Intrathecal gabapentin does not act as a hyperpolarization-activated cyclic nucleotide-gated channel activator in the rat formalin test

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Background and objective **Gabapentin, an anticonvulsant with analgesic effect, has been reported to be an activator of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. In this study, we tested the effect of intrathecal ZD7288, an HCN channel inhibitor, and its interaction with intrathecal gabapentin in the rat formalin test.**

Methods **Male Sprague–Dawley rats (250–300 g) with an intrathecal catheter were intraplantarly injected with formalin (5% formaldehyde, 50 μl) in the right hindpaw. Ten minutes before formalin injection, gabapentin (100 or 200 μg) was given intrathecally. ZD7288 (50 μg) was administered intrathecally 10 min before paw formalin injection or intrathecal gabapentin. The paw flinch numbers in 1 min were counted at the first minute and every 5 min for 1 h after formalin injection.**

Results **Biphasic flinching responses were induced by formalin and monitored at 0–9 min (phase 1) and 10–60 min (phase 2) after formalin injection. Gabapentin (100 and 200 μg), given intrathecally 10 min before formalin injection, attenuated the flinching response during phase 2 of the formalin test. ZD7288 (50 μg), given intrathecally 10 min before formalin injection or intrathecal gabapentin injection, did not attenuate the formalin-induced flinching response or reverse gabapentin-induced analgesia.**

Conclusion **Our data suggest that activation of spinal or dorsal root ganglion HCN channels or both is not involved in formalin-induced pain, and intrathecal gabapentin does not act as an HCN channel activator to achieve its antinociceptive effect in the formalin test.** ***Eur J Anaesthesiol*** **26:821–824 © 2009 European Society of Anaesthesiology.**

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Introduction

Gabapentin is an anticonvulsant with analgesic effects in various clinical and animal studies [1,2]. In the past two decades, lots of studies have been performed to unveil the working mechanisms of gabapentin [3,4]. Among all the possible mechanisms, binding to the $\alpha_2\delta$ subunit of Ca^{2+} channels and reducing excitatory neurotransmitter release is the most frequently mentioned one [1]. A point mutation of arginine 217 of the Ca^{2+} channel $\alpha_2\delta$-1 subunit, which is critical for gabapentin binding, was found to cause a loss of gabapentin-induced analgesia [5]. Apart from binding to the $\alpha_2\delta$ subunit, other actions possibly underlying the analgesic effect of gabapentin, such as activating spinal cholinergic [6] and descending noradrenergic systems [7], have recently been reported.

The hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels are responsible for the functional hyperpolarization-activated current (I_h), which plays a distinct role in rhythmic activities in a variety of tissues, including neurons and cardiac cells [8]. These channels have been found in superficial spinal dorsal horn neurons [9], dorsal root ganglion (DRG) neurons [10] and peripheral sensory structures such as Meissner's corpuscles and Merkel cells [11], suggesting a possible involvement in pain processing.

In the study by Surges *et al.* [12] using rat hippocampal slices, gabapentin was found to increase the I_h amplitude in CA1 pyramidal neurons. Up to now, it is not known whether HCN channel activation is involved in the antinociceptive effect of gabapentin. In this study, we examined the effect of intrathecal ZD7288, an HCN channel inhibitor [13], in the rat formalin test and whether ZD7288 could reverse intrathecal gabapentin-induced analgesia.

Materials and methods

All experiments conformed to the guidelines of and were approved by the Institutional Laboratory Animal Care and

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Use Committee of Mackay Memorial Hospital. All efforts were made to minimize the number of animals used.

Intrathecal drug administration

Male Sprague–Dawley rats (250–300 g) were anaesthetized with isoflurane and received intrathecal catheterization as previously reported [14]. A PE-5 catheter was inserted spinally through an incision in the atlantooccipital membrane to a position 8.5 cm caudal to the cisterna at the level of lumbar enlargement of the rat spinal cord [15]. The catheter was secured on the top of the skull by a steel wire, and the wound was closed with sutures. Rats were allowed to recover for 5 days before the formalin test. Animals showing neurological deficits after intrathecal catheterization were euthanized. On the day of the experiments, isotonic saline or gabapentin (100 and 200 μg) was injected intrathecally 10 min before paw formalin injection. To test the effects of ZD7288 on formalin-induced flinching response and gabapentin-induced analgesia, ZD7288 (50 μg) was given intrathecally 10 min before paw formalin injection or intrathecal gabapentin injection. Drugs or isotonic saline were intrathecally delivered in a volume of 5 μl, followed by an additional 10 μl of isotonic saline to flush the intrathecal catheter.

Formalin test

Rats were placed in open Plexiglas boxes to acclimatize for 30 min and then gently restrained in a tube to receive subcutaneous injection of 5% formaldehyde (50 μl) at the plantar surface of the right hindpaw with a 27-gauge needle. A successful injection can be identified by the gross swelling of the injected hindpaw and typical flinching response. Rats showing improper injections were excluded from the study. The flinch numbers were counted by an observer blinded to the treatment groups.

After paw formalin injection, two phases of flinching response were observed as described previously [16]. Phase 1 was initiated within seconds after formalin injection and lasted for 5–10 min. After a quiescent interval of several minutes, a second phase of flinching response appeared. The number of flinches in 1 min was counted at the first minute and every 5 min for 1 h after formalin injection. The time–response data were presented as the number of flinches. The flinch numbers during 0–9 and 10–60 min after formalin injection were summed as the responses of phases 1 and 2, respectively [17].

Motor function evaluation

To assess the possible motor impairment induced by the drugs, two motor function tasks, the placing/stepping reflex and righting reflex, were examined as reported [18]. The placing/stepping reflex, an upward lifting of the hindpaw of the rat when its dorsum was placed over the edge of a tabletop, was used to evaluate whether there was hindlimb flaccidity in the rat. The righting reflex is an immediate coordinated twisting of the body around its longitudinal axis to regain its normal position on its feet when a rat was placed horizontally with its back on the table.

Drug

Gabapentin was a gift from Pfizer Inc. (Groton, Connecticut, USA), and ZD7288 was purchased from Tocris (Bristol, UK). Both drugs were dissolved in isotonic saline. The doses of gabapentin (100 and 200 μg) and ZD7288 (50 μg) were chosen on the basis of previous studies [19,20].

Data and statistics

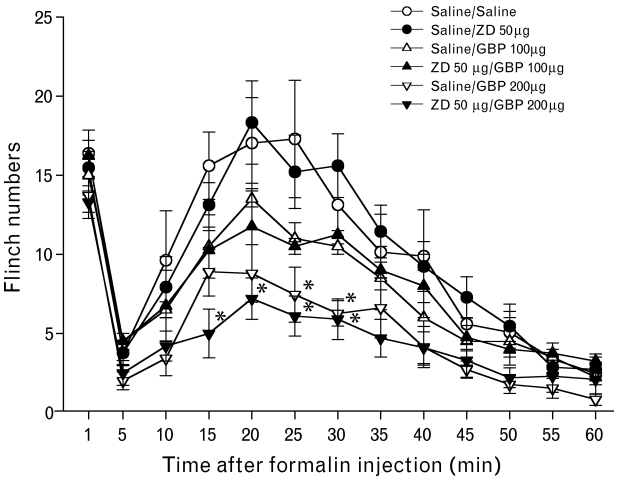
Data are expressed as means ± SEM. In each treatment group, eight rats were tested. The flinch numbers at each time point and total flinch numbers of phases 1 and 2 were compared between groups using one-way analysis of variance with post-hoc Tukey test. The software SigmaStat 2.0 (SPSS Science, Chicago, Illinois, USA) was used for statistical analysis. A P value of less than 0.05 was considered statistically significant.

Results

Formalin-induced biphasic flinching response

Two phases of flinching behaviour were observed after paw formalin injection in the rats. The first phase was initiated immediately after formalin injection and subsided gradually until about 10 min after injection. Thereafter, the second phase of flinching response appeared, which peaked at approximately 20–30 min after injection (Fig. 1). The total flinch numbers in the saline/saline

Fig. 1



Time courses of the effects of intrathecal gabapentin, ZD7288 and their interaction on flinching responses in the rat formalin test. The ordinates are the flinch numbers in 1 min at each time point. Two phases, phase 1 (0–9 min) and phase 2 (10–60 min), of flinching responses were induced after paw formalin injection. Each line represents the mean ± SEM of eight rats. GBP, gabapentin; ZD, ZD7288. *$P < 0.05$ compared with the saline/saline group by one-way analysis of variance with the post-hoc Tukey test.

group in phase 1 (0–9 min) and phase 2 (10–60 min) were 20 ± 2 and 109 ± 19 ($n = 8$), respectively.

ZD7288 did not affect formalin-induced flinching response

ZD7288 (50 μg), administered intrathecally 10 min before paw formalin injection, did not enhance or attenuate the formalin-induced flinching response in either phase 1 or 2 (Fig. 1). The total flinch numbers in the saline/ZD7288 group in phases 1 and 2 were 19 ± 2 and 109 ± 7 ($n = 8$), respectively ($P > 0.05$ when compared with the saline/saline group).

Gabapentin attenuated phase 2 flinching response

At 15, 20, 25 and 30 min after formalin injection, there were significant differences among the treatment groups ($P < 0.05$, degrees of freedom = 5, F values being 5.2, 4.4, 3.7 and 5.4, respectively). Intrathecal administration of gabapentin (100 or 200 μg), 10 min before paw formalin injection, attenuated the flinching response in phase 2 but not in phase 3 (Fig. 1). The saline/gabapentin (200 μg) group was different from the saline/saline group at 25 and 30 min after formalin injection ($P < 0.05$, Fig. 1). The total flinch numbers in the saline/gabapentin (100 μg) and saline/gabapentin (200 μg) groups in phase 2 were 81 ± 8 and 54 ± 7, respectively ($P < 0.05$ when compared with the phase 2 total flinch numbers in the saline/saline group, $n = 8$). No change in motor function was seen after intrathecal administration of gabapentin. This suggests that the inhibitory effect of gabapentin on the formalin-induced flinching response is not due to motor impairment.

ZD7288 failed to reverse gabapentin-induced analgesia

ZD7288 (50 μg), administered intrathecally 10 min before gabapentin injection, did not modulate the analgesic effect of gabapentin (100 or 200 μg) (Fig. 1). The total flinch numbers of the ZD7288/gabapentin (100 μg) and ZD7288/gabapentin (200 μg) groups in phase 2 were 83 ± 5 and 47 ± 8, respectively [$P > 0.05$ when compared with the saline/gabapentin (100 μg) and saline/gabapentin (200 μg) groups, respectively, $n = 8$]. The ZD7288/gabapentin (200 μg) group was different from the saline/saline group at 15, 20, 25 and 30 min after formalin injection ($P < 0.05$, Fig. 1). Motor weakness was noted when the dose of ZD7288 was increased to 80 μg (data not shown).

Discussion

In this study, we found that intrathecal ZD7288, an HCN channel inhibitor, did not affect formalin-induced nociceptive behaviour, implying that spinal or DRG HCN channels or both are not critically involved in the formalin test. In addition, ZD7288 failed to reverse the antinociceptive effect of intrathecal gabapentin in the formalin test. It is suggested that intrathecal gabapentin does not act as an HCN channel activator to achieve its antinociceptive effect in the formalin test.

HCN channels, particularly HCN-1, are abundantly expressed in rat primary afferent somata [19]. HCN-1, HCN-2 and HCN-4 have been detected in spinal dorsal horn, with HCN-1 in lamina I, III and IV and both HCN-2 and HCN-4 in lamina I–IV [9,10]. Upregulation of I_h has been observed in medium-size DRG neurons after chronic ganglion compression [21]. The distribution of HCN channels in the pain pathway suggests a possible involvement of HCN channels in the modulation of nociceptive transmission. The HCN channel inhibitor, ZD7288, nonselective among HCN-1–4 [19], could inhibit spontaneous firing in ligation-injured primary afferent neurons and reverse nerve ligation-induced mechanical allodynia, when administered systemically [19]. Dalle and Eisenach [22] have reported that perineural administration of ZD7288 could reverse paw incision-induced and partial sciatic nerve injury-induced mechanical allodynia in rats, suggesting a role of HCN channels in peripheral nerve block. In a recent study by Luo *et al.* [11], intraplantar and systemic administration of ZD7288 was found to reverse thermal injury-induced spontaneous pain and spinal nerve ligation-induced mechanical allodynia.

In the present study, intrathecal pretreatment with ZD7288 (50 μg) neither enhanced nor attenuated the formalin-induced flinching response. In the study by Masuda *et al.* [23], intrathecal injection of ZD7288 (3 μg) in rats can eliminate bladder contractions for more than 30 min, which is longer than the 10 min pretreatment time of ZD7288 in this study. To our knowledge, this is the first study examining the effect of intrathecal HCN channel inhibitor in the formalin test. In a recent study by Wan [24], intrathecal injection of ZD7288 (30 μg) attenuated spinal nerve ligation-induced mechanical allodynia in rats and motor deficits were observed when the dose of ZD7288 was higher than 60 μg, similar to our finding with ZD7288 (80 μg). ZD7288 (50 μg) has been reported to be the maximally tolerable dose not to induce hindlimb motor weakness, with estimated cerebrospinal fluid concentration in excess of 500 $\mu mol\,l^{-1}$ [19], much higher than the 10 or 100 $\mu mol\,l^{-1}$ of ZD7288 used to block the I_h of rat DRG neurons [25,26]. Our finding suggests that spinal or DRG HCN channels or both do not play an important role in the induction of formalin-induced pain.

Gabapentin is an anticonvulsant and analgesic with several possible mechanisms of action, including binding to the $\alpha_2\delta$ subunit of voltage-dependent Ca^{2+} channels [1]. In the study by Surges *et al.* [12], gabapentin was found to enhance I_h in rat hippocampal CA1 pyramidal neurons. The enhancement of I_h by an HCN channel activator could lead to cell membrane depolarization and, hence, increased neuronal excitability. Spinal inhibitory interneurons might be the possible target of intrathecal gabapentin if gabapentin acts as an HCN channel

activator to achieve its antinociceptive effect. In this study, however, the analgesic effect of intrathecal gabapentin was not reversed by the HCN channel inhibitor ZD7288. Furthermore, ZD7288 did not enhance formalin-induced nociceptive behaviour. It is therefore suggested that HCN channel activation does not contribute to gabapentin-induced analgesia in the present formalin test.

In the study by Chaplan *et al.* [19] using an L5–6 spinal nerve ligation pain model, the large neurons in nerve-ligated ganglia showed increased I_h, and the nerve ligation-induced tactile allodynia was blocked by systemic ZD7288. Their findings suggest that the injured large DRG neurons express more HCN channels and could possibly fire spontaneously and lead to neuropathic pain behaviour. It is important to note that gabapentin is also effective in the L5–6 spinal nerve ligation pain model, given either intrathecally [27] or systemically [28]. Taken together with our finding, it is possible that gabapentin-induced antinociception is not mediated via HCN channel activation. Whether HCN channel activation is involved in other therapeutic actions of gabapentin, such as anxiolysis and anticonvulsion, remains to be elucidated.

Conclusion

In summary, the present study reveals that intrathecal ZD7288 neither affected formalin-induced nociceptive behaviour nor reversed intrathecal gabapentin-induced analgesia in the rat formalin test. It is suggested that spinal or DRG HCN channels or both are not critically involved in formalin-induced pain, and intrathecal gabapentin does not act as an HCN channel activator to achieve its antinociceptive effect in the formalin test.

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