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Effect of K^+ channel modulators on the antiallodynic effect of gabapentin

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Abstract

The effect of K^+ channel inhibitors on the antiallodynic activity induced by spinal gabapentin was assessed in rats. Ligation of L5 and L6 spinal nerves made the rats allodynic, whereas that intrathecal administration of gabapentin (25–200 μg) reduced tactile allodynia in a dose-dependent manner. Spinal pretreatment with glibenclamide (12.5–50 μg, ATP-sensitive K^+ channel inhibitor), charybdotoxin (0.01–1 ng) or apamin (0.1–3 ng, large-and small-conductance Ca^{2+}-activated K^+ channel blockers, respectively), but not margatoxin (0.01–10 ng, voltage-dependent K^+ channel inhibitor), significantly prevented gabapentin-induced antiallodynia. Pinacidil (1–30 μg, K^+ channel opener) significantly reduced nerve ligation-induced allodynia. Intrathecal glibenclamide (50 μg), charybdotoxin (1 ng) and apamin (3 ng), but not margatoxin (10 ng), significantly reduced pinacidil-induced antiallodynia. K^+ channel inhibitors alone did not modify allodynia produced by spinal nerve ligation. Results suggest that gabapentin and pinacidil may activate Ca^{2+}-activated and ATP-sensitive K^+ channels in order to produce part of its spinal antiallodynic effect in the Chung model.

Keywords: Gabapentin; K^+ channel; Pinacidil; Apamin; Charybdotoxin; Glibenclamide

1. Introduction

Peripheral nerve injury is associated with spontaneous pain, allodynia and hyperalgesia. These neuropathic pain symptoms are often poorly relieved by conventional analgesics, such as opioids and non-steroidal anti-inflammatory drugs (NSAIDs) (MacFarlane et al., 1997). In the search for alternatives, anticonvulsants have been found to be a valuable pharmacological tool for patients with neuropathic pain. Gabapentin, a structural analog of γ-aminobutyric acid (GABA), is a novel anticonvulsant used effectively for the treatment of epilepsy and neuropathic pain in humans (Mellick et al., 1995; Rice and Maton, 2001). Although the clinical efficacy of gabapentin is well established, the sites and mechanisms responsible for its antiallodynic effect remain unclear (Taylor et al., 1998). Studies in rats have shown that systemic or intrathecal administration of gabapentin attenuates nociceptive behaviors that arise following nerve (Gillin and Sorkin, 1998; Hwang and Yaksh, 1997; Chapman et al., 1998; Field et al., 1999) or tissue injury (Singh et al., 1996; Field et al., 1997; Jun and Yaksh, 1998; Partridge et al., 1998; Chizh et al., 2000). However, gabapentin is ineffective in models of acute pain (Hunter et al., 1997).

The reduction of excitatory neurotransmission produced by gabapentin (Shimoyama et al., 2000; Chizh et al., 2000) can be reached, among others, through the blockade of Ca^{2+} and Na^+ channels or activation of K^+ channels. There is evidence to support that blockade of voltage-dependent Ca^{2+} channels (Gee et al., 1996; Taylor et al., 1998; Stefani et al., 2001) could be involved in the antinociceptive effect of gabapentin. In addition, some data indicate that gabapentin does not affect Na^+ channels (Taylor et al., 1998; Stefani et al., 2001; Freiman et al., 2001). On the other hand, there is evidence about the possible participation of K^+ channels in the pharmacological activity of

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gabapentin. A recent report suggests the involvement of K^+ channels in the modulation of K^+-evoked [^{3}H]-noradrenaline release (Freiman et al., 2001) from rat and human brain slices by gabapentin as this effect was antagonized by glibenclamide, a ATP-sensitive K^+ channel inhibitor. Therefore, this work was undertaken to determine the effect of several K^+ channel inhibitors on the antiallodynic activity of spinal gabapentin. We tested the actions of glibenclamide (an ATP-sensitive K^+ channel blocker; Edwards and Weston, 1993), charybdotoxin (an inhibitor of large-and intermediate-conductance Ca^{2+}-activated K^+ channels; Stretton et al., 1992), apamin (an inhibitor of small-conductance Ca^{2+}-activated K^+ channels; Romey et al., 1984) and margatoxin (a voltage-gated K^+ channel inhibitor; Garcia-Calvo et al., 1993) on the antiallodynic activity of spinal gabapentin in the Chung model of neuropathy (Kim and Chung, 1992).

2. Material and methods

2.1. Animals

Female Wistar rats aged 6–7 weeks (weight range, 120–140 g) from our own breeding facilities were used in this study. Animals had free access to food and drinking water before experiments. Efforts were made to minimize animal suffering and to reduce the number of animals used. Rats were used once only. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). Additionally, the study was approved by the Institutional Animal Care and Use Committee (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México, D.F., Mexico).

2.2. Measurement of antiallodynic activity

Rats were prepared according to the method of Kim and Chung (1992). Animals were anesthetized with a mixture of ketamine/xylazine (45/12 mg/kg, i.p.). After surgical preparation and exposure of the dorsal vertebral column, the left L5 and L6 spinal nerves were exposed and tightly ligated with 6–0 silk suture distal to the dorsal root ganglion. For sham operated rats, the nerves were exposed but not ligated. The incisions were closed, and the animals were allowed to recover for 15 days. Rats exhibiting motor deficiency (such as paw-dragging) were discarded from testing.

Tactile allodynia was determined by measuring paw withdrawal in response to probing with a series of calibrated fine filaments (von Frey filaments). The strength of the von Frey stimuli ranged from 0.4 to 15 g. Withdrawal threshold was determined by increasing and decreasing stimulus strength eliciting paw withdrawal (Chaplan et al., 1994). The stimulus intensity required to produce a response in 50% of the applications for each animal was defined as "50% withdrawal threshold". All nerve-ligated rats were verified to be allodynic (responding to a stimulus of less than 4 g). Rats not demonstrating allodynia were not further studied (less than 5%).

2.3. Spinal surgery

Twelve days after surgery rats were submitted to a second surgery for insertion of a spinal catheter. Rats were anesthetized with a ketamine/xylazine mixture (45/12 mg/kg, i.p.), placed in a stereotaxic head holder, and the atlantooccipital membrane exposed (Yaksh and Rudy, 1976). The membrane was pierced, and a PE-10 catheter (7.5 cm) was passed intrathecally to the level of the thoracolumbar junction and the wound was sutured. Rats

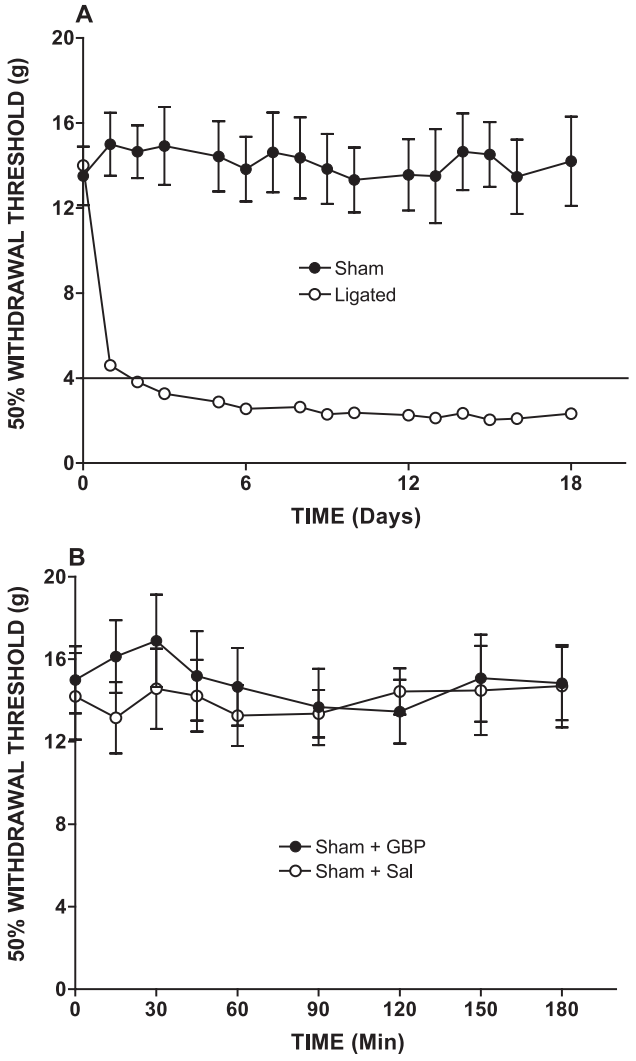

Fig. 1. (A) Time course of paw withdrawal threshold in rats submitted to the ligation of L5 and L6 spinal nerves tactile compared to sham-operated rats. (B) Lack of effect of gabapentin (GBP, 100 μg, i.t.) on sham-operated rats. Data are presented as mean ($n = 6$) ± S.E.M.

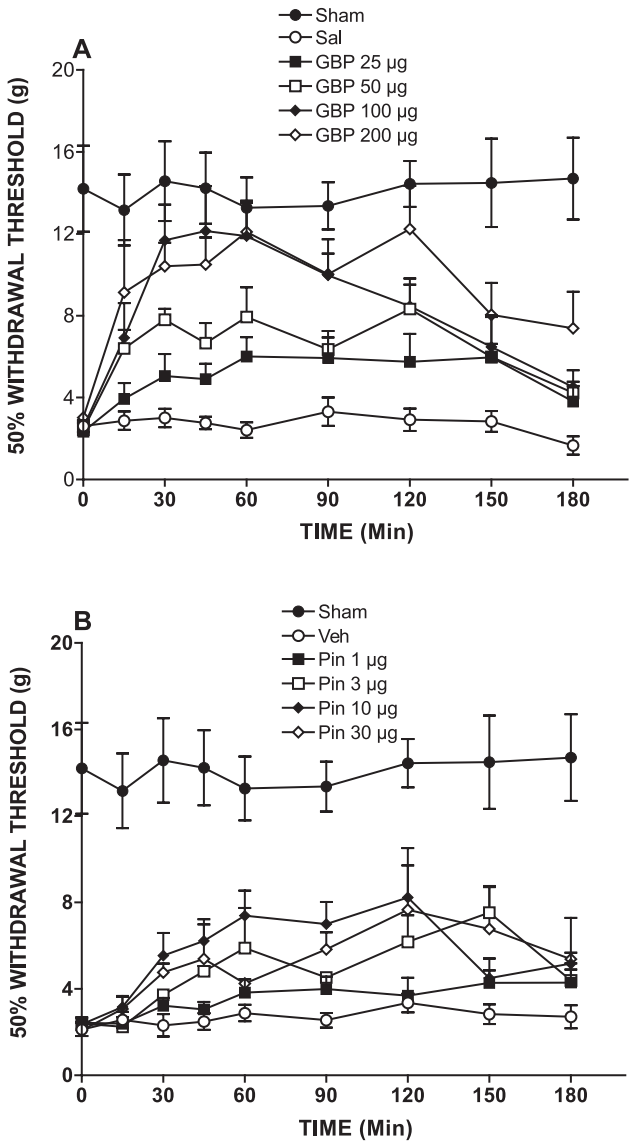

Fig. 2. Antiallodynic effect of spinal gabapentin (GBP, panel A) and pinacidil (Pin, panel B) in rats submitted to ligation of L5 and L6 spinal nerves. Rats were treated with intrathecal saline or increasing doses of gabapentin or pinacidil 15 min before starting thresholds evaluations. Data are the mean ± S.E.M. for six to seven animals. A significant difference ($P<0.05$, by ANOVA, followed by the Tukey's test) was observed between saline (control) and either 25, 50, 100 and 200 µg gabapentin groups (panel A) or 3, 10 and 30 µg pinacidil groups (panel B) 30 min after administration. Asterisks indicating significant difference were omitted for the sake of clarity.

were allowed to recover from surgery for at least 5 days before use. Animals showing any signs of motor impairment were euthanized in a CO_2 chamber.

2.4. Drugs

Gabapentin was purchased from Research Biochemicals International (Natick, MA, USA). Glibenclamide (glyburide), pinacidil, charybdotoxin, apamin and margatoxin were purchased from Sigma (St. Louis, MO, USA). Gabapentin, margatoxin, charybdotoxin and apamin were dissolved in saline. Glibenclamide was dissolved in dimethylsulfoxide (DMSO) 50%. Pinacidil was dissolved in DMSO 25%.

2.5. Study design

Rats received an intrathecal injection of vehicle (saline for gabapentin or DMSO 25% for pinacidil, 10 µl) or increasing doses of gabapentin (25–200 µg in 10 µl) or pinacidil (1–30 µg in 10 µl) 15 min before evaluation of withdrawal threshold in nerve injured rats. To determine whether K^+ channel blockers affect gabapentin-induced antinociception, effect of pretreatment (−5 min) with the appropriate vehicle (DMSO 50% for glibenclamide or saline for charybdotoxin, apamin, and margatoxin) or glibenclamide (12.5–50 µg), charybdotoxin (0.01–1 ng), apamin (0.1–3 ng) or margatoxin (0.01–10 ng) on the antiallodynic effect induced by gabapentin (100 µg, i.t.) or pinacidil (10 µg, i.t.) was assessed. Initially greater doses of K^+ channel blockers were tried (for example: 75–100 µg of glibenclamide), however, since side effects were presented, these doses were reduced until no side effects were observed. Drugs were injected in a volume of 10 µl. Each rat received two intrathecal injections and appropriate controls for the injections and vehicles were performed before starting the formal study. Doses and drug administration schedule of K^+ channel inhibitors and gabapentin for spinal administration were selected based on previous reports (Ocaña et al., 1990; Ortiz et al., 2002; Granados-Soto et al., 2002; Ortiz et al., 2003) and on pilot experiments in our laboratory. In order to exclude a possible

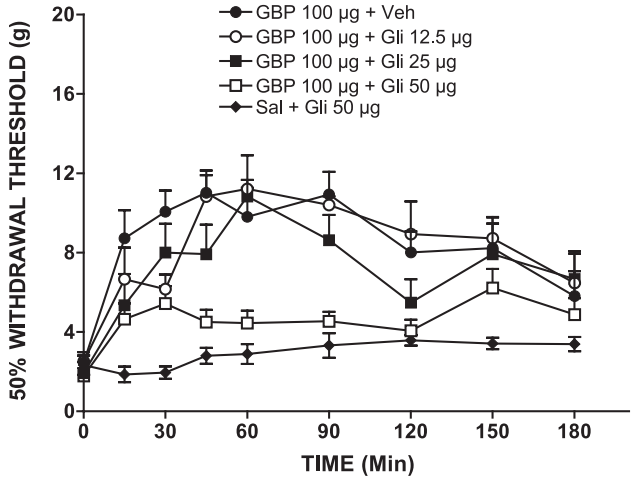

Fig. 3. Effect of spinal treatment with glibenclamide (Gli) on the antiallodynic activity induced by gabapentin (GBP, 100 µg, i.t.). Rats were pretreated with gabapentin and then vehicle or increasing doses of glibenclamide. Data are the mean ± S.E.M. for six to seven animals. A significant difference ($P<0.05$, by ANOVA, followed by the Tukey's test) was observed between gabapentin (100 µg) and gabapentin (100 µg) + glibeclamide (50 µg) 15 min after administration. Asterisks indicating significant difference were omitted for the sake of clarity.

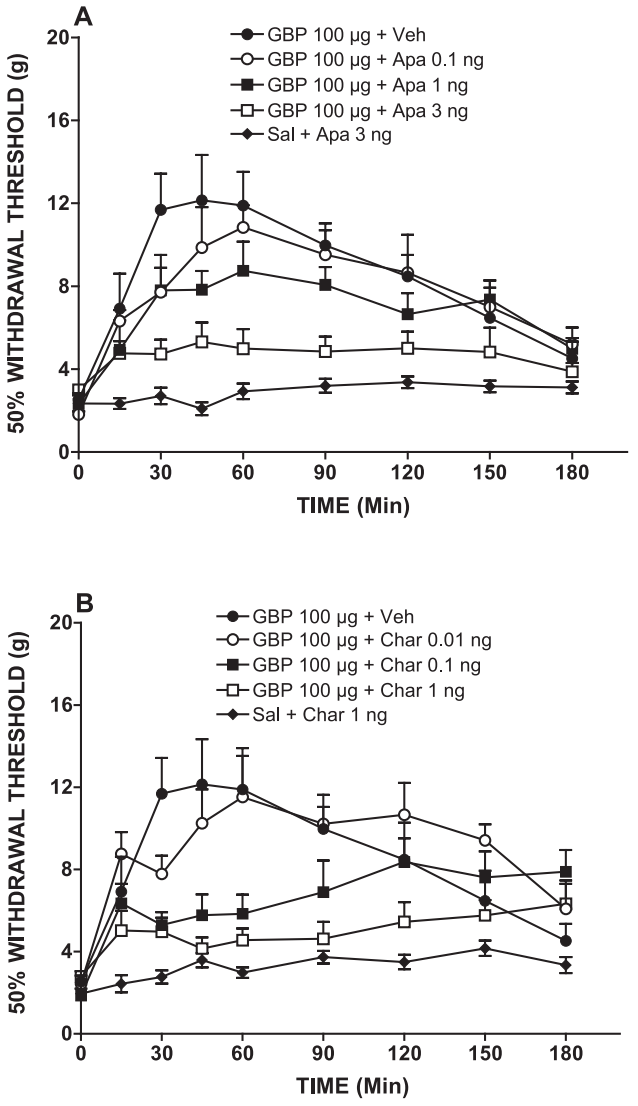

Fig. 4. Effect of spinal treatment with apamin (Apa, panel A) or charybdotoxin (Char, panel B) on the antiallodynic activity of gabapentin (GBP, 100 µg, i.t.). In panel A rats were pretreated with gabapentin and then vehicle or increasing doses of apamin. In panel B rats were pretreated with gabapentin 100 µg and then vehicle or increasing doses of charybdotoxin. Data are the mean ± S.E.M. for six animals. A significant difference ($P<0.05$, by ANOVA, followed by the Tukey's test) was observed between gabapentin (100 µg) and either the 1 and 3 ng apamin groups (panel A) or the 0.1 and 1 ng charybdotoxin groups (panel B) after 30 min. Asterisks indicating significant difference were omitted for the sake of clarity.

antinociceptive effect of gabapentin, a group of sham operated rats (not allodynic rats) received a gabapentin dose (100 µg, i.t.) and the withdrawal threshold was assessed. Pinacidil was used as positive control as there is evidence that its effect is mainly due to opening of K^+ channels (Edwards and Weston, 1993; Ortiz et al., 2002; Granados-Soto et al., 2002). Observer was unaware of the treatment in each animal. Rats in all groups were tested for possible behavioral side effects observed as a reduction of righting, stepping, corneal and pinna reflexes as previously described (Malmberg and Yaksh, 1992) before and after drug treatment.

2.6. Data analysis and statistics

All experimental results are given as the mean ± S.E.M. for six to seven animals per group. Time course data are presented as mean 50% paw withdrawal threshold (gram) ± S.E.M. Curves were constructed plotting the threshold for paw withdrawal as a function of time. Analysis of variance (ANOVA), followed by Tukey's test was used to compare differences between treatments. Differences were considered to reach statistical significance when $P<0.05$.

3. Results

3.1. Antiallodynic activity of spinal gabapentin

Ligation of L5 and L6 spinal nerves produced a clear-cut allodynia in rats submitted to the surgery compared to the sham operated rats (Fig. 1A). Ligation of spinal nerves or intrathecal catheter implantation did not modify weight gain in these rats compared to the sham operated rats (data not shown). In addition, gabapentin was not able to modify withdrawal threshold in sham-operated rats (Fig. 1B). Intrathecal administration of gabapentin (25–200 µg) or pinacidil (1–30 µg), but not vehicle (saline or DMSO 25%, respectively), reduced in a dose-dependent manner tactile allodynia ($P<0.05$) induced by ligation of L5 and L6 nerves (Fig. 2). Maximal antiallodynic effect was

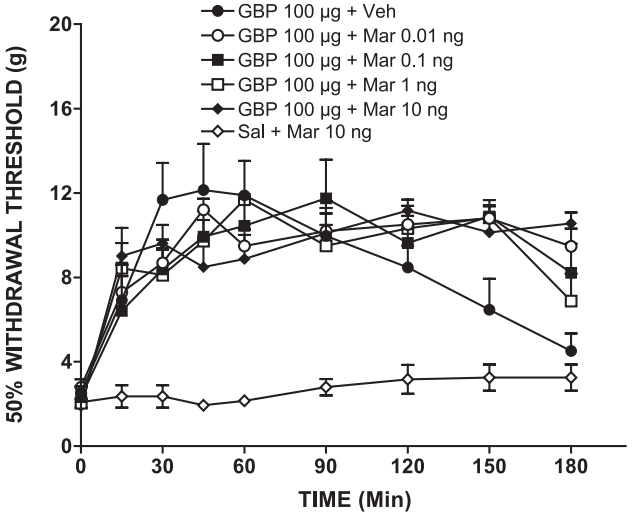

Fig. 5. Effect of spinal treatment with margatoxin (Mar) on the antiallodynic activity induced by the spinal administration of gabapentin (GBP, 100 µg, i.t.). Rats were pretreated with gabapentin 100 µg and then vehicle or increasing doses of margatoxin. Data are the mean ± S.E.M. for six to seven animals.

reached with 100 μg of gabapentin and 10 μg of pinacidil and higher doses of either drug did not produce greater effects (Fig. 2). Therefore, we decided to use these doses in the following studies. No reduction in the assessed reflexes was observed in either group, control or treated (data not shown).

3.2. Effect of glibenclamide, charybdotoxin, apamin and margatoxin on the antiallodynic activity of gabapentin

Spinal pretreatment with the ATP-sensitive K^+ channel inhibitor glibenclamide (12.5–50 μg), but not vehicle, significantly reversed ($P<0.05$) the antiallodynic effect induced by the spinal administration of gabapentin (100 μg) (Fig. 3). In addition, spinal apamin (0.1–3 ng) or charybdotoxin (0.01–1 ng) (small- and large-conductance Ca^{2+}-activated K^+ channel inhibitors, respectively) (Fig. 4), but not margatoxin (0.01–10 ng) (voltage-dependent K^+ channel inhibitor) (Fig. 5), dose-dependently ($P<0.05$) prevented the antiallodynic activity of gabapentin. Given alone, at the greatest dose tested K^+ channel inhibitors did not modify nerve ligation-induced allodynia.

3.3. Effect of glibenclamide, charybdotoxin, apamin and margatoxin on pinacidil-induced antiallodynia

Intrathecal administration of glibenclamide (50 μg), apamin (3 ng) or charybdotoxin (1 ng), but not margatoxin (10 ng), significantly reduced the antiallodynic effect produced by pinacidil ($P<0.05$) (Fig. 6).

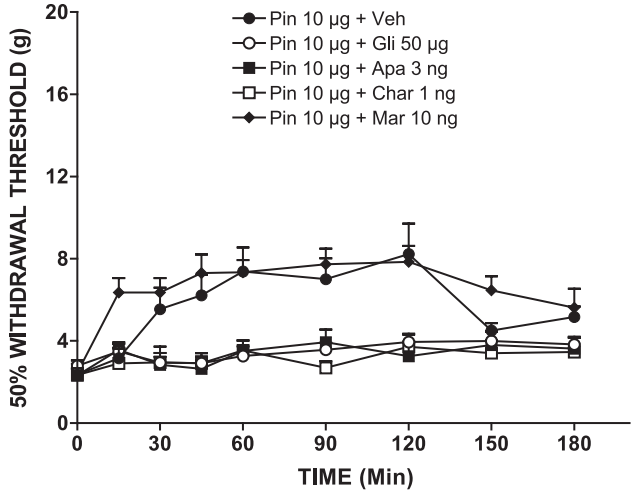

Fig. 6. Effect of spinal treatment with glibenclamide (Gli), apamin (Apa), charybdotoxin (Char) and margatoxin (Mar) on the antiallodynic activity of pinacidil. Data are the mean ± S.E.M. for six to seven animals. A significant difference ($P<0.05$, by ANOVA, followed by the Tukey's test) was observed between pinacidil (10 μg) + vehicle and either pinacidil (10 μg) + glibenclamide (50 μg), pinacidil (10 μg) + apamin (3 ng) and pinacidil (10 μg) + charybdotoxin (1 ng) groups 30 min after administration. Asterisks indicating significant difference were omitted for the sake of clarity.

4. Discussion

In the present investigation we were able to observe a reduction of neuropathic pain after spinal administration of gabapentin in rats. These results agree with previous observations showing an antiallodynic effect of intrathecal gabapentin (Hwang and Yaksh, 1997; Jun and Yaksh, 1998; Field et al., 1999; Patel et al., 2001; Wallin et al., 2002; Cheng et al., 2003). The antiallodynic effect of gabapentin was blocked by pretreatment with intrathecal glibenclamide, an ATP-sensitive K^+ channel inhibitor (Amoroso et al., 1990; Davies et al., 1991; Edwards and Weston, 1993), thus suggesting that gabapentin may activate this channel in order to reduce tactile allodynia in nerve ligated rats. Moreover, the intrathecal administration of small-and large-conductance Ca^{2+}-activated K^+ channel inhibitors apamin and charybdotoxin, respectively, also prevented the antiallodynic effect produced by gabapentin, suggesting the possible participation of both types of Ca^{2+}-activated K^+ channels in gabapentin-induced effect. It is known that besides its effects on large-conductance Ca^{2+}-activated K^+ channel, charybdotoxin is also able to inhibit intermediate-conductance Ca^{2+}-activated and voltage-gated K^+ channels (Kv), in particular Kv1.3 (Price et al., 1989; Ouadid-Ahidouch et al., 1999). Therefore, blockade of gabapentin-induced antiallodynic effect by apamin and charybdotoxin suggest that gabapentin may be producing its antiallodynic effect through activation of small-, intermediate- and large-conductance Ca^{2+}-activated K^+ channel as well as voltage-gated K^+ channels (Kv1.3). However, since the selective inhibitor of voltage-gated K^+channels Kv1.3 margatoxin (Garcia-Calvo et al., 1993), at concentrations able to inhibit Kv1.3 (Garcia-Calvo et al., 1993), was not able to reduce the effect produced by gabapentin (this work), data suggest that gabapentin may only activate Ca^{2+}-activated K^+ channels, but not voltage-gated K^+ channels (Kv1.3). Lack of effect of margatoxin on gabapentin-induced antiallodynia can not be attributed to a reduction in Kv1.3 expression since this channel is not modified by neuropathy (Ishikawa et al., 1999) as it is the case for other Kv channels (Rasband et al., 2001; Kim et al., 2002). Taken together, results suggest that the antiallodynic effect of gabapentin may result from activation of ATP-sensitive and small-, intermediate- and large-conductance Ca^{2+}-activated K^+ channels.

At the concentrations used in this work, the K^+ channel blockers used (glibenclamide, charybdotoxin, apamin, and margatoxin) did not modify allodynia induced by ligation of L5 and L6 spinal nerves in comparison with that of control rats. The lack of effect of the K^+ channel blockers is consistent with the results of studies in which these compounds did not modify the nociceptive activity of thermal noxious stimuli and mechanical hyperalgesia (Welch and Dunlow, 1993; Ortiz et al., 2002), thus excluding the possibility that the prevention of gabapentin antiallodynia could be due to a hyperalgesic or nociceptive effect of the K^+ channel blockers used. The lack of modification of the withdrawal

thresholds by the K^+ channel modulators at concentrations able to prevent gabapentin effect might also indicate that the K^+ channels of primary afferent neurons involved in the modulation of allodynia are not tonically activated.

In this study we have used pinacidil as positive control because there is evidence that this drug is a K^+ channel opener (Edwards and Weston, 1993). Pinacidil produced a dose-dependent antiallodynic effect in the neuropathic rats. Previously pinacidil produced antinociception or increased that induced by morphine, H_1-anti-histamines or tricyclic antidepressants in the hot-plate, tail-flick and formalin test (Vergoni et al., 1992; Galeotti et al., 2001; Ortiz et al., 2002; Zushida et al., 2002). However, to the best of our knowledge this is the first report about the antiallodynic property of pinacidil by itself in a model of neuropathic pain in the rat. In our study, the antiallodynic effect of pinacidil was completely blocked by glibenclamide (50 μg, i.t.), suggesting that effectively it is an ATP-sensitive K^+ channel opener. However, the effect of pinacidil was also reversed by spinal charybdotoxin (1 ng) or apamin (3 ng), but not by margatoxin (10 ng). Then, these results suggest that the antiallodynic effect of pinacidil, as well as that of gabapentin, is not only produced by ATP-sensitive, but by Ca^{2+}-activated, but not voltage-gated (Kv1.3), K^+ channels. The profile of pinacidil as a K^+ channel opener in this study is similar to that previously reported for this drug in formalin-induced pain (Ortiz et al., 2002). However, it is interesting to note that pinacidil-induced antiallodynic effect was considerably lower than that produced by gabapentin (see Fig. 2). This difference in efficacy could be attributed to the different mechanisms activated by gabapentin versus pinacidil (see below).

A body of literature has shown that gabapentin has several mechanisms of action (for a review, see Taylor et al., 1998). Gabapentin may interact indirectly with NMDA receptors because the glycine-NMDA receptor agonist D-serine reverses the antihyperalgesic action of gabapentin (Singh et al., 1996). Furthermore, gabapentin is able to block thermal hyperalgesia induced by intrathecal NMDA (Partridge et al., 1998). More recently, an electrophysiological study has shown that gabapentin is able to presynaptically inhibit glutamatergic neurotransmission preferentially in the lamina superficial of the dorsal horn (Shimoyama et al., 2000). This effect would possibly reduce the release of excitatory amino acids. However, other studies have found only a modest reduction in glutamate release (Dooley et al., 2000). Moreover, low affinity of (+)-MK-801 for the [^{3}H] gabapentin binding site suggest that [^{3}H] gabapentin does not interact with NMDA receptors (Suman et al., 1993). Other studies have found that gabapentin is able to increase GABA synthesis and release in brain regions (Loscher et al., 1991; Götz et al., 1993). In vitro gabapentin reduces release of several monoamine neurotransmitters such as dopamine, serotonin and noradrenaline from brain slices (Reimann, 1983). It has been suggested that gabapentin action could also result from its binding to the α2δ subunit of L-type voltage-dependent Ca^{2+} channels (Gee et al., 1996; Cheng et al., 2003) reducing Ca^{2+} currents in about 25%. The physiological role of the α2δ subunit is not well understood; therefore so far it is unclear whether this binding site is indeed involved in the antinociceptive actions of gabapentin. However, despite the different hypothesis to explain the actions of gabapentin, the exact mechanism of this drug is at present unknown. In this work we suggest that gabapentin may be able to open K^+ channels, which would lead to hyperpolarization of sensory neurons and to a reduction of tactile allodynia in nerve injured rats. Previous evidence indicates that gabapentin activates K^+ currents in CA1 pyramidal cell in situ via $GABA_B$ receptors (Ng et al., 2001). This effect is produced by activation of the $GABA_B$ gb1a-gb2 heterodimer subtype coupled to K_{IR} channels. It is suggested that activation of this receptor leads to hyperpolarization and to a reduction in excitability of pre- and postsynaptic neurons. In the same way, other study has shown that either gabapentin or pinacidil inhibits [^{3}H]-noradrenaline release elicited by a low concentration of K^+ (15 mM) through opening of ATP-sensitive K^+ channels, as this inhibition was blocked by the ATP-sensitive K^+ channel blocker glibenclamide (Freiman et al., 2001). These results are in line with our data as the antiallodynic effect of the ATP-sensitive K^+ channel opener pinacidil as well as that of gabapentin was partially blocked by glibenclamide, charybdotoxin or apamin, but not by margatoxin. Taken together, there is evidence to suggest that the antiallodynic effect of gabapentin may result from several mechanisms of action, including the opening of K^+ channels.

In conclusion, gabapentin reduced tactile allodynia in neuropathic rats. The antiallodynic effect of gabapentin was antagonized by glibenclamide, charybdotoxin and apamin, but not by margatoxin. These results suggest that modulation of ATP-sensitive as well as small-, intermediate-and large-conductance Ca^{2+}-activated, but not voltage-sensitive (Kv1.3), K^+ channels at the spinal cord neurons could play a role in the antiallodynic effect of gabapentin in this model of neuropathy.

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