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Different role of spinal 5-HT(hydroxytryptamine)7 receptors and descending serotonergic modulation in inflammatory pain induced in formalin and carrageenan rat models

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Editor's key points

- The spinal serotonergic system may be involved in inflammatory pain conditions.
- This study examined whether the role of serotonin differed between inflammatory models
- 5-HT7 had antinociceptive effects in the formalin model, with limited effects in the carrageenan model.
- 5-HT3 effects were predominantly pronociceptive.

Background. Spinal serotonin (5-HT) receptors 3 (5-HT3R) and 7 (5-HT7R) are differentially involved in facilitatory or inhibitory descending modulation, respectively. Electrophysiological studies of the spinal cord have demonstrated that 5-HT3R is involved in nociception induced by intraplantar injection of formalin, but not carrageenan. In addition, depletion of spinal serotonin has been shown to attenuate pain behaviour in the formalin test, but there have been no such reports regarding the carrageenan model. This study compared the role of 5-HT7R and the influence of descending serotonergic modulation between formalin- and carrageenan-induced inflammatory pain.

Methods. Effects of intrathecal (i.t.) AS-19 (5-HT7R agonist) and SB-269970 (5-HT3R antagonist) on flinching response in the formalin test and mechanical allodynia in the carrageenan model were evaluated in male Sprague–Dawley rats. The effect of serotonin depletion by i.t. 5,7-dihydroxytryptamine was also examined in the two models.

Results. Intrathecal AS-19 significantly reduced the flinching responses in the formalin test ($P<0.01$), which was reversed by i.t. SB269970. However, neither AS-19 nor SB269970 produced a significant change in mechanical allodynia in the carrageenan model. Depletion of spinal serotonin attenuated the flinching response in phase 2 of the formalin test ($P<0.01$), but increased mechanical allodynia in the carrageenan model compared with controls ($P<0.01$).

Conclusions. Spinal 5-HT7R plays a significant inhibitory role in descending serotonergic modulation in pain induced by formalin but not carrageenan. Descending serotonergic modulation is differentially involved in inflammatory pain induced by formalin and carrageenan, with facilitatory and inhibitory effects, respectively.

Keywords: carrageenan; formalin; serotonin receptor 7; spinal cord

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It has been noted that the role of spinal serotonin (5-hydroxytryptamine, 5-HT) in nociceptive processing is different depending on the modality of pain stimuli and activated receptor subtypes. Accordingly, spinal 5-HT can modulate nociceptive processing in either a facilitatory or inhibitory manner.[1–4]

The 5-HT7 receptor (5-HT7R), the most recently identified 5-HT receptor subtype, has been shown to be involved in nociceptive processing. Activation of 5-HT7R has a significant antinociceptive effect on capsaicin- and nerve injury-induced pain, and blockade of 5-HT7R reduces the analgesic effects of several drugs,[5–11] although a pronociceptive role of 5-HT7R has also been reported in a few studies involving the formalin test or spinal nerve ligation.[12–14] Unlike 5-HT7R, there is a great deal of evidence supporting a facilitatory role of the 5-HT3 receptor (5-HT3R) in descending pain modulation in various pain states; however, some controversy remains.[15–17]

Recently, a clear distinction between 5-HT3 and 5-HT7Rs was demonstrated in a study in which allodynia and hyperalgesia elicited by spinal nerve ligation or cholecystokinin injection into the rostroventral medulla was reduced by spinal administration of a 5-HT3R antagonist, but not by a 5-HT7R antagonist.[18] In addition, an antagonist of 5-HT7R blocked the antinociceptive effect of morphine administered systemically

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or into the rostroventral medulla, but 5-HT3R antagonist did not influence the effect of morphine. However, 5-HT7R was also shown to be pronociceptive in the formalin test, although confirmatory data on 5-HT7R are still lacking.[12 14] Interestingly, electrophysiological studies have shown that 5-HT3R is involved in nociception induced by intraplantar injection of formalin, but not carrageenan, where no significant difference is observed between naïve and carrageenan-injected rats in the neuronal response to mechanical and thermal stimuli in the spinal cord pre-treated with the 5-HT3R antagonist ondansetron.[19–21] The findings outlined above suggest that the role of spinal 5-HT7R could be different from that of 5-HT3R in formalin- and carrageenan-induced pain, and it is more likely that the activation of 5-HT7R could be inhibitory rather than facilitatory.

In addition, molecular depletion of 5-HT in the spinal cord may attenuate the pain behaviour elicited by intraplantar injection of formalin,[22] suggesting that the facilitatory role is predominant over the inhibitory role in descending serotonergic modulation of formalin-induced pain, probably mediated by 5-HT3R. However, the relative contributions of inhibitory and facilitatory serotonergic modulation have not been examined in carrageenan-induced pain.

The present study compared the role of spinal 5-HT7R in inflammatory pain induced by formalin and carrageenan. We also evaluated the differences in the nature of descending serotonergic modulation between the two pain models.

Methods

Animals and intrathecal catheter implantation

Male Sprague–Dawley rats weighing 225–250 g were used, and all the animals were housed in a room maintained at a constant temperature of 22–23°C with an alternating 12 h light/dark cycle. Free access was given to both water and food. All experiments were performed in accordance with the International Association for the Study of Pain guidelines for the Use of Animals in Research. The protocol (CNU IACUC-H-2011-10) was approved by the Institutional Animal Care and Use Committee, Chonnam National University Medical School, Republic of Korea.

A polyethylene-5 (PE-5) catheter was implanted into the intrathecal (i.t.) space for experimental drug administration as described previously.[23 24] Under general anaesthesia using sevoflurane (adequate anaesthesia assessed using response to skin pinch), a PE-5 catheter was introduced through the atlantooccipital membrane and advanced caudally 8.5 cm to the level of the lumbar enlargement. The other end of the PE-5 catheter, which was connected to a short PE-10 catheter, was tunnelled subcutaneously, externalized through the skin of the top of the head, and plugged with a stainless steel wire for drug administration. Any rat with a neurological deficit after catheter implantation was killed immediately with an overdose of inhalation anaesthetic. Four rats, ~2% of the animals implanted with the i.t. catheter, were excluded due to motor impairment after i.t. catheter implantation. Animals were housed in individual cages after surgery. Ketorolac 0.3 mg kg^{-1}, dissolved in 5 ml of lactated Ringer's solution was given subcutaneously immediately after the surgery. Upon completion of the following experiments and euthanasia (using high concentrations of sevoflurane, and confirming death by lack of breathing or heart beat, and cyanotic change of the skin), the lumbar spine of each animal was cut and dissected to ensure correct placement of the i.t. catheter.

Drugs

The following drugs were used in this study: AS-19 (5-HT7R agonist; Tocris, UK); SB269970 (5-HT7R antagonist; Tocris, UK). The doses tested were selected based on previous studies using AS-19 and SB269970 and adjusted according to the body weight of the animals and the route of administration used.[5 6 13 18] The selectivity of the drugs for 5-HT7R was demonstrated in a binding affinity study, in which the affinity for 5-HT7R was 149.5-fold higher than for the 5-HT1A receptor.[6] The drugs were dissolved in dimethyl sulphoxide and diluted with saline. They were delivered in a volume of 10 μl, followed by an additional 10 μl saline to flush the catheter.

5,7-Dihydroxytryptamine creatinine sulphate salt (5,7-DHT; a serotonergic neurotoxin; Sigma-Aldrich, USA) and desipramine hydrochloride (Sigma-Aldrich, USA) were also used. 5,7-DHT was dissolved in saline containing 0.1% ascorbic acid and injected intrathecally in a volume of 20 μl followed by flushing with a 10 μl vehicle. Desipramine was dissolved in saline and injected intraperitoneally.

Nociceptive test and behavioural study

Intraplantar injection of formalin or carrageenan, which are well characterized and highly reproducible rodent inflammatory pain models, was used in this study.[25 26] Animals were randomly allocated, using a random integer generator, to subcutaneous injection of either 50 μl 5% formalin or 100 μl 2% carrageenan (degraded λ-carrageenan; Sigma Aldrich, USA) into the centre of the plantar surface of the hind paw using a 30 G needle. The formalin test was conducted with the rats restrained in a cylinder, while carrageenan was injected under sevoflurane anaesthesia. Carrageenan was dissolved in saline to form a 2% solution and stored at room temperature for 24 h before use.

Nociceptive behaviour in response to intraplantar injection of formalin was quantified by counting the number of flinching responses at 1 and 5 min (phase 1, 0–9 min), and thereafter every 5 min up to 60 min after formalin injection (phase 2, 10–60 min). Counting was performed for 1 min each time. Phase 1 behaviour originates essentially from the direct stimulation of nociceptors and results in acute and rapid flinching responses, but dissipates within a few minutes.[27] Following phase 1, phase 2 response begins to increase gradually and involves a period of sensitization during which inflammatory phenomena occur. Although the origin of phase 2 response remains debatable, it has been shown to be closely related to the peripheral inflammatory mechanisms including peripheral sensitization, ongoing input from primary afferent fibres, and the sensitization within the dorsal horn.[27–30]

Mechanical allodynia produced by the intraplantar injection of carrageenan was assessed using von Frey filaments. The paradigm was based on the up–down method of determining the 50% probability paw withdrawal threshold.[31] Paw withdrawal threshold was measured using von Frey filaments after a 30 min acclimation period in a cage with a wire mesh floor, and was considered the baseline withdrawal threshold. Then the animals were injected with carrageenan and withdrawal threshold was measured every 1 h for 4 h. Filaments with forces between 0.41 and 15.2 g were applied perpendicular to the middle of the plantar surface through the wire mesh floor, starting with one having a force of 2 g. Each application was maintained for 5 s or until paw lifting or licking, which were considered positive responses. Rats showing a paw baseline withdrawal threshold below 10 g were excluded from the study, which totalled eight in the present study. The behavioural testing was performed in a manner blinded with regard to the treatment groups.

Depletion of spinal serotonin using the serotonergic neurotoxin 5,7-DHT

Serotonin in the spinal cord was depleted using 5,7-DHT, which has been shown to ablate serotonergic nerve fibres in the spinal cord.[32 33] After insertion of a catheter into the i.t. space of lumbar enlargement, 5,7-DHT (60 μg per 20 μl) was injected through the catheter and flushed with 10 μl saline. The catheter was removed 20 min after 5,7-DHT injection. This dose has been reported to significantly deplete endogenous spinal 5-HT. Desipramine (30 mg kg^{-1}) was injected intraperitoneally 45 min before i.t. injection of 5,7-DHT to prevent non-specific uptake of 5,7-DHT by noradrenergic nerve fibres. After 5,7-DHT injection, motor function was assessed by evaluating righting and the placing–stepping reflex daily for 3 days. Pinna reflex and corneal reflex were also examined to check for sensory deficits. Animals without sensory or motor deficits were used for behavioural study or immunohistochemistry. No rats showed motor or sensory deficit on the day of 5,7-DHT injection, but one out of 24 animals treated with 5,7-DHT was found dead in the cage the day after 5,7-DHT injection; the cause was unknown.

Depletion of serotonin was evaluated by comparing 5-HT immunoreactivity of the dorsal horn of the lumbar spinal cord.[27] Rats were deeply anaesthetized with pentobarbital and ketamine and perfused transcardially with heparinized 0.9% saline followed by chilled 4% paraformaldehyde in 0.1 M phosphate buffer. After removal of the spinal cord and 6 h post-fixation in perfusate, it was transferred to 20% sucrose for 12–24 h and then 30% sucrose for cytoprotection. After snap-freezing, transverse sections (30 μm) of the lumbar enlargement were obtained. At least four random sections per animal were mounted and stained, where each section was incubated with rat monoclonal anti-5-HT antibody (1:100; Santa Cruz Biotechnology, USA) overnight at 4°C. Then, using the ABC staining system (Santa Cruz Biotechnology, USA), the sections were incubated with biotinylated secondary antibodies and visualized with avidin and biotinylated HRP and diaminobenzidine.

Study paradigm

First, the antinociceptive effects of i.t. 5-HT7R agonist (AS-19) and antagonist were tested to examine the role of spinal 5-HT7R in inflammatory pain induced by formalin and carrageenan. Animals were given AS-19 (3, 10, 30, 100 μg), SB269970 (3, 10, 30 μg), or vehicle 10 min before formalin or carrageenan injection. We also evaluated the reversal of antinociceptive effect of AS-19 by SB269970 in the formalin test, but not in the carrageenan model in which AS-19 did not exhibit a significant antinociceptive effect against mechanical allodynia.

The second set of experiments were designed to evaluate the role of serotonergic modulation on formalin- or carrageenan-evoked pain behaviour, in which animals were administered vehicle or 5,7-DHT i.t. to deplete serotonin in the spinal cord. Three days later, they were injected with formalin or carrageenan and subjected to behavioural study.

Statistical analysis

Data are expressed as means (SEM) for each group. The percentage of control (% of control={[sum of phase 1(2) count with drug]/[sum of phase 1(2) count of control]}×100) was used to compare the differences among groups in flinching responses induced by formalin. Mechanical allodynia observed in carrageenan-injected rats was compared using the hyperalgesic area under the curve (AUC), which was the sum of the percentage of hyperalgesic effect {=[(pre–post-injury withdrawal threshold)/pre-injury withdrawal threshold]×100} below the baseline over the 4 h duration of the test.

Differences among the groups or treatments were analysed by the *t*-test or one-way analysis of variance followed by Bonferroni's correction. In all analyses, $P<0.05$ was taken to indicate statistical significance.

Results

Antinociceptive effects of i.t. 5-HT7R agonist on formalin-induced flinching responses

Intraplantar injection of formalin resulted in typical biphasic flinching responses. Animals treated with AS-19 i.t. 10 min before formalin injection showed a significant decrease in flinching responses in both phases of the formalin test compared with controls (Fig. 1A, C, and E). The antinociceptive effect was dose-dependent, but reached a maximum at a dose of 30 μg with no further effect at the higher dose of 100 μg. In contrast, i.t. SB269970 did not alter the flinching responses (Fig. 1B, D, and F).

Pre-treatment with SB269970 (3 μg) i.t. 10 min before i.t. injection of AS-19 (30 μg) completely reversed the antinociceptive effect of i.t. AS-19 during phase 2 (Fig. 2). In phase 1, however, SB269970 pre-treatment partially but significantly reversed the antinociceptive effect of AS-19.

Lack of effects of i.t. 5-HT7R agonist and antagonist on carrageenan-induced mechanical allodynia

Paw withdrawal threshold was decreased significantly in response to intraplantar injection of carrageenan in controls.

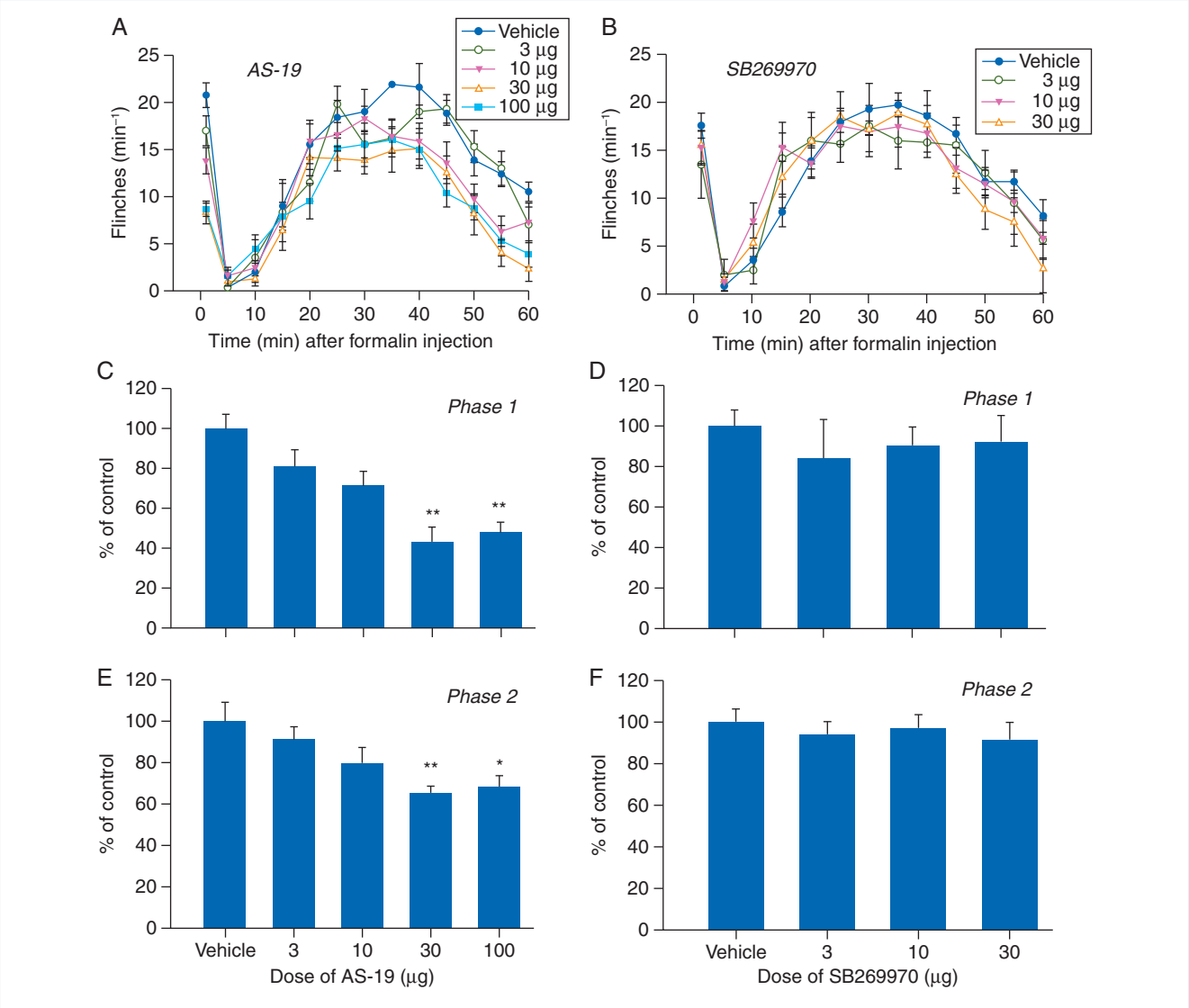

Fig 1 Effects of i.t. AS-19 (A, C, and E) and SB269970 (B, D, and F) on formalin-induced flinching responses. Time course after formalin injection (A and B) and per cent (%) of control at each dose (C–F) are illustrated. Each treatment group consisted of six to eight animals.*$P<0.05$, **$P<0.01$ *vs* vehicle.

Treatment with i.t. AS-19 10 min before injection of carrageenan did not increase withdrawal threshold even at the highest dose administered (Fig. 3A and C). In addition, i.t. SB269970 did not produce any significant change in paw withdrawal threshold compared with controls (Fig. 3B and D). Although the behavioural study showed a trend towards an increase in withdrawal threshold with i.t. AS-19 and a decrease with i.t. SB269970 compared with controls, these effects did not reach the level of statistical significance.

Different roles of descending serotonergic modulation in formalin- and carrageenan-induced nociception

Immunohistochemical analysis revealed a marked decrease in 5-HT immunoreactivity in serotonin-depleted rats, as described previously (Fig. 4).[34] Serotonin depletion using i.t. 5,7-DHT treatment had no effect on sensory or motor function and normal behaviour.

Animals treated with i.t. 5,7-DHT showed a significant decrease in flinching responses during phase 2 of the formalin test, but not during phase 1, when compared with the control group (Fig. 5A and C). In contrast to the antinociceptive effect seen in the formalin test, serotonin depletion with i.t. 5,7-DHT increased mechanical allodynia to a small but significant extent compared with the control group after intraplantar carrageenan administration (Fig. 5B and D).

Discussion

The main finding of the present study was that activation of 5-HT7R had a significant antinociceptive effect on formalin-induced pain, but no effect on carrageenan-induced pain, indicating differences in the involvement of 5-HT7R according to

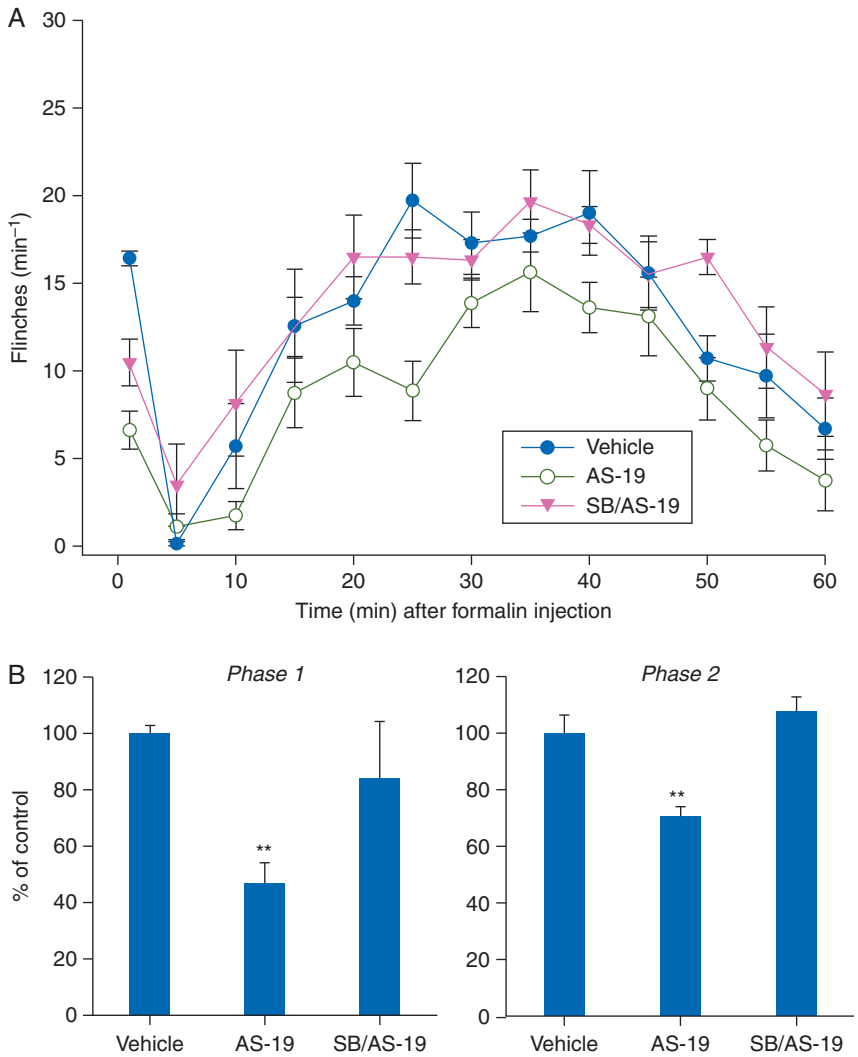

Fig 2 Antagonism of the antinociceptive effect of i.t. AS-19 by pretreatment with i.t. SB269970. Time course after formalin injection (A) and per cent (%) of control in each treatment (B) are shown. Each treatment group consisted of seven to eight animals. $^{**}P<0.01$ *vs* vehicle.

pain modality. Secondly, depletion of serotonin in the spinal cord reduced flinching responses in the formalin test, but increased mechanical allodynia in the carrageenan model, suggesting that the predominant roles of descending serotonergic modulation are facilitation in the formalin test and inhibition in the carrageenan model.

Inconsistent with the antinociceptive role of 5-HT7R in the formalin test of the current study, the pronociceptive role of 5-HT7R in the formalin test has been indicated in two previous studies.[12 14] However, one of these examined the role of 5-HT7R in mechanical secondary hyperalgesia and allodynia observed 7 days after formalin injection.[12] The other study used 5-carboxytryptamine (5-CT), a non-selective agonist of 5-HT7/1A receptor, which produced a pronociceptive effect during both phases at a lower dose and antinociceptive effects during phase 2 at a higher dose.[14] The pronociceptive effect was reversed by SB269970, but not by 5-HT1A receptor antagonist, suggesting a pronociceptive role of 5-HT7R and antinociceptive role of 5-HT1A receptor; the effect of SB269970 on the antinociceptive effect of 5-CT was not evaluated in this study. However, the current study provides direct evidence of the antinociceptive role of 5-HT7R in the formalin test by using its agonist and antagonist. In addition, both studies used a lower concentration of formalin (0.5% or 1%) in contrast with the concentration of 5% used in the present study. Interestingly, no significant effect of 5-HT7R antagonist, SB269970, on pain behaviour during phase 1 or 2 was seen in one previous study,[14] similar to the present study. However, dose-dependent inhibition of secondary mechanical hyperalgesia and allodynia was produced by spinal i.t. SB269970 in the other study.[12] These conflicting observations suggest that the role of 5-HT7R in acute and facilitated pain could be different from that in the late secondary hyperalgesia and allodynia.

The majority of evidence supports the inhibitory role of 5-HT7R in nociceptive processing of many pain models. In

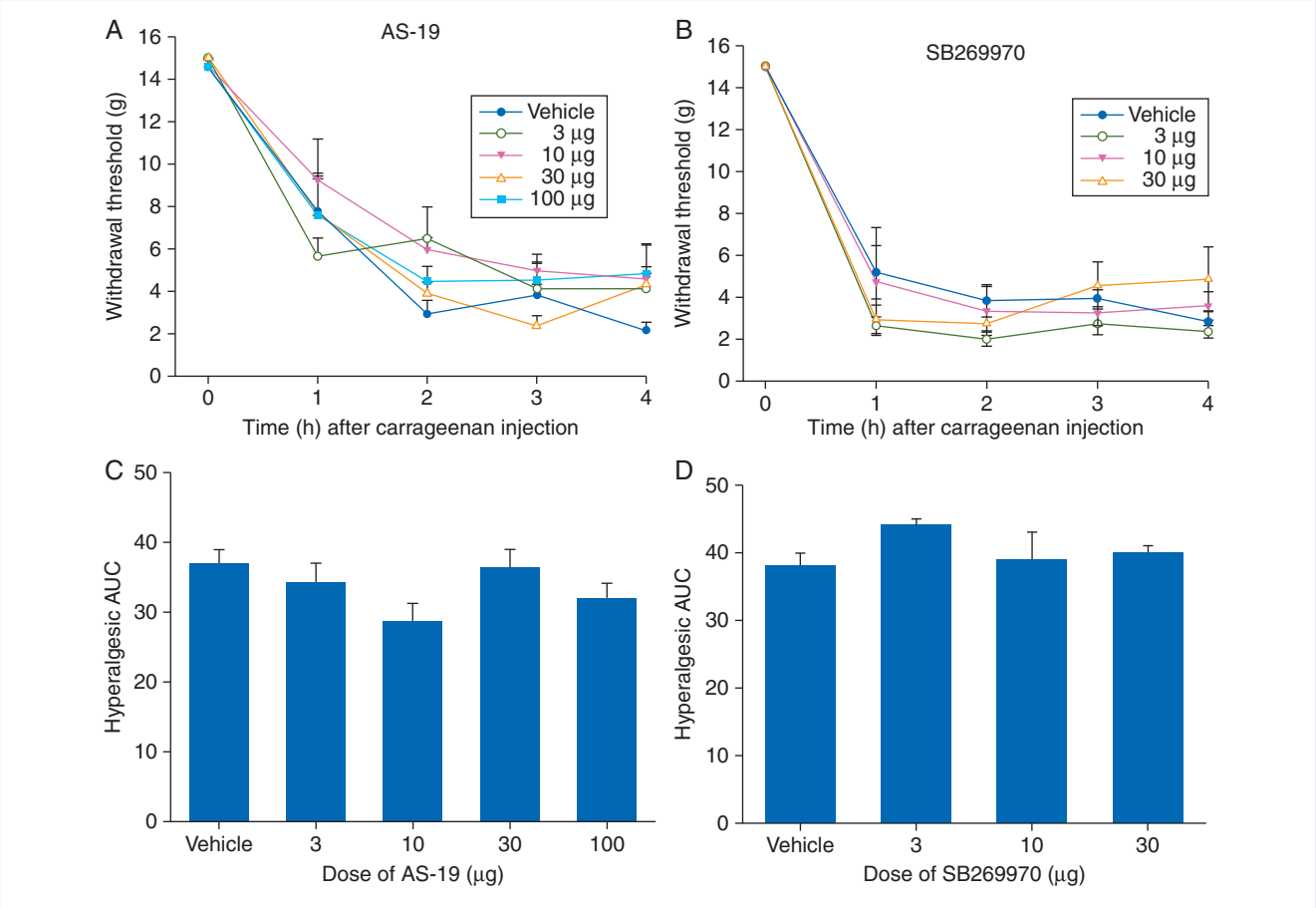

Fig 3 Effects of i.t. AS-19 (A and C) and SB269970 (B and D) on carrageenan-induced mechanical allodynia. Time course after carrageenan injection (A and B) and hyperalgesic AUC of each dose (C and D) are illustrated. No significant differences were observed among control and animals treated with AS-19 or SB269970. Each treatment group consisted of seven to eight animals.

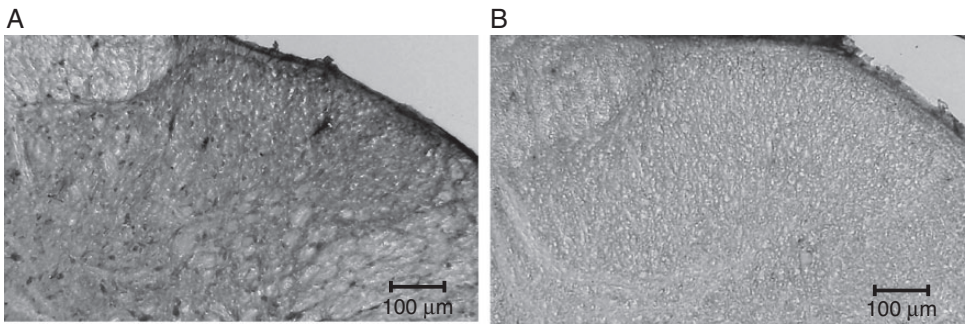

Fig 4 Representative sections of lumbar spinal cord of animals treated with i.t. vehicle (n=4, A) or i.t. 5,7-DHT (n=4, B) for 5-HT immunoreactivity, 3 days after i.t. injection. A marked reduction in 5-HT reactivity was observed in the dorsal horn of 5,7-DHT-treated rats compared with vehicle controls.

this study, i.t. AS-19 showed an antinociceptive effect on phase 1 of the formalin test, suggesting the importance of descending modulation via 5-HT7R in acute pain. This effect may be related to the significant role of 5-HT7R in mediating the antinociceptive effects of morphine, cannabinoids, and tramadol in acute pain models.[9–11 35] However, further investigations using acute pain modalities are needed to clarify the role of 5-HT7Rs in acute nociception. Consistent with previous studies,[22 33] serotonin depletion produced a significant decrease in nociceptive behaviour of phase 2, but not phase 1. However, these findings provide indirect evidence that spinal 5-HT7 plays a less significant role than other receptors known to

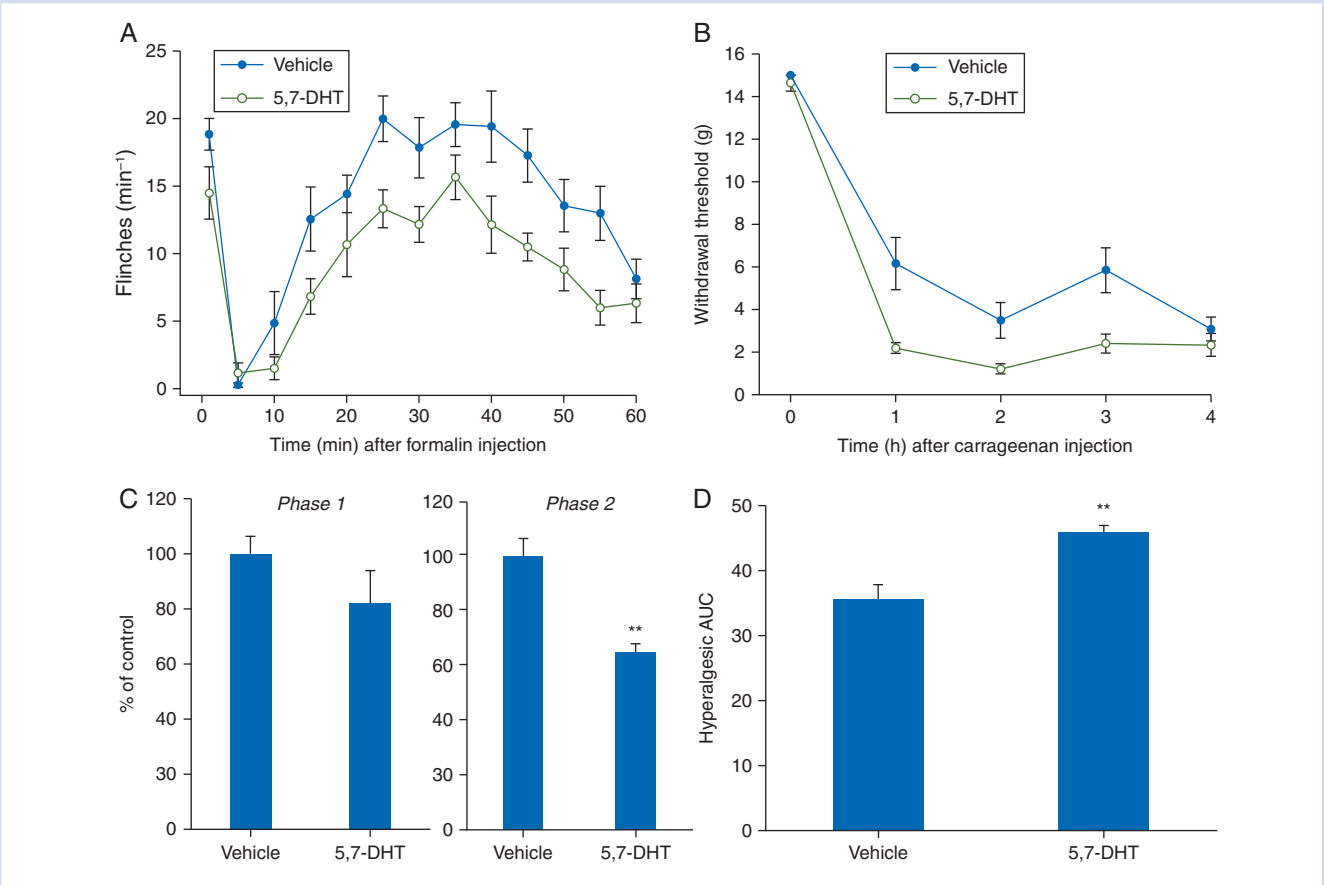

Fig 5 Effects of intrathecal treatment with 5,7-DHT on pain behaviour induced by intraplantar injection of formalin (A and C) and carrageenan (B and D). Time course (A and B) and per cent (%) of control after formalin injection or hyperalgesic AUC after carrageenan injection (C and D) are shown. Each treatment group consisted of seven to eight animals. $^{**}P<0.01$ *vs* vehicle.

have pronociceptive roles, such as 5-HT3R, in descending serotonergic modulation in the formalin test.

To the best of our knowledge, this is the first study to investigate the role of spinal 5-HT7Rs in carrageenan-induced inflammatory pain. The results of the present study were unexpected considering the inhibitory role of 5-HT7R in various pain models and action of analgesics,[5–7 9–11 18 35] no involvement of facilitatory 5-HT3R in the carrageenan model,[19–21] and difference in nature of pain modality between the formalin test and the carrageenan model. Compared with the formalin test, limited information on the role of spinal 5-HT in the carrageenan model exists, and no report has assessed the role of 5-HT7R in the carrageenan model. Collectively, both this and previous studies demonstrated that neither 5-HT7R nor 5-HT3R plays a significant role in carrageenan-induced inflammatory pain in contrast to formalin-induced pain.

Our data are insufficient to define the mechanisms underlying the different effects of the 5-HT7R agonist in the two models, which exhibit quite different nociceptive pain behaviours (spontaneous in the formalin test *vs* elicited in the carrageenan model) and chronicity (rapid nociceptive response *vs* subacute response) characteristics. The injection of carrageenan results in slowly developing and longer lasting pain, which is distinct from a briefer barrage of afferent drive in the formalin model. Therefore, different sensitizing mechanisms, which involve multiple pathways depending on the pain modality, may contribute to the different responses to 5-HT7R activation. Meanwhile, the different effect of i.t. AS-19 between the formalin and carrageenan models observed in the current study suggests that 5-HT release or 5-HT7R expression in the spinal cord may differ depending on the stimuli applied, that is, formalin or carrageenan injection. The increase in the 5-HT in the spinal cord in both the formalin and carrageenan models was observed in previous studies.[36 37] However, explaining the overall effect of spinal 5-HT or the role of 5-HT7R in descending modulation in terms of changes in the release or content of spinal 5-HT alone is difficult.

5-HT7R in the spinal cord is expressed mainly in the superficial lamina of the dorsal horn, specifically in the primary afferent fibres and interneurones in the naïve rat.[38] A decrease of 5-HT7R expression in the spinal cord was demonstrated in the spinal nerve ligation model, in which the blockade of 5-HT7R with SB269970 reduced the allodynia and hyperalgesia, suggesting a pronociceptive role of 5-HT7R.[13] Interestingly, in another study using the same model, the 5-HT content in the ipsilateral

dorsal spinal cord was not different after spinal nerve ligation compared with that in the sham rat.[39] Conflicting results were also reported for nerve-injury pain produced by partial sciatic nerve ligation, in which 5-HT7R immunoreactivity of the spinal cord dorsal horn was increased significantly compared with that in sham-operated mice, and activation of 5-HT7R with AS-19 reduced the nerve-injury-induced pain behaviour.[5] These results suggest that the change in the release of 5-HT or the extent of 5-HT7R expression may not be a key factor in determining the role of 5-HT7R in nociceptive processing.

A recent study more specifically indicated excitation of GABAergic interneurone of the spinal dorsal horn as the mediator of inhibitory control of 5-HT7R in chronic constriction injury of the rat.[40] The antihyperalgesic effect of systemic 5-HT7R agonist was significantly reduced by i.t. $GABA_A$ receptor antagonist but not by an opioid antagonist. In support of the GABAergic action of 5-HT7R agonist, the expression of 5-HT7R on the GABAergic interneurone of the spinal dorsal horn was verified in a mouse partial sciatic nerve ligation model.[5] These findings are also consistent with the stimulatory nature of 5-HT7R activity.[41] The pronociceptive role of 5-HT7R observed in spinal nerve ligation might be related to the increase in 5-HT7R expression in primary afferent fibres and a decrease in GABAergic interneurones, but this has yet to be demonstrated. Therefore, the difference between formalin and carrageenan in the effect of AS-19 may be a result of the diversity of the location and cell type in which 5-HT7R is expressed in the spinal cord, and so further investigation is required to determine the complex role of 5-HT7R in descending pain modulation.

The mechanism underlying the increase in mechanical allodynia observed in serotonin-depleted rats, which was the opposite of the result in the formalin test, was not explored further in this study. Owing to the lack of significant roles of 5-HT3R- and 5-HT7R-mediated descending facilitation in the carrageenan model and the balance between descending inhibition and facilitation,[1 15 19–21] the results of the present study indicate that serotonergic inhibition is predominant in the carrageenan model. However, the role of spinal 5-HT receptors in the carrageenan model has not been investigated extensively, and the results of previous studies were inconsistent. Other than 5-HT3R and 5-HT7R, the pronociceptive roles of spinal 5-HT1A and 5-HT1B receptors were demonstrated in a electrophysiological study of the spinal cord, although behavioural data are still lacking.[42] In contrast, stimulation of 5-HT1B/D receptor with sumatriptan attenuated the hypersensitivity to thermal stimuli after carrageenan injection,[43] suggesting that this receptor is an important mediator of descending inhibitory modulation in the carrageenan model.

One of the weak points of this study was that AS-19 is a partial agonist because E-55888, the full 5-HT7R agonist,[6] is not available in our country. AS-19 was shown to have relatively higher affinity and potency, but lower efficacy than E-55888 (E_{max}=99.7% *vs* 77%) in stimulating c-AMP production of human embryonic kidney (HEK)-293F cells expressing the human 5-HT7R by Brenchat and colleagues.[6] The action of AS-19, a partial agonist, on 5-HT7R may have an antagonistic effect, blocking the action of full agonist. The graded level of 5-HT7R expression in the spinal dorsal cord of normal rodents is lower than those of 5-HT1R or 5-HT3R, but comparable with those of other 5-HT receptors.[44] However, the action of a partial agonist (AS-19) would be determined by both its concentration and endogenous 5-HT relative to its receptor and also expression level of the 5-HT7 receptor. Until now, the expression of 5-HT7R in the spinal cord in carrageenan- and formalin-induced inflammatory pain models has not been investigated. Furthermore, the maximal effect of AS-19 against capsaicin-induced mechanical allodynia was similar to that of E55888.[6] Considering the findings of the current study, that is, that the antinociceptive action of AS-19 was blocked by pre-treatment of SB269970, which did not affect the pain behaviours in the formalin and carrageenan models, its effects are likely due to an agonistic effect of AS-19 on 5-HT7R rather than an antagonistic action.

In addition, this study adopted injection of 5% formalin as a pain modality, but 0.5% or 1.0% formalin should have been used for direct comparison with the previous two studies of 5-HT7R. Formalin concentrations ranging from 0.5% to 15% have been used in experiments regarding inflammatory pain, and the intensities of the pain behaviours have been shown to be dependent on the formalin concentration.[26] Previous studies showed differences in the role of spinal 5-HT3R in inflammatory pain induced by formalin and carrageenan, which were pronociceptive and not significant, respectively.[19–21] Therefore, this study was designed to test our hypothesis that the role of 5-HT7R activation in descending pain modulation differs between the formalin and carrageenan models, and we used 2% carrageenan and 5% formalin, used in the previous electrophysiological studies.

With regard to the different pain measurement modality used in this study, we measured the non-evoked spontaneous response for the formalin model, but the evoked-pain response for the carrageenan model. Our results may have been influenced by the behavioural assessment method used and the type of pain stimulus applied. Measurement of mechanical allodynia by the von Frey test in formalin-injected animals after the flinching behaviour disappeared would represent a pain modality different from phase 1 or 2. As mentioned earlier, the opposite role was reported in previous studies using the von Frey test for behavioural assessment, in which 5-HT7R was shown to play a pronociceptive role in the formalin-induced secondary allodynia and spinal nerve ligation model,[12 13] but an antinociceptive role in pain elicited by capsaicin or partial sciatic nerve ligation.[5 6] Furthermore, our findings suggest that 5-HT7R plays only a minor role in mechanical allodynia induced by carrageenan. Hence, the authors suggest that the type of assessment used could be a confounding factor in comparisons of the role of spinal 5-HT7R between the two models. However, the type of pain stimulus applied is a more important cause of such a difference.

In addition, immunohistochemistry was used to assess the depletion of spinal serotonin instead of quantitative analysis, which measures the content of spinal serotonin, and could provide more accurate data on the changes in serotonin levels.

However, the variations in 5-HT7R expression and 5-HT release according to pain modality suggest that the most important determinant of the role of 5-HT7R is the location and cell type expressing this factor in the spinal cord. Thus, the role of the 5-HT7R subtype in inflammatory pain, including that induced by formalin and carrageenan, should be investigated.

The results of the present study suggest that spinal 5-HT7R has an inhibitory role in formalin-induced pain in which facilitatory serotonergic modulation is predominant, but has no significant role in carrageenan-induced pain in which serotonergic inhibition is predominant.

Authors' contributions

J.Y., H.B.B., and H.G.K. performed experiments, assisted in the design, statistical analysis, and the writing. J.M.O. assisted in performing the immunohistochemistry and associated analyses. W.M.K., H.G.L., and M.H.Y. contributed to the design and data analysis. J.I.C. performed experiments, contributed to the design, data analysis, and writing of the manuscript.

Declaration of interest

None declared.

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