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Analgesic Properties of Umbellatine from *Psychotria umbellata*

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Abstract

Interesting analgesic activity has been previously identified in alkaloids isolated from the genus *Psychotria* (Rubiaceae). We here report the analgesic activity of umbellatine, a new glycoside indole monoterpene alkaloid isolated from *Psychotria umbellata.* Umbellatine produced a dose-dependent (100–300 mg/kg) analgesic effect, partially reversed by naloxone, in the tail-flick and hot-plate models. These results suggest an analgesic effect at least in part associated with the activation of opioid receptors. In the formalin- and capsaicin-induced pain models, umbellatine (100–300 mg/kg) elicited significant and dose-related antinociception. Umbellatine acts synergistically when co-administered with the NMDA antagonist MK-801. These results indicate the involvement of NMDA receptors in the umbellatine mechanism of action. Such a combined mode of action can be of relevance for developing analgesics useful in neurophatic pain.

Keywords: Analgesia, opioid, *Psychotria*, *P. umbellata*, umbellatine, NMDA.

Introduction

Natural products have been instrumental in the evolution of pharmacology and biochemistry, resulting in many useful drugs. Medicinal plants in particular are regarded as an important source of new chemical substances with potential therapeutic applicability (Balandrin et al., 1985). Regarding pain management, well-known and clinically useful substances such hyoscyamine, salicilic acid and morphine were all isolated from plant species used in traditional medicine as analgesics.

Management of most pain states has relied heavily on the use of non-steroidal and opioid analgesics; nevertheless, their usefulness is limited by unwanted effects (Perkins & Dray, 1996). Moreover, various pain states are unresponsive to available medication (Wiesenfeld-Hallin, 1998). This reality has stimulated a continuing search for new analgesics, ideally hoping to find effective and potent compounds devoid of undesired effects.

The genus *Psychotria* L. (Rubiaceae) has attracted a considerable amount of chemical/pharmacological investigation, due to the presence of bioactive alkaloids (Schultes & Raffauf, 1990). Analgesic activity has been reported for the pyrrolidinoindoline-type alkaloids, hodgkinsine and psychotridine, isolated from *Psychotria colorata* (Amador et al., 2000, 2001). Considering the interest in finding new potent analgesics, devoid of associated side effects and abuse liability, we engaged in a continuous investigation for analgesic compounds by associating ethnopharmacology with chemotaxonomy (Elisabetsky et al., 1997). Throughout the study, the crude ethanol extract of *Psychotria umbellata* Vell. (former *Psychotria brachypoda* Muell.Arg) was found to possess morphine like analgesic properties, as evaluated in mice with the tail-flick test (Leal & Elisabetsky, 1996).

The present work was undertaken to study umbellatine, the major alkaloid isolated from *P. umbellata* (nearly 1.3% w/w of the dry plant) (Fig. 1). Analgesic activity was evaluated through thermal and chemically induced pain models.

Materials and methods

Plant material

Leaves were collected by Gert Hatscbash, in February 1995; a voucher (No. 48571) has been deposited at the herbarium of the Museu Municipal de Curitiba, Paraná (Brazil).

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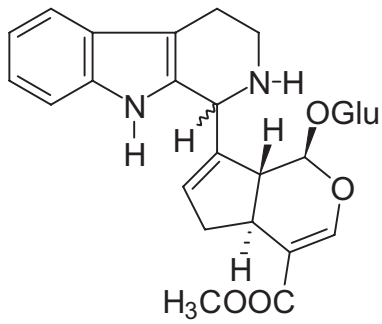

Figure 1. Structure of umbellatine.

Alkaloid isolation

Dried leaves (100 g) were extracted with EtOH at room temperature 3-times, each for a week. The extract was concentrated under vacuum at 40 °C to a dark green syrup. The syrup was dissolved in HCl 2% (0.5 l) and partitioned with CH_2Cl_2. The acid solution was alkalinized with NH_4OH 25% until pH = 10 and extracted with CH_2Cl_2. From the CH_2Cl_2 extract 954 mg of a colorless amorphous compound was precipitated. Purity of the compound was checked by TLC with silica gel 60 F_{254} ($CHCl_3$: MeOH/NH_3 vapor – 85 : 15 – RF = 0.2) and HPLC (column: NOVAPACK C_{18} 150 mm × 3.9 mm – Waters; MeOH : H_2O – 50 : 50 as eluent and a Photo Diode Array as detector; R_t = 2.13 min).

Animals

Male albino adult (25–35 g) mice (CF-1 strain), bred at the Fundação Estadual de Produção e Pesquisa em Saúde (Porto Alegre, RS, Brazil), were used in all experiments. Mice were kept in our own facilities on a 12 light/dark cycle, at a room temperature of 22 °C, with free access to food and water for at least 1.5 months before the experiments.

Forty-five minutes before analgesia experiments, the animals were individually placed in acrylic cages (20 × 20 × 20), which also served as observation chambers. These habituation periods aim to reduce exploratory behavior and reduce the novelty factor known to release opioids (Netto et al., 1987).

Drugs

Morphine sulfate, naloxone, capsaicin, formalin and MK-801 (dizocilpine) were acquired from Sigma (USA).

Tail-flick

The tail-flick latency was assessed by applying radiant heat on a selected spot on the tail using a tail-flick analgesymeter (Albarsch Electronics). A light sensor (0.01 sec sensitivity) located under the tail indicated the latency for its withdrawal. Experiments were carried out according to Amador et al. (2000). Baseline latency (reaction time) was obtained with three measures (after each measure, animals were returned to the observation chambers for two minutes), the mean considered as pre-drug latency. At this point, animals presenting two measures ≥6 sec were discarded. A cut-off time of 10 sec was established to prevent tissue damage. Treatments were administered (i.p.) immediately after the third pre-drug measure (6–8 per group). Thirty minutes later, another set of three measures was taken and the mean considered as post-drug reaction time. Morphine (6 mg/kg, i.p.) and saline were used as controls. Reversibility by naloxone (i.p., 15 mg/kg) was tested by administering naloxone 10 min before treatments.

Data are expressed as percentage of maximum possible effect (%MPE) according to the following formula: %MPE = post drug latency – pre drug latency/cut-off time – pre drug latency × 100 (Gardmark et al., 1998).

Hot-plate

Analgesia was assessed with a hot-plate apparatus (Ugo Basile) following the method of Woolfe and Mac Donald (1944) and Eddy et al. (1950). Twenty minutes before the test each animal was habituated to the apparatus for 1 min. After thorough cleaning of the apparatus, animals were placed in the heated surface (55.0 ± 1.0 °C) and the time elapsed between placing the animal and licking the forepaw or jumping was recorded as response latency. Latency measures were taken before (pre-drug) and 30 min after drug administration (i.p.). Animals exhibiting latency times greater than 12 sec in the pre-drug measure were excluded. A 30 sec cut-off time was used to prevent tissue damage. Morphine (6 mg/kg) and saline were used as controls. Reversibility by naloxone (i.p., 15 mg/kg) was tested by administering naloxone 10 min before treatments. Data are expressed as %MPE as for tail-flick.

Formalin test

The method described by Hunskaar and Hole (1987) was used. Twenty microliters of 1% formalin were injected into the dorsal surface of the left hindpaw. Mice were observed in the chambers with a mirror mounted on three sides to allow an unobstructed view of the paws; time spent licking the injected paw (licking time) was recorded. Animals were observed for the first 5 min post-formalin (early phase) or for 10 min starting at the 20th min post-formalin (late phase).

Capsaicin-induced pain

The methodology has been detailed elsewhere (Sakurada et al., 1992; Amador et al., 2000): after the adaptation period, 20 µl of capsaicin (1.6 µg/paw) were injected under the dorsal skin of the right hindpaw using a Hamilton microsyringe with a 26-gauge needle. Treatments were administered i.p. 30 min before capsaicin and animals were individually observed for 5 min after capsaicin administration. The time

spent in licking the injected paw was recorded and taken as the pain index.

Rota rod test

To evaluate if nonspecific muscle-relaxant or sedative effects were induced by umbellatine, mice were tested on the rota-rod apparatus (Ugo Basile). Experiments were carried out according to Dalmmeier and Carlini (1981) and adapted as follows: mice were initially trained to remain on the rota-rod apparatus (18 rpm) for 90 sec; those that did not remain on the bar for at least two out of three 90 sec consecutive trials were discarded. Umbellatine (100 and 200 mg/kg) or saline were administrated i.p. 24 h after this initial training. The latency to fall from the rota-rod (one 60 sec trial) was determined 30, 60, 90 and 120 min after administration of saline or umbellatine. Latency results are expressed as mean ± SEM.

Statistical analysis

Statistical analysis involved an initial one-way analysis of variance (ANOVA), followed by Student Newman Kewus (SNK).

Results

Figure 2a shows the results obtained with the tail-flick model. Umbellatine was clearly active at 100 mg/kg. The analgesic activities of 200 and 300 mg/kg of umbellatine were comparable in efficacy to those of 6 mg/kg of morphine, and the effects were partially reversed by naloxone. Hot plate results are presented in Fig. 2b. Umbellatine was dose-dependently active, and its activity was diminished but not completely reversed by naloxone. As with tail-flick, 200 and 300 mg/kg activity was comparable in efficacy to that obtained with 6 mg/kg of morphine.

The effects of umbellatine in the formalin model are shown at Fig. 3. In the acute phase (Fig. 3a) of the model, umbellatine produced a significant ($p < 0.01$) and dose-dependent inhibition, with an almost complete suppression (99.4% inhibition with 300 mg/kg) of licking. In the tonic phase (Fig. 3b) umbellatine (200 and 300 mg/kg) completely abolished the formalin-induced licking response ($p < 0.05$).

The effects of umbellatine in the capsaicin model are shown at Fig. 4. Umbellatine significantly ($p < 0.01$) inhibited capsaicin-induced pain. The effect was dose-dependent, detectable at 100 mg/kg, coming to a plateau at 200 mg/kg (89.4% inhibition). MK-801 also presented a clear dose-response effect (88.9% inhibition at 0.3 mg/kg) and a distinct synergism with umbellatine.

Figure 5 shows results obtained with the rota-rod. Umbellatine induced motor coordination deficits at 200 mg/kg, but not 100 mg/kg. The deficit was noted at all post-drug times studied.

Discussion

The present study established that umbellatine, a glycoside alkaloid presenting structural features similar to that of β-carboline alkaloids, possesses analgesic properties in mouse models using diverse algogenic stimuli. Given that umbellatine is the major component of *P. umbellata* and its ethanol or alkaloid extracts (Kerber, 1999), it can be concluded that

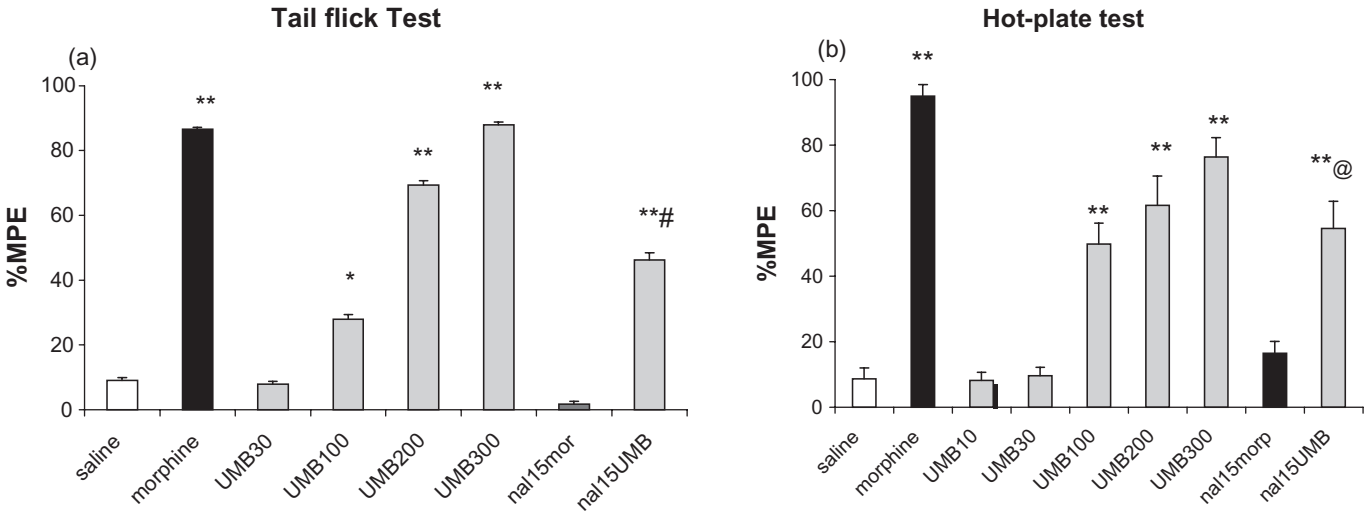

Figure 2. Umbellatine effects in the tail-flick (2a) and hot plate (2b) models. UMB = umbellatine (10–300 mg/kg); nalmorp = naloxone 15mg/kg + morphine; nalUMB = naloxone 15 mg/kg + umbellatine (200 mg/kg 2a; 300 mg/kg 2b). n = 6–8. Columns represent % of Maximum Possible Effect (%MPE) and vertical bars ± SD. * = $p < 0.05$, and ** = $p < 0.01$ compared to saline; # = $p < 0.01$ compared to umbellatine 200 mg/kg; @ = $p < 0.01$ × umbellatine 300 mg/kg ANOVA/SNK.

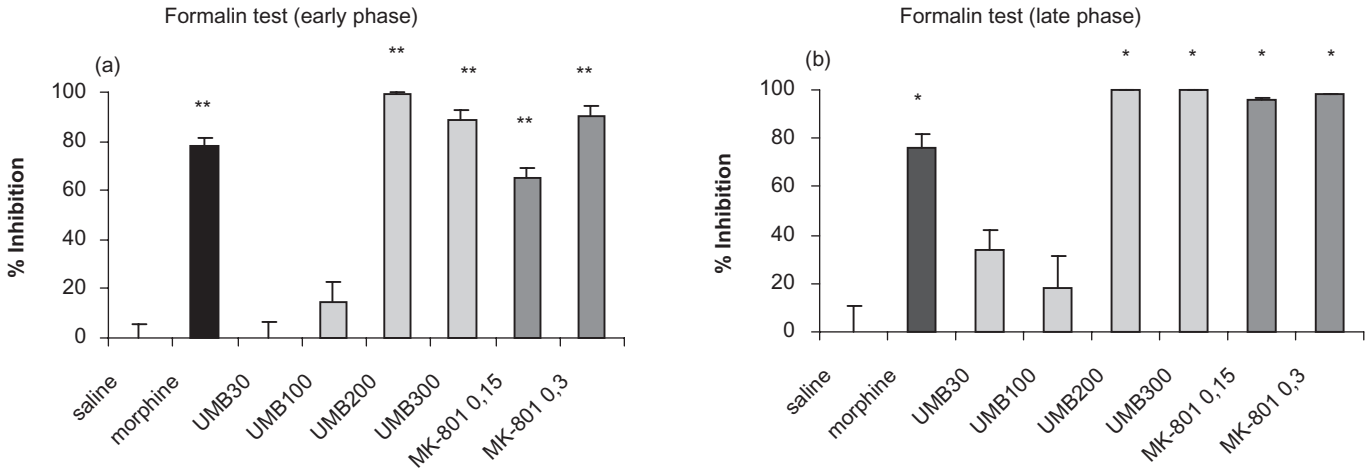

Figure 3. Analgesic effect of umbellatine (UMB) in the early (3a) and late (3b) phases of the formalin test. The columns represent percentage of inhibition and the vertical bars ± SE. UMB = umbellatine (30–300 mg/kg) n = 7–10, ** = $p < 0.01$, * = $p < 0.05$, ANOVA/SNK.

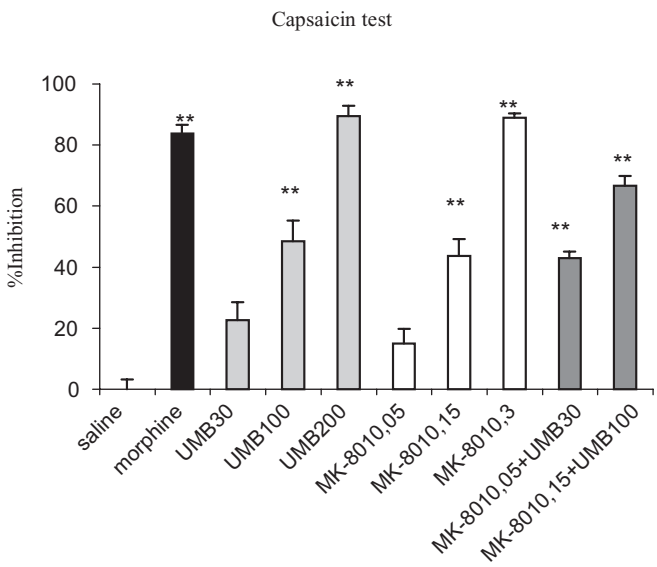

Figure 4. Effects of umbellatine (UMB) and the co-administration of the MK-801 with umbellatine in capsaicin-induced pain. The columns represent percentage of inhibition and the vertical bars ± SE. UMB = umbellatine (30–300 mg/kg); MK-801 + UMB = MK-801 + umbellatine. n = 7–10, ** = $p < 0.01$, ANOVA/SNK.

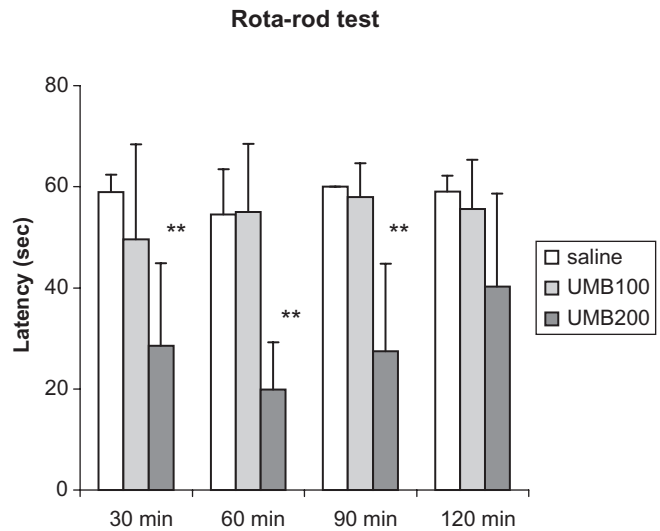

Figure 5. Effects of umbellatine in the rota rod test. UMB = umbellatine. Columns represent the latency (30, 60, 90 and 120 min) in seconds and vertical bars ± SD. n = 10 − 11. ** = $p < 0.01$, ANOVA/SNK.

umbellatine is the active compound responsible for the analgesic activity earlier detected with the crude extract (Leal & Elisabetsky, 1996).

As verified by this study, umbellatine produces a dose-dependent analgesic activity, partially reversed by naloxone, both in the tail-flick and the hot-plate models. These results suggest the involvement of opioid receptors, inasmuch as thermal nociceptive tests are more sensitive to opioid μ-agonists (Abbot & Young, 1988), and that activity is modified by naloxone.

Umbellatine presented a marked and dose-dependent analgesic effect in the capsaicin and formalin models of pain, with efficacy comparable to or greater than morphine or dizocilpine, although less potent that the latter. The data clear indicate the participation of NMDA glutamate receptors, since capsaicin elicits a significant release of glutamate and aspartate and activates NMDA receptors at the dorsal spinal cord *in vivo* and *in vitro* (Sakurada et al., 1998). Moreover, it has been shown that subcutaneous injection of formalin produces an increased release of glutamate and aspartate in the spinal corn dorsal horn (Coderre & Melzack, 1992). In addition, co-administration of umbellatine and MK801 in non-effective doses per se (30 and 0.05 mg/kg, respectively), result in analgesia comparable to umbellatine alone (100 mg/kg) or

MK801 (0.15 mg/kg) alone. Likewise, effective doses of umbellatine and MK801 (100 and 0.15 mg/kg, respectively) when co-administered result in a greater effect, comparable to morphine.

The formalin test possesses two distinctive phases, possibly reflecting different types of pain. The earlier acute phase (0–5 min) reflects direct effect of formalin on nociceptors (non-inflammatory pain) (Hunskaar & Hole, 1987), whereas the late tonic phase is also accompanied by the onset of a persistent inflammatory response that lasts well beyond the cessation of the nociceptive response (Hunter & Singh, 1994). Both earlier and late phases are known to release glutamate in the spinal corn dorsal horn (Coderre & Melzack, 1992). Nevertheless, NMDA antagonists (such as MK801) reduce the slowly developing tonic nociception after formalin injury, but not the immediate response to formalin. This observation suggests that antagonism of NMDA receptors specifically suppresses the development of the plasticity needed to the establishment of the late phase response, rather than producing a generalized analgesic effect (Coderre & Melzack, 1992). Contrasting with MK-801, umbellatine was active in both phases of the formaline test, a pattern that may result from the activation of opioid receptors by umbellatine.

Rota rod was used to evaluate if umbellatine induced motor deficits at doses found to be analgesic (Stamford, 1995). Motor deficit, in terms of coordination at the rotating bar, was observed with 200 mg/kg (but not with 100 mg/kg) of umbellatine, whereas analgesic activity is already observed with 100 mg/kg. Although the highest analgesic effect (obtained with 200 and 300 mg/kg) may have been somewhat enhanced by motor deficits or tainted by an eventual impairment of motor reflexes, we believe that analgesia in this study should not be considered a false positive result since animals treated with the highest dose of umbellatine do not loose righting reflex, the ability to jump, lick their paws or move their tail, being always responsive to touch. It is noteworthy that in the rota rod umbellatine shares with MK801 the same pattern of activity (i.e., ataxia with higher doses); therefore, results are consistent with the hypothesis that activation of NMDA receptors is part of the mode of action of umbellatine.

There is abundant evidence for a major role of the widely distributed glutamatergic NMDA receptors, in a large number of processes including development, learning and memory, degenerative diseases, drug addiction, pain and plasticity in general (Trujillo & Akil, 1991). The excessive activation of NMDA receptors that leads to cell death and neuronal degeneration, may also have an important role in the development of acute and chronic pain (Mao et al., 1992; Wiesenfeld-Hallin, 1998). Accordingly, a number of studies have attempted to define a clinical role for NMDA antagonists in neurophatic pain. Despite the well documented cognitive side effects, usually psychotomimetic in nature, there is an increasing body of evidence suggesting that NMDA antagonists may be useful in managing certain aberrant pain states (Chaplan et al., 1997).

Recent studies found a remarkable potentiation and prolongation of morphine-induced behavioral antinociception by NMDA antagonists in rats. The co-administration of NMDA antagonists with morphine also reduced the development of tolerance. It has been further suggested that in opiate tolerance the administration of NMDA antagonists could reestablish the antinociceptive effect of opioids, i.e., reverse tolerance (Wiesenfeld-Hallin, 1998), which is experience-dependent and in many respects is similar to learning. NMDA antagonism is therefore of clinical relevance for pain management, since a number of human studies have now supported the ideas that came from animals models (Dickenson, 1997). In this context, it is of interest that umbellatine apparently combines opioid activation and NMDA antagonism properties. The exact mode of interaction of umbellatine with these receptor types are therefore worth investigating.

Interesting enough, in the genus *Psychotria*, analgesic activity was identified in alkaloids with diverse molecular structures, such as the indole monoterpene alkaloid umbellatine, and the pyrrolidinoindoline-type alkaloids hodgkinsine and psychotridine. Further characterization of analgesic profile, as well as molecular modeling studies, will reveal the basis for such pattern of biological activity and the possibilities for its applicability in medicinal chemistry and drug development.

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