Expression of TRPM8 in the distal cerebrospinal fluid-contacting neurons in the brain mesencephalon of rats
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Abstract

Background: It has been shown that distal cerebrospinal fluid-contacting neurons (dCSF-CN) exist near the ventral midline of the midbrain aqueduct and also in the grey matter of the inferior third ventricle and the fourth ventricle floor in the superior segment of the pons. The dCSF-CN communicate between the cerebrospinal fluid (CSF) and the brain parenchyma and may participate in the transduction and regulation of pain signals. The cold sensation receptor channel, TRPM8 is involved in analgesia for neuropathic pain, but whether the TRPM8 receptor exists on dCSF-CN remains unknown. However, there is preliminary evidence that TRPM8 is expressed in dCSF-CN and may participate in the transmission and regulation of sensory information between brain parenchyma and cerebrospinal fluid (CSF) in rats.

Methods: Retrograde tracing of the cholera toxin subunit B labeled with horseradish peroxidase (CB-HRP) injected into the lateral ventricle was used to identify dCSF-CN. A double-labeled immunofluorescent technique and laser scanning confocal microscopy were used to identify the expression of TRPM8 in dCSF-CN. Software Image-Pro Plus was used to count the number of neurons in three sections where CB-HRP positive neurons were located in the mesencephalon of six rats.

Results: The cell bodies of CB-HRP-positive dCSF-CN were found in the brain parenchyma near the midline of the ventral Aq, also in the grey of the 3V, and the 4V floor in the superior segment of the pons. In the mesencephalon their processes extended into the CSF. TRPM8 labeled neurons were also found in the same area as were CB-HRP/TRPM8 double-labeled neurons. CB-HRP/TRPM8 double-labeled neurons were found in 42.9 ± 2.3% of neurons labeled by TRPM8, and all CB-HRP-labeled neurons were also labeled with TRPM8.

Conclusion: This study has demonstrated that the cold sensation receptor channel, TRPM8, is localized within the dCSF-CN of the mesencephalon. TRPM8 acts as receptor of dCSF-CN for sensation transmission and pain regulation.
Background
The transient receptor potential (TRP) channel is a transmembrane protein which is a thermo-sensitive channel that is expressed on sensory neurons and skin epithelial cells in vertebrate and non-vertebrate animals [1,2]. A subtype of TRP, TRPM8 which was originally cloned as a prostate-specific protein, has been widely known as a cold- and menthol-activated channel implicated in thermosensation. Recent studies have revealed that TRPM8 is not only necessary for cold sensation [3,4], but also mediates the process of analgesia for neuropathic pain activated by cold [5]. The results indicate that TRPM8 is related to cold-induced pain relief and may have some potential for pharmacological applications.

There are three types of CSF-CNs: intraependymal neurons which project into the ventricle lumen and the central canal of the spinal cord, supraependymal cells which are subjacent to the ependyma, and distal CSF-CNs [6,7]. Many studies have been made on the distribution of CSF-CNs in the parenchyma of the brain with horseradish peroxidase (HRP) and autoradiography. However, a significant amount of data has shown that both HRP and radiolabeled substances can pass freely through the ependymal lining of the ventricles into the parenchyma of the brain [7]. Because of this property, it has been necessary to develop an alternative neuronal marker that does not pass through the ependymal layer and will only label synaptic functions in the brain parenchyma distal to the ventricle system and their processes extend into the CSF. This suggests that they transmit signals between brain parenchyma and CSF. It is not known whether the signaling directions of the neuron are from CSF to the parenchyma, the parenchyma to CSF or both. Our previous experiment found that there were different synapses between CSF-CNs in mesencephalon region ventral to the aqueduct [7]. There were not only axo (-)-dendritic (+) synapses, but also dendo (-)-dendritic (+) synapses. However, their common characteristics were that the presynaptic elements were formed by non-CSF contacting neurons in the brain parenchyma, and the postsynaptic elements formed by the neurons in DR contacting the CSF. According to the general rule of chemical synapses, impulses are conducted from the presynaptic membrane to the postsynaptic membrane. Hence it was suggested that the signaling direction of the CSF-CNs in mesencephalon region ventral to the aqueduct occurs only from the brain parenchyma to the ventricular CSF [7]. Our previous studies indicated that dCSF-CNs may participate in transduction and regulation of pain signals [7,8]. TRPM8 function is related to the process of analgesia for neuropathic pain [5]; however, whether the specific pain signal receptor TRPM8 exists in dCSF-CNs remains unknown.

This paper, presents the first description and preliminary results of TRPM8 expressed on dCSF-CNs.

To determine whether the distal cerebrospinal fluid-contacting neurons possess the membrane receptor, TRPM8, we combined the cholera toxin B horseradish peroxidase (CB-HRP) retrograde tracing with TRPM8 immunofluorescence double-labeling technique to investigate the function of the dCSF-CNs.

Methods
Animal care and treatment
All experiments were conducted in accordance with the guidelines of the International Association for the Study of Pain (IASP) and approved by the Committee for the Ethical Use of Laboratory Animals, Xuzhou Medical College, license number: SYXK (Jiangsu) 2002–0038. Six male Sprague-Dawley rats (250 ± 50 g) were obtained from the experimental animal center, Xuzhou Medical College. The SPF grade rats were maintained in climate and light-controlled (23 ± 1°C, 12/12 h dark/light cycle with light on at 08:00 h) for at least one week prior to the experiments.
CB-HRP injection
Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and the head fixed in a stereotaxic instrument (Narishige Scientific Instruments, Tokyo Japan). A 3-μl volume of 30% CB-HRP (Sigma) was injected into one LV according to stereotaxic coordinates (Bregma: -1.2 ± 0.4 mm, Depth: 3.2 ± 0.4 mm, Right of median sagittal plane: 1.4 ± 0.2 mm).

Tissue processing
Forty-eight h following tracer injection, rats were deeply anesthetized again with intraperitoneal pentobarbital sodium (50 mg/kg) and transcardially perfused with 150 ml of phosphate buffered saline (0.01 M PBS, pH 7.4), followed without interruption by 4% paraformaldehyde in 0.2 M phosphate buffer (300 ml, pH 7.4). The brainstem was removed immediately and post-fixed for 4–48 h at 4°C, then cryoprotected by immersion for 24–48 h in sucrose gradients (5%, 10%, 15%, 20%, and 30%) with 0.01 mol/L PBS at 4°C. The brainstem embedded with OCT, at -20°C and sectioned on a cryostat (Leica CM1900, Germany) at 25 μm in the transverse plane.

Immunofluorescence procedures and confocal microscopy technique
The frozen sections were collected in PBS. Following 3 washes in PBS, sections were incubated in PBS with 0.3% triton X-100 (PBST) for 48–72 h at 4°C with an goat anti-cholera toxin B-subunit in (1:200, 227040, Calbiochem, San Diego, USA) and rabbit Anti-TRPM8 (ab3243, Abcam, Cambridge, UK). After rinsing in PBS, sections were incubated and in donkey anti-goat IgG-conjugated to tetraethyl rhodamine isothiocyanate (sc-2094 Santa Cruz Biotechnology), and in donkey anti-rabbit IgG conjugated to fluorescein isothiocyanate (sc-2090, Santa Cruz Biotechnology, Santa Cruz, USA), in the dark for 1 h at room temperature. Finally, sections were rinsed, mounted, and coverslipped with glycerol containing 2.5% of anti-fading agent DABCO (1, 4-di-aza-bi-cyclo-2, 2, 2-tetraoctane, Sigma, USA) and stored at -20°C in the dark. Tissue sections were examined using laser scanning confocal microscopy (TCS SP2, Leica, Wetzlar, Germany) to identify dCSF-CNs labeled with TRITC and TRPM8 labeled with FITC.

Image analysis
Software Image-Pro Plus Version 6.0 (MediaCybernetics, Bethesda, USA) was used to count the number of neurons. Three sections with centralized CB-HRP positive neurons were chosen from the same aspect of brain parenchyma in each rat. Under 100× magnification sections 846.8 μm × 655.3 μm in area were selected to count the number of CB-HRP, TRPM8 and double-labeled CB-HRP/TRPM8-positive neurons. Data are expressed as mean ± SD.

Results

Location of labeled neurons
The positive cells with labeling from the ventricular CB-HRP were observed as described by Zhang et al. [7,8]. These dCSF-CNs were found near the midline of the ventral Aq (Figure 1A and Figure 1B), also in the grey of the 3V, and the 4V floor in the superior segment of the pons.

Image analysis and morphology
Positive labeling of the CB-HRP-traced neurons in the ventral aqueduct region was mainly located in cytoplasm, and the majority were multipolar and round or oval in shape. The cell bodies of these dCSF-CNs were in the brain parenchyma and the processes extended into CSF. A tissue section, CB-HRP immunoreactivity in red TRITC is illustrated in Figure 2A. TRPM8-positive neurons (FITC, green) were more numerous (Figure 2B). CB-HRP/TRPM8 double-labeled neurons were found in only a proportion of neurons labeled for TRPM8. On the other hand, all neurons labeled for CB-HRP were also CB-HRP/TRPM8-double-labeled neurons (Figure 2C).

Neuron counting
Neurons were counted on three sections from each rat and the results are reported in Table 1. The mean number of double-labeled neurons in an area of 846.8 μm × 655.3 μm from the mesencephalon of six rats was 380 +/- 22. The mean number of TRPM8-labeled neurons for the same area was 884 +/- 19 or 42.9% of the double-labeled neurons.

Discussion
TRPM8 is a transient receptor potential cation channel which, after activation, is permeable to Ca2+ influx [9]. A previous study has suggested that TRPM8 not only conducts cold sensation but also has an important role in cold-induced pain relief [5]. TRPM8 was originally thought to be expressed almost exclusively in the prostate and in a number of non-prostatic primary tumours of breast, colon, lung and skin [10]. However, later studies detected TRPM8 mRNA or protein (or both) in a subset of sensory neurons from the DRG (dorsal root ganglion), trigeminal ganglia [11-13], nodose ganglion cells innervating the upper gut [14], gastric fundus [15], vascular smooth muscle [16], liver [17], in bladder urothelium, and different tissues of the male genital tract [18]. Recent studies have revealed that TRPM8 is not only necessary for cold sensation [3,4], but also mediates the process of analgesia for neuropathic pain after being activated by cold [5].

The dCSF-CNs were found near the midline of the ventral midbrain aqueduct (Aq) and also found in the grey of the third ventricle (3V) in the inferior and the fourth ventricle (4V) floor in the superior segment of the pons where neu-
Immunofluorescence of distal CSF-contacting neurons in rat mesencephalon

Figure 1
Immunofluorescence of distal CSF-contacting neurons in rat mesencephalon. Neurons labeled with tetraethyl rhodamine isothiocyanate (TRITC, red) after intraventricular administration of CB-HRP, were found in the mesencephalon ventral to the aqueduct. The number of labeled neurons varied in different sections (A, B). AQ: midbrain aqueduct. Scale bar: 100 μm.

Dual labelling of neurons with CB-HRP/TRPM8 fluorescent immunohistochemistry in rats

Figure 2
Dual labelling of neurons with CB-HRP/TRPM8 fluorescent immunohistochemistry in rats. A: CB-HRP positive neurons (red). The cell bodies of dCSF-CN5s were in the brain parenchyma and the processes were extended into CSF. B: the same section showing TRPM8 positive neurons (green). C: same section showing CB-HRP/TRPM8 double-labeled neurons (arrow, yellow). All CB-HRP/TRPM8 double-labeled neurons were coincident with the neurons labeled by CB-HRP. Scale bar: 100 μm.
rons for pain regulation are concentrated [7]. Without
special tracing method, the dCSF-CNs were hard to iden-
tify from the non-CSF-CNs in parenchyma of the brain.
Up to present, their structure and function have been little
investigated.

Both clinical practice and animal experiments indicate
that the chemical composition of the CSF can change in
pathological or special physiological conditions [19-22].
The reasons, origin and receptors for these chemical
changes remain unclear. Our tracing experiment with
intraventricular CB-HRP has shown that the bodies of
CSF-CN in DR were in the brain parenchyma and their
processes extended into CSF in the ventricle system. Our
previous electron microscope observation showed that
there were both excitatory and inhibitory synapses
between non-CSF-CNs and CSF-CN in DR and found that
the axon terminals of CSF-CN labeled by CB-HRP
extended directly into the cavity of 3V [7]. Hence we can
presume that when the CSF contacting neurons are stim-
ulated in the brain by receiving signals, their axon termi-
nals extend into the cavity of brain ventricles possibly to
release some chemical substances into CSF and change
the CSF composition. We speculate that the dCSF-CNs are
closely linked to inflammatory and neuropathic pain, and
morphine dependency and withdrawal [7,8]. However, no
previous studies have focused on the relationship
between dCSF-CNs and cold and pain sensation. There-
fore, we speculate that the dCSF-CNs participate in pain
modulation via the cold sensation receptor TRPM8. This
study demonstrates that the cold sensation channel,
TRPM8, is expressed in the distal CSF-contacting neurons
of mesencephalon ventral to the cerebral aqueduct. The
existence of TRPM8 immunoreactivity suggests that CSF-
CNs may transmit thermal and pain sensation to non-
CSF-CNs in the central nervous system neurons via
TRPM8.

Using the HRP tracing method [23,24], investigators
found that the neurons in the mesencephalon region ven-
tral to the aqueduct received many axis-cylinder contacts
from many regions of the brain. These regions include the
locus coeruleus nucleus, the solitary tract nucleus, the
raphe magnus nucleus, the substantia nigra, the thalamo-
central medial nucleus, the parafascicular nucleus, the
gigantocellular reticular nucleus, the preoptic area and the
cortex. Many studies have indicated that the neurons in
the mesencephalon region ventral to the aqueduct have
many physiological functions including thermoregula-
tion [25], sleep [26-28], cardiovascular functions [29],
hormone-endocrine functions [30,31], multiple arousal
systems [32], obesity [33], anxiety and depression

| Rat number | CB-HRP and TRPM8 double-labeled (yellow) | TRPM8 (green) | CB-HRP/TRPM8(%) |
|------------|----------------------------------------|---------------|-----------------|
| R1         | 405                                    | 909           | 44.6            |
| R2         | 366                                    | 892           | 41.0            |
| R3         | 389                                    | 861           | 45.0            |
| R4         | 344                                    | 876           | 39.3            |
| R5         | 395                                    | 898           | 44.0            |
| R6         | 378                                    | 868           | 43.5            |
| Mean ± SD  | 380 ± 22                               | 884 ± 19      | 42.9 ± 2.3      |

All the brain tissue sections counted had CB-HRP/TRPM8 double-labeled neurons.
[34,35], conditioned-fear and stress [36], sexual behaviors [37] and mood disorders [38] and are also important in pain modulation and anti-nociceptive function [39,40]. Zhang et al. [7] indicated that the mesencephalon region ventral to the aqueduct was a locus which had the largest number of distal CSF contacting neurons and the most concentrated distribution. It is suggested that the neurons in the mesencephalon region ventral to the aqueduct also play an important role in signaling between the brain and CSF. In 1992, Wang & Zhang first used CB-HRP in retrograde tracing to label dCSF-CNs and obtained satisfactory results [41].

CB-HRP is an ideal dCSF-CN tracer which is absorbed by neurons via their receptors [8]. The sensitivity is 10 to 100 times higher compared to HRP [41]. A small dose applied to the peripheral nerves is sufficient to show the axis, dendrite, and neurons around peripheral nerve and its degeneration time is of long duration. However, labeling the CNS using CB-HRP is poor, thus it has been rarely used. Based on our prior experiment [7], we assumed that the dCSF-CNs are different from the general central nervous neurons and their function is similar to that of peripheral neurons. TRPM8 is a cation channel that is specifically located on the peripheral receptors and is expressed in the dCSF-CNs. Our results indicate that the dCSF-CNs may be similar in function to peripheral sensory neurons. In addition, these findings provide a novel method for exploring the function and nature of dCSF-CNs.

Conclusion

Our data suggest that the cold sensation receptor channel, TRPM8, is expressed in the nucleus of dCSF-CNs. The dCSF-CNs may play the important roles in neuromodulation and neuroendocrine regulation between brain parenchyma and CSF.

List of abbreviations

CB-HRP: cholera toxin B conjugated to horseradish peroxidase; CSF-CNs: Cerebrospinal fluid-contacting neurons; dCSF-CNs: distal CSF-CNs; TRP: Transient receptor potential.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

JD and XY contributed equally to this work. JD carried out the experiments, performed data acquisition and part drafted the manuscript. XY was involved with interpretation and manuscript revision. LZ conceived and designed the project, revised experiments and drafted the manuscript. YZ was involved with manuscript revision. All authors read and approved the final manuscript.

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References

1. Seebacher F, Murray SA: Transient receptor potential ion channels control thermoregulatory behaviour in reptiles. PLoS ONE 2007, 2:e281.
2. Frederick J, Buck ME, Matson D, Cortright D: Increased TRPA1, TRPM8, and TRPV2 expression in dorsal root ganglia by nerve injury. Biochem Biophys Res Commun 2007, 358:1058-1064.
3. Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL: The menthol receptor TRPM8 is the principal detector of environmental cold. Nature 2007, 448:204-208.
4. Dhaka A, Murray AN, Mathur J, Earley TJ, Petrus MJ, Patapoutian A: TRPM8 is required for cold sensation in mice. Neuron 2007, 54:371-378.
5. Vigh B, Silva MJM, Frank CL, Vincze C, Czirok SJ, Szabo A: The system of cerebrospinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain. Histol Histopathol 2004, 19:607-628.
6. Zhang LC, Zeng YM, Ting J, Cao JP, Wang MS: The distributions and signaling directions of the cerebrospinal fluid contacting neurons in the parenchyma of a rat brain. Brain Res 2003, 979:1-8.
7. Lu X, Geng X, Zhang L, Zeng Y: The methodology for labeling the distal cerebrospinal fluid-contacting neurons in rats. J Neurosci Methods 2008, 168:98-103.
8. Voets T, Owsiannik G, Nilius B, TRPM8. Handb Exp Pharmacol 2007, 179:239-44.
9. Tsavaler L, Shapero MH, Morkowski S, Laus R: 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 2001, 61:3760-3769.
10. Peier AM, Mogrich A, Hergarden AC, Reese AJ, Andersson DA, Story GM: A TRP channel that senses cold stimuli and menthol. Cell 2002, 108:705-715.
11. McKemy DD, Neuhausser WM, Julius D: Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 2002, 416:52-58.
12. Nealen ML, Gold MS, Thut PD, Caterina MJ: TRPM8 mRNA is expressed in a subset of cold-responsive trigeminal neurons from rat. J Neurophysiol 2003, 90:515-520.
13. Zhang L, Jones S, Brody K, Costa M, Brookes SJ: Thermosensitive transient receptor potential channels in vagal afferent neurons of the mouse. Am J Physiol Gastrointest Liver Physiol 2004, 286(4):G983-G991.
14. Mustafa S, Oriowo M: Cooling-induced contraction of the rat gastric fundus: mediation via transient receptor potential (TRP) cation channel TRPM8 receptor and Rho-kinase activation. Clin Exp Pharmacol Physiol 2005, 32:822-838.
15. Yang X, R Li M, Mclnrosh LS, Sham JS: Functional expression of transient receptor potential melastatin-(TRPM) and vaniloid-related (TRPV) channels in pulmonary arterial and aortic smooth muscle. Am J Physiol Lung Cell Mol Physiol 2006, 290:L1267-L1276.
16. Henshall SM, Afar DE, Hiller J, Horvath LG, Quinn DI, Rassiah KK: Survival analysis of genome-wide gene expression profiles of prostate cancers identifies new prognostic targets of disease relapse. Cancer Res 2003, 63:4196-4203.
17. Stein RJ, Santos S, Nagatomi Y, Hayashi Y, Minnery BS, Xavier M: Cool (TRPM8) and hot (TRPV1) receptors in the bladder and male genital tract. J Urol 2004, 172:1175-1178.
18. Segen J, Kemmler JE: Increased levels of Met-enkephalin-like immunoreactivity in the spinal cord CSF of rats with adrenal medullary transplants. Brain Res 1989, 502:1-10.
19. Spaziante R, Merola B, Colao A: Beta-endorphin concentrations both in plasma and in cerebrospinal fluid in response to acute painful stimuli. J Neurosurg Sci 1990, 34:99-106.
21. Strittmatter M, Grauer M, Isenberg E: Cerebrospinal fluid neuropeptides and monoaminergic transmitters in patients with trigeminal neuralgia. Headache 1997, 37:211-216.
22. Zubrzycka M, Janecka A: Substance P content in the cerebrospinal fluid and fluid perfusing cerebral ventricles during elicitation and inhibition of trigemino-hypoglossal reflex in rats. Brain Res 2002, 941:29-33.
23. Marchand JE, Hagnino N: Afferents to the periaqueductal gray in the rat. Neuroscience 1983, 9:95-106.
24. Saka K, Salvert D, Touret M: Afferent connections of the nucleus raphe dorsalis in the cat as visualized by the horseradish peroxidase technique. Brain Res 1977, 137:1-35.
25. Gottschlich KW, Werner J: Effects of medial midbrain lesions on thermoreceptive neurons in the thalamus of the rat. Exp Brain Res 1985, 57:355-361.
26. Gao J, Zhang JX, Xu TL: Modulation of serotonergic projection from dorsal raphe nucleus to basolateral amygdala sleep-waking cycle of rats. Brain Res 2002, 945:60-70.
27. Monti JM, Jantos H, Monti D: Increased REM sleep after intradorsal raphe nucleus injection of foesinodox or 8-OHDPAT: prevention with WAY 100635. Eur Neuropsychopharmacol 2002, 12:47-55.
28. Shima K, Nakahama H, Yamamoto M: Firing properties of two types of nucleus raphe dorsalis neurons during the sleep-waking cycle and their responses to sensory stimuli. Brain Res 1986, 399:317-326.
29. Morilak DA, Fornal C, Jacobs BL: Single unit activity of noradrenergic neurons in locus coeruleus and serotonergic neurons in the nucleus raphe dorsalis of freely moving cats in relation to the cardiac cycle. Brain Res 1986, 399:262-270.
30. Han FY, Xu YZ: Morphological observation in the median rapheal nuclei of the rabbit. Acta of 2nd Academiae medicine. Beijing 1981, 4:93-106.
31. Koibuchi N, Kato M, Egawa TK: Suppression of human growth hormone (GH)-releasing hormone induced Gh secretion in pentobarbital-anesthetized rats after electrical stimulation of the midbrain central gray and several raphe nuclei. Endocrinology 1988, 122:659-664.
32. Brown RE, Sergeeva OA, Eriksson KS, Haas HL: Convergent excitation of dorsal raphe serotonin neurons by multiple arousal systems. J Neurosci 2002, 22:8850-8859.
33. Ohlinger FP, Horowitz J, Horwitz B: Enhanced adrenergic excitation of serotonergic dorsal raphe neurons in genetically obese rats. Neurosci Lett 2002, 332:107.
34. Clark MS, Sexton Tj, Clain M: Overexpression of 5-HT1B receptor in dorsal raphe nucleus using Herpes Simplex Virus gene transfer increases anxiety behavior after inescapable stress. J Neurosci 2002, 22:4550-4562.
35. Picciotto MR, Picciotto MR, Brunzell DH, Caldarone B: Effect of nicotine and nicotinic receptors on anxiety and depression. Neuroreport 2002, 13:1097-1106.
36. Ishida Y, Hashiguchi H, Takeda R: Conditioned-fear stress increases Fos expression in monoaminergic and GABAergic neurons of the locus coeruleus and dorsal raphe nuclei. Synapse 2002, 45:46-51.
37. Keyama M, Yamanouchi K: Female sexual behaviors in male rats with dorsal raphe nucleus lesions. Brain Res Bull 1993, 30:705-709.
38. Baumann B, Bielau H: Circumscribed numerical deficit of dorsal raphe neurons in mood disorders. Psychol Med 2002, 32:93-103.
39. Chuma T, Tanaka K, Kato M: Modulation of noradrenergic and serotonergic transmission by noxious stimuli and intrathecal morphine differs in the dorsal raphe nucleus of anesthetized rats: in vivo voltammetric studies. Neurosci Res 2002, 44:37-44.
40. Wang QQ, Na K: The dorsal raphe: an important nucleus in pain modulation. Brain Res Bull 1994, 34:575-585.
41. Wang HS, Zhang LC: Methodological comparison on the tracing CSF-CN with HRP and CB-HRP. Acad Med 1992, 12:286-287.