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Strictosidinic acid, isolated from *Psychotria myriantha* Mull. Arg. (Rubiaceae), decreases serotonin levels in rat hippocampus

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ABSTRACT

Psychotria is a complex genus whose neotropical species are known by the presence of glucosidic monoterpene indole alkaloids. These compounds are able to display a large range of effects on the central nervous system, such as anxiolytic, antidepressant, analgesic, and impairment of learning and memory acquisition. The aims of this study were to investigate the effects displayed by strictosidinic acid, isolated from *Psychotria myriantha* Mull. Arg. (Rubiaceae) leaves, on monoamine levels in rat hippocampus and on monoamine oxidase activity. A significance ($p<0.01$) of 83.5% reduction in 5-HT levels was observed after intra-hippocampal injection (20 μg/μl). After treatment by intraperitoneal route (10 mg/kg), a 63.4% reduction in 5-HT levels and a 67.4% reduction in DOPAC values were observed. The results indicate that strictosidinic acid seems to act on 5-HT system in rat hippocampus, possibly inhibiting precursor enzymes of 5-HT biosynthesis. The decrease verified in DOPAC levels suggests a role of strictosidinic acid in the dopaminergic transmission, probably due to an inhibition of monoamine oxidase activity, confirmed by the enzymatic assay, which demonstrated an inhibitory effect on MAO A in rat brain mitochondria.

Introduction

Psychotria (Rubiaceae) is a taxonomically complex genus [1]. In the folk medicine *Psychotria* species are employed all over the world in the treatment of several diseases such as: diarrhea and intestinal parasites [2], snake bites [3], viral and bacterial infections [4,5], hypertension, cardiovascular dysfunctions, mental disturbs and alimentary disorders [6]. *Psychotria viridis* is one of the most cited species, probably due to its effects on central nervous system. This specie, along with *P. carthagenensis* and *Banisteriopsis caapi*, is constituent of the hallucinogenic "ayahuasca" beverage, traditionally used for religious practice in Amazonian region [7].

Neotropical species, belonging to the subgenus *Heteropsychotria*, have been subjected to chemical and pharmacological investigations, revealing the presence of bioactive glucosidic monotepene indole alkaloids (MIAs) [8–14]. Psychollatine, the major MIA isolated from *Psychotria umbellata* Vell., exhibited mild analgesic effects against a number of algogenic stimuli [15], anxiolytic (7.5 and 15 mg/kg) and antidepressant effects (3 and 7.5 mg/kg) in mice models [16,17]. In higher doses (100 mg/kg), psychollatine impaired the acquisition of learning and memory

Abbreviation: MIA, monoterpene indole alkaloids.

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consolidation [16], suggesting the modulation of different neurotransmitter systems, such as glutamate, opioid and serotonergic pathways. Alkaloid extract from *Psychotria myriantha* Mull. Arg., a shrub occurring in southern Brazil, showed dose-dependent analgesic effect (200 mg/kg), partially reversed by naloxone in the hot plate model [18], suggesting the involvement of NMDA receptors in its mechanism of action. Strictosidinic acid, a glycoside indole monoterpene alkaloid isolated from leaves of this specie, is able to inhibit in vitro polymorphonuclear leukocytes (PMN) chemotaxis [13] and it has shown peripheral analgesic and antipyretic activities in mice after oral administration [19]. Moreover, strictosidinic acid (10 mg/kg) seems to act on 5-HT and DA systems in rat striatum, increasing the monoamines metabolism in this brain area [20]. Fractions of *P. suterella* and *P. laciniata* were able to inhibit rat brain monoamine oxidase A (MAO-A) in concentrations ranging from 0.5 to 135 μg/mL. The chemical analysis of the active fractions suggested that the enzymatic inhibition could be attributed to the alkaloids E/Z-vallesiachotamine [21], which were previously described in other *Psychotria* species.

Dopamine (DA), serotonin (5-HT) and their main metabolites (3,4-hydroxyindoleacetic acid, DOPAC; homovanilic acid, HVA; and 5-hydroxyindole acetic acid, 5-HIAA) represent important monoamines and metabolites-derived neurotransmitters. DA is one of the most important excitatory neurotransmitters, being widely distributed in the mammalian brain, including the hippocampus. The central dopaminergic transmission is involved in a variety of behaviors and brain functions, including motor activity, cognition, emotion, positive reinforcement, food intake and endocrine regulation [22]. The serotonergic transmission on the CNS has been related with multiple behaviors such as food intake, endocrine regulation, activity rhythm, sexual behavior, sleep, and emotional states [23]. Alterations in 5-HT transmission are related with some neurological and psychiatric illness including migraine, hallucinations, anxiety and depression [24]. In addition, changes on DA transmission are associated with Parkinson disease and schizophrenia [25].

Monoamine oxidases (MAOs) are mitochondrial outer membrane-bound flavoenzymes which catalyze the oxidative deamination of several important neurotransmitters, including 5-hydroxytryptamine (5-HT, or serotonin), histamine and the catecholamines dopamine, norepinephrine and epinephrine [26]. Two subtypes of MAO, MAO-A and MAO-B, are similar in their primary sequences but have different substrate and inhibitor affinities. MAO-A is inhibited by low concentrations of clorgyline and catalyzes the oxidation of 5-HT, whereas MAO-B is inhibited by low concentrations of *l*-deprenyl or pargyline and is active towards benzylamine and 2-phenylethylamine. Dopamine, norepinephrine, tryptamine and tyramine are oxidized by both forms of the enzyme in most species [26,27]. MAO-A inhibitors have been proven to be effective in the pharmacological treatment of depression and further developments have provided reversible inhibitors of MAO-A, which offer antidepressant activity without the serious side effects of the earlier inhibitors. On the other hand, selective inhibitors of MAO-B have found a therapeutic role in the treatment of Parkinson's disease [28].

Considering the relevance of monoamines (DA and 5-HT) in brain functions and the previous results found to *P. myriantha* and strictosidinic acid, the aims of this study were (i) to investigate the effect of intra-hippocampal and acute intraperitoneal (i.p.) strictosidinic acid treatment in the monoamine levels and their metabolites in rat hippocampus, and (ii) to evaluate the effect displayed by strictosidinic acid on MAO-A and MAO-B activities, employing rat brain mitochondria as enzymatic source.

Experimental methods

Chemicals

Dopamine (DA), 3,4-dihydroxyphenyl acetic acid (DOPAC), 3-metoxytyramine (3-MT), homovanillic acid (HVA), serotonin (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA), kynuramine dihydrobromide, pargyline hydrochloride, clorgyline hydrochloride, 4-hydroxyquinoline (4-OH), dimethyl sulfoxide (DMSO), bovine albumin (BSA), HEPES and D-mannitol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 3,4-Dihydroxybenzylamine (DHBA) was obtained from Aldrich Chemical Company Inc. (USA). Sucrose, potassium chloride and sodium chloride were acquired from Labsynth (Diadema, SP, Brazil). Sodium phosphate monobasic monohydrate and sodium phosphate dibasic dodecahydrate were purchased from Merck (Darmstadt, Germany). All remaining chemicals used were of analytical grade and were purchased from F. Maia (Cotia, SP, Brazil). Stock solutions of kynuramine, pargyline, clorgyline and 4-OH were prepared in PBS buffer (pH 7.4) and maintained at −20 °C for until six months.

Plant material

P. myriantha was collected in Reserva Estadual do Turvo, Derrubadas, Rio Grande do Sul, Brazil and identified by M. Sobral. A voucher specimen (M. Sobral et al., 8913) was deposited in the ICN Herbarium (Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil).

Extraction and isolation

The alkaloid was extracted from leaves of *P. myriantha* as previously described by Simões-Pires et al. [13]. Briefly, dried leaves were extracted with EtOH at room temperature. The extract was concentrated under vacuum at 40 °C and the alkaloids fraction was obtained by acid/base extraction, being the alkaline extracts partitioned with dichloromethane and n-butanol. The butanolic extract was purified by semi-preparative HPLC using Symmetry-Prep column (7 μm, 19×150 mm,

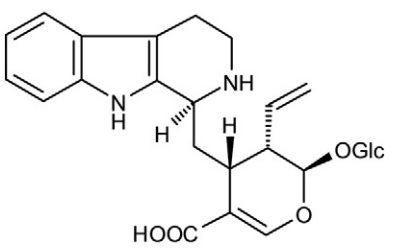

Fig. 1. Chemical structure of the alkaloid strictosidinic acid.