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Identification of brain areas sensitive to the toxic effects of sparteine

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ABSTRACT

Sparteine is one of the most toxic quinolizidine alkaloids found in leguminous plants. Several studies have demonstrated that sparteine affects the nervous system, blocking the nervous ganglion, producing antimuscarinic effects, depressing the central nervous system and causing neuronal necrosis. However, there are no reports identifying the areas of the brain that are sensitive to the toxic effects of this alkaloid. 32 adult Wistar rats were on study, sixteen were implanted with an intracerebral stainless steel cannula and randomly assigned to a control or experimental group (n = 8). Animals, control and experimental, received daily intraventricular (ICV) injections of a sparteine or a sterile water solution for five consecutive days. Additionally, two groups of animals (8 rats each) received daily intraperotineal injections (IP) of a sparteine or sterile water solution for five consecutive days. 72 h after the last dose, the animals were sacrificed, their brains removed, fixed and embedded in paraffin to obtain 10 μm tissue slices. Brain slices were stained with H&E and evaluated under a light microscope. The main brain structures sensitive to sparteine were the cerebral cortex (frontal, fronto-parietal and striate) olfactory and amygdaloid areas, the ventromedial hypothalamic nucleus, the Purkinje cells in the cerebellum, and the CA1, CA3 and dentate gyrus regions of the hippocampus.

Administration of sparteine, via ICV or IP, caused neuronal necrosis in brain structures, mainly related with cholinergic pathways.

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1. Introduction

Sparteine is a quinolizidine alkaloid (QA) widely distributed in some species of the *Fabacea* family (Schmeller and Wink, 1998). QA allows plants, including lupins, to defend themselves against depredators such as mammals, insects, and snails (Wink and Hartmann, 1982; Wink, 1993, 1992). Sparteine is obtained from the leaves of *Cytisus scoparius Linn* for pharmacological use and is widely distributed in species of the *Lupinus* genus (Kim et al., 1982).

Several studies have shown that sparteine affects the autonomic nervous system, causing, among other things, ganglion blocking and antimuscarinic effects (Cohen and Jacquot, 2008; Lechat, 1978; Piere and Kirkiacharian, 1986; Schmitt, 1980). QA also depresses the central nervous system (Piere and Kirkiacharian, 1986; Schmitt, 1980) and has oxytocic, (Lechat, 1978; Piere and Kirkiacharian, 1986; Cohen and Jacquot, 2008) diuretic and local anesthetic effects (Schmitt, 1980; Cohen and Jacquot, 2008). For many years, sparteine was used as an oxytocic, and antiarhythmic drug (Cohen and Jacquot, 2008; Lechat, 1978; Piere and Kirkiacharian, 1986); however, it fell into disuse due to its side effects, consisting of cardiovascular events (arrhythmia, tachycardia), neurological (weakness, fatigue, blurred vision and lack of coordination) and gastrointestinal symptoms (nausea and vomiting). These disorders occur in 10% of the patients, who are considered poor QA metabolizers (Schmeller and Wink, 1998). Acute sparteine intoxication in humans has also been reported, characterized by neurotoxic effects such as decreasing cardiac contractility, blocking of ganglionic transmission and contraction of uterine smooth muscle (Robbins et al., 1996; Yovo et al., 1984).

Therefore, there is evidence that both, the central and peripheral, nervous systems are the main target tissue for sparteine. In the peripheral nervous system, this compound inhibits the ganglionic signal transmission (Lechat, 1978; Piere and Kirkiacharian, 1986; Cohen and Jacquot, 2008), and in high doses reduces carotid sinus and coronary flow, as well as cardiac contraction and amplitude (Agid et al., 1988). The proposed mechanism by which sparteine causes cerebral damage includes

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activation of muscarinic and nicotinic receptors of "Ach" (Bañuelos Pineda et al., 2005; Flores-Soto et al., 2006; Schmeller et al., 1994), as well as the inactivation of Na+ and K+ channels (Körper et al., 1998; Schauf et al., 1976; Schmeller et al., 1994).

There are few reports on the effect of sparteine on the central nervous system of experimental animals. Acute toxicity symptoms, of a purified spartein extract injection, include reduced locomotor activity (Pothier et al., 1998) and repeated subcutaneous administration of sparteine to rat neonates causes neuronal necrosis in the motor cortex with presence of eosinophilic neurons (Flores-Soto et al., 2006) and necrotic neurons with shrunken eosinophilic cytoplasm and strongly stained pyknotic nuclei. The administration of crude extracts of lupin QA (containing 70% sparteine) resulted in damage to several cerebral structures (Bañuelos Pineda et al., 2005).

Therefore, the objective of this study was to identify rats's brain areas sensitive to intracranial or intraperitoneal sparteine administration

2. Materials and methods

32 male Wistar rats weighing 250–300 g were housed in individual cages in an environmentally controlled room. Water and commercial rat ration were provided *ad libitum* (Ralston-rations, USA). The animals were randomly divided into two groups of 16 rats each. Using a stereotaxic apparatus (David-Kopf-Instruments), the animals in one group (IVC) were implanted with an intracerebral stainless steel cannula (0.72 mm in outer diameter, 0.37 mm internal diameter and 9.0 mm in length) into the right lateral ventricle using the following coordinates: 1.4 mm mediolateral, 8.2 mm above lambda, and −4.0 mm dorsoventral (Paxinos and Watson, 1982). The cannula was adjusted with a plastic ring to achieve the desired depth (4 mm). The other 16 animals were used to evaluate the intraperitoneal administration route (IP). After the postoperative recovery, the ICV animals were given 10 ng of sparteine (Sigma Chemical Co., St. Louis, MO, USA) or sterile water daily for five consecutive days. This dose is considered sublethal for this administration route (Lechat, 1978). Animals in the IP group were administrated, intraperitoneally, 1.85 mg/kg (5% of the DL_{100} for sparteine) of sparteine daily for five consecutive days. The administration of sparteine was done between 9:00 and 10:00 a.m. on each day. 72 h after the last sparteine dose, the animals were anesthetized with an IP injection of sodium pentobarbital (50 mg/kg) and sacrificed by intracardiac perfusion (100 ml of saline solution at 37 °C for 4 min), followed by 250 ml of 4% paraformaldehyde in 0.1 M, pH 7.4 phosphate buffered saline solution. The brains were extracted and post fixed in the same fixing solution for 24 h, washed with phosphate-buffered saline solution, dehydrated by placing them in alcohol solutions of increasing concentrations, and finally embedded in paraffin. 10 μm thick sagittal and coronal sections were prepared, from each brain, using a rotary microtome; they were placed on slides and stained with hematoxylin and eosin. The stained tissue slices were dehydrated, clarified and covered with resin (Entellan, Merck). Microscopic examination was performed on the cerebral hemisphere contralateral to the hemisphere where the cannula was placed, in both ICV groups, using the 40× objective. Regions with signs of necrosis l (pyknosis, cavitation and eosinophilia), were identified based on the descriptions previously published by (Bañuelos Pineda et al., 2005). The proportion of necrotic cells, for each of the structures in which damage was present, was recorded in 20 different microscopic fields (0.78 mm^2/field) using a Leica microscope coupled to an image analyzer (QWIN-500). Only neurons that showed pyknosis, cytoplasmic eosinophilia and shrinkage (eosinophilic neurons) were considered for quantification. Brain structures were identified based on the terminology of the atlas of Paxinos and Watson.

Statistical analysis of the data, obtained from the quantification of "eosinophilic cells", was done using Student's *t* test, according to the administration routes. Differences between treatments groups were considered statistically significant when $p < 0.05$ using SPSS statistical program version 10.0 (SPSS Inc, Chicago IL, USA).

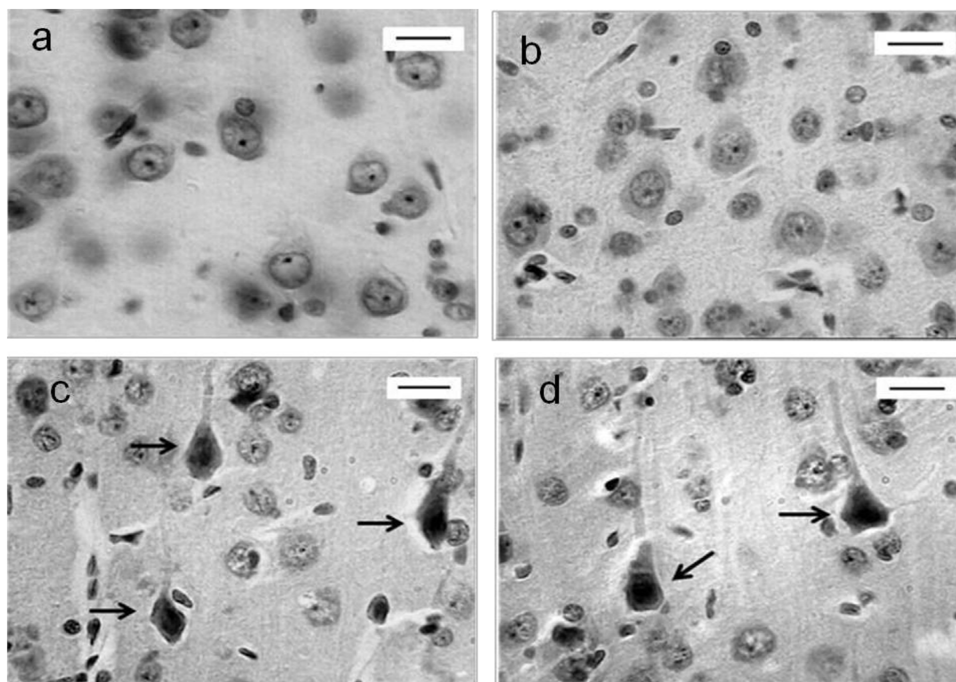

Fig. 1. Coronal slices of rat brain stained with H&E show normal and altered neurons. (a) Tissues from control IP rats show normal appearance, the parenchyma appears compact and the neurons show a spherical nucleus with scattered heterochromatin; (b) control ICV tissues, characterized by light gliosis; (c) eosinophilic neurons (arrows) are marked (red cells) with a strong reddish color by an alteration of pH (IP sparteine group); (d) similar red neurons with cytoplasm containing dissolved organelles and eccentric nucleus (arrows); the nervous parenchyma shows a spongy appearance (ICV sparteine group). Bar: 20 μm.

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