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The antinociceptive effect of acetylsalicylic acid is differently affected by a CB_1 agonist or antagonist and involves the serotonergic system in rats

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ABSTRACT

Aims: Combinations of non-steroidal anti-inflammatory drugs (NSAIDs) and cannabinoids are promising because of their potential synergistic effects in analgesia, resulting in a reduction in dosage and minimizing adverse reactions. The analgesic effect of acetylsalicylic acid (ASA), probably due to a central mechanism, also implicates changes in the central monoaminergic system. Therefore, we decided to evaluate the antinociceptive interaction between the CB_1 receptor agonist, HU210, and ASA in tests involving central pain in rats as well as the implication of the central serotonergic system thereon.
Main methods: The selective CB_1 antagonist SR141716A and the potent cannabinoid agonist HU210 were evaluated alone and in combination with ASA in both algesimetric tests (hot-plate and formalin tests) and for 5-HT activity and 5-HT_2 receptor density and affinity.
Key findings: ASA or HU210 alone showed a dose-dependent effect in both tests. HU210, at an inactive dose, significantly increased the antinociceptive effect of the sub-active dose of ASA. SR141716A (1.5 mg/kg i.p.) was ineffective *per se* and failed to modify antinociception induced by the HU210 plus ASA combination in either test. HU210 plus ASA significantly decreased the 5-HIAA/5-HT ratio and the 5-HT_2 receptor density in the frontal cortex, changes not antagonized by SR141716A.
Significance: The present study provides evidence that mutual potentiation of the antinociceptive effects of HU210 and ASA may, at least partly, depend on serotonergic mechanisms, with an indirect participation of cannabinodiergic mechanism. In conclusion, combinations of low doses of cannabinoids and NSAIDs may be of interest from the therapeutic point of view.

Introduction

Combinations of analgesic drugs from different pharmacological classes are often used in pain management to improve analgesic efficacy and lowering the incidence of severe side effects.

Non-steroidal anti-inflammatory drugs (NSAIDs) are a heterogeneous group of compounds, often used clinically for their anti-inflammatory, analgesic, antipyretic and antithrombotic effects (Vane and Botting 1987). Acetylsalicylic acid (ASA), the prototype NSAID, irreversibly inhibits COX-1 and COX-2 (de Leval et al. 2000). Furthermore, the analgesic effect of NSAIDs is probably also due to a central mechanism involving both prostaglandin synthesis inhibition (McCormack 1994) and changes in the monoaminergic system in the brain (Pini et al. 1995; Sandrini et al. 1995) and in the spinal cord (Björkman 1995). Moreover, the analgesic effect of ASA is accompanied by an increase in serotonin (5-HT) concentration and a decrease in the 5-HT_2 receptor number at supraspinal level in the rat (Vitale et al. 1998; Sandrini et al. 2002). Behavioural and biochemical modifications induced by ASA were completely abolished by a pre-treatment with the serotonin synthesis inhibitor para-chlorophenylalanine (Pini et al. 1995), once more indicating the involvement of the serotonergic system.

It is well known that pain control is a complex network, owing to possible multiple interactions between some neurotransmitter systems whose effects could be mediated by a plethora of receptor subtypes modulating and producing many behavioural changes, including nociception (Millan 2002, for a review).

In particular, there has been evidence that the cannabinoid system can contribute to the pharmacological effects of NSAIDs (Anand et al. 2009), as opioids also do (Miranda and Pinardi 2009). Indeed, the importance of cannabinoid system-mediated analgesia in nociceptive processing has been demonstrated (Walker and Huang 2002). Exogenous cannabinoids reduce responsiveness to noxious thermal (Lichtman and Martin 1991), mechanical and chemical stimuli (Moss and Johnson 1980) in rats and mice, with a potency similar to that

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of morphine (Bloom et al. 1977). Indeed, cannabinoids produce antinociceptive effects at various supraspinal sites implicated in the modulation of nociception (Herkenham et al. 1991; Hohmann et al. 1999).

Additive or synergistic antinociceptive effects are likely to occur after coadministration of cannabinoids and NSAIDs, because each group of drugs acts through a distinct site of action, but both groups activate, through specific mechanisms, the descending monoaminergic inhibitory antinociceptive pathways. There are only few data on this antinociceptive interaction (Ulugöl et al. 2006), a field of great interest for therapeutic potential, limiting possible side effects.

Therefore, the first aim was to investigate the potential antinociceptive interaction between the potent cannabinoid agonist HU210 and ASA, and possible interference by a selective CB_1 antagonist (SR141716A) using two types of noxious tests in rats: the hot-plate (model of thermal pain) and formalin tests (chemical-induced nociception). Full doses of ASA or HU210 were used as active antinociceptive compounds in the behavioural tests utilized.

The second aim was to assess the possible implication of the serotonergic system in the behavioural effects displayed by the above-described combinations, by evaluating the possible changes in 5-HT and 5-hydroxyindolacetic acid (5-HIAA), as well as in the 5-HT_2 receptor density and binding affinity in the rat frontal cortex.

Materials and methods

Animals

Male Wistar rats weighing 180–200 g at the beginning of the experiments were housed in Plexiglas® cages in groups of two or three under controlled conditions (free access to food and water, 12-h dark/light cycle, temperature of 22 ± 1 °C, and 60% humidity). The ethical guidelines for the investigation of experimental pain in conscious animals were followed, and procedures were carried out according to the EC ethical regulations for animal research (EEC Council 86/609; D.L. 27/01/1982, No. 116).

Drugs and treatments

Acetylsalicylic acid, as lysine salt, was purchased from Sanofi-Wintrop (Milan, Italy). SR141716A was kindly provided by Sanofi Recherche (Montepellier, France). HU210 was from Tocris Cookson Ltd. (Bristol, UK). Formalin was obtained through Bracco Chemical Co. (Milan, Italy) and all other reagents used for biochemistry were supplied by Sigma Chemicals Co. (Milan, Italy). 5-HT, 5-HIAA, DHBA and methysergide were purchased from Sigma-Aldrich, as well. [^{3}H] ketanserin was from Du Pont NEN, Co. Ltd (Milan, Italy).

Experiment 1

The rats were randomly divided into groups of eight animals each and intraperitoneally (i.p.) injected with acetylsalicylic acid at a dose of 100, 200, and 400 mg/kg, dissolved in sterile saline, or sterile saline alone, and subjected to the hot-plate or formalin tests 30 min thereafter.

HU210 (HU, 6.25, 12.5 and 25 µg/kg, i.p.) freshly dissolved in Tween 80 (0.17–5%) in sterile saline or vehicle (consisting in equivolume amounts of Tween 80 in sterile saline) was administered 20 min before ASA (200 mg/kg) or sterile saline (in combination experiments only the dose of 12.5 µg/kg of HU210 was administered before ASA, 200 mg/kg); the rats were subjected to the hot-plate or formalin tests 30 min thereafter.

SR141716A (SR, 0.5 and 1.5 mg/kg, i.p.) or vehicle (same preparation as above-described for HU) was administered 30 min before ASA (400 mg/kg) or sterile saline, and the rats were subjected to the hot-plate or formalin tests 30 min after the final treatment.

Experiment 2

SR141716A (1.5 mg/kg, i.p.) or vehicle was injected 30 min before HU210 (25 µg/kg, i.p.) or HU (12.5 µg/kg) + ASA (200 mg/kg) combination and the animals were subjected to both behavioural tests 30 min after the final treatment.

Immediately after the last pain threshold measurement, the animals were anaesthetized with ethyl ether and decapitated. The brains were removed, the areas weighed and stored at −80 °C until required for assay.

The frontal cortex of the rats used in our experiments was processed to evaluate 5-HT and 5-HIAA concentrations, by means of high performance liquid chromatography (HPLC), and 5-HT_2 receptor density and binding affinity, using a radioligand binding technique. This area was chosen on the basis of previous results indicating its implication in the modulation of the nociceptive system.

Nociceptive testing

Hot-plate test

The hot-plate consisted of an electrically-heated surface (Socrel DS-35, Ugo Basile, Comerio, VA, Italy) kept at a constant temperature of 52.0 ± 0.4 °C. The baseline latency in the hot-plate ranged from 0.5 ± 0.6 to 1.8 ± 0.7 s (ANOVA, $P>0.05$). The latencies for paw licking or jumping were recorded for each animal. The analgesic efficacy of the drug was evaluated as a percentage of the maximum possible effect (%MPE), according to the formula $(TL-BL)/(45-BL)\times 100$, where TL = Test Latency, BL = Baseline Latency, and 45 = cut-off time in seconds.

Formalin test

Two hours before testing, the animals were placed individually in standard cages and, after the adaptation period, 50 µl of 5% formalin solution were injected s.c. into the dorsal surface of the right hind paw using a microsyringe with a 26 gauge needle. Pain behaviour was monitored over a period of 40 min. The number of flinches/shakes of the injected paw was measured at 5 min intervals, starting at time 0. Two phases of spontaneous flinching were observed: phase 1, immediately after formalin injection until 10 min thereafter; phase 2 (11–40 min after injection), having a maximum response around 25 to 35 min after formalin injection.

Biochemical assays

Serotonin and 5-hydroxyindolacetic acid determination

The frontal cortex was assayed for 5-HT and 5-HIAA determinations by reverse phase HPLC, according to Grossi et al. (1990) with modifications. The thawed areas were homogenized with an ultrasonic dismembranator in 0.1 N $HClO_4$ containing 4 mM $NaHSO_4$ (10 µl per mg of wet weight) and centrifuged at 2000 × *g* for 15 min at 4 °C. After centrifugation the acid supernatant was filtered on 0.22 µm filters before analysis. 5-HT and 5-HIAA assays were performed with a Beckman System Gold high performance liquid chromatograph (Beckman Instruments Inc., San Ramon, CA, USA), equipped with an ESA Coulochem II Multi-Electrode high sensitivity electrochemical detector (ESA Inc., Bedford, MA, USA) with conditioning cell set at −0.75 V, detector 1 set at +0.05 V and detector 2 set at +0.25 V, response 2, gain 10 × 5. A reverse phase C18 10 cm × 4.6 Hypersil Column (Labservice Analytical, BO, Italy) packed with 3 µm octadecylsilane (ODS) was used. The mobile phase, consisting of methanol 15%, acetonitrile 8% and 50 mM NaH_2PO_4 pH 2.8 with 0.2 mM ethylenediaminetetraacetic acid disodium salt and 200 mg/l octyl sulphate, was pumped at a rate of 1 ml/min. 3,4-Dihydroxybenzylamine (DHBA), the internal standard, 5-HT and 5-HIAA were used as standards. Standards and samples were evaluated according to the analyte/DHBA ratio in the calibration curve.