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Possible activation of the NO–cyclic GMP–protein kinase G–K^+ channels pathway by gabapentin on the formalin test

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Abstract

The effect of modulators of the nitric oxide–cyclic GMP–protein kinase G–K^+ channels pathway on the local peripheral antinociceptive action induced by gabapentin was assessed in the rat 1% formalin test. Local peripheral administration of gabapentin produced a dose-dependent antinociception in the second phase of the test. Gabapentin-induced antinociception was due to a local action as its administration in the contralateral paw was ineffective. Local peripheral pretreatment of the paws with N^G-L-nitro-arginine methyl ester (L-NAME, a nitric oxide synthesis inhibitor), 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ, a soluble guanylyl cyclase inhibitor) and KT-5823 (a protein kinase G inhibitor) dose-dependently reduced gabapentin-induced antinociception. Likewise, glibenclamide or tolbutamide (ATP-sensitive K^+ channel inhibitors), 4-aminopyridine or tetraethylammonium (non-selective inward rectifier K^+ channel inhibitors) or charybdotoxin (large-conductance Ca^{2+}-activated-K^+ channel blocker), but not apamin (small-conductance Ca^{2+}-activated-K^+ channel blocker) or naloxone (opioid receptor antagonist), reduced the antinociception induced by gabapentin. Our data suggest that gabapentin could activate the nitric oxide–cyclic GMP–protein kinase G–K^+ channels pathway in order to produce its peripheral antinociceptive effect in the rat 1% formalin test.

Keywords: Gabapentin; ODQ; L-NAME; KT-5823; Glibenclamide; Tolbutamide; Charybdotoxin; Antinociception; K^+ channel; Formalin test

1. Introduction

Gabapentin is a relatively new antiepileptic drug that is structurally similar to the neurotransmitter γ-aminobutyric acid (GABA) and the endogenous amino acid L-leucine (Taylor, 1994). Although gabapentin is a structural analogue of GABA, which does not cross the blood–brain barrier, this drug penetrates into the central nervous system and is able to produce anticonvulsant effect (McLean and Gidal, 2003). It is well known that antiepileptic drugs have antinociceptive and antihyperalgesic effects and some are used in the control of clinical pain (Attal et al., 1998; Nicholson, 2000; Werner et al., 2001). In this respect, the properties of gabapentin have been attributed to an action on the central and peripheral nervous system (Field et al., 1997; Shimoyama et al., 1997; Partridge et al., 1998; Laughlin et al., 2002). The local peripheral administration of gabapentin has been demonstrated to be useful in the formalin-induced nociceptive behavior and peripheral thermal nociception in rats (Carlton and Zhou, 1998; Todorovic et al., 2003; Granados-Soto and Argüelles, 2005).

The mechanism of action of gabapentin has not been totally elucidated. It has been reported that gabapentin increases GABA accumulation in several regions of rat brain (Loscher et al., 1991; Czuczwar, 2000). Likewise, gabapentin may

Abbreviations: ODQ, 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one; L-NAME, N^G-L-nitro-arginine methyl ester; cGMP, cyclic guanosine 3,5-monophosphate; PKG, protein kinase G; NO, nitric oxide; 4-AP, 4-aminopyridine; TEA, tetraethylammonium.

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significantly reduce the glutamate levels to inhibit the branched-chain amino acid transferase and stimulate glutamate dehydrogenase activity (Goldlust et al., 1995). Additionally, Gee et al. (1996) characterized a high affinity [3H]gabapentin-binding protein from pig brain membranes. This site was identified as an $\alpha_2\delta$ subunit of a voltage-dependent Ca^{2+} channel. After this finding, Stefani et al. (1998) reported a large inhibition of calcium currents by gabapentin in pyramidal neocortical cells.

On the other hand, in hippocampal neurons, it has been found that gabapentin is able to activate the $GABA_B$ receptor and to induce inwardly rectifying K^+ currents (K_{ir}), sensitive to Ba^{2+} and Cs^+ (Bertrand et al., 2003). Furthermore, it has also been demonstrated in human neocortical slices that gabapentin is able to decrease the K^+-evoked [3H]noradrenaline release (Freiman et al., 2001). This inhibitory effect was modified by glibenclamide, suggesting the possible participation of ATP-sensitive K^+ channels. In support of this last data, Mixcoatl-Zecuatl et al. (2004) suggested that gabapentin may activate Ca^{2+}-activated and ATP-sensitive K^+ channels in order to produce part of its spinal antiallodynic effect in a model of neuropathic pain in the rat.

It has been suggested that, in hyperalgesic conditions, there is a concomitant activation of adenylyl cyclase and protein kinase C (Taiwo and Levine, 1991; Aley et al., 2000; Cunha et al., 1999), while that the cyclic GMP–protein kinase G (PKG) pathway is involved in antinociceptive states (Cunha et al., 1999; Sachs et al., 2004). In a perfused slice, preparation from the rat caudal trigeminal nucleus was demonstrated that gabapentin is able to reduce stimulation of protein kinase C by phorbol 12-myristate 13-acetate and adenylyl cyclase by forskolin (Maneuf and McKnight, 2001). More recently, Sachs et al. (2004) demonstrated that the administration of the specific PKG inhibitor KT-5823 (Kase et al., 1987) was able to block the antinociceptive effect of morphine and dipyrone in hypernociception. Therefore, this work was undertaken to determine whether drugs affecting the nitric oxide (NO)–cyclic GMP–PKG–K^+ channels pathway (L-NAME, ODQ, KT-5823 and K^+ channel blockers) have any effect on the peripheral antinociception induced by gabapentin in the formalin test. Additionally, we tested the effect of the opioid receptor antagonist naloxone on the effect induced by gabapentin.

2. Materials and methods

2.1. Animals

Female Wistar rats aged 8–10 weeks (weight range: 180–200 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 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 (Departamento de Farmacobiología, México, D.F., Mexico). At the end of the experiment, the rats were sacrificed in a CO_2 chamber.

2.2. Measurement of antinociceptive activity

Antinociception was assessed using the 1% formalin test. Rats were placed in open plexiglass observation chambers for 30 min to allow them to accommodate to their surroundings, then they were removed for formalin administration. 50 μl of diluted formalin (1%) were injected subcutaneously (s.c.) into the dorsal surface of the right hind paw with a 30-gauge needle. Animals were then returned to the chambers and nocifensive behavior was observed immediately after formalin injection. Mirrors were placed to enable unhindered observation. Nocifensive behavior was quantified as the number of flinches of the injected paw during 1-min periods every 5 min up to 60 min after injection (Wheeler-Aceto and Cowan, 1991; Malmberg and Yaksh, 1992). Flinching was readily discriminated and was characterized as rapid and brief withdrawal or flexing of the injected paw. Formalin-induced flinching behavior is biphasic. The initial acute phase (0–10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged persistent response (15–60 min). Both phases of the formalin test were registered.

2.3. Drugs

Gabapentin was purchased from Research Biochemical International (Natick, MA, USA). N^G-L-Nitro-arginine methyl ester (L-NAME), 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ), KT-5823, glibenclamide, tolbutamide, 4-aminopyridine (4-AP), tetraethylammonium (TEA), charybdotoxin and apamin were purchased from Sigma (St. Louis, MO, USA). Gabapentin, TEA, 4-AP, charybdotoxin, apamin and L-NAME were dissolved in saline. Glibenclamide, tolbutamide, ODQ and KT-5823 were dissolved in 20% dimethylsulfoxide.

2.4. Study design

Rats received a subcutaneous injection (50 μl/paw) into the dorsal surface of the right hind paw of vehicle or increasing doses of gabapentin (30–300 μg/paw) 20 min before formalin injection into the same paw (ipsilateral). To determine whether the antinociceptive effect was due to a local action, an additional set of rats was injected with gabapentin in the left (contralateral, 300 μg/paw) paw 20 min before formalin was injected into the right paw and the effect assessed.

To determine whether gabapentin-induced peripheral antinociception was mediated by the NO–cyclic GMP–PKG pathway or by activation of opioid receptors, effect of treatment (10 min before) with the appropriate vehicle (20% DMSO for ODQ and KT-5823 or saline for L-NAME and naloxone) or L-NAME (25–100 μg/paw), ODQ (25–100 μg/paw), KT-5823 (5–500 ng/paw) and naloxone (50 μg/paw) on the antinociceptive effect induced by gabapentin (300 μg/paw, 20 min before) was assessed.

To determine whether gabapentin-induced antinociception was mediated by the K^+ channel activation, effect of treatment (10 min before) with the subcutaneous injection into the dorsal surface of the right hind paw of appropriate vehicle (20% DMSO for glibenclamide and tolbutamide or saline for 4-AP, TEA, charybdotoxin and apamin) or glibenclamide (12.5–50 μg/paw),

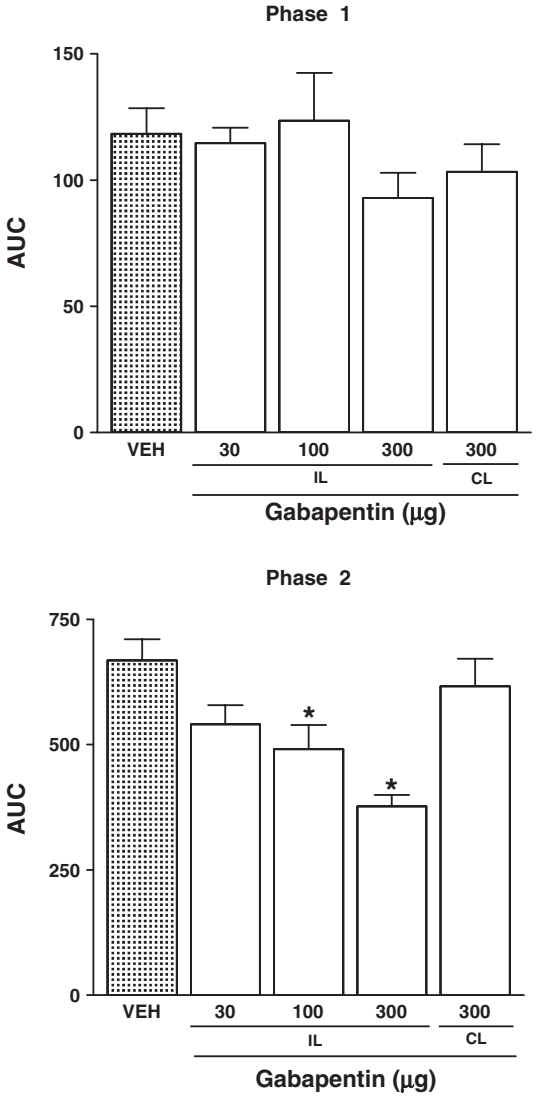

Fig. 1. Local peripheral antinociceptive effect of gabapentin on the 1% formalin test. Rats were pretreated with a local injection of vehicle (VEH) or gabapentin into the right (ipsilateral, IL) or left paw (contralateral, CL), 20 min before formalin injection. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean±S.E.M. of the data obtained in six animals. *Significantly different from the vehicle (VEH) group ($P<0.05$), as determined by analysis of variance followed by Tukey's test.

tolbutamide (12.5–50 μg/paw), 4-aminopyridine (10–50 μg/paw), tetraethylammonium (25–100 μg/paw), charybdotoxin (0.125–0.5 μg/paw) or apamin (0.1–2 μg/paw) on the antinociceptive effect induced by gabapentin (300 μg/paw, 20 min before) was assessed.

Drugs were injected in a volume of 50 μl. Doses and drug administration schedules of inhibitors and gabapentin for peripheral administrations were selected based on previous reports (Rodrigues and Duarte, 2000; Ortiz et al., 2003; Granados-Soto et al., 2004) and on pilot experiments in our laboratory. Rats in all groups were observed regarding behavioral or motor function changes induced by the treatments. This was assessed, but not quantified, by testing the animals' ability to stand and walk in a normal posture.

2.5. Data analysis and statistics

All experimental results are given as the mean±S.E.M. for six animals per group. Curves were constructed plotting the number of flinches as a function of time. The area under the number of flinches against time curves (AUC), an expression of the duration and intensity of the effect, was calculated by the trapezoidal rule. Reduction of number of flinches or AUC of the second phase is

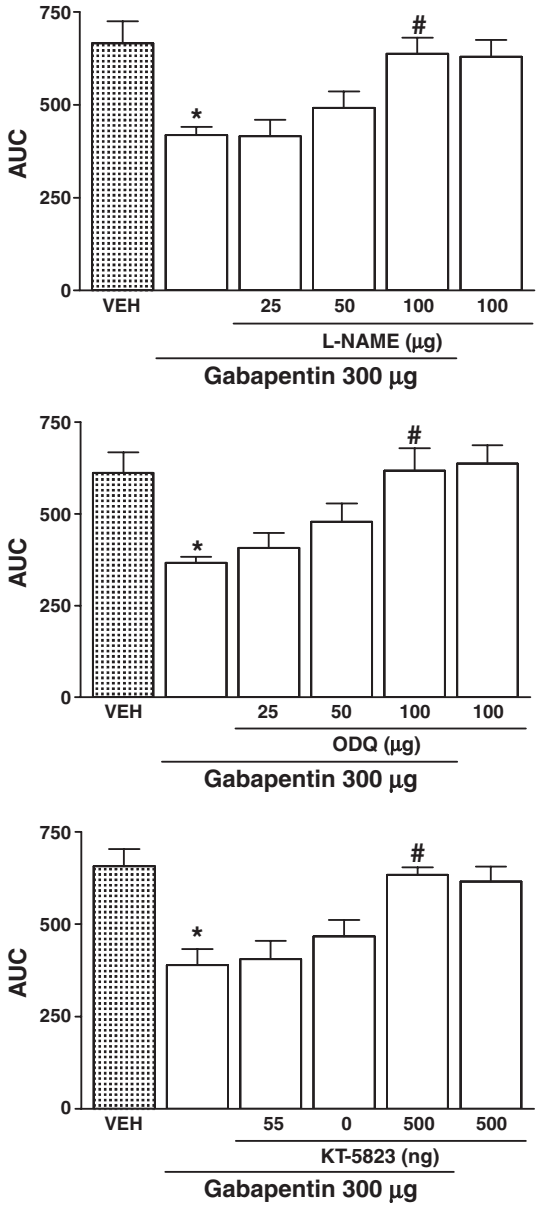

Fig. 2. Effect of N^G-L-nitro-arginine methyl ester (L-NAME, top panel), 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ, middle panel) and KT-5823 (bottom panel) on the local peripheral antinociception produced by gabapentin during the second phase of the formalin test. Rats were pretreated with a local injection of gabapentin (−20 min) and then L-NAME, ODQ or KT-5823 (−10 min) into the right paw. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean±S.E.M. of the data obtained in six animals. *Significantly different from the vehicle (VEH) group ($P<0.05$) and #significantly different from the gabapentin group ($P<0.05$), as determined by analysis of variance followed by Tukey's test.

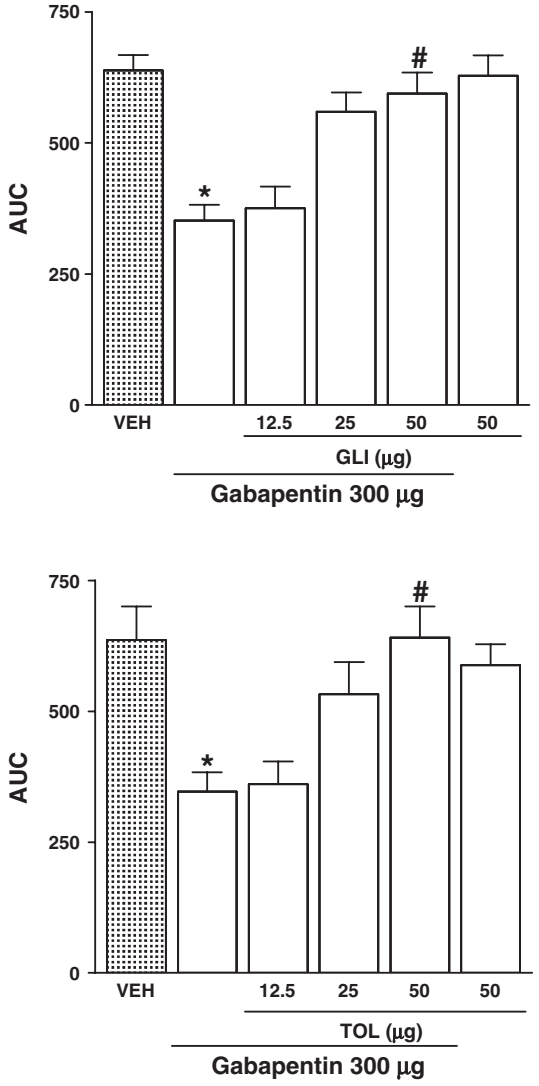

Fig. 3. Effect of glibenclamide (GLI, top panel) or tolbutamide (TOL, bottom panel) on the local peripheral antinociception produced by gabapentin during the second phase of the formalin test. Rats were pretreated with gabapentin (−20 min) and then a local injection of glibenclamide or tolbutamide (−10 min) into the right paw. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean±S.E.M. of the data obtained in six animals. *Significantly different from the vehicle (VEH) group ($P<0.05$) and #significantly different from the gabapentin group ($P<0.05$), as determined by analysis of variance followed by Tukey's test.

reported only, since we were not able to observe effect on phase 1. Analysis of variance, followed by Tukey's test, was used to compare differences between treatments. Differences were considered to achieve statistical significance when $P<0.05$.

3. Results

3.1. Peripheral antinociceptive effect of gabapentin

Local injection of 1% formalin produced a typical pattern of flinching behavior. Flinching behavior was biphasic. The first phase started immediately after formalin injection, decreasing gradually in about 10 min. The second phase started at about 15 min and lasted until 60 min. Ipsilateral, but not contralateral, local peripheral administration of gabapentin produced a dose-dependent reduction in the flinching behavior otherwise observed after formalin injection (Fig. 1). Gabapentin significantly reduced the number of flinches during phase two ($P<0.05$), but not during phase one (Fig. 1). No side effects were observed in any of the studied groups of animals.

3.2. Effect of L-NAME, ODQ and KT-5823 on gabapentin-induced peripheral antinociception

Local peripheral injection with the NO synthesis inhibitor L-NAME, the soluble guanylyl cyclase inhibitor ODQ or the

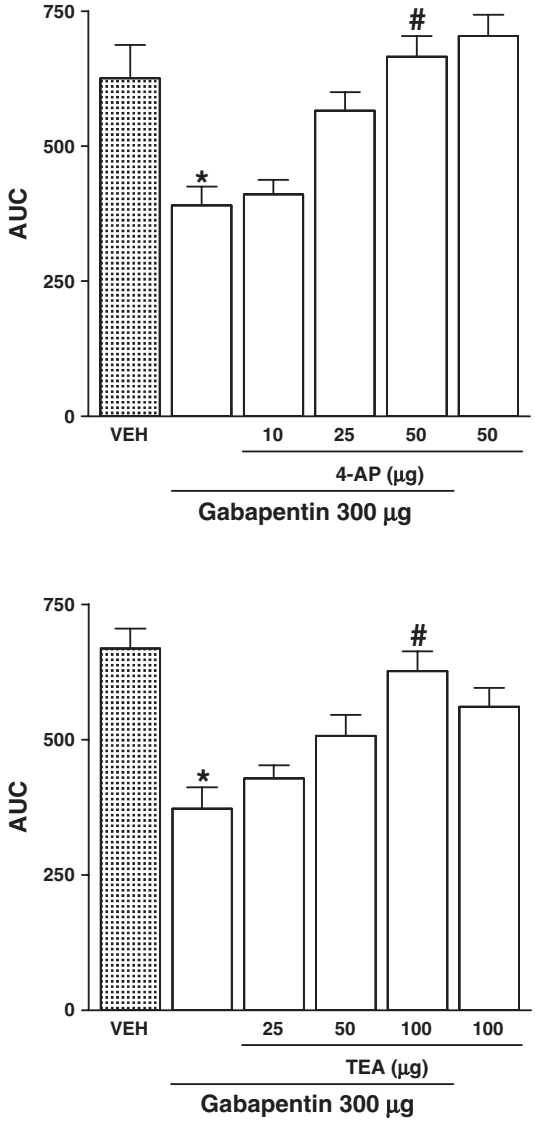

Fig. 4. Effect of 4-aminopyridine (4-AP, top panel) or tetraethylammonium (TEA, bottom panel) on the peripheral antinociception produced by gabapentin during the second phase of the formalin test. Rats were pretreated with gabapentin (−20 min) and then a local injection of 4-AP or TEA (−10 min) into the right paw. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean±S.E.M. of the data obtained in six animals. *Significantly different from the vehicle (VEH) group ($P<0.05$) and #significantly different from the gabapentin group ($P<0.05$), as determined by analysis of variance followed by Tukey's test.

inhibitor of PKG KT-5823 was able to significantly reduce the local effect of gabapentin in a dose-dependent manner ($P<0.05$) (Fig. 2). Given alone, the inhibitors did not modify formalin-induced nociceptive behavior ($P>0.05$).

3.3. Effect of glibenclamide, tolbutamide, 4-AP, TEA, charybdotoxin and apamin on gabapentin-induced peripheral antinociception

Local pretreatment with the ATP-sensitive K^+ channel inhibitors glibenclamide or tolbutamide was able to reduce the local effect of gabapentin in a dose-dependent manner ($P<0.05$) (Fig. 3). In addition, 4-AP and TEA (non-selective inward rectifier K^+ channel inhibitors) as well as charybdotoxin (large conductance Ca^{2+}-activated K^+ channel inhibitor), but not apamin (small conductance Ca^{2+}-activated K^+ channel inhibitor), reduced in a dose-dependent manner ($P<0.05$) the gabapentin-induced antinociception (Figs. 4 and 5). Given alone, K^+ channel inhibitors did not modify formalin-induced nociceptive behavior ($P>0.05$).

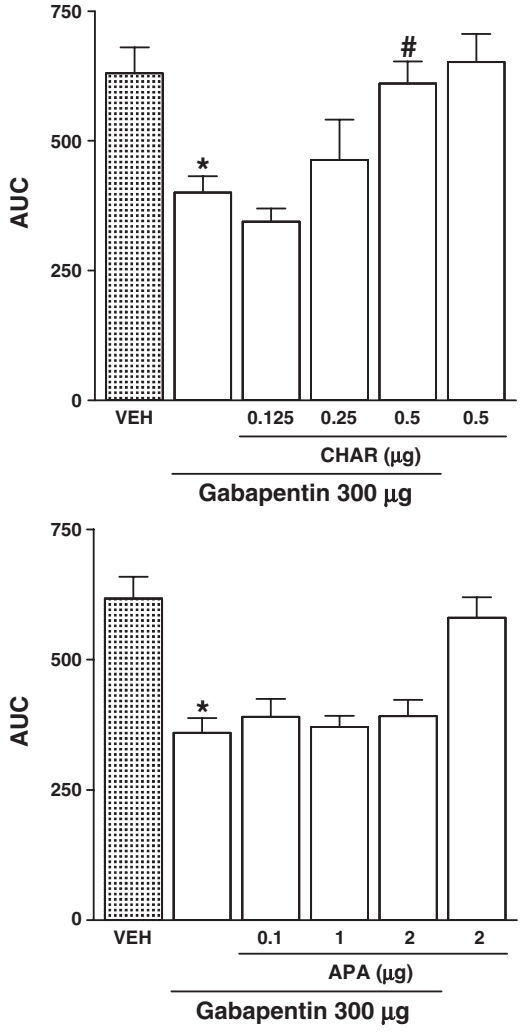

Fig. 5. Effect of charybdotoxin (CHAR, top panel) or apamin (APA, bottom panel) on the peripheral antinociception produced by gabapentin during the second phase of the formalin test. Rats were pretreated with gabapentin (−20 min) and then a local injection of charybdotoxin or apamin (−10 min) into the right paw. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean±S.E.M. of the data obtained in six animals. *Significantly different from the vehicle (VEH) group ($P<0.05$) and #significantly different from gabapentin group ($P<0.05$), as determined by analysis of variance followed by Tukey's test.

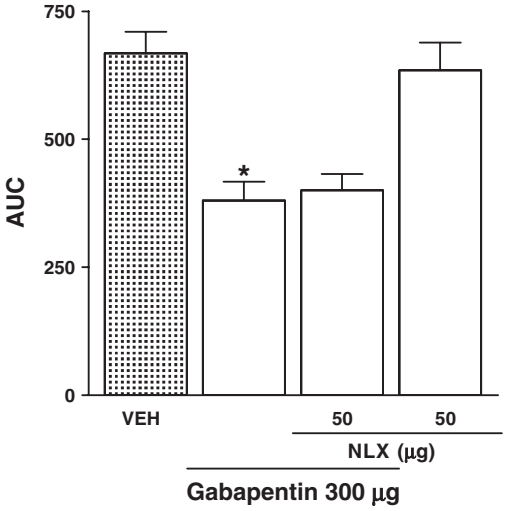

Fig. 6. Effect of naloxone (NLX) on the local peripheral antinociception produced by gabapentin during the second phase of the formalin test. Rats were pretreated with gabapentin (−20 min) and then a local injection of naloxone (−10 min) into the right paw. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean±S.E.M. of the data obtained in six animals. *Significantly different from the vehicle (VEH) group ($P<0.05$) as determined by analysis of variance followed by Tukey's test.

3.4. Effect of naloxone on gabapentin-induced peripheral antinociception

Local peripheral administration of naloxone (50 μg/paw) did not modify formalin-induced nociceptive behavior nor the antinociceptive action induced by gabapentin ($P>0.05$) (Fig. 6). No reduction in the assessed reflexes were observed in either group, control or treated.

4. Discussion

Several animal and human studies have shown that gabapentin is effective in a wide variety of pain syndromes. In our study, the local peripheral administration of gabapentin produced a dose-dependent reduction of formalin-induced nociceptive behavior. This antinociceptive effect appeared to be due to a local action, as gabapentin injection in the contralateral paw did not produce any significant modification in flinching behaviour. These results agree with those reported by Carlton and Zhou (1998) and Granados-Soto and Argüelles (2005), which demonstrated that, besides its spinal effect, gabapentin has a peripheral site of action in the formalin-induced nociceptive behavior. Likewise, another study demonstrated a peripheral action of gabapentin (Todorovic et al., 2003). Using a model of acute thermal nociception in rats, the authors found that gabapentin injected intradermally into the hindpaw was able to induce dose-dependent analgesia. Taken

together, data suggest that the mechanism for the antihyperalgesic effects of gabapentin includes its binding to peripheral structures.

In the current work, the NO synthesis inhibitor L-NAME (Rees et al., 1990) and the soluble guanylyl cyclase inhibitor ODQ (Moro et al., 1996) were able to reduce the antinociceptive effect produced by gabapentin. The participation of the L-arginine–NO–cyclic GMP pathway in peripheral antinociception is supported by several observations. It has been demonstrated that the local peripheral administration of NO donors or membrane permeable analogs of cyclic GMP produce antinociception (Cunha et al., 1999; Soares et al., 2000; Soares and Duarte, 2001). In addition, these drugs are able to potentiate the peripheral response of other analgesics (Aguirre-Bañuelos et al., 1999; Lázaro-Ibáñez et al., 2001; Alves et al., 2004). In support of this, the systemic administration of the nitric oxide-releasing derivative of gabapentin NCX 8001 alleviated pain-like behaviours in two rat models of neuropathic pain (Wu et al., 2004).

However, other studies in the literature indicate that the NO–cyclic GMP pathway can have pronociceptive rather than antinociceptive effects (Aley et al., 1998). Oka et al. (2003) demonstrated that gabapentin was able to inhibit depolarization-induced NO synthase activation in murine cortical neuronal culture. The authors suggested that this inhibition was mediated via blockade of both P/Q-type and L-type Ca^{2+} channels. This discrepancy may be due to the different experimental pain models used, diverse tissue level and the variant NO and cGMP intracellular content (Kawabata et al., 1994; Pehl and Schmid, 1997; Tegeder et al., 2002). Nevertheless, it is important to point out that, in the rat formalin and rat paw pressure models, the production of NO and cyclic GMP in subcutaneous tissue is involved in antinociceptive states (Duarte et al., 1992; Aguirre-Bañuelos et al., 1999; Soares et al., 2000; Soares and Duarte, 2001; Lázaro-Ibáñez et al., 2001; Ortiz et al., 2003; Alves et al., 2004; Sachs et al., 2004).

Some studies suggest that peripheral sensitization of nociceptors is mediated by the adenylyl cyclase–cyclic AMP–protein kinase pathway (Taiwo et al., 1989; Taiwo and Levine, 1991; Cunha et al., 1999), while that the cyclic GMP–PKG pathway is involved in antinociceptive effects (Cunha et al., 1999; Sachs et al., 2004). The mechanisms by which NO and cyclic GMP modulate the neuronal function have not been totally studied. Actually, it has been suggested three families of cyclic GMP receptors: cyclic nucleotide-gated ion channels, cyclic GMP-regulated phosphodiesterases and PKG (Wang and Robinson, 1997). Activation of PKG would lead to phosphorylation and regulation of ion channels, protein phosphatase and cytoskeletal protein (Wang and Robinson, 1997). In the present study, local peripheral treatment with the inhibitor of PKG KT-5823 (Kase et al., 1987) was able to reduce in a dose-dependent manner gabapentin-induced antinociception. This result suggest for the first time that PKG activation could be an important step in the peripheral effects produced by gabapentin. This event implies that phosphorylation is a necessary action of this process. Our results are in agreement with previous observation in the constant rat-paw pressure test (Sachs et al., 2004). In this last study, the local peripheral application of L-NMMA, ODQ and KT-5823 was able to decrease the peripheral antinociceptive actions of morphine and dipyrone. Thus, data support the participation of the NO–cyclic GMP–PKG pathway in the peripheral antinociception produced by gabapentin.

The standardised nomenclature for potassium channels recognizes four different types of K^+ channels known as voltage-gated (K_v), calcium-activated (K_{Ca}), inward rectifier (K_{ir}) and finally two-pore (K_{2P}) K^+ channels (Gutman et al., 2003). Besides Ca^{2+}, ATP and voltage, K^+ channels are also modulated by other messengers such as protein kinase (Ewald et al., 1985), cyclic GMP (Yao et al., 1996), phosphatases (White et al., 1993) and G proteins (Gutman et al., 2003). The inward rectifier K^+ channels have been organised into three groups, namely the inward rectifier channels, the ATP-sensitive channels and the G protein-coupled channels (Doupnik et al., 1995; Kubo et al., 2002). In the present study, local peripheral administration of glibenclamide and tolbutamide, ATP-sensitive K^+ channel inhibitors (Edwards and Weston, 1993), was able to reduce the antinociceptive action of gabapentin, suggesting that this drug could activate these channels. These results agree with previous observations showing that glibenclamide reduced the decrease of K^+-evoked [3H]noradrenaline release induced by gabapentin in human neocortical slices (Freiman et al., 2001). In line with this assumption, our group recently has observed that gabapentin may activate ATP-sensitive K^+ channels in order to produce part of its spinal antiallodynic effect in the Chung model of neuropathic pain (Mixcoatl-Zecuatl et al., 2004).

At the moment, there are not selective blockers of inward rectifier and G protein-coupled K^+ channels (Kubo et al., 2002). However, Ba^{2+}, Cs^+, Mg^{2+}, polyamines, 4-aminopyridine (4-AP) and tetraethylammonium (TEA) are indicated as blockers of these channels (Kubo et al., 2002). In the present study, we were able to find that the local peripheral administration of 4-AP and TEA dose-dependently reduced the antinociceptive effect produced by gabapentin. Previously, it has been demonstrated that gabapentin is able to induce inwardly rectifying K^+ currents (K_{ir}), sensitive to Ba^{2+} and Cs^+ (Bertrand et al., 2003). Therefore, the participation of inward rectifier K^+ channels and/or G protein-coupled K^+ channels is strongly suggested, but since there are no selective blockers the data awaits supplementary validation.

On the other hand, in this study, the local peripheral administration of a large conductance Ca^{2+}-activated K^+ channel inhibitor (charybdotoxin) (Reinhart et al., 1989) dose-dependently reduced the antinociceptive effect produced by gabapentin, suggesting that large-conductance Ca^{2+}-activated K^+ channels are involved on gabapentin-induced antinociceptive activity. Previously, our group published that the spinal pretreatment with charybdotoxin significantly reduced gabapentin-induced antiallodynia in the rat (Mixcoatl-Zecuatl et al., 2004). In opposite direction, local peripheral administration of the small-conductance Ca^{2+}-activated K^+ channel inhibitor apamin was not able to reduce the antinociceptive action induced by gabapentin at the same doses that in other experiments blocked the antinociception produced by diclofenac, pinacidil or atrial natriuretic peptide (Ortiz et al., 2003).

Then, our data do not support the participation of small-conductance Ca^{2+}-activated K^+ channel in the peripheral antinociception induced by gabapentin. The lack of effect of the small-conductance Ca^{2+}-activated K^+ channel blocker has been previously observed in other studies in which the antinociceptive actions of indomethacin, morphine, dibutyryl-cyclic GMP or fentanyl (Rodrigues and Duarte, 2000; Soares and Duarte, 2001; Ortiz et al., 2003; Rodrigues et al., 2005) were not influenced by apamin.

The mechanism responsible for the activation of the NO–cyclic GMP–PKG–K^+ channel peripheral pathway has not been elucidated previously and we considered several potential pathways. First, it has been found that gabapentin is able to activate the $GABA_B$ receptor (Ng et al., 2001; Bertrand et al., 2003; Parker et al., 2004). $GABA_B$ receptors are coupled to Gi/Go proteins and its activation can inhibit adenylyl cyclase activity (Franek, 2004). Activation or increase in expression of nitric oxide synthase has been shown to be mediated by Gi/Go proteins (Hare et al., 1998; Wyckoff et al., 2001). Therefore, these data suggest that gabapentin may be able to activate $GABA_B$ receptors, which in turn would activate nitric oxide synthase to produce NO.

Our data demonstrated that the local peripheral administration of the opioid receptor antagonist naloxone was not able to reduce the antinociceptive action induced by gabapentin, suggesting that activation of opioid receptors are not involved in the peripheral mechanism of action of gabapentin. The lack of action of naloxone in our study was similar to that previously published in other models of pain (Field et al., 1997; Dixit and Bhargava, 2002; Granados-Soto et al., 2004).

When applied alone, neither the NO–cyclic GMP–PKG pathway inhibitors nor the K^+ channel blockers affected formalin-induced nociceptive behavior. The lack of effect of these compounds is consistent with results of studies in which these drugs were not able to modify the nociceptive activity of thermal noxious stimulus, formalin-induced nociception and mechanical hyperalgesia (Welch and Dunlow, 1993; Rodrigues and Duarte, 2000; Ortiz et al., 2003; Sachs et al., 2004), thus excluding the possibility that the inhibition of gabapentin antinociception could be due to a hyperalgesic or nociceptive effect of the blockers used. The lack of modification of the flinching behavior by the different modulators at concentrations able to reduce gabapentin antinociception might also indicate that the NO–cyclic GMP–PKG–K^+ channel pathway in subcutaneous tissue involved in the modulation of pain is not tonically activated.

Results show that gabapentin was able to produce peripheral antinociception on the 1% formalin test. Likewise, this study suggests that gabapentin might activate K^+ channels in subcutaneous tissue by an indirect mechanism that involves activation of the NO–cyclic GMP–PKG pathway. As it was already mentioned, gabapentin is able to inhibit calcium currents in neurons isolated of the adult rat brain (Stefani et al., 1998). Taken together, the results obtained demonstrate that, besides the inhibition of Ca^{2+} channels, gabapentin is able to produce peripheral antinociception by the activation of the NO–cyclic GMP–PKG–K^+ channels pathway.

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