Antinociceptive Effect of Hydroxydihydrocarvone

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Hydroxydihydrocarvone (HC) is a synthetic intermediate obtained from R-(-)-carvone hydration. Due to the chemical and structural similarity between HC and other monoterpenes with psychopharmacological activity, this study was carried out to investigate the possible central antinociceptive effect of intraperitoneally administered HC, and to evaluate its effect on the opioid system in mice. In the tail immersion test, the time of the response to the thermal noxious stimulus was longer in the animals that received HC (200 mg/kg). In the hot plate test, HC (100—200 mg/kg) significantly increased the time mice stayed on the apparatus. In the formalin test, HC was effective in both phases of the test with significant dose-dependent response (50—200 mg/kg), showing central antinociceptive activity. In addition, HC (25—200 mg/kg) did not induce catalepsy in mice. In an attempt to evaluate the mechanism of action of HC, the mice were pretreated with nalozone (5 mg/kg, s.c.). The effect of HC on the formalin and hot plate tests was not blocked by nalozone. Therefore, HC has an antinociceptive effect on the central nervous system without causing catalepsy. These results suggest the nonparticipation of the opioid system in the modulation of pain by HC.

Key words hydroxydihydrocarvone; monoterpen; essential oil; antinociceptive activity; catalepsy test; nalozone

MATERIAL AND METHODS

Preparation of Hydroxydihydrocarvone

HC was prepared in this laboratory as previously described. Next, it was dissolved in 5% Tween 80, which was used as an emulsion.

Animals

Albino male Swiss mice (25—35 g) were obtained from the Prof. Dr. Thomas George, Laboratory of the Federal University of Paraiba and were subsequently separated into groups of 8 animals. The animals were kept under standard environmental temperature conditions (21 ± 0.5 °C) with 12-h light/dark periods, light beginning at 06:00 h. Food and water were provided ad libitum until 1 h prior to the experimental procedures. The animals were acclimatized to the laboratory for 1 h prior to the experiments and the studies were conducted between 12:00 and 17:00 h. All experimental protocols were approved by the Institution’s Ethics Committee for the Care and Use of Animals (approval #0611/05).

Tail Immersion Test

The lower two-thirds of the tail was immersed in a beaker containing water kept at 50 ± 0.5 °C. The time in seconds until tail withdrawal from the water was considered the reaction time. Mice that had a reaction time of less than 4 s were selected. The reaction time was then measured 30, 60 and 120 min after intraperitoneal administration of HC (25—200 mg/kg), vehicle (control) and morphine (10 mg/kg). The mice were exposed to hot water for no longer than 12 s to avoid tissue injury.

Hot Plate Test

This test was used to measure response latencies according to the method previously described. Animals were placed on a hot plate maintained at 47 ± 0.5 °C. The time elapsed between placing the animal on the hot plate and the animal either licking its fore or hind paw or jumping on the surface was considered the response latency. Mice with baseline latencies of more than 15 s were excluded from the study. Response latency testing was measured prior to intraperitoneal administration (baseline) of HC (25—200 mg/kg), vehicle (control) and morphine (10 mg/kg) and at

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30, 60 and 120 min after each treatment. The cut-off time for the hot-plate test latency was set at 30 s. 25)

**Formalin Test** Nociceptive response was evaluated according to a previously described model. 26—28) The animals were injected with 20 μl of formalin 2.5% (0.92% formaldehyde diluted in saline) in the subplantar area of the right hind paw. The duration of paw licking was measured at 1—5 min (first phase) and 15—30 min (second phase) after the formalin injection. The amount of time spent licking the injected paw was considered as the nociceptive response. Animals were submitted to intraperitoneal administration of HC (25—200 mg/kg), vehicle (control) and morphine (10 mg/kg), 30 min prior to the injection of formalin. 29)

**Catalepsy Test** For evaluation in a horizontal bar test, forepaws were placed on a 6 cm high horizontal bar, while the hind paws remained on the floor. Latency to step down was recorded before the intraperitoneal administration of HC (25—200 mg/kg), vehicle (control) and haloperidol (5 mg/kg) and at 30, 60 and 120 min following administration. Animals were placed on the bar for a series of three tests. 30)

**Possible Antagonism of the Antinociceptive Effect of HC by Pretreatment with Naloxone** First, naloxone was administered to all the animals at a dose of 5 mg/kg (s.c.). After 15 min, the test groups were given 100 and 200 mg/kg of HC intraperitoneally, while the control group received the vehicle and the standard group was given morphine (10 mg/kg). The evaluations were made by submitting the animals to the hot plate test and the formalin test, a model used to clarify the possible mechanisms of the antinociceptive effect of a substance. 31)

**Statistical Analysis** The data obtained in the various experiments were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. The results obtained were considered significant when \( p < 0.05 \).

**RESULTS AND DISCUSSION**

The objective of the present study was to evaluate the antinociceptive effect of HC on several pain models. In the tail immersion test, which consists of a thermal stimulus, an increase in the reaction time is generally considered an important parameter for evaluating central antinociceptive activity. 32) This test is able to differentiate between central opioid-like analgesics and peripheral analgesics. 33) The antinociceptive activity of HC was observed at a dose of 200 mg/kg, showing a similar effect to that of morphine (Fig. 1). This test also revealed that the antinociceptive effect of HC on mice remained present up to 60 min after administration of the compound. The tail flick response is believed to be a spinally mediated reflex. 34) Moreover, Grumbach 35) has shown that the effectiveness of analgesic agents in the tail flick pain model is highly correlated with relief of human pain. HC was found to have antinociceptive activity in the hot plate test, which is a specific central antinociceptive test. 36) This effect was observed in animals treated with doses of 100 and 200 mg/kg of HC up to 60 min post-treatment (Fig. 2). The antinociceptive effect of HC involves supraspinal as well as spinal components as demonstrated by the use of the hot plate 37) and tail immersion 38) tests, respectively. The results suggest that HC has a central antinociceptive effect as shown by the prolonged delay in response when mice were subjected to a nociceptive stimulus in the tail immersion test and also by the increase in reaction time of the mice in the hot plate test.

The formalin test produced a distinct biphasic response, and different analgesics may act differently in this test. 39) The first phase results from direct chemical stimulation of the nociceptive afferent fibers, mainly C fibers, and the release of substance P, 40) and may be inhibited by centrally acting analgesics such as morphine. The second phase results from the...
action of inflammatory mediators released locally, such as prostaglandins, serotonin, histamine and bradykinin, and also from enhanced synaptic transmission in the spinal cord. Drugs whose principal mode of action is central, inhibit both phases of this test, whereas peripherally acting drugs only inhibit the second phase. As other substances that act on the central nervous system, HC dose-dependently inhibited both phases of the test in a manner similar to that of morphine (Figs. 3A, B). Moreover, the results of this test are in agreement with those obtained in the tail immersion and hot plate tests, confirming the central antinociceptive effect of HC.

HC did not produce catalepsy in mice (Fig. 4). This behavior is also observed with many depressants of the CNS and there have been reports of adverse effects during opioid use. This finding shows that, unlike opioids and antipsychotics, the antinociceptive effect of HC does not involve catalepsy. The next step in evaluating HC was to investigate its mechanism of action. The animals were pretreated with naloxone, an opioid antagonist that opposes the effects of opioid agonists such as morphine. The results of these tests showed that naloxone was unable to cancel the antinociceptive effect of HC in the hot plate (Fig. 5) and formalin tests (Figs. 6A, B). On the other hand, the effect of morphine was blocked, suggesting nonparticipation of the opioid system in the modulation of pain by HC. The present study showed the efficacy of HC in different antinociceptive responses generated by exposure to thermal noxious stimulus in the tail immersion and hot plate tests or by a chemical noxious stimulus in the tissue injury produced by formalin injection. The antinociceptive effect of HC would appear to occur at doses that evoked no visible modification in the overall behavior of the animals.

Therefore, HC produces a central antinociceptive effect without causing catalepsy that appears to be unrelated to the opioid system. However, further studies should be carried out to investigate the molecular mechanism of action of HC and its participation in the inhibitory mechanisms of pain in the CNS.

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