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Expression of TRPM8 in diabetic rats and its relationship with visceral pain stimulation

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ABSTRACT. Transient receptor potential cation channel, subfamily M, member 8 (TRPM8) is a nonselective cation channel and a candidate for cold sensation signaling, but the relationship between TRPM8 and diabetes remains unclear. In the present study, we determined the expression levels of TRPM8 messenger RNA (mRNA) and the levels of the TRPM8 protein in the bladder tissue of diabetic rats. We also investigated the correlation between TRPM8 expression and the visceral pain stimulation-related factor, calcitonin gene-related peptide (CGRP) in diabetic rats. The rats were sacrificed 3, 5, 7, and 15 days after streptozotocin injection, and blood was collected from their tail veins to determine the blood glucose levels. Bladder tissue was removed to assess the expression of TRPM8 mRNA by reverse transcription-polymerase chain reaction, and the expression of the TRPM8 protein by western blotting. After administering electrical stimulation (5 V/1 Hz), the expression levels of TRPM8 and CGRP proteins were determined. Our results revealed that the blood glucose level, and TRPM8 mRNA and

TRPM8 protein expression levels increased significantly in the diabetic rats. Spinal tissue protein expression levels of both TRPM8 and CGRP also increased significantly following electrical stimulation. This possibly indicates that TRPM8 is closely associated with visceral pain stimulation, and could be an independent prognostic biomarker for diabetes.

Key words: Diabetic rats; TRMP8; Visceral pain stimulation; Bladder; Calcitonin gene-related peptide

INTRODUCTION

Temperature sensitivity is essential for thermoregulation and for the recognition of potentially damaging hot or cold stimuli in the environment (Premkumar and Abooj, 2013). Defects in the ability to sense cold can lead to cold allodynia and cold hyperalgesia, which are clinically important symptoms in patients suffering from neuropathic pain, complex regional pain syndrome, chemotherapy-induced allodynia, and bladder pain syndrome (BPS) (Liu and Qin, 2011). Various molecular ion channels have been identified as candidates for cold sensation signaling, one of which is transient receptor potential cation channel, subfamily M, member 8 (TRPM8) (Liu and Qin, 2011). TRPM8 is a nonselective cation channel that is localized in subsets of rat A-δ and C-fiber sensory neurons and mouse C-fiber neurons. It is known to have multiple modes of activation, including low temperature (<28°C), menthol, icilin, eucalyptol, and modulation of intracellular pH (Liu and Qin, 2011). Upon activation, TRPM8 allows the entry of sodium and calcium ions to the cell, which leads to depolarization and the generation of an action potential; this ultimately results in the sensation of cold and cold pain (Liu and Qin, 2011).

A great deal of evidence suggests that TRPM8 may also be an important player in various chronic conditions such as inflammatory and neuropathic pain (Proudfoot et al., 2006; Xing et al., 2007; Caspani et al., 2009; Liu and Qin, 2011; Su et al., 2011; Straub, 2014). TRPM8 messenger RNA (mRNA) and protein have also been detected in the urothelia of both rat and human bladders (Stein et al., 2004; Mukerji et al., 2006). Additionally, TRPM8-immunoreactive staining has been observed in the urothelium and in nerve fibers distributed throughout the suburothelium, and a marked increase in the number of fine-caliber nerve fibers immunoreactive for TRPM8 was found in patients with BPS compared with controls (Mukerji et al., 2006). BPS is a chronic pain condition that typically presents suprapubic pain related to bladder filling, and is accompanied by other symptoms such as more frequent urination and nocturia in the absence of a definable etiology (Chai, 2002). Furthermore, BPS is a severe comorbidity of diabetes with high prevalence, and can lead to kidney injury in diabetic patients (Schmid et al., 2011; Chung et al., 2013). However, the relationship between TRPM8 and diabetes is still unclear.

In the present study, we determined the expression levels of TRPM8 mRNA and protein in bladder tissue from diabetic rats. We also investigated the correlation between TRPM8 expression and the visceral pain stimulation-related factor, calcitonin gene-related peptide (CGRP) in diabetic rats. This research will provide new insights into, and evidence for, the molecular mechanism of visceral pain in diabetic patients, and should ultimately lead to clinical therapy.

MATERIAL AND METHODS

Animals and visceral pain stimulation

Male Sprague-Dawley rats (40), aged 7-8 weeks and weighing 190-210 g were purchased

from the Animal Center of the Key Cardiology Research Center, Qilu Hospital, Shandong University. The rats were randomly divided into two groups: a control group and a diabetic group. Each rat was housed in a plastic compartment under controlled environmental conditions (25° ± 1°C; relative humidity 40-60%; a 12/12-h light-dark cycle from 7:00 am to 7:00 pm), with access to food and water *ad libitum*. Diabetic rats (N = 20) were divided into four groups at random and received an intraperitoneal injection of 65 mg/kg streptozotocin (STZ) (Sigma, USA) in 0.1 M sterile citric acid buffer solution, pH 4.5 (4 mg/mL) (Beijing Deyuan Co. Ltd., China). The control rats (N = 20) were also divided into four groups at random and received an intraperitoneal injection of sterile citric acid buffer solution only. The diabetic rats were sacrificed 3, 5, 7, and 15 days after STZ injection, and blood was collected from their tail veins to determine blood glucose levels. Bladder tissue was removed and stored at -80°C until analysis. All the procedures in the present study were performed in accordance with the guidelines for animal research provided by Shandong University.

For the visceral pain stimulation study, the diabetic rats were fixed in the supine position. The carotid artery and vagus nerve (3-4 cm) were separated from the carotid sheath. The distal end of the vagus nerve was connected to a platinum electrode. After administering electric stimulation at 5 V/1 Hz, the rats were sacrificed and the spinal cord was quickly removed and stored in liquid nitrogen.

Reverse transcription-polymerase chain reaction (RT-PCR)

The expression of TRPM8 mRNAs in the bladder was measured by RT-PCR. Briefly, total RNA from the bladder tissue was extracted using TRIzol Reagent and complementary DNAs (cDNAs) were synthesized using iScript Reverse Transcription Supermix for RT quantitative PCR (RT-qPCR) according to the manufacturer instructions (Bio-Rad Laboratories Ltd., USA). The cDNAs were amplified using SsoAdvanced SYBR Green Supermix (Bio-Rad Laboratories Ltd.). The primers were designed and synthesized by Invitrogen and the sequences are listed in Table 1. Relative mRNA expression was determined after normalizing to β-actin and calculated using the $2^{-\Delta\Delta Ct}$ equation (Ct was defined as the PCR cycle where the fluorescence signal from the specific amplified product first increased above the background), setting the blank control group as 1. Each sample was tested in triplicate.

Table 1. Primer sequences for TRPM8.

Primers	Sequence
β-actin forward	CCTCTATGCCAACACAGTGC
β-actin reverse	ACATCTGCTGGAAGGTGGAC
TRPM8 forward	CTGATCCTCTATGCCCTGGT
TRPM8 reverse	GTCCCAGTGTGCCATAACG

TRPM8 = transient receptor potential cation channel, subfamily M, member 8.

Western blotting analysis

Protein expression levels of TRPM8 and CGRP were analyzed by western blotting. Bladder or spinal tissues (5 mg) were ground and resuspended in radio-immunoprecipitation assay (RIPA) lysis buffer with protease inhibitor (RIPA/cocktail = 100/1 (v/v) (1 mL) (Abcam Co. Ltd., UK), sonicated, and centrifuged at 14,000 rpm for 10 min at 4°C. Supernatant protein concentrations were quantified using a BCA (bicinchoninic acid) Protein Assay Kit (Bio-Rad Laboratories Ltd.). Equal amounts of protein samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and were transferred to polyvinylidene difluoride membranes (Millipore).

After blocking with 5% nonfat milk in TBST (a mixture of Tris-buffered saline and Tween 20), the membranes were incubated for 2 h at room temperature with primary rabbit anti-mouse polyclonal TRPM8, CGRP, and β-actin (1:1000; Santa Cruz Co., USA). After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary goat anti-rabbit IgG antibodies (1:1000; Beijing Zhongshan Biotechnology Co., China) for 1 h at room temperature. Enhanced chemiluminescence reagent (Millipore Limited Co., USA) was used for protein detection. TRPM8 was normalized to the loading control β-actin.

Statistical analysis

Statistical analyses were performed with the SPSS 19.0 software package (SPSS Inc., Chicago, IL, USA). Data are reported as means ± standard deviation (SD). A paired *t*-test was used to assess the differences between the control and diabetic groups, and analysis of variance (ANOVA) was used to assess the differences among different groups. The threshold for statistical significance was defined as $P < 0.05$.

RESULTS

Body weight and blood glucose levels in the diabetic rats

The weight and blood glucose levels were detected in the control and diabetic groups at 3, 5, 7, and 15 days after intraperitoneal injection of sterile citric acid buffer solution or STZ injection (Table 2 and Figure 1). Compared with the control group, the diabetic rats had significantly higher blood glucose levels on day 5 (18.06 ± 2.50 *vs* 5.13 ± 0.20, $P < 0.01$), day 7 (27.77 ± 3.30 *vs* 5.50 ± 1.30, $P < 0.01$), and day 15 (34.23 ± 3.40 *vs* 5.30 ± 0.87, $P < 0.01$). Additionally, body weight was significantly lower in the diabetic group on day 7 (209.7 ± 12.1 *vs* 237.7 ± 9.6, $P < 0.05$) and day 15 (223.3 ± 5.5 *vs* 277.0 ± 8.0, $P < 0.01$). ANOVA revealed significant differences among the different groups in both body weight and blood glucose level ($P < 0.05$).

Table 2. Difference in body weight and blood glucose level between the control and diabetic groups (N = 5).

	Day 3	Day 5	Day 7	Day 15
Body weight (g)				
Control	206.7 ± 7.5	215.0 ± 7.8	237.7 ± 9.6	277.0 ± 8.0
Diabetic	201.3 ± 8.0	216.0 ± 8.7	209.7 ± 12.1*	223.3 ± 5.5**[1]
Blood glucose level (mM)				
Control	5.67 ± 0.50	5.13 ± 0.20	5.50 ± 1.30	5.30 ± 0.87
Diabetic	10.03 ± 0.38*	18.06 ± 2.50**[1]	27.77 ± 3.30**[1,2]	34.23 ± 3.40**[1,2,3]

Data are reported as means ± standard deviation (SD); *$P < 0.05$, compared with the control by paired *t*-test; **$P < 0.01$, compared with the control by paired *t*-test; [1]$P < 0.05$, compared with day 3 by ANOVA; [2]$P < 0.05$, compared with day 5 by ANOVA; [3]$P < 0.05$, compared with day 7 by ANOVA.

Expression of TRPM8 mRNA and protein in the bladder tissue of the diabetic rats

In the bladder tissue, the expression of TRPM8 mRNA was measured by RT-PCR and the expression of the TRPM8 protein was measured by western blotting after normalizing to β-actin (Table 3 and Figure 2). Gene expression and protein expression of TRPM8 did not vary greatly in the control group from day 0 to day 15. In the diabetic group, the expression of TRPM8 mRNA in the bladder was not significantly different than that in the control group on day 3, but was significantly

greater on days 5, 7, and 15 (P < 0.05), when it was approximately two times (2.60 ± 0.56 *vs* 1.17 ± 0.35, P < 0.05), three times (4.27 ± 0.40 *vs* 1.30 ± 0.40, P < 0.01), and six times (6.10 ± 1.05 *vs* 1.00 ± 0.27, P < 0.01) higher than that in the control, respectively. In contrast, the band for the TRPM8 protein became significantly more intense on day 5 (0.66 ± 0.01 *vs* 0.35 ± 0.03, P < 0.05), day 7 (1.26 ± 0.06 *vs* 0.47 ± 0.11, P < 0.05), and day 15 (1.51 ± 0.03 *vs* 0.39 ± 0.05, P < 0.01), respectively. This was approximately consistent with the gene expression results for TRPM8 in the diabetic rats. ANOVA revealed significant differences among the different groups (P < 0.05).

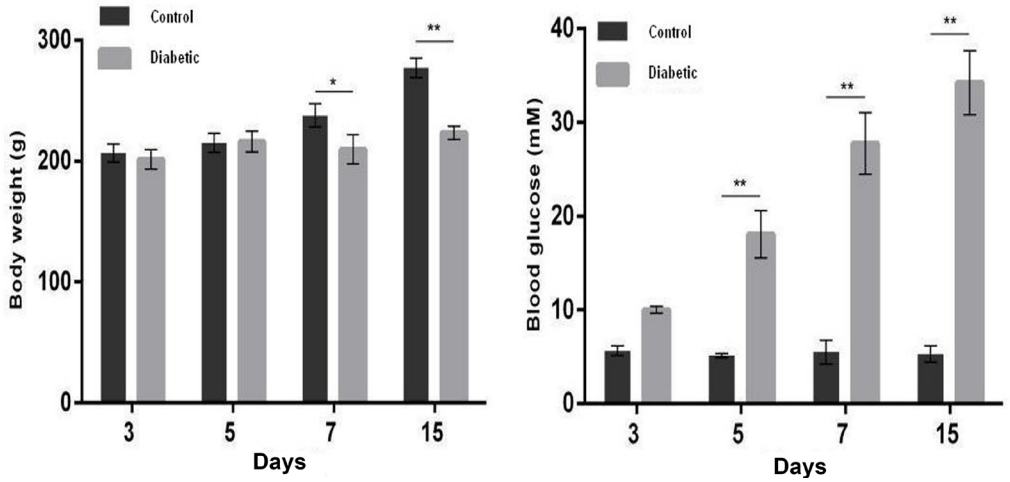

Figure 1. Differences in body weight and blood glucose levels between the control and diabetic groups (N = 5). *P < 0.05. **P < 0.01.

Table 3. Relative expression levels of TRPM8 mRNA and protein in the bladders of diabetic rats and controls after normalizing to β-actin (N = 5).

	Day 3	Day 5	Day 7	Day 15
mRNA expression				
Control	0.93 ± 0.26	1.17 ± 0.35	1.30 ± 0.40	1.00 ± 0.27
Diabetic	1.30 ± 0.53	2.60 ± 0.56*[1]	4.27 ± 0.40**[1,2]	6.10 ± 1.05**[1,2,3]
Protein expression				
Control	0.76 ± 0.01	0.35 ± 0.03	0.47 ± 0.11	0.39 ± 0.05
Diabetic	0.82 ± 0.03	0.66 ± 0.01*	1.26 ± 0.06**[1,2]	1.51 ± 0.03**[1,2,3]

TRPM8 = transient receptor potential cation channel, subfamily M, member 8. Data are reported as means ± standard deviation (SD); *P < 0.05, compared with the control by paired *t*-test; **P < 0.01, compared with the control by paired *t*-test; [1]P < 0.05, compared with day 3 by ANOVA; [2]P < 0.05, compared with day 5 by ANOVA; [3]P < 0.05, compared with day 7 by ANOVA.

Relationship between TRPM8 and CGRP protein expression levels in the diabetic rats after visceral pain stimulation

Furthermore, TRPM8 and CGRP protein expression levels in the spinal tissue of diabetic rats were assessed to investigate the relationship between TRPM8 and visceral pain stimulation. Compared with the control, protein expression of both TRPM8 and CGRP in the spinal tissue of the

diabetic rats increased significantly after electrical stimulation (5 V/1 Hz) (Figure 3).

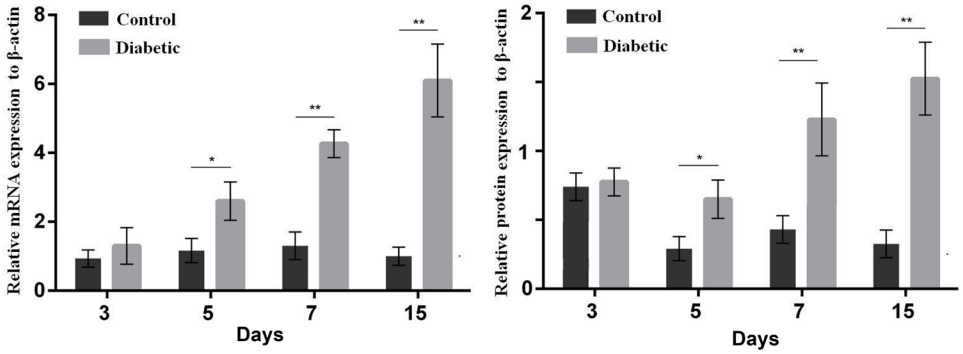

Figure 2. Relative expression levels of TRPM8 mRNA and protein in the bladders of diabetic rats and controls after normalizing to β-actin (N = 5). *P < 0.05. **P < 0.01. TRPM8: transient receptor potential cation channel, subfamily M, member 8.

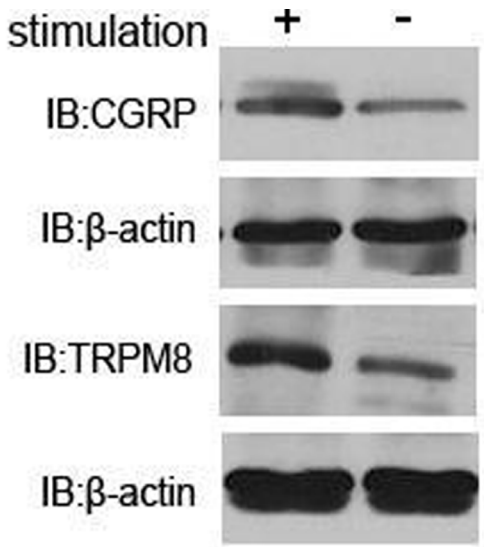

Figure 3. Relationship between TRPM8 and CGRP protein expression levels in diabetic rats with and without visceral pain stimulation. TRPM8: transient receptor potential cation channel, subfamily M, member 8; CGRP: calcitonin gene-related peptide.

DISCUSSION

Transient receptor potential channels include about 28 channels that share some structural similarity to each other, and mediate a variety of sensations such as pain, sensitivity to heat or cold, taste, pressure, and vision (Liu and Qin, 2011). Among them, TRPM8 acts as a cold sensor and can be activated by low temperatures (<28°C), menthol, icilin, eucalyptol, and the modulation of intracellular pH (Liu and Qin, 2011). It has been reported that TRPM8 can mediate the detection of

cold thermal stimuli by primary afferent sensory neurons (McKemy et al., 2002; Peier et al., 2002). Studies with TRPM8 knockout mice and selective TRPM8 channel blockers have demonstrated a lack of cold sensitivity and reduced cold pain in various rodent models (Thut et al., 2003; Chung and Caterina, 2007; Colburn et al., 2007; Dhaka et al., 2007; Caspani et al., 2009; Eberle et al., 2009; Liu and Qin, 2011). In contrast, some studies have questioned the role of TRPM8 in cold detection, or proposed that other excitatory or inhibitory channels are more important to this sensory modality *in vivo* (Viana et al., 2002; McKemy, 2005; Madrid et al., 2006). However, TRPM8 is still a target for the mechanism for cold compress.

Recently, TRPM8 has been considered a major contributor to various chronic conditions such as inflammatory and neuropathic pain (Proudfoot et al., 2006; Xing et al., 2007; Caspani et al., 2009; Liu and Qin, 2011; Su et al., 2011; Straub, 2014). TRPM8 mRNA and protein have been detected in the urothelia of both rat and human bladders (Stein et al., 2004; Mukerji et al., 2006). Additionally, TRPM8-immunoreactive staining has been observed in the urothelium and in nerve fibers distributed throughout the suburothelium, and a marked increase in the number of fine-caliber nerve fibers immunoreactive for TRPM8 was found in patients with BPS compared with controls ($P < 0.05$) (Mukerji et al., 2006). Furthermore, TRPM8 antagonists lower body temperature in rats, and might be used to treat cold hypersensitivity and hyperalgesia in several neuropathic conditions, including complex regional pain syndrome and trigeminal neuralgia, peripheral nerve injury, and chemotherapy-induced neuropathy (Almeida et al., 2012; Gavva et al., 2012; Patel et al., 2014; Winchester et al., 2014). Moreover, TRPM8 has higher expression in prostate cancer tissue than in normal prostate tissue, and has also been detected in tumors of the breast, colon, lung, skin, and pancreas (Tsavaler et al., 2001; Yee et al., 2010; Okamoto et al., 2012). The expression levels of TRPM8 mRNA in bladder tissue from urothelial carcinoma subjects were also significantly higher than those in matched noncancerous tissues ($P = 0.016$) (Xiao et al., 2014). Expression of the TRPM8 protein in bladder tissue from urothelial carcinoma subjects was significantly and positively associated with histological grade ($P = 0.039$) and tumor stage ($P = 0.037$) (Xiao et al., 2014). Significant correlation has been found between high TRPM8 expression and poor cumulative survival in patients with urothelial carcinoma of the bladder, suggesting that overexpression of TRPM8 may play a role in the pathogenesis and progression of the disease (Xiao et al., 2014). However, the relationship between TRPM8 and diabetes is still unclear.

In the present study, we determined the expression profile of the TRPM8 mRNA and the TRPM8 protein in bladder tissue from diabetic rats. Significantly high blood glucose levels and low body weight were found in the rats that received an intraperitoneal STZ injection on days 5, 7, and 15 ($P < 0.05$; Figure 1 and Table 2). The expression of TRPM8 mRNA in the bladder tissue was significantly higher on days 5, 7, and 15 in the diabetic group compared with the control group ($P < 0.05$), with 6-fold higher expression on day 15 ($P < 0.01$; Figure 2 and Table 3). Furthermore, the band for the TRPM8 protein in the bladder tissue of the diabetic rats was significantly more intense on days 7 and 15 ($P < 0.05$; Figure 3), which was approximately consistent with the gene expression results for TRPM8 in the diabetic rats. Subsequently, the expression levels of TRPM8 and CGRP proteins in spinal tissue from the diabetic rats were assessed to investigate the relationship between TRPM8 and visceral pain stimulation in diabetic rats. CGRP is produced in both peripheral and central neurons; it acts as a potent peptide vasodilator and can function in the transmission of pain (McCoy et al., 2013; Russo, 2015). Compared with the control, the expression levels of both TRPM8 and CGRP proteins in spinal tissue from diabetic rats were significantly greater after electrical stimulation (5 V/1 Hz). These results provide the first convincing evidence that TRPM8 may serve as an independent prognostic biomarker for diabetes, and may play an

important role in visceral pain in diabetic patients.

In conclusion, our results showed that blood glucose levels, and TRPM8 mRNA and protein expression levels were significantly increased in the diabetic rats. Additionally, expression levels of both TRPM8 and CGRP proteins in the spinal tissue from diabetic rats were significantly higher than those in controls. This might indicate that TRPM8 has a close relationship with visceral pain stimulation, and could be an independent prognostic biomarker for diabetes.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Almeida MC, Hew-Butler T, Soriano RN, Rao S, et al. (2012). Pharmacological blockade of the cold receptor TRPM8 attenuates autonomic and behavioral cold defenses and decreases deep body temperature. *J. Neurosci.* 32: 2086-2099. http://dx.doi.org/10.1523/JNEUROSCI.5606-11.2012

Caspani O, Zurborg S, Labuz D, Heppenstall PA (2009). The contribution of TRPM8 and TRPA1 channels to cold allodynia and neuropathic pain. *PLoS One* 4: e7383. http://dx.doi.org/10.1371/journal.pone.0007383

Chai TC (2002). Diagnosis of the painful bladder syndrome: current approaches to diagnosis. *Clin. Obstet. Gynecol.* 45: 250-258. http://dx.doi.org/10.1097/00003081-200203000-00026

Chung MK and Caterina MJ (2007). TRP channel knockout mice lose their cool. *Neuron* 54: 345-347. http://dx.doi.org/10.1016/j.neuron.2007.04.025

Chung SD, Keller J and Lin HC (2013). A nationwide population-based study on bladder pain syndrome/interstitial cystitis and ED. *Int. J. Impot. Res.* 25: 224-228. http://dx.doi.org/10.1038/ijir.2013.15

Colburn RW, Lubin ML, Stone DJ Jr, Wang Y, et al. (2007). Attenuated cold sensitivity in TRPM8 null mice. *Neuron* 54: 379-386. http://dx.doi.org/10.1016/j.neuron.2007.04.017

Dhaka A, Murray AN, Mathur J, Earley TJ, et al. (2007). TRPM8 is required for cold sensation in mice. *Neuron* 54: 371-378. http://dx.doi.org/10.1016/j.neuron.2007.02.024

Eberle T, Doganci B, Kramer HH, Geber C, et al. (2009). Warm and cold complex regional pain syndromes: differences beyond skin temperature? *Neurology* 72: 505-512. http://dx.doi.org/10.1212/01.wnl.0000341930.35494.66

Gavva NR, Davis C, Lehto SG, Rao S, et al. (2012). Transient receptor potential melastatin 8 (TRPM8) channels are involved in body temperature regulation. *Mol. Pain.* 8: 36. http://dx.doi.org/10.1186/1744-8069-8-36

Liu Y and Qin N (2011). TRPM8 in health and disease: cold sensing and beyond. *Adv. Exp. Med. Biol.* 704: 185-208. http://dx.doi.org/10.1007/978-94-007-0265-3_10

Madrid R, Donovan-Rodriguez T, Meseguer V, Acosta MC, et al. (2006). Contribution of TRPM8 channels to cold transduction in primary sensory neurons and peripheral nerve terminals. *J. Neurosci.* 26: 12512-12525. http://dx.doi.org/10.1523/JNEUROSCI.3752-06.2006

McCoy ES, Taylor-Blake B, Street SE, Pribisko AL, et al. (2013). Peptidergic CGRPalpha primary sensory neurons encode heat and itch and tonically suppress sensitivity to cold. *Neuron* 78: 138-151. http://dx.doi.org/10.1016/j.neuron.2013.01.030

McKemy DD (2005). How cold is it? TRPM8 and TRPA1 in the molecular logic of cold sensation. *Mol. Pain* 1: 16. http://dx.doi.org/10.1186/1744-8069-1-16

McKemy DD, Neuhausser WM and Julius D (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416: 52-58. http://dx.doi.org/10.1038/nature719

Mukerji G, Yiangou Y, Corcoran SL, Selmer IS, et al. (2006). Cool and menthol receptor TRPM8 in human urinary bladder disorders and clinical correlations. *BMC Urol.* 6: 6. http://dx.doi.org/10.1186/1471-2490-6-6

Okamoto Y, Ohkubo T, Ikebe T, Yamazaki J (2012). Blockade of TRPM8 activity reduces the invasion potential of oral squamous carcinoma cell lines. *Int. J. Oncol.* 40: 1431-1440.

Patel R, Goncalves L, Newman R, Jiang FL, et al. (2014). Novel TRPM8 antagonist attenuates cold hypersensitivity after peripheral nerve injury in rats. *J. Pharmacol. Exp. Ther.* 349: 47-55. http://dx.doi.org/10.1124/jpet.113.211243

Peier AM, Moqrich A, Hergarden AC, Reeve AJ, et al. (2002). A TRP channel that senses cold stimuli and menthol. *Cell* 108: 705-715. http://dx.doi.org/10.1016/S0092-8674(02)00652-9

Premkumar LS and Abooj M (2013). TRP channels and analgesia. *Life Sci.* 92: 415-424. http://dx.doi.org/10.1016/j.lfs.2012.08.010

Proudfoot CJ, Garry EM, Cottrell DF, Rosie R, et al. (2006). Analgesia mediated by the TRPM8 cold receptor in chronic neuropathic pain. *Curr. Biol.* 16: 1591-1605. http://dx.doi.org/10.1016/j.cub.2006.07.061

Russo AF (2015). Calcitonin Gene-Related Peptide (CGRP): A New Target for Migraine. *Annu. Rev. Pharmacol. Toxicol.* 55: 533-552. http://dx.doi.org/10.1146/annurev-pharmtox-010814-124701

Schmid C, Berger K, Muller M, Silke J, et al. (2011). Painful bladder syndrome: management and effect on sexual function and quality of life. *Ginekol. Pol.* 82: 96-101.

Stein RJ, Santos S, Nagatomi J, Hayashi Y, et al. (2004). Cool (TRPM8) and hot (TRPV1) receptors in the bladder and male genital tract. *J. Urol.* 172: 1175-1178. http://dx.doi.org/10.1097/01.ju.0000134880.55119.cf

Straub RH (2014). TRPV1, TRPA1, and TRPM8 channels in inflammation, energy redirection, and water retention: role in chronic inflammatory diseases with an evolutionary perspective. *J. Mol. Med.* 92: 925-937. http://dx.doi.org/10.1007/s00109-014-1175-9

Su L, Wang C, Yu YH, Ren YY, et al. (2011). Role of TRPM8 in dorsal root ganglion in nerve injury-induced chronic pain. *BMC Neurosci.* 12: 120. http://dx.doi.org/10.1186/1471-2202-12-120

Thut PD, Wrigley D and Gold MS (2003). Cold transduction in rat trigeminal ganglia neurons *in vitro*. *Neuroscience* 119: 1071-1083. http://dx.doi.org/10.1016/S0306-4522(03)00225-2

Tsavaler L, Shapero MH, Morkowski S, Laus R (2001). Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. *Cancer Res.* 61: 3760-3769.

Viana F, de la Pena E and Belmonte C (2002). Specificity of cold thermotransduction is determined by differential ionic channel expression. *Nat. Neurosci.* 5: 254-260. http://dx.doi.org/10.1038/nn809

Winchester WJ, Gore K, Glatt S, Petit W, et al. (2014). Inhibition of TRPM8 channels reduces pain in the cold pressor test in humans. *J. Pharmacol. Exp. Ther.* 351: 259-269. http://dx.doi.org/10.1124/jpet.114.216010

Xiao N, Jiang LM, Ge B, Zhang TY, et al. (2014). Over-expression of TRPM8 is associated with poor prognosis in urothelial carcinoma of bladder. *Tumour Biol.* 35: 11499-11504. http://dx.doi.org/10.1007/s13277-014-2480-1

Xing H, Chen M, Ling J, Tan W, et al. (2007). TRPM8 mechanism of cold allodynia after chronic nerve injury. *J. Neurosci.* 27: 13680-13690. http://dx.doi.org/10.1523/JNEUROSCI.2203-07.2007

Yee NS, Zhou Wa and Lee M (2010). Transient receptor potential channel TRPM8 is over-expressed and required for cellular proliferation in pancreatic adenocarcinoma. *Cancer Lett.* 297: 49-55. http://dx.doi.org/10.1016/j.canlet.2010.04.023