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Spinal antinociception evoked by the triterpene 3β, 6β, 16β-trihydroxylup-20(29)-ene in mice: Evidence for the involvement of the glutamatergic system via NMDA and metabotropic glutamate receptors

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ABSTRACT

The present study investigated the possible involvement of the glutamatergic and neurokinin systems in the antinociception caused by triterpene 3β, 6β, 16β-trihydroxylup-20(29)-ene (TTHL) in mice. TTHL given by intraperitoneal (i.p., 2.1–65.5 μmol/kg), intraplantar (i.pl., 6.5–65.5 nmol/paw) or intrathecal (i.t., 21.8–655 nmol/site) routes, produced dose-dependent inhibition of glutamate-induced nociception with ID_{50} values of 12 μmol/kg; 34.2 nmol/paw; 233.8 nmol/site and inhibitions of 78 ± 6; 82 ± 4 and 77 ± 8%, respectively. I.t. injection of TTHL (6.5–218 nmol/site, co-administered) also caused significant and dose-dependent reduction of nociceptive response induced by i.t. injection of glutamate (175 nmol/site), with ID_{50} value of 54.5 nmol/site and inhibition of 51 ± 6%. Moreover, TTHL (65.5 nmol/site) co-injected by i.t. route with agonist caused marked reduction of nociceptive responses induced by N-methyl-D-aspartate (NMDA, 450 pmol/site), (±)-1-aminocyclopentane-trans-1,3 dicarboxylic acid (trans-ACPD, 10 nmol/site) and substance P (100 pmol/site), with inhibitions of 81 ± 7; 79 ± 7; 81 ± 11%, respectively. Conversely, TTHL had no effect on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, 135 pmol/site) and kainic acid (kainate, 110 pmol/site)-induced nociception. Moreover, the association of sub-effective doses of TTHL (6.5 nmol/site, i.t.) and MK-801(1 nmol/site, i.t.; non-competitive NMDA antagonist) or (RS)-MCPG (30 nmol/site, i.t.; non-selective group I/group II metabotropic glutamate receptor antagonist) produced a synergic antinociceptive effect in the nociception induced by NMDA or trans-ACPD, respectively. Together, these results provide experimental evidence for the involvement of the glutamatergic system (NMDA and metabotropic glutamate receptors) in the antinociceptive action caused by TTHL in mice.

1. Introduction

Combretum leprosum Mart., a member of the Combretaceae family, is widely distributed in the North of Brazil and has been used in folk medicine to heal wounds, to treat hemorrhages or as a sedative (Facundo et al., 1993, 2005). Phytochemical studies have revealed the presence of flavonols 3-o-metilquercetina and 3-o-α-L-ramnopirano-silquercitrina and triterpenes mollic acid, arjunolic acid and 3β, 6β, 16β-trihydroxylup-20(29)-ene in this plant (Facundo et al., 1993).

It is well established that the excitatory amino acid glutamate plays an important role on nociceptive pathways, either in central or peripheral nervous system, due to the fact that glutamate is stored in sensory C fibers which are responsible for the transmission of nociceptive stimulus in the spinal cord (Bleakman et al., 2006; Fundytus, 2001; Millan, 1999). Glutamate exerts its effects through both ionotropic and metabotropic receptors.

Several studies have shown, by using glutamatergic-selective receptor agonists and antagonists, that all subtypes of ionotropic and metabotropic glutamate receptors play significant roles in the establishment of pain (Jesse et al., 2008; Neugebauer, 2001; Sawynok, 2003; Varney and Gereau, 2002). In this regard, it has been reported that modulation of glutamate receptors may have potential therapeutic activity, since this modulation exerts an antinociceptive effect in several mammalian species including humans (Gadotti et al., 2006; Lutfy et al., 1997; Rosa et al., 2005; Wiech et al., 2004). Nevertheless, the clinical use of these drugs as analgesics is restricted due to the undesirable side effects (Millan, 1999).

Moreover, it was reported by Biasi and Rustioni (1988) that the activation of nociceptors resulting from tissue damage, inflammation or

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nerve injury evokes co-releasing of glutamate and substance P from primary afferents neurons either peripherally (Carlton et al., 1995;1998) or centrally (Dougherty and Willis, 1991; Randic et al., 1990) (for review see Chizh, 2002). It has also been demonstrated that substance P increases glutamate-induced nociception and that the neurotransmitters glutamate and substance P can act synergistically in the transmission of pain (Battaglia and Rustioni, 1988; Beirith et al., 2003; Malcangio et al., 1998; Teoh et al., 1996; Siebel et al., 2004).

In a previous study, we reported that the ethanolic extract obtained from the flowers of *C. leprosum* caused dose-related antinociception in several models of chemical and thermal pain (Pietrovski et al., 2006). Additionally, it was demonstrated that 3β, 6β, 16β-trihydroxylup-20 (29)-ene (TTHL, Fig. 1), given by oral route, produced a significant and dose-dependent inhibition of glutamate-induced nociception (Pietrovski et al., 2006).

Regarding that glutamate plays important role in both peripheral and central pain transmission, this study aimed: i) to evaluate the antinociceptive effect of TTHL, administered either peripherically (by intraplantar route), systemically (by intraperitoneal route), or centrally (by intrathecal route), against nociception induced by intraplantar injection of glutamate in the mouse hind paw, which causes a direct stimulation of peripheral nociceptors; ii) we also investigated the involvement of the spinal glutamatergic system by stimulation of spinal cord (central) neurons through intrathecal injection of glutamate receptor agonists or antagonists and neurokinin substance P.

2. Material and methods

2.1. Animals

Experiments were conducted using Swiss mice of both sexes (25–35 g), housed at 22 ± 2 °C under a 12-h light/12-h dark cycle (lights on at 06:00) and with access to food and water *ad libitum*. Animals (male and female mice were equally distributed among groups) were acclimatized to the laboratory for at least 1 h before testing and were used only once throughout the experiments. The experiments were performed after protocol approval by the Institutional Ethics Committee (protocol number: PP0162) and were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). The numbers of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effect of the treatments.

2.2. Glutamate-induced nociception

In an attempt to evaluate the involvement of TTHL with the glutamatergic system, we investigated the effect of TTHL administered by intraperitoneal (i.p.), intraplantar (i.pl.) or intrathecal (i.t.) routes, on glutamate-induced nociception. The procedure used was similar to that previously described (Beirith et al., 2002). Mice were pre-treated with TTHL i.p. (2.1–65.5 μmol/kg, 30 min before), i.pl. (6.5–65.5 nmol/paw, co-administered with glutamate) or i.t. (21.8–655 nmol/site, 15 min before). Control animals received an equal volume of vehicle by i.p. (10 ml/kg), i.pl. (20 μl/paw) or i.t. (5 μl/site) routes. A volume of 20 μl glutamate (10 μmol/paw prepared in saline) was injected i.pl. into the right hindpaw. Animals were observed individually for 15 min following glutamate injection. The amount of time spent licking the injected paw was recorded with a chronometer and was considered as indicative of nociception.

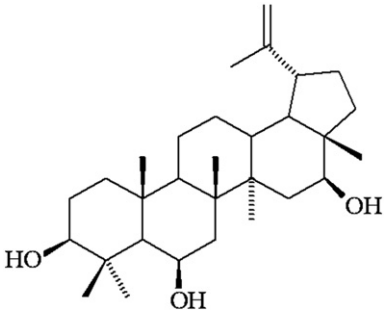

Fig. 1. Molecular structure of triterpene 3β, 6β, 16β-trihydroxylup-20(29)-ene (TTHL).

2.3. Algogen-induced overt nociception

2.3.1. Intrathecal (i.t.) injection

As previously described by Hylden and Wilcox (1980), animals were manually restrained, and a 30-gauge needle connected by polyethylene tubing to a 25 μL Hamilton gas-tight syringe (Hamilton, Birmingham, UK), was inserted through the skin and between the vertebras into the subdural space of the L5–L6 spinal segments. The tail reflex movement was considered as indicative of success of administration and the injections were given over a period of 5 s.

2.3.2. Spinal injection of excitatory aminoacids agonists and substance P

In parallel experiments, in order to investigate the spinal involvement of TTHL with the central glutamatergic and neurokinin systems, TTHL was co-administered with ionotropic and metabotropic agonists of glutamate receptors or substance P by i.t. route. In a first set of experiments, TTHL (dose range: 6.5–218 nmol/site) was co-administered via i.t. with glutamate (an excitatory amino acid, 175 nmol/site; Urca and Raigorodsky, 1988). In subsequent experiments, TTHL (65.5 nmol/site) was given by i.t. route and co-injected with N-methyl-D-aspartate (NMDA, a selective agonist of NMDA subtype of ionotropic glutamate receptors, 450 pmol/site; Urca and Raigorodsky, 1988), (±)-1-aminocyclopentane-trans-1,3 dicarboxylic acid (trans-ACPD, a non-selective metabotropic glutamate receptor agonist, 10 nmol/site; Boxall et al., 1998), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, a selective agonist of AMPA subtype of ionotropic glutamate receptors, 135 pmol/site; Brambilla et al., 1996), kainic acid (kainate, a selective agonist of kainate subtype of ionotropic glutamate receptors, 110 pmol/site; Urca and Raigorodsky, 1988) or substance P (a neurokinin 1-receptor selective agonist, 100 pmol/site; Sakurada et al., 1990). In another group, vehicle (5 μl/site) was administered by i.t. route.

Immediately after i.t injection of the substances, mice were individually placed in observation chambers and the amount of time (seconds) the animal spent biting, licking or scratching the caudal region (flanks, hindlimbs and tail) was taken as index of nociception. The ceiling for observation of nociceptive behavior was variable to the differents agonists: glutamate 3 min; NMDA and substance P 5 min; AMPA 1 min; kainate 4 min; and trans-ACPD 15 min (Boxall et al., 1998; Brambilla et al., 1996; Sakurada et al., 1990; Scheidt et al., 2002; Urca and Raigorodsky, 1988).

2.3.3. I.t injection of NMDA and metabotropic glutamate receptors antagonists

We also investigated if the commercially available antagonists of NMDA and metabotropic receptors, such as MK-801 (selective non-competitive NMDA antagonist) and (RS)-MCPG (non-selective group I/group II metabotropic glutamate receptor antagonist) are able to reverse the nociceptive response induced by i.t injection of glutamate, NMDA and trans-ACPD. Then, both antagonists MK-801 (3 nmol/site) and (RS)-MCPG (100 nmol/site) were intrathecally co-injected with glutamate (175 nmol/site), NMDA (450 pmol/site) or trans-ACPD (10 nmol/site). The nociceptive response (seconds) was quantified as described above.