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Central and peripheral antinociceptive activity of 3-(2-oxopropyl)-3-hydroxy-2-oxindoles

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ABSTRACT

Convolutamydine A has been shown to develop a significant antinociceptive effect. Here we demonstrated that new analogues (5-iodo-3-(2-oxopropyl)-3-hydroxy-2-oxindole (5-Iisa), 5-fluoro-3-(2-oxopropyl)-3-hydroxy-2-oxindole (5-Fisa), 5-chloro-3-(2-oxopropyl)-3-hydroxy-2-oxindole (5-Clisa) and 5-methyl-3-(2-oxopropyl)-3-hydroxy-2-oxindole (5-Meisa)), at 0.1–10 mg/kg doses, have significant peripheral and central antinociceptive effects in thermal and chemical models of nociception. Oral administered analogues demonstrated more pronounced antinociceptive effects than that obtained with the classical opioid drug morphine (5 mg/kg) in the first and second phases of formalin-induced licking. In the tail flick model, 5-Clisa and 5-Meisa antinociceptive effect was almost twice as that observed with the same dose of morphine. The concomitant administration of diverse antagonists and the analogues indicates that 5-Iisa effects involve the activation of opioid pathway. On the other hand, 5-Fisa and 5-Clisa have the participation of opioid, nitrergic, cholinergic adrenergic and serotoninergic pathways and 5-Meisa has the involvement of opioid, serotoninergic and cholinergic pathways. In conclusion, our results suggest that the new four analogues from Convolutamydine A have significant antinociceptive effects in thermal and chemical induced nociception and could be used in development of new drugs to be used in pain treatment with reduced side effects.

1. Introduction

Pain is a hallmark of inflammation, and the therapeutic approaches to pain relief are based upon non-steroidal anti-inflammatory drugs or opiates. The improper use of either class of drugs gives rise to several unwanted effects such as ulcers, vomiting, tolerance, and dependence (Nakagawa and Kaneko, 2010; Zhao et al., 2011). The inevitable side effects associated with the use of these classes of drugs have led to the search for and synthesis of new efficient analgesics presenting little or no side effects in pain control.

Convolutamydine A is a member of a family of oxindole alkaloids isolated from the Floridian marine bryozoan *Amathia convoluta*. This compound is interesting, as it has been described in the literature as having significant pharmacological activity. Several analogues have been synthesised from Convolutamydine A that demonstrate a diversity of effects (Kamano et al., 1995; Garden et al., 1997; Luppi et al., 2006; Cravotto et al., 2006). Our group described anti-inflammatory and/or antinociceptive effects for a diversity of analogues (Matheus et al, 2007; Figueiredo et al, 2013; Fernandes et al., 2014).

In this study we examine the antinociceptive effects of four synthesised 3-(2-oxopropyl)-3-hydroxy-2-oxindoles using thermal and inflammatory pain models in mice. We further evaluate the possible mechanisms of action of these substances with the hope of suggesting them as potential antinociceptive therapeutic agents.

2. Materials and methods

2.1. Animals

All experiments were performed with male Swiss Webster mice (20–25 g) donated by Instituto Vital Brazil (Niterói, Brazil). The animals were maintained in a room with controlled temperature (22 ± 2 °C) on a 12-h light/dark cycle, with free access to food and water. All efforts were made to minimise animal suffering, to reduce the number of animals used. All animal experimental protocols are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH

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Publications No. 8023, revised 1978). The experimental protocols were also in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA). Research protocols were approved by the Ethical Committee for Animal Research (Biomedical Science Institute/UFRJ) and received the approval number DFBCICB015-04/16.

2.2. General

Acetylsalicylic acid (ASA), dexamethasone, atropine sulphate monohydrate, capsaicin, L-glutamic acid (glutamate), L-nitro arginine methyl ester (L-NAME), and carrageenan were purchased from Sigma (St. Louis, MO, USA), and formalin was purchased from Merck, Inc. Morphine sulphate and naloxone hydrochloride were kindly provided by Cristália (São Paulo, Brazil). All drugs were dissolved in phosphate buffer saline (PBS) just before use. Capsaicin was dissolved in 80% (v/v) ethanol plus 20% PBS. ASA (200 mg/kg, po) and dexamethasone (5 mg/kg, ip) were used as reference drugs. The negative control group was treated with the vehicle.

2.3. Synthesis of substances

Isatins were suspended in acetone, and drops of diethylamine were added at room temperature, leading to 5-iodo-3-(2-oxopropyl)-3-hydroxy-2-oxindole (5-Iisa), 5-fluoro-3-(2-oxopropyl)-3-hydroxy-2-oxindole (5-Fisa), 5-chloro-3-(2-oxopropyl)-3-hydroxy-2-oxindole (5-Clisa) and 5-methyl-3-(2-oxopropyl)-3-hydroxy-2-oxindole (5-Meisa) (Fig. 1). The structures of the final compounds were determined by ^{1}H-NMR and ^{13}C-NMR, as described by Garden et al. (1997).

2.4. Administration of substances

5-Iisa, 5-Fisa, 5-Clisa and 5-Meisa were dissolved in dimethylsulphoxide (DMSO) to prepare stock solutions at 100 mg/ml. All substances were administered by oral gavage at doses of 0.1 to 10 mg/kg in a final volume of 0.1 ml of Tween 80 per animal. The control group was treated with vehicle (Tween 80/DMSO). In all experiments, the final concentration of DMSO or Tween 80 had no effect *per se*.

2.5. Acute toxicity

Acute toxicity was determined according to the experimental model described by Lorke (1983). A single oral dose (150 mg/kg) of a substance was administered to a group of ten mice (five males and five females). Behavioural parameters, including convulsion, hyperactivity, sedation, grooming, loss of righting reflex, increased or decreased respiration, and food and water intake, were observed over a period of 5 days. After this period, the animals were sacrificed by cervical dislocation, their stomachs were removed, an incision was made along the greater curvature, and the number of ulcers (single or multiple erosions, ulcers or perforations) and degree of hyperaemia were counted.

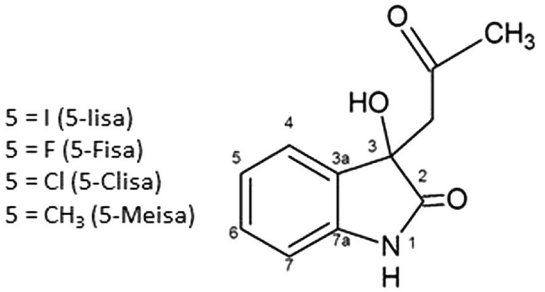

Fig. 1. General structure of oxopropyl-oxindoles.

2.6. Formalin-induced licking model

The procedure was similar to the method described by Hunskaar and Hole (1987) and with some modifications performed by Gomes et al. (2007). Animals received 20 μl of formalin (2.5% v/v) into the dorsal surface of the left hind paw. The time of licking the formalin-injected paw was immediately recorded during two phases: first phase (neurogenic pain response) between the moment of injection and 5 min and second phase (inflammatory pain response) between 15 and 30 min post-injection. The animals were pretreated with oral doses of each compound (0.1, 1 or 10 mg/kg), morphine (5 mg/kg), ASA (200 mg/kg), or vehicle (PBS) 60 min before the administration of formalin.

2.7. Glutamate- or capsaicin-induced nociception

The procedure used for glutamate-induced nociception was described by Beirith et al. (2002) and adopted by Pinheiro et al. (2013), and for capsaicin-induced nociception the protocol described by Sakurada et al. (1998) was adopted by Pinheiro et al. (2013). Briefly, an intraplantar injection of glutamate (10 μl, 3.7 ng/paw) or capsaicin (20 μl; 1.6 μg/paw) was administered to the right hind paw. The animal was immediately placed in an individual box, and the time that the animal sustained licking or biting of the glutamate- or capsaicin-injected paw was recorded for 15 or 5 min, respectively. Mice were orally pretreated 60 min before the intraplantar injection of glutamate or capsaicin with each substance (0.1, 1 or 10 mg/kg) or vehicle.

2.8. Tail flick

This test was performed according to Bem-Bassat et al. (1959) and adapted by Pinheiro et al. (2010). Briefly, mice were placed in a container tube, and one third of the tail was immersed in a water bath set to 50 ± 1 °C. The reaction time necessary for the animal to withdraw the tail was registered at several intervals of 20 min after the administration of a substance (0.1 to 10 mg/kg), morphine (5 mg/kg) or vehicle. Baseline was designated as the average of the reaction times obtained at 40 and 20 min before the administration of the substance, morphine or vehicle and defined as the normal reaction of the animal to thermal stimulus. If the animal did not withdraw their tail from the hot water for a period of time greater than three times the baseline (cut-off), their tail was manually removed to avoid any possible damage. Antinociception was quantified as an increase in baseline (IB) (%) calculated by the formula: IB = ((reaction time × 100) / baseline) − 100 or area under the curve (AUC) calculated by the formula based on the trapezoid rule of responses from 20 min after drug administration until the end of the experiment (Matthews et al., 1990): AUC = 20 × IB [(min 20) / 2 + (min 20 + min 40) / 2 + (min 40 + min 60) / 2... + (min 120) / 2]. The baselines values varied between 4 and 7 s.

2.9. Evaluation of the possible mechanism(s) of action of substances in the tail flick test

To assess the possible participation of the opioid, cholinergic, noradrenergic, nitrergic, serotoninergic and cannabinoid systems in the antinociceptive effect of the substances, mice were pretreated with naloxone (opioid antagonist, 5 mg/kg, ip), mecamylamine (nicotinic antagonist, 2 mg/kg, ip), atropine (muscarinic antagonist, 1 mg/kg, ip), ondansetron (5-HT3 serotoninergic antagonist, 0.5 mg/kg, ip), yohimbine (alpha-2 adrenergic antagonist, 2 mg/kg, ip), or L-nitro arginine methyl ester (L-NAME, inhibitor of nitric oxide synthase, 3 mg/kg, ip) 15 min before treatment with a compound (10 mg/kg, po). Dose response curves for each antagonist were previously constructed and the dose that reduced the response to the agonist by 50% was chosen for these assays (Pinheiro et al., 2010; Figueiredo et al, 2013).