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Spinal gabapentin is antinociceptive in the rat formalin test

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Abstract

Gabapentin is a novel anticonvulsant that may be of value for the relief of clinical pain. To determine whether gabapentin is antinociceptive after spinal administration, the drug was given via an intrathecal catheter in doses from 6 to 200 μg/rat 10 min prior to intraplantar formalin. Five percent formalin injected subcutaneously in the right hind paw produced a biphasic reaction consisting of flinching and licking behaviors (phase 1, 0–10 min; phase 2, 10–60 min). Gabapentin dose-dependently reduced the numbers of flinches and the duration of licking during phase 2 of the formalin test. The highest dose of gabapentin (200 μg/rat) did not affect the tail-flick response. These results demonstrate that spinal gabapentin is antinociceptive in the formalin test.

Keywords: Gabapentin; Spinal administration; Formalin; Antinociception

Anticonvulsants have antinociceptive effects and some are used in the management of clinical pain [3]. Gabapentin, a structural analog of γ-aminobutyric acid (GABA), is a novel anticonvulsant that anecdotally has analgesic properties in the treatment of postherpetic neuralgia [8] and reflex sympathetic dystrophy [5]. Development of new drugs to treat such refractory pain syndromes is of urgent clinical interest.

The formalin test is an experimental pain model involving central sensitization [2]. Central sensitization plays an important role in many pathological pain processes [1]. We examined the effects of spinal gabapentin in the formalin test, to determine whether gabapentin can produce antinociception with spinal administration.

Male Sprague–Dawley rats weighing 300–350 g were used. The animals were housed in groups of four prior to intrathecal catheterization and individually after the surgery on a 12 h light/dark schedule with food and water ad libitum.

For the spinal administration of drugs to the rat, a catheter was placed in the intrathecal space. Under halothane anesthesia, a PE-10 tube was inserted through a small hole made in the atlanto-occipital membrane, and threaded 8.5 cm down the intrathecal space to the lumbo-sacral level of the spinal cord [11]. The catheterized rats were observed for 24 h postoperatively, and those with any signs of paralysis were excluded from the study. At the end of the study, 5 μl of a 1% methylene blue solution was introduced into the catheter followed by 10 μl of saline to confirm the position of the catheter and the spread of the dye in the intrathecal space.

Gabapentin (1-(aminomethyl)-cyclohexaneacetic acid; Park Davis, MI, USA) was dissolved in normal saline. Gabapentin at a dose of 6, 20, 60 or 200 μg/rat or saline in a volume of 5 μl was administered spinally 10 min prior to the administration of formalin. The number of animals in the treatment groups averaged eight.

Formalin was diluted to 5% from a stock solution of 100% (formaldehyde solution 37% w/w; Fisher Scientific Co., Fairlawn, NJ, USA). Formalin was injected subcutaneously into the right hindpaw in a volume of 50 μl with the use of a 50 μl glass syringe and a new disposable 30-gauge needle. Immediately following the formalin injec-

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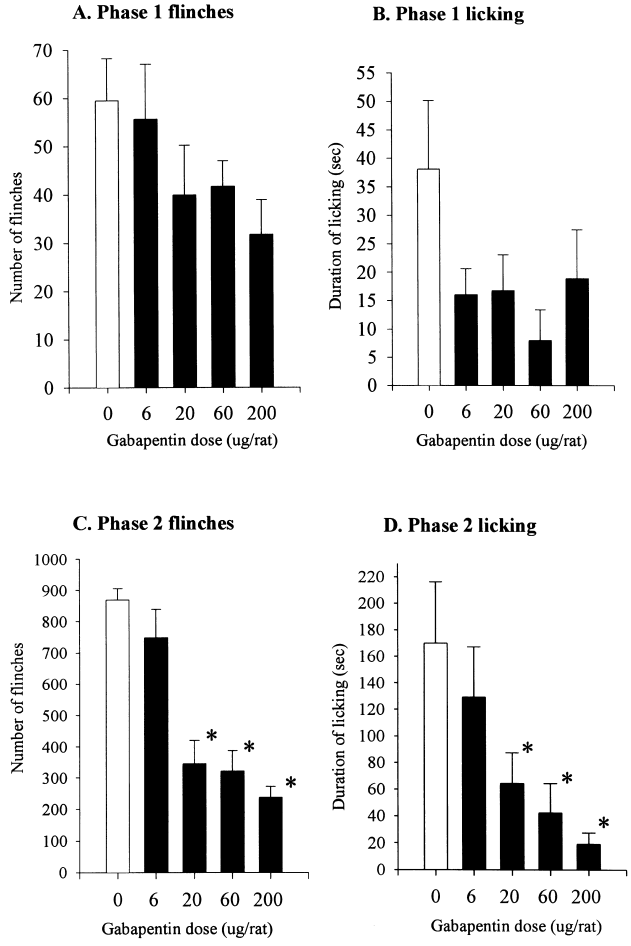

Fig. 1. Spinal gabapentin dose-dependently reduces flinching and licking behaviors in rats during phase 2 of the formalin test. Gabapentin at a dose of 6, 20, 60 or 200 μg/rat, or saline (0 μg) was administered to rats 10 min prior to the intraplantar injection of 50 μl of 5% formalin. (A) The number of flinches (mean + SEM) observed during phase 1 (0–10 min after formalin). (B) The duration of licking (mean + SEM) observed during phase 1. (C) The number of flinches (mean + SEM) observed during phase 2 (10–60 min after formalin). (D) The duration of licking (mean + SEM) observed during phase 2. *Significantly different ($P < 0.05$) from the saline (0 μg) treatment group.

tion, the rat was placed in a test chamber and was observed continuously by a blinded observer for the next 60 min. The formalin injection resulted in a biphasic reaction consisting of flinching and licking behaviors (phase 1, 0–10 min; phase 2, 10–60 min). The total number of flinches, defined as quick shakes of the injected hindpaw, and the total number of seconds spent licking the affected hindpaw were recorded for each phase.

Data were analyzed by a one-way analysis of variance (ANOVA). Multiple comparisons versus the saline control group were made by the Dunnett's Method. Statistical significance was accepted at $P < 0.05$.

During phase 1, gabapentin produced decreases in flinching and licking, but these effects were not dose-dependent and did not reach statistical significance (Fig. 1A,B; $P = 0.190$ and $P = 0.109$, respectively). During phase 2, gabapentin reduced nociceptive behaviors in a dose-dependent manner (Fig. 1C,D). At 6 μg/rat of gabapentin, no difference was observed as compared to saline controls. At 20, 60 and 200 μg/rat both flinching and licking behaviors were significantly reduced. At the highest dose (200 μg/rat), gabapentin reduced flinching by 73% and licking by 89%.

In a separate experiment, gabapentin at a dose of 200 μg/rat was given to rats ($n = 9$) and the tail-flick latencies were measured before (baseline latencies) and every 15 min up to 90 min after the administration. The intensity of thermal stimulus was adjusted so that the baseline latencies were in the range of 2.5–3.5 s. Animals were tested for their ability to negotiate a 60° vertical mesh [10] immediately after each tail-flick latency measurement. No differences were seen between baseline latencies and tail-flick latencies measured at any time point after the administration of gabapentin (Fig. 2).

Behaviorally, the 20 and 60 μg/rat doses of gabapentin were without overt central nervous system (CNS) effects, while the 200 μg/rat dose appeared to have a calming effect on the usual exploratory behavior of the rat. The 200 μg/rat dose had no effect on the rats' ability to negotiate the 60° vertical mesh.

These results demonstrate that spinal gabapentin is antinociceptive in the formalin test (Fig. 1). This report confirms the previous anecdotal clinical reports on gabapentin's analgesic effects [5,8]. Clinical use of gabapentin as an antiepileptic has shown that it has minimal side effects and its chronic use is well tolerated by patients [6,16]. Thus, gabapentin may also be a safe and useful drug to be used as an analgesic to treat chronic pain. Con-

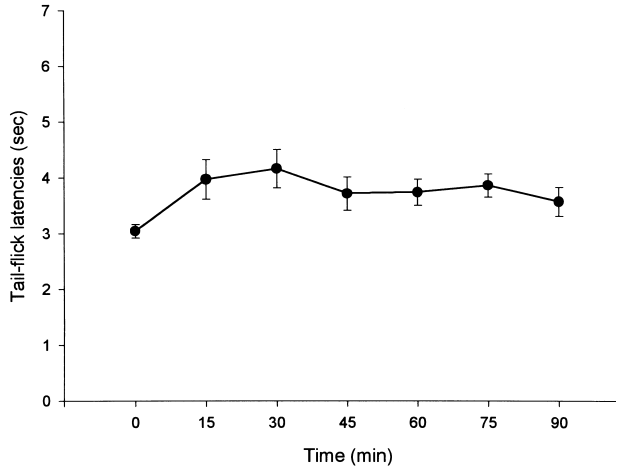

Fig. 2. Spinal gabapentin at the dose of 200 μg/rat does not affect the tail-flick response. Tail-flick latencies were measured before (baseline) and every 15 min up to 90 min after the administration of spinal gabapentin. No differences were seen between the baseline latencies and the tail-flick latencies measured at any point after gabapentin. Values are in mean ± SEM.

trolled clinical trials are required to determine its efficacy and safety in patients with pain syndromes.

In spite of extensive research, the mechanism of action of gabapentin is still unclear. Although it is a structural analog of GABA, it does not bind to GABA receptors or any known neurotransmitter receptor [15] but binds to a novel site in the rat brain, which is unique to the CNS [12,13]. A number of structural analogs of gabapentin and 3-alkyl substituted GABA derivatives including 3-isobutyl GABA also bind to this novel site [12]. The S(+) enantiomer of 3-isobutyl GABA has high affinity to the binding site and possesses potent anticonvulsant properties, while, the R(−) enantiomer shows low potency in both binding to the novel site and anticonvulsant action [14]. This suggests that this novel binding site is involved in the anticonvulsant action of gabapentin. Recently, the high-affinity binding protein for [^{3}H]gabapentin has been isolated from pig cerebral cortex membranes and characterized as the $\alpha_2\delta$ subunit of a voltage-dependent calcium channel [4]. This finding suggests that the voltage-dependent neuronal calcium channels may play an important role in mechanism of action of gabapentin. However, in another study, gabapentin did not affect voltage-dependent calcium channel currents in cultured rodent neurons [7].

Phase 2 of the formalin test is thought to reflect central sensitization [2]. Sensitization of the dorsal horn neuron involves the activation of *N*-methyl-d-aspartate (NMDA) and NK-1 receptors, the influx of Ca^{2+} into the cell and subsequent intracellular events including the activation of secondary messenger systems. Suppressing any of these events may block sensitization of the dorsal horn neuron. Known agents that suppress these events block phase 2 response of the formalin test while having little effect on phase 1 and on acute pain responses such as the tail-flick response [17]. On the other hand, agents such as morphine, that suppress afferent input, block the phase 1 response of the formalin test and the tail-flick response as well as the phase 2 response of the formalin test [9,18]. In the present study, spinal gabapentin produced dose-dependent reduction of formalin-induced nociceptive behaviors during phase 2 (Fig. 1C,D), while its effects were not statistically significant nor dose-dependent during phase 1 (Fig. 1A,B). Furthermore, the highest dose that showed significant effects in the formalin test had no effect in the tail-flick test (Fig. 2). These antinociceptive effects of spinal gabapentin are similar to those of the agents that block central sensitization by suppressing the activation of the NMDA or NK-1 receptor, or the subsequent events that lead to central sensitization. These observations suggest that gabapentin may act directly or indirectly on the dorsal horn neuron to block central sensitization.

In conclusion, we have demonstrated the dose-dependent antinociceptive effects of spinal gabapentin in the rat. Further controlled studies on the analgesic effects of gabapentin are needed to determine whether it may be a useful analgesic in patients.

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