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Analgesic activity of piracetam: Effect on cytokine production and oxidative stress

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ABSTRACT

Piracetam is a prototype of nootropic drugs used to improve cognitive impairment. However, recent studies suggest that piracetam can have analgesic and anti-inflammatory effects. Inflammatory pain is the result of a process that depends on neutrophil migration, cytokines and prostanoids release and oxidative stress. We analyze whether piracetam has anti-nociceptive effects and its mechanisms. Per oral pretreatment with piracetam reduced in a dose-dependent manner the overt pain-like behavior induced by acetic acid, phenyl-*p*-benzoquinone, formalin and complete Freund's adjuvant. Piracetam also diminished carrageenin-induced mechanical and thermal hyperalgesia, myeloperoxidase activity, and TNF-α-induced mechanical hyperalgesia. Piracetam presented analgesic effects as post-treatment and local paw treatment. The analgesic mechanisms of piracetam were related to inhibition of carrageenin- and TNF-α-induced production of IL-1β as well as prevention of carrageenin-induced decrease of reduced glutathione, ferric reducing ability and free radical scavenging ability in the paw. These results demonstrate that piracetam presents analgesic activity upon a variety of inflammatory stimuli by a mechanism dependent on inhibition of cytokine production and oxidative stress. Considering its safety and clinical use for cognitive function, it is possible that piracetam represents a novel perspective of analgesic.

1. Introduction

Acute inflammatory pain is characterized by sensitization of nociceptors resulting in hyperalgesia and allodynia, which is exacerbated pain intensity in response to painful stimuli, and pain to stimuli that is not normally painful, respectively (Millan, 1999). Inflammatory stimuli such as carrageenin induce a cascade of inflammatory cytokines resulting in inflammatory hyperalgesia. For instance, carrageenin induces the production of TNF-α, which triggers IL-1β production, which in turn, activates the synthesis of PGE_2 (Cunha et al., 2005). These inflammatory mediators are responsible for sensitization of nociceptors and activation of second messenger pathway (cAMP, PKA, and PKC) which reduce the nociceptor threshold and increase neuronal membrane excitability, facilitating the primary nociceptor activation and impulse transmission, resulting in hyperalgesia (Cury et al., 2011; Verri et al., 2006a; Villarreal et al., 2009).

Neutrophils are great contributors to acute inflammatory hyperalgesia. There is evidence that neutrophils recruited by cytokines, endothelin-1, complement component C5a and leukotriene B_4 contribute to hyperalgesia by further producing nociceptive mediators such as PGE_2 (Cunha et al., 2008; Guerrero et al., 2008; Ting et al., 2008; Verri et al., 2009). Therefore, blockade of neutrophil recruitment reduces inflammatory hyperalgesia (Cunha et al., 2008). Another important component of inflammatory pain is the oxidative stress with the generation of molecules such as hydrogen peroxide, superoxide anion, and peroxinitrite, which are produced in response to stimuli and can promote hyperalgesia (Keeble et al., 2009; Ndengele et al., 2008; Wang et al., 2004). Under normal conditions, oxidative stress products are counteracted by endogenous antioxidant systems, which include enzymes (superoxide dismutase, glutathione reductase, and catalase) and other molecules (GSH, bilirubin, and uric acid) (Limón-Pacheco and Gonsebatt, 2009). During inflammatory processes, the levels of oxidative stress products exceed endogenous antioxidant system overwhelming their capacity and producing an imbalance that mediates tissue injury and inflammatory pain (Keeble et al., 2009; Ndengele et al., 2008; Wang et al., 2004).

Nootropic drugs improve cognitive functions such as learning and memory (Genkova-Papazova and Lazarova-Bakarova, 1996; Winblad, 2005). Piracetam, 2-oxo-1-pyrrolidine acetamide, is the prototype of nootropic drug derived from GABA, however its mechanism of action does not seem to be related to GABA and the precise mechanism of

Abbreviations: ABTS, 2,2V-azinobis (3-ethylbenzothiazoline 6-sulfonate); AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; cAMP, cyclic adenosine monophosphate; CFA, complete Freund's adjuvant; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); FRAP, ferric reducing/antioxidant power; GABA, γ-aminobutyric acid; GSH, reduced glutathione; HTAB, hexadecyltrimethylammonium bromide; MPO, myeloperoxidase; PBQ, phenyl-*p*-benzoquinone; PKA, proteins kinase A; PKC, protein kinase C.

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action remains unknown (Winblad, 2005). There are evidences that piracetam acts by restoring the membrane fluidity and by promoting AMPA receptor allosteric modulation (Winblad, 2005).

To our knowledge, the only evidence that piracetam inhibits nociceptive behavior demonstrated that it reduces acetic acid-induced writhing response by mechanisms involving adenosine receptors, and that blockade of muscarinic, adrenergic and opioid receptors potentiates the antinociceptive effect of piracetam (Abdel-Salam, 2006). In contrast, levetiracetam, an antiepileptic drug α-etil analogue of piracetam, did not affect formalin-induced nociceptive response (Munro et al., 2007) while it reduced carrageenin-induced hyperalgesia by mechanisms mediated at least in part by direct/indirect activation of $GABA_A$, opioid, serotoninergic and adrenergic receptors (Micov et al., 2010). It is noteworthy to mention that these studies applied models of inflammatory pain with differences in the nociceptive endpoint and nociceptive mechanisms, which explain why the antinociceptive spinal/central mechanisms of piracetam (Abdel-Salam, 2006) contrast with the results demonstrated for levetiracetam (Micov et al., 2010). It is also important to mention that levetiracetam reduces the IL-1β production in astrocytes culture (Haghikia et al., 2008) and decreases oxidative stress in hippocampus (Oliveira et al., 2007). These results suggest that levetiracetam and possibly piracetam can modulate additional mechanisms such as the production of inflammatory/nociceptive molecules that eventually would contribute to their analgesic mechanism. Furthermore, piracetam inhibits the exudation induced by formalin injection in the peritoneal cavity and cell proliferation induced by cotton pellet (Nikolova et al., 1984), indicating that it reduces peripheral inflammation. Thus, the effect of piracetam seems not restricted to the central nervous system.

Therefore, considering that the spinal/central antinociceptive effect of piracetam was demonstrated in only one study (Abdel-Salam, 2006) and that it can also reduce peripheral inflammation (Nikolova et al., 1984) as presented above, we propose to investigate whether piracetam has antinociceptive effects in models of inflammatory pain and address its peripheral antinociceptive mechanisms of action focusing on cytokines and oxidative stress.

2. Materials and methods

2.1. Animals

Male Swiss mice, from Universidade Estadual de Londrina, Londrina, Paraná, Brazil, weighing approximately 25 ± 5 g served as experimental animals. Mice were housed in standard clear plastic cages with free access to food (Nuvilab® from Nuvital Nutrientes, Colombo, PR, Brazil) and water, with a light/dark cycle of 12:12 h, at 21 °C. All behavioral testing was performed between 9 a.m. and 5 p.m. in a temperature-controlled room. Mice were placed in a quiet room 15–30 min before the start of testing. The mice were used only once. Animals' care and handling procedures were in accordance with the International Association for Study of Pain (IASP) guidelines and with the approval of the Ethics Committee of the Universidade Estadual de Londrina. All efforts were made to minimize the number of animals used and their suffering. It is noteworthy that different experimenters prepared the solutions, made the administrations and performed the evaluation of pain-like behavior.

2.2. Drug and reagents

The following materials were obtained from the sources indicated: piracetam (Nootropil®) from Sanofi-Aventis (Suzano, SP, Brazil); acetic acid from Mallinckrodt Baker S.A (Mexico City, Mexico); Trolox® (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), PBQ and CFA from Sigma Aldrich Co. (St. Louis, MO, USA); carrageenin from FMC Corp (Philadelphia, PA, USA); TNF-α and ELISA kits from e-Bioscience Inc (San Diego, CA, USA); and formaldehyde from Merck (Rio de Janeiro, RJ, Brazil).

2.3. Writhing response tests

PBQ and acetic acid-induced writhing models were performed as previously described (Collier et al., 1968; Valério et al., 2007; Verri et al., 2008). In brief, PBQ (diluted in DMSO 2%/saline, 1890 μg/kg), acetic acid (0.6% v/v, diluted in saline, 10 mL/kg), or vehicle was injected into the peritoneal cavities of mice. Each mouse was placed in a large glass cylinder and the intensity of nociceptive behavior was quantified by counting the total number of writhings (contractions of abdominal muscles accompanied by an elongation of the body and extension of the hind limbs) occurring between 0 and 20 min after stimulus injection.

2.4. The formalin and CFA tests

The time spent licking the paw was determined between 0 and 30 min after intraplantar (i.pl.) injection of 2.5% formalin diluted in saline 0,9% (25 μL/paw) or CFA (10 μL/paw), in similar ways and as previously described (Dubuisson and Dennis, 1977; Mizokami et al., 2012; Valério et al., 2009). Results were presented at 5 min interval up to 30 min for formalin test and total time spent licking the paw between 0 and 30 min in CFA test. CFA (10 μL/paw) was also used as stimulus to induce mechanical and thermal hyperalgesia (Mizokami et al., 2012; Valério et al., 2009).

2.5. Electronic pressure-meter test

Mechanical hyperalgesia was tested in mice, as previously reported (Cunha et al., 2004). Briefly, mice were placed in acrylic cages (12 × 10 × 17 cm) in a quiet room 15–30 min before the start of testing. The test consisted of evoking a hindpaw flexion reflex with a hand-held force transducer (electronic anesthesiometer, Insight Equipamentos, Ribeirao Preto, SP, Brazil) adapted with a 0.5 mm^2 polypropylene tip. The investigator was trained to apply the tip perpendicularly to the central area of the hindpaw with a gradual increase in pressure. The end point was characterized by the removal of the paw and flinching movements. The stimulation of the paw was repeated until the animal presented two similar measurements. The results are expressed by delta (Δ) withdrawal threshold (in g) calculated by subtracting the zero-time mean measurements from the mean measurements of 1, 3, or 5 h after stimulus. Withdrawal threshold was 9.4 ± 0.2 g (mean ± SEM; n = 6) before injection of the hyperalgesic agents (e.g. carrageenin, TNF-α or CFA).

2.6. Hot plate test

Thermal hyperalgesia was evaluated before and after stimulus. The test was performed as previously reported (Verri et al., 2005). In brief, mice were placed in a 10 cm wide glass cylinder on a hot plate (Hot Plate HP-2002, Insight Equipamentos, Ribeirao Preto, SP, Brazil) maintained at 55 °C. The reaction time was scored when the animal jumped, flinched or licked its paws. The normal latency (reaction time) was 12 ± 0.7 s (mean ± SEM; n = 6). A maximum latency (cutoff) was set at 30 s to avoid tissue damage.

2.7. Neutrophil migration to the paw skin tissue (myeloperoxidase activity)

The neutrophil migration to paw was indirectly evaluated by the MPO activity kinetic-colorimetric assay (Bradley et al., 1982; Casagrande et al., 2006). Briefly, paw skin sample was collected in 50 mM K_2PO_4 buffer (pH 6.0) containing 0.5% HTAB and were homogenized using Ultra-Turrax® (IKA T10 Basic, CQA Química, Paulínea, SP). Then the homogenates were centrifuged at 16100 *g* for 2 min at 4 °C. 15 μL of resulting supernatant was mixed with 200 μL of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/mL o-dianisidine dihydrochloride and 0.05% hydrogen peroxide and was assayed spectrophotometrically for MPO activity determination at 450 nm (BEL SP2000UV, Photonics, São