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Resin glycosides evoke the Gaba release by sodium- and/or calcium-dependent mechanism

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ABSTRACT

Ipomoea tyrianthina Lindley (syn. *I. orizabensis* Pelletan, Lebed. ex Steud., Convolvulaceae) is known as a purgative, but it has been also used in Mexican traditional medicine in the treatment of seizures and pain for their anticonvulsive, hypnotic and sedative properties. Some glycolipids isolated from this plant have shown significant effects on Central Nervous System, modifying inhibitory or excitatory processes. The mechanism for such activity it is not clear; studies with these metabolites have suggested that a pore-forming mechanism is involved in their activity. Therefore, the present work explores a possible not pore-forming mechanism related to the effect of four resin glycosides, Scammonin 1 (S-1), tyrianthin C (T-C), tyrianthin A (T-A) and tyrianthinic acid VI (TA-VI), isolated from Ipomoea tyrianthina root on GABAergic transmission system in cerebral cortex slices of mouse brain in an in vitro model. The results obtained show that all glycolipids tested evoked endogenous GABA release and increased its concentration within the incubation medium compared with controls; T-C demonstrated a dose-dependent effect. Sodium absence and guvacine presence did not affect significantly the activity of S-1 and T-C in contrast to T-A and TA-VI. S-1 and T-C effects were calcium-dependent, where GABA concentrations were considerably reduced. These results suggest that the increase of endogenous γ-aminobutyric acid (GABA) released evoked by these glycolipids is possibly done through a Na^+- and/or Ca^{2+}-dependent mechanisms, discarding a pore-forming mechanism.

1. Introduction

In the brain function, there is a balance between excitatory and inhibitory mechanisms, where a sudden imbalance might generate cell damage and lately large functional changes, resulting in several neurological diseases in humans. Currently, drugs used for treatments produce a variety of direct and indirect, or compensatory effects on any of the neurotransmitter systems [1], even with numerous adverse reactions (from minimal alterations on CNS to aplastic anemia and hepatic failure). On the other hand, secondary metabolites of medicinal plants could be eliminated easier than synthetic drugs, diminishing the side effects. In this context, the study of these metabolites represents an alternative for overcome adverse effects and will give us new insights in the development of new potential neuroactive drugs [2].

In Mexico, some species of *Ipomoea* genus have been used for the treatment of several diseases on CNS [3]. Herbal extracts, active fractions, and pure compounds from this genus have been studied for their pharmacological properties [4–9].

Ipomoea tyrianthina Lindley (syn. *I. orizabensis* Pelletan, Lebed. ex Steud., Convolvulaceae) is a perennial twining herb with a large root. This root has been used in Mexican traditional medicine as a mild purgative, to treat seizures and sedative [10–12]. *Ipomoea tyrianthina* root is popularly known in Mexico as “escamonea” (mexican scammony) or locally named “*quiebra platos*”, and it is available as dried root, crude resin, or alcoholic extract [7–10]. Previous reports have described the isolation and characterization of resin glycosides with different chemical structures (macrolactones, glycosidic acids, or dimers) [5–9,13–16].

The effect of these resin glycosides has been related with a pore-forming mechanism [17]. It has been described that intraperitoneal administration to mice of some pure glycolipids from *I. tyrianthina* showed activities associated to CNS (antidepressant, relaxing, hypnotic,

Abbreviations: TA-VI, tyrianthinic acid VI; T-A, tyrianthin A; T-C, tyrianthin C; S-1, scammonin 1; Na^+, sodium; Ca^{2+}, calcium; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; RP-HPLC, reverse phase HPLC

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sedative, anxiolytic, anticonvulsant and neuroprotective), and in *in vitro* assays, evoked endogenous GABA and glutamic acid release in brain cortex [5–9], however, the mechanism of such activity has not been studied.

Therefore, the aim of the present work was to explore the possible mechanism of four resin glycosides (*tyrianthinic acid VI*, *tyrianthin A*, *tyrianthin C* and *scammonin 1*) on the GABA release processes in cerebral cortex slices of mouse brain in an *in vitro* model.

2. Materials and methods

2.1. Plant material

Ipomoea thyrianthina root was collected in the state of Morelos (August 2005), and Mexico City (July 2004), Mexico. Botanical classification and identification was conducted by Biologist M. Castro, Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM). Specimens were deposited in the Herbarium of Instituto Mexicano del Seguro Social (vouchers IMSS-15076 and IMSS-15075, respectively). The name of the plant was checked and confirmed according the official website www.theplantlist.org.

2.2. Resin glycosides

All resin glycosides (Fig. 1) used in this work were isolated from the jalapin and convolvulin of *Ipomoea tyrianthina* root.

The dried, powdered roots of *Ipomoea tyrianthina* (250.0 g) were extracted by maceration with CH_2Cl_2 (500 mL × 3) and CH_3OH (500 mL × 3), to obtain a solid dichoromethane-soluble extract (jalapin, 26 g) and 15 g of a dark syrup methanol-soluble extract. The jalapin was separated by column chromatography on Silica gel eluting with dichloromethane-methanol (9:1), yielded scammonin 1.

The metanol-soluble extract was washed with distilled water (3 × 50 mL) and CH_2Cl_2 (3 × 50 mL), to afford a dark solid (9.3 g). The solid (1.0 g) was submitted to a C18 column (Supelco, 10 × 15 mm) with a gradient of CH_3OH-H_2O (0:100 to 100:0, at increments of 10%/mL), fractions were collected and pooled. After elimination of the solvent, 0.7 g of a resinous material was obtained. The resinous solid was percolated on an activated charcoal column, eluting with CH_3OH. Fractions of 5 mL were collected and reunited giving the convolvulin (0.42 g). Tyrianthin A, tyrianthin C, and tyrianthinic acid VI were separated from the convolvulin and purified by reverse phase HPLC.

The chemical characterization of *T-A, TA-VI,* S-1, and *T-C* was performed by NMR and Mass spectrometry [5,7,9,14].

2.3. Animals

Twenty CD1 adult mice (20–30 g) were provided by the Animal Laboratory Unit of Instituto de Biotecnología, UNAM. Mice were kept under controlled conditions (12 light hours/12 dark hours at 25 °C, and free access to water and food). The ethical authorization for this research was obtained through the protocol approved by the Institutional Research Committee, conducting animal experiments in accordance with the Mexican Official Norm (NOM-062-ZOO-1999).

2.4. Pharmacological evaluation

2.4.1. Cerebral cortex slices preparation

Mice were sacrificed by cervical dislocation and brain dissection was performed on a plate with crushed ice. Subsequently, cortex slices (250–300 μm) were manually obtained with a razor blade and guide coverslips according to Gutiérrez and Delgado-Coello. For each condition, a cerebral cortex slice (500–600 μg of protein) was placed in 2 ml of modified Krebs-Ringer buffer (basal medium): 118.0 mM NaCl, 1.18 mM KH_2PO_4, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.18 mM $MgSO_4$, 20.0 mM $C_4H_{11}NO_3$ (Trizma ® base) and 5.6 mM $C_6H_{12}O_6$, at a pH 7.4 and 4 °C in constant aeration. Aminoxyacetic acid (AOA) 10.0 μM was added to the medium to prevent GABA metabolism. Trizma ® base and AOA were purchased from Sigma Aldrich (St. Louis, MO, USA), and the rest of the reagents were purchased from J. T. BAKER (Mallinckrodt Baker, S.A. de C.V., México).

2.4.2. GABA release assays

In vitro GABA release assays were performed through an incubation system [18]. Cerebral cortex slices were placed into in vial with 2 ml of basal medium at 37 °C during 10 min with constant aeration. Thereafter, resin glycosides were added at a final concentration of 10 μg/mL (***T-A*** and ***TA-VI***) or 20 μg/mL (***T-A***, ***TA-VI***, ***S-1*** and ***T-C***). Aliquots of 200 μl were taken out at different times (0, 30, 60, 90, 120, 180 and 300 s) throughout the assay. To determine if the effect of resin glycosides on GABA release was sodium- or calcium-dependent, experiments were conducted in a similar manner as before replacing NaCl for 118.0 mM $C_5H_{14}NOCl$ (choline chloride) or by adding 100 μM EGTA instead of $CaCl_2$. Moreover, the effect of resin glycosides on either GABA uptake process or the modulation of extracellular GABA concentration through $GABA_B$ receptors was determined by adding at the test medium 100 μM guvacine hydrochloride (a GABA transport inhibitor), 100 μM β-alanine (a GABA transport inhibitor in glia), or 100 μM 2-hydroxy-saclofen (2-OH-S, selective $GABA_B$ receptor antagonist). As positive control, incubation medium was modified increasing potassium up to 47.0 mM and diminishing sodium concentration (75.7 mM). Negative control was conducted in the presence of the basal medium. Aliquots were store at −20 °C for GABA measurement. Choline chloride, EGTA, guvacine hydrochloride, β-alanine and 2-hydroxy-saclofen were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.4.3. GABA quantification

GABA content of samples was determined by RP-HPLC in a Merck-Hitachi chromatograph equipped with a fluorescence detector (wavelength of 330 nm of excitation and 450 nm of emission), using a Cartridge-LiChorospher® 100 RP-C18 chromatographic column of 5 μm in particle size and a linear gradient of 80% solvent A (CH_3COONa 0.1 M, pH 7.0) to 100% solvent B (CH_3OH) in a running time of 10 min with a flow of 1.5 ml/min. 20 μl of each collected aliquot was previously derivatized with O-phthaldialdehyde (OPA, Merck-Schuchardt), according to Jones and Gilligan. Quantification of GABA was achieved by using a standard curve of known concentrations of GABA (Sigma Aldrich) derivatised. A linear regression equation was used for the calculation of the concentration of GABA. Protein content in brain slices was determined after its homogenization in 1 ml of water according to the Lowry, using bovine serum albumin (BSA, Irvine Scientific) as protein calibration. GABA concentration in each aliquot was corrected for volume changes in the incubation medium and the data were expressed as pmol of GABA/μg of protein. All solvents used in the chromatography protocol were HPLC grade and purchased from J. T. BAKER (Mallinckrodt Baker, S.A. de C.V., México).

2.5. Statistical analysis

Data obtained from GABA release assays were analyzed using GraphPad Prism statistical platform (Prism 6 for Windows version 6.01, GraphPad Software Inc., La Jolla, CA) under ANOVA one tail and t-Student tests, ($p < 0.05$). All data were expressed as mean and standard error ($\overline{X} \pm$ SEM).

3. Results

3.1. Effect of resin glycosides on endogenous GABA release

Resin glycosides ***T-A***, ***TA-VI***, ***S-1*** and ***T-C*** (10 or 20 μg/mL)