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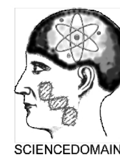

The Antidepressant-like Effect of Hyperbrasilol B, A Natural Dimeric Phloroglucinol Derivative is Prevented by Veratrine, a Sensitive-Voltage Na^+ Channel Opener

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Authors' contributions

This work was carried out in collaboration between all authors. Author FBC contributed with the design of the study; performed the In vivo experiments, statistical analysis an literature searches; wrote the protocol and the first draft of the manuscript. Author SS performed all chemical procedures. Author LGM contributed with In vivo experiments and statistical analysis. Author PMC performed In vitro experiments. Author GVP planned and supervised all chemical experiments. Author CWN planned and supervised all in vitro experiments. Author SMKR designed the whole study, managed the literature searches, statistical analyses and revised the final version of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: South Brazilian *Hypericum* species are a source of dimeric structures, constituted of filicinic acid and phloroglucinol moieties, which present antidepressant-like effects mediated by monoaminergic neurotransmission in rodents. Here, we show that hyperbrasilol B, a phloroglucinol derivative from *Hypericum caprifoliatum*, presents

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antidepressant-like activity in mice forced swimming test (FST). The aim of this study was to determinate if Na+ channels are important to the antidepressant-like effect of hyperbrasilol B and also verify the effect of this compound on Na+, K+ ATPase activity in the cerebral cortex and hippocampus of mice.
Methodology: We assessed the effects of veratrine, a Na^+ channel opener on antidepressant-like effect of hyperbrasilol B by using mice FST. Veratrine (0.06 mg/kg) and hyperbrasilol B (10 mg/kg) were given i.p. 60 and p.o. 30 min, respectively, before the test. In another batch of experiments different groups of mice were treated with hyperbrasilol B 10 mg/kg, p.o. (Single administration or once a day during 3 days). Two hours after the acute or after the last of the three treatments, the brain structures were removed for measuring Na+, K+ ATPase activity.
Results: Veratrine was able to prevent the anti-immobility effect of hyperbrasilol B on the FST, suggesting that its antidepressant-like effect might be due to Na+ influx modifying properties. Animals treated for 3 consecutive days with hyperbrasilol B presented a significant increase in the hippocampus Na+, K+ ATPase activity. The acute treatment was ineffective.
Conclusion: Alterations in the Na^+ gradient may be implicated in the antidepressant-like effect of hyperbrasilol B.

Keywords: Hypericum caprifoliatum; phloroglucinol derivatives; depression; hyperbrasilol B; Na+ channels; Na+, K+ ATPase.

1. INTRODUCTION

Hypericum perforatum (St. John's wort) is one of the best-known and best-selling herbal therapies for depression. In various models of depression, *H. perforatum* extracts have been shown to be as effective as conventional antidepressant drugs [1,2]. Study of the genus *Hypericum* could not only lead to more rational uses of *H. perforatum* for the treatment of depression, but could also be helpful in the search for an alternative source of antidepressant molecules.

Brazilian *Hypericum* species occur predominantly at southern regions, where about 20 of them have been identified [3]. Regarding chemical composition, these species are rich in flavonoids [4] and dimeric phloroglucinol structures consisting of an acylfilicinic acid and phloroglucinol moieties linked by a methylene bridge [5]. These phloroglucinol derivatives are proposed as chemotaxonomic markers for the southern Brazilian species [6] and differ from hyperforin (Fig. 1A), one of the most known phloroglucinol derivative occurring in *H. perforatum*, which is a polyprenylated bicyclic acylphloroglucinol derivative [7].

Among the native species studied, *H. caprifoliatum* extracts showed promising results in the forced swimming test (FST), which predicts antidepressant activity [3,8,9]. Also, a cyclohexane extract from *H. polyanthemum* administered orally produced a significant antidepressant-like effect in the FST in rats and mice. This effect was also observed with uliginosin B (Fig. 1B) which is likely to be the main active substance of *H. polyanthemum* [10]. These effects seem to be due to an activation of the three monoaminergic systems*: H. caprifoliatum* [8] and *H. polyanthemum* [10] extracts as well as HC1 (an enriched phloroglucinol fraction obtained from *H. caprifoliatum*) and uliginosin B inhibit synaptosomal monoamine uptake without specific binding to each of the different monoamine transporters.

These data indicate that these products act differently than classical antidepressant drugs and suggest that their mechanism of action involves neurotransmitters transport in general.

Hyperforin dissipates the pH gradient across the synaptic vesicle membrane thereby interfering with vesicular monoamines storage [11] and also increases monoamines synaptic concentrations by an indirect and yet unknown mechanism [12,13,14]. Hyperforin seems to exert its antidepressant action by mechanisms dependent on changes in Na^+ ions conductance [15]. Indeed, Marsh and Davies [16] have already demonstrated that hyperforin increases the flow of neurotransmitters of mice cerebral cortex, possibly by facilitating the entry of Na^+ ions in neurons. Interestingly, it was already demonstrated that a decrease or reversal of the electrochemical Na^+ gradient triggers the DA transporter (DAT)-mediated release of dopamine [17].

Antidepressants are a therapeutic group with a high incidence of Na^+ channel inhibitory activity [18]. Accordingly, Na^+ channel blockade has been reported for a number of tricyclic antidepressants, and antidepressants interact predominantly with the inactivated state of the channel, and interactions with this state are thought to be the most pharmacologically relevant [19]. Na^+ channels are also molecular targets for antiepileptic drugs, such as lamotrigine, topiramate, phenytoin, carbamazepine and valproic acid, which are also mood stabilizers [20,18]. Investigations into the pathophysiology of bipolar illness have consistently revealed altered homeostasis of metal ions. Most notably, acutely manic patients show increased Na^+ retention and these changes can result from a primary reduction in Na^+, K^+-ATPase activity [21,22].

The enzyme Na^+, K^+-ATPase is the major determinant of cytoplasmic Na^+ concentration, hydrolyzing ATP and using the free energy to drive the transport of K^+ into the cell and Na^+ out of the cell, against their electrochemical gradients [23]. Accumulating evidence has suggested that brain Na^+, K^+-ATPase activity may be involved in the etiology of depressive disorders in humans. Na^+, K^+-ATPase activity in erythrocytes correlates with mood in bipolar depression; Na^+, K^+-ATPase activity is decreased during manic and depressive states and normal during healthy states [20]. Furthermore, decreased expression and function of the Na^+, K^+-ATPase is associated with depressive disorders in humans and the Na^+, K^+-ATPase α3 gene expression is decreased in major and bipolar depression in the prefrontal cortex [24].

Another compound with dimeric structure consisting of filicinic acid and a phloroglucinol moieties was isolated from *H. caprifoliatum*: hyperbrasilol B (Fig. 1C) [6]. As we have data that allow us to state that phloroglucinol derivatives present a promising molecular pattern for the development of antidepressant drugs with different mode of action, we decided to investigate the potential antidepressant effect of hyperbrasilol B and search for its mechanism of action out of monoaminergic neurotransmission. The aim of this study was to investigate the potential antidepressant effect of hyperbrasilol B by using the FST, and also to investigate if Na^+ influx is important for the hyperbrasilol B antidepressant-like activity, by assessing the effects of veratrine (a Na^+ channel opener) on its effect in the FST. We also evaluated the effect of acute and repeated administration of hyperbrasilol B on Na^+, K^+-ATPase activity in the cerebral cortex and hippocampus of mice.

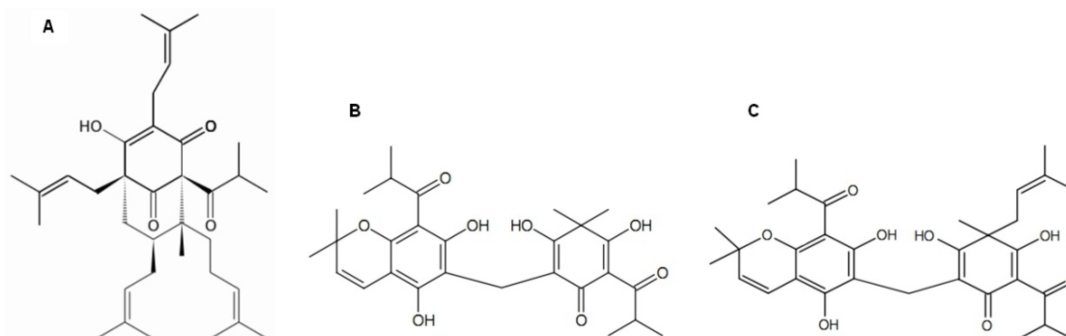

Fig. 1. Structures of hyperforin (A), uliginosin B (B) and hyperbrasilol B (C)

2. MATERIALS AND METHODS

2.1 Plant Material

The aerial parts of *H. caprifoliatum* were collected in the flowering period in the city of Guaíba, Rio Grande do Sul – Brazil (October/2009). The voucher specimens were deposited at the herbarium of Universidade Federal do Rio Grande do Sul (ICN Bordignon, 3118 Herbário do Departamento de Botânica – Instituto de Biociências – UFRGS). The plant collection was authorized by IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) (Protocol 02000.001717/2008-60). The plant material was dried at room temperature and powdered.

2.2 Preparation of *H. caprifoliatum* Extract

To obtain the lipophilic extract of *H. caprifoliatum*, plant material (200 g) was extracted with *n*-hexane (plant/solvent ratio 1:5 w/v) by static maceration (5 x 48h), followed by evaporation to dryness under reduced pressure at 45ºC yielding a crude *n*-hexane extract. In order to obtain purified extract, this extract was treated with acetone, according to Rocha et al. [25], producing an insoluble fatty residue, which was eliminated through a paper filter, yielding 2.04% of a wax free extract.

2.3 Isolation of Hyperbrasilol B

The wax free *n*-hexane extract was submitted to silica gel column chromatography Acrôs organics (0.06–0.200 mm 60Å) using *n*-hexane: dichloromethane gradient system as the mobile phase. The hyperbrasilol B enriched fraction obtained was purified by preparative Thin Layer Chromatography (TLC) performed on 20 cm × 20 cm glass-supported plates covered with 0.5 mm layers of silica gel GF254 (Merck®), with *n*-hexane: dichloromethane (1:1 v/v) as chromatographic eluent, yielding 50 mg of hyperbrasilol B. Band was detected using UV light (254 nm) and the purity of hyperbrasilol B (81%) was confirmed by high performance liquid chromatography (HPLC) analysis. Prior to the analysis, the compound was dissolved in HPLC grade MeOH and filtered (0.22 μm pore size, Merck). The HPLC system consisted of a Shimadzu liquid chromatography instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a DGU-20A5 degasser, LC-6AD pumps, SIL-10AD auto sampler, CTO-20AC column oven, SPD-20AV UV/VIS detector and CBM-20A communications module. The chromatographic data were recorded and processed by LC

Solution software version 1.24 SP2. For separations, a Waters Nova-Pack C18 column (4 μm, 3.9 9 150 mm) and a Waters Nova-Pack C18 60 Å guard column (3.9 9 20 mm) were used. Hyperbrasilol was eluted using an isocratic elution program with 95% CH3CN, 5% H2O, 0.01% TFA, monitoring at 220 nm. Peak was identified by comparison of its retention time (tR) with standard compound (authentic sample), previously identified by 1H and 13C NMR [6]. The pick area was determined using a calibration curve previously prepared in the laboratory [26]. The hyperbrasilol B retention time was compared with standard compound (authentic sample), previously identified by ^{1}H and ^{13}C NMR [6].

2.4 Animals

Behavioral and biochemical tests were carried-out with male CF1 mice (25–30 g) purchased from Fundação Estadual de Produção e Pesquisa em Saúde – RS (Brazil). The animals were housed by eight mice in plastic cages (17 x 28 x 13 cm) and all animals were kept under a 12 hours light/dark cycle (lights on at 7:00 a.m.) at constant temperature of 23º±1ºC with free access to standard certified rodent diet and tap water. All experimental protocols were approved by The Animal Care Local Ethical Committee (CEUA UFRGS; Protocol 18203) and performed according to Brazilian law [27], which are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and International Guiding Principles for Biomedical Research Involving Animals [28].

2.5 Behavioral Experiments

2.5.1 Forced swimming test

The FST was carried out according to Porsolt et al. [29] with minor modifications standardized and validated in our laboratory [8]. Mice were adapted to the laboratory conditions 1 hour before being exposed to FST. The animals were individually forced to swim in a cylinder pool (10 cm diameter, 13 cm height, water at 22±1ºC) and the total duration of immobility during a 6 minutes test was scored (in seconds) by a human observer blinded to the treatments. Each mouse was recorded as immobile when it remained floating motionless or making only the movements necessary to keep its head above water. Hyperbrasilol B was suspended at concentrations of 1.0 and 1.5 mg/mL in saline with 2% of polysorbate 80. Different groups of animals (n=8 or 9) were treated with hyperbrasilol B (10 and 15 mg/kg, p.o.) or 2% polysorbate 80 solution in saline (NaCl 0.9%) 30 min before the test. The doses of hyperbrasilol B were chosen based on active doses of uliginosin B, an analogous dimeric phloroglucinol that presents antidepressant-like activity in the FST [10]. A control experiment was carried out in parallel by testing imipramine (tricyclic antidepressant), fluoxetine (selective serotonin reuptake inhibitor) and bupropion (dual inhibitor of dopamine and norepinephrine reuptake) administered at 20, 30 and 15 mg/kg, p.o., respectively. All treatments were administered at 10 mL/kg body weight

In order to investigate the potential contribution of Na^{+} channels to antidepressant-like effects of hyperbrasilol B, we investigated the influence of a pre-treatment with veratrine on its effect in the FST. Dose-response experiments were performed in the FST and locomotor activity apparatus to determine the appropriate dose of veratrine to be used in the interaction studies. A subactive dose was defined as it did not, by itself, reduce mobility in the FST and had no effect on locomotor activity. Veratrine was diluted in saline 0.9% and administered i.p. 60 min before the test at doses of 0.06, 0.125 and 0.5 mg/kg [30]. A vehicle-treated

group was introduced as a control. In the association studies, veratrine or vehicle was administered 60 min before the test and HC1 was administered 30 min before testing.

2.5.2 Locomotor activity

The measurement of spontaneous locomotor activity was performed in the open-field. After the drug administration (pre-treatment/or treatment), mice were replaced in their holding cages for the required administration-test interval and then individually transferred to a transparent acrylic box measuring 45 X 30 X 30 cm with a dark bottom divided into 24 equal quadrants. The animals were observed for 15min, and the first 5 min were regarded as the period of habituation. The number of crossings was recorded by a human observer blinded to treatments. These animals were different from those used in the FST.

2.6 Biochemical Experiments

2.6.1 Treatments and procedures

Acute treatment: Different groups of mice (n=6) were treated with a single administration of hyperbrasilol B (10 mg/kg, p.o.) or vehicle (saline with 2% of polysorbate 80).

Repeated treatment: Different groups of mice (n=6) were treated once a day with hyperbrasilol B (10 mg/kg, p.o.) or vehicle (saline with 2% of polysorbate 80), during 3 days between 9:00 and 12:00 am.

Two hours after the single or the last of the third treatments, animals were killed by decapitation and the hippocampus and cerebral cortex were removed and stored at -80ºC until use. All procedures (mice isolation, forced swimming session and decapitation) were performed in separated rooms. The time interval between treatments and euthanasia of mice was chosen based on previous results from our group [10].

2.6.2 Tissue preparation

The samples of hippocampus and cerebral cortex were homogenized in 50 mM Tris–HCl, pH 7.4 (1:5, w/v) and centrifuged at 2.400 and 4.000 × g for 10 min at 4ºC to obtain the low-speed supernatant (S1). S1 freshly prepared was used for the enzyme assays.

2.6.3 Na^+, K^+-ATPase activity assay

For Na^+, K^+- ATPase activity assay, a reaction mixture containing 3 mM $MgCl_2$, 125 mMNaCl, 20 mMKCl, 50 mM Tris-HCl, pH 7.4 and S1 in a final volume of 500 µL was used. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Control samples were carried out under the same conditions with the addition of 0.1 mM ouabain. The samples were incubated at 37ºC for 30 min, the incubation was stopped by adding trichloroacetic acid solution (10%) with 10 mM $HgCl_2$. Na^+, K^+-ATPase activity was calculated by the difference between the two assays. SKF 82526 (1 mM) was used as a positive control. Released inorganic phosphate (Pi) was measured by the method of Fiske and Subbarow [31]. Enzyme activity was expressed as nmol Pi/mg protein/min.

2.6.4 Protein determination

Protein was measured by the method of Bradford [32] using bovine serum albumin as standard.

2.7 Statistical Analysis

Data were expressed as mean + SEM of the mean, and were analyzed by Student's t test for biochemical analysis and one-way ANOVA followed by Dunnett's test for behavioral experiments. Differences were considered statistically significant at P<0.05.

3. RESULTS AND DISCUSSION

3.1 Behavioral Experiments

Fig. 2 shows a control experiment, which demonstrated the effect of the antidepressant drugs imipramine (a tricyclic antidepressant), bupropion (a selective norepinephrine and dopamine reuptake inhibitor) and fluoxetine (a serotonin selective reuptake inhibitor) on FST at our laboratory conditions [One-Way ANOVA followed by Student Newman-Keuls comparisions, $F_{(3.31)=}$12.815, P<0.001].

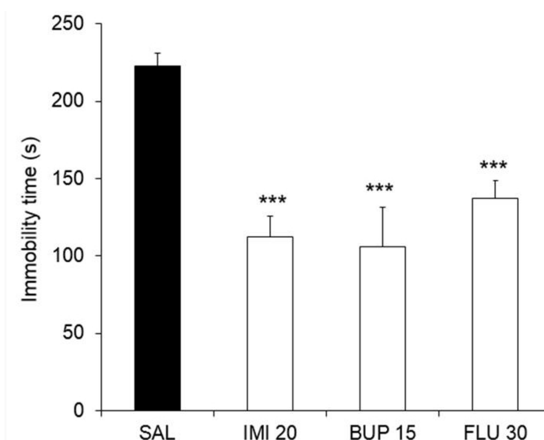

Fig. 2. Effects of imipramine (IMI 20mg/kg p.o.), bupropion (BUP 15 m/kg p.o) and fluoxetine (FLU 30 mg/kg p.o) on immobility time in the forced swimming test in mice
*Results are expressed as mean ± SEM. ***P<0.001 compared to control (SAL – saline) group (one-way ANOVA followed by Student Newman-Keuls comparisons)*

When tested on FST, hyperbrasilol B (hyper, 10 and 15 mg/kg, p.o.) demonstrated an anti-immobility effect [One way ANOVA, F(2.25)=11.236, P<0.001] (Fig. 3), which can be considered as an antidepressant-like activity of this phloroglucinol derivative. This anti-immobility effect was not related to a non-specific behavioral stimulation, given that hyperbrasilol B did not affect locomotor activity (Fig. 3). This observation supports the proposition that this product could be a potential antidepressant drug since antidepressants reduce immobility in FST at doses that do not stimulate locomotion [29].

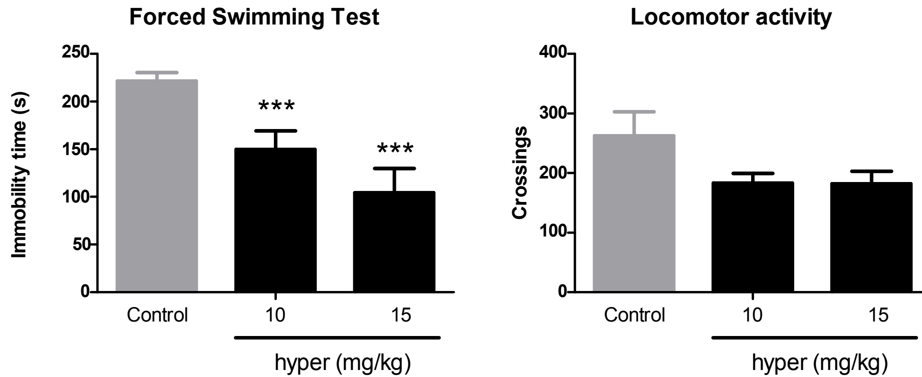

Fig. 3. Effects of hyperbrasilol B (hyper, 10 and 15 mg/kg p.o.) on immobility time in the forced swimming test and number of crossings in open-field test in mice

*Results are expressed as mean ± SEM. ***P<0.001 compared to control groups (one-way ANOVA followed by Dunnett's test)*

The FST, developed by Porsolt et al. [29] is widely used for assessing potential antidepressant activity preclinically due its simplicity and predictive validity. In this test, the acute and single drug administration has been considered good enough to predict antidepressant-like effect. Veratrine was tested at doses of 0.06, 0.125 and 0.5 mg/kg (i.p.) in the FST and spontaneous locomotion in order to evaluate whether it would present an effect per se. At doses of 0.125 and 0.5 mg/kg veratrine reduced the immobility time [One way ANOVA, $F(3.28)=4.890$, $P<0.01$] (Fig. 4) and the spontaneous locomotion (Fig. 4) [One way ANOVA, $F(3.28)=5.255$, $P<0.05$] when compared to the respective control groups; at 0.06 mg/kg (i.p.) it did not have any effect (Fig. 4). Thus, this dose was chosen to be co-administered with the lowest tested dose of hyperbrasilol B (10 mg/kg, p.o.): veratrine 0.06 mg/kg prevented the hyperbrasilol B anti-immobility effect [One way ANOVA, $F(3.34)= 6.352$; $P=0.01$] (Fig. 5) without affecting the spontaneous locomotion (Fig. 5), suggesting that the antidepressant-like effect of hyperbrasilol B could be related to Na^+ influx inhibiting properties.

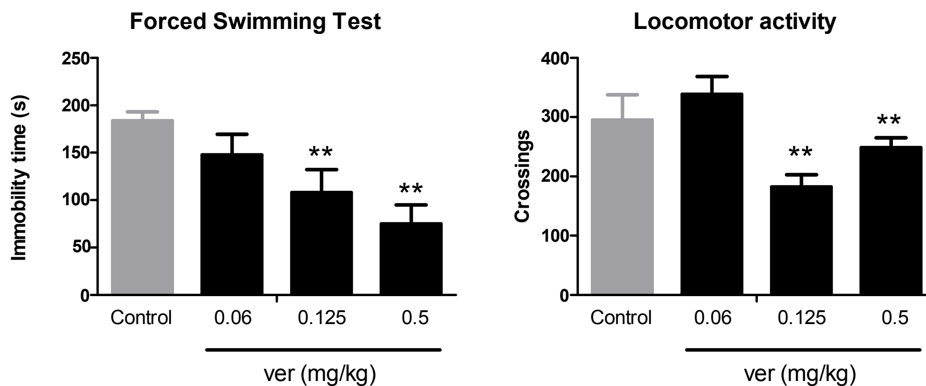

Fig. 4. Effects of veratrine (ver, 0.06, 0.125 and 0.5 mg/kg i.p.) on immobility time in the forced swimming test and number of crossings in open-field test in mice

*Results are expressed as mean ± SEM. **P<0.01 compared to control groups (one-way ANOVA followed Dunnett's test)*

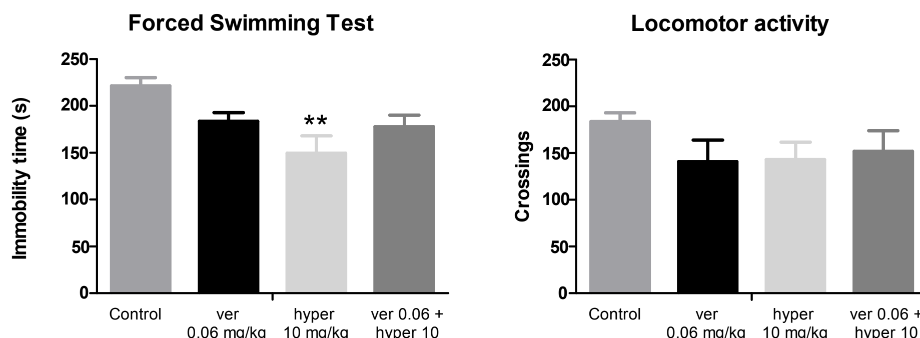

Fig. 5. Effects of co-administration of hyperbrasilol B (10 mg/kg, p.o.) and veratrine (0.06 mg/kg i.p.) on immobility time in the forced swimming test and number of crossings in open-field test in mice

*Results are expressed as mean ± SEM. **P=0.01 compared to control groups (one-way ANOVA followed by Dunnett's test)*

Tarnawa et al. [33] assumed that voltage-gated Na^+ channels modulating effects may play a role in the antidepressant effects of tricyclic antidepressants drugs, such as amytriptiline and desipramine and the selective serotonin reuptake inhibitor (SSRI) fluoxetine. Nevertheless, the antidepressant-like effect of paroxetine, desipramine and imipramine was not reversed by veratrine [30]. On the other hand, most of the anticonvulsants used in the treatment of bipolar disorders are blockers of voltage-gated Na^+ channels and the lamotrigine, topiramate and phenytoin anti-immobility effects in mice FST were reversed by veratrine [30,34]. Although the mechanism of action of these anticonvulsants is still not completely elucidated, this fact suggests that the mechanism of action underlying the antidepressant-like effect of anticonvulsants is different from that of classical antidepressants.

The entry of Na^+ from the extracellular space into the cell takes place through multiple routes consisting of voltage-sensitive and ligand-gated ion channels as well as various transporters. Central to this Na^+-regulating capacity is the Na^+, K^+-ATPase, which can rapidly activate or deactivate in response to changes in intracellular concentration of Na^+ [35]. Small changes in the cytoplasmic Na^+ concentrations secondary to activation of either various Na^+-dependents transporters or Na^+ channels can have serious effects on Na^+, K^+-ATPase activity. Manic subjects exhibit an increase of intracellular Na^+ [36] and a reduction of membrane Na^+, K^+-ATPase expression and activity [37].

Animals killed 2 hours after the single administration of hyperbrasilol B (10 mg/kg, p.o.) did not present any alteration in Na^+, K^+-ATPase activity in the cerebral cortex (Fig. 6A) nor in the hippocampus (Fig. 6B) in relation to the animals treated with vehicle. When animals were treated for 3 consecutive days with hyperbrasilol B (10 mg/kg, p.o.) and killed 2 hours after the third administration, they presented a significant increase in the enzyme activity, in hippocampus (Fig. 6D) [t (9) = 3.220, P=0.01] but not in the cerebral cortex (Fig. 6C).

In the nervous system, Na+, K+-ATPase activity facilitates the generation of action potentials [38] and affects neurotransmitter release [39,40] and reuptake [41], which is crucial to neuronal function. In the present study, repeated hyperbrasilol B administration (10 mg/kg, p.o. during 3 days) caused a significant increase in Na+, K+-ATPase activity in hippocampus but not in cerebral cortex of mice, when compared to vehicle-treated animals. Different morphological and neurochemical effects have been reported in hippocampus after

depressive states and post-mortem and neuroimaging studies of depressed patients have revealed reductions in gray-matter volume and glial density in the hippocampus [42]. In addition, the systems mediating the regulatory mechanisms on the Na+, K+-ATPase activity have been shown to depend on the brain area and on the particular physiological conditions [43], being possible that different effects may be found in different brain regions.

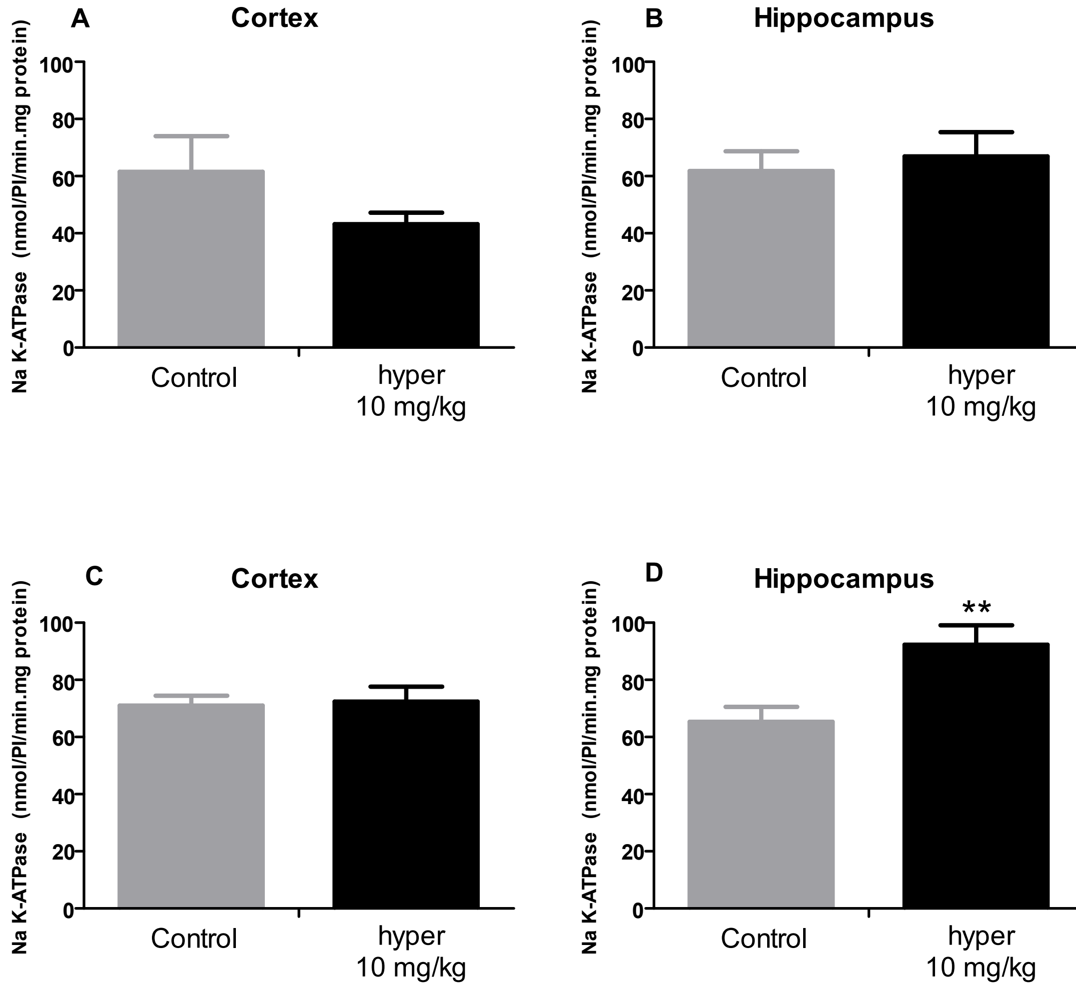

Fig. 6. Effects of hyperbrasilol B (10 mg/kg, p.o.) acute treatment (a single administration) on Na^{+},K^{+}-ATPase activity in the cerebral cortex (A) and in the hippocampus (B) of mice. Panels C and D shows the effects of hyperbrasilol B (10 mg/kg, p.o.) repeated treatment on Na^{+},K^{+}-ATPase activity in the cerebral cortex and in the hippocampus of mice, respectively

*Results expressed as mean $\pm$ SEM. T-test. **P=0.01 compared to control groups*

However, is worth to note that the reduction in FST immobility time after the acute administration of hyperbrasilol B appears not to be linked to an increase in Na+, K+-ATPase activity, since when administered acutely hyperbrasilol B had no effect in enzyme activity. The regulation of Na+, K+-ATPase activity is a complex matter and is subjected to both

short- and long-term regulation by a variety of hormones. The long-term regulatory mechanisms generally affect de novo Na+, K+-ATPase synthesis or degradation [44]. Although the exact mechanism underlying hyperbrasilol B effects is still unknown, it is realizable that the long-term control is involved, since only repeated treatment affected the Na+, K+-ATPase activity.

Viana et al. [8] demonstrated that *H. caprifoliatum* extracts increase dopamine synaptosomal release and inhibit synaptosomal monoamine uptake without specific binding on each of the different monoamine transporters, suggesting that *H. caprifoliatum* mechanism of action involves neurotransmitters transport in general. The fact that *H. caprifoliatum* and *H. polyanthemum* phloroglucinol derivatives influence monoamine uptake through a different mechanism than typical transporter inhibitors is coherent with their chemical structures. As hyperbrasilol B, these molecules do not have a nitrogen atom and structure activity relationship studies for monoamine transporters have revealed that the presence of an amine group is important for both binding affinity and uptake activity [45]. In this study, we demonstrated that hyperbrasilol B seems to affect intracellular Na^+ concentrations by acting on voltage-sensitive Na^+ channels as well as by activating Na^+, K^+-ATPase. The Na^+ gradient is a key driving force of the neurotransmitter transporters [46]. Dopamine, norepinephrine and serotonin entry into the nerve terminal via the carrier is accompanied by Na^+ ions [47].

The phloroglucinol derivative hyperforin inhibits neuronal uptake of several neurotransmitters including serotonin, norepinephrine, dopamine, GABA and glutamate into neurons and astrocytes [12,48] and it has been suggested that this could be the consequence of an elevated intracellular sodium concentration [14]. However, unlike hyperbrasilol B, hyperforin affects neither Na^+, K^+-ATPase nor voltage-gated sodium channels [15]. It was hypothesized that hyperforin activates nonselective cation channels (NSCCs) [49] and in fact, Leuner et al. [7] demonstrated that hyperforin selectively activates TRPC6, a subtype of transient receptor potential channels, resulting in immediately increased intracellular Na^+ and Ca^{2+} concentrations.

4. CONCLUSION

In conclusion, our results reinforce the usefulness of dimeric phloroglucinol structures in the search of new antidepressant molecules. Furthermore, seeing that hyperbrasilol B had its antidepressant-like effect prevented by co-administration of veratrine, a Na^+ channel activator and it was able to increase Na^+, K^+-ATPase brain activity in mice, we can consider it as a putative candidate to possess mood-stabilizing or anticonvulsant properties. Further studies are needed to bear out this hypothesis.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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