Biomedicine & Pharmacotherapy 91 (2017) 155–161

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Sauroxine reduces memory retention in rats and impairs hippocampal long-term potentiation generation

Mariana Vallejo[a,1], Valeria Carlini[b,1], Laura Gabach[c,1], M.G. Ortega[a], José L. Cabrera[a], Susana Rubiales de Barioglio[c], Mariela Pérez[c], Alicia M. Agnese[a,*]

[a] IMBIV-CONICET and Farmacognosia, Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[b] INICSA-CONICET and Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[c] IFEC-CONICET and Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form 1 April 2017
Accepted 10 April 2017

Keywords:
Sauroxine
Lycopodium alkaloid
Phlegmariurus saururus
Long-term potentiation
Inhibitory avoidance
Blood-brain barrier

ABSTRACT

In the present paper it was investigated the role of sauroxine, an alkaloid of *Phlegmariurus saururus*, as a modulator of some types of learning and memory, considering the potential nootropic properties previously reported for the alkaloid extract and the main alkaloid sauroine. Sauroxine was isolated by means of an alkaline extraction, purified by several chromatographic techniques, and assayed in electrophysiological experiments on rat hippocampus slices, tending towards the elicitation of the long-term potentiation (LTP) phenomena. It was also studied the effects of intrahippocampal administration of sauroxine on memory retention *in vivo* using a Step-down test. Being the bio distribution of a drug an important parameter to be considered, the concentration of sauroxine in rat brain was determined by GLC–MS. Sauroxine blocked LTP generation at both doses used, 3.65 and $3.6 10^{-2}$ μM. In the behavioral test, the animals injected with this alkaloid ($3.65 10^{-3}$ nmol) exhibited a significant decrease on memory retention compared with control animals. It was also showed that sauroxine reached the brain (3.435 μg/g tissue), after an intraperitoneal injection, displaying its ability to cross the blood-brain barrier. Thus, sauroxine demonstrated to exert an inhibition on these mnemonic phenomena. The effect here established for **1** is defeated by other constituents according to the excellent results obtained for *P. saururus* alkaloid extract as well as for the isolated alkaloid sauroine.

© 2017 Elsevier Masson SAS. All rights reserved.

1. Introduction

Phlegmariurus saururus (Lam.) B. Øllg. [(=*Huperzia saururus* (Lam.) Trevis.; *Lycopodium saururus* Lam.; *H. sanctae-barbarae* (Rolleri) Rolleri & Deferrari; Lycopodiaceae)] is a Lycophyta that can be found in South America, from Peru to northern and central region of Argentina, growing at high altitudes [1,2]. Its commercial importance has jeopardized its natural survival, becoming a species at risk of extinction [3]. Belonging to the Lycopodiaceae family, it has an extensive ethnomedical use, mainly because of its aphrodisiac properties [4], and the literature also reported its use in folk medicine for memory improvement [5]. We have previously demonstrated that Lycopodium alkaloids are the bioactive components in this species [6–8], being sauroine [(8*R*,15*S*)-7,8-dihydroxy-15-methyllycopodan-5-one], sauroxine [(4a*S*,5*S*,12*R*)-1,12-dimethyl-2,3,4,4a,5,6,9,10-octahydro-1*H*-5,10b-propano-1,7-phenanthrolin-8(7*H*)-one, **1**] and 6-hydroxylycopodine [(6α,15*R*)-6-hydroxy-15-methyllycopodan-5-one] the main alkaloids of the purified *P. saururus* alkaloid extract (PSAE). In turn, PSAE presented marked inhibition of acetylcholinesterase (AChE) [6].

Hippocampus is a brain structure implicated in spatial and context-dependent learning. Long-term potentiation (LTP) is a form of synaptic plasticity in the hippocampus characterized by an enduring increase in the efficacy of glutamatergic synaptic transmission. This phenomenon is accepted as a molecular mechanism for learning and memory in the brain, in which contextual cues are relevant [9,10]. Acetylcholine (ACh) modulates this phenomenon and also participates in learning and memory processes [11]. *In vitro* studies from our laboratories, demonstrated that the PSAE increased synaptic transmission in the hippocampus

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; ACSF, artificial cerebro-spinal fluid; BBB, blood-brain barrier; CA1, cornus ammonis 1; fEPSP, field excitatory post synaptic potentials; GLC–MS, gas liquid chromatography–mass spectrometry; HFS, high-frequency stimulation; i.p., intraperitoneal; LTP, Long-term potentiation; NMR, nuclear magnetic resonance; PSAE, *Phlegmariurus saururus* alkaloid extract; PP, perforant path.

* Corresponding author.
E-mail address: magnese@fcq.unc.edu.ar (A.M. Agnese).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biopha.2017.04.016
0753-3322/© 2017 Elsevier Masson SAS. All rights reserved.

[12], and enhanced memory retention in rats, when it was administered into the hippocampus [13]. The main PSAE constituent sauroine also presented these effects [14]. Thus, sauroine showed to be one of the contributing alkaloids for the PSAE *in vitro* effects upon hippocampal synaptic plasticity [12], and in *in vivo* memory improvement [13,14]. These results explain the effects claimed by the ethnomedicine, even when it did not exert inhibitory effect on AChE [15], stating the potential use of sauroine as a nootropic agent.

On the other hand, the alkaloid **1** is the second main component of PSAE, and its inhibitory action on AChE was even validated by docking and molecular dynamics studies. Nevertheless, the inhibition level of **1** was lower compared to PSAE effect [15].

As it is already known, the miscellaneous nature of plant extracts gives as result a potential chemical diversity. So, the bioactivities of each individual agent could be spread in a wide range of effects, affording synergism, antagonism or no-interaction phenomena, summarizing the total effect of the extract. Usually, the active isolated components are expected to exert stronger activity than the extracts, but sometimes their effects are lacking, weaker or totally opposite to that of the extract. An example can be taken from *Cannabis sativa* components, cannabidiol would tend to reduce some of the acute and subchronic effects of Δ^9-tetrahydrocannabinol [16].

Considering all the diverse evidences presented above, the aim of the present investigation was to evaluate if the effects of **1** upon hippocampal synaptic transmission and memory were similar to PSAE and sauroine, especially considering its inhibitory action on AChE. Additionally, we examined the blood-brain barrier (BBB) crossing of **1** when it was administered intraperitoneally (i.p.) in rats, in order to determine the alkaloid level in the target tissue.

2. Material and methods

2.1. Plant material

Aerial parts of *P. saururus* were collected in Pampa de Achala, San Alberto Department, Province of Córdoba, Argentina, in October 2012 (Spring in Argentina) at 2300 m in high, and they were 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.

2.2. Extraction, isolation and identification

Aerial parts of *P. saururus* (2.0 kg) were dried, ground, and then alkalinized with NaOH reduced to a powder (160 g). This mixture was hydrated with distilled water until pH 12, and extracted with $CHCl_3$ using a Soxhlet extractor. The organic solvent was evaporated under reduced pressure until dryness. This crude total extract (59 g) was dissolved in 0.01 N HCl to pH 2, filtered and partitioned twice with $CHCl_3$. The acidic aqueous extracts were combined and then alkalinized with 0.1 N NaOH to pH 12 and

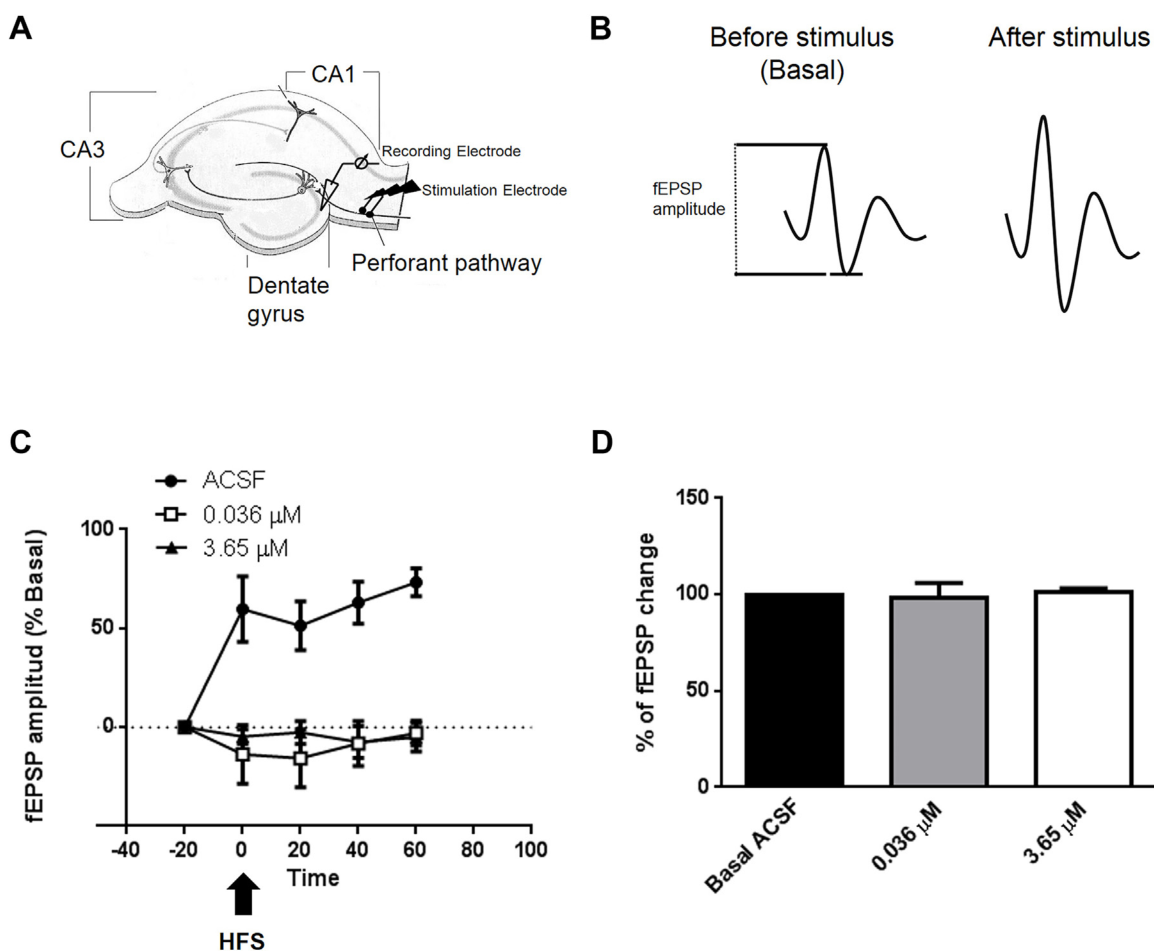

Fig. 1. Effect of **1** on LTP generation. A. Recording and stimulation electrodes positions in rat hippocampal slice. B. Typical average fields potentials for control and **1**, before and after effective tetanus. C. Time course of the LTP after perfusion with 3.65 μM (open squares) and 0.0365 μM (solid squares) of **1** during 60 min and LTP induced by tetanus under control condition (solid circles). D. Fast synaptic transmission expressed as % of fEPSP change.

subsequently partitioned with $CHCl_3$ in a liquid-liquid extractor. The chloroform extract obtained (3.03 g) was purified by a Sephadex LH-20 (Pharmacia) 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 1.85 g of an alkaloid extract. This residue was submitted to a Sephadex LH-20 column and acetone was used as mobile phase affording four fractions. Fraction 3 (355.6 mg) was subjected to a Sephadex G_{10} (Pharmacia) column and eluted with 5% EtOH. Fraction 3.3 afforded **1,** which was purified by preparative TLC on silica gel GF_{254} (Merck), using cyclohexane/$CHCl_3$/diethylamine (5:4:1) as mobile phase, yielding 9.7 mg of **1**. Identification of **1** was carried out using one- and two-dimensional ^1H and ^{13}C NMR spectra and were measured on a 400 MHz Bruker Advance II NMR spectrometer, using $CDCl_3$ as solvent (^1H = 400.16 MHz; ^{13}C = 100 MHz), and comparing the spectra with the scientific literature and to those spectra of authentic samples previously obtained in our laboratory [8,17,18].

2.3. Animals

Male Wistar rats (60–75 days old, 190–300 g) were obtained from the Department of Pharmacology vivarium (Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina). The animals were maintained under standard laboratory conditions (12 h light-dark cycle, lights on at 07:00 h, temperature 21 ± 1 °C), housed in groups of five in their home boxes, with free access to food and water.

2.4. Electrophysiological procedures

Electrophysiological experiments were carried out using the *in vitro* hippocampal slice preparation [19]. Rats were sacrificed between 11.00 a.m. and noon in order to prevent variations caused by circadian rhythms or nonspecific stressors [20]. Briefly, hippocampal formation was dissected, and transverse slices of approximately 400 μm thick were placed in a recording chamber (BSC-BU Harvard Apparatus), perfused with standard Krebs solution (124.3 mM NaCl, 4.9 mM KCl, 1.3 mM $MgSO_47H_2O$, 1.25 mM H_2KPO_4, 25.6 mM $HNaCO_3$, 10.4 mM glucose, and 2.3 mM $CaCl_2{\cdot}2H_2O$), saturated with 95% O_2 and 5% CO_2. The rate of perfusion was 1.6 mL/min, and the bathing solution temperature was kept at 28 °C by the use of a Temperature Controller (TC-202A Harvard Apparatus) for the duration of the experiment. A stimulating electrode made of two twisted wires, which were insulated except for the cut ends (diameters 50 μm), was placed in the perforant path (PP) and the recording electrode, made with a glass micropipette (10–20 μm tip), was inserted in the dentate granule cell body layer (Fig. 1A). Only slices showing a stable response were included in this study. Field excitatory post synaptic potentials (fEPSP) that responded to 0.2 Hz stimuli were sampled twice, during 4 s, each 5 min, within a 40 min period until fEPSP stabilization (baseline). Once no further changes were observed in the fEPSP amplitude, the stimulation protocol was applied. LTP was generated using the classical tetanization paradigm consisting of three 100-Hz high-frequency stimulation (HFS) trains (of 1 s duration each) given at 20 s intervals, delivered by an A310 accupulser pulse generator (World Precision Instruments Inc.). LTP was considered to have occurred when the fEPSP amplitude

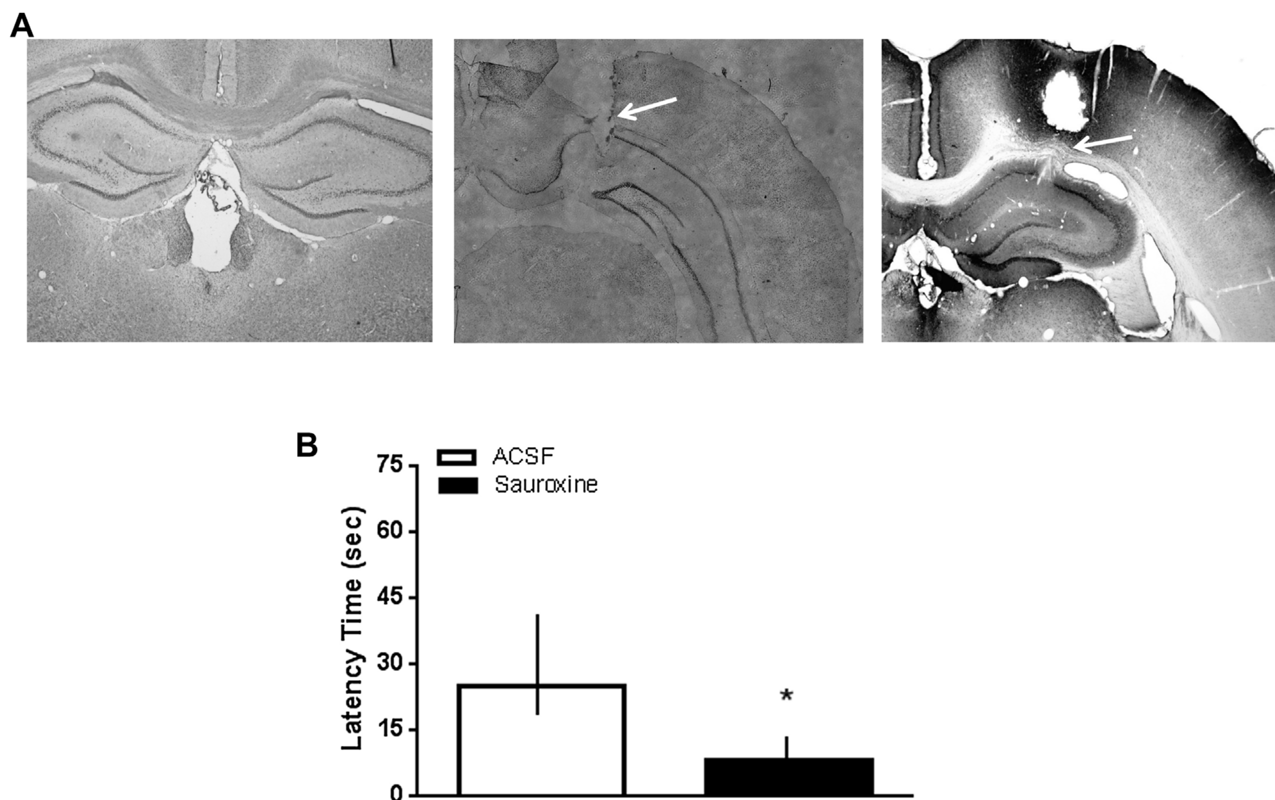

Fig. 2. Effect of **1** on memory retention in the step-down test. A. Hippocampal frozen brain slices of no implanted, control and treatment animal (from left to right). Arrows indicate cannula's position in implanted rats. B. Latency time in the test for control and 1 group (3.65 10^{-3} nmoles). The (*) shows the significant differences in comparison to control animals ($p < 0.05$).

recorded after the stimulus (at 0.2 Hz) had risen at least 30% from baseline and persisted for 60 min (Fig. 1B). When the effects of **1** were tested, the alkaloid hydrochloride at concentrations of 3.65 and $3.65\,10^{-2}$ μM were added to the perfusion buffer, and after 5 min of perfusion a new fEPSP was measured and then the HFS was applied. All collected data were recorded and stored for future analysis.

2.5. Behavioral assays

Rats were anesthetized with 55 mg/kg ketamine HCl (Vetanarcol König: Laboratorios König S.A, Bs As, Argentina) and 11 mg/kg xylazine (Kensolkönig: Laboratorios König S.A., Bs As, Argentina) and placed in a stereotaxic apparatus. The rats were bilaterally implanted into CA1 area of the hippocampus with steel guide cannula, following the atlas of Paxinos and Watson [21]. The coordinates for CA1 relative to bregma were anterior: −4.3 mm; lateral: ±4.0 mm; vertical: −3.4 mm. Cannulas were fixed to the skull surface with dental acrylic cement. Animals were allowed to a 7 days of recovery period, and they were handled daily to habituate them to the injection procedures. Seven days after surgery, animals were then injected with a solution of **1** as hydrochloride in artificial cerebrospinal fluid (ACSF), 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. **1** or ACSF solution (control) were administered at a volume of 0.5 μL per hemisphere ($3.65\,10^{-3}$ nmol), immediately after training. Each animal was used in only one experiment. The number of animals ranged between 7 and 9 per treatment.

The apparatus of Step-Down Test (inhibitory avoidance) was a $50 \times 25 \times 25$ cm plastic box with a 2.5 cm height, 7.0 cm width, and a platform on 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 from each other. The animals were placed on the platform. Latency to step down placing the four paws on the grid was measured. In the training session, immediately upon stepping down, the rats received a 0.4-mA, 2 s scrambled shock to the foot, and were then immediately removed from the box and placed in their home cages. Twenty four hours later, the test session was performed, procedurally identical to the training session, except that no shock was given. A ceiling of 180 s was imposed on the test measures. Latency time was taken as a measure of memory retention. The retention test was carried out 24 h after training in order to measure long term memory retention. At the end of the experiments, the animals were immediately sacrificed by guillotine decapitation, in order to confirm the correct injection site. The cannula's position was assessed histologically on frozen brain slices (−20 °C) (Fig. 2A). Only results obtained from animals in which the tips of the cannulas were placed into the hippocampus CA1 were included in this study.

2.6. Brain tissue analysis

Three groups of animals were administered *via* i.p. (n = 3 per group), Control and two doses of treatment: 3, and 30 mg/kg of **1** as hydrochloride. Thirty min later, animals were sedated with CO_2, decapitated and their brains removed. Brain tissue was homogenized in HCl 0.1 M and then centrifuged at 5000 rpm. The obtained precipitate was rinsed several times with HCl 0.1 M, and all the supernatants were reunited. After that, supernatant was alkalinized and partitioned five times with $CHCl_3$. Organic phases were reunited and $CHCl_3$ was eliminated under reduced pressure. This brain extract was analyzed by gas liquid chromatography-mass spectrometry (GLC–MS). **1** was used as external standard, thus, three chloroform solutions of 0.36, 3.65 and 7.30 mM were injected (n = 3) for the calibration curve. Dry brain extract was solubilized in $CHCl_3$ (30 μL) and injected three times in GLC–MS equipment (Shimadzu QP5050A, Kyoto, Japan), with a VF–5 ms (5% phenyl and 95% dimethyl-polysiloxane) column of 30 m in length, 0.25 mm of diameter and 0.25 μm thickness of the film. Carrier was He and flux was adjusted to 1.2 mL/min. Injector and detector temperatures were 280 °C. The following GLC temperature (T) program was used: Starting T = 230 °C (2 min); rate 1 = 20 °C/min;

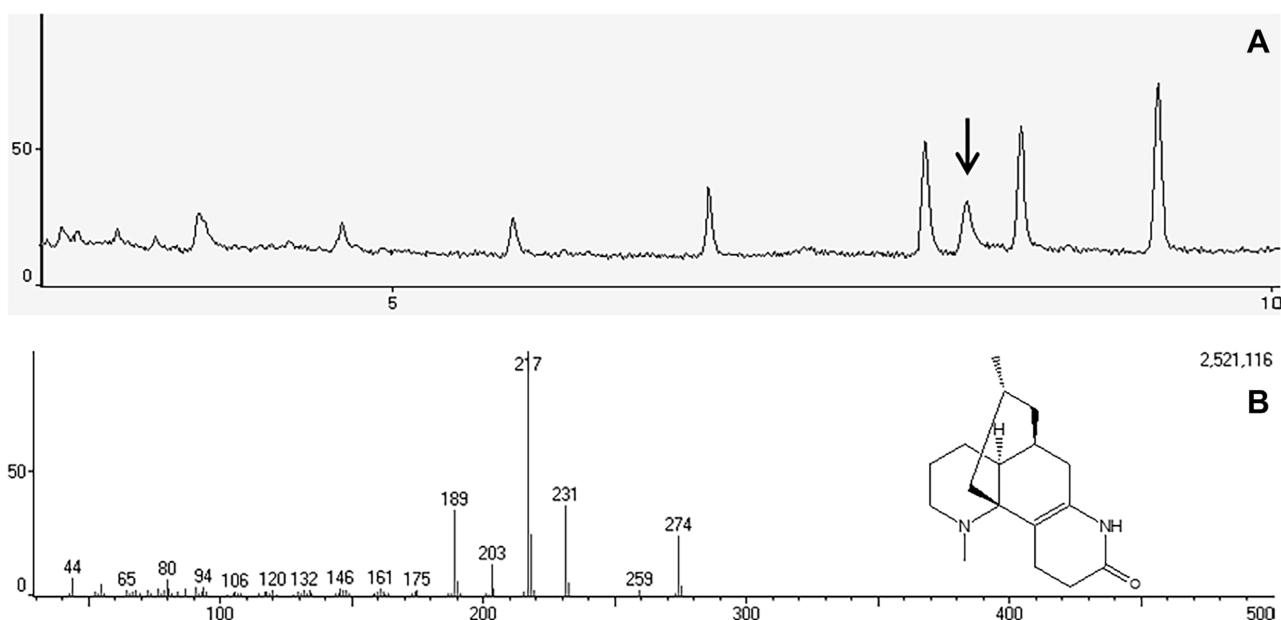

Fig. 3. Gas liquid chromatography–mass spectrometry (GLC–MS) analysis of brain tissue. A. Chromatogram of the chloroform extract obtained by GLC [arrow shows sauroxine (**1**) peak and its retention time]. B. Chemical structure and MS of **1** ($M^+ = 274$).

T1 = 250 °C (2 min); rate 2 = 5 °C/min; Final T: 280 °C (2 min). Class 5000, 2.01 MS, 3.02 GC 2003 programs were employed.

2.7. Statistics

Data from LTP induction were analyzed by repeated measured two way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. Data from basal fEPSP under ACSF or **1** perfusion were analyzed by Paired Student T-test. The statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA). Data from inhibitory avoidance are expressed as medians (inter-quartile range) and analyzed by non-parametric tests (Mann-Whitney or Kruskal–Wallis), followed by Dunn's post hoc comparisons, because the variables being analyzed do not follow a normal distribution and its variance does not fulfill the assumption of homoscedasticity. For both analysis $p < 0.05$ was accepted as a statistical significant value. Brain tissue **1** quantification results are expressed as the media ± SEM; GraphPadInStat (GraphPad Software Inc., San Diego, California, USA) software version 3.01 was used in this analysis.

3. Results

The results presented in this paper showed that **1**, the second main constitutive alkaloid present in PSAE, inhibited *in vitro* Long-term-potentiation (LTP) generation when it was perfused in hippocampal slices at a concentration of 3.65 μM, compared to artificial cerebrospinal fluid (ACSF) perfused slices (Fig. 1C). After a lower **1** concentration ($3.6\,10^{-2}$ μM) was perfused, similar inhibitory response was obtained [F (8, 36) = 5.381; p = 0.0002], (Fig. 1C). Our results also showed that under our experimental conditions, **1** did not affected fast synaptic transmission: the fEPSP observed in slices perfused with ACSF did not differ from the fEPSP after 5 min of **1** perfusion, at both concentrations tested (Fig. 1D) (using Paired Student *T* test). Oppositely, slow transmission was impaired when a standard protocol to generate LTP was used, since no changes in the fEPSP amplitude were observed after frequency stimulation protocol (HFS) application, as it was described above [22,23]. Considering the electrophysiological results, we investigated whether **1** administration into the hippocampus would induce changes on memory consolidation in the step-down test. Fig. 2B shows latency time as an index of memory retention after intra-hippocampal **1** administration. The animals injected with **1** (0.5 μL/side; $3.65\,10^{-3}$ nmol), presented a significant reduction in latency time indicating a decrease on memory retention compared to control animals (ACSF infused) [H (9,19) = 7.74, p = 0.00].

As a complementary study, we examined the BBB crossing of **1** following to an i.p. administration. Brain tissue analysis revealed that **1** was not detected by using GLC–MS in brain homogenate from animals administered systemically (i.p.) with 3 mg/kg. When animals received *via* i.p. 30 mg/kg of **1**, profile shown in Fig. 3A and B confirmed its presence in those brain homogenates. By using linear regression analysis, the homogenate concentration of **1** was determined as 0.34 ± 0.05 mg/mL. Therefore, the **1** content in the brain was 3.435 ± 0.002 μg/g, representing a 0.0674 ± 0.006% of the i.p. administered dose. These results demonstrate that **1** reaches the brain, crossing the BBB to exert its pharmacological action.

4. Discussion

Results presented in this paper, surprisingly showed that the effects of **1** here are opposite to those obtained with the PSAE and sauroine, that demonstrated a facilitating effect on learning and memory processes in both, *in vivo* and *in vitro* procedures [12–14].

It is well known that, fast hippocampal synaptic transmission in dentate gyrus is mediated mainly by AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) type glutamatergic receptors, while slow transmission is mediated by NMDA (*N*-methyl-D-aspartate) type glutamatergic receptors [22,23]. Results of our electrophysiological experiments showed that **1** did not affect the fast synaptic transmission, as it can be seen in Fig. 1D, where the fEPSP is similar for the slices perfused with ACSF as well as for the both assayed concentrations of **1**. However, when a standard protocol to generate LTP was used, a detriment in the slow transmission was observed (Fig. 1C). These results could be interpreted as decreased NMDA-glutamatergic transmission induced by **1**. In addition, the fact that **1** decreased latency time in behavioral paradigm, is in accordance with this notion.

Another possible explanation for the present results could be a correlation with AChE activity. In a previous work, we have demonstrated that **1** elicited an AChE inhibitory activity. In consequence, our results could probably be attributed to an accumulation of extracellular ACh in hippocampus induced by **1,** *via* inhibition of AChE at both concentrations tested, activating receptors that interfere with facilitation of LTP generation, as it was described for muscarinic agonist at elevated concentrations [24]. Data from scientific literature indicate that ACh regulation of hippocampal synaptic plasticity is a complex mechanism, and depends on the subarea studied, the stimulation protocols used, in both *in vitro* and *in vivo* experiments, as well as the cholinergic receptors involved in those effects. For instance, a pharmacological AChE inhibition causes a prolonged decrease in presynaptic glutamate release at CA3-CA1 synapses [25], and muscarinic receptors activation can enhance synaptic inhibition in the CA1 [26], while m1-muscarinic receptor gene product modulates excitatory synaptic transmission by potentiation of NMDA-receptor currents in CA1 [27], or AChE inhibitors enhances LTP in CA1 of the behaving rat [28].

The effects described above were observed when **1** was administered directly into the central nervous system (behavior) or tested *in vitro* (electrophysiology). Taken into account that sauroine and PSAE showed opposite results upon these effects, it is reasonable to consider that these differences could probably be attributed to the prevalent sauroine concentration in the PSAE, or to the fact that **1** could not cross the blood-brain barrier (BBB) when PSAE is administered orally. Nevertheless, our results demonstrate that **1** reaches the brain following the i.p. administration, crossing the BBB to exert its pharmacological action.

In the wide spectrum of neurological disorders, new therapeutic approaches are acutely searched to several of them. We have proposed that memory decline, evinced in pathologies like Alzheimer's disease (AD), could be counteracted by the highly active alkaloid sauroine [14]. As well as for AD, the posttraumatic stress disorder (PTSD) following to accidents, explosions, rapes, and other events, is lacking of effective treatments. Nowadays, the pharmacotherapy used for fear-related disorders mainly include anxiolytic (*e.g.* benzodiazepines) and antidepressive drugs (serotonin- or other monoamine-reuptake inhibitors) are mostly employed for phobias and PTSD [29]. Nevertheless, symptomatology of PTSD persists despites the current medications received by the patients [30]. These agents are required to relieve chronic stress and anxiety symptoms [31] but they do not modify underlying aversive-memory [32]. In this state of the issue, rising of natural amnesic agents which can exhibit novel mechanisms of action, underlying in their structural diversity, could lead to new therapies in PTSD and their study should be considered. In fact, the potential of **1** in the PTSD treatment needs to be investigated, even when the action of this alkaloid in the PSAE is perhaps nullified by the others. All the set of our previous and present results emphasize the incredible ability of natural extracts to have

complex branches regarding the bioactivities of their isolated components, including those opposites, as we demonstrated.

5. Conclusions

Combined, all these results showed a detrimental effects upon memory and impairment in LTP generation induced by **1**. They could be attributed to high ACh levels in hippocampus induced by **1** at the concentrations tested. Besides, we cannot rule out that **1** exerts these effects by different mechanisms rather than AChE inhibition, including blockade of NMDA receptors or interference with intracellular signaling pathways activated by them, enhancement of the inhibitory actions of GABA, or potassium channels activation, among others. Further experiments need to be addressed in order to explore the possible mechanisms by which **1** exerts its effects upon learning and memory processes.

Taking into account the relevance of emotionally aversive memories following traumatic events and the limited current treatment options for some fear-related disorders [33–35], we highlight the importance of our findings, based on the effects elicited by sauroxine. This Lycopodium alkaloid could be regarded as a potential substance useful to counteract the negative effects left by those kind of traumatic events.

Conflict of interest

The authors declare no conflict of interest

Ethical standard

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. In the present study efforts were made to minimize animal suffering and to reduce the number of animals used. Experimentation was carried out in accordance to the NIH Guide for the Care and Use of Laboratory Animals as approved by the Animal Care and Use Committee of the Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina (Res. Decanal 48/2015). All procedures were handled minimizing the number of animals used and their suffering,

Acknowledgments

This work was supported by ANPCyT-FONCYT (PICT 2011 No. 2476 and PICT 2014 No. 2204), CONICET (PIP 2013–2015, GI 4316/13), and Secretaría de Ciencia y Tecnología-Universidad Nacional de Córdoba (05/C548; 05/C712; 05/C701). Author thanks to Dr. G. Barboza for the species identification.

ReferencesIn the present proof, reference style has been changed. We sent the revised article according to the Guide for Authors as well as recently published articles. Nevertheless, we detected two mistakes in the references included in the revised article. Please, see these corrected references and their respective comments:[19] M.F. Perez, L.A. Gabach, R.S. Almirón, V.P. Carlini, S. Rubiales de Barioglio, O.A. Ramírez, Different chronic cocaine administration protocols induce changes on dentate gyrus plasticity and hippocampal dependent behavior, Synapse 64 (10) (2010) 742-753.Issue number is now between parenthesis.[24] E. Burgard, J. Sarvey, Scopolamine Administration Modulates Muscarinic, Nicotinic and NMDA Receptor Systems, Neurosci. Lett. 16 (1990) 34-39.Year of publication is now after issue number.

[1] B. Øllgaard, Neotropical Lycopodiaceae, an overview, Ann. Mo. Bot. Gard. 79 (1996) 686–717.

[2] M. Ponce, Pteridophyta, in: F. Zuloaga, O. Morrone (Eds.), Catálogo de las Plantas Vasculares de la República Argentina I. Pteridophyta, Gymnospermae y Angiospermae (Monocotiledoneae), 60, Monograp. Syst. Bot. Mo. Bot. Gard. St. Louis, 1996, pp. 1–79.

[3] Ministerios de Salud y Acción Social, Manual de intoxicaciones para agentes de atención primaria, Buenos Aires, 2002. Resolución Nº 652. Expediente 1-2002-11726-01-3. Acceso 21/06/07. http://www.msal.gov.ar/htm/site/pngcam/normas/r%2002_652%20.pdf (Accessed 07 January 2017).

[4] E. de la Sota, Pteridophyta, in: A. Cabrera (Ed.), Flora de la Provincia de Jujuy, INTA, Buenos Aires, 1977, pp. 19–27.

[5] R. Martínez Croveto, Las plantas utilizadas en medicina popular en el Noroeste de Corrientes, República Argentina, Miscelanea 69 (1981) 15–15.

[6] M.G. Ortega, A.M. Agnese, J.L. Cabrera, Anticholinesterase activity in an alkaloid extract of *Huperzia saururus*, Phytomedicine 11 (2004) 539–543.

[7] M.G. Ortega, A.M. Agnese, J.L. Cabrera, Sauroine—a novel Lycopodium alkaloid from *Huperzia saururus*, Tetrahedron Lett. 45 (2004) 7003–7005.

[8] M.G. Vallejo, M.G. Ortega, J.L. Cabrera, A.M. Agnese, *N*-Demethyl-sauroxine, a novel Lycodine Group alkaloid from *Huperzia saururus*, Tetrahedron Lett. 54 (2013) 5197–5200.

[9] R. Phillips, J. LeDoux, Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning, Behav. Neurosci. 106 (1992) 274–285.

[10] S.J. Martin, P.D. Grimwood, R.G. Morris, Synaptic plasticity and memory: an evaluation of the hypothesis, Annu. Rev. Neurosci. 23 (2000) 649–711.

[11] D. Jerusalinsky, E. Kornisiuk, I. Izquierdo, Cholinergic neurotransmission and synaptic plasticity concerning memory processing, Neurochem. Res. 22 (1997) 507–515.

[12] M.G. Ortega, M.G. Vallejo, J.L. Cabrera, M.F. Pérez, R.S. Almirón, O.A. Ramírez, A. M. Agnese, *Huperzia saururus*, activity on synaptic transmission in the hippocampus, J. Ethnopharmacol. 104 (2006) 374–378.

[13] M.G. Vallejo, M.G. Ortega, J.L. Cabrera, V.P. Carlini, S. Rubiales de Barioglio, A.M. Agnese, *Huperzia saururus* increases memory retention in rats, J. Ethnopharmacol. 111 (2007) 685–687.

[14] M.G. Vallejo, M.G. Ortega, J.L. Cabrera, V.P. Carlini, S. Rubiales de Barioglio, R.S. Almirón, O.A. Ramirez, A.M. Agnese, Sauroine, an alkaloid from *Huperzia saururus* with activity in Wistar rats in electrophysiological and behavioral assays related to memory retention, J. Nat. Prod. 72 (2009) 156–158.

[15] M. Puiatti, J.L. Borioni, M.G. Vallejo, J.L. Cabrera, A.M. Agnese, M.G. Ortega, A.B. Pierini, Study of the interaction of *Huperzia saururus* Lycopodium alkaloids with the acetylcholinesterase enzyme, J. Mol. Graph. Model. 44 (2013) 136–144.

[16] S.M. Todd, C. Zhou, D.J. Clarke, T.W. Chohan, D. Bahceci, J.C. Arnold, Interactions between cannabidiol and $\triangle$9 -THC following acute and repeated dosing: rebound hyperactivity, sensorimotor gating and epigenetic and neuroadaptive changes in the mesolimbic pathway, Eur. Neuropsychopharmacol. 27 (2017) 132–145.

[17] T.T. Nakashima, P.P. Singer, L.M. Browne, W.A. Ayer, Carbon-13 nuclear magnetic resonance studies of some Lycopodium alkaloids, Can. J. Chem. 53 (1975) 1936–1942.

[18] D.A. Cifuente, M.G. Vallejo, M.G. Ortega, J.L. Cabrera, V.S. Martin, C.E. Tonn, A.M. Agnese, C. Ardanaz, Mass spectrometry studies of Lycopodium alkaloids lycodine-type: sauroxine and N-demethyl-sauroxine, Rapid Commun. Mass Spectrom. 28 (2014) 2690–2694.

[19] M.F. Perez, L.A. Gabach, R.S. Almirón, V.P. Carlini, S. Rubiales de Barioglio, O.A. Ramírez, Different chronic cocaine administration protocols induce changes on dentate gyrus plasticity and hippocampal dependent behavior, Synapse 64 (10) (2010) 742–753.

[20] T.J. Teyler, P. Discena, Long-term potentiation, Annu. Rev. Neurosci. 10 (1987) 131–161.

[21] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, second ed., Academic Press, San Diego, 1986.

[22] M. Andreasen, J. Lambert, M. Jensen, Effects of new non-*N*-methyl-D-aspartate antagonists on synaptic transmission in the *in vitro* rat hippocampus, J. Physiol. 414 (1989) 317–336.

[23] B. Keller, A. Konnerth, Y. Yaari, Patch clamp analysis of excitatory synaptic currents in granule cells of rat hippocampus, J. Physiol. 435 (1991) 275–293.

[24] E. Burgard, J. Sarvey, Scopolamine administration modulates muscarinic, nicotinic and NMDA receptor systems, Neurosci. Lett. 16 (1990) 34–39.

[25] R. Mans, B. Warmus, C. Smith, L. McMahon, An acetylcholinesterase inhibitor, eserine, induces long-term depression at CA3-CA1 synapses in the hippocampus of adult rats, J. Neurophysiol. 112 (2014) 2388–2397.

[26] S. Domínguez, D. Fernández de Sevilla, W. Buño, Postsynaptic activity reverses the sign of the acetylcholine-induced long-term plasticity of $GABA_A$ inhibition, Proc. Natl. Acad. Sci. 111 (2014) 2741–2750.

[27] M. Marino, S. Rouse, A. Levey, L. Potter, P. Conn, Activation of the genetically defined m1 muscarinic receptor potentiates *N*-methyl-D-aspartate (NMDA) receptor currents in hippocampal pyramidal cells, Proc. Natl. Acad. Sci. 95 (1998) 11465–11470.

[28] S. Doralp, L. Leung, Cocaine enhancement of Long-Term Potentiation in the CA1 region of rat hippocampus: lamina-specific mechanisms of action, Neurobiol. Learn. Mem. 90 (2008) 382–388.

[29] B. Bandelow, L. Sher, R. Bunevicius, E. Hollander, S. Kasper, J. Zohar, H.J. Möller, WFSBP Task Force on Mental Disorders in Primary Care, WFSBP Task Force on Anxiety Disorders, OCD and PTSD, Guidelines for the pharmacological treatment of anxiety disorders, obsessive-compulsive disorder and posttraumatic stress disorder in primary care, Int. J. Psychiatry Clin. Pract. 16 (2012) 77–84.

[30] S. Barton, C. Karner, F. Salih, D.S. Baldwin, S.J. Edwards, Clinical effectiveness of interventions for treatment-resistant anxiety in older people: a systematic review, Health Technol. Assess. 18 (2014) 1–59.
[31] J. Ipser, S. Seedat, D.J. Stein, Pharmacotherapy for post-traumatic stress disorder—a systematic review and meta-analysis, S. Afr. Med. J. 96 (2006) 1088–1096.
[32] C.C. Lin, C.S. Tung, Y.P. Liu, Escitalopram reversed the traumatic stress-induced depressed and anxiety-like symptoms but not the deficits of fear memory, Psychopharmacology 233 (2016) 1135–1146.
[33] N. Hait, L. Wise, J. Allegood, M. O'Brien, D. Avni, T. Reeves, P. Knapp, J. Lu, C. Luo, M. Miles, S. Milstien, A. Lichtman, S. Spiegel, Active, phosphorylated fingolimod inhibits histone deacetylases and facilitates fear extinction memory, Nat. Neurosci. 17 (2014) 971–983.
[34] J. Otis, C. Werner, D. Mueller, Noradrenergic regulation of fear and drug-associated memory reconsolidation, Neuropsychopharmacology 40 (2015) 793–803.
[35] D. de Quervain, L. Schwabe, B. Roozendaal, Stress, glucocorticoids and memory: implications for treating fear-related disorders, Nat. Rev. Neurosci. 18 (2017) 7–19.