Repeated intra-articular injections of acidic saline produce long-lasting joint pain and widespread hyperalgesia

N. Sugimura¹, M. Ikeuchi¹, M. Izumi¹, T. Kawano², K. Aso¹, T. Kato¹, T. Ushida³, M. Yokoyama², T. Tani¹

¹ Department of Orthopaedic Surgery, Kochi Medical School, Kochi University, Nankoku, Japan
² Department of Anesthesiology and Intensive Care Medicine, Kochi Medical School, Kochi University, Nankoku, Japan
³ Multidisciplinary Pain Center, Aichi Medical School, Nagakutecho, Japan

Abstract

Background: Synovial fluid in inflamed joint shows a drop in pH, which activates proton-gated ion channels in nociceptors. No studies have ever tried to develop and characterize acid-induced joint pain.

Methods: Rats were injected intra-articularly with pH 4.0 acidic saline twice, 5 days apart. Pain-related behaviour tests including weight-bearing asymmetry, paw withdrawal threshold and knee compression threshold were conducted. To clarify the roles of proton-gated ion channels, rats were injected intra-articularly with selective antagonists for ASIC1a, ASIC3 and TRPV1 on day 5 (before the second injection) or on day 14. Underlying peripheral and central pain mechanisms were evaluated using joint histology, interleukin-1β concentrations in the synovium, single-fibre recording of the knee afferent and expression of phosphorylated cyclic adenosine monophosphate-responsive element-binding protein (p-CREB) in the spinal dorsal horn.

Results: Repeated injections of acidic saline induced weight-bearing asymmetry, decrease in paw withdrawal threshold and knee compression threshold bilaterally, which lasted until day 28. Early administration of ASIC3 antagonist reduced the bilateral and long-lasting hyperalgesia. Neither articular degeneration nor synovial inflammation was observed. C-fibre of the knee afferent was activated by acidic saline, which was attenuated by pre-injection of ASIC3 antagonist. p-CREB expression was transiently up-regulated bilaterally on day 6, but not on day 14.

Conclusion: We developed and characterized a model of acid-induced long-lasting bilateral joint pain. Peripheral ASIC3 and spinal p-CREB played important roles for the development of hyperalgesia. This animal model gives insights into the mechanisms of joint pain, which is helpful in developing better pain treatments.

1. Introduction

Chronic joint pain is currently one of the most frequent health problems, particularly osteoarthritis and rheumatoid arthritis. Chronic joint pain leads to disability, psychological distress and impaired quality of life. Despite its frequency and impact, the mechanisms of chronic joint pain are largely unknown (Dieppe and Lohmander, 2005). Joint pain is uniquely different from cutaneous pain and characterized as diffuse, long-lasting and unpleasant paw (Sluka, 2002). It is often accompanied by referred pain and secondary hyperalgesia (Sluka, 2002), i.e., increased nociceptive response to noxious stimuli outside the joint, suggesting that sensitization of central nervous system underlies chronic joint pain. For the better understanding of
pain mechanisms and future progress in treatment, it is helpful to develop an animal model of chronic joint pain predominantly driven by nervous system. Synovial fluid in inflamed joint shows a drop in pH to levels around 6.6–6.8 (Goldie and Nachemson, 1969; Shishkin et al., 2005). Local acidity activates nociceptors through proton-gated cation channels such as acid-sensing ion channels (ASICs) and transient receptor potential vanilloid1 (TRPV1). There is sufficient experimental evidence in rodents and humans to confirm acid-induced cutaneous and muscle pain (Steen et al., 1995; Issberner et al., 1996; Hamamoto et al., 1998; Ugawa et al., 2002a; Karczewski et al., 2010). Interestingly, repeated intra-muscular injections of acidic saline produce a bilateral and long-lasting hyperalgesia without significant tissue damage (Sluka et al., 2001), which is considered as an animal model of fibromyalgia (Desantana et al., 2013). To date, however, no studies have ever tried to develop acid-induced joint pain. It was hypothesized that repeated intra-articular injections of acidic saline could produce a bilateral, long-lasting hyperalgesia. The purpose of this study was (1) to develop an animal model of acid-induced chronic joint pain and (2) to clarify underlying peripheral and central pain mechanisms of this model.

2. Materials and methods

2.1 Animals and experimental design

Male Sprague–Dawley rats (8 weeks old, weight 250–400 g) were used. Rats received intra-articular injections of acidic or normal saline and divided into three groups: acid–acid, acid–saline and saline–saline groups. Pain behaviours including weight-bearing asymmetry, mechanical sensitivity of the paw and the knee were compared among groups until day 35. In order to characterize the involvement of proton-gated ion channels, rats with acid-induced chronic joint pain were treated with selective antagonists for ASIC1a, ASIC3 and TRPV1. Joint histology, inflammatory cytokines in the synovium, single-fibre recording of the knee afferent, immunohistochemistry of the spinal cord and intra-articular pH measurement were also evaluated. All experiments were approved by the Animal Care and Use Committee of Kochi University.

2.2 Acid-induced joint pain model

Rats were anaesthetized briefly with an intraperitoneal injection of sodium pentobarbitone (30 mg/kg). Left knee joint was injected with 100 μL of acidic (pH 4.0) or normal (pH 7.4) saline using a 27-gauge needle. The pH of saline was measured immediately before the injections. Five days later, the rats were reanaesthetized and the ipsilateral knee joint was re-injected with the same volume of acidic or normal saline. The rats were divided into three groups: acid–acid (n = 10), acid–saline (n = 5) and saline–saline groups (n = 5). Pain-related behaviour tests were assessed on the following schedule: before the first injection, 4 and 24 h after the first injection, before the second injection, 4 and 24 h after the second injection, and weekly after the second injection. The final assessment was conducted 4 weeks after the second injection (Fig. 1).

2.3 Pain-related behaviour tests

2.3.1 Weight-bearing asymmetry

The weight distribution was measured using a hind paw limb weight-bearing apparatus (Linton incapacitance tester, 2014 European Pain Federation - EFIC®
Linton Instrumentation, Norfolk, UK). The apparatus consists of two force transducers capable of measuring the body weight that the animal places on each hind paw. Rats were placed on the capacitance tester with their hind paws centred on the two force transducers; the average body weight distribution in grams was calculated over a period of 3 s. Weight placed through the ipsilateral paw was expressed as a ratio of the weight of ipsilateral paw to the sum of the weight of both paws, with a ratio of 0.5 resulting from equal weight distribution across both hind paws.

2.3.2 Paw withdrawal reflex

Mechanical sensitivity of the paw was examined using von Frey filaments. Rats were put inside a plexiglass cage placed on an elevated mesh steel platform and acclimated for 15 min before the pain behaviour tests. von Frey filaments of varying bending forces (26, 15, 10, 8, 6, 4, 2, 1.4, 1, 0.6, 0.4 g) were applied to the plantar surface of the bilateral paw. Minimum bending force to induce leg withdrawal was recorded three times. The median value was recorded as mechanical threshold of the paw.

2.3.3 Knee compression threshold

Joint hyperalgesia was assessed by a forceps compression device similar to a previous report (Yu et al., 2002). The device consists of a forceps, a strain-gauge sensor, a signal amplifier and a laptop computer. Rats were placed in a glove, each hindlimb extended and acclimated for 15 min before the pain behaviour tests. Next, a pair of forceps applied to the knee joint until the rats withdrew from the stimulus or vocalized. Three trials of squeezing the knee joint were applied at each testing period. A computer program was used to measure the magnitude of each squeeze in gram. The mean value was recorded as knee compression threshold.

2.4 Selective antagonists for proton-gated ion channels

Rats given repeated acid injections received an intra-articular injection of selective antagonists for ASIC3 (APETx2) (Diochot et al., 2004), ASIC1a (PcTx1) (Chen et al., 2005; Salinas et al., 2006), TRPV1 (BCTC) (Pomonis et al., 2003; Valenzano et al., 2003) or vehicle at day 5 or day 14. The injection of antagonists at day 5 was performed immediately before the second injection of acidic saline. The concentration of each antagonist was determined according to previous reports: 2.2 mM for APETx2 (Karczewski et al., 2010), 120 nM for PcTx1 (Deval et al., 2011) and 30 μM for BCTC (Benko et al., 2012). The rats were divided into six groups: APETx2 at day 5 (early APETx2 group), PcTx1 at day 5 (early PcTx1 group), BCTC at day 5 (early BCTC group), APETx2 at day 14 (late APETx2 group), PcTx1 at day 14 (late PcTx1 group), BCTC at day 14 (late BCTC group) (Fig. 1).

2.5 Joint histology

To evaluate potential tissue damage, rats given repeated injections of acidic or normal saline were euthanized with an overdose of sodium pentobarbitone (150 mg/kg, intraperitoneal) at day 14 (n = 5 in each group). Knee joints were excised and fixed with 10% formaldehyde, decalcified by 12.5% formic acid and 1% formaldehyde in phosphate-buffered water for 7 days and embedded in paraffin. Cross-sections were cut on a microtome REM-700 (Yamato, Saitama, Japan) at 7–10 μm, stained with 0.1% safranin O and haematoxyline eosine and examined by light microscopy. Histopathologic classification on the severity of joint damage was graded using the modified Mankin scoring system (van der Sluijs et al., 1992). The scale evaluates the loss of safranin O staining (scale 0–4), cell appearance (scale 0–3), invasion of the tidemark by blood vessels (scale 0–1) and structural changes of the cartilage (scale 0–6, where 0 = normal cartilage structure and 6 = erosion of the cartilage down to the subchondral bone).

2.6 IL-1β measurement

Concentrations of interleukin (IL)-1β, a proinflammatory cytokine in the knee joint (synovium), were quantified using enzyme-linked immunosorbent assay (ELISAs) at day 14 (n = 5 in each group). As a positive control, rats received intra-articular injection of 3% carrageenan (100 μL) and acutely inflamed knee joints were excised 24 h after the injection (n = 4). The trimmed samples were cut into pieces, well-rinsed in phosphate-buffered saline (PBS) and then crushed to powder and homogenized in a 200-μL lysis/extraction reagent (Sigma-Aldrich, St. Louis, MO, USA). The rat joint homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C twice. Supernatants were extracted for the assay. The concentration of the IL-1β was measured using ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. The limits of sensitivity for IL-1β measuring were 5 pg/mL. The total protein concentration in all samples was measured using the Lowry method using a modified Lowry protein assay kit (Thermo Scientific, Chicago, IL, USA). After the measurements with a microplate reader, the absolute cytokine concentrations were converted into corresponding concentrations per mg total protein.

2.7 Single-fibre recordings

To investigate the reactivity of rat knee joint afferents activity to intra-articular acidic saline injection, and its role of ASIC3, we next conducted the experiments of in vivo single C-fibre recording as previously described (Brenn et al., 2007) (Pogatzki et al., 2002) with some modifications. Briefly, rats were anaesthetized using intraperitoneal injection of sodium pentobarbitone (50 mg/kg). Supplementary doses of pentobarbitone sodium (20 mg/kg) were given as necessary to maintain areflexia. The animals breathed spontaneously during surgery and the recording period. Body temperature
 Acid-induced long-lasting joint pain

N. Sugimura et al.

was kept at 37 °C with a heating pad positioned under the animal (KN-475, Natsume Seisakusho, Tokyo, Japan). A longitudinal skin incision was made along the medial aspect of the hind limb. The resulting skin flaps were secured to a metal ring to create a pool, which was filled with warm mineral oil to prevent drying of the tissue. The saphenous nerve projecting from the knee joint was identified and transected proximally. After placing a platinum electrode on the proximal cut end of the fine filament, the knee joint afferent fibre was explored by the responses to noxious outward torque of the knee (approximately 40 mN·m) (Brenn et al., 2007). Then, the afferent was repeatedly subdivided until single-unit activity could be discriminated. A reference electrode was pinned onto the adjacent muscle tissue. Single-unit potentials were amplified with an AC/DC differential amplifier (Model 3000, A-M Systems Inc., Sequim, WA, USA) with low-frequency cut-off at 0.1 Hz and high-frequency cut-off at 10 kHz. Single C-fibre activities were continuously recorded using a data acquisition system (PowerLab, AD Instrument, Australia), and analysed at 1-s intervals over an interval of 120 s throughout the recording period. Conduction velocity (CV) of an individual fibre was calculated from the response latency determined using an electric stimulator (SEN-5201, Nihon Koden, Japan). Afferent fibre was classified as C-fibre if their CV was less than 2.0 m/s. The C-fibre activities after intra-articular acidic saline injection were evaluated with or without pre-injection of APETx2.

2.8 p-CREB in the spinal dorsal horn

Involvement of the central mechanism of this pain model was verified using spinal phosphorylated cyclic adenosine monophosphate (cAMP) response element-binding protein (p-CREB). p-CREB has been reported as an important transcription factor in spinal activation of the cAMP pathway that produce mechanical hyperalgesia (Sluka, 1997, 2002; Dolan and Nolan, 2001).

Standard immunohistochemical labelling was used to assess the location of cells in which CREB had been phosphorylated after induction of hyperalgesia (Sluka and Westlund, 1993; Messersmith et al., 1998). Rats were euthanized by sodium pentobarbitone (350 mg/kg, intraperitoneal) on day 6 and day 14 (acid–acid group, n = 5; saline–saline group, n = 4) and perfused through the left ventricle with 250 mL of 3.8% sodium citrate in PBS (pH 7.4) followed by 350 mL of 4% paraformaldehyde in PBS, pH 7.4, RT. Segments L3 to L4 of the spinal cord were removed and placed in 4% paraformaldehyde in PBS for 30 min followed by 30% sucrose solution overnight. Tissue was cut on a cryostat at 30-μm thickness and mounted onto APS-coated slides. These sections underwent a step-wise procedure that included 3% H2O2, and 3% normal goat serum (NGS). Between each step, tissue was rinsed in PBS. Next, sections were incubated overnight in primary antibody in 1% NGS/PBS containing 0.75% Triton X-100 at room temperature as follows: anti-p-CREB (Millipore, Billerica, MA, USA; 1:5000). The anti-p-CREB recognizes phosphorylation at the PKA site, Ser 133 (Gonzalez and Montminy, 1989). Preliminary dilution series for p-CREB determined appropriate concentrations for the one antibody. Sections were washed in PBS followed by incubation for 1 h in the secondary antibody biotinylated-goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA; 1:200). After they were washed in PBS, sections were incubated in avidin horseradish peroxidase (1% NGS with 0.75% Triton X-100; 1:50) for 1 h. This was followed by 6 min in 0.05% diaminobenzidine (DAB) and 0.01% H2O2. Sections were rinsed in PBS, dehydrated and coverslipped. To minimize differences in staining between animals, each group was run simultaneously, i.e., pH 4.0 and pH 7.2 saline. Furthermore, between groups the exact staining protocol was used, including incubation in primary and secondary antibodies and DAB.

Immunohistochemically stained tissue sections were examined under a light microscope at 100× magnification. The images were analysed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) by a single examiner (N.S.). Neurons with distinct nuclear staining were counted positive in two subregions: superficial layers (laminae I–II) and deeper layers (laminae III–VI) of spinal cord. Margin of each subregion was plotted manually, and the area was given automatically by the software. Density of p-CREB was defined as the number of positive neurons divided by the area of each subregion. The average of the density was calculated in 6–10 sections of L3–L4 spinal cord from each rat.

2.9 Intra-articular pH measurement

Intra-articular pH was measured with a needle electrode (Precision Digital pH Meter, Chemical Instruments Co., Ltd., Tokyo, Japan). Rats were anaesthetized using intraperitoneal injection of sodium pentobarbitone (35 mg/kg). A needle electrode and a 27-gauge needle connected to a Hamilton syringe with 100 μL of pH 4.0 or 7.2 acidic saline were inserted into the left knee. Measurements were performed before, soon after and at the following periods after injection of saline: every 10 s for the first 1 min, every 30 s for the next 4 min, then every 1 min until the 10 min.

2.10 Statistical analysis

Statistical analysis was carried out using JMP, version 10 (SAS Institute, Cary, NC, USA). Two-way analysis of variance followed by Tukey’s test was used to evaluate pain-related behaviour tests, single-fibre recording and intra-articular pH measurement. Kruskal–Wallis test with Steel–Dwass test was used for comparing histological evaluations of knee joints and p-CREB, and inflammatory cytokines. p < 0.05 was considered significant.

3. Results

Single injection of acidic saline (acid–saline group) decreased mechanical threshold in the ipsilateral paw until day 5. Short-term weight-bearing asymmetry
was also observed in acid–saline group. On the other hand, repeated injections of acidic saline (acid–acid group) induced significant decrease in mechanical threshold in the bilateral paws, which lasted until day 28. The ipsilateral paw showed greater threshold decrease compared with the contralateral paw. Weight-bearing asymmetry in acid–acid group was greater and lasted longer (until day 28) than acid–saline group (Fig. 2).

Fig. 3 showed the effects of an intra-articular injection with proton-gated ion channel antagonists on pain behaviour test. Acid-induced pain behaviour was significantly reduced only in rats given selective ASIC3 antagonist at day 5 (early APETx2 group). On the other hand, pain reduction was not achieved by late administration of ASIC3 antagonist (late APETx2 group). Antagonists for ASIC1a (PcTx1) and TRPV1 (BCTC) produced no significant effects regardless of the time point of administration.

Primary hyperalgesia of the knee was assessed with a forceps compression device (Fig. 4). In saline–saline group, the knee compression threshold increased.
bilaterally over time, suggesting that animals became acclimated to the test. In contrast, acid–acid group showed temporal decrease of the compression threshold in the ipsilateral knee after first injection. Bilateral long-lasting decrease after second injection was observed until day 28. Intra-articular injection of APETx2 before the second injection of acidic saline significantly reduced the bilateral long-lasting hyperalgesia, but did not reduce the transient hyperalgesia immediately after the second injection of acidic saline.

Histology of knee joints obtained at day 14 showed no evidence of cartilage damage due to acid injections (Fig. 5A and B). All examined joints were graded as 0 (normal appearance) in modified Mankin scoring system. There was also no evidence of synovial inflammation such as synovial proliferation, leukocyte infiltration or vasodilation (Fig. 5C and D). In addition, IL-1β concentration in acid–acid group was extremely low (mean ± standard error: 0.59 ± 0.53 pg/mg) compared with carrageenan-induced inflamed joint (31.1 ± 2.4 pg/mg, \( p < 0.001 \)).

Electrical stimulation-evoked action potentials were recorded at the beginning of all recordings to measure the CV for the subsequent identification of C-type afferent (Fig. 6A). The mean CV value of all recording C-fibres was 1.1 ± 0.3 m/s. During the single-fibre recording, spontaneous fibre activity at low frequency (1.0–2.0 imp/s) was observed in all fibres (Fig. 6B and C). However, upon intra-articular injection of acidic saline, the fibre activity was markedly increased,
reaching peak levels at 4–6 min after injection, and then slowly decreased thereafter (Fig. 6B upper trace and Fig. 6C). In addition, identical control experiments in the absence of acidic saline showed that injections of the same amount of saline solution had no influence in C-fibre activities during the same time periods (n = 3, data not shown). These findings suggest that intra-articular stimulation with acidic saline itself, rather than the effects of volume expansion of the joint space, could activate the C-fibre response from rat knee joint afferents. On the other hand, pretreatment with APETx2 resulted in the attenuation of acidic saline-induced C-fibre activation (p < 0.05 vs. no APETx2 pretreated), while APETx2 alone had no influence on baseline spontaneous fibre activity (Fig. 6B lower trace and Fig. 6C).

Fig. 7A shows representative photos of dorsal horn with immunohistochemical staining. Bilateral up-regulation in p-CREB expression was demonstrated in the L3–4 superficial and deep dorsal horn 24 h after intra-articular injection of pH 4.0 saline compared with pH 7.2 (p < 0.05) (Fig. 7B). In contrast,
there was no significant up-regulation in the superficial and deep dorsal horn on day 14 (data not shown).

A significant drop of intra-articular pH was shown for 90 s following injection of pH 4.0 acidic saline, which was returned to the baseline approximately 8 min after the injection (*p < 0.05 vs. saline).

**4. Discussion**

Repeated injections of acidic saline into the knee joint produced a bilateral long-lasting hyperalgesia of the paw without joint damage, which was significantly reduced by early intra-articular administration of selective ASIC3 antagonist. In spite of no significant peripheral tissue damage, there was an increase of p-CREB reactivity in the bilateral spinal dorsal horn. Although central sensitization has been demonstrated in patients with arthritis (Imamura et al., 2008; Arendt-Nielsen et al., 2010; Meeus et al., 2012), there have been no animal models to directly address central sensitization associated with joint insults. To the best of our knowledge, this is the first study to develop an animal model of chronic joint pain predominantly driven by nervous system.

Among proton-gated ion channels, ASIC3 is the most sensitive to such a pH change (Wemmie et al., 2006; Lingueglia, 2007), abundantly expressed in dorsal root ganglia (Wemmie et al., 2006) including joint afferents (Ikeuchi et al., 2009), and strongly correlated with pain (Ugawa et al., 2002a; Sluka et al., 2003, 2007; Wemmie et al., 2013). In particular, peripheral ASIC3 in joint afferents is a key to the development, but not the maintenance, of chronic widespread pain in arthritis models (Ikeuchi et al., 2008; Izumi et al., 2012). In the current study, long-lasting bilateral hyperalgesia was prevented by pre-injection of APETx2 prior to second acidic saline injection. Along with the pain-related behaviour tests, the C-fibre activity in primary afferent of the knee was markedly increased by intra-articular injection of acidic saline and attenuated by pre-injection of APETx2. These results are in agreement with previous reports, and activation of ASIC3 in joint afferents was responsible for initiating the bilateral, long-lasting mechanical hyperalgesia induced by repeated injections of acidic saline. In contrast to ASIC3, ASIC1a and TRPV1 did not appear to be involved in the development and maintenance of hyperalgesia in this study. However, previous studies showed significant roles of not only ASIC3 but also ASIC1a and TRPV1 in several animal models of musculoskeletal pain (Chen et al., 2009; Walder et al., 2010; Kelly et al., 2013; Ota et al., 2013). Peripherally located proton-gated ion channels possibly play different roles in hyperalgesia after musculoskeletal insults.

Bilateral, long-lasting hyperalgesia after unilateral intra-articular acid injections suggests that the underlying mechanisms involve changes in the central nervous system. Our study showed that p-CREB expression in the spinal dorsal horn was up-regulated bilaterally on the day after second acidic saline injection. This result suggests that involvement of cAMP pathway immediately after second acid injection is essential for widespread chronic hyperalgesia. Spinal mechanisms maintaining the hyperalgesia were previously examined in chronic muscle pain model induced by repeated acid injections (Sluka et al., 2001). Blockade of glutamate receptors spinally reversed the hyperalgesia once developed (Skyba et al., 2005). Release of glutamate increased in the spinal dorsal horn in response to the second intramuscular injection of acidic saline, and increased concentrations of glutamate in the dorsal horn were observed at 1 week after the second injection (Skyba et al., 2005). Wide, dynamic range neurons in the dorsal horn were sensitized with the expansion of receptive fields to include the contralateral limb and increased their responses to mechanical stimuli bilaterally (Sluka et al., 2003). Blockade of the cAMP pathway reversed the mechanical hyperalgesia as well as the increases in p-CREB (Hoeger-Bement and Sluka, 2003). It is likely that these changes in the central nervous system also underlie bilateral, long-lasting hyperalgesia in this model.

Several studies showed that primary mechanical hyperalgesia in inflammatory models was comparable between ASIC3−/− and ASIC3+/+ mice (Price et al., 2001; Chen et al., 2002; Mogil et al., 2005; Ikeuchi et al., 2008), indicating that primary hyperalgesia is
not mainly associated with ASIC3. In our study, primary hyperalgesia of the knee immediately after second acidic saline injection was not reduced by pre-injection of APETx2, consistent with previous reports. However, the primary hyperalgesia of the knee after second acid injection persisted until day 28, which was attenuated by APETx2. A plausible explanation is that the long-lasting primary hyperalgesia after repeated intra-articular acid injection has a different mechanism compared with the knockout studies of inflammatory models. It was also reported that repeated intramuscular injection of acid increased the primary mechanical threshold of muscle, which was bilateral (Yokoyama et al., 2007; Tillu et al., 2008) and long-lasting (Sharma et al., 2009). Tillu et al. (2008) mentioned that muscle hyperalgesia induced by repeated intramuscular injection of acid was considered as primary: however, it may also be secondary as there is no observed tissue injury in this model and the hyperalgesia is maintained independent of the afferent input. According to this theory, the difference of our results between the transient knee hyperalgesia and the long-lasting knee hyperalgesia would be understandable. Although further research to clarify the mechanism is required, peripheral ASIC3 possibly plays a role for developing long-lasting primary hyperalgesia in repeated acid injection models.

There are several limitations in this study. Firstly, histology and IL-1β assay were performed only at day 14. Although there was no obvious inflammation present, minor inflammation could have occurred earlier. It is necessary to examine the time course of histological changes and pro-inflammatory cytokine release. Secondly, the expression of ASIC3 was not assessed in this study. Previous study using whole-cell patch clamp technique demonstrated that repeated intramuscular injection of acidic saline did not provide significant change in ASIC3 in DRG after the second injection (Gautam et al., 2012). Because muscle and joint are similar deep somatic structures, significant change of ASIC3 expression in DRG was not expected much. Instead, single-fibre recording of primary afferent of the knee was performed with or without pre-injection of APETx2 in this study. Thirdly, species-specific differences of ASIC3 were not considered. It is uncertain whether repeated acidification of human joint causes chronic hyperalgesia identical to our rodent chronic pain model. Although there would be some differences between human and rodent ASIC3, possible common mechanisms of human pain associated with tissue acidosis, such as intra-dermal acid injection and intestine inflammation (Yiangou et al., 2001; Ugawa et al., 2002b), were reported.

In conclusion, we developed and characterized a model of acid-induced joint pain. Repeated intra-articular injections of acidic saline produced a bilateral, long-lasting hyperalgesia along with the activation of primary afferent of the knee without joint damage. Peripheral ASIC3 and spinal p-CREB played important roles for the development of hyperalgesia. This animal model gives insights into the mechanisms of joint pain, which is helpful in developing better pain treatments.

Author contributions

N.S., M.I. and M.I. designed experiments, analysed and interpreted results, and wrote the manuscript. N.S. did histology. T.K. conducted and analysed single-fibre recording. M.I., M.I., K.A. and T.K. analysed and interpreted results. T.T., T.U. and M.Y. directed the study.

Acknowledgements

We thank Ms R Shiraiishi and Mrs M Morioka for excellent technical assistance.

References


Acid-induced long-lasting joint pain

N. Sugimura et al.


