Evaluation of Gabapentin and S-\((+)-3\)-Isobutylgaba in a Rat Model of Postoperative Pain

MARK J. FIELD, ELIZABETH F. HOLLOMAN, SCOTT McCLEARY, JOHN HUGHES and LAKHBIR SINGH

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

Accepted for publication May 8, 1997

ABSTRACT

Gabapentin and S-\((+)-3\)-isobutylgaba are anticonvulsant agents that selectively interact with the \(\alpha_2\delta\) subunit of voltage-dependent calcium channels. This report describes the activities of these two compounds in a rat model of postoperative pain. An incision of the plantaris muscle of a hind paw induced thermal hyperalgesia and tactile allodynia lasting at least 3 days. Postoperative testing was carried out using the plantar test for thermal hyperalgesia and von Frey hairs for tactile allodynia. A single s.c. dose of gabapentin, 1 h before surgery, dose-dependently (3–30 mg/kg) blocked the development of allodynia and hyperalgesia with a minimum effective dose (MED) of 10 and 30 mg/kg, respectively. The highest dose of gabapentin prevented development of hyperalgesia and allodynia for 24 and 49 h, respectively. Similar administration of S-\((+)-3\)-isobutylgaba also dose-dependently (3–30 mg/kg, s.c.) prevented development of hyperalgesia and allodynia with MED of 3 and 10 mg/kg, respectively. The highest dose of S-\((+)-3\)-isobutylgaba completely blocked development of both nociceptive responses for 3 days. The administration of S-\((+)-3\)-isobutylgaba (30 mg/kg s.c.) 1 h after surgery also completely blocked the development of hyperalgesia and allodynia, but its duration of action was much shorter (3 h). The administration of morphine (1–6 mg/kg s.c.) 0.5 h before surgery prevented the development of thermal hyperalgesia with a MED of 1 mg/kg. However, unlike gabapentin and S-\((+)-3\)-isobutylgaba, it had little effect on the development of tactile allodynia. It is suggested that gabapentin and S-\((+)-3\)-isobutylgaba may be effective in the treatment of postoperative pain.

Gabapentin (NEURONTIN®) is an antiepileptic agent currently in clinical use as an add-on therapy in patients with partial seizures resistant to conventional therapies (see Goa and Sorkin, 1993, for review). Although gabapentin was originally designed as a GABA analog which would penetrate into the central nervous system, it does not interact with GABA receptors (Bartoszyk and Reimann, 1985). A single highly specific \(^{3}H\)gabapentin binding site \((K_D = 38 \pm 2.8 \text{ nM})\) in the brain has been described (Suman-Chauhan et al., 1993). More recently this recognition site was identified as the \(\alpha_2\delta\) subunit of voltage-dependent calcium channels (Gee et al., 1996). In binding studies, gabapentin \((IC_{50} = 80 \text{ nM})\) and \((RS)-3\)-isobutylgaba were the most active compounds identified for this site. \((RS)-3\)-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. It remains to be seen whether this site is involved in the mediation of the anticonvulsant action of gabapentin.

Recent studies have shown that gabapentin possesses antihyperalgesic actions in animal models of inflammatory and neuropathic pains. Thus, it has been reported that gabapentin selectively blocks the second phase of the formalin response and carrageenan-induced thermal and mechanical hyperalgesia (Singh et al., 1996; Field et al., in press, 1997). Other studies have shown that it can also block hyperalgesia and allodynia in rat models of neuropathic pain (Hwang and Yaksh, in press, 1997; Xiao and Bennett, 1995). The antihyperalgesic actions of gabapentin are centrally mediated and do not involve an opiate mechanism. Further work has shown that tolerance does not develop to this effect of gabapentin and that morphine tolerance does not cross-generalize to gabapentin (Field et al., in press, 1997). Preliminary clinical data suggest that gabapentin is effective in the treatment of various forms of neuropathic pain (Mellick et al., 1995; Rose, 1996).

Recently, a rat model of postoperative pain was described (Brennan et al., 1996). It involves an incision of the skin, fascia, and muscle of the plantar aspect of the hind paw. This leads to an induction of reproducible and quantifiable mechanical hyperalgesia lasting several days. It has been suggested that this model displays some similarities to the human postoperative pain state. In the present study we have examined and compared the activities of gabapentin and...
(S)-(+)-3-isobutylgaba with morphine in this model of postoperative pain.

**Methods**

Male Sprague Dawley rats (250—300 g), obtained from Bantin and Kingman, (Hull, U.K.) were used in all experiments. Before surgery animals were housed in groups of 6 under a 12-h light/dark cycle (lights on at 7:00 A.M.) with food and water ad libitum. Postoperatively animals were housed in pairs on “Aqua-sorb” bedding consisting of air laid cellulose (Beta Medical and Scientific, Sale, U.K.) under the same conditions. All experiments were carried out by an observer blind to drug treatments.

**Surgery.** The surgery was based on the procedure recently described by Brennan et al. (1996). Animals were anesthetized with 2% isoflurane and 1:4 O2/N2O mixture which was maintained during surgery via a nose cone. The plantar surface of the right hind paw was prepared with 50% ethanol, and a 1-cm longitudinal incision was made through skin and fascia, starting 0.5 cm from the edge of the heel and extending toward the toes. The plantaris muscle was elevated by use of forceps and incised longitudinally. The wound was closed with two simple sutures of braided silk with a FST-02 needle. The wound site was covered with Terramycin spray and Aureomycin powder. Postoperatively, none of the animals displayed any signs of infection with the wounds healing well after 24 h. The sutures were removed after 48 h.

**Evaluation of thermal hyperalgesia.** Thermal hyperalgesia was assessed by the rat plantar test (Ugo Basile, Italy) following a modified method of Hargreaves et al. (1988). Rats were habituated to the apparatus which consisted of three individual Perspex boxes on an elevated glass table. A mobile radiant heat source was located under the table and focused onto the hind paw and PWL were recorded. There was an automatic cut-off point of 22.5 s to prevent tissue damage. PWL were taken two to three times for both hind paws of each animal, the mean of which represented base lines for right and left hind paws. The apparatus was calibrated to give a PWL of approximately 10 s. PWL were reassessed with the same protocol as above 2, 24, 48 and 72 h postoperatively.

**Evaluation of tactile allodynia.** Tactile allodynia was measured with Semmes-Weinstein von Frey hairs (Stoelting, Wood Dale, IL). Animals were placed into wire mesh bottom cages allowing access to the underside of their paws. The animals were habituated to this environment before the start of the experiment. Tactile allodynia was tested by touching the plantar surface of the animal’s hind paw with von Frey hairs in ascending order of force (0.7, 1.2, 1.5, 2, 3.6, 5.5, 8.5, 11.8, 15.1 and 29 g) until a paw-withdrawal response was elicited. Each von Frey hair was applied to the paw for 6 s, or until a response occurred. Once a withdrawal response was established, the paw was retested, starting with the next descending von Frey hair until no response occurred. The highest force of 29 g lifted the paw as well as eliciting a response, thus representing the cut-off point. Each animal had both hind paws tested in this manner. The lowest amount of force required to elicit a response was recorded as withdrawal threshold in grams. When compounds were administered before surgery, the same animals were used to study drug effects on tactile allodynia and thermal hyperalgesia, with each animal being tested for tactile allodynia 1 h after thermal hyperalgesia.

**Separate groups of animals were used for examination of tactile allodynia and thermal hyperalgesia when S-(+)-3-isobutyrgaba was administered after surgery.**

**Drugs.** Gabapentin and S-(+)-3-isobutylgaba were synthesized at Parke-Davis Research Laboratories (Ann Arbor, MI). Morphine sulfate was obtained from Savory and Moore (Cambridge, U.K.). All compounds were dissolved in 0.9% w/v NaCl (isotonic saline) and administered in a dosing volume of 1 ml/kg s.c. Gabapentin and S-(+)-3-isobutylgaba were dosed 1 h before surgery. S-(+)-3-isobutylgaba was also examined in a separate experiment administered 1 h after surgery.

**Statistics.** Data obtained for thermal hyperalgesia was subjected to a one-way ANOVA followed by a Dunnett’s t test. Tactile allodynia results obtained with the von Frey hairs were subjected to an individual Mann-Whitney U test.

**Results**

An incision of the rat plantaris muscle led to an induction of thermal hyperalgesia and tactile allodynia. Both nociceptive responses peaked within 1 h after surgery and were maintained for 3 days. During the experimental period all animals remained in good health.

**Effect of gabapentin, S-(+)-3-isobutylgaba and morphine administered before surgery on thermal hyperalgesia.** The single-dose administration of gabapentin 1 h before surgery dose-dependently (3—30 mg/kg s.c.) blocked development of thermal hyperalgesia with a MED of 30 mg/kg (fig. 1b). The highest dose of 30 mg/kg gabapentin prevented the hyperalgesic response for 24 h (fig. 1b). Similar administration of S-(+)-3-isobutylgaba also dose-dependently (3—30 mg/kg s.c.) prevented development of thermal hyperalgesia with a MED of 3 mg/kg (fig. 1c). The 30 mg/kg dose of S-(+)-3-isobutylgaba was effective up to 3 days (fig. 1c). The administration of morphine 0.5 h before surgery dose-dependently (1—6 mg/kg s.c.) antagonized the development of thermal hyperalgesia with a MED of 1 mg/kg (fig. 1a). This effect was maintained for 24 h (fig. 1a).

**Effects of gabapentin, S-(+)-3-isobutylgaba and morphine administered before surgery on tactile allodynia.** The effect of drugs on development of tactile allodynia was determined in the same animals used for thermal hyperalgesia above. One hour was allowed between thermal hyperalgesia and tactile allodynia tests. Gabapentin dose-dependently prevented development of tactile allodynia with a MED of 10 mg/kg. The 10 and 30 mg/kg doses of gabapentin were effective for 25 and 49 h, respectively (fig. 2b). S-(+)-3-Isobutylgaba also dose-dependently (3—30 mg/kg) blocked development of the allodynic response with a MED of 10 mg/kg (fig. 2c). This blockade of the nociceptive response was maintained for 3 days by the 30 mg/kg dose of S-(+)-3-isobutylgaba (fig. 2c). In contrast, morphine (1—6 mg/kg) only prevented the development of tactile allodynia for 3 h post-surgery at the highest dose of 6 mg/kg (fig. 2a).

**Effect of S-(+)-3-isobutylgaba administered 1 h after surgery on tactile allodynia and thermal hyperalgesia.** The allodynia and hyperalgesia peaked within 1 h in all animals and was maintained for the ensuing 5 to 6 h. The s.c. administration of 30 mg/kg S-(+)-3-isobutylgaba 1 h after surgery blocked the maintenance of tactile allodynia and thermal hyperalgesia for 3 to 4 h. After this time both nociceptive responses returned to control levels, which indicated disappearance of antihyperalgesic and antiallodynic actions (fig. 3).

Gabapentin and S-(+)-3-isobutylgaba did not affect PWL in the thermal hyperalgesia test or tactile allodynia scores in the contralateral paw up to the highest dose tested in any of the experiments. In contrast, morphine (6 mg/kg s.c.) increased PWL of the contralateral paw in the thermal hyperalgesia test (data not shown).
Discussion

The results presented here show that incision of the rat plantaris muscle induces thermal hyperalgesia and tactile allodynia lasting at least 3 days. The major findings of this study are that gabapentin and S-(+)-3-isobutyrlgaba are equally effective at blocking both nociceptive responses. In contrast, morphine is more effective against thermal hyperalgesia than tactile allodynia. Furthermore, S-(+)-3-isobutyrlgaba completely blocks induction and maintenance of allodynia and hyperalgesia.

The duration of action of the antihyperalgesic and antiallodynic actions appear to depend on the time of administration of the compound. It is surprising that single doses of
Gabapentin and Postoperative Pain

1245

study indicate that prevention of the induction phase can produce long-lasting antihyperalgesic and antiallodynic actions.

It is known that chronic pain induced by either tissue damage or neuropathy can lead to an increased state of excitability in the spinal cord (Coderre et al., 1993). This sensitization of dorsal horn neurons is widely thought to contribute to abnormal pain sensitivity. Recently, it has been shown that the antihyperalgesic action of gabapentin is centrally mediated (Field et al., in press, 1997) and that it can block the maintenance of carrageenan-induced sensitization of dorsal horn neurons (Stanfa et al., 1997). It has been suggested that the induction and maintenance of sensitization of dorsal horn involves different mechanisms (Woolf, 1994). This study shows that there was no difference in the magnitude of the block of hyperalgesia and allodynia by S(-)-3-isobutylgaba when administered either before or after surgery. This indicates that this chemical class of compounds are probably capable of blocking induction and maintenance of sensitization of dorsal horn neurons. The only other class of compounds currently known to block both phases of central sensitization are N-methyl-D-aspartate receptor antagonists (Woolf and Thompson, 1991). This action involves antagonism of calcium influx through the N-methyl-D-aspartate receptor-ion channel complex. It remains to be seen whether the interaction of gabapentin with the alpha_3 subunit of voltage-dependent calcium channels mediates its antihyperalgesic and antiallodynic actions. Such an interaction with channels located on primary afferents will inhibit neurotransmitter release from primary afferents. Similar interaction at postsynaptic dorsal horn neurons will decrease the secondary messenger action of calcium by reducing the activation of protein kinases. Both of these effects will inhibit the hypersensitivity of dorsal horn neurons and will lead to the elimination of hyperalgesia and allodynia.

The present data show that morphine possesses a limited antiallodynic action, being more effective at blocking hyperalgesia than allodynia. A similar profile of morphine has previously been documented in animal models of neuropathic pain (Lee et al., 1994; Yaksh, 1989). In contrast, gabapentin and S(-)-3-isobutylgaba were equally effective at blocking the hypersensitivity of dorsal horn neurons and will lead to the elimination of hyperalgesia and allodynia.

Previous studies have shown that gabapentin is inactive in transient models of pain (Field et al., in press, 1997). Taken together with the failure of gabapentin and S(-)-3-isobutylgaba to affect the contralateral paw in the present study, these data suggest that these compounds do not block physiological pain. They only appear to be effective against hypersensitivity induced by tissue damage or neuropathy, and as such, should be referred to as antihypersensitive agents.

Fig. 3. Effect of S(-)-3-isobutylgaba on the maintenance of (a) thermal hyperalgesia and (b) tactile allodynia in the rat postoperative pain model. S(-)-3-isobutylgaba (S(-)-IBG) was administered 1 h after surgery. Thermal PWL, determined by the rat plantar test, and paw-withdrawal thresholds to von Frey hair filaments, were determined in separate groups of animals for both ipsilateral and contralateral paws. For clarity only the ipsilateral paw data are shown. Base-line (BL) measurements were taken before surgery and withdrawal thresholds were reassessed up to 6 h postsurgery. For thermal hyperalgesia the results are expressed as the mean PWL(s) of six animals per group (vertical bars represent +-S.E.M.), *P < .05; **P < .01 significantly different (unpaired t test), comparing ipsilateral paw of drug-treated group to ipsilateral paw of vehicle (Veh)-treated group at each time point. For tactile allodynia the results are expressed as median force (g) required to induce a withdrawal of six animals per group (vertical bars represent first and third quartiles). ***P < .05 significantly different (Mann-Whitney U test), comparing ipsilateral paw of drug-treated group to ipsilateral paw of vehicle-treated group at each time point.

gabapentin and S(-)-3-isobutylgaba administered before surgery blocked the development of hyperalgesia and allodynia for up to 3 days. In contrast, S(-)-3-isobutylgaba showed much shorter duration of action when it was administered after induction of allodynia and hyperalgesia. The long duration of action of gabapentin and S(-)-3-isobutylgaba observed after pretreatment is inconsistent with other studies. Thus, it has been shown that in rat anticonvulsant models the duration of action of a 30 mg/kg dose of each compound is about 3 h, and moreover their half-lives are between 4 and 5 h (Taylor et al., 1993). These observations suggest that gabapentin and S(-)-3-isobutylgaba can not be present in the animal for 3 days. The input from primary afferent fibers during and up to 1 h postsurgery appears to be the major stimulus for the induction of allodynia and hyperalgesia. The input from primary afferents after this time appears to be insufficient for the induction but adequate to maintain these nociceptive responses. The results of this

1997 Gabapentin and Postoperative Pain 1245

Downloaded from jpet.aspetjournals.org at ASPET Journals on November 7, 2016
This profile of action is very different from morphine, which is analgesic and blocks both physiological and clinical pain. It will be interesting to see whether this selective antihyperalgesic profile of gabapentin and S-(+)-3-isobutylgaba will allow the detection of postoperative complications which sometimes remain undetected with morphine because of its powerful analgesic action. The present results indicate that prevention of the induction of hyperalgesia and allodynia is of paramount importance for the effective treatment of postoperative pain. However, S-(+)-3-isobutylgaba was also effective at blocking maintenance of hyperalgesia and allodynia. It may be optimal to administer a compound such as S-(+)-3-isobutylgaba before, during and after surgery to provide maximal relief from postoperative pain.

**References**


Send reprint requests to: Dr L. Singh, Department of Biology, Parke-Davis Neuroscience Research Centre, Cambridge University Forvie Site, Robinson Way, Cambridge, CB2 2QB, United Kingdom.