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Gabapentin (neurontin) and S-(+)-3-isobutylgaba represent a novel class of selective antihyperalgesic agents

M.J. Field, R.J. Oles, A.S. Lewis, S. McCleary, J. Hughes & [1]L. Singh

Department of Biology, Parke-Davis Neuroscience Research Centre, Cambridge University Forvie Site, Robinson Way, Cambridge, CB2 2QB

1 Gabapentin (neurontin) is a novel antiepileptic agent that binds to the $\alpha_2\delta$ subunit of voltage-dependent calcium channels. The only other compound known to possess affinity for this recognition site is the (**S**)-(+)-enantiomer of 3-isobutylgaba. However, the corresponding (**R**)-(−)-enantiomer is 10 fold weaker. The present study evaluates the activity of gabapentin and the two enantiomers of 3-isobutylgaba in formalin and carrageenan-induced inflammatory pain models.

2 In the rat formalin test, **S**-(+)-3-isobutylgaba (1–100 mg kg^{-1}) and gabapentin (10–300 mg kg^{-1}) dose-dependently inhibited the late phase of the nociceptive response with respective minimum effective doses (MED) of 10 and 30 mg kg^{-1}, s.c. This antihyperalgesic action of gabapentin was insensitive to naloxone (0.1–10.0 mg kg^{-1}, s.c.). In contrast, the **R**-(−)-enantiomer of 3-isobutylgaba (1–100 mg kg^{-1}) produced a modest inhibition of the late phase at the highest dose of 100 mg kg^{-1}. However, none of the compounds showed any effect during the early phase of the response.

3 The s.c. administration of either **S**-(+)-3-isobutylgaba (1–30 mg kg^{-1}) or gabapentin (10–100 mg kg^{-1}), after the development of peak carrageenan-induced thermal hyperalgesia, dose-dependently antagonized the maintenance of this response with MED of 3 and 30 mg kg^{-1}, respectively. Similar administration of the two compounds also blocked maintenance of carrageenan-induced mechanical hyperalgesia with MED of 3 and 10 mg kg^{-1}, respectively. In contrast, **R**-(−)-3-isobutylgaba failed to show any effect in the two hyperalgesia models.

4 The intrathecal administration of gabapentin dose-dependently (1–100 μg/animal) blocked carrageenan-induced mechanical hyperalgesia. In contrast, administration of similar doses of gabapentin into the inflamed paw was ineffective at blocking this response.

5 Unlike morphine, the repeated administration of gabapentin (100 mg kg^{-1} at start and culminating to 400 mg kg^{-1}) over 6 days did not lead to the induction of tolerance to its antihyperalgesic action in the formalin test. Furthermore, the morphine tolerance did not cross generalize to gabapentin. The s.c. administration of gabapentin (10–300 mg kg^{-1}), **R**-(−) (3–100 mg kg^{-1}) or **S**-(+)-3-isobutylgaba (3–100 mg kg^{-1}) failed to inhibit gastrointestinal motility, as measured by the charcoal meal test in the rat. Moreover, the three compounds (1–100 mg kg^{-1}, s.c.) did not generalize to the morphine discriminative stimulus. Gabapentin (30–300 mg kg^{-1}) and **S**-(+)-isobutylgaba (1–100 mg kg^{-1}) showed sedative/ataxic properties only at the highest dose tested in the rota-rod apparatus.

6 Gabapentin (30–300 mg kg^{-1}, s.c.) failed to show an antinociceptive action in transient pain models. It is concluded that gabapentin represents a novel class of antihyperalgesic agents.

Keywords: Formalin; carrageenan; thermal and mechanical hyperalgesia; $\alpha_2\delta$ subunit of voltage dependent calcium channels; side effects

Introduction

Gabapentin (neurontin), is an antiepileptic agent structurally related to γ-aminobutyric acid (GABA) with an unknown mechanism of action. It is currently in clinical use as an add-on therapy in patients with partial seizures resistant to conventional therapies (see Goa & Sorkin, 1993, for review). Although gabapentin was originally designed as a GABA analogue which would penetrate into the CNS, it does not interact with either $GABA_A$ or $GABA_B$ receptors (Bartoszyk & Reimann, 1985). A single highly specific [^{3}H]-gabapentin binding site ($K_D = 38 \pm 2.8$ nM) in the brain has been described (Suman-Chauhan *et al*., 1993) and more recently this has been identified as the $\alpha_2\delta$ subunit of voltage-dependent calcium channels (Gee *et al*., 1996). In binding studies, gabapentin ($IC_{50} = 80$ nM) and (**RS**)-3-isobutylgaba were the most active compounds identified. (**RS**)-3-isobutylgaba stereoselectively inhibited [^{3}H]-gabapentin binding to brain membranes with the (**S**)-(+)-enantiomer showing similar affinity as gabapentin, whereas the corresponding (**R**)-(−)-enantiomer was found to be 10 times weaker.

The two chemical irritants formalin and carrageenan are widely used in studies of tonic pain following peripheral inflammation. The s.c. administration of dilute formalin into the plantar surface of the rodent paw produces a biphasic nocifensive behavioural response. The early phase consists of intense licking and biting of the injected paw and lasts up to 10 min, but the late tonic phase occurs over 20–60 min after injection (Dubuisson & Dennis, 1977; Wheeler-Aceto & Cowan, 1991) and is a state of facilitated pain processing (hyperalgesia) associated with inflammation. This behavioural response has been shown to correlate with a biphasic increase in the activation of C-fibre primary afferents after formalin injection (McCall *et al*., 1996). It has been shown that carrageenan elicits little or no spontaneous nocifensive behaviour (Wheeler-Aceto *et al*., 1990) but induces a period of hyperalgesia to peripheral thermal and mechanical stimulation peaking at 2–3 h after injection (Winter & Flataker, 1965; Kayser & Guilbaud, 1987; Hargreaves *et al*., 1988). The formalin and carrageenan induced behavioural and hyperalgesia responses involve central mechanisms re-

[1] Author for correspondence.

lated to sensitization of dorsal horn neurones that occur following tissue injury or intense activation of C-afferents (Woolf, 1983; Woolf & Wall, 1986; Dickenson & Sullivan, 1987; Stanfa *et al*., 1992). In the present study we examined the relationship between binding affinity for the $\alpha_2\delta$ subunit and the antihyperalgesic actions of gabapentin, (**R**)-(−) and (**S**)-(+)-3-isobutylgaba in the rat formalin test, and the carrageenan-induced thermal and mechanical hyperalgesia models. The side effect profile of gabapentin was also compared with that of morphine.

Methods

Animals

Male Sprague-Dawley rats (70–90 g or 180–250 g), were obtained from Bantin and Kingman, (Hull, U.K.) and male Hooded Lister rats (200–250 g) from Interfauna Universal (Huntingdon, U.K.). Animals were housed in groups of 6–10 under a 12 h light/dark cycle (lights on at 07h 00 min) with food and water *ad libitum*, except Hooded Lister rats which were maintained at 80–85% of their free feeding body weight.

Drug administration

Drugs were administered s.c. in a volume of 2 ml kg^{-1} for animals weighing <100 g and 1 ml kg^{-1} for animals >100 g. The intrathecal injections (i.t.) were made in a volume of 5 μl with a 50 μl Hamilton syringe by exposing the spine of the rats under isoflurane anaesthesia. Injections were made between lumbar region 5–6 with a 3.5 mm long 27 gauge needle. The wound was sealed with an autoclip.

Procedures

Rat paw formalin test Male Sprague Dawley rats (70–90 g) were habituated to perspex observation chambers (24 cm × 24 cm × 24 cm) for at least 15 min before testing. A mirror was placed behind the box to aid observation. Formalin-induced hind paw licking and biting was initiated by a 50 μl subcutaneous injection of a 5% formalin solution (5% formaldehyde in isotonic saline) into the plantar surface of the left hind paw. Immediately following the formalin injection, licking/biting of the injected hind paw was scored in 5 min bins for 45 min. Test compounds were administered s.c. 1 h before formalin. The 1 h pretreatment times were selected from preliminary studies addressing the duration of action of gabapentin and **S**-(+)-3-isobutylgaba. These showed that the peak effects for both of these compounds occur within 30 min and are maintained for several hours. The results are expressed as mean licking/biting time for the early phase (0–10 min) and the late phase (10–45 min).

Carrageenan-induced thermal hyperalgesia in the rat Thermal hyperalgesia was assessed by the rat plantar test (Ugo Basile, Italy) following a modified method of Hargreaves *et al*. (1988). Male Sprague-Dawley rats (70–90 g) were habituated to the apparatus which consisted of three individual perspex boxes on an elevated glass table. A mobile radiant heat source located under the table was focused onto the desired paw and paw withdrawal latencies (PWL) recorded. PWL were taken 3 times for both hind paws of each animal, the mean of which represented baselines for right and left hind paws. At least 5 min were allowed between each PWL for an animal. The apparatus was calibrated to give a PWL of approximately 10 s. The heart rate was on average 1.33°C s^{-1} and the maximum temperature obtained to yield the mean latency of 10 s was 33.6°C. There was an automatic cut off point of 20 s to prevent tissue damage. After baseline PWL were determined, animals received an intraplantar injection of carrageenan (100 μl of 20 mg ml^{-1}) into the right hind paw. PWL were reassessed following the same protocol as above 2 h post carrageenan (this time point represented the start of peak hyperalgesia) to ascertain that hyperalgesia had developed. Test compounds were then administered s.c. 2.5 h post carrageenan and PWL taken again at 3, 3.5 and 4 h post carrageenan.

Carrageenan-induced mechanical hyperalgesia Nociceptive pressure thresholds were measured in the rat paw pressure test (Randall & Selitto, 1957) by an analgesymeter (Ugo Basile, Italy). Male Sprague Dawley rats (70–90 g) received six training sessions on the day before test. Pressure was gradually applied to the hind paw of each rat and nociceptive thresholds were determined as the pressure (g) required to elicit paw withdrawal. A cut off point of 250 g was used to prevent any tissue damage to the paw. On the test day 2–3 baseline measurements were taken before animals were administered carrageenan (100 μl of 20 mg ml^{-1}) by intraplantar injection into the right hind paw. Nociceptive thresholds were taken again 3 h after carrageenan to establish that animals were exhibiting hyperalgesia. This time point represented the start of peak hyperalgesia. Test compounds or saline was administered at 3.5 h after carrageenan and nociceptive thresholds were examined at 4, 4.5, and 5 h post carrageenan.

Effect of naloxone on the antihyperalgesic activity of gabapentin in the formalin test The effect of naloxone on the actions of morphine, enadoline and gabapentin was examined in male Sprague-Dawley rats (70–90 g) during the late phase of the formalin response. Naloxone was administered (s.c.) 5 min before morphine (4 mg kg^{-1}) or 10 min before enadoline (0.01 mg kg^{-1}) and, 25 min later animals received an intraplantar injection of 50 μl 5% formalin. Gabapentin (100 mg kg^{-1}, s.c.) was administered 35 min before naloxone (0.1–10 mg kg^{-1}) and 25 min later animals received a similar injection of formalin. The late phase licking response to formalin was measured as described above.

Tolerance studies The late phase of the formalin response was used to examine whether tolerance develops to the antihyperalgesic action of morphine and gabapentin as previously described (Singh *et al*., 1996b). Briefly, morphine was administered s.c. over 6 days beginning with 1 and culminating to 16 mg kg^{-1} on days 5 and 6 (1, 2, 4, 8, 16, 16 mg kg^{-1}). Similarly, gabapentin was administered s.c. over 6 days beginning with 100 and culminating to 400 mg kg^{-1} on days 5 and 6 (100, 100, 200, 200, 400, 400 mg kg^{-1}). Other groups of animals received chronic dosing of saline. On the 7th day, animals treated with chronic morphine received either morphine (4 mg kg^{-1}, s.c. 20 min before formalin) or gabapentin (100 mg kg^{-1}, s.c. 1 h before formalin). Chronic vehicle treated rats received either vehicle or a similar administration of morphine. In a second study chronically treated gabapentin animals received gabapentin (100 mg kg^{-1}, s.c. 1 h before formalin) on the 7th day, whilst two chronically dosed vehicle groups received either a similar administration of gabapentin or vehicle 1 h before formalin.

Gastrointestinal transit Male Sprague Dawley rats (180–250 g) were given a 1.0 ml suspension of charcoal meal (10% w/v charcoal in 5% w/v gum acacia) orally by gavage and were killed by cervical dislocation 15 min later. The abdomen was opened and the intestine was dissected out from the pyloric end up to the ileocaecal junction. Gastrointestinal transit was expressed as the distance travelled by the charcoal as a percentage of the total length of the small intestine. Morphine was administered s.c. 25 min before charcoal. Test compounds were administered s.c. 1 h before charcoal.

Drug discrimination Male Hooded Lister rats (200–250 g at start of training) were trained to discriminate between the s.c. administration of morphine (3.0 mg kg^{-1}) and saline, by

a two-lever, operant drug discrimination paradigm with a fixed ratio FR10 schedule of food reinforcement as previously described (Tricklebank *et al*., 1989). Training sessions were of 15 min duration and commenced 30 min after drug administration. Lever selection was considered correct if the animal made five or fewer responses on the inappropriate lever before completing 10 presses on the appropriate one. Generalization tests were carried out in the presence of reinforcement when the animals had achieved a correct choice in at least nine out of 10 consecutive training sessions. Test compounds were administered s.c. 1 h before a generalized test.

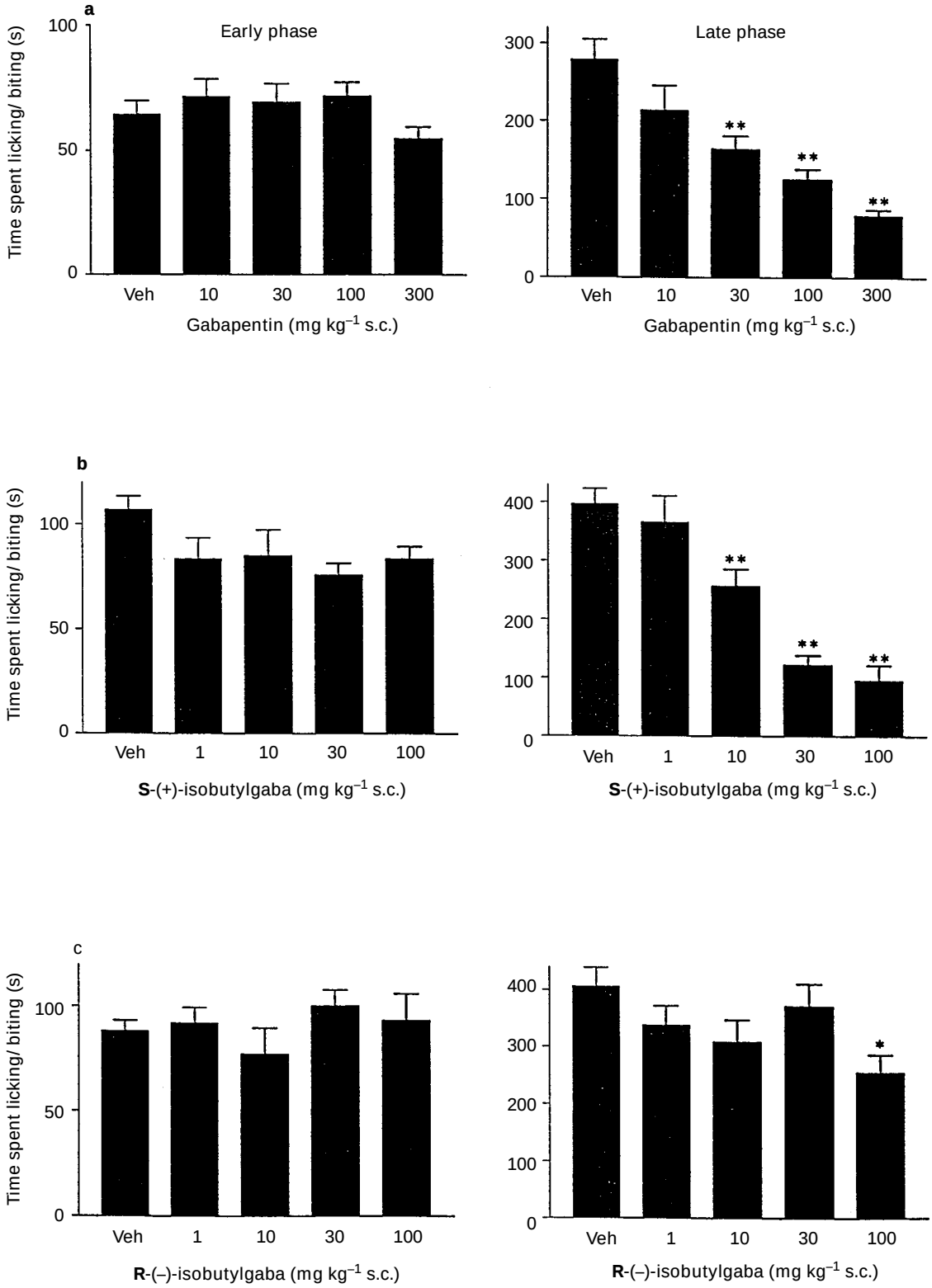


Figure 1 Effect of (a) gabapentin, (b) **S**-(+)-3-isobutylgaba and (c) **R**-(−)-3-isobutylgaba in the rat formalin test. Test compounds were administered s.c. 1 h before animals received 50 μl of 5% formalin into the left hind paw. Time spent licking/biting the injected paw was recorded for 45 min post formalin. Results are expressed as mean ($n=8-10$ animals per group) time spent licking and biting the injected paw during the early (0–10 min) and late (10–45 min) phases of the test (vertical lines represent s.e.mean). $^{*}P<0.05$, $^{**}P<0.01$, significantly different from vehicle (Veh)-treated controls (one-way ANOVA followed by Dunnett's t test).

Rat rota-rod Male Sprague Dawley rats (70–90 g) were trained to stay on an accelerating rota-rod (Ugo Basile, Italy) for 120 s. On the following day the animals were retested after s.c. administration of test compound 1 h before test.

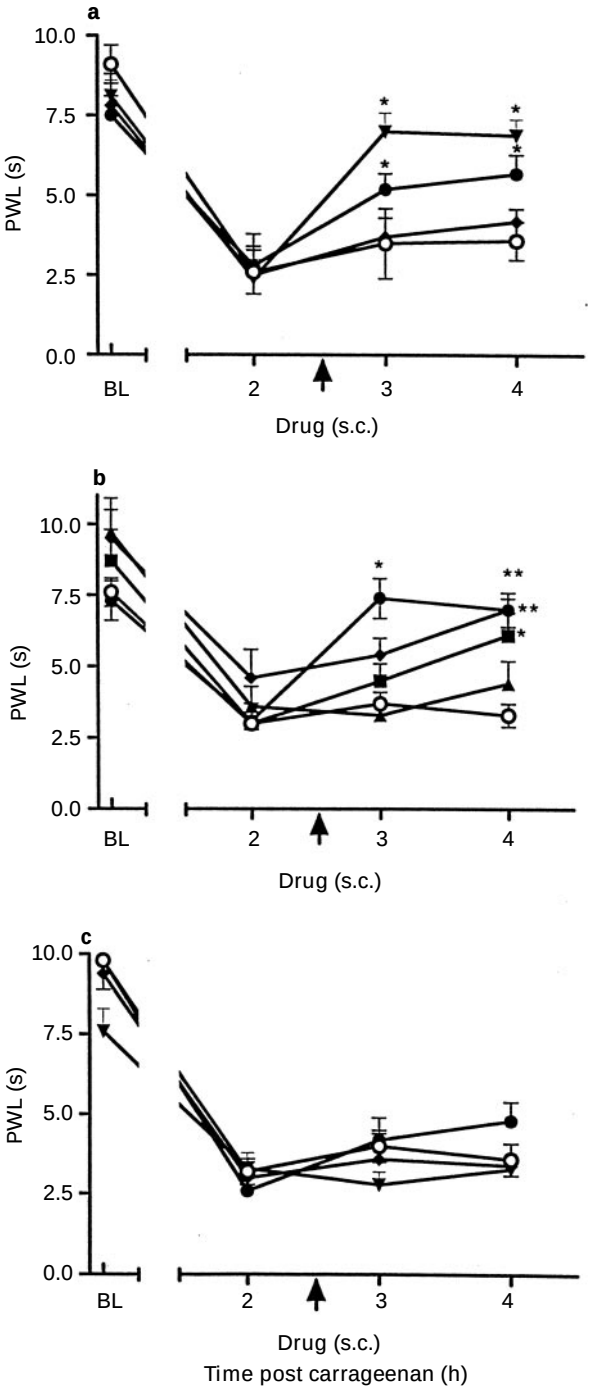


Figure 2 Effect of (a) gabapentin, (b) **S**-(+)-3-isobutylgaba and (c) **R**-(−)-3-isobutylgaba on carrageenan-induced thermal hyperalgesia. Paw withdrawal latencies (PWL) were determined by the rat plantar test. Baseline (BL) measurements were taken before animals received an intraplantar injection of carrageenan (100 μl of 20 mg ml^{-1}). PWLs were re-assessed at 2 h post carrageenan. Vehicle (open circles) or test compound: 1 mg kg^{-1} (solid triangles), 3 mg kg^{-1} (solid squares), 10 mg kg^{-1} (solid diamonds), 30 mg kg^{-1} (solid circles) or 100 mg kg^{-1} (solid inverted triangles), were administered s.c. 2.5 h after carrageenan. PWLs were assessed again at 3, 3.5 and 4 h post carrageenan. Results are expressed as PWL (s) of 8–10 animals per group (vertical lines represent s.e.mean). $*P<0.05$, $**P<0.01$, significantly different from vehicle (Veh)-treated controls (two-way ANOVA followed by Dunnett's t test).

Drugs and solutions

Gabapentin, **S**-(+)-3-isobutylgaba and **R**-(−)-3-isobutylgaba were synthesized at Parke-Davis Research Laboratories (Ann Arbor, U.S.A.). Morphine sulphate was obtained from Savory and Moore (Cambridge, U.K.). λ-Carrageenan, diazepam and naloxone were obtained from Sigma (Poole, U.K.). All compounds were dissolved in 0.9% w/v NaCl (isotonic saline) except diazepam which was suspended in 1% w/v carboxymethycellulose containing 0.1% Tween 80 with the aid of ultrasound sonification. Carrageenan was dissolved by use of a whirly mixer for 15 min.

Statistics

All data were analysed by either a one-way or two-way analysis of variance (ANOVA). A *post hoc* Dunnett's t test was carried out following a significant ANOVA. For data subjected to a two-way ANOVA, dose and time were factors with Dunnett's t test being carried out at each separate time point comparing the drug treatments to the vehicle group.

Results

*Effect of gabapentin, **S**-(+)-3-isobutylgaba and **R**-(−)-3-isobutylgaba in the rat formalin test*

The s.c. administration of gabapentin (10–300 mg kg^{-1}) and **S**-(+)-3-isobutylgaba (1–100 mg kg^{-1}) 1 h before formalin dose-dependently antagonized the late phase of the formalin response with respective minimum effective doses (MED) of 30 and 10 mg kg^{-1} (gabapentin: $F(4,39)=11.6$; **S**-(+)-3-isobutylgaba ($F(5,47)=13.8$) (Figure 1). However, similar administration of **R**-(−)-3-isobutylgaba (1–100 mg kg^{-1}) only produced a modest blockade of the second phase at the highest dose of 100 mg kg^{-1} ($F(4,39)=1.4$) (Figure 1). None of the compounds had any effect on the early phase of the formalin response (gabapentin: $F(4,39)=1.3$; **S**-(+)-3-isobutylgaba $F(5,47)=1.58$: **R**-(−)-3-isobutylgaba $F(4,39)=1$) (Figure 1). Data were analysed by a one-way ANOVA.

*Effect of gabapentin, **S**-(+)-3-isobutylgaba and **R**-(−)-3-isobutylgaba in the rat carrageenan-induced thermal hyperalgesia model*

Animals exhibited a baseline paw withdrawal latency (PWL) of approximately 10 s. Carrageenan induced a significant reduction of PWL in all animals at 2 h following injection, indicating the induction of thermal hyperalgesia (Figure 2). This hyperalgesia was maintained in vehicle-treated animals for at least 4 h post carrageenan. The s.c. administration of gabapentin (10–100 mg kg^{-1}) or **S**-(+)-3-isobutylgaba (1–30 mg kg^{-1}) at 2.5 h after carrageenan, dose-dependently antagonized the maintenance of the thermal hyperalgesia with respective MED of 30 and 3 mg kg^{-1} (gabapentin $F(9.72)=6.15$; **S**-(+)-3-isobutylgaba $F(12,96)=5.21$) (Figure 2). The highest dose of both gabapentin and **S**-(+)-3-isobutylgaba produced a complete antagonism of the inflammation-induced hyperalgesia. In contrast, similar administration of **R**-(−)-3-isobutylgaba (10–100 mg kg^{-1}) failed to demonstrate any antihyperalgesic action in this model, $F(9,87)=1.09$ (Figure 2). Data were analysed by two-way ANOVA.

*Effect of gabapentin, **S**-(+)-3-isobutylgaba and **R**-(−)-3-isobutylgaba in the rat carrageenan-induced mechanical hyperalgesia model*

On the test day animals exhibited baselines of approximately 150 g. Carrageenan reduced the nociceptive threshold of all animals 3 h following injection, indicating the induction of

mechanical hyperalgesia (Figure 3). This hyperalgesia was maintained in vehicle-treated animals for at least 5 h post carragenan. The s.c. administration of gabapentin (3–300 mg kg^{-1}) or **S**-(+)-3-isobutylgaba (1–100 mg kg^{-1}) at 3.5 h post carrageenan, dose-dependently antagonized the maintenance of mechanical hyperalgesia with MED of 10 and 3 mg kg^{-1}, respectively (gabapentin $F(24,224)=5.4$; **S**-(+)-3-isobutylgaba $F(24,264)=7.06$) (Figure 3). The highest dose of gabapentin and **S**-(+)-3-isobutylgaba produced complete antagonism of the inflammation-induced hyperalgesia similar to that seen with 3 mg kg^{-1} morphine (Figure 3). In contrast, **R**-(−)-3-isobutylgaba (10–100 mg kg^{-1}) failed to demonstrate any antihyperalgesic action in this model up to doses of 100 mg kg^{-1}, $F(12,128)=0.41$ (Figure 3). Data were analysed by two-way ANOVA.

Site of action of gabapentin

Gabapentin when administered intrathecally 3.5 h post carrageenan, dose-dependently (1–100 μg/animal) antagonized the mechanical hyperalgesia with a MED of 100 μg, $F(12,96)=2.94$ (Figure 4). However, it failed to demonstrate any antihyperalgesic action following intraplantar administration of similar doses into the inflamed paw $F(8,71)=0.31$ (Figure 4). Data were analysed by two-way ANOVA.

Effect of gabapentin in thermal and mechanical models of nociception in the absence of inflammation

For experiments in the absence of inflammation gabapentin was administered 1 h, and morphine 0.5 h before test. Data were analysed by one-way ANOVA.

Paw pressure test Vehicle-treated animals demonstrated a mean (±s.e.mean) nociceptive threshold of 110±7 g. The s.c. administration of gabapentin failed to increase nociceptive thresholds significantly, $F(3,39)=0.38$, with respective means for 30, 100 and 300 mg kg^{-1} of 122±23, 143±27 and 137±23 g. However, morphine (3 mg kg^{-1}, s.c.)-treated animals exhibited a significant antinociceptive effect, $F(4,49)=8$, with a nociceptive threshold of 273±19 g ($n=10$ for all groups).

Thermal plantar test Vehicle-treated animals demonstrated a mean (±s.e.mean) paw withdrawal latency (PWL) of 8.3±0.5 s. The s.c. administration of morphine (3 mg kg^{-1}) significantly increased paw withdrawal latency ($F(4,49)=32.9$) to 18.1±0.5 s. In contrast, similar administration of gabapentin failed to alter paw withdrawal latency, $F(3,39)=0.9$, with respective PWLs of 8±0.5, 7.2±0.5 and 7.7±0.5 s for 30, 100 and 300 mg kg^{-1} ($n=8$ for all groups).

Effect of naloxone on the antihyperalgesic action of gabapentin

The s.c. administration of morphine (4 mg kg^{-1}), enadoline (0.01 mg kg^{-1}) or gabapentin (100 mg kg^{-1}) antagonized the late phase of the formalin response (Figure 5). The s.c. administration of naloxone (0.1–1.0 mg kg^{-1}) dose-dependently antagonized the action of morphine, $F(5,60)=14.4$ (Figure 5). Similarly, s.c. administration of a higher dose of naloxone (10 mg kg^{-1}) completely antagonized the action of the κ-opioid receptor agonist enadoline, $F(2,36)=4$ (Figure 5). In contrast, similar administration of naloxone (0.1–10.0 mg kg^{-1}) failed to block the action of gabapentin, $F(4,38)=1.8$ (Figure 5). Data were analysed by a one-way ANOVA.

Tolerance studies

Morphine (4 mg kg^{-1}, s.c.) antagonized the late phase of the formalin response in chronic vehicle-treated animals. How-

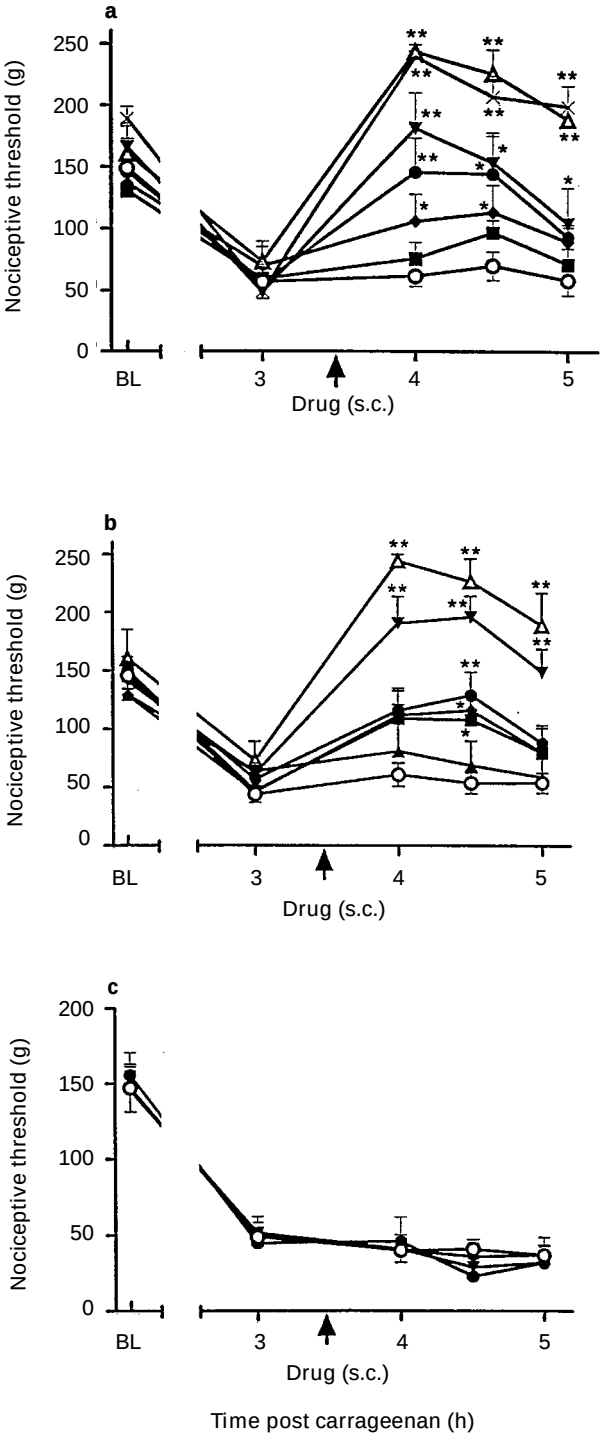


Figure 3 Effect of (a) gabapentin, (b) **S**-(+)-3-isobutylgaba and (c) **R**-(−)-3-isobutylgaba on carrageenan-induced mechanical hyperalgesia. Nociceptive thresholds were determined by the rat paw pressure test. Baseline (BL) measurements were taken before animals received an intraplantar injection of carrageenan (100 μl of 20 mg ml^{-1}). Nociceptive thresholds were re-assessed at 3 h post carrageenan. Vehicle (open circles) or test compounds: 1 mg kg^{-1} (solid triangles), 3 mg kg^{-1} (solid squares), 10 mg kg^{-1} (solid diamonds), 30 mg kg^{-1} (solid circles), 100 mg kg^{-1} (solid inverted triangles), 300 mg kg^{-1} (x) or 3 mg kg^{-1} morphine (open triangles), were administered s.c. 3.5 h post carrageenan. Nociceptive thresholds were assessed again at 4, 4.5 and 5 h following carrageenan. Results are expressed as nociceptive threshold (g) of 8–10 animals per group (vertical lines represent s.e.mean). *$P<0.05$, **$P<0.01$, significantly different from vehicle (Veh)-treated controls (two-way ANOVA followed by Dunnett's t test).

ever, the same dose of morphine failed to show such action in animals subjected to chronic morphine treatment, indicating development of tolerance (Figure 6). In contrast, gabapentin (100 mg kg^{-1}, s.c.) still demonstrated antihyperalgesic activity in chronic morphine treated animals, indicating no cross tolerance exists between morphine and gabapentin ($F(3,30) = 22.7$). Gabapentin also showed a comparable action in rats given chronic administration of either gabapentin or vehicle indicating a lack of development of tolerance, $F(3,28) = 16.1$ (Figure 6). Data were analysed by a one-way ANOVA.

*Effect of gabapentin, **S**-(+)-3-isobutylgaba and **R**-(−)-3-isobutylgaba on gastrointestinal transit*

The oral administration of 1 ml charcoal via gavage in control animals, travelled approximately (mean ± s.e.mean) 59 ± 1.7% of the length of the intestine during 15 min. This was significantly reduced to 32.6 ± 2.9% by the s.c. administration of morphine (5 mg kg^{-1}) 25 min before the charcoal meal, $F(5,30) = 7.82$. In contrast, the s.c. administration of gabapentin (10–300 mg kg^{-1}), **S**-(+)-3-isobutylgaba (3–100 mg kg^{-1}) or **R**-(−)-3-isobutylgaba (3–100 mg kg^{-1}) 1 h before the charcoal meal did not produce inhibition of gastrointestinal motility at any of the doses tested (gaba-

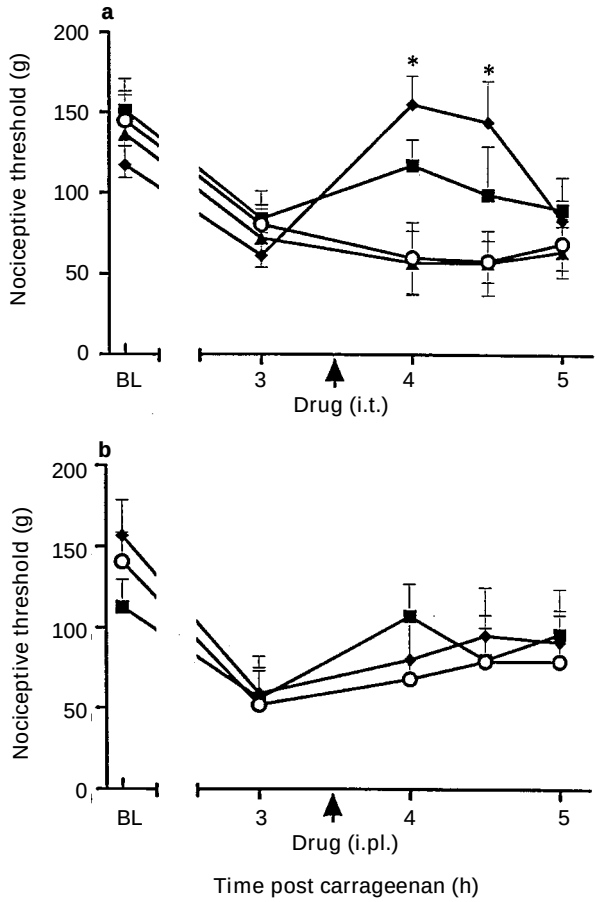
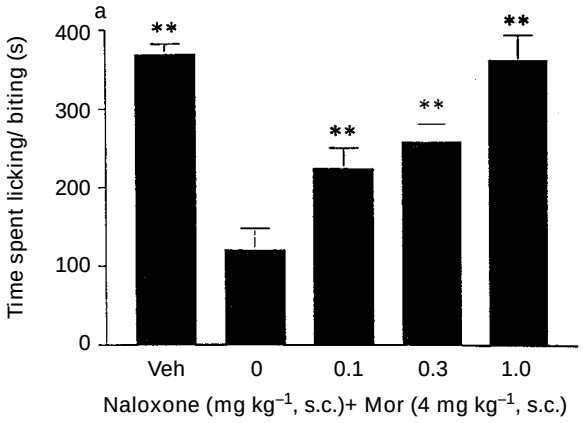
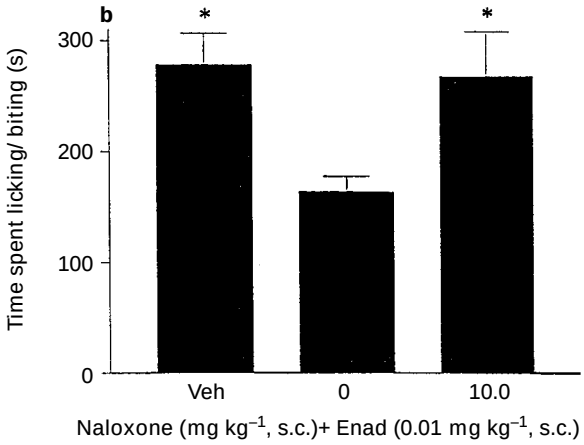


Figure 4 Effect of (a) intrathecal (i.t.) or (b) intraplantar (i.pl.) administration of gabapentin on carrageenan-induced mechanical hyperalgesia. Nociceptive thresholds were determined by the rat paw pressure test. Baseline (BL) measurements were taken before animals received an intraplantar injection of carrageenan (100 μl of 20 mg ml^{-1}). Nociceptive thresholds were re-assessed at 3 h post carrageenan. Vehicle (open circles) or gabapentin: 1 μg (solid triangles), 10 μg (solid squares), or 100 μg (solid diamonds), were administered either i.t. or i.pl. at 3.5 h post carrageenan. Nociceptive thresholds were assessed again at 4, 4.5 and 5 h after carrageenan. Results are expressed as nociceptive threshold (g) of 8–10 animals per group (vertical lines represent s.e.mean). $^*P<0.05$ significantly different from vehicle (Veh)-treated controls (two-way ANOVA followed by Dunnett's t test).





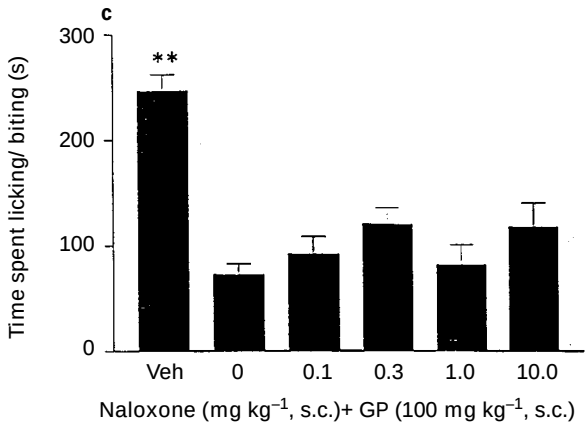


Figure 5 Lack of effect of naloxone on the antihyperalgesic action of gabapentin in the rat formalin test. Naloxone was administered 5 min before (a) morphine (Mor; 4 mg kg^{-1}) or 10 min before (b) enadoline (Enad; 0.01 mg kg^{-1}) and 25 min later animals were given formalin. (c) Gabapentin (GP) was administered 35 min before naloxone and 25 min later animals were given formalin. Time spent licking/biting the injected paw during the late phase was scored. Results are shown as the mean (vertical lines show s.e.mean) of 6–8 animals per group. $^*P<0.05$, $^{**}P<0.01$ significantly different from vehicle + drug group (one-way ANOVA followed by Dunnett's t test).

pentin $F(4,25)=0.25$; **S**-(+)-3-isobutylgaba $F(4,28)=2.29$; **R**-(−)-3-isobutylgaba $F(4,27)=0.74$), with transit values of 61.4 ± 6.2, 55 ± 5.2 and $59.5\pm2.7\%$ for 300 mg kg^{-1} gabapentin, 100 mg kg^{-1} **S**-(+)-3-isobutylgaba and 100 mg kg^{-1} **R**-(−)-3-isobutylgaba, respectively. Groups of 6–8 animals were used in these studies and data were analysed by a one-way ANOVA.

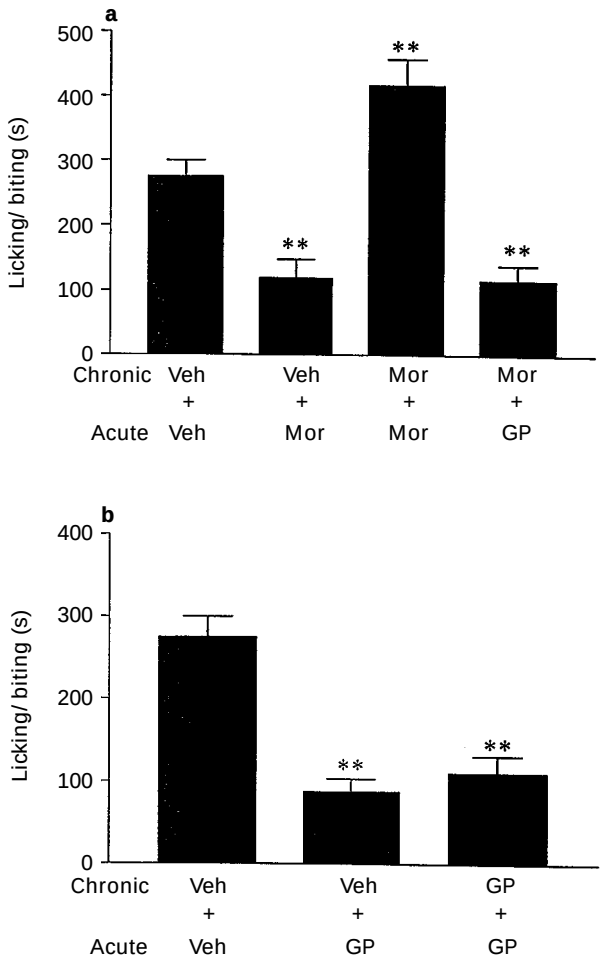


Figure 6 Lack of tolerance to the antihyperalgesic action of gabapentin in the rat formalin test. (a) Morphine (Mor) was administered over 6 days beginning with 1 and culminating to 16 mg kg^{-1} on days 5 and 6 (1, 2, 4, 8, 16 and 16 mg kg^{-1}, s.c.). (b) Gabapentin (GP) was also administered over 6 days beginning with 100 and culminating to 400 mg kg^{-1} on days 5 and 6 (100, 100, 200, 200, 400 and 400 mg kg^{-1}). The vehicle (Veh) groups received saline over the same time period. The day after the last administration animals received a single dose of either morphine (4 mg kg^{-1}, s.c. 20 min before formalin) or gabapentin (100 mg kg^{-1}, s.c. 1 h before formalin). The time spent licking/biting the injected paw during the late phase was scored. Results are expressed as the mean (vertical lines show s.e.mean) of 6–9 animals per group. $^*P<0.05$, $^{**}P<0.01$ significantly different from chronic vehicle + acute Veh group (one-way ANOVA followed by Dunnett's t test).

Morphine drug discrimination

Stable discrimination between s.c. injections of morphine (3 mg kg^{-1}) and saline was obtained after approximately 20 training sessions. Under the conditions of stimulus generalization, morphine dose-dependently (0.3–3.0 mg kg^{-1}, s.c.) induced drug lever responding, with the dose of 3.0 mg kg^{-1} morphine producing 100% responding on the drug appropriate lever. Gabapentin, **S**-(+)-3-isobutylgaba or **R**-(−)-3-isobutylgaba (1–100 mg kg^{-1}, s.c.) failed to induce responding on the drug lever, with 100% of animals responding on the saline appropriate lever at all doses examined, except the top dose of 100 mg kg^{-1} **R**-(−)-3-isobutylgaba at which 83% of animals responding on the saline appropriate lever. Groups of 6 animals were used in these studies.

Rota-rod

The mean (±s.e.mean) time spent on the accelerating rota-rod by vehicle-treated animals was 111.5 ± 6 s. Diazepam (10 mg kg^{-1}) administered 30 min before testing, significantly reduced time spent on the rota-rod to 67.5 ± 14.8 s, indicating the presence of sedation/ataxia, $F(4,33)=3.8$. Gabapentin and **S**-(+)-3-isobutylgaba administered s.c. 1 h before test, only decreased rota-rod performance at the highest doses examined with times of, 111.5 ± 6, 112.8 ± 4.9, 103.3 ± 8 and 69 ± 18 for 0, 30, 100 and 300 mg kg^{-1} gabapentin ($F(3,26)=3.7$), respectively, and 115.6 ± 3.7, 96 ± 9.9, 107.6 ± 6.6, 104.6 ± 6.5 and 78 ± 8.3 s for 0, 1, 10, 30 and 100 mg kg^{-1} **S**-(+)-3-isobutylgaba ($F(4,35)=3.9$), respectively. Following similar administration, **R**-(−)-3-isobutylgaba also failed to show a significant sedative/ataxic action, except at the top dose of 100 mg kg^{-1} with times of 116.4 ± 8.3, 104.9 ± 5.4, 103.4 ± 8.7 and 86.4 ± 8.9 s for 0, 10, 30 and 100 mg kg^{-1} ($F(3,28)=2.42$), respectively. Groups of 7–10 animals were used in these studies and data were analysed by one-way ANOVA.

Discussion

The results presented here show that gabapentin and the **S**-(+)-isomer of 3-isobutylgaba possess antihyperalgesic actions in inflammatory pain models. Thus, both compounds dose-dependently and selectively blocked the development of the second phase of the formalin response and the maintenance of carrageenan-induced mechanical and thermal hyperalgesia. The failure of gabapentin to reduce the effects of transient application of noxious thermal or mechanical stimuli suggests that these compounds are antihyperalgesic and not antinociceptive agents. The results further indicate that these antihyperalgesic actions occur in the absence of side effects associated with morphine.

Table 1 Comparison of antihyperalgesic actions of **S**-(+)-3-isobutylgaba, gabapentin and **R**-(−)-3-isobutylgaba with their side effect profiles

	Minimum effective dose (mg kg^{-1})					
Compound	*Formalin test*	*Carrageenan mechanical hyperalgesia*	*Carrageenan thermal hyperalgesia*	*Morphine drug discrimination*	*GI transit*	*Rota-rod*
S-(+)-3-isobutylgaba	10	3	3	>100	>100	100
Gabapentin	30	10	30	>100	>300	300
R-(−)-3-isobutylgaba	100	>100	>100	>100	>100	>100

It has previously been shown that gabapentin binds with high affinity to the $\alpha_2\delta$ subunit of a voltage-dependent calcium channel (Gee *et al*., 1996). These studies further revealed that 3-isobutylgaba binds to the $\alpha_2\delta$ protein in a stereoselective manner. (**S**)-(+)-3-isobutylgaba was found to show a similar affinity as gabapentin, but the (**R**)-(−)-isomer was 10 fold weaker. The present results show that the (**S**)-(+)-isomer of 3-isobutylgaba displays similar antihyperalgesic potency to gabapentin in both carrageenan and formalin-induced hyperalgesia models. In contrast, (**R**)-(−)-3-isobutylgaba was either inactive or only weakly active at 10 fold higher doses than the (**S**)-(+)-isomer. Previously, a similar rank order of potency was obtained for these three compounds in experimental models of epilespy (Taylor *et al*., 1993). It is interesting to note that in these models the potency of gabapentin and (**S**)-(+)-3-isobutylgaba was similar to that observed in the present study. These observations suggest that the $\alpha_2\delta$ subunit may play an important role in the mediation of the anticonvulsant and antihyperalgesic actions of gabapentin. Recent results suggest that the physiological role of the $\alpha_2\delta$ subunit is to increase the functional expression of calcium channel complexes (Williams *et al*., 1992; Brust *et al*., 1993; Isom *et al*., 1994; Gurnett *et al*., 1996). The $\alpha_2\delta$ subunit appears to be common to all voltage-dependent calcium channels studied to date (Isom *et al*., 1994; Hofmann *et al*., 1994). Therefore, it is conceivable that the antihyperalgesic actions of gabapentin and (**S**)-(+)-3-isobutylgaba involve more than one type of calcium channel.

The results presented here indicate that the antihyperalgesic actions of gabapentin and (**S**)-(+)-3-isobutylgaba are mediated at the level of spinal cord. This is consistent with previous studies which failed to show any evidence of a peripheral anti-inflammatory action of gabapentin (Singh *et al*., 1996a). Our recent autoradiographic studies with spinal cord tissue have shown that there is a high density of gabapentin binding ($\alpha_2\delta$) sites located particularly in the superficial laminae of the dorsal horn (R. Williams, personal communication). However, the exact location of these sites is not clear at present. It remains to be determined whether these sites are located presynaptically on primary afferent terminals or postsynaptically on dorsal horn neurones. It is thought that sensitization of dorsal horn neurones during prolonged pain is the underlying mechanism responsible for the development of hyperalgesia (see Woolf, 1994, for review). A recent study has shown that calcium channel antagonists of P- and particularly N-type can block the inflammation-induced sensitization of dorsal horn neurones (Diaz & Dickenson, 1997). Other studies have shown that calcium channel antagonists are also active in the formalin test (Malmberg & Yaksh, 1994). In particular, it has been shown that the P-type calcium channel blocker ω-agatoxin IVA, like gabapentin and (**S**)-(+)-3-isobutylgaba, selectively blocks the second phase of the formalin response (Malmberg & Yaksh, 1994). It remains to be established whether the interaction of gabapentin and (**S**)-(+)-3-isobutylgaba at the $\alpha_2\delta$ subunit can inhibit inflammation-induced wind-up of dorsal horn neurones. Such an effect would explain their anithyperalgesic actions.

Gabapentin and (**S**)-(+)-3-isobutylgaba antagonized the development as well as the maintenance of inflammatory hyperalgesia. This implies that as well as antagonizing established inflammatory hyperalgesia, these compounds may exhibit a pre-emptive antihyperalgesic action. However, further studies involving surgical pain models are required to substantiate such an action. It is interesting to note that gabapentin and (**S**)-(+)-3-isobutylgaba were equally effective at blocking inflammation-induced thermal and mechanical hyperalgesia. It is thought that thermal and mechanical hyperalgesia involve activation of different excitatory amino acid receptors and intracellular cascades in the spinal cord (see Meller & Gebhart, 1994, for review). Thus, it has been shown that the N-methyl-D-aspartate (NMDA) receptor plays an important role in the induction and maintenance of thermal hyperalgesia (Meller *et al*., 1992; 1994). In contrast, coactivation of α-amino-3-hydroxy-5-methyl-4-isoxazolapropionate (AMPA) and metabotropic receptors is necessary and sufficient for the development of mechanical hyperalgesia (Meller & Gebhart, 1994). It has been established that voltage-dependent calcium channels particularly of the N- and P-type (Turner *et al*., 1992; 1993; Uchitel *et al*., 1992; Gaur *et al*., 1994; Olivera *et al*., 1994) are located on nerve terminals and have been shown to modulate neurotransmitter release. It remains to be seen whether gabapentin and (**S**)-(+)-3-isobutylgaba by interacting at the $\alpha_2\delta$ subunit of these calcium channels can inhibit release of excitatory amino acids from primary afferents. Such an action would be expected to reduce the availability of glutamate at the NMDA, AMPA and metabotropic receptors and may explain the ability of gabapentin and (**S**)-(+)-3-isobutylgaba to block both the thermal and mechanical hyperalgesia observed in the present study.

The results of the present study suggest that the antihyperalgesic action of $\alpha_2\delta$ ligands does not involve opiate pathways. Furthermore, the antihyperalgesic actions of gabapentin appear not to be compromised by the serious side effects associated with opiate therapy (constipation, sedation, respiratory depression, tolerance and dependence). Thus, unlike morphine, gabapentin and (**S**)-(+)-isobutylgaba did not reduce gut motility. Sedation/ataxia was only observed at 10 times higher doses of each compound than those inducing antihyperalgesic actions. Moreover, repeated administration of gabapentin for 6 days did not lead to development of tolerance to its antihyperalgesic action. However, it remains to be seen whether administration for a longer period leads to tolerance. The present study further shows that morphine tolerance does not cross generalize to gabapentin. The failure of gabapentin and (**S**)-(+)-3-isobutylgaba to generalize to the morphine discriminative stimulus suggests that these compounds do not share the subjective properties of the μ-opioid receptor agonist. This suggests that gabapentin-like compounds are unlikely to be abused by patients dependent on morphine. Gabapentin has been in clinical use for several years now for the treatment of epilepsy and there has been no evidence indicating development of tolerance, dependence or respiratory depression. In fact gabapentin is well tolerated and, unlike morphine, any side effects tend to be mild to moderate in intensity and are transient, resolving with prolonged treatment (Ramsay, 1994; McLean, 1995). However, further studies are necessary to evaluate fully the side effect profile of gabapentin regarding treatment of pain.

Recently, it has been shown that gabapentin blocks hyperalgesia and allodynia induced by nerve injury in the rat (Xiao & Bennett, 1995). Preliminary clinical data suggest that gabapentin is effective in the treatment of neuropathic pain in patients suffering from reflex sympathetic dystrophy (Mellick *et al*., 1995). The present results indicate that gabapentin does not possess an antinociceptive effect in transient models of pain. The ability of gabapentin to block inflammatory- and neuropathy-induced hyperalgesia indicates that this class of compounds is effective only in sensitized pain models. Therefore, we suggest that gabapentin and (**S**)-(+)-3-isobutylgaba should be referred to as antihypersensitive agents rather than analgesics. As such they are not expected to abolish physiological pain but should reduce abnormal hypersensitivity induced by chronic pain. Taken together with their good side effect profiles, gabapentin and (**S**)-(+)-3-isobutylgaba offer a possibility of improved treatment for chronic pain. The results of the present study are consistent with but do not prove beyond doubt that the $\alpha_2\delta$ subunit of voltage-dependent calcium channels mediate the antihyperalgesic actions of gabapentin and (**S**)-(+)-3-isobutylgaba. Our previous studies suggest a possible indirect involvement of the NMDA receptor in mediating the actions of gabapentin. The elucidation of the exact mechanism mediating the antihyperalgesic action of

gabapentin should lead to the discovery and development of more potent and effective drugs for the treatment of chronic pain.

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