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Pharmacological evidence for the participation of NO–cyclic GMP–PKG–K^+ channel pathway in the antiallodynic action of resveratrol

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Abstract

The possible participation of the nitric oxide (NO)–cyclic GMP–protein kinase G (PKG)–K^+ channels pathway in the antiallodynic action of resveratrol and YC-1 in spinal nerve injured rats was assessed. Ligation of L5/L6 spinal nerves produced a clear-cut tactile allodynia in the rats. Intrathecal administration of resveratrol (100–600 μg) and 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (0.1–2.7 μg, YC-1, a soluble guanylyl cyclase activator) decreased tactile allodynia induced by ligation of L5/L6 spinal nerves. Intrathecal treatment with N^G-L-nitro-arginine methyl ester (10–100 μg, L-NAME, a NO synthase inhibitor), 1H-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (1–10 μg, ODQ, a soluble guanylyl cyclase inhibitor), KT-5823 (5–500 ng, a PKG inhibitor) and iberiotoxin (5–500 ng, a large-conductance Ca^{2+}-activated K^+ channel blocker), but not N^G-D-nitro-arginine methyl ester (100 μg, D-NAME, an inactive isomer of L-NAME), glibenclamide (12.5–50 μg, ATP-sensitive K^+ channel blocker) or vehicle, significantly diminished resveratrol (300 μg)- and YC-1 (2.7 μg)-induced spinal antiallodynia. These effects were independent of prostaglandin synthesis inhibition as indomethacin did not affect resveratrol-induced antiallodynia. Results suggest that resveratrol and YC-1 could activate the proteins of the NO–cyclic GMP–PKG spinal pathway or large-conductance Ca^{2+}-activated, but not ATP-sensitive, K^+ channels at the spinal cord in order to produce at least part of their antiallodynic effect in this model of neuropathy.

Keywords: Neuropathic pain; Resveratrol; YC-1; K^+ channel; L-NAME; ODQ; Iberiotoxin; Glibenclamide

1. Introduction

Resveratrol (3, 4′, 5-trihydroxystilbene) is a naturally occurring phytoalexin present in grapes and wine. It is believed that resveratrol produces its affects *via* different mechanisms (for a review see Granados-Soto, 2003). Resveratrol exhibits antioxidant activity (Chanvitayapongs et al., 1997; Hung et al., 2004; King et al., 2005). In addition, resveratrol induces up-regulation of endothelial nitric oxide (NO) synthase gene expression and activity (Wallerath et al., 2002, 2005). This drug also increases cyclic GMP levels by activating particulate guanylyl cyclase (El-Mowafy, 2002). Resveratrol also stimulates the activity of large-conductance Ca^{2+}-activated K^+ channels in cultured endothelial cells (Li et al., 2000) and this effect has been associated with its antinociceptive effect in the formalin test (Granados-Soto et al., 2002). Taken together it seems that resveratrol activates the nitric oxide–cyclic GMP–K^+ channel pathway in order to produce its effects.

Previous studies have shown that NO and cyclic GMP can activate several targets including PKG and different types of K^+ channels (Bolotina et al., 1994; Lucas et al., 2000; Han et al., 2002; Levy and Strassman, 2004). Other studies have shown that endogenous NO in the spinal cord is important for the antiallodynic action of intrathecal clonidine (an α_2-adrenoceptor agonist),

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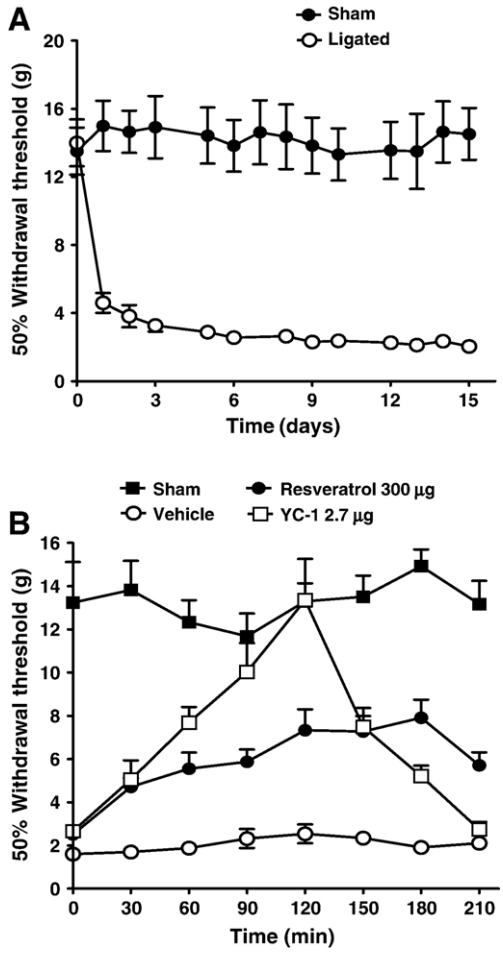

Fig. 1. A) Time course of development of tactile allodynia in rats with ligation of L5 and L6 spinal nerves compared to sham operated rats. B) Time course of the spinal antiallodynic effect of resveratrol and YC-1 in rats submitted to ligation of L5 and L6 spinal nerves. Rats were treated with intrathecal vehicle, resveratrol or YC-1 30 min before starting paw withdrawal threshold evaluations. Data are the mean ± S.E.M. for six to seven animals. A significant difference ($P<0.05$, by two-way ANOVA, followed by the Tukey's test) was observed between vehicle (control) and either resveratrol or YC-1 groups 30 min after starting threshold evaluations. Asterisks indicating significant difference were omitted for the sake of clarity.

neostigmine (an acetylcholinesterase inhibitor) and DPDPE (a δ opioid receptor agonist) (Pan et al., 1998; Chen et al., 2001; Chen and Pan, 2003). Our group (Lázaro-Ibáñez et al., 2001; Ortiz et al., 2002; Ambriz-Tututi et al., 2005; Mixcoatl-Zecuatl et al., 2006) and others (Soares et al., 2000; Soares and Duarte, 2001; Sachs et al., 2004) have reported that drugs which activate the NO–cyclic GMP pathway seem to modulate the opening of K^+ channels in order to produce antinociception.

Based on the above considerations, this work was undertaken to determine whether the NO–cyclic GMP–PKG–K^+ channel pathway participates on the antiallodynic effect induced by spinal resveratrol in the Kim and Chung model of neuropathy. YC-1, a NO-independent soluble guanylyl cyclase activator, was assayed as positive control as there is evidence that this drug increases the expression of guanylyl cyclase (Wu et al., 2004a, b), production of cyclic GMP (Friebe et al., 1996; Demirkoprulu et al., 2005) and activation of PKG (Deguchi et al., 2005). In addition, YC-1 can behave as a K^+ channel opener (Seitz et al., 1999; Wu et al., 2000). In addition, to test the possible participation of the cyclo-oxygenase pathway in the resveratrol's antiallodynic activity, the effect of indomethacin on resverastrol-induced antiallodynic activity was assessed.

2. Materials and methods

2.1. Animals

Female Wistar rats aged 6–7 weeks (weight range, 140–160 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. Experiments were carried out at the same hours of the day (10:00–16:00 h). All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). Additionally, the Institutional Animal Care and Use Committee approved the study (Centro de Investigación y de Estudios Avanzados, 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 10 days. Rats exhibiting motor deficiency (such as paw-dragging) were discarded from testing.

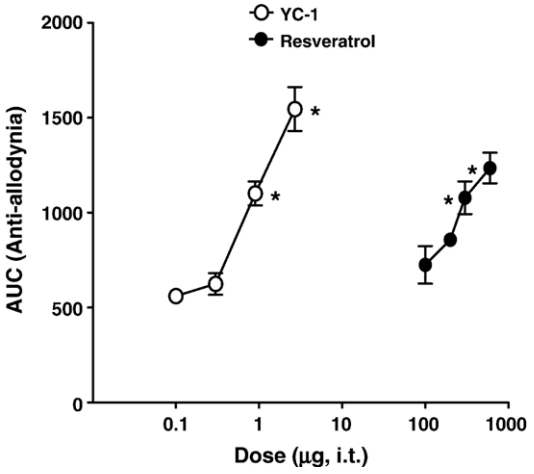

Fig. 2. Dose–response curves for the antiallodynic effect of resveratrol and YC-1 in rats submitted to ligation of L5 and L6 spinal nerves. Rats were treated with vehicle or increasing doses of resveratrol or YC-1 30 min before starting thresholds evaluations. Data are expressed as the area under the 50% withdrawal threshold against time curve (AUC). Symbols are the mean ± S.E.M. for six to seven animals. *Significantly different from the vehicle group, as determined by one-way ANOVA followed by the Tukey's test.

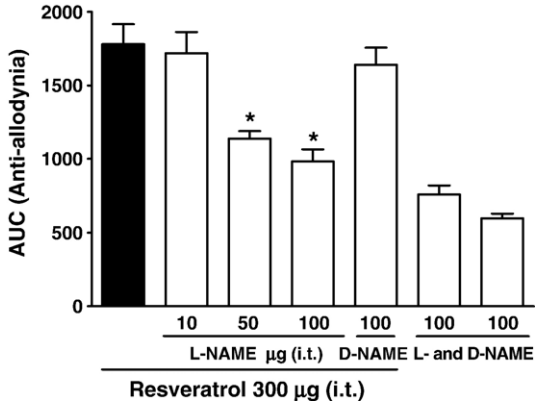

Fig. 3. Effect of N^G-L-nitro-arginine methyl ester (L-NAME) or N^G-D-nitro-arginine methyl ester (D-NAME) on resveratrol-induced spinal antiallodynic activity in rats submitted to ligation of L5 and L6 spinal nerves. Rats were treated with intrathecal vehicle or resveratrol (−30 min) and increasing doses of L-NAME (−20 min) before starting threshold evaluations. Data are expressed as the area under the 50% withdrawal threshold against time curve (AUC). Bars are the mean ± S.E.M. for six to seven animals. *Significantly different from the resveratrol group, as determined by one-way ANOVA followed by the Tukey's test.

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

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

2.4. Drugs

Resveratrol, indomethacin, iberiotoxin, glibenclamide, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1), (9S,10R,12R)-2,3,9,10,11,12-hexahydro-10-methoxy-2,9-dimethyl-1-oxo-9,12-epoxy-1H-diindolo-[1,2,3-fg:3′,2′,1′-kl] pyrrolo[3,4-*i*][1,6]-benzodiazocine-10-carboxylic acid methyl ester (KT-5823) and 1H-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ) were obtained from Sigma (St. Louis, MO, USA). N^G-L-nitro-arginine methyl ester (L-NAME) and N^G-D-nitro-arginine methyl ester (D-NAME) were purchased from Research Biochemical International (Natick, MA, USA). L-NAME and D-NAME were dissolved in saline. Iberiotoxin and KT-5823 were dissolved in 20% dimethylsulfoxide (DMSO). ODQ was dissolved in 50% DMSO. Resveratrol, YC-1, indomethacin and glibenclamide were dissolved in 100% DMSO.

2.5. Experimental protocols

Rats received a spinal injection of vehicle or increasing doses of resveratrol (100–600 µg) 30 min before evaluation of withdrawal threshold in spinal nerve injured rats. To determine whether resveratrol-induced antiallodynia was mediated by NO–cyclic GMP–PKG–K^+ channel pathway activation, effect of treatment (−20 min) with the appropriate vehicle (see above) or L-NAME (10–100 µg), D-NAME (100 µg), ODQ (1–10 µg), KT-5823 (5–500 ng), iberiotoxin (5–500 ng) and glibenclamide (12.5–50 µg) on the antiallodynic effect induced by resveratrol (−30 min, 300 µg) was assessed. In order to discharge the possible participation of the inhibition of cyclo-oxygenase in resveratrol-induced antiallodynic effect, effect of treatment (−10 or +10 min) with the appropriate vehicle (see above) or indomethacin (100 µg) on the antiallodynic effect induced by resveratrol (300 µg) was assessed.

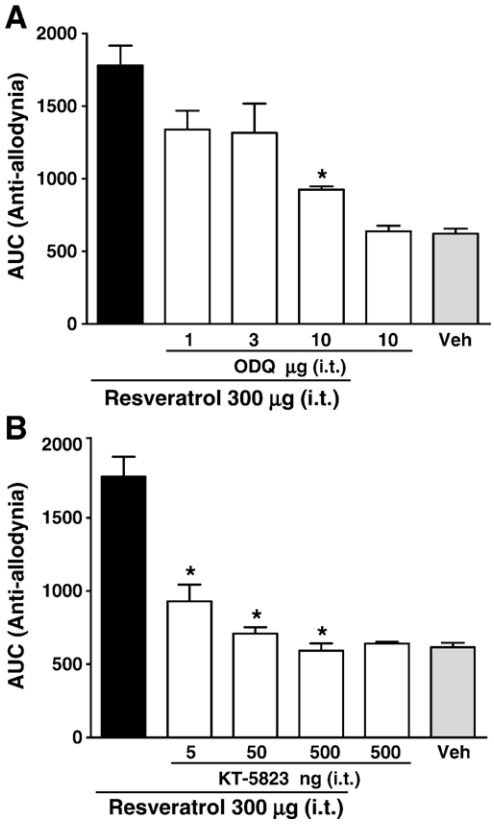

Fig. 4. Effect of 1H-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ) (panel A) and KT-5823 (panel B) on resveratrol-induced spinal antiallodynic activity in rats submitted to ligation of L5 and L6 spinal nerves. Rats were treated with intrathecal vehicle or resveratrol (−30 min) and increasing doses of ODQ or KT-5823 (−20 min) before starting thresholds evaluations. Data are expressed as the area under the 50% withdrawal threshold against time curve (AUC). Bars are the mean ± S.E.M. for six to seven animals. *Significantly different from the resveratrol group, as determined by one-way ANOVA followed by the Tukey's test.

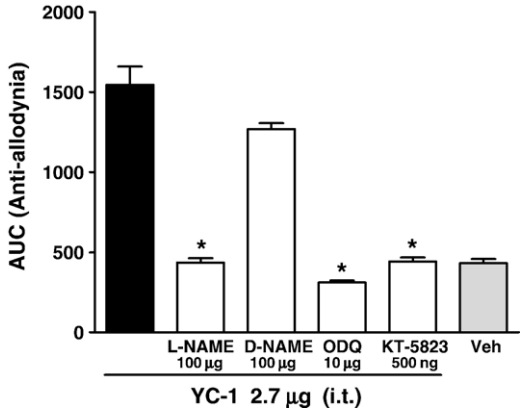

Fig. 5. Effect of vehicle, N^G-L-nitro-arginine methyl ester (L-NAME), N^G-D-nitro-arginine methyl ester (D-NAME), 1H-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ) and KT-5823 on YC-1-induced spinal antiallodynic activity in rats submitted to ligation of L5 and L6 spinal nerves. Rats were treated with intrathecal vehicle or YC-1 (−30 min) and fixed doses of L-NAME, D-NAME, ODQ or KT-5823 (−20 min) before starting thresholds evaluations. Data are expressed as the area under the 50% withdrawal threshold against time curve (AUC). Bars are the mean±S.E.M. for six to seven animals. *Significantly different from the YC-1 group, as determined by one-way ANOVA followed by the Tukey's test.

YC-1 (0.1–2.7 µg, − 30 min) was used as positive control as there is evidence that their effects are mainly due to cyclic GMP (Friebe et al., 1996; Seitz et al., 1999; Wu et al., 2004a,b; Demirkoprulu et al., 2005) and opening of K^+ channels (Seitz et al., 1999; Wu et al., 2000). The dose of YC-1 (2.7 µg) producing the maximum antiallodynic effect was chosen for co-administration with L-NAME (100 µg), ODQ (10 µg), KT-5823 (500 ng), iberiotoxin (500 ng) and glibenclamide (50 µg).

Drugs were injected in a volume of 10 µl. Each rat received 2 spinal injections and appropriate controls for the injections and vehicles were performed before starting the formal study. Doses and drug administration schedule of different inhibitors and resveratrol for spinal administration were selected based on previous reports (Mixcoatl-Zecuatl et al., 2004; Lozano-Cuenca et al., 2005) and on pilot experiments in our laboratory. Observer was unaware of the treatment in each animal. 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, as proposed elsewhere (Chen and Pan, 2001).

2.6. Data analysis and statistics

All experimental results are given as the mean±S.E.M. for six to seven animals per group. Curves were constructed plotting the threshold for paw withdrawal as a function of time. An increase of 50% withdrawal threshold was considered as antiallodynic effect. Area under the 50% withdrawal threshold against time curve (AUC) for a period of 210 min was calculated by the trapezoidal method. One- or two-way 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. Spinal antiallodynic effect of resveratrol and YC-1

Ligation of L5/L6 spinal nerves produced a clear-cut allodynia in rats submitted to surgery compared to the sham operated rats (Fig. 1A). Intrathecal administration of resveratrol (300 µg) and YC-1 (2.7 µg), but not vehicle, reduced tactile allodynia induced by ligation of L5/L6 spinal nerves (Fig. 1B). The antiallodynic effects of resveratrol and YC-1 were dose-dependent ($P<0.05$) (Fig. 2). No changes in behavioral or motor function were observed in either group, control or treated (data not shown).

3.2. Effect of L-NAME, D-NAME, ODQ and KT-5823 on the intrathecal antiallodynic activity of resveratrol and YC-1

Intrathecal treatment with the non-specific inhibitor of NO synthase L-NAME (10–100 µg), but not with the inactive isomer of L-NAME, D-NAME (100 µg) or vehicle, significantly ($P<0.05$) reversed the antiallodynic effect induced by the spinal administration of resveratrol 300 µg (Fig. 3). At the greatest tested

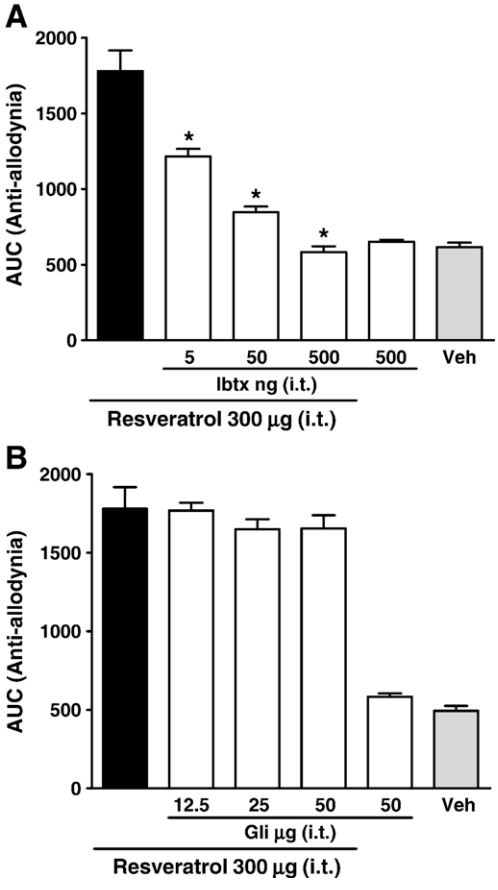

Fig. 6. Effect of iberiotoxin (Ibtx) and glibenclamide (Gli) on resveratrol-induced spinal antiallodynic activity in rats submitted to ligation of L5 and L6 spinal nerves. Rats were treated with intrathecal resveratrol (−30 min) and iberiotoxin or glibenclamide (−20 min) before starting threshold evaluations. Data are expressed as the area under the 50% withdrawal threshold against time curve (AUC). Bars are the mean±S.E.M. for six to seven animals. *Significantly different from the resveratrol group, as determined by one-way ANOVA followed by the Tukey's test.

dose, L-NAME did not modify tactile allodynia in the rats (Fig. 3). Moreover, intrathecal treatment with the guanylyl cyclase inhibitor ODQ (1–10 μg, Fig. 4A) and the PKG inhibitor KT-5823 (5–500 ng, Fig. 4B), but not vehicle, significantly diminished the antiallodynic effect of intrathecal resveratrol ($P<0.05$). In contrast, intrathecal treatment with the greatest tested doses of ODQ (10 μg) and KT-5823 (500 ng) did not produce any effect on spinal nerves ligation-induced tactile allodynia (Fig. 4A and B).

As with resveratrol, intrathecal administration of L-NAME (100 μg), ODQ (10 μg) and KT-5823 (500 ng), but not D-NAME (100 μg) or vehicle, significantly reduced YC-1 (2.7 μg)-induced spinal antiallodynia ($P<0.05$, Fig. 5).

3.3. Effect of iberiotoxin and glibenclamide on the intrathecal antiallodynic activity of resveratrol and YC-1

Intrathecal treatment with iberiotoxin (5–500 ng, a large-conductance Ca^{2+}-activated K^+ channel blocker), but not vehicle, significantly reversed ($P<0.05$) the antiallodynic effect of intrathecal resveratrol 300 μg (Fig. 6A). Contrariwise, spinal treatment with glibenclamide (12.5–50 μg, an ATP-sensitive K^+ channel blocker) was not able to modify ($P>0.05$) resveratrol (300 μg)-induced spinal antiallodynic effect (Fig. 6B). Given alone K^+ channel blockers did not produce any effect on spinal nerve ligation-induced tactile allodynia (Fig. 6A and B).

As with resveratrol, intrathecal administration of iberiotoxin (500 ng), but not glibenclamide (50 μg) or vehicle, significantly ($P<0.05$) reduced YC-1 (2.7 μg)-induced spinal antiallodynia ($P<0.05$, Fig. 7).

3.4. Effect of indomethacin on the intrathecal antiallodynic activity of resveratrol

Intrathecal administration of indomethacin (100 μg) was not able to reduce tactile allodynia in neuropathic rats. Moreover, indomethacin, given as pretreatment (−10 min) or postreatment (+10 min), did not modify resveratrol-induced antiallodynic effect (Table 1).

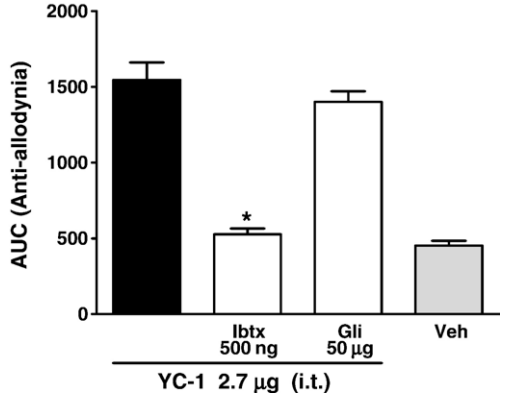

Fig. 7. Effect of iberiotoxin (Ibtx) and glibenclamide (Gli) on YC-1-induced spinal antiallodynic activity in rats submitted to ligation of L5 and L6 spinal nerves. Rats were treated with intrathecal YC-1 (−30 min) and fixed doses of iberiotoxin or glibenclamide (−20 min) before starting threshold evaluations. Data are expressed as the area under the 50% withdrawal threshold against time curve (AUC). Bars are the mean±S.E.M. for six to seven animals. *Significantly different from the YC-1 group, as determined by one-way ANOVA followed by the Tukey's test.

Table 1
Effect of indomethacin on the spinal antiallodynic activity of resveratrol in spinal nerve injured rats

Treatment (μg, it)	Effect (AUC, anti-allodynia)
Vehicle (DMSO 100%)	598.3±38.0
Resveratrol 300+Vehicle	1780.7±136.0 *
Resveratrol 300+Indomethacin 100 [a]	1952.7±65.6 *
Indomethacin 100+Resveratrol 300 [b]	1686.8±61.3 *
Indomethacin 100	631.7±33.1

[a] Indomethacin was administered 10 min after resveratrol.
[b] Indomethacin was given 10 min before resveratrol.
* Significantly different from vehicle ($P<0.05$) by one-way ANOVA followed by the Tukey's Test.

4. Discussion

4.1. Spinal antiallodynic effect of resveratrol and YC-1

In this study we have shown that intrathecal administration of resveratrol is able to produce antiallodynic effect in rats with neuropathic pain. Previously it has been reported that resveratrol produces antinociceptive effect in acute (Gupta et al., 2004) and inflammatory (Gentilli et al., 2001; Torres-López et al., 2002; Granados-Soto et al., 2002; Elmali et al., 2005) pain models after peripheral or systemic administration. Thus, we have extended these observations by showing that spinal resveratrol significantly reduced spinal nerve ligation-induced tactile allodynia. To our knowledge, this is the first report about the spinal antiallodynic effect of resveratrol in a neuropathic pain model. Resveratrol has several effects (Granados-Soto, 2003) which could be responsible for the antiallodynic effects observed in this study. Particularly, resveratrol has been reported to increase the expression and activity of endothelial and inducible NO synthase (Wallerath et al., 2002; Imamura et al., 2002) and particulate guanylyl cyclase (El-Mowafy, 2002) thus activating the NO–cyclic GMP pathway. However, the exact mechanism of action of resveratrol has not been assessed.

In this study we have used the NO-independent soluble guanylyl cyclase activator YC-1 as positive control as there is evidence that this drug increases the expression of guanylyl cyclase (Wu et al., 2004a,b) and consequently increases cyclic GMP (Friebe et al., 1996; Demirkoprulu et al., 2005). More recently it has been suggested that YC-1 could directly or indirectly activate PKG (Deguchi et al., 2005). Therefore, it seems that this drug may be able to activate the cyclic GMP–PKG pathway. In our conditions, YC-1 produced a dose-dependent antiallodynic effect in neuropathic rats. This is the first report about the antiallodynic properties of YC-1. The reduction of tactile allodynia could result from activation of the above mentioned pathway.

It has been recently reported that resveratrol (Li et al., 2000; Granados-Soto et al., 2002) and YC-1 (Seitz et al., 1999; Wu et al., 2000) can behave as K^+ channel openers. Since previous evidence has shown that K^+ channels can be activated by NO

(Bolotina et al., 1994; Archer et al., 1994), cyclic GMP (Lucas et al., 2000) or cyclic GMP-dependent PKG (Han et al., 2002), the possible participation of the NO–cyclic GMP–PKG–K^+ channels spinal pathway in resveratrol-, and YC-1-induced spinal antiallodynic activity was assessed in this study.

4.2. Effect of L-NAME, D-NAME, ODQ and KT-5823 on the intrathecal antiallodynic activity of resveratrol and YC-1

The spinal antiallodynic effect of resveratrol and YC-1 was diminished by the non-specific inhibitor of NO synthase L-NAME (Gibson et al., 1990), soluble guanylyl cyclase inhibitor ODQ (Moro et al., 1996) and specific PKG inhibitor KT-5823 (Grider, 1993), but not by the inactive isomer of L-NAME, D-NAME. These results suggest that the NO–cyclic GMP–PKG spinal pathway is partially involved in resveratrol- or YC-1-induced spinal antiallodynic effect. Our data agree with evidence showing that the relaxant effects produced by resveratrol (Li et al., in press) and YC-1 (Che et al., 2005; Demirkoprulu et al., 2005) are blocked by L-NAME and ODQ. Accordingly, resveratrol (Wallerath et al., 2002; Das et al., 2005) and YC-1 (Che et al., 2005) are able to increase the NO release and expression of NO synthase. Thus data suggest that YC-1 is not a soluble guanylyl cyclase direct activator as its effect depends on NO production. However, this characteristic makes this drug more suitable to be used as positive control in this study. Moreover, resveratrol (El-Mowafy, 2002) and YC-1 (Ko et al., 1994; Friebe et al., 1996; Friebe and Koesling, 1998; Seitz et al., 1999; Demirkoprulu et al., 2005) have been shown to increase cyclic GMP by stimulation of particulate and soluble guanylyl cyclase, respectively. YC-1 is also able to activate PKG (Deguchi et al., 2005), further contributing to the activation of the pathway. In line with these observations it has been reported the presence of all components of this pathway in the spinal cord (Tao and Johns, 2002).

Activation, by resveratrol or YC-1, of the NO–cyclic GMP–PKG spinal pathway could reduce tactile allodynia by a direct effect on the electrical activity of spinal neurons of lamina I and II (Pehl and Schmid, 1997; Levy and Strassman, 2004; Sung et al., 2004) or by decreasing the activity of voltage-gated tetrodotoxin-resistant Na^+channels (Liu et al., 2004). In addition, resveratrol could synergize this effect by directly inhibiting sodium currents (Kim et al., 2005). Our group has recently reported that activation of this spinal pathway also participates in the spinal antiallodynic effect of gabapentin (Mixcoatl-Zecuatl et al., 2006), as well as in the peripheral and spinal antinociception of sildenafil and lumiracoxib in the formalin test (Ambriz-Tututi et al., 2005; Araiza-Saldaña et al., 2005; Lozano-Cuenca et al., 2005).

Other studies in the literature indicate that the NO (Inoue et al., 1998; Yoon et al., 1998) or the NO–cyclic GMP (Salter et al., 1996) pathway can have pronociceptive rather than antinociceptive effects in neuropathic pain. This discrepancy may be due to the different experimental pain models used, diverse tissue level and the variant NO and cyclic GMP intracellular content (Pehl and Schmid, 1997; Sousa and Prado, 2001). Nevertheless, it is important to point out that up-regulation of neuronal nitric oxide synthase is not responsible for the development and/or maintenance of allodynia after nerve injury (Luo et al., 1999). In addition, in the L5/L6 spinal nerve ligation model (Pan et al., 1998; Chen et al., 2000) and other models (Chen et al., 2001; Wu et al., 2004a,b) of neuropathic pain, production of NO and cyclic GMP is involved in antiallodynia.

4.3. Effect of iberiotoxin and glibenclamide on the intrathecal antiallodynic activity of resveratrol and YC-1

Based on the observations that resveratrol may induce local peripheral antinociception in the formalin test by activation of Ca^{2+}-activated K^+ channels (Granados-Soto et al., 2002) and the fact that YC-1-induced relaxation of vascular smooth muscle depends on the K^+ channel activation (Seitz et al., 1999), we decided to assess the possible participation of K^+ channels on resveratrol- and YC-1-induced antiallodynia. The specific large-conductance Ca^{2+}-activated K^+ channel blocker iberiotoxin (Galvez et al., 1990), but not the ATP-sensitive K^+ channel inhibitor glibenclamide (Amoroso et al., 1990), significantly diminished the antiallodynic effect of resveratrol and YC-1. These results suggest the participation of large-conductance Ca^{2+}-activated, but not ATP-sensitive, K^+ channels at the spinal cord in the antiallodynic effects of resveratrol and YC-1. Data agree with an electrophysiological study in which resveratrol was able to increase the amplitude of K^+ outward current by increasing mean open time of large-conductance Ca^{2+}-activated K^+ channels in cultured endothelial cells (Li et al., 2000). Data also agree with a functional study showing that the relaxant effect of YC-1 was blocked by the large-conductance Ca^{2+}-activated K^+ channel blockers charybdotoxin and iberiotoxin, but not by the ATP-sensitive K^+ channel blocker glibenclamide (Seitz et al., 1999). Our data are in line with those showing that YC-1 is able to increase the activity and K^+ current of large-conductance Ca^{2+}-activated K^+ channel (Seitz et al., 1999; Wu et al., 2000) in a soluble guanylyl cyclase-independent way.

Taken together data suggest that resveratrol and YC-1 could produce their spinal antiallodynic effect by activation of the NO–cyclic GMP–PKG pathway which in turn would phosphorylate large-conductance Ca^{2+}-activated K^+ channels at the spinal cord, as reported in other tissues (Archer et al., 1994; Han et al., 2002).

4.4. Effect of indomethacin on the intrathecal antiallodynic activity of resveratrol

Previous evidence has shown that resveratrol may block prostaglandin synthesis by inhibiting cyclooxygenase-1 and 2 (COX-1 and COX-2) (Subbaramaiah et al., 1998). Resveratrol and the combination of resveratrol + indomethacin (a non-selective COX inhibitor) were administered after neuropathic pain was established (15 days). In these conditions, pretreatment (−10 min) or postreatment (+10 min) with indomethacin did not modify resveratrol-induced spinal antiallodynic activity thus suggesting that spinal inhibition of prostaglandins do not participate in the antiallodynic effect of resveratrol in this model. These results agree with data showing that indomethacin and

other anti-inflammatory drugs (Lashbrook et al., 1999; Takeda et al., 2005; LaBuda and Little, 2005), given after neuropathic pain is established, do not affect tactile allodynia in this model. However, some evidence suggests that anti-inflammatory drugs may have a prophylactic effect in neuropathic pain if they are administered before o immediately after nerve injury (Hefferan et al., 2003; Takahashi et al., 2004).

In conclusion, resveratrol and YC-1 reduced tactile allodynia in neuropathic rats. The spinal antiallodynic effect of resveratrol and YC-1 was partially diminished by L-NAME, ODQ, KT-5823, iberiotoxin, but not by D-NAME or glibenclamide. These results suggest that resveratrol and YC-1 may activate the NO–cyclic GMP–PKG-large-conductance Ca^{2+}-activated K^+ channel spinal pathway in this model of neuropathy. The effects of resveratrol are independent of the prostaglandin synthesis inhibition.

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