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Research report

The distribution of kisspeptin and its receptor GPR54 in rat dorsal root ganglion and up-regulation of its expression after CFA injection

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ABSTRACT

Kisspeptin/GPR54 system plays a crucial role in the control of puberty onset and reproductive function. In the present study, we gave the first report that kisspeptin and GPR54 were expressed in the small- to large-sized neurons, and co-localized with *Bandeiraea simplicifolia isolectin* B4 (IB4), calcitonin-gene-related peptide (CGRP) and neurofilament 200 (NF200) in the L4/5 dorsal root ganglion (DRG) of naïve rats, detected by the double immunofluorescent staining. Interestingly, a marked elevation in the levels of KiSS-1 and GPR54 mRNA as well as protein was observed in the spinal dorsal horn and DRG 4 and 14 days following intra-articular injection of complete Freund's adjuvant (CFA), indicating a possible involvement of the kisspeptin/GPR54 system in chronic inflammatory pain.

1. Introduction

Kisspeptins, the peptide products of the KiSS-1 gene, were identified in 2001 as natural ligands of the previously orphan G protein-coupled receptor, GPR54 [9,10], which is also named AXOR12 [17] or OT7T175 [18]. They include, among others, kisspeptin-54 (or metastin), kisspeptin-14, kisspeptin-13 and kisspeptin-10, which share the same C terminal Arg-Phe-NH_2 structure [9]. The expression of KiSS-1 and its peptide was presented at every level of neuraxis studied, being higher in the hypothalamus, midbrain, and spinal cord. Kisspeptin has also been shown to be expressed in several peripheral tissues, most notably, the placenta, pancreas and liver [3].

The GPR54 receptor shared a significant homology with the rat galanin receptor [10] and it is highly conserved between humans and rodents [4]. GPR54 expression is found in the central nervous system, including the hypothalamus, hippocampus, spinal cord and in peripheral tissues similar to kisspeptin. However, to our knowledge, the expression as well as the distribution of kisspeptin and GPR54 in the rat dorsal root ganglion (DRG) has yet not been studied.

The known biological functions of kisspeptin were initially restricted to their ability to suppress tumor metastasis, hence the name of metastin. Numerous recent studies were focused on the role of the kisspeptin/GPR54 system in the control of puberty onset and reproductive function [8,20,24]. Kisspeptin has also been implicated as a novel endogenous factor which is dynamically regulated by neuronal activity and increases synaptic transmission in dentate granule cells in the hippocampus [1]. Some other reports suggested that the kisspeptin/GPR54 system might participate in sensory neural signaling and nociception. In the spinal cord, metastin-like immunoreactive stainings formed a dense plexus in superficial layers I and II of the dorsal horn, the primary center in pain transmission [6]. The topographic distribution of metastin-like immunoreactivity in various brain areas is in many respects parallel to that of endomorphin 1 and endomorphin 2 [14], as well as that of mu-opioid receptors [13], raising the possibility that metastin interacts with the opioid

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system, which is known to play an important role in pain modulation.

Complete Freund's adjuvant (CFA) has been utilized to induce an arthritic immunopathological disease that displays many of the pathological features of human rheumatoid arthritis [5,19]. CFA induced monoarthritis (MA) could remain stable hyperalgesia state up to 2–5 weeks [15], and hence was applied as one of the experimental models of chronic inflammatory pain. In the present study, we observed the cellular distribution of kisspeptin and GPR54 in the DRG of naïve rat as well as an up-regulation of kisspeptin and GPR54 mRNA and protein level after CFA injection, using the CFA induced monoarthritic rat model.

2. Materials and methods

2.1. Animals

Experiments were performed on adult male Sprague Dawley rats weighing 200–220 g, supplied by the Experimental Animal Center, Chinese Academy of Sciences, Shanghai. Prior to experimental manipulation, rats were allowed to acclimate for 1 week in groups of four rats per cage, and maintained under controlled conditions (22 ± 1 °C, 6 a.m. to 6 p.m. alternate light–dark cycles) with food pallets and water *ad libitum*. All experiments were conducted strictly in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the guidelines of the International Association for the Study of Pain [25]. All efforts were made to minimize the number of animals used and their suffering.

2.2. Induction of monoarthritis

Monoarthritis was induced by an intra-articular injection of CFA (Sigma, St. Louis, USA; 1 mg/ml) as previously described [15]. Briefly, the sterilized left foot of the anesthetized rat was held and the fossa of the lateral malleolus of the fibula was located. A 28-gauge needle was inserted vertically to penetrate the skin, and turned distally to insert into the articular cavity from the gap between the tibiofibular and tarsus bone until a distinct loss of resistance was felt. A volume of 50 μl CFA was then injected.

2.3. Behavioral test

The paw withdrawal latency (PWL) to radiant heat was examined as previously described [15]. Briefly, rats were placed into an inverted, clear plastic cage upon an elevated floor of window glass. After an accommodation period of 30 min, using IITC Model 390 Paw Stimulator Analgesia Meter (Life Science Instruments, USA), a constant intensity radiant heat source (50 W, 8 V bulb) was aimed at the ankle joint until the rat lifted its paw. The time from onset of radiant heat application to paw withdrawal was defined as PWL. The intensity of radiant heat was adjusted to elicit the response around 12 s in normal rats, and a cut-off time was set at 20 s in order to avoid tissue injury. Both hind paws were tested independently with a 15 min interval between tests. All the experiments were carried out every other day at the same time of the day between 8 a.m. and 12 a.m. to avoid diurnal variation in behavioral tests. The PWLs were measured before the CFA injection and the 6 h, 4 days and 14 days following CFA injection.

2.4. Real-time quantitative RT-PCR

The mRNA changes of expression of KiSS-1 and GPR54 were examined by real-time RT-PCR. The time points of analysis were selected as normal as well as 6 h, 4 days, and 14 days following CFA injection. At each time point, four animals of each group were used for every analysis.

Rats were sacrificed with an overdose of sodium pentobarbital (50 mg/kg, i.p.) and the lumbar spinal cord and the L4/5 DRGs were collected in dry ice. A real-time RT-PCR method was used to quantify the relative expression of KiSS-1 and GPR54 mRNA. Briefly, total RNA was extracted using Trizol (Invitrogen, USA) and reverse transcribed with oligo (dT) and M-MLV reverse transcriptase (Promega, Madison, WI, USA). PCR reactions were performed in the presence of the oligonucleotide primers for KiSS-1 (F: 5′CTCGCTGGCTTCTTGGCA3′; R: 5′GGGTTCAGGGTTCACCACA3′) and GPR54 (F: 5′CACTTTCCTTCTGTGCTGCGT3′; R: 5′ACCGAGACCTGCTGGATGTAGT3′), and quantified by Sybergreen I (Molecular Probes, USA). And the GAPDH (F: 5′AAGAAGGTGGTGAAGCAGGC3′; R: 5′TCCACCACCCTGTTGCTGTA3′) housekeeping gene was chosen as an internal control. End point PCR products were initially assessed on ethidium bromide-stained agarose gels that gave a single band of the expected size for each assay. The lightcycler software Version 4.0 (Roche, Germany) was used for instrument control, automated data collection, and data analysis. Relative quantification of the mRNA expression levels of target genes was calculated. All samples were run in duplicate and the average values were used for the relative quantification of the mRNA expression.

2.5. Immunohistochemistry

Sixteen deeply anaesthetized rats were perfused via the aorta with 200 ml of normal saline followed by 200 ml 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). Of the rats, four naïve rats were used for the double immunofluorescent staining and four rats in the 6 h, 4 days, 14 days group each were used to determine changes in kisspeptin or GPR54 expression after CFA injection. The L4/5 DRGs and lumbar spinal cord were removed, postfixed in the fixative solution for 4 h at 4 °C, and immersed in 30% sucrose in PB for 24 h at 4 °C for cryoprotection. Frozen sections (30 μm) were cut and collected in cryoprotectant solution (0.05 M PB, 30% sucrose, 30% ethylene glycol) and then stored at −20 °C until use.

Free-floating tissue sections were processed for kisspeptin and GPR54 protein immunocytochemistry by the avidin–biotin method. Sections were brought to room temperature and rinsed in 0.1 M PBS followed by blocking in 0.3% H_2O_2 for 10 min. Following three 15 min rinses in 0.01 M PBS, the sections were pre-incubated for 30 min at room temperature in a blocking solution of 3% normal rabbit serum in 0.01 M PBS with 0.3% Triton-X 100 (NGST). The sections were then incubated in polyclonal goat anti-(KiSS-1) antibody (1:50, C-20, Santa Cruz Biotechnology, USA) or goat anti-GPR54 (1:50, Santa Cruz, USA) diluted in 1% NGST at 4 °C for 24 h. The incubated sections were washed three times in 1% NGST and incubated in biotinylated rabbit anti-goat immunoglobulin G (IgG) (1:200, Jackson ImmunoResearch, USA) for 1 h at room temperature, washed three times in 1% NGST and incubated for 1 h in avidin–biotin–peroxidase complex (1:100, Vector Laboratories) at room temperature. Finally, the sections were washed three times in 0.01 M PBS, and the immunoreactive products was visualized by catalysis of 3,3-diaminobenzidine (DAB) by horseradish peroxidase in the presence of 0.01% H_2O_2. The sections were then mounted, dehydrated and covered. The sections were observed and analyzed using Leica Q500IWimage analysis system. The total density of immunoreactive signals in the spinal dorsal horn as well as DRG per section was measured. For each animal, four sections were taken from the lumbar spinal cord segments or DRG and the mean value was calculated. The investigator responsible for image analysis was blind to the experimental condition of each rat.

For double immunofluorescent staining, the sections from the naïve rats were blocked with 5% normal donkey serum in NGST for 1 h at 37 °C. Sections were then incubated overnight at 4 °C with primary antibody goat anti-KiSS-1 or goat anti-GPR54 antibody. Next, the primary antibody was combined with one of the following antibodies: mouse anti-neurofilament 200 (NF200, 1:1000, Sigma, St. Louis, USA), FITC-conjugated *Bandeiraea simplicifolia isolectin* B4 (IB4, 1:1000, Sigma, St. Louis, USA), or rabbit anti-calcitonin-gene-related peptide (CGRP, 1:5000, Sigma, St. Louis, USA). Then, incubation with the secondary antibodies (rabbit anti-goat TRITC (for visualizing KiSS-1 and GPR54), donkey anti-mouse FITC (for NF200) and donkey anti-rabbi FITC (for CGRP)) was carried out for 1 h at 37 °C. Photographs were taken using a confocal laser scanning microscope in sequential scan mode to avoid channel crosstalk (TCS SP2 Leica Microsystems) and analyzed using Optimas 6.2 software. Only neurons positively classified in both individual images were recorded as co-expressing cells.

Control experiments were carried out to determine the amount of non-specific binding by omitting the primary antiserum from the incubation media. Pre-incubation of primary antibody with the blocking peptide overnight reduced the labeling to background levels.

For analysis, randomly selected photographs (six per animal) were taken with a confocal laser scanning microscope in sequential scan mode to avoid channel crosstalk (TCS SP2 Leica Microsystems).

The area of each neuron was measured using Optimas 6.2 software (Optimas Corporation). The mean diameter was calculated assuming a symmetric round cell. Only neurons positively classified in both individual images were categorized to co-express proteins. The investigator responsible for image analysis was blind to the experimental condition of each rat.

2.6. Statistical analysis

Data are presented as mean ± S.E.M. and analyzed by statistical software SPSS 11.5. Repeated measures analysis of variance (ANOVA) followed by Student–Newman–Keuls test was used for post hoc analysis for differences between groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Co-localization of kisspeptin and GPR54 with IB4, CGRP and NF200 in the DRG of naïve rat

In order to identify the cell types in the DRG, double immunostaining of kisspeptin and GPR54 with either IB4, CGRP or NF200 was performed, respectively (Fig. 1). IB4 and CGRP labeling was observed in the cytoplasm of small-sized (<30 μm) to medium-sized (30–40 μm) neurons, whereas NF200 labeling in the

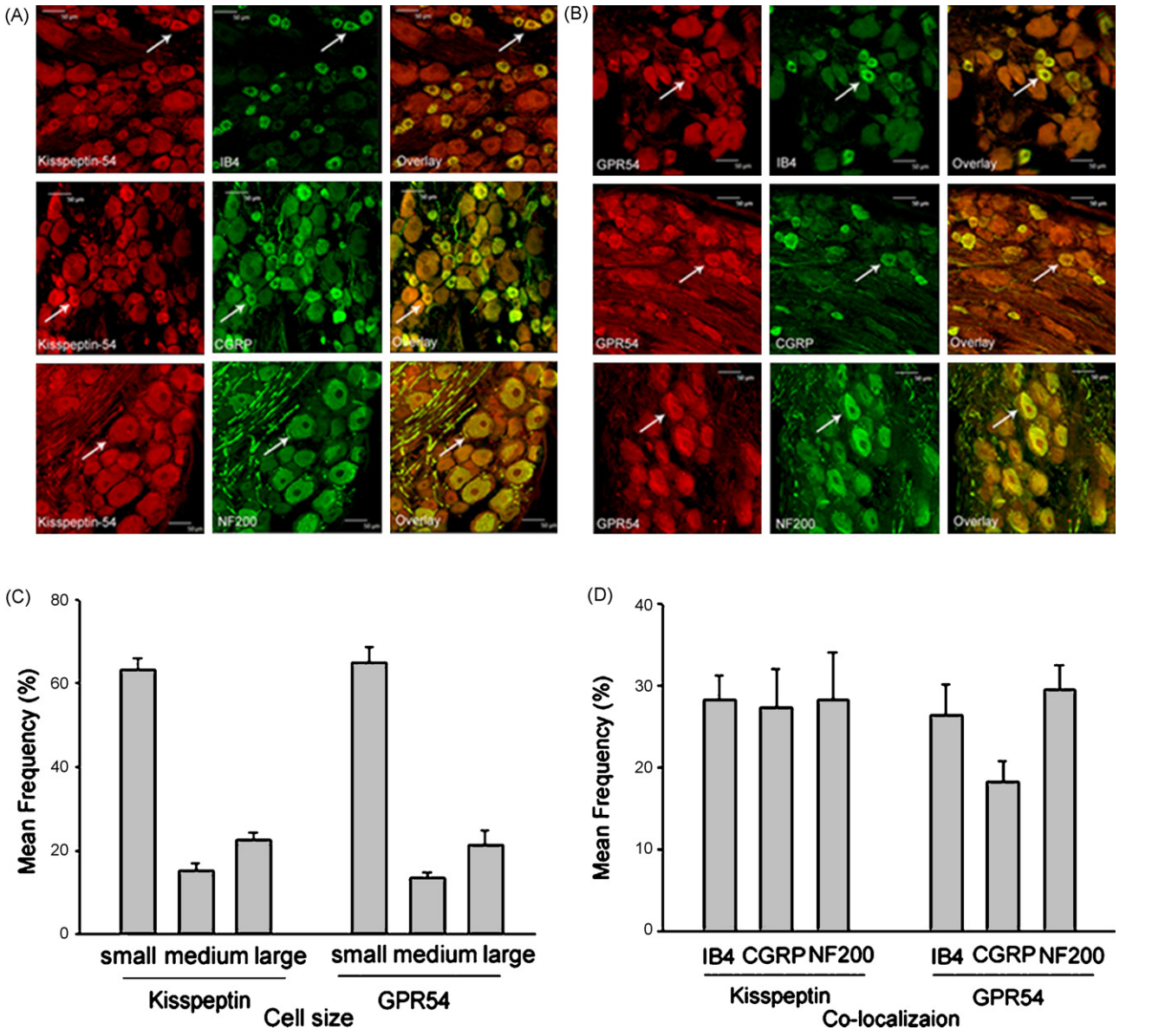


Fig. 1. Immunohistochemical co-localization of red reaction product for kisspeptin (A), GPR54 (B) and green product for IB4, CGRP or NF200 in the L4/5 DRG of naïve rats. Double staining appears yellow. In all rows, an example of double labeling is indicated with an arrow. (C) Size distribution of kisspeptin and GPR54-labeled neurons in the L4/5 DRG. Small size: the diameter <30 μm; medium size: 30–40 μm; large size: >40 μm. (D) Quantification of the percentage of co-localization of IB4, CGRP and NF200 with kisspeptin and GPR54 in the L4/5 DRG neurons. Scale bar = 50 μm.

cytoplasm and membrane of mainly medium-sized to large-sized (>40 μm) neurons. Kisspeptin-like immunoreactive (kisspeptin-LI) labeling was observed in the cytoplasm of small- to large-sized neurons, but it is mainly expressed in the small-sized neurons, with 63 ± 3% of the diameter of the kisspeptin-positive neurons less than 30 μm (Fig. 1A and C). And kisspeptin-LI labeling was co-expressed with IB4, CGRP as well as NF200 with similar proportion (Fig. 1A and D). Very similar expression patterns were found for GPR54 in the DRG (Fig. 1B and C), and the same holds true for the co-expression of GPR54 and IB4, CGRP or NF200 (Fig. 1B and D).

3.2. Thermal hyperalgesia induced by intra-articular CFA injection in rats

Before CFA injection, the basal withdrawal latencies of all rats were not distinctly different. The PWL in the normal rats remained stable over the observation period, while ipsilateral PWL markedly decreased at 6 h, 4 days and 14 days in the MA rats, compared with the normal rats ($P < 0.01$) (Fig. 2).

3.3. Up-regulation of KiSS-1 and GPR54 mRNA levels in the lumbar spinal cord and L4/5 DRG in MA rats

Low levels of KiSS-1 mRNA were presented in the normal rat lumbar spinal cord (Fig. 3A) and L4/5 DRG (Fig. 3B). Intra-articular injection of CFA produced a marked elevation in the mRNA levels of KiSS-1 in the spinal cord as well as DRG from 6 h to 14 days, compared with the normal group ($P < 0.05$). Similarly, the GPR54 mRNA levels in both spinal cord and DRG were remarkably elevated 4 days and 14 days following CFA injection, compared with the low level normal group (Fig. 3C and D) ($P < 0.05$).

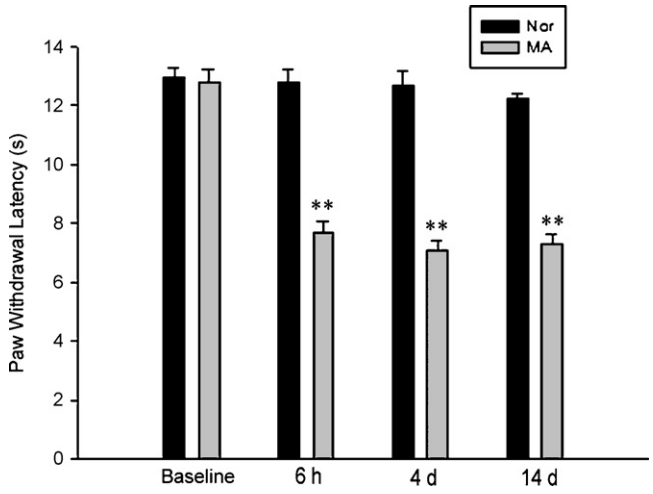


Fig. 2. Unilateral intra-articular injection of complete Freund's adjuvant (CFA) induces significant decreases in PWLs to thermal stimulation in the ipsilateral hindpaw. Data were presented as mean ± S.E.M. ($n = 8$ in each group) $^{**}P < 0.01$ vs. normal group.

3.4. Up-regulation of kisspeptin and GPR54 in the lumbar spinal cord and L4/5 DRG in MA rats

Immunohistochemistry revealed that intra-articular administration of CFA produced a significant increase in the expression for the kisspeptin in the spinal dorsal horn as well as DRG after the CFA injection, compared with the normal control group ($P < 0.05$) (Fig. 4I). Mild expression of the kisspeptin-LI labeling signals was observed in the nerve-fiber-like structures of the superficial layers of spinal dorsal horn in the naïve rats (Fig. 4A and C). On day 4 after CFA administration, dense kisspeptin-LI signals were observed in the spinal dorsal horn (Fig. 4B). The kisspetin-LI cells in the DRG also remarkably increased 4 days after CFA injection, compared with the normal control (Fig. 4C and D). Similarly, the GPR54 expression was markedly elevated in the spinal dorsal horn and DRG (Fig. 4E–H).

4. Discussion

To characterize the fiber types and the localization of DRG neurons expressing kisspeptin and GPR54, markers for subpopulations of a small diameter, unmyelinated nonpeptidergic C-type fibers (IB4), a small diameter, unmyelinated peptidergic C-type fibers (CGRP) and a large diameter, myelinated A-type fibers (NF200) were used for double labeling. The results in the present study showed for the first time that kisspeptin and GPR54 were co-

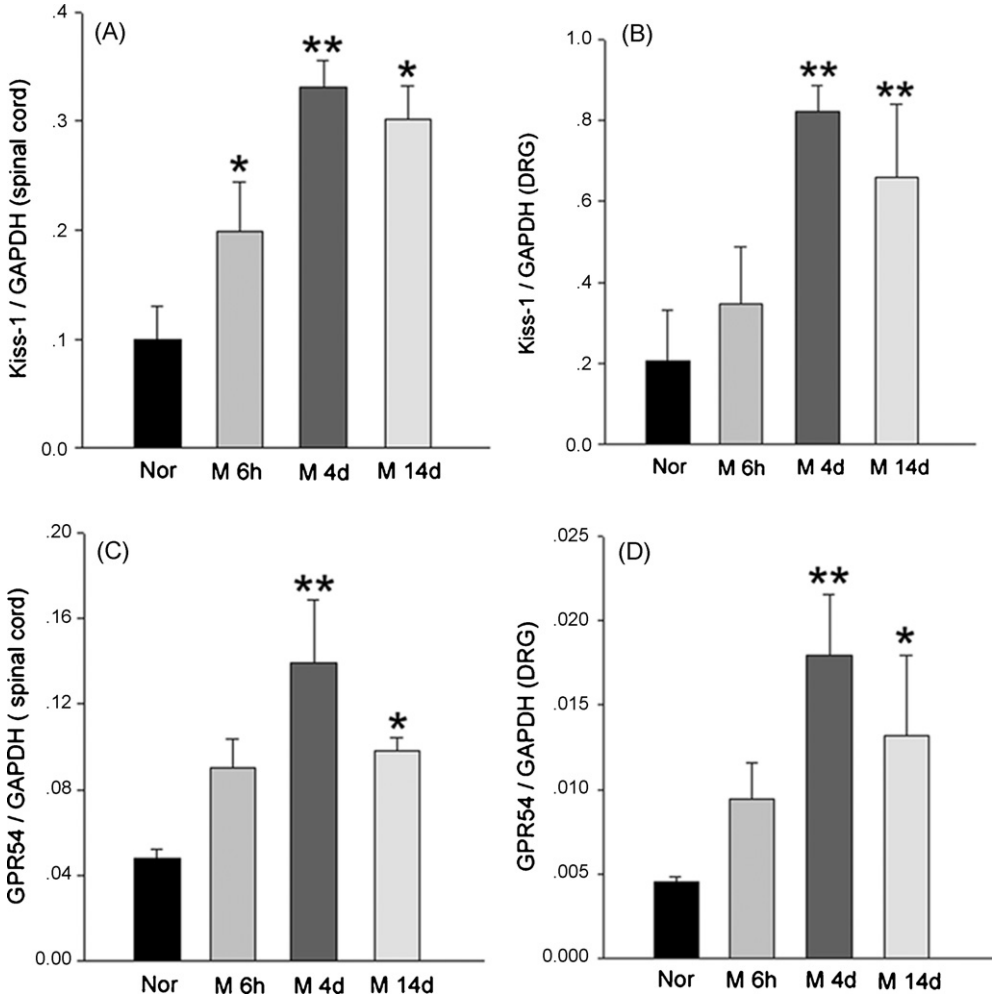
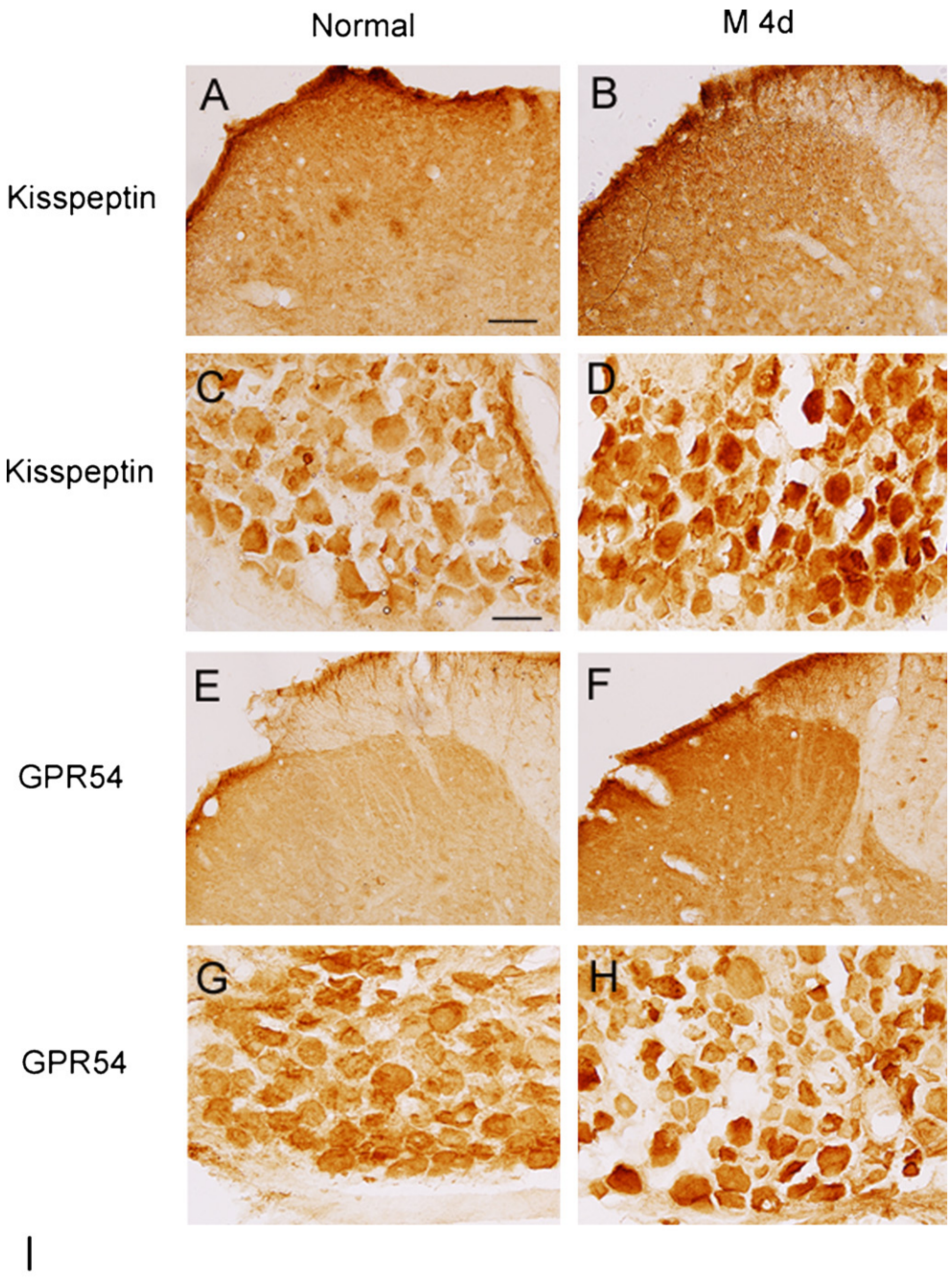


Fig. 3. Up-regulation of KiSS-1 mRNA levels in the rat lumbar spinal cord (A) and L4/5 DRG (B) as well as GPR54 in the spinal cord (C) and DRG (D) 4 and 14 days after intra-articular injection of CFA. Data were presented as mean ± S.E.M. ($n = 4$ in each group at each time point) $^{*}P < 0.05$, $^{**}P < 0.01$ vs. normal group.



Treatment		Normal	M6h	M4d	M14d
Kisppeptin	Spinal cord	100.00±10.47	176.47±13.8*	205.88±14.00**	194.12±12.48*
	DRG	100.00±2.78	127.91±7.3*	154.07±10.71**	140.70±8.91*
GPR54	Spinal cord	100.00±7.62	135.71±6.94	160.00±8.24**	158.57±5.64**
	DRG	100.00±18.26	160.00±25.82	340.00±29.44##	260.00±14.72##

*P < 0.05, **P < 0.01, vs. Normal group (kisppeptin spinal cord);

* P < 0.05, ** P < 0.01, vs. Normal group (kisppeptin DRG);

**P < 0.01, vs. Normal group (GPR54 spinal cord);

##P < 0.01, vs. Normal group (GPR54 DRG).

Fig. 4. Changes of expression of kisspeptin and GPR54 immunoreactivity in the dorsal horn of the spinal cord and L4/5 DRG during various phases of CFA induced peripheral inflammation detected by immunohistochemistry. Representative images were shown for kisspeptin (A–D) and GPR54 (E–H) immunostaining in the spinal dorsal horn of control (naïve) group and CFA group at the time point of 4 days after the injection. As compared with control group, after intra-articular CFA administration, a large number of kisspeptin-positive and GPR54-positive cells exhibited intense immunoreactivity (B, D, F, H). Quantification of average intensity in the superficial layers of the spinal dorsal horn and the DRG is shown in (I). The optical density was expressed as a percentage to that of the normal group (100%). Data were represented as mean ± S.E.M. ($n = 4$ in each group at each time point). Scale bar = 100 μm.

localized with IB4-expressing neurons, CGRP-expressing neurons and NF200-expressing primary afferent neurons, suggesting their universal expression in small-, medium- and large-sized neurons in DRG. IB4 and CGRP positive neurons form two groups of neurons that have unmyelinated axons and are thought to be nociceptors [16,22,26], and so these characteristics of kisspeptin and GPR54 labeling in the L4/5 DRG indicating their possible involvement in nociception.

Given that both kisspeptin and it receptor GPR54 are expressed in the small to large DRG neurons, kisspeptin most likely acts locally in an autocrine manner to activate its receptor GPR54 to play its role in nociception. This is in accordance with the previous reports that kisspeptin acted in the autocrine mode of action in the dentate gyrus in the hippocampus to increase the efficacy of transmission at afferent synapses [1,2] and in the pituitary to exert it effect in the reproductive function [7,24]. Moreover, the KiSS-1/GPR54 system has also been shown to be affecting cancer cell growth and metastasis includes complex endocrine, paracrine and autocrine actions [12]. Therefore, the paracrine roles of locally produced kisspeptins at the DRG cannot be ruled out, as the investigation of the expression of kisspeptin in the glial cells (schwann cell or satellite cell) has not been conducted.

Consistent with previous reports [11,15,21], the present study demonstrated that following intra-articular injection of CFA, themal hyperalgesia developed within hours and lasted for more than 2 weeks in the injected paw. However, the mechanisms underlying thermal hyperalgesia are still not fully elucidated at present.

DRG and the dorsal horn of the spinal cord are very important processing stations of the senses and nociception. The present study showed the increase of the expression of kisspeptin and GPR54 in the DRG following CFA injection, but in what type of the DRG neurons still need to be further examined, and the mechanism by which CFA induces changes in kisspeptin expression in DRG neurons is unclear. The up-regulation in kisspeptin expression in the spinal dorsal horn in the present study, which parallels to the changes in the DRG, implies a characteristic response of nociceptive information signaling in the inflammation at the spinal cord level. This up-regulation may due to the anterograde transportation from the DRG neurons following CFA treatment.

It is demonstrated that in the hippocampus, activation of GPR54 with kisspeptin causes a rapid and large increase in the amplitude of excitatory synaptic responses in granule cells, inducing the synaptic potentiation [1]. Moreover, mRNA for BDNF was markedly increased when hippocampal slice cultures were treated with kisspeptin for 6 h [2]. Therefore, it is possible that the kisspeptin in the spinal cord induced the change of the neuronal activity and the up-regulation of the BDNF, which caused the nociception in the CFA induced inflammation.

In addition, increased expression of the kisspeptin and GPR54 was observed throughout the phases of inflammation, demonstrating the time course of the up-regulation of kisspeptin and GPR54 paralleled the hyperalgesia during the phases of inflammation. This raise the possibility that the kisspeptin/GPR54 system might be involved in the CFA-induced inflammatory pain. However, the GPR54 antagonist or GPR54−/− mice should be used to confirm the role of the kisspeptin/GPR54 system in the chronic inflammatory pain.

Kisspeptin has been shown to be biologically active on central neurons, in that it induces an increase of $[Ca^{2+}]_i$ in a population of cultured hippocampal neurons, which are known to express GPR54 [3]. Previous studies also showed that metastin increased inositol1,4,5-trisphosphate (IP3) formation in Cos-7 cells [23], which mobilizes $[Ca^{2+}]_i$. Upon ligand–receptor interaction, the major intracellular signaling systems recruited by GPR54 include activation of phospholipase C and PIP2 hydrolysis, which is followed by the accumulation of IP3, Ca^{2+} mobilization, arachidonic acid release and phosphorylation of ERK1/2 and p38 MAP kinases [9]. And whether the activation of phospholipase C and PIP2 hydrolysis or other cellar signaling pathways meditated the effects of kisspeptins/GPR54 system in the inflammatory pain remains to be further investigated.

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References

[1] A.C. Arai, The role of kisspeptin and GPR54 in the hippocampus, Peptides (2008), Aug 13 (Epub ahead of print).
[2] A.C. Arai, N. Orwig, Factors that regulate KiSS1 gene expression in the hippocampus, Brain Res. 1243 (2008) 10–18.
[3] G.C. Brailoiu, S.L. Dun, M. Ohsawa, D. Yin, J. Yang, J.K. Chang, E. Brailoiu, N.J. Dun, KiSS-1 expression and metastin-like immunoreactivity in the rat brain, J. Comp. Neurol. 481 (2005) 314–329.
[4] M.K. Clements, T.P. McDonald, R. Wang, G. Xie, B.F. O'Dowd, S.R. George, C.P. Austin, Q. Liu, FMRFamide-related neuropeptides are agonists of the orphan G-protein-coupled receptor GPR54, Biochem. Biophys. Res. Commun. 284 (2001) 1189–1193.
[5] F.C. Colpaert, Evidence that adjuvant arthritis in the rat is associated with chronic pain, Pain 28 (1987) 201–222.
[6] S.L. Dun, G.C. Brailoiu, A. Parsons, J. Yang, Q. Zeng, X. Chen, J.K. Chang, N.J. Dun, Metastin-like immunoreactivity in the rat medulla oblongata and spinal cord, Neurosci. Lett. 335 (2003) 197–201.
[7] J.S. Jacobi, C. Martin, G. Nava, M.C. Jeziorski, C. Clapp, G. Martinez de la Escalera, 17-Beta-estradiol directly regulates the expression of adrenergic receptors and kisspeptin/GPR54 system in GT1-7 GnRH neurons, Neuroendocrinology 86 (2007) 260–269.
[8] A.S. Kauffman, D.K. Clifton, R.A. Steiner, Emerging ideas about kisspeptin-GPR54 signaling in the neuroendocrine regulation of reproduction, Trends Neurosci. 30 (2007) 504–511.
[9] M. Kotani, M. Detheux, A. Vandenbogaerde, D. Communi, J.M. Vanderwinden, E. Le Poul, S. Brezillon, R. Tyldesley, N. Suarez-Huerta, F. Vandeput, C. Blanpain, S.N. Schiffmann, G. Vassart, M. Parmentier, The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54, J. Biol. Chem. 276 (2001) 34631–34636.
[10] D.K. Lee, T. Nguyen, G.P. O'Neill, R. Cheng, Y. Liu, A.D. Howard, N. Coulombe, C.P. Tan, A.T. Tang-Nguyen, S.R. George, B.F. O'Dowd, Discovery of a receptor related to the galanin receptors, FEBS Lett. 446 (1999) 103–107.
[11] H. Luo, J. Cheng, J.S. Han, Y. Wan, Change of vanilloid receptor 1 expression in dorsal root ganglion and spinal dorsal horn during inflammatory nociception induced by complete Freund's adjuvant in rats, Neuroreport 15 (2004) 655–658.
[12] A. Makri, N. Pissimissis, P. Lembessis, C. Polychronakos, M. Koutsilieris, The kisspeptin (KiSS-1)/GPR54 system in cancer biology, Cancer Treat. Rev. 34 (2008) 682–692.
[13] A. Mansour, C.A. Fox, S. Burke, H. Akil, S.J. Watson, Immunohistochemical localization of the cloned mu opioid receptor in the rat CNS, J. Chem. Neuroanat. 8 (1995) 283–305.
[14] S. Martin-Schild, A.A. Gerall, A.J. Kastin, J.E. Zadina, Differential distribution of endomorphin 1- and endomorphin 2-like immunoreactivities in the CNS of the rodent, J. Comp. Neurol. 405 (1999) 450–471.
[15] W.L. Mi, Q.L. Mao-Ying, Q. Liu, X.W. Wang, Y.Q. Wang, G.C. Wu, Synergistic antihyperalgesia of electroacupuncture and low dose of celecoxib in monoarthritic rats: involvement of the cyclooxygenase activity in the spinal cord, Brain Res. Bull. 77 (2008) 98–104.
[16] D.C. Molliver, M.J. Radeke, S.C. Feinstein, W.D. Snider, Presence or absence of TrkA protein distinguishes subsets of small sensory neurons with unique cytochemical characteristics and dorsal horn projections, J. Comp. Neurol. 361 (1995) 404–416.
[17] A.I. Muir, L. Chamberlain, N.A. Elshourbagy, D. Michalovich, D.J. Moore, A. Calamari, P.G. Szekeres, H.M. Sarau, J.K. Chambers, P. Murdock, K. Steplewski, U. Shabon, J.E. Miller, S.E. Middleton, J.G. Darker, C.G. Larminie, S. Wilson, D.J. Bergsma, P. Emson, R. Faull, K.L. Philpott, D.C. Harrison, AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1, J. Biol. Chem. 276 (2001) 28969–28975.
[18] T. Ohtaki, Y. Shintani, S. Honda, H. Matsumoto, A. Hori, K. Kanehashi, Y. Terao, S. Kumano, Y. Takatsu, Y. Masuda, Y. Ishibashi, T. Watanabe, M. Asada, T. Yamada, M. Suenaga, C. Kitada, S. Usuki, T. Kurokawa, H. Onda, O. Nishimura, M. Fujino, Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor, Nature 411 (2001) 613–617.

[19] H.G. Schaible, A. Ebersberger, G.S. Von Banchet, Mechanisms of pain in arthritis, Ann. N. Y. Acad. Sci. 966 (2002) 343–354.
[20] S.B. Seminara, Kisspeptin in reproduction, Semin. Reprod. Med. 25 (2007) 337–343.
[21] S. Shan, M.Y. Qi-Liang, C. Hong, L. Tingting, H. Mei, P. Haili, W. Yan-Qing, Z. Zhi-Qi, Z. Yu-Qiu, Is functional state of spinal microglia involved in the anti-allodynic and anti-hyperalgesic effects of electroacupuncture in rat model of monoarthritis? Neurobiol. Dis. 26 (2007) 558–568.
[22] J.D. Silverman, L. Kruger, Selective neuronal glycoconjugate expression in sensory and autonomic ganglia: relation of lectin reactivity to peptide and enzyme markers, J. Neurocytol. 19 (1990) 789–801.
[23] L.J. Stafford, C. Xia, W. Ma, Y. Cai, M. Liu, Identification and characterization of mouse metastasis-suppressor KiSS1 and its G-protein-coupled receptor, Cancer Res. 62 (2002) 5399–5404.
[24] M. Tena-Sempere, GPR54 and kisspeptin in reproduction, Hum. Reprod. Update 12 (2006) 631–639.
[25] M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals, Pain 16 (1983) 109–110.
[26] M. Zwick, B.M. Davis, C.J. Woodbury, J.N. Burkett, H.R. Koerber, J.F. Simpson, K.M. Albers, Glial cell line-derived neurotrophic factor is a survival factor for isolectin B4-positive, but not vanilloid receptor 1-positive, neurons in the mouse, J. Neurosci. 22 (2002) 4057–4065.