a grant from the Michigan Economic Development Corporation.

References

- 1 Vlachova V, Teisinger J, Susankova K, Lyfenko A, Ettrich R, Vyklicky L: Functional role of C-terminal cytoplasmic tail of rat vanilloid receptor 1. *J Neurosci* 2003;23:1340–1350.
- 2 Sasamura T, Kuraishi Y: Peripheral and central actions of capsaicin and VR1 receptor. *Jpn J Pharmacol* 1999;80:275–280.
- 3 Vulcu SD, Liewald JF, Gillen C, Rupp J, Nawrath H: Proton conductance of human transient receptor potential-vanilloid type-1 expressed in oocytes of *Xenopus laevis* and in Chinese hamster ovary cells. *Neuroscience* 2004;125:861–866.
- 4 Julius D, Basbaum AI: Molecular mechanisms of nociception. *Nature* 2001;413:203–210.

- 5 Pedersen SF, Owsianik G, Nilius B: TRP channels: an overview. *Cell Calcium* 2005; 38:233–252.
- 6 Xie C, Sachs JR, Wang DH: Interdependent regulation of afferent renal nerve activity and renal function: role of transient receptor potential vanilloid type 1, neurokinin 1, and calcitonin gene-related peptide receptors. *J Pharmacol Exp Ther* 2008;325:751–757.
- 7 Feng NH, Lee HH, Shiang JC, Ma MC: Transient receptor potential vanilloid type 1 channels act as mechanoreceptors and cause substance P release and sensory activation in rat kidneys. *Am J Renal Physiol* 2008; 294:F316–F325.
- 8 Mezey E, Toth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, Guo A, Blumberg PM, Szallasi A: Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proc Natl Acad Sci USA* 2000;97:3655–3660.
- 9 Szallasi A, Nilsson S, Farkas-Szallasi T, Blumberg PM, Hokfelt T, Lundberg JM: Vanilloid (capsaicin) receptors in the rat: distribution in the brain, regional differences in the spinal cord, axonal transport to the periphery, and depletion by systemic vanilloid treatment. *Brain Res* 1995;703:175–183.
- 10 Ahluwalia A, Vallance P: Evidence for functional responses to sensory nerve stimulation of rat small mesenteric veins. *J Pharmacol Exp Ther* 1997;281:9–14.
- 11 Akerman S, Kaube H, Goadsby PJ: Vanilloid type 1 receptors (VR1) on trigeminal sensory nerve fibres play a minor role in neurogenic dural vasodilatation, and are involved in capsaicin-induced dural dilation. *Br J Pharmacol* 2003;140:718–724.
- 12 De Schepper HU, De Winter BY, Van Nas-sauw L, Timmermans JP, Herman AG, Pelckmans PA, De Man JG: TRPV1 receptors on unmyelinated C-fibres mediate colitis-induced sensitization of pelvic afferent nerve fibres in rats. *J Physiol* 2008;586:5247–5258.
- 13 Kollarik M, Undem BJ: Mechanisms of acid-induced activation of airway afferent nerve fibres in guinea-pig. *J Physiol* 2002;543:591–600.
- 14 Tan LL, Bornstein JC, Anderson CR: Distinct chemical classes of medium-sized transient receptor potential channel vanilloid 1-immunoreactive dorsal root ganglion neurons innervate the adult mouse jejunum and colon. *Neuroscience* 2008;156:334–343.
- 15 Zhong B, Wang DH: N-oleoyldopamine, a novel endogenous capsaicin-like lipid, protects the heart against ischemia-reperfusion injury via activation of TRPV1. *Am J Physiol Heart Circ Physiol* 2008;295:H728–H735.
- 16 Rolle U, Brylla E, Tillig B: Immunohistochemical detection of neuronal plexuses and nerve cells within the upper urinary tract of pigs. *BJU Int* 1999;83:1045–1049.
- 17 Li J, Kaminski NE, Wang DH: Anandamide-induced depressor effect in spontaneously hypertensive rats: role of the vanilloid receptor. *Hypertens* 2003;41:757–762.
- 18 Wang L, Wang DH: TRPV1 gene knockout impairs postischemic recovery in isolated perfused heart in mice. *Circulation* 2005; 112:3617–3623.
- 19 Zhong B, Wang DH: TRPV1 gene knockout impairs preconditioning protection against myocardial injury in isolated perfused hearts in mice. *Am J Physiol Heart Circ Physiol* 2007;293:H1791–H1798.
- 20 Zhu Y, Wang Y, Wang DH: Diuresis and natriuresis caused by activation of VR1-positive sensory nerves in renal pelvis of rats. *Hypertens* 2005;46:992–997.
- 21 Wang Y, Wang DH: A novel mechanism contributing to development of Dahl salt-sensitive hypertension: role of the transient receptor potential vanilloid type 1. *Hypertens* 2006;47:609–614.
- 22 Li J, Wang DH: Role of TRPV1 channels in renal haemodynamics and function in Dahl salt-sensitive hypertensive rats. *Exp Physiol* 2008;93:945–953.
- 23 Zhu Y, Xie C, Wang DH: TRPV1-mediated diuresis and natriuresis induced by hypertonic saline perfusion of the renal pelvis. *Am J Nephrol* 2007;27:530–537.
- 24 Li JP, Wang DH: Increased GFR and renal excretory function by activation of TRPV1 in the isolated perfused kidney. *Pharmacol Res* 2008;57:239–246.
- 25 Zhu Y, Wang DH: Segmental regulation of sodium and water excretion by TRPV1 activation in the kidney. *J Cardiovasc Pharmacol* 2008;51:437–442.
- 26 Wang DH, Zhao Y: Increased salt sensitivity induced by impairment of sensory nerves: is nephropathy the cause? *J Hypertens* 2003;21: 403–409.
- 27 Li J, Wang DH: Function and regulation of the vanilloid receptor in rats fed a high salt diet. *J Hypertens* 2003;21:1525–1530.
- 28 Kopp UC, Cicha MZ, Smith LA: Endogenous angiotensin modulates PGE(2)-mediated release of substance P from renal mechanosensory nerve fibers. *Am J Physiol Regul Integr Comp Physiol* 2002;282:R19–R30.
- 29 Kopp UC, Farley DM, Cicha MZ, Smith LA: Activation of renal mechanosensitive neurons involves bradykinin, protein kinase C, PGE(2), and substance P. *Am J Physiol Regul Integr Comp Physiol* 2000;278:R937–R946.
- 30 Huang W, Wang H, Galligan JJ, Wang DH: Transient receptor potential vanilloid subtype 1 channel mediated neuropeptide secretion and depressor effects: role of endoplasmic reticulum associated Ca^{2+} release receptors in rat dorsal root ganglion neurons. *J Hypertens* 2008;26:1966–1975.
- 31 Guo A, Vulchanova L, Wang J, Li X, Elde R: Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. *Eur J Neurosci* 1999;11: 946–958.
- 32 Robinson DR, McNaughton PA, Evans ML, Hicks GA: Characterization of the primary spinal afferent innervation of the mouse colon using retrograde labelling. *Neurogastroenterol Motil* 2004;16:113–124.
- 33 Sharif Naeini R, Witty MF, Séguéla P, Bourque CW: An N-terminal variant of Trpv1 channel is required for osmosensory transduction. *Nat Neurosci* 2006;9:93–98.
- 34 Haddy FJ: Role of dietary salt in hypertension. *Life Sci* 2006;79:1585–1592.
- 35 He FJ, Marciniak M, Visagie E, Markandu ND, Anand V, Dalton RN, MacGregor GA: Effect of modest salt reduction on blood pressure, urinary albumin, and pulse wave velocity in white, black, and Asian mild hypertensives. *Hypertension* 2009;54:482–488.
- 36 Rodriguez-Iturbe B, Romero F, Johnson RJ: Pathophysiological mechanisms of salt-dependent hypertension. *Am J Kidney Dis* 2007;50:655–672.
- 37 Dahly-Vernon AJ, Sharma M, McCarthy ET, Savin VJ, Ledbetter SR, Roman RJ: Transforming growth factor-beta, 20-HETE interaction, and glomerular injury in Dahl salt-sensitive rats. *Hypertension* 2005;45:643–648.
- 38 Kassab S, Novak J, Miller T, Kirchner K, Granger J: Role of endothelin in mediating the attenuated renal hemodynamics in Dahl salt-sensitive hypertension. *Hypertension* 1997;30:682–686.
- 39 Kobori H, Nishiyama A, Abe Y, Navar LG: Enhancement of intrarenal angiotensinogen in Dahl salt-sensitive rats on high salt diet. *Hypertension* 2003;41:592–597.
- 40 Roman RJ, Alonso-Galicia M, Wilson TW: Renal P450 metabolites of arachidonic acid and the development of hypertension in Dahl salt-sensitive rats. *Am J Hypertens* 1997;10:63S–67S.
- 41 Scotland RS, Chauhan S, Davis C, De Felipe C, Hunt S, Kabir J, Kotsonis P, Oh U, Ahluwalia A: Vanilloid receptor TRPV1, sensory C-fibers, and vascular autoregulation: a novel mechanism involved in myogenic constriction. *Circ Res* 2004;95:1027–1034.
- 42 Xie C, Wang DH: Ablation of transient receptor potential vanilloid 1 abolishes endothelin-induced increases in afferent renal nerve activity: mechanisms and functional significance. *Hypertens* 2009;54:1298–1305.