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Differential modulation of human GABAC- $\rho 1$ receptor by sulfur-containing compounds structurally related to taurine

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RESEARCH ARTICLE

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Differential modulation of human GABA_C-p1 receptor by sulfur-containing compounds structurally related to taurine

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Abstract

Background: The amino acid taurine (2-Aminoethanesulfonic acid) modulates inhibitory neurotransmitter receptors. This study aimed to determine if the dual action of taurine on GABA_C-p1R relates to its structure. To address this, we tested the ability of the structurally related compounds homotaurine, hypotaurine, and isethionic acid to modulate GABA_C-p1R.

Results: In *Xenopus laevis* oocytes, hypotaurine and homotaurine partially activate heterologously expressed GABA_C-p1R, showing an increment in its deactivation time with no changes in channel permeability, whereas isethionic acid showed no effect. Competitive assays suggest that hypotaurine and homotaurine compete for the GABA-binding site. In addition, their effects were blocked by the ion-channel blockers picrotoxin and Methyl(1,2,5,6-tetrahydropyridine-4-yl) phosphinic acid. In contrast to taurine, co-application of GABA with hypotaurine or homotaurine revealed that the dual effect is present separately for each compound: hypotaurine modulates positively the GABA current, while homotaurine shows a negative modulation, both in a dose-dependent manner. Interestingly, homotaurine diminished hypotaurine-induced currents. Thus, these results strongly suggest a competitive interaction between GABA and homotaurine or hypotaurine for the same binding site. "In silico" modeling confirms these observations, but it also shows a second binding site for homotaurine, which could explain the negative effect of this compound on the current generated by GABA or hypotaurine, during co-application protocols.

Conclusions: The sulfur-containing compounds structurally related to taurine are partial agonists of GABA_C-p1R that occupy the agonist binding site. The dual effect is unique to taurine, whereas in the case of hypotaurine and homotaurine it presents separately; hypotaurine increases and homotaurine decreases the GABA current.

Keywords: Receptor modulation, GABA receptor, *Xenopus* oocytes, Sulfur-containing compounds, Taurine, Homotaurine, Hypotaurine, Isethionic acid

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Background

Incorporation of sulfur into amino acids, proteins, enzymes, vitamins, and other biomolecules makes sulfur essential for biological systems [1]. In mammals, methionine is an essential amino acid, whereas cysteine, homocysteine, and taurine are semi-essential amino acids because they can be synthesized from methionine, sulfur, and serine via trans-sulfuration [1, 2].

Taurine (2-ethanesulfonic acid) is a ubiquitous, non-protein β -amino acid that abounds in different mammalian tissues. In the central nervous system (CNS) and retina, taurine is the second most abundant amino acid after glutamic acid, with a concentration in different species that ranges from 10 to 90 mM [3]. Taurine plays a role in different cell functions, as well as in neuronal migration and CNS development. In general, extensive experimental evidence reported relates taurine with cell volume and osmolality regulation [4–6]. In addition, taurine is involved in other physiological processes of the CNS and retina, such as modulation of Ca^{2+} channels and neurotransmission [7–11]. Other physiological functions of taurine not described in retina are antioxidant defense, phase II detoxification reactions and a role as a neurotrophic factor. GABA (γ -aminobutyric acid) plays a key role in neurotransmission. For example, in the retina, GABA modulates transmission of information flowing from photoreceptors to the brain. GABA performs its inhibitory action via two types of receptors: (1) ionotropic, designated GABA_A and GABA_C (also known as GABA_{A-p}); and, (2) metabotropic, named GABA_B , all of them with different molecular and pharmacological properties [12–15]. GABA_C receptor is particularly interesting because it is insensitive to barbiturates, benzodiazepines, bicuculline, and baclofen (modulators and inhibitors of GABA_A and GABA_B receptors, respectively) [16–20] but sensitive to picrotoxin and Methyl(1,2,5,6-tetrahydropyridine-4-yl) phosphinic acid (TPMPA) [21].

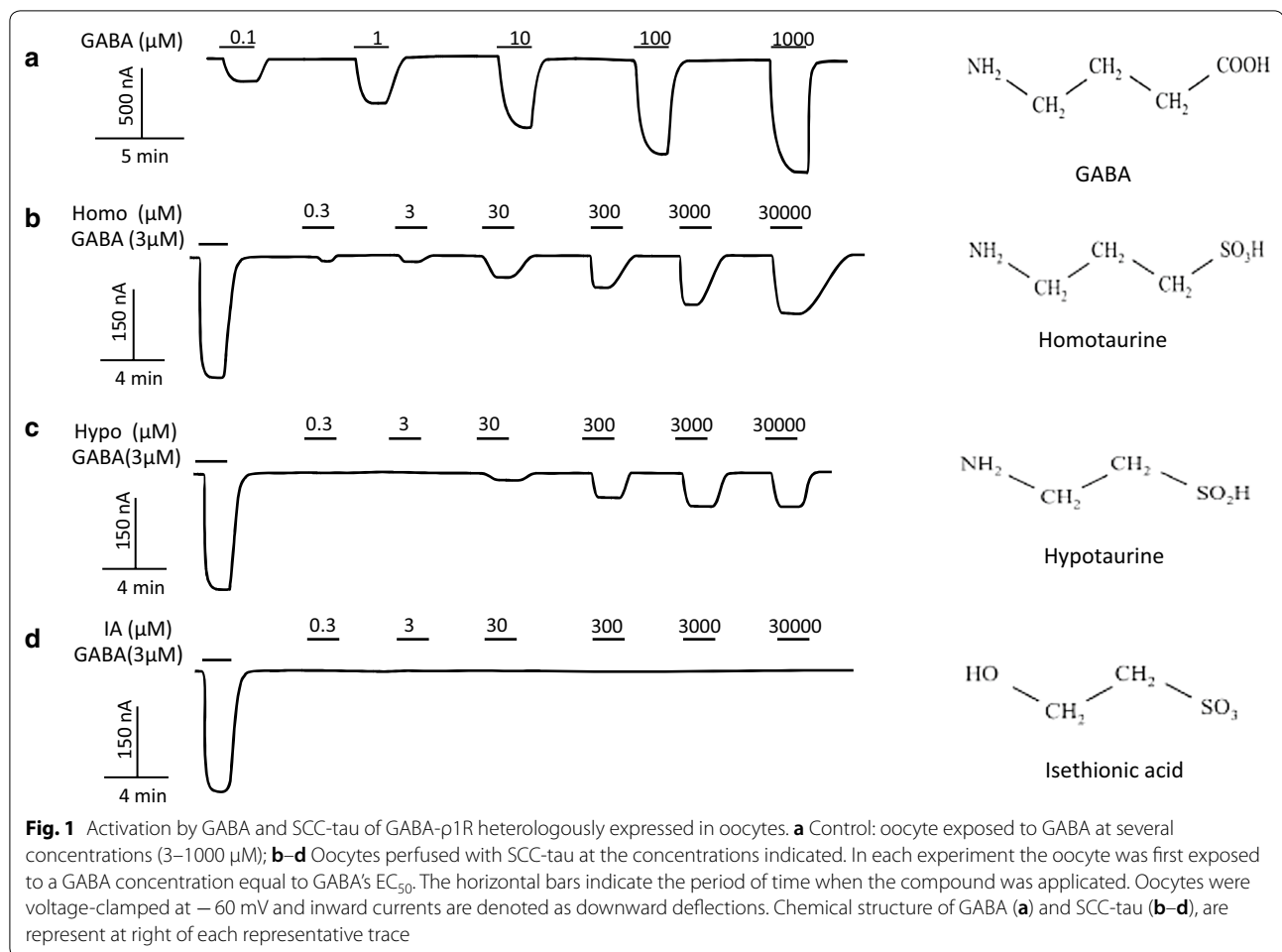
GABA_A receptors are heteropentamers, constituted by α , β , γ , and δ subunits; the combinations of these subunits determine the pharmacological and physiological properties of the receptor, and the $\alpha 1/\beta 2/\gamma 2$ is the most common combination found in the CNS and retina [22, 23]. GABA_C receptors are composed of the $\rho 1$, $\rho 2$, and $\rho 3$ subunits, each of them capable of forming homomeric functional receptors when expressed heterologously. However, the native composition of these receptors in neurons is unknown. In the CNS, evidence of the expression of GABA_C receptors has been demonstrated in cerebellar Purkinje neurons and in the amygdala [24–26]. Experiments “in vivo” using specific antagonists of GABA_C receptors directly applied into the amygdala, suggest that GABA_C -mediated activity participates in the modulation of fear and anxiety [26]. Although the role of

GABA_C receptors in the retina is still poorly understood, its function is tightly modulated by intracellular cascades triggered by neuroactive molecules and their receptors, and they are thought to play a major role in visual signaling [19, 20, 27, 28]. While GABA_A receptors ($\alpha 1/\beta 2/\gamma 2$) are distributed in all neuronal types of the retinal circuit, GABA_C conformed by $\rho 1$ and $\rho 2$ subunits are located in bipolar neurons, where they downregulate NMDA receptors and, consequently, modulate transient glutamate release in response to light. In addition, amacrine and ganglion cells express GABA_C receptors [29–31].

Taurine exerts its inhibitory effect via activation of GABA_A and glycine receptors, but has less affinity compared to the specific agonists of each receptor [10, 11]. However, the mechanisms by which taurine regulates ionotropic GABA receptors remain to be determined. A negative and positive modulation of the GABA-induced current in oocytes heterologously expressing human $\text{GABA}_C-\rho 1$ receptor, exposed at lower and higher taurine concentrations was reported [32]. These results suggest that taurine acts in a dual way and may compete with GABA for the same binding site. Our study aimed to determine if the dual effect of taurine observed on $\text{GABA}_C-\rho 1R$ was associated with the molecular structure of taurine. To confirm this hypothesis, we used homotaurine (Homo), hypotaurine (Hypo), and isethionic acid (IA), all sulfur-containing compounds structurally related to taurine (SCC-tau), but with some differences in their chemical structure. For instance, unlike taurine, Homo has one additional carbon and the sulfonic group (SO_3H) is in *Cis* position with respect to the amino (NH_2) group; Hypo has a sulfinic group (SO_2H) in *Trans* position with respect to the NH_2 group, and IA has a hydroxyl group instead of NH_2 . Some of these compounds have been widely used to better understand the physiological functions of taurine and, likewise, to evaluate compounds that may be therapeutically applied in some diseases such as diabetes, alcoholism, ischemia, and others [33–35]. Although a variety of taurine analogues have been developed, their effects are still unknown in terms of structure–function in various systems [36].

Results

$\text{GABA}_C-\rho 1$ receptor ($\text{GABA}_C-\rho 1R$) heterologously expressed in oocytes generated typical non-desensitizing GABA currents (Fig. 1a) when SCC-tau was perfused onto the same oocyte; Homo- and Hypo- elicited a response in a concentration dependent manner. Both compounds gate the ion-channel at micromolar concentrations (Fig. 1b, c). In contrast, IA did not show any effect at the concentrations tested (Fig. 1d). Similar to the currents induced by GABA, Homo- and Hypo-induced well-maintained currents and did not desensitize, even



after long exposure. Despite the lack of differences between the magnitude of the current elicited by Homo- and Hypo-, these SCC-tau show a clear difference in the EC_{50} of 70 ± 1.1 μ M for Homo and 3 ± 1.5 mM for Hypo (Fig. 2a). The response induced by Homo and Hypo did not correlate with changes in the activation time of the currents (τ_{act}). Nevertheless, Homo showed an increase in deactivation time (τ_{deac}). The τ_{act} and τ_{deac} for GABA alone were 1 ± 0.2 min and 1.7 ± 0.5 min, respectively. In the presence of Hypo, τ_{act} was 1.5 ± 0.1 min and t_{deact} was 3.4 ± 1.1 min, whereas for Homo τ_{act} was 1.8 ± 0.2 min and τ_{deac} was 6.7 ± 2.2 min (Fig. 2b). However, we did not observe any permeability changes of the channel activated, as suggested by their inversion potential, which was -21 ± 1.0 mV for 3.5 μ M GABA and -25 ± 2.1 mV and -26 ± 1.0 mV for 70 μ M Homo and 3 mM Hypo, respectively (Fig. 2c). In all cases, the current–voltage (I–V) relationship was linear within the range explored (-120 to $+40$ mV), indicating that activation of GABA $_C$ -p1R by SCC-tau was voltage-independent and did not change the ion selectivity of the channel.

We also analyzed if the currents activated by Homo and Hypo were sensitive to TPMPA, a highly specific and selective antagonist of GABA $_C$ -p1R [21]. Figure 3 shows an IC_{50} of 1.2 ± 0.1 μ M, 1.4 ± 0.1 μ M or 2.0 ± 0.2 μ M TPMPA when the receptor was activated by 3 μ M GABA, 70 μ M Homo or 3 mM Hypo, respectively (Fig. 3a). Hill coefficients were 1.3 ± 0.2 μ M (GABA), 1.0 ± 0.1 μ M (Homo), and 1.1 ± 0.8 μ M (Hypo). It is well known that TPMPA competes with GABA for the same binding-site, so since the IC_{50} of TPMPA values are similar; it is probable that SCC-tau, Homo and Hypo, share the same binding site as GABA in the GABA $_C$ -p1R. As expected, picrotoxin, an allosteric antagonist of ionotropic GABA receptors [37, 38], irreversibly inhibits the currents activated by 3 μ M GABA, 70 μ M Homo or 3 mM Hypo in a dose-dependent manner, with an IC_{50} of 100 ± 0.1 μ M, 105 ± 0.3 μ M, and 100 ± 0.8 μ M, respectively (Fig. 3b).

Figure 4 shows that during GABA-induced activation of the receptor, Homo reduced the currents when co-applied at different concentrations (3 μ M to 30 mM). The effect of Homo on the GABA-induced current changes

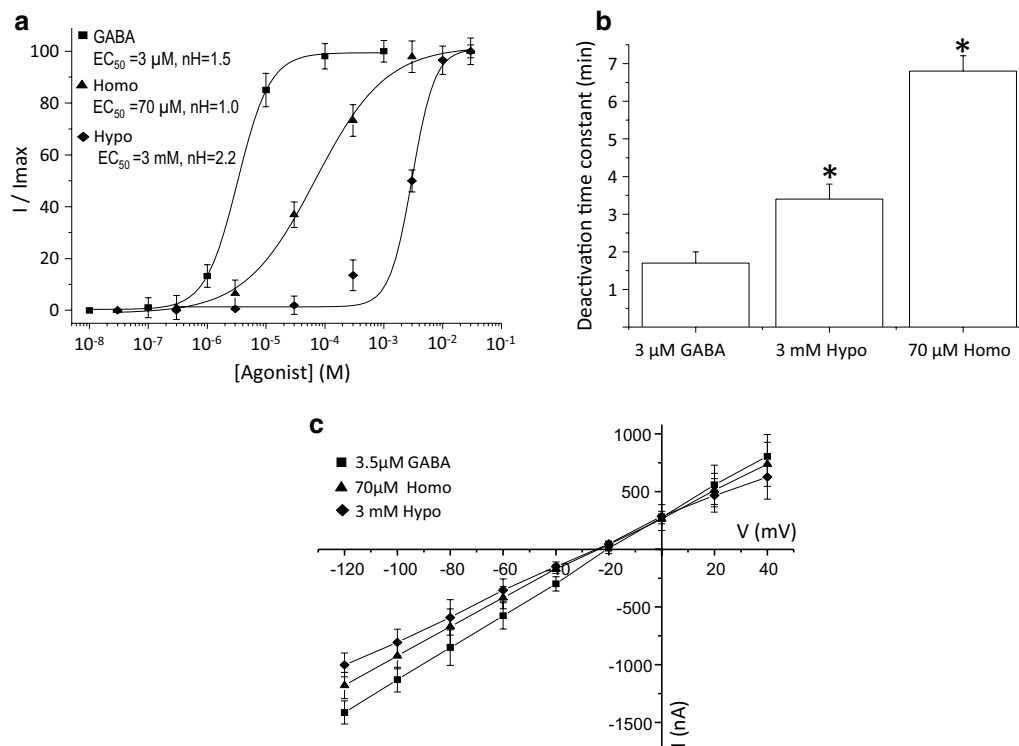


Fig. 2 GABA- and SCC-tau-induced currents in oocytes heterologously expressing GABA_C-p1R. **a** Dose-response relationship showing the EC_{50} and Hill coefficient for each agonist. Data were normalized to the maximal current (I) obtained for each agonist. **b** Deactivation constant (τ_{deac}) of currents activated by GABA, Hypo, and Homo. The difference in the deactivation constants between, GABA-, Hypo- or Homo-induced currents, was significant when $P < 0.05$. **c** Current-voltage relationship of GABA-, Homo-, and Hypo-induced currents at the indicated concentrations. Dose-response relationship (**a**) was constructed by measuring the maximum response evoked by each agonist concentration (see methods), for **b** and **c**, data are given as the mean \pm S.E. from at least 8 oocytes ($n=8$) from 3 frogs ($N=3$)

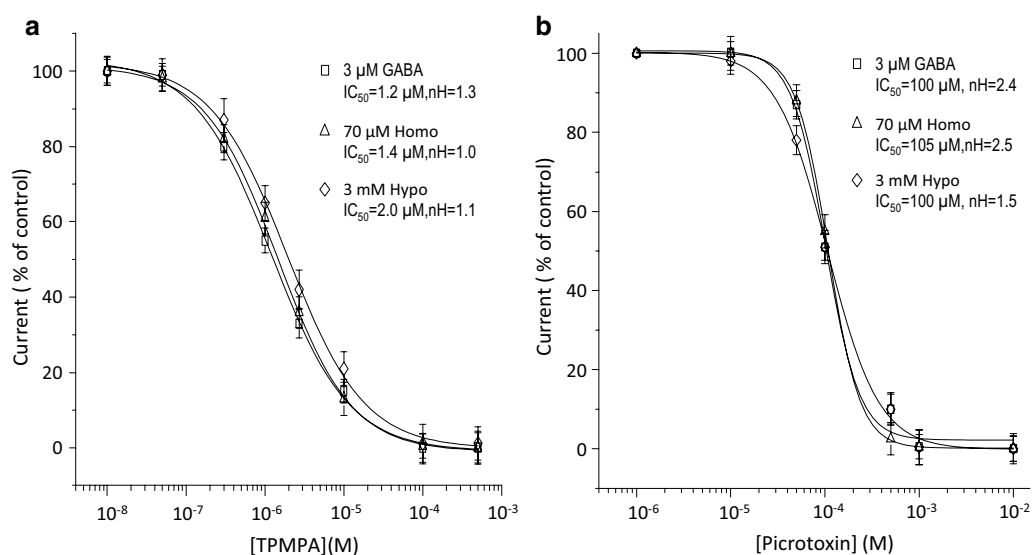
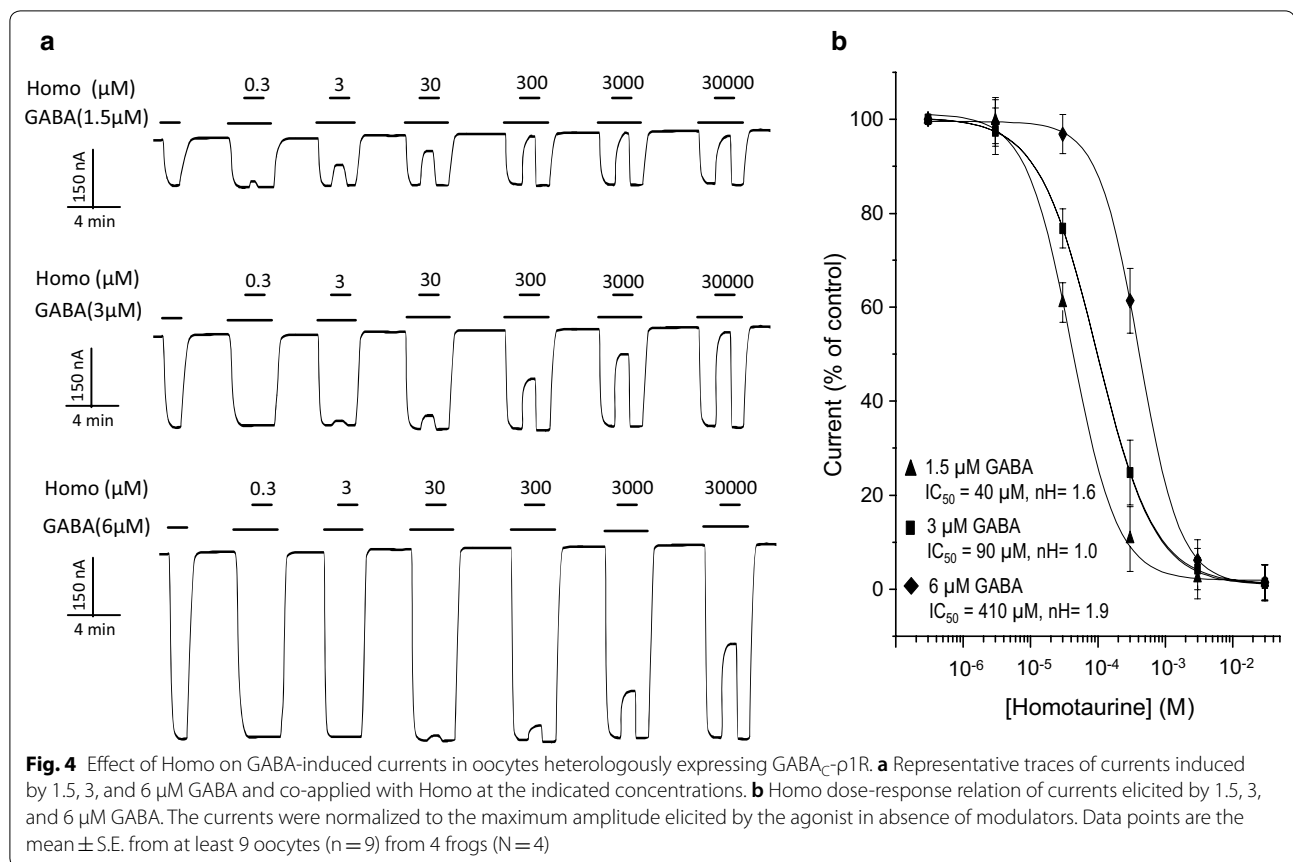


Fig. 3 Pharmacological modulation of GABA- and SCC-tau-induced currents by TPMPA (**a**) and picrotoxin (**b**). Currents were normalized to the maximum amplitude elicited by GABA or SCC-tau in absence of inhibitors. Data points are the mean \pm S.E. from at least 7 oocytes ($n=7$) from 3 frogs ($N=3$)



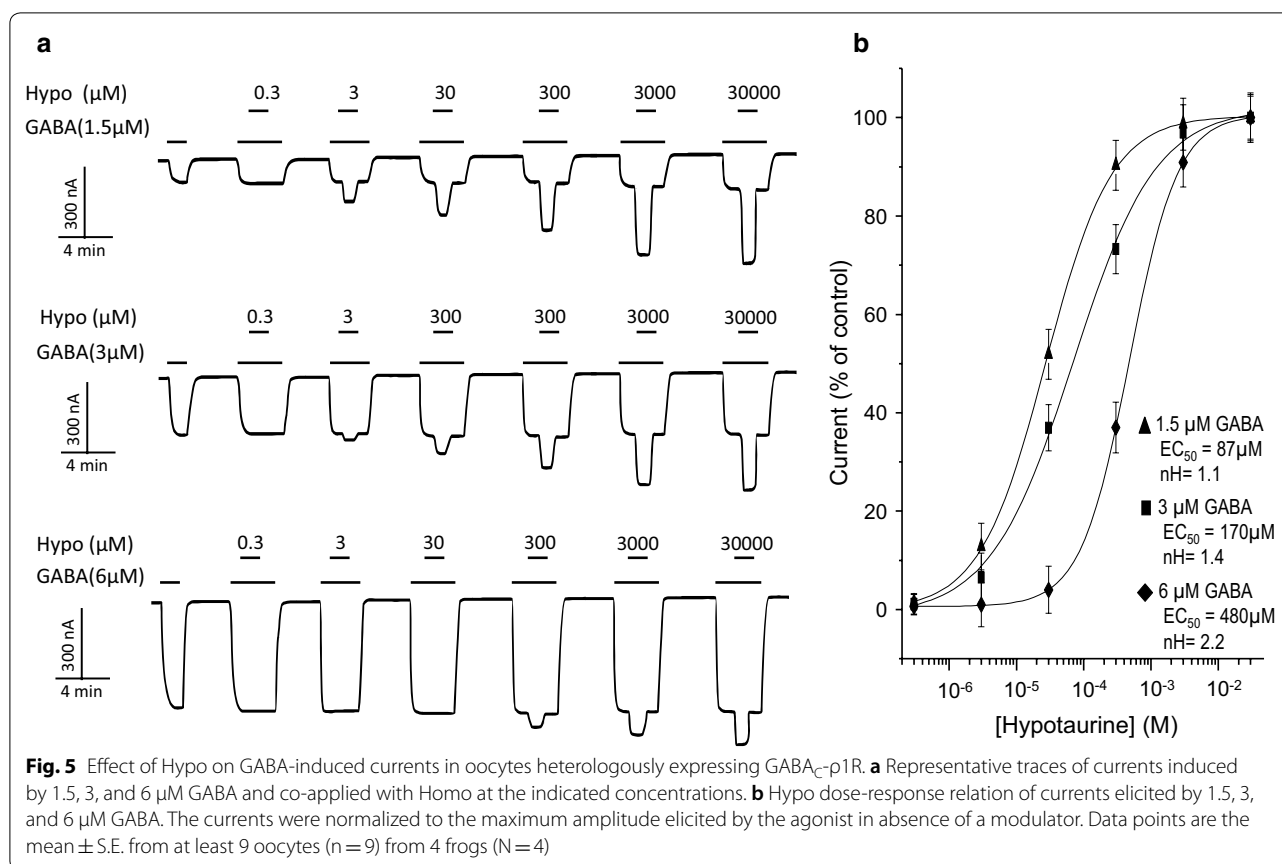
depended on GABA concentrations. Figure 4a shows that at a concentration of 1.5 μM and 3 μM of GABA, Homo decreased GABA-activated currents. However, at a higher concentration of GABA (6 μM), the effect of Homo diminished; the IC₅₀ for Homo were 40 ± 1.0 μM, 90 ± 1.5 μM, and 410 ± 2.9 μM at 1.5 μM, 3 μM and 6 μM GABA, respectively (Fig. 4b). In all cases, the GABA-induced currents without SCC-tau, increased in a dose-concentration fashion.

We also analyzed whether the stimulatory effect of Hypo changed by the extracellular GABA concentration and we found that unlike Homo, Hypo potentiated GABA currents. As observed in Fig. 5a, at 1.5 μM GABA, Hypo induced a current increase, that was even greater than the current activated by GABA alone. At 3 μM GABA, the effect of Hypo was still present almost with the same characteristics as 1.5 μM GABA; however, at 6 μM GABA, the increase induced by Hypo on GABA currents diminished. GABA-induced current increased in a dose-concentration fashion. The EC₅₀ values obtained for Hypo were 87 ± 1.5 μM, 170 ± 1.7 μM, and 480 ± 2.1 μM for 1.5 μM, 3 μM, and 6 μM GABA, respectively (Fig. 5b). These results suggest a competitive action of SCC-tau with GABA for the same binding site

(Additional file 1: Figure S1). In the case of IA, we did not observe any effect (data not shown).

Molecular modeling

To prove theoretical studies on the interactions of the different ligands with the human GABA_C-p1receptor, we performed homology modeling. We selected as target the structure of the GABA_C-p1 R-beta3 homopentamer, since it has coverage of around 70% and an identity of 48%. The original sequence of this gene (GenBank: AAA52509.1) has an initial segment of 44 amino acids with a disordered/unstructured identity. We constructed this segment using an “ab initio” modeling, which shows good structural correlation. With this segment and the crystallographic structure, the GABA_A-p1R model was constructed and validated (Additional file 2: Figure S2). This model presents an initial segment corresponding to the transmembrane section, which may have an effective recognition function. Subsequently, using this constructed model, we performed docking studies with different ligands (GABA, Homo, Hypo), IA, and one GABA_A receptor antagonist (picrotoxin). For docking probes, we included taurine to compare the binding sites with SCC-tau and GABA. Figure 6 shows the results obtained after

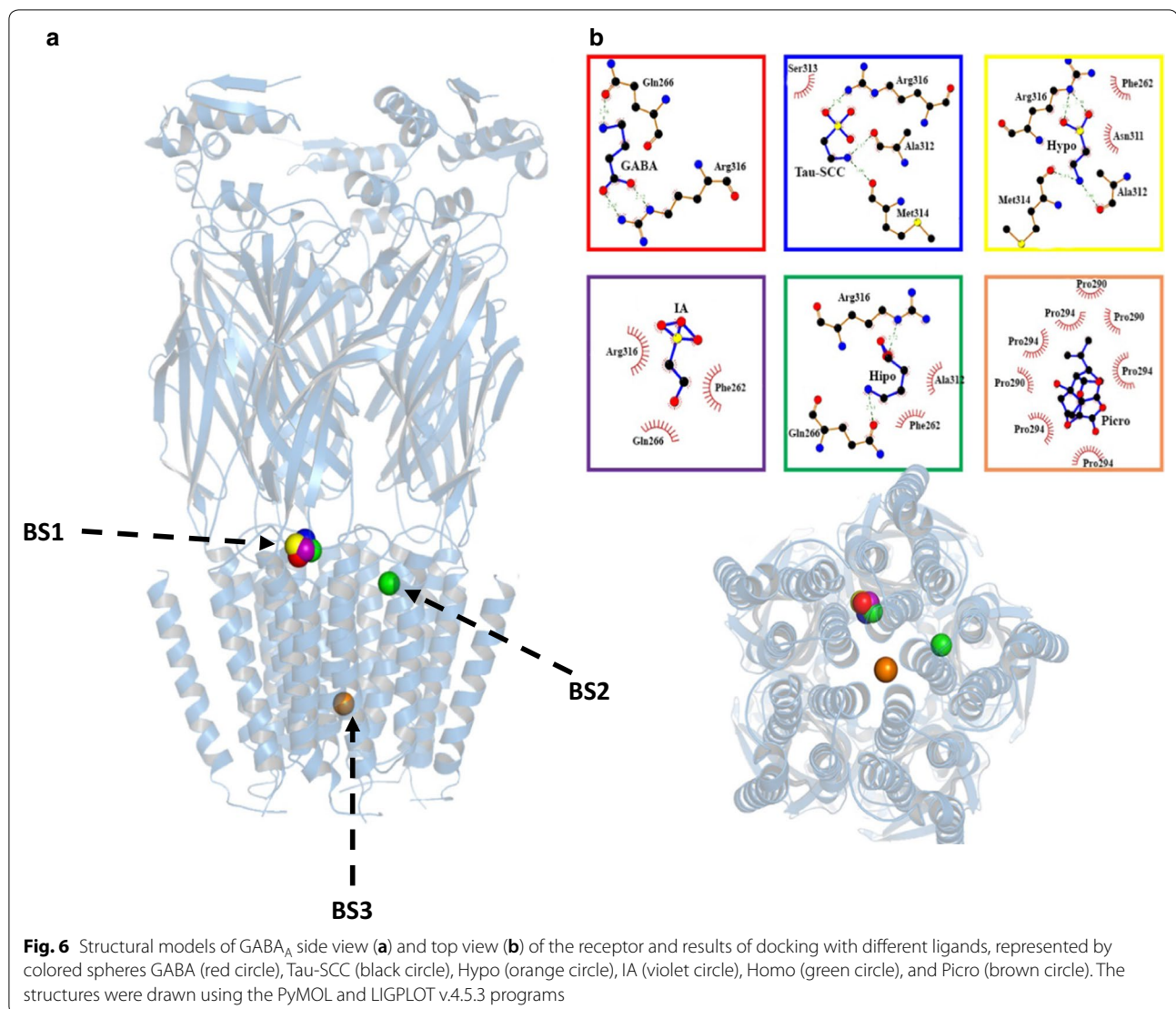


performing 1000 runs for each compound, observing three different binding sites. In the first one we located (BS1) GABA, taurine, Homo, Hypo, and IA; the second binding site (BS2) apparently is exclusive for Homo, additional to the site it shares with GABA and SCC-tau, and the third one (BS3) corresponds to picrotoxin, located inside the receptor. The binding parameters (K_i) obtained by AutoDock4 are in the mM range, except for picrotoxin that is of a lesser order of magnitude. The order of affinity predicted is as follows: picrotoxin (0.012 mM) < IA (3.25 mM) < Homo (3.56 mM) < GABA (8.48 mM) < Hypo (10.50 mM) < taurine (10.86 mM). There are different binding sites at the GABA_C-p1R that function as modulators of the activity of this receptor. Therefore, we complemented the experimental results with theoretical studies, observing some correlation, such as: (1) the structural relationship between Homo and Hypo to activate GABA_C-p1R, demonstrates that Homo is a more potent agonist than Hypo; also, it has a lower K_i than Hypo; (2) competition experiments between TPMPA and GABA by GABA_C-p1R indicate that Homo and Hypo (even taurine), interact at the same site (BS1), supporting the

data obtained by docking; (3) Homo has a second binding site (BS2), that is not used by GABA or SCC-tau; and (4) picrotoxin, an allosteric antagonist of ionotropic GABA receptors, its binding was predicted in the anion channel of the receptor (BS3), a unique site not used by the SCC-tau compounds with a strong affinity according to literature reports for this blocker [37, 38].

Discussion

The use of SCC-tau, that possesses only some of the properties of taurine, has increased our understanding about the role of this β -amino acid in the cell physiology. Previous reports show that SCC-tau modulates the inhibitory action triggered by GABA [39–41]. However, the structural characteristics that determine these effects are unclear. In this work, we report that even though Homo, Hypo, and IA share similar core structures, Homo was a potent partial agonist of GABA_C-p1R compared to Hypo. This observation could indicate that the presence of an additional carbon and the SO₃H group in *Cis* position with respect to the NH₂ group in Homo's structure allows it to form a moiety that enhances the affinity and binding of Homo for GABA_C-p1R. In contrast, it is probable that the absence of an NH₂ group and the short



carbon skeleton are responsible for the non-response effect with IA.

Oocytes that heterologously express the GABA_C-p1R can generate currents when exposed to GABA, Homo, or Hypo. When SCC-tau and GABA were co-applied, we observed a concentration-dependent decrease (Homo) or increase (Hypo) of GABA induced-currents. Interestingly, in both cases, we did not observe a *dual* action of Homo and/or Hypo in the modulation of GABA-induced currents, as in the case of taurine [32]. In this sense, unlike the human glycine receptor (Gly- α 1R), where taurine and other β -amino acids act like agonists or antagonists [42], in GABA_A-p1R only taurine follows this pattern. One interpretation for this lack of *dual* effect of Homo and Hypo is that probably its action it is not associated to a change in the molecular structure of the compounds [42], and the positive and negative modulation on

the GABA-induced current could be a result of the occupation of the agonist binding sites in the binding pocket by each of these compounds. A second possibility is and effect of either negative or positive cooperativity between GABA-Homo or GABA-Hypo.

In the case of taurine, it was previously reported that it generates a *dual* effect on GABA-induced currents, suggesting that this β -amino acid (at low concentrations) can compete with GABA for the same binding site [32]. This hypothesis is reinforced with our molecular model, where Hypo, Homo, IA, taurine and GABA share the same binding site. The fact that taurine inhibits GABA response at high concentrations [32], just like Homo in this work, could be due to an interaction of taurine and Homo with a second binding site.

These data suggest a competitive behavior of SCC-tau and, thus, reflects an antagonistic binding within

a common ligand-binding pocket. The Hill coefficient obtained in both the negative and positive modulations generated by Homo and Hypo, respectively, indicates a contribution of GABA displacement despite its affinity to the binding site. This structural phenomenon would allow the interaction of SCC-tau in different binding conformations, inducing agonist or antagonist effects.

GABA induced-currents were modulated when SCC-tau and GABA were co-applied, while Homo reduced, Hypo increased the currents. This effect is explained with the data obtained from the docking, where Homo presents a second site with a greater binding probability, different from the binding site it shares with GABA; these two sites are located at different places of the receptor. Therefore, considering that GABA shares the same binding site as Hypo and Homo, this would confirm the fact that they compete for the same binding site as reflected in the experimental protocol. However, in the case of Homo, it does not share the second binding site with GABA, so there is no competition between them. Therefore, it is likely that this second Homo binding site may act as a negative regulatory site of the GABA_C-p1R, when GABA activates the receptor.

Homo acts like an antagonist of GABA- and Hypo-induced currents; however, is a partial agonist at high concentrations without GABA or Hypo. This could be explained by negative cooperativity between Homo and GABA or Hypo, and this interaction is possibly due to a dependence of the second Homo binding site on the action of the agonist, that is available only when the first site (BS1) is previously occupied by GABA or Hypo. Therefore, it is likely that the affinity of Homo depends on the previous binding of a first agonist, which changes the conformational structure of the receptor.

Conclusions

Interest in receptors for γ -aminobutyric acid, the major inhibitory transmitter in the central nervous system, has been development over the last four decades. Given their widespread distribution, lower abundance and relative simplicity compared to GABA_A and GABA_B receptors, GABA_C receptors are attractive drug targets. GABA_C receptors pharmacology is different from GABA_A and GABA_B receptors, therefore, the development or characterization of novel compounds for GABA_C receptor is imperative. This will allow to determine with certainty the function of this receptor in the central nervous system. In this sense, small molecules such as SCC-tau are compounds with great potential for this purpose. Here, we observed that SCC-tau are partial agonists of the GABA_C-p1R that occupy the agonist binding site. The dual effect observed with SCC-tau was present in a separate way: while Hypo increases, Homo decreases the

GABA-currents. These observations suggest that taurine could induce the dualistic effect by a change in the molecular structure in response to taurine concentration. However, the fact that Hypo and Homo act differently on GABA-currents could potentially be used to design pharmacological tools for the modulation of GABAergic receptors.

Methods

Expression of homomeric human GABA_C-p1 receptors in *Xenopus laevis* oocytes

All the animals were handled in accordance with guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals and with the approval of the Institutional Animal Care and Use Committee of the National University of Mexico. We used Ethyl 3-aminobenzoate methanesulfonate (MS-222) at 0.17% for 30 min, to anesthetize the *Xenopus laevis* frogs. The oocytes were manually removed and enzymatically defolliculated at room temperature using collagenase type I (0.3 μ g/ μ l) for 45 min. Later, oocytes were kept in Barth's medium (in mM): 88, NaCl; 1, KCl; 0.33, Ca₂ (NO)₃; 0.41, CaCl₂; 0.82, MgSO₄; 2.4, NaHCO₃; 5, HEPES; pH 7.4 and 0.1 mg/ml gentamicin sulfate. After 24 h, 0.5 μ g/ μ l of human GABA_C-p1R mRNA was injected in vegetal hemisphere of the oocyte, and electrophysiological recordings were obtained 2–3 days after injection.

Voltage-clamp recordings

Membrane currents elicited by GABA and SCC-tau were recorded using the two-microelectrode voltage-clamp technique. Oocytes were placed in a chamber, with a volume of 500 μ l, and impaled with two microelectrodes previously filled with 3 M KCl (0.5–2.5 M Ω) and clamped at –60 mV. To determine the equilibrium membrane potential of the agonist action, I–V relationships were constructed by stepping the oocyte's membrane potential from –60 to –120 mV for 1 s and then from –60 to +40 mV (in 20 mV steps) in the absence or presence of GABA or SCC-tau (Homo, Hypo, IA). All recordings were done at 20–23 °C in a chamber continuously perfused at 5–10 ml/min of Ringer solution (in mM): 115, NaCl; 2, KCl; 1.8, CaCl₂; 5, HEPES, pH 7.4. All drugs were purchased from SIGMA-ALDRICH (San Louis Missouri, USA). The stock solution of GABA (1 M), Homo (0.5 mM), Hypo (0.5 mM) or IA (0.5 mM) was kept frozen until thawed for the experiments. The pH of all solutions was adjusted to 7.4.

Data analysis

Results are reported as mean \pm S.E. of the values obtained from several cells, each value being the average of measurements in different cells. Data from each experiment

were collected from at least seven oocytes. Agonist concentration–response curves were constructed by measuring the maximum response evoked by each agonist concentration. The half-maximal concentration (EC_{50}) and Hill coefficient (n_H) of GABA, Hypo, and Homo were estimated for each curve by fitting the data to the logistic type equation (Origin 6.0, Northampton, MA): $A = A_{max}/(1 + 10^{[\log EC_{50} - [\log [agonist]] n_H]})$. The half-inhibitory concentration (IC_{50}) of Homo was estimated by fitting the following equation: $A = A_{max}/(1 + 10^{[[agonist] - \log EC_{50}]})$. Differences among groups were statistically analyzed by ANOVA and Tukey–Kramer post-test, and were considered significant when $P < 0.05$. Control responses to GABA were obtained before and after each drug application to account for possible shifts in the amplitude of the control current. To determine the time constants for the activation (τ_{act}) and deactivation (τ_{deac}) of GABA-, Homo- and Hypo-currents responses, a decay function of the form: $I(\tau) = \exp(-t/\tau_d) + C$, where I is the current and t is time, was fit to the experimental data (Origin 6.0 software; Northampton, MA). Differences between groups were statistically analyzed by ANOVA and a Tukey–Kramer post-test, and were considered significant at the level $P < 0.05$.

Homology modeling of Type A gamma-aminobutyric acid (GABA_A) receptors (Homo sapiens)

This homology modeling started with the retrieval of the amino acid sequence of GABA_A (GenBank: AAA52509.1). The mature protein, predicted from this cDNA sequence in 458 amino acids, displays between 30 and 38% amino acid similarity to the previously identified GABA_A subunits [29]. Using the program Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) [43], and the database of the Protein Data Bank, the structural model was built based on the structure of a human gamma-aminobutyric acid receptor, the Gaba(a) r-beta3 homopentamer (4COF.pdb) [44], which revealed a coverage of 70% and the highest sequence identity (48%) with the target. A multiple alignment was performed using the constraint-based multiple alignment tool (COBALT; https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi) server, where an initial segment of 44 amino acids with no structural identity appears. An ab initio model of this fragment was made using the Rosetta 3.4 program [45]; 1000 models of this segment were made. Subsequently, using MODELER 9.17 r10881 with the multiple models protocol, 500 GABA_A models were constructed, using the most stable model of the peptide and the 4COF.pdb file as a template. Later, a simple structural refinement of full atom was performed using “relax” application of Rosetta. The final model was validated using the Verify-3D (structure

evaluation software) and Whatcheck (protein verification tools software) computer programs [46, 47].

Docking protocol

Docking was made with the previously constructed three-dimensional structure (Type A gamma-aminobutyric acid receptors—GABA_A), and the ligands were built with HyperChem 8 software. The ligands were minimized employ Gaussian 09, revision A.02 (Gaussian Inc., Wallingford, CT) at DTF B3LYP/3-21G level of theory. AutoDockTools 1.5.4 (<http://mgltools.scripps.edu/>), it was used to prepare the structures before carrying out the docking. The preparation of the structures consisted in adding all hydrogen atoms as well as the Kollman united-atom partial charges to the receptor, while Gasteiger–Marsili charges and rotatable groups were assigned automatically to the ligands. AutoGrid4 was occupying to generate the electrostatic grid maps for each atom type. The initial grid box size was 60 Å × 60 Å × 60 Å in the x, y, and z dimensions. Docking was produced out with AutoDock4 version 4.2 (<http://autodock.scripps.edu/>) [48, 49] using default parameters; for the Lamarckian genetic algorithm with local search, number of individuals in population (150), maximum number of energy evaluations (2.5 million), maximum number of generations (27,000), rate of gene mutation (0.02), rate of crossover (0.8), and 1000 runs for docking. Finally, the docking was analyzed with AutoDockTools using cluster analysis and PyMOL software [50].

Additional files

Additional file 1: Figure S1. Effect of Homo on Hypo-induced currents in oocytes heterologously expressing GABA_A-p1R. (A) Representative traces of currents induced by 30 μM, 3 mM and 30 mM Hypo and co-applied with Homo at the indicated concentrations. (B) Homo dose-response relation of currents elicited by 30 μM, 3 mM and 30 mM Hypo. The currents were normalized to the maximum amplitude elicited by the agonist in absence of modulators. Data points are the means ± S.E. from at least 9 oocytes (n = 9) from 4 frogs (N = 4).

Additional file 2: Figure S2. Three-dimensional models of GABA_A and template. In slate blue cartoon (GABAA), splitpea green cartoon (template) (Protein Data Bank [PDB] code: 4COF). The structures were drawn using the PyMOL program.

Abbreviations

GABA: γ-aminobutyric acid; Taurine: 2-Aminoethanesulfonic acid; Homotaurine: 3-Aminopropane-1-sulfonic acid; Hypotaurine: 2-Aminoethanesulfonic acid; Isethionic acid: 2-hydroxyethanesulfonic acid; TPMPA: Methyl(1,2,5,6-tetrahydropyridine-4-yl) phosphinic acid; MS-222: Ethyl 3-aminobenzoate methanesulfonate; NMDA: N-methyl-D-aspartate; HEPEs: 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid; SCC-tau: sulfur-containing compounds structurally related to taurine.

Authors' contributions

LDOP conceived the project and coordinate the experiments, and did the electrophysiology and pharmacology assays in oocytes; RF did microinjections of mRNA into the oocytes; MG-A did the “in silico” model and docking probes;

RZ-A, HP-M, and RG-S. did the purification and extraction of the DNA, and synthesized the mRNA; EZ, HQ-M, and RG-C did the statistics probes. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data set during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All the animals were handled in accordance with guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals and with the approval of the Institutional Animal Care and Use Committee of the National University of Mexico.

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