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Huperzia saururus increases memory retention in rats

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Abstract

Huperzia saururus is reported in Argentinean popular medicine as a memory improver. Chemical studies have shown that the main constituents of the species are *Lycopodium* alkaloids. On the basis of this information, a purified alkaloid extract was obtained by alkaline extraction of the aerial parts. The aim of this work was to investigate the effects of intra-hippocampal administration of the purified alkaloid extract (AE) on memory retention in vivo, using a step down test, in order to correlate with previous results obtained in vitro in an electrophysiological model. The AE administration significantly increased the latency time in comparison to control animals. For treated animals the latency time was 37.61 ± 2.84, 80.94 ± 2.37, and 180.00 ± 5.74 s for 1, 5, and 10 ng/rat, respectively versus 14.89 ± 2.38 s for controls. According to these results there is a good relationship between the ethnopharmacological use and the effects hereby showed.

Keywords: *Huperzia saururus*; *Lycopodiaceae*; Memory retention; Step-down test

1. Introduction

Huperzia saururus (Lam.) Trevis. (Lycopodiaceae) is a fern that grows from the north of Peru to Argentina, in South America, and also in Africa, Madagascar and the Mascarenes (Øllgaard, 1992). In Argentina, its habitat extends from high altitudes in the north-west (Jujuy, Salta and Catamarca) to the central part of the country (San Luis, Córdoba and Buenos Aires) (Ponce, 1996). *Huperzia saururus* named as "cola de quirquincho" is used mainly as an aphrodisiac (Amorín, 1974; de la Sota, 1977; Ratera and Ratera, 1980), but it has been also reported that it could improve memory (Martinez Crovetto, 1981).

Many studies have demonstrated the participation of the hippocampus in learning and memory processes (Bliss and Colingridge, 1993). Long-term potentiation (LTP) is a persistent increase in synaptic efficacy, and several properties of LTP have led to the suggestion that is a synaptic mechanism of mnemonic functions in the mammalian brain (Maren and Baudry, 1995). In previous studies performed in our laboratory, we have demonstrated in an electrophysiological in vitro model of rat hippocampal slices that the purified alkaloid extract (AE) of *Huperzia saururus* induces and maintains the LTP (Ortega et al., 2006).

With respect to the ethnopharmacological use on memory, the aim of this work was to investigate the effects of the intra-hippocampal administration of AE of *Huperzia saururus* on memory retention in vivo, using a step down test, in order to compare them with previously results obtained in vitro (Ortega et al., 2006).

2. Materials and methods

2.1. Plant material

Plant material was collected in Pampa de Achala, Departamento San Alberto, provincia de Córdoba, Argentina, in November 2001, and identified by Dr. Gloria Barboza, Instituto Multidisciplinario de Biología Vegetal, Universidad Nacional de Córdoba. A voucher specimen is deposited at the herbarium of the Museo Botánico de Córdoba (CORD) as CORD 684.

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2.2. Extraction and purification

Aerial parts of *Huperzia saururus* (350 g) were dried at shadow, ground and then alkalinized with NaOH reduced to powder (30 g). This mixture was hydrated with distilled water until pH 12 and extracted with $CHCl_3$ by using a Soxhlet extractor. The organic solvent was evaporated under reduced pressure until acquiring half of its original volume. This crude total extract was partitioned twice with 2% tartaric acid. The acidic aqueous extracts were combined and then alkalinized with 0.1 N NaOH to pH 12 and subsequently partitioned with $CHCl_3$ by using a liquid–liquid extractor. The chloroform extract obtained was purified through Sephadex LH-20 in a glass column employing $CHCl_3$/EtOH (1:1) as the mobile phase. All fractions positive to Dragendorff's reagent were combined and evaporated under reduced pressure to yield 11 g of the alkaloid-purified extract (AE) (3.14%, w/w, calculated relative to dry starting material). 1 mg of AE was dissolved in 10 μl of pH 5 HCl. Saline was then added up to a volume of 1000 μl. Finally, aliquots of 1, 5 and 10 μl were taken up again to a volume of 1000 μl with saline. This way, concentrations of 1, 5 and 10 μg/ml were used to develop the test.

2.3. Animals

The experiment was performed on adult male Wistar rats (60 days old at the beginning of the treatment, weighing 240–260 g) with food and water ad libitum. The colony room was maintained under controlled temperature (21–23 °C) and light (12-h light:12-h dark). The animals were placed in individual cages.

2.4. Experimental protocol

All procedures performed in the present work were conducted according to the guidelines of NIH Guide for the Care and Use of Laboratory Animals as approved by the School of Chemical Sciences, National University of Córdoba Animal Care and Use Committee.

2.5. Surgery

The animals were anesthetized with 55 mg/kg ketamine HCl and 11 mg/kg xylazine (both from Laboratorios König S.A, Buenos Aires, Argentina) and placed in a stereotaxic apparatus. The rats were divided into two groups and bilaterally implanted into the hippocampus with steel guide cannula, following the atlas of Paxinos and Watson, 1986. The coordinates relative to bregma were anterior: −4.3 mm; lateral: ± 2.4 mm; vertical: −2.6 mm for hippocampus CA1 region, according to the atlas of Paxinos. Cannulas were fixed to the skull surface with dental acrylic cement. During the 7 days of recovery period, the animals were handled daily to habituate them to the injection procedures. They were then injected with AE or saline, using a 10 μl Hamilton syringe connected by Pe-10 polyethylene tubing to a 30-gauge needle extending 0.75 mm beyond the guide cannula.

Each infusion was delivered over a 1 min period and AE or saline were injected at a volume of 0.5 μl per side.

2.6. Step-down test (inhibitory avoidance)

The inhibitory avoidance training apparatus was a 50 cm × 25 cm × 25 cm plastic box with 2.5 cm high, 7.0 cm wide, and platform to the left of the training box apparatus. The floor of the apparatus was made of parallel 0.1 cm caliber stainless steel bars spaced 1.0 cm apart. The animals were placed on the platform. Latency to step down placing the four paws on the grid was measured, with AE being administered immediately after training. During the training session, immediately upon stepping down, the rats received a 0.4-mA, 2 s scrambled foot shock. A retention test was evaluated 24 h post-training. This test session was procedurally identical except that no foot shock was given. The step down latency was stopped after 180 s and taken as a measure of memory retention.

2.7. Histology

At the end of the experiments, the animals were immediately killed by decapitation. Methylene blue injection (0.5 μl) was used to confirm the correct injection site. The cannula's position was assessed histologically on frozen brain slices (−20 °C). Only results obtained from animals in which the tips of the cannulas were placed into the hippocampus were included in this study.

2.8. Statistics

All results are expressed as medians (interquartile range). The statistical significance was calculated by a one-way Kruskal–Wallis analysis followed by an individual two-tailed Mann–Whitney U test; $p < 0.05$ was accepted as a statistically significant value.

3. Results and discussion

Fig. 1 shows the effect of the three doses of AE (1, 5 and 10 ng/rat) on memory retention. The AE intra-hippocampal administration significantly increased the latency time in a dose-related manner with respect to the control animals $H(6.1) = 12.16$; $p = 0.00$.

This result is in agreement with previous electrophysiological studies in rat hippocampus slices (in vitro) showing that AE diminished the threshold for LTP induction from 86 ± 0.92 in the controls to 22 ± 1.01 Hz (Ortega et al., 2006). Huperzine A and B are *Lycopodium* alkaloids among the compounds with known activity on memory and learning (Zhu and Tang, 1988; Zhang et al., 1991).

In a previous investigation, some eight alkaloids were isolated and identified from the AE of the Argentinean species of *Huperzia saururus,* with the presence of lycodine, *N*-methyllycodine, *N*-acetyllycodine, and sauroxine (Flabellidane group), sauroine, 6-hydroxylycopodine, lycopodine, and

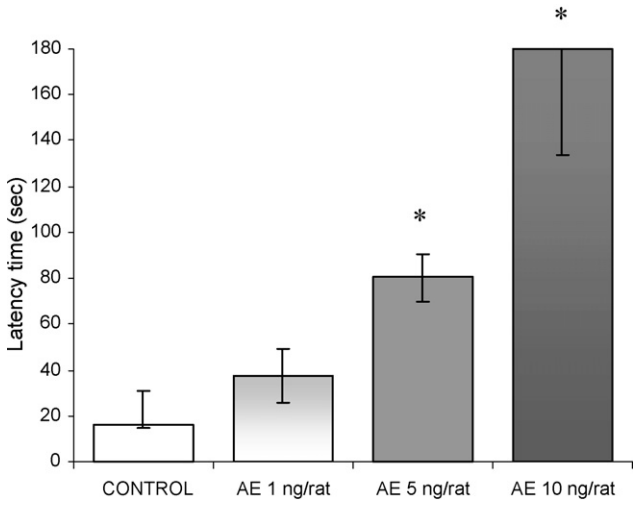

Fig. 1. Effect of hippocampal AE administration upon long-term memory retention in rats. The animals were injected with saline (control) or with different doses of AE of 1, 5 or 10 ng/rat. The latency time (in seconds) was quantified. The results are expressed as medians (interquartile range). $N = 7$–9 animals/group. The symbol (*) shows the significant differences in comparison to control animals; $p < 0.05$.

clavolonine (Lycopodane group) being detected (Ortega et al., 2004a,b). Their relative proportions were established as well.

As neither Huperzine A nor Huperzine B are present in AE, it seems to be a new source of natural products having activity on memory and learning. Further studies will be developed with each isolated alkaloid in order to evaluate which is/are responsible for the extract activity herein demonstrated.

The relevance of our findings base on that the obtained results confirm the ethnopharmacologal use of *Huperzia saururus* as memory improver, suggesting that alkaloids are the responsible compounds for its action.

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