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The ruthenium NO donor, $[Ru(bpy)_2(NO)SO_3](PF_6)$, inhibits inflammatory pain: Involvement of TRPV1 and cGMP/PKG/ATP-sensitive potassium channel signaling pathway

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ABSTRACT

The activation of nitric oxide (NO) production is an analgesic mechanism shared by drugs such as morphine and diclofenac. Therefore, the controlled release of low amounts of NO seems to be a promising analgesic approach. In the present study, the antinociceptive effect of the ruthenium NO donor $[Ru(bpy)_2(NO)SO_3](PF_6)$ (complex I) was investigated. It was observed that complex I inhibited in a dose (0.3–10 mg/kg)-dependent manner the acetic acid-induced writhing response. At the dose of 1 mg/kg, complex I inhibited the phenyl-p-benzoquinone-induced writhing response and formalin- and complete Freund's adjuvant-induced licking and flinch responses. Additionally, complex I also inhibited transient receptor potential cation channel subfamily V member 1 (TRPV1)-dependent overt pain-like behavior induced by capsaicin. Complex I also inhibited the carrageenin-induced mechanical hyperalgesia and increase of myeloperoxidase activity (MPO) in paw skin samples. The inhibitory effect of complex I in the carrageenin-induced hyperalgesia, MPO activity and formalin was prevented by the treatment with ODQ, KT5823 and glybenclamide, indicating that complex I inhibits inflammatory hyperalgesia by activating the cGMP/PKG/ATP-sensitive potassium channel signaling pathway. The present study demonstrates the efficacy of a novel ruthenium NO donor and its analgesic mechanisms.

1. Introduction

The animal models evaluate two main symptoms of pain: i) overt nociception/overt pain-like behavior or ii) hyperalgesia and allodynia. In overt nociception/overt pain-like behavior, an inflammatory stimulus induces a declared behavior such as paw flinch or licking and abdominal contortions (writhing) without further mechanical or thermal external stimuli. This declared behavior occurs because the overt nociceptive stimuli activate or induce fast production of endogenous mediators that activate the nociceptors (Collier et al., 1968; Dubuisson and Dennis, 1977; Pavao-de-Souza et al., 2012; Ribeiro et al., 2000; Verri et al., 2008, 2006).

Hyperalgesia is defined as an increase of pain sensation caused by a stimulus that normally induces pain and allodynia as pain due to a stimulus that does not normally provoke pain. Both result from the sensitization of nociceptors in which, for instance, inflammatory mediators activate second messenger pathways resulting in the lowering of the nociceptor threshold and increasing neuronal membrane excitability (Pavao-de-Souza et al., 2012; Verri et al., 2006).

Experimentally, peripheral pharmacological control of inflammatory hyperalgesia is based on two strategies. The first is the prevention of nociceptor sensitization, which can be achieved with non-steroidal anti-inflammatory (aspirin-like) drugs that inhibit prostaglandin synthesis (Ferreira, 1972). The second strategy is the direct blockade of the current nociceptor sensitization, which can be achieved by the use

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CFA, complete Freund's adjuvant; cGMP, cyclic monophosphate guanosine; GLY, glybenclamide; HTAB, hexadecyltrimethylammonium bromide; KT5823, 2, 3, 9,10, 11, 12-hexahydro-10R-methoxy-2, 9-dimethyl-1-oxo-9S, 12R-epoxy-1H-diindolo [1, 2, 3-fg:3′, 2′, 1′-kl]pyrrolo[3, 4-i][1, 6]benzodiazocine-10-carboxylic acid, methyl este; MPO, myeloperoxidase; NO, nitric oxide; NOS, nitric oxide synthase; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase, nNOS, neuronal nitric oxide synthase; NSAIDS, nonsteroidal anti-inflammatory drugs; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; PBQ, phenyl-p-benzoquinone; PGE2, prostaglandin E2; PKG, cGMP-dependent protein kinase; TRP channels, transient receptor potential channels; TRPV1 channel, transient receptor potential vanilloid 1.

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of peripheral opioids, analgesics (e.g. dipirone) and NO donors (Cunha et al., 2010; Ferreira et al., 1991b; Lorenzetti and Ferreira, 1985; Sachs et al., 2004). These drugs are able to inhibit the already established hyperalgesia induced by PGE_2 (Cunha et al., 2010, 2012; Ferreira et al., 1991a; Lorenzetti and Ferreira, 1985; Sachs et al., 2004). Moreover, several studies have demonstrated that the antinociceptive mechanism of these drugs depends on the activation of the L-arginine/NO/guanosine 3, 5-cyclic monophosphate (cGMP)/protein kinase G (PKG)/ATP-sensitive potassium channel pathway (Cunha et al., 2010, 2012; Ferreira et al., 1991a; Mizokami et al., 2012; Napimoga et al., 2008; Sachs et al., 2004; Santodomingo-Garzon et al., 2006; Vivancos et al., 2003). However, the control of inflammatory pain is still a major challenge because of the deleterious side effects attributed to the prolonged use of NSAIDs and opioids, their ineffectiveness in some cases and receptor desensitization in the case of opioids.

Nitric oxide is produced in mammalian cells from L-arginine and oxygen by three isoforms of the enzyme NO synthase (NOS): neuronal (nNOS or type I), inducible (iNOS or type II) and endothelial (eNOS or type III) (Alderton et al., 2001; Moncada et al., 1991). An important difference between the isoforms of NOS is that the constitutive type produces low NO concentrations in the nmol L^{-1} range, while the inducible form produces NO in the μmol L^{-1} range (Pagliaro, 2003). Accordingly, nitric oxide plays an important role in a variety of physiological functions, such as blood pressure control (Moncada and Higgs, 1993), neurotransmission (Bredt et al., 1990; Garthwaite, 1991), immunological and inflammatory responses (Hibbs et al., 1988; Rees et al., 1989; Silva et al., 2007) and antioxidant action (McCleverty, 2004; Wink and Mitchell, 2003). These effects are quite dependent on the local NO bioavailability and concentration (Wink and Mitchell, 1998). Therefore, intense efforts have been devoted to develop new compounds that deliver NO efficiently and in a controlled manner (Tfouni et al., 2012). In models of nociception, it is consensus that NO activates the cGMP/PKG/ATP-sensitive potassium channel signaling pathway (Kawabata et al., 1994; Li and Qi, 2010; Sousa and Prado, 2001; Tegeder et al., 2002; Vivancos et al., 2003), which is also the signaling pathway activated by the peripheral action of opioids (Cunha et al., 2010, 2012). Therefore, NO donors could be used to achieve similar effects as of peripheral opioids without the side effects related to opioid receptor activation.

In this context, our group has synthesized a nitrosyl ruthenium complex that donates NO, $[Ru(bpy)_2(NO)SO_3](PF_6)$ (Silva et al., 2007) here named as complex I. We have previously demonstrated that mice treated with complex I are more resistant to paracoccidioidomycosis infection, which was observed as prolonged survival with reduced leukocyte recruitment and TNFα production in the lung and liver as well as increased production of the anti-inflammatory cytokine IL-10 (Pavanelli et al., 2011). Furthermore, the complex I presents additional biological activities against several diseases such as in vitro and in vivo trypanocidal activities (Silva et al., 2007) and beneficial effects in a model of ischemia/reperfusion in the brain (Campelo et al., 2012).

Some interesting characteristics of ruthenium NO donors are the release of NO through electrochemical, photochemical and chemical processes (Silva et al., 2007). The rate constant for the NO release induced by thiols is pH-dependent and increases by raising the pH, due to the acid–base equilibrium of thiol group of the cysteine residue (Silva et al., 2011). In this respect, ruthenium compounds could be useful as delivery or scavenging agents for molecules that have a crucial role in physiology (Tfouni et al., 2012). Moreover, NO-donors are of great interest due to their outstanding properties, such as the low cytotoxicity against host cells, water solubility, stability to air oxidation and tailoring reactivity possibilities of the coordinated NO (Borges et al., 1998; Lopes et al., 1998; Toledo et al., 2002; Tfouni et al., 2012).

Considering the information above, in the present study we investigated the mechanism involved in the antinociceptive effect of $[Ru(bpy)_2(NO)SO_3](PF_6)$ in models of overt pain-like behavior and in carrageenin-induced mechanical inflammatory hyperalgesia. The role of cGMP/PKG/ATP-sensitive potassium channel signaling pathway was also investigated.

2. Material and methods

2.1. Animals

Male Swiss mice (25–30 g) from the Universidade Estadual de Londrina, Paraná, Brazil, were used in this study. Mice were housed in standard clear plastic cages with free access to food and water and a light/dark cycle of 12:12 h and kept at 21 °C. Different investigators prepared the solutions, treated the mice, injected the stimulus and quantified the nociceptive behaviors. All behavioral testing was performed between 9 am and 5 pm in a temperature-controlled room. Animal care and handling procedures were in accordance with the International Association for the 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. Drugs

The following materials were obtained from the sources indicated: acetic acid from Mallinckrodt Baker, S.A. (Mexico City, Mexico); glybenclamide, capsaicin, KT5823 (2,3,9,10,11,12-hexahydro-10R-methoxy-2,9-dimethyl-1-oxo-9S,12R-epoxy-1diindolo [1,2,3-fg:3′,2′, 1′-kl] pyrrolo [3,4-i][1,6]benzodiazocine-10-carboxylic acid, methyl ester) were obtained from Sigma-Aldrich (St. Louis, MO, USA). ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) was obtained from Calbiochem (San Diego, CA, USA). Carrageenin from FMC Corporation (Philadelphia, PA), phenyl-p-benzoquinone and CFA from Sigma (St. Louis, MO), formalin from Merck (Darmstadt, Germany). The selected doses of drugs were chosen based on pilot studies and previous data of our laboratory. We detected that the doses of drugs used do not alter the nociceptive response per se, and inhibit the respective standard stimulus (Cunha et al., 2010; Mizokami et al., 2012; Vale et al., 2007; Valerio et al., 2009; Verri et al., 2008).

2.3. Synthesis of ruthenium NO donor $[Ru(bpy)_2(NO)SO_3](PF_6)$ (complex I)

The ruthenium NO donor $[Ru(bpy)_2(NO)SO_3](PF_6)$ namely complex I was synthesized and characterized as we previously described (Silva et al., 2007). Elemental analysis of hydrogen, carbon, and nitrogen was carried out using an EA 1110 CHNS-O CE instrument. Analysis of ruthenium was performed as described elsewhere (Clarke, 1978), using a Polarized Zeeman atomic absorption spectrophotometer, Hitachi (model Z-8100), with a Hitachi Hollow Cathode Lamp, 12 mA, and $k = 349.9$ nm. UV visible measurements were performed in a 1.0 cm quartz cell in a Hewlett-Packard diode array model 8452A spectrophotometer. IR spectra were recorded with a Bomem FTIR, model MB-102, spectrophotometer in the 400–4000 cm^{-1} range, with the sample supported in potassium bromide pellets. A polarographic analyzer/stripping voltammeter model 264A from Princeton Applied Research attached to a microcomputer and employing Microquimica Eletrochemical software was used for the electrochemical measurements. The electrochemical cell used was a conventional three-electrode type with an aqueous saturated calomel electrode as a reference electrode and a glassy carbon and platinum wire as working and auxiliary electrodes, respectively (Pavanelli et al., 2011).

2.4. Writhing response tests

The PBQ (Emele and Shanaman, 1967) and acetic acid (Collier et al., 1968) induced writhing models were performed as previously described (Pavao-de-Souza et al., 2012). Mice were pretreated with