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Mechanism for the uncoupling of oxidative phosphorylation by juliprosopine on rat brain mitochondria

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ABSTRACT

Prosopis juliflora, popularly known as Algaroba, is a major problem because the lack of food during the driest times of the year and its high palatability and nutritional value make its fruits (pods) much appreciated by cattle, goats, sheep and other animals. However, the consumption of this plant for long periods can cause a disease called *cara-torta* (pie face), which is characterized by cranial nerve dysfunction, mainly due to the degeneration and disappearance of neurons in the trigeminal motor nucleus. Algaroba contains piperidine alkaloids that have been suggested as being responsible for its toxicity; one of these alkaloids is juliprosopine. This study was conducted to evaluate the mechanisms of action of juliprosopine in isolated rat brain mitochondria to evaluate the potential mechanisms that lead to neurotoxicity in animals intoxicated by algaroba. Juliprosopine stimulated state-4 respiration at concentrations of 10–25 μM, affected the membrane potential at all concentrations studied (5–25 μM) and affected ATP production only at higher concentrations (15 and 25 μM). Juliprosopine cannot be classified as a member of the protonophoric class of uncouplers, such as 2,4-dinitrophenol or CCCP (m-chlorophenylhydrazone), due to its inability to promote mitochondrial swelling in the hyposmotic medium of potassium acetate. In addition, carboxyatractyloside, Mg^{2+}, cyclosporine A and dithiothreitol did not protect the uncoupling induced by juliprosopine. Because juliprosopine increased the fluorescence responses of mitochondria labeled with 1-aniline-8-naphthalene sulfonate (ANS) and DPH (1,6-diphenyl-1,3,5-hexatriene), we suggested that its uncoupling action must be attributed to a modification of the arrangement of the inner mitochondrial membrane.

1. Introduction

The plant *Prosopis juliflora*, popularly known as algaroba or algarobeira, is a shrub belonging to the family Leguminosae, subfamily Mimosoideae. The genus *Prosopis* contains 44 species distributed in the arid and semiarid regions of the Americas, North Africa and East Asia. Some piperidine alkaloids are present in these species, such as juliprosopine, julifloricine, julifloridine, and juliprosinene (Tabosa et al., 2000); according to Ahmad et al. (1991), juliprosopine (Fig. 1) is present in all parts of the plant, including the fruit.

The intoxication after consuming *P. juliflora* pods has been reported in cattle and goats in the USA (Dollahite and Anthony, 1957; Dollahite, 1964) and Brazil (Figueiredo et al., 1996; Lima et al., 2004), and in goats in Peru (Baca et al., 1966). In Brazil, the algaroba is a major problem because the lack of food during the driest times of the year and its high palatability and nutritional value make the fruits of

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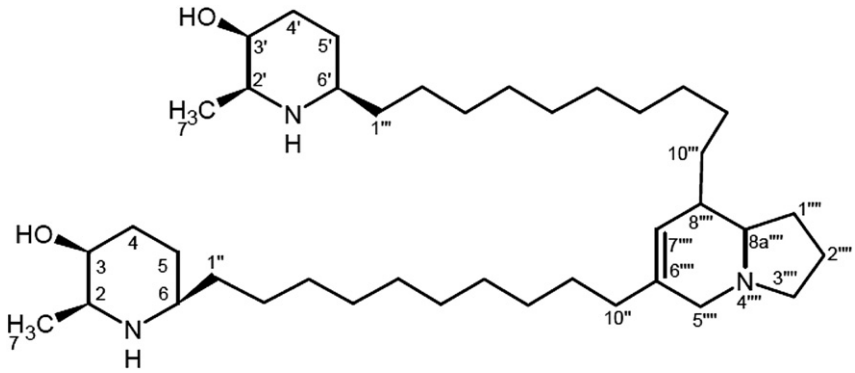

Fig. 1. Chemical structure of the piperidine alkaloid juliprosopine.

algaroba (pods) much appreciated by cattle, goats, sheep and other animals (Silva, 1989; Tabosa et al., 2004; Mahgoub et al., 2005). It has also been used for human consumption in breads, biscuits, jellies, sweets, and spirits (Tabosa et al., 2004).

In farm animals, the dietary intake of *P. juliflora* pods in large quantities for prolonged periods can cause a disease called *cara-torta* (pie face) (Figueiredo et al., 1996), which is characterized by cranial nerve dysfunction, mainly due to the degeneration and disappearance of neurons in the trigeminal motor nucleus (Tabosa et al., 2006). In a histological analysis of the neurons of the trigeminal nuclei of animals poisoned by the plant *P. juliflora*, Tabosa et al. (2006) observed a marked swelling of the mitochondria and that the mitochondrial crest was peripherally displaced and disintegrated.

These changes in the mitochondrial morphology may prevent its proper operation, which is detrimental to the cell because the mitochondria perform a variety of biochemical processes and produce a majority (>90%) of the cellular ATP via oxidative phosphorylation (Mitchell, 1961).

Uncouplers of oxidative phosphorylation in the mitochondria prevent the coupling between the electron transport and phosphorylation reactions, thereby inhibiting the synthesis of ATP (Terada, 1990; Rahn et al., 1991). By increasing the permeability of the inner mitochondrial membrane to protons over a continuous gradient from the intermembrane space to the mitochondrial matrix, these compounds prevent the organelle from maintaining ATP synthesis (Kadenbach, 2003).

Given the lack of knowledge regarding the exact molecular and biochemical mechanisms of action for alkaloids present in *P. juliflora* and the results obtained in our recent studies suggesting that mitochondria are a major target organelle of toxic compounds isolated from toxic plants (Mingatto et al., 2007; Santos et al., 2009; Garcia et al., 2010), this study was conducted to evaluate the effects of the piperidine alkaloid, juliprosopine, on the bioenergetics of mitochondria isolated from the rat brain. Using the fluorescent probes, ANS (1-anilino-8-naphthalene sulfonate) and DPH (1,6-diphenyl-1,3,5-hexatriene), we propose that the uncoupling of oxidative phosphorylation promoted by juliprosopine may be due to an interaction of the compound with the mitochondrial membrane.

2. Material and methods

2.1. Plant material

P. juliflora (family Leguminosae, subfamily Mimosoideae) pod samples were collected in a rural area from Patos (07° 01′ 28″S, 37° 16′ 48″W), Paraíba, Brazil.

2.2. Extraction and isolation of juliprosopine

The juliprosopine extraction was performed according to the methodology described by Tabosa et al. (2000). After purification, the alkaloid was subjected to identification by ^{1}H NMR and ^{13}C and was confirmed as the piperidine alkaloid juliprosopine.

2.3. Animals

Male Wistar rats weighing approximately 200 g were used in this study. The animals, obtained from the Central Bioterium of UNESP–Univ Estadual Paulista, Campus de Botucatu, SP, Brazil, were maintained with a maximum of 4 rats per cage under standard laboratory conditions with water and food provided *ad libitum*. The experimental protocols were approved by the Ethical Committee for the Use of Laboratory Animals of the UNESP–Univ Estadual Paulista, Campus de Dracena, SP, Brazil.

2.4. Isolation of rat brain mitochondria

Mitochondria were isolated by a modified procedure based on the method previously described by Rosenthal et al. (1987). The rats were euthanized by decapitation, and the brain was immediately removed. The brain slices were placed into 10 mL of isolation buffer containing 0.21 M mannitol, 70 mM sucrose, 1 mM EGTA, 1 mg/mL BSA and 5 mM HEPES–KOH, pH 7.4, and were homogenized three times for 15 s at 1-min intervals with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 3000 × *g* for 2 min. The resulting supernatant was centrifuged at 12,000 × *g* for 20 min. The pellet was