Effects of Diene Valepotriates from *Valeriana glechomifolia* on Na^+/K^+-ATPase Activity in the Cortex and Hippocampus of Mice

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Key words
- *Valeriana glechomifolia*
- Valerianaceae
- valepotriates
- antidepressant
- forced swimming test
- Na^+/K^+-ATPase activity

Abstract

Diene valepotriates obtained from *Valeriana glechomifolia* present antidepressant-like activity, mediated by dopaminergic and noradrenergic neurotransmissions. Also, previous studies have shown inhibitory activity of diene valepotriates towards Na^+/K^+-ATPase from the rat brain *in vitro*. Nevertheless, *in vivo* studies regarding the action of diene valepotriates on this enzyme are still lacking. Considering that Na^+/K^+-ATPase cerebral activity is involved in depressive disorders, the aim of this study was to investigate the effects of acute (5 mg/kg, p.o.) and repeated (5 mg/kg, p.o., once a day for three days) diene valepotriate administration on Na^+/K^+-ATPase activity in the cortex and hippocampus of mice submitted or not submitted to the forced swimming test. In addition, the protein expression of Na^+/K^+-ATPase α1, α2, and α3 isoforms in the cortex of mice repeatedly treated with diene valepotriates (and submitted or not submitted to the forced swimming test) was investigated. Diene valepotriates significantly decreased mice immobility time in the forced swimming test when compared to the control group. Only the animals repeatedly treated with diene valepotriates presented increased Na^+/K^+-ATPase activity in the cerebral cortex, and the exposure to the forced swimming test counteracted the effects of the diene valepotriates. No alterations in the hippocampal Na^+/K^+-ATPase activity were observed. Repeated diene valepotriate administration increased the cortical content of the α2 isoform, but the α3 isoform protein expression was augmented only in mice repeatedly treated with diene valepotriates and forced to swim. Mice treated with the vehicle and submitted to the forced swimming test also presented an increase in the content of the α2 isoform, but no alterations in Na^+/K^+-ATPase activity. These results suggest that cortical Na^+/K^+-ATPase may represent a molecular target of the diene valepotriates *in vivo* and long-term regulatory mechanisms are involved in this effect. Also, the forced swimming test *per se* influences the protein expression of Na^+/K^+-ATPase isoforms and counteracts the effects of the diene valepotriates on cortical Na^+/K^+-ATPase.

Abbreviations

CAPOS:	cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss
FST:	forced swimming test
IMI:	imipramine
OFT:	open field test
PMSF:	phenylmethylsulfonyl fluoride
$SCCO_2$:	supercritical carbon dioxide
SISBIO-IBAMA:	Sistema de Autorização e Informação em Biodiversidade, of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis
VAL:	diene valepotriates fraction

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Introduction

The *Valeriana* genus (Valerianaceae) comprises about 300–400 species [1] having a worldwide distribution. Species from this genus are widely recognized by their mild sedative, antispasmodic, and relaxing properties and represent the most popular herbal supplement for the treatment of anxiety and mild sleep disorders [2, 3]. In fact, *Valeriana officinalis* is proposed by the World Health Organization as an alternative or a possible substitute to benzodiazepines [4]. Along with its sedative and antianxiety properties, some preclinical studies have focused on the antidepressant-like

Table 1 Diene valepotriates content (mg/g extract, expressed as mean ± SD) of the supercritical CO2 *V. glechomifolia* extract determined through HPLC.

Valepotriate	Concentration (mg/g extract)
Valtrate	643 ± 56
Acevaltrate	172 ± 33
1-*β*-Acevaltrate	87 ± 9
1-*β*-Aceacevaltrate	39 ± 5
Isovaltrate	37 ± 6

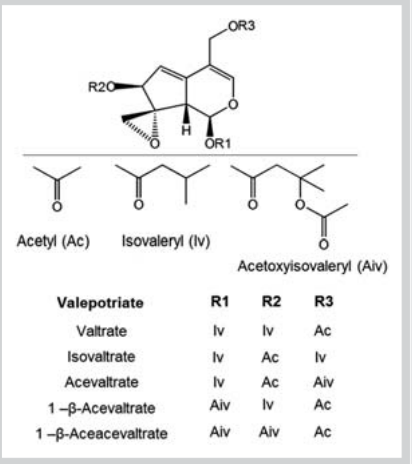

Fig. 1 Diene valepotriates from *V. glechomifolia*.

activity of species from the *Valeriana* genus, like *Valeriana officinalis* [2], *Valeriana wallichii* [5,6], *Valeriana prionophylla* [7], and *Valeriana fauriei* [8].

The pharmacological properties of plants belonging to the *Valeriana* genus are attributed to different constituents, including monoterpene valerenic acid [9], flavonoids [10], and epoxy iridoid esters known as valepotriates [1,11–13], which are restricted to the Valerianaceae family [14].

In line with these findings, our research group has been studying the pharmacological properties of one species of *Valeriana* genera native to south of Brazil, *Valeriana glechomifolia* Meyer, which is especially enriched in valepotriates [14,15]. Recently, we have shown that a VAL obtained from the aerial and subterranean parts of *V. glechomifolia* by $SCCO_2$ extraction presents an antidepressant-like effect, which is mediated by noradrenergic and dopaminergic neurotransmission [16]. Additionally, Maurman and colleagues demonstrated that a valepotriate fraction from *V. glechomifolia* displays anxiolytic and sedative effects and impairs behavioral parameters related to recognition memory in rodents [13]. Moreover, we have shown that diene valepotriates isolated from *V. glechomifolia* (valtrate, acevaltrate, 1-*β*-acevaltrate, and 1-*β*-aceacevaltrate) inhibit Na^+/K^+-ATPase from the rat brain *in vitro* [17].

Na^+/K^+-ATPase is a membrane-bound enzyme made up of a catalytic *α* subunit and a regulatory *β* subunit [18]. Na^+/K^+-ATPase pumps maintain electrochemical gradients in neurons and astrocytes by the active exchange of intracellular Na^+ and extracellular K^+ and are necessary for neuronal excitability and regulation of neuronal cell volume [19]. Clinical studies have associated alterations in cerebral Na^+/K^+-ATPase activity with depression and other psychiatric disorders [20,21]. In this sense, some authors demonstrated that animal models of depression involving stress are related to decreased Na^+/K^+-ATPase activity in the brain of rodents [22–24], and that repeated treatment with antidepressants [20] and mood stabilizers [23] is able to restore the Na^+/K^+-ATPase activity in the hippocampus of rats subjected to experimental models of depression.

Three Na^+/K^+-ATPase *α* isoforms are abundant in the brain. Both neurons and glia express the ubiquitous α1 isoform; α2 is primarily expressed in glial cells and α3 is only neuronal [25,26]. Of note, mutations in Na^+/K^+-ATPase isoforms have been related to the etiology of some neurological disorders [27,28]. Familial hemiplegic migraine type II, an autosomal dominant disorder, is related to mutations in the ATP1A2 gene, encoding the α2 isoform of the enzyme [29]. Also, mutations in the ATP1A3 gene, which encodes the Na^+/K^+-ATPase α3 isoform, were identified as the primary cause of some neurological disorders, such as rapid-onset dystonia-parkinsonism, which is associated with decreased α3 isoform expression [30], and alternating hemiplegia of childhood, which is related to decreased Na^+/K^+-ATPase activity [31]. Recently, Demos and colleagues found that an allelic mutation of ATP1A3 is also related to CAPOS syndrome, a rare neurological disorder [32]. Interestingly, some clinical and preclinical studies have related alterations in the protein expression of the α3 isoform as well as in its activity with the neurobiology of psychiatric disorders, including depression [33,34].

Considering this background, the present study was undertaken with the aim of investigating the effects of acute and repeated administration of VAL on Na^+/K^+-ATPase activity and protein expression of the Na^+/K^+-ATPase α1, α2, and α3 isoforms in the cerebral cortex and hippocampus of mice submitted or not submitted to a forced swimming session. These brain regions are considered to be closely related to the neurobiology of depression [35,36].

Results

Table 1 depicts the diene valepotriates constitution of VAL, and the chemical structure of each compound is presented in **Fig. 1**. The valepotriate found in a larger quantity was valtrate, followed by acevaltrate, 1-*β*-acevaltrate, 1-*β*-aceacevaltrate, and isovaltrate.

The effects of acute and repeated administration of VAL and IMI in the FST are shown in **Fig. 2 A, C**, respectively. One-way ANOVA revealed a significant main effect of both schedules of treatment on the FST [acute treatment $F(2, 27) = 17.39$, $p < 0.001$; repeated treatment $F(2, 31) = 17.11$, $p < 0.001$]. Post hoc analysis indicated a significant decrease in mice immobility time caused by the acute or repeated administration of the positive control imipramine or VAL when compared to the vehicle-treated group. The immobility time of the vehicle-treated mice is in accordance to previous studies published elsewhere by our group [16,37,38] and others [39], which have used the same mouse strain.

The acute treatment with VAL (**Fig. 2 B**) did not affect mice locomotor activity assessed by the OFT [acute treatment – crossings: $t(18) = 0.1853$, $p = 0.8550$; rearings: $t(18) = 0.8519$, $p = 0.4055$; groomings: $t(18) = 1.061$, $p = 0.3029$]. On the other hand, repeated treatment with VAL decreased the number of rearings, but not crossings and groomings, in the OFT (**Fig. 2 D**) [repeated treatment – crossings: $t(18) = -0.886$, $p = 0.338$; rearings: $t(18) = -2.240$, $p < 0.05$; groomings: $t(18) = 0.136$, $p = 0.894$].

Fig. 3 depicts the activity of Na^+/K^+-ATPase in the hippocampus and cortex of mice acutely or repeatedly treated with VAL (or ve-

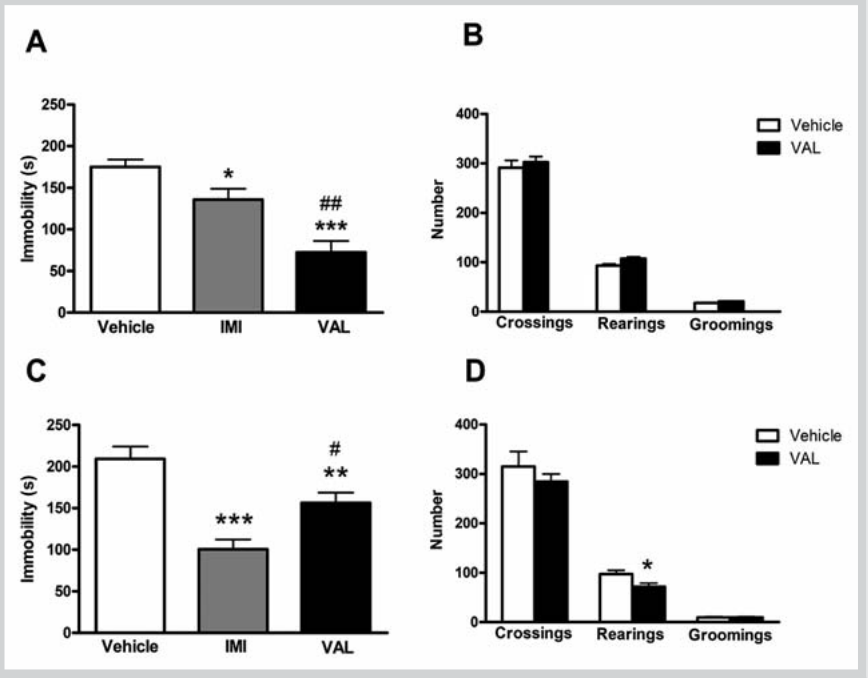

Fig. 2 Effects of acute and repeated administration of diene valepotriates from *V. glechomifolia* obtained by supercritical CO_2 extraction (diene valepotriates fraction) in the mice FST and OFT. The animals were acutely (5 mg/kg, p. o.) or repeatedly (5 mg/kg, p. o.; once a day, during three days) treated with VAL and submitted to the tests 1 h (acute treatment: **A** and **B**, for the FST and OFT, respectively) or 24 h after (repeated treatment: **C** and **D** for the FST and OFT, respectively) the administration. The antidepressant imipramine (IMI) was used as a positive control for the FST (acute treatment: 20 mg/kg, p. o.; repeated treatment 20 mg/kg, p. o., once a day, during 3 days). Each column represents the mean ± SEM. One-way ANOVA followed by Student-Newman-Keuls: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the respective vehicle-treated group; # $p < 0.05$, ## $p < 0.01$ when compared to the IMI-treated group.

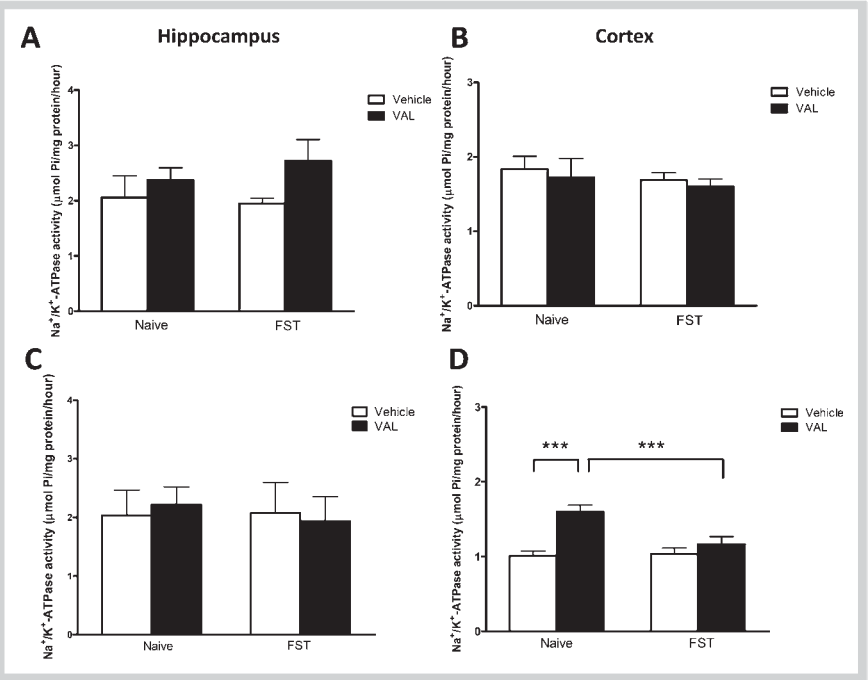

Fig. 3 Effects of acute and repeated administration of diene valepotriates from *V. glechomifolia* obtained by supercritical CO_2 extraction (diene valepotriates fraction) on Na^+/K^+-ATPase activity from the hippocampus (**A** and **C**) and cortex (**B** and **D**) of the mice. The animals were acutely (5 mg/kg, p. o.) (**A** and **B**) or repeatedly (5 mg/kg, p. o., once a day, during three days) (**C** and **D**) treated with VAL and submitted (or not) to the FST 1 h or 24 h after VAL administration. Each column represents the mean ± SEM. Two-way ANOVA followed by Student-Newman-Keuls; *** $p < 0.001$.

hicle) and submitted or not to the FST. Two-way ANOVA did not reveal any effect of VAL acute treatment, FST exposition, or VAL acute treatment x FST interaction, neither in the hippocampus (**Fig. 3A**) [$F_{VAL}(1,8) = 3.232$, $p = 0.110$; F_{FST} $(1,8) = 0.157$, $p = 0.703$; $F_{VALxFST}(1,8) = 0.550$, $p = 0.480$] nor in the cortex (**Fig. 3B**) [F_{VAL} $(1,44) = 0.326$, $p = 0.571$; $F_{FST}(1,44) = 0.610$, $p = 0.439$; $F_{FSTxVAL}(1,44) = 0.688$, $p = 0.934$] of mice. No alterations on Na^+/K^+-ATPase activity were detected in the hippocampus of mice repeatedly treated with VAL and submitted or not to the FST (**Fig. 3C**). Indeed, the two-way ANOVA did not reveal an effect of VAL repeated treatment [$F(1,20) = 0.00279$, $p = 0.958$], FST exposure [$F(1,20) = 0.0828$, $p = 0.777$], or a VAL x FST interaction [$F(1,20) = 0.140$, $p = 0.712$]. In contrast, the repeated administration of VAL significantly increased Na^+/K^+-ATPase activity in the cortex of naïve mice but not in mice repeatedly treated with VAL and submitted to the FST (**Fig. 3D**). Two-way ANOVA revealed a significant main effect of VAL repeated treatment [$F(1,44) = 17.317$; $p < 0.001$], FST exposure [$F(1,44) = 5.521$, $p < 0.05$], and a VAL x FST interaction [$F(1,44) = 7.065$, $p < 0.05$].

The protein expression of different Na^+/K^+-ATPase isoforms in the cortex of the experimental groups repeatedly treated with VAL (or vehicle) by gavage and submitted or not to the FST is shown in **Fig. 4**. With respect to the $\alpha 1$ isoform, two-way ANOVA revealed a global significant effect of VAL repeated treatment [*F*

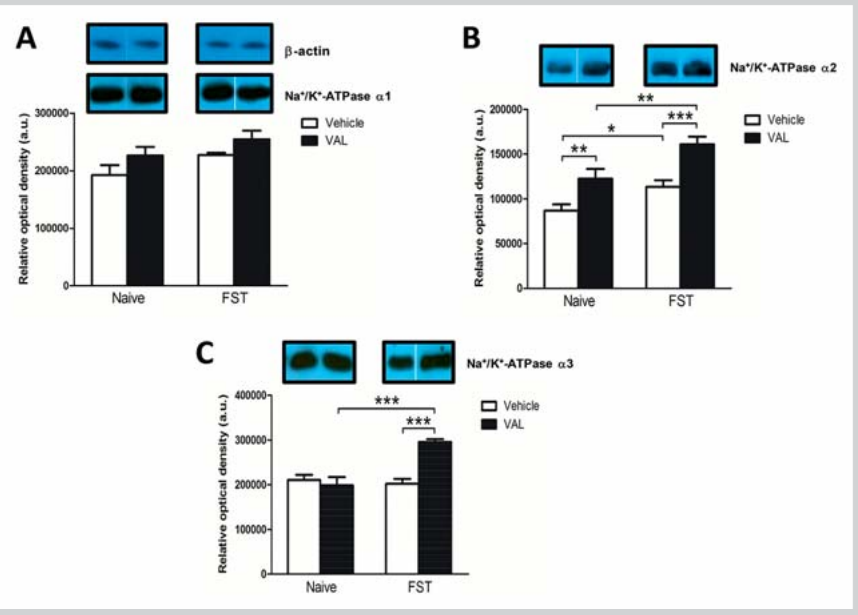

Fig. 4 Effects of repeated administration of diene valepotriates from *V. glechomifolia* obtained by supercritical CO_2 extraction (diene valepotriates fraction) on Na^+/K^+-ATPase $\alpha1$ (**A**), $\alpha2$ (**B**), and $\alpha3$ (**C**) isoform protein expression in the cortex of the mice. The animals were repeatedly (5 mg/kg, p.o., once a day, during three days) treated with VAL and submitted (or not) to the FST 24 h after VAL administration. Each column represents the mean ± SEM. of n animals. Representative Western blot signals of each group are shown. β-actin was used as a loading control. Two-way ANOVA followed by Student-Newman-Keuls; * $p < 0.05$; ** $p < 0.01$; ** $p < 0.001$. (Color figure available online only.)

(1,16) = 5.357, $p < 0.05$] and FST exposure [$F(1,16) = 5.088$, $p < 0.05$], but not a VAL x FST interaction [$F(1,16) = 0.0617$, $p = 0.807$] (**Fig. 4A**). The repeated treatment with VAL as well as the exposure to the FST significantly increased the content of Na^+/K^+-ATPase $\alpha2$ isoform in the cortex of mice (**Fig. 4B**). Two-way ANOVA revealed a significant main effect of VAL repeated treatment [$F(1,16) = 5.357$, $p < 0.05$] and FST exposure [$F(1,16) = 23.084$, $p < 0.001$], but no significant VAL x FST interaction [$F(1,16) = 0.466$, $p = 0.505$]. The content of the $\alpha3$ isoform was increased only in the cortex of mice both repeatedly treated with VAL and submitted to the FST (**Fig. 4C**). Two-way ANOVA revealed a significant main effect of VAL repeated treatment [$F(1,16) = 11.959$; $p < 0.05$], FST exposure [$F(1,16) = 10.077$; $p < 0.01$], and a VAL x FST interaction [$F(1,16) = 17.143$, $p < 0.001$].

Discussion

In the present study, we have replicated previous findings of our laboratory, which have shown that the diene valepotriates obtained from *V. glechomifolia* present an antidepressant-like effect [16] in the mouse FST, a test predictive of antidepressant activity [40]. This effect was not related to a nonspecific behavioral stimulation once the animals acutely treated with VAL did not present locomotor alterations in the OFT. These results are in accordance to the proposal by Porsolt and colleagues who postulated that a potential antidepressant must reduce immobility in the FST at doses that do not stimulate locomotion [41].

Most important, this study further supports the hypothesis that the enzyme Na^+/K^+-ATPase may constitute one of the valepotriates' molecular targets. In a previous work, we have demonstrated that diene valepotriates (valtrate, acevaltrate, and 1-β-acevaltrate) isolated from *V. glechomifolia* presented inhibitory activity towards rat cerebral Na^+/K^+-ATPase *in vitro* [17]. Herein, we show for the first time that VAL increases the activity of Na^+/K^+-ATPase and protein expression of the $\alpha2$ isoform in the cerebral cortex of mice after a repeated treatment. The effects of VAL on the global activity of the enzyme are similar to those found with fluoxetine by Zanatta and colleagues [42]. These authors showed that rats chronically treated with fluoxetine presented an increase in the cortical Na^+/K^+-ATPase global activity, which may contribute to its antidepressant effects, whereas the Na^+/K^+-ATPase activity *in vitro* was reduced by this drug [42]. Effects on cortical Na^+/K^+-ATPase could be particularly relevant, since this brain structure is involved in depression neurobiology [36] and *post mortem* studies have demonstrated alterations on Na^+/K^+-ATPase activity in the cortex of depressed patients [20]. On the other hand, none of the VAL treatment schedules altered the enzyme activity in the hippocampus. As Na^+/K^+-ATPase activity varies in different brain regions [43], these data suggest that VAL could cause region-specific changes in this enzyme activity.

The pathophysiology of depression has been associated with decreased Na^+/K^+-ATPase activity [44]. Moreover, clinical evidence is in agreement with observations in animals, since the submission of rats to the unpredictable chronic mild stress, an experimental model of depression [45], results in decreased Na^+/K^+-ATPase activity in the hippocampus [22,23]. What is noteworthy is that repeated treatment with fluoxetine [20] and lithium [23] prevents the inhibition of Na^+/K^+-ATPase activity in depressed animals. In the present study, the animals were not submitted to that experimental model of depression, but our results demonstrate that VAL *per se* was able to increase the cortical activity of the enzyme as well as the protein expression of the $\alpha2$ isoform. Thus, we suggest that these effects of VAL on Na^+/K^+-ATPase could contribute to its antidepressant-like properties.

The association of repeated VAL treatment with the FST exposure affected the total enzyme activity and the protein expression of Na^+/K^+-ATPase $\alpha1$, $\alpha2$, and $\alpha3$ isoforms in cerebral cortex differently. Repeated VAL administration to naive mice significantly increased cortical Na^+/K^+-ATPase activity and protein expression of the $\alpha2$ isoform. Surprisingly, the mice treated with the vehicle and who were submitted to the FST also presented an increase in the content of the $\alpha2$ isoform, but did not present any alteration on Na^+/K^+-ATPase activity. Furthermore, the mice treated with VAL and submitted to the FST displayed a higher content of the $\alpha2$ isoform when compared to naive mice treated with VAL. On the other hand, $\alpha3$ isoform protein expression increased only when the mice were submitted to both conditions: VAL treat-

ment and forced swimming. Of note, the content of the cortical α1 isoform was not affected in any experimental group. In fact, some authors have demonstrated that the housekeeping α1 isoform appears to be less sensitive to different types of insults than the other ones [46,47]. Altogether, these data strongly suggest that the FST affects the functioning of α2 and α3 Na+/K+-ATPase isoforms by itself, which in turn influences the effects of VAL on the expression and activity of this enzyme.

Goto and colleagues already suggested the influence of forced swimming on Na^+/K^+-ATPase activity [48]. These authors demonstrated that rats submitted to a short forced swimming session (10 min) presented increased corticosterone plasma levels and ouabain-like compound levels in plasma and adrenal, which had inhibitory effects towards Na^+/K^+-ATPase. In line with that, Viana and colleagues demonstrated, in experimental conditions quite similar to ours, that the acute swim stress (6 min) increases corticosterone levels, both in plasma and the cortex of mice [37]. Therefore, considering the regulatory role of corticosteroids on Na^+/K^+-ATPase activity [49], it is plausible that the corticosterone increase induced by FST counteracts, at least in part, the effects of VAL on the global activity of Na^+/K^+-ATPase.

In addition, we speculate that the acute swim stress may disrupt Na^+/K^+-ATPase isoforms, causing a loss of function of the enzymes, so that the increase in the protein expression could represent a compensatory upregulation of these isoforms and, therefore, the global activity of the enzyme would remain unchanged [50,51]. Jamme and colleagues reported a decrease in the cortical Na^+/K^+-ATPase activity 30 min after inducing focal cerebral ischemia in mice. Interestingly, they observed a tendency towards an increase of α2 and, mainly, α3 isoform mRNA expression in the same animals [52], and an augment of α protein expression can also be observed by Western blot [53]. The authors suggested that these changes could be due to adaptive responses to stress, which is in line with our proposition. Furthermore, studies performed with a non-mammalian species demonstrated that environmental stressors, such as salinity, pH, and temperature, stimulate Na^+/K^+-ATPase α [54] and β [55] subunits.

Mutations in Na^+/K^+-ATPase α1, α2, and α3 isoforms have been identified in the cortex of patients with bipolar depression, and these polymorphisms may involve a broad spectrum of changes in enzymatic activity, which remain not completely elucidated [21,56,57]. Kirshenbaum and colleagues demonstrated that Na^+/K^+-ATPase α3 heterozygous mice [Atp1a3(±)] have reduced neuronal Na^+/K^+-ATPase activity and are more vulnerable to present depression-like behavior when submitted to chronic variable stress compared to wild-type littermates [33]. The expression of the α3 isoform gene is decreased in the prefrontal cortex of patients with major and bipolar depression [34]. These data suggest that the Na^+/K^+-ATPase α3 isoform plays an important role in the susceptibility to the development of the depression-like endophenotypes. Therefore, this isoform may become a novel target for antidepressants and a putative new player in the pathophysiology of depression. From this perspective, our results about the combined effect of VAL plus FST on Na^+/K^+-ATPase α3 isoform protein expression are exciting and deserve further investigation.

The regulation of Na^+/K^+-ATPase activity is very complex and can be divided into short-term and long-term mechanisms, which are controlled by diverse factors, including hormones and neurotransmitters [49,58,59]. The long-term regulatory mechanisms involve alterations in protein synthesis and/or the degradation rate, and take some hours or days to occur [49]. Considering that the acute treatment of mice with VAL did not alter Na^+/K^+-ATPase neither in the cortex nor in the hippocampus of mice, we suggest that VAL might affect the long-term regulatory mechanisms of this enzyme and, therefore, only the repeated treatment is able to affect mice cortical Na^+/K^+-ATPase. In addition, we cannot rule out the hypothesis that the lack of an acute effect of VAL on Na^+/K^+-ATPase activity might be due to the need of the repetitive doses to reach the VAL steady state concentration associated with this specific activity. Noteworthy, the reduction in the immobility time of mice in the FST after the acute administration of VAL is probably not associated to an effect on Na^+/K^+-ATPase activity, since the acute administration of VAL did not alter this enzyme activity. In a previous study, we have demonstrated that the acute antidepressant-like effect of VAL in the mice FST is mediated by dopaminergic and noradrenergic neurotransmissions [16]. Thus, we may suggest that the acute anti-immobility effect of VAL observed in the present study may be associated to the action of VAL on the monoaminergic system.

In conclusion, the results discussed so far confirm the antidepressant-like potential of diene valepotriates from *V. glechomifolia*, which could represent a new chemical scaffold with antidepressant properties. These compounds increased cortical Na^+/K^+-ATPase activity, as well as the cortical protein expression of the α2 isoform, which may contribute to its antidepressant-like activity, since decreased Na^+/K^+-ATPase activity has been associated with depression. Also, the FST counteracted the effects of the diene valepotriates on cortical Na^+/K^+-ATPase activity and augmented α3 isoform protein expression. The mechanisms whereby the FST affects enzyme expression deserve further studies.

Materials and Methods

Chemical reagents

For the extract characterization, HPLC grade acetonitrile and methanol were purchased from Merck®. For the behavioral experiments, imipramine hydrochloride (purity > 99%) was purchased from Henrifarma®. Materials used in *ex vivo* experiments were ouabain (Sigma-Aldrich), rat polyclonal primary antibodies anti-Na^+/K^+-ATPase α1 and α2 (Merck Millipore), and mouse monoclonal antibody anti-Na^+/K^+-ATPase α3 (Santa Cruz Biotechnology). Anti-rat or anti-mouse peroxidase-conjugated antibodies were purchased from Santa Cruz Biotechnology. For the behavioral experiments, VAL was dissolved in normal saline (NaCl 0.9%) with an additional 1% polysorbate 80 (vehicle). All the solutions were freshly prepared on the day of the test and administered via the oral route (gavage) at 10 mL/kg.

Plant material

V. glechomifolia Meyer (Valerianaceae) was collected during its flowering stage in São José dos Ausentes, Rio Grande do Sul state, Brazil, in January 2012, and identified by Dr. M. Sobral (Universidade Federal de São João del-Rei, Minas Gerais, Brazil). A voucher specimen (Sobral, 7733) was deposited at the Herbarium of the Universidade Federal do Rio Grande do Sul (ICN). The plant material was freeze-dried, powdered, and kept frozen until analyzed. Plant collection was authorized by SISBIO-IBAMA, approval No. 29495–1.

Supercritical carbon dioxide extraction

The $SCCO_2$ extraction was conducted according to Müller and colleagues [16,60]. Powdered aerial and underground plant material (100 g dry weight) were used, and the extraction was per-

formed using Pilot equipment [61]. $SCCO_2$ flowed at a rate of 6.67×10^{-4} kg·s^{-1} through the extraction vessel, and the extraction was carried out at a constant temperature (40 °C) and pressure (90 bar). The $SCCO_2$ extraction yield was 2.96 g.

Determination of diene valepotriates content

Diene valepotriates characterization and quantification in the fraction were performed by HPLC analysis, using an isocratic mobile phase (acetonitrile: water; 50 : 50 v/v) and detection at 254 nm, as described elsewhere [15, 16, 60]. The experiments were performed in triplicate. All diene valepotriates were quantified in mg of valtrate equivalent/g extract.

Animals

Male CF1 mice (P35–P45; 25–30 g) were purchased from Fundação Estadual de Produção e Pesquisa em Saúde – RS (Brazil). The animals were housed in plastic cages (17 × 28 × 13 cm), five mice per cage, and kept under a 12-h light/dark cycle (lights on between 7 a.m. and 7 p.m.) at constant temperature of 23 ± 1 °C with free access to standard certified rodent diet and tap water. All behavioral experiments were approved by The Animal Care Local Ethical Committee (CEUA-UFRGS; project approval No. 22 648; approval date: May 3, 2012) and performed according to Brazilian law [62–64] and the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Forced swimming test

The FST was carried out according to Porsolt and colleagues [65] with minor modifications standardized and validated in our laboratory [66]. Mice were adapted to the laboratory conditions 1 h before being exposed to the FST. The animals were individually forced to swim in a cylinder pool (10 cm diameter, 13 cm high; water temperature 22 ± 1 °C), and the total duration of immobility during a 6 min test was scored (in seconds). Each mouse was recorded as immobile when it remained floating motionless or making only the movements necessary to keep its head above water. The immobility behavior was recorded by human observers blind to the treatment.

Treatments

Independent groups of mice (n = 8–12 per group) were acutely (5 mg/kg, p.o.) or repeatedly (5 mg/kg, p.o., once a day, during three days) treated with VAL by gavage. The antidepressant imipramine was used as a positive control for the acute (20 mg/kg, p.o.) and repeated treatment (20 mg/kg, p.o., once a day, during three days). The doses were chosen on the basis of previous results obtained by our research group [16]. Control groups were submitted to the same treatment schedules and orally treated with the vehicle. One h after the acute treatment or 24 h after the repeated one, mice were evaluated in the FST. Immediately after the test, mice treated with VAL were sacrificed by decapitation, and brain structures were removed and stored at − 80 °C until analysis. Separate groups of mice acutely or repeatedly treated with VAL but not submitted to the FST were also euthanized in order to perform enzymatic activity and Western blotting, as described in the next sections.

Open field test

The OFT was performed in order to evaluate the possible effects of VAL on the locomotor and exploratory activities. For this purpose, mice (n = 9–12) were submitted to acute and repeated treatments previously described before being observed in the open field. Animals were individually placed in the open field arena (acrylic box – 40 × 30 × 30 cm), with the floor divided into 24 equal squares. After habituation to the arena for 5 min, the number of squares crossed with the four paws (crossing) and rearing was recorded in a 15-min session by human observers blind to the treatment. The apparatus was cleaned with 10% ethanol after each mouse exposition.

Tissue preparation

Crude homogenates were performed as previously described, with a few adaptations [67]. The brain cortex (one for each preparation) and hippocampus (two or three pooled for each preparation) from the control and treated animals were homogenized in a 250 mM sucrose, 10 mM EGTA, 1 mM EDTA, 0.5 mM dithiothreitol, 0.1 mM PMSF, and 50 mM Tris–HCl buffer (pH 7.4) using a motorized Potter apparatus (Fisatom). Ultracentrifugation was carried out at 100 000 *g* for 60 min in an Optima XE-90 ultracentrifuge (Beckman Coulter). The pellet was resuspended in the same buffer without PMSF and stored at − 80 °C. Protein concentration was determined by the Lowry protein assay method.

Na^+/K^+-ATPase activity assay

ATPase activity was evaluated colorimetrically by measuring the amount of inorganic phosphate (Pi) released from ATP hydrolysis as reported [68]. The assay was performed at 37 °C for 1 h. The reaction was started by adding 10 µg protein of each preparation to a medium consisting of 84 mM NaCl, 3 mM $MgCl_2$, 3 mM $ATPNa_2$, 10 mM NaN_3, 1 mM EGTA, and 20 mM maleate-Tris buffer, pH 7.4, with 10 mM KCl (total activity) or 1 mM ouabain (ouabain-resistant activity). Specific Na^+/K^+-ATPase activity was the difference between total and ouabain-resistant ATPase activities.

Western blot

SDS-PAGE (7.5% gels) was used to run 10 µg of protein of each preparation for 1 h and then electrotransferred to nitrocellulose papers. After Ponceau red staining in order to check homogeneous protein transfer, membranes were incubated for 1 h in 5% dry skim milk dissolved in Tris–buffered saline solution containing 0.1% Tween 20 (TTBS), followed by 1 h at room temperature with rat polyclonal primary antibodies anti-Na^+/K^+-ATPase $\alpha 1$ and $\alpha 2$, and mouse monoclonal antibody anti-Na^+/K^+-ATPase $\alpha 3$. Membranes were further incubated for 1 h with anti-rat or anti-mouse peroxidase-conjugated antibodies [69]. Proteins were detected with SuperSignal West Pico chemiluminescent substrate (Thermo Scientific), and blots were analyzed using Image J software (NIH). β-actin protein levels were used as a loading control. The same membrane was stripped and reprobed with successive anti-Na^+/K^+-ATPase α antibodies to ensure equal conditions.

Statistical analysis

Results obtained in the OFT were analyzed by Student's t test. Data from the FST and the biochemical and Western blot assays were analyzed by one- or two-way ANOVA followed by Student-Newman-Keuls test when appropriate. The level for statistical significance was set as $p < 0.05$. The statistical procedures were performed using Sigma Stat software 2.03 (Jandel Scientific Corporation).

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Conflict of Interest

All authors declare that there are no conflicts of interest.

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References

1 *Backlund A, Moritz T.* Phylogenetic implications of an expanded valepotriate distribution in the Valerianaceae. Biochem System Ecol 1998; 26: 309–335
2 *Hatesohl M, Feistel B, Sievers H, Lehnfeld R, Hegger M, Winterhoff H.* Extracts of *Valeriana officinalis* L. show anxiolitic and antidepressant effects but neither sedative nor myorelaxant properties. Phytomedicine 2008; 15: 2–15
3 *Murphy K, Kubin ZJ, Shepherd JN, Ettinger RH. Valeriana officinalis* root extracts have potent anxiolytic effects in laboratory rats. Phytomedicine 2010; 17: 674–678
4 *Subhan F, Karim N, Gilani AH, Sewell RDE.* Terpenoid content of *Valeriana wallichii* extracts and antidepressant-like response profiles. Phytother Res 2010; 24: 686–691
5 *Sah SP, Mathela CS, Chopra K.* Antidepressant effect of *Valeriana wallichii* patchouli alcohol chemotype in mice: behavioural and biochemical evidence. J Ethnopharmacol 2011; 135: 197–200
6 *World Health Organization.* WHO Monographs on selected medicinal Plants. Malta: WHO Graphics; 1999
7 *Holzmann I, Filho VC, Mora TC, Cáceres A, Martínez JV, Cruz SM, Souza MM.* Evaluation of behavioral and pharmacological effects of hydroalcoholic extract of *Valeriana prionophylla Standl.* from Guatemala. Evid Based Complement Alternat Med 2011; 2011: 1–9
8 *Liu XG, Gao PY, Wang GS, Song SJ, Li LZ, Li X, Yao XS, Zhang ZX. In vivo* antidepressant activity of sesquiterpenes from the roots of *Valeriana fauriei* Briq. Fitoterapia 2012; 83: 599–603
9 *Benke D, Barberis A, Kopp S, Altmann KH, Schubiger M, Vogt KE, Rudolph U, Mohler H.* $GABA_A$ receptors as *in vivo* substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts. Neuropharmacology 2009; 56: 174–181
10 *Marder M, Viola H, Wasowski C, Fernández S, Medina JH, Paladini AC.* 6-Methylapigenin and hesperidin: new *Valeriana* flavonoids with activity on the CNS. Pharmacol Biochem Behav 2003; 75: 537–545
11 *Andreatini R, Leite JR.* Effect of valepotriates on the behavior of rats in the elevated plus-maze during diazepam withdrawal. Eur J Pharmacol 1994; 1: 233–235
12 *Andreatini R, Sartori VA, Seabra ML, Leite JR.* Effect of valepotriates (valerian extract) in generalized anxiety disorder: a randomized placebo-controlled pilot study. Phytoter Res 2002; 165: 650–654
13 *Maurmann N, Reolon GK, Rech SB, Fett-Neto AG, Roesler R.* A valepotriate fraction of *Valeriana glechomifolia* shows sedative and anxiolytic properties and impairs recognition but not aversive memory in mice. Evid Based Complement Alternat Med 2011; 2011: 1–7
14 *Salles LA, Silva AL, Rech SB, Zanatta N, Von Poser GL.* Constituents of *Valeriana glechomifolia* Meyer. Biochem Syst Ecol 2000; 28: 907–910
15 *Silva AL, Rech SB, von Poser GL.* Quantitative determination of valepotriates from *Valeriana* native to south Brazil. Planta Med 2002; 68: 570–572
16 *Müller LG, Salles LA, Stein AC, Betti AH, Sakamoto S, Cassel E, Vargas RF, von Poser GL, Rates SMK.* Antidepressant-like effect of *Valeriana glechomifolia* Meyer (Valerianaceae) in mice. Prog Neuropsychopharmacol Biol Psychiatry 2012; 36: 101–109
17 *Bettero GM, Salles L, Rosário RMF, von Poser GL, Rates SMK, Noël F, Quintas LEM. In vitro* effect of valepotriates isolated from *Valeriana glechomifolia* on rat P-type ATPases. Planta Med 2011; 77: 1702–1706
18 *Kaplan JH.* Biochemistry of Na^+,K^+-ATPase. Annu Rev Biochem 2002; 71: 511–535
19 *Dobretsov M, Stimers JR.* Neuronal function and α3 isoform of the Na^+/K^+-ATPase. Front Biosci 2005; 10: 2373–2396
20 *Goldstein I, Levy T, Galili D, Ovadia H, Yirmiya R, Rosem H, Lichtstein D.* Involvement of Na^+,K^+-ATPase and endogenous digitalis-like compounds in depressive disorders. Biol Psychiatry 2006; 60: 491–499
21 *Goldstein I, Lerer E, Laiba E, Mallet J, Mujaheed M, Laurent C, Rosen H, Ebstein RP, Lichtstein D.* Association between sodium- and potassium-activated adenosine triphosphatase α isoforms and bipolar disorders. Biol Psychiatry 2009; 65: 985–991
22 *Gamaro GD, Streck EL, Matté C, Prediger ME, Wyse ATS, Dalmaz C.* Reduction of hippocampal Na^+,K^+-ATPase activity in rats subjected to an experimental model of depression. Neurochem Res 2003; 28: 1339–1344
23 *Vasconcellos APS, Zugno AI, Santos AHDP, Nietto FB, Crema LM, Gonçalves M, Franzon R, Wyse ATS, Rocha ER, Dalmaz C.* Na^+,K^+-ATPase activity is reduced in hippocampus of rats submitted to an experimental model of depression: effect of chronic lithium treatment and possible involvement in learning deficits. Neurobiol Learn Mem 2005; 84: 102–110
24 *Crema L, Schlabitz M, Tagliari B, Cunha A, Simão F, Krolow R, Pettenuzzo L, Salbego C, Vendite D, Wyse AT, Dalmaz C.* Na^+,K^+-ATPase activity isreduced in amygdala of rats with chronic stress-induced anxiety-like behavior. Neurochem Res 2010; 35: 1787–1795
25 *Mcgrail KM, Phillips JM, Sweadner KJ.* Immunofluorescent localization of three Na^+,K^+ ATPase isozymes in the rat central nervous system: both neurons and glia can express more than one Na^+,K^+ ATPase. J Neurosci 1991; 11: 381–391
26 *Moseley AE, Lieske SP, Wetzel RK, James PF, He S, Shelly DA, Paul RJ, Boivin GP, Witte DP, Ramirez JM, Sweadner KJ, Lingrel JB.* The Na^+,K^+ ATPase α2 isoform is expressed in neurons, and its absence disrupts neuronal activity in newborn mice. J Biol Chem 2003; 278: 5317–5324
27 *Gritz SM, Radcliffe RA.* Genetic effects of ATP1A2 in familial hemiplegic migraine type II and animal models. Hum Genomics 2013; 7: 8–14
28 *Heinzen EL, Arzimanoglou A, Brashear A, Clapcote SJ, Gurrieri F, Goldstein DB, Jóhannesson SH, Mikati MA, Neville B, Nicole S, Ozelius LJ, Poulsen H, Schyns T, Sweadner KJ, van den Maagdenberg A, Vilsen B.* Distinct neurological disorders with ATP1A3 mutations. Lancet Neurol 2014; 13: 503–514
29 *De Fusco M, Marconi R, Silvestri L, Atorino L, Rampoldi L, Morgante L, Ballabio A, Aridon P, Casari G.* Haploinsufficiency of ATP1A2 encoding the Na/K pump 2 subunit associated with familial hemiplegic migraine type 2. Nat Genet 2003; 33: 192–196
30 *De Carvalho Aguiar P, Sweadner KJ, Penniston JT, Zaremba J, Liu L, Caton M, Linazasoro G, Borg M, Tijssen MA, Bressman SB, Dobyns WB, Brashear A, Ozelius LJ.* Mutations in the Na^+/K^+-ATPase α3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. Neuron 2004; 43: 169–175
31 *Heinzen EL, Swoboda KJ, Hitomi Y, Gurrieri F, Nicole S, de Vries B, Tiziano FD, Fontaine B, Walley NM, Heavin S, Panagiotakaki E, Fiori S, Abiusi E, Di Pietro L, Sweney MT, Newcomb TM, Viollet L, Huff C, Jorde LB, Reyna SP, Murphy KJ, Shianna KV, Gumbs CE, Little L, Silver K, Ptáček LJ, Haan J, Ferrari MD, Bye AM, Herkes GK, Whitelaw CM, Webb D, Lynch BJ, Uldall P, King MD, Scheffer IE, Neri G, Arzimanoglou A, van den Maagdenberg AM, Sisodiya SM, Mikati MA, Goldstein DB.* De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. Nat Genet 2012; 44: 1030–1034
32 *Demos MK, van Karnebeek CD, Ross CJ, Adam S, Shen Y, Zhan SH, Shyr C, Horvath G, Suri M, Fryer A, Jones SJ, Friedman JM.* A novel recurrent mutation in ATP1A3 causes CAPOS syndrome. Orphanet J Rar Dis 2014; 9: 15–23
33 *Kirshenbaum GS, Saltzman K, Rose B, Petersen J, Vilsen B, Roder JC.* Decreased neuronal Na^+,K^+-ATPase activity in ATP1A3 heterozygous mice increases susceptibility to depression-like endophenotypes by chronic variable stress. Genes Brain Behav 2011; 10: 542–550
34 *Tochigi M, Iwamoto K, Bundo M, Sasaki T, Kato N, Kato T.* Gene expression profiling of major depression and suicide in the prefrontal cortex of postmortem brains. Neurosci Res 2008; 60: 184–191
35 *Berton O, Nestler EJ.* New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 2006; 7: 137–141

36 *Price JL, Drevets WC.* Neurocircuitry of mood disorders. Neuropsychopharmacology 2010; 35: 192–216
37 *Viana AF, Rates SMK, Naudin B, Janin F, Costentin J, do Rego JC.* Effects of acute or 3-day treatments of *Hypericum caprifoliatum* Cham. & Schltdt. (Guttifereae) extract of two established antidepressants on basal and stress induced increase in serum and brain corticosterone levels. J Psychopharmacol 2008; 22: 681–690
38 *Stein AC, Viana AF, Müller LG, Nunes JM, Stolz ED, Do Rego JC, Costentin J, von Poser GL, Rates SM.* Uliginosin B, a phloroglucinol derivative from *Hypericum polyanthemum*: a promising new molecular pattern for the development of antidepressant drugs. Behav Brain Res 2012; 228: 66–73
39 *Erhardt E, Zibetti LC, Godinho JM, Bacchieri B, Barros HM.* Behavioral changes induced by cocaine in mice are modified by a hyperlipidic diet or recombinant leptin. Braz J Med Biol Res 2006; 39: 1625–1635
40 *Petit-Demouliere B, Chenu F, Bourin M.* Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology 2005; 177: 245–255
41 *Porsolt RD, Anton G, Blavet N, Jafre M.* Behavioral despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978; 47: 379–391
42 *Zanatta LM, Nascimento FC, Barros SV, Silva GR, Zugno AI, Netto CA, Wyse ATS. In vivo* and *in vitro* effect of imipramine and fluoxetine on Na,K-ATPase activity in synaptic plasma membranes from the cerebral cortex of rats. Braz J Med Biol Res 2001; 34: 1265–1269
43 *Yallapragada PR, Butler J, Kumar BK, Rajanna B. In vitro* effect of lead on Na^+,K^+-ATPase activity in different regions of adult rat brain. Drug Chem Toxicol 2003; 26: 117–124
44 *El-Mallakh RS, Wyatt RJ.* The Na^+K^+ ATPase hypothesis for bipolar illness. Biol Psychiatry 1995; 37: 235–244
45 *Willner P.* Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 2005; 52: 90–110
46 *Azuma KK, Hensley CB, Putnam DS, Mcdonough AA.* Hypokalemia decreases Na^+-K^+-ATPase α2- but not α1-isoform abundance in heart, muscle, and brain. Am J Physiol 1991; 260: C958–C964
47 *Ostadal P, Elmoselhi AB, Zdobnicka I, Lukas A, Elimban V, Dhalla NS.* Role of oxidative stress in ischemia-reperfusion-induced changes in Na^+, K^+-ATPase isoform expression in rat heart. Antioxid Redox Signal 2004; 6: 914–923
48 *Goto A, Yamada K, Nagoshi H, Terano Y, Omata M.* Stress-induced elevation of ouabain-like compound in rat plasma and adrenal. Hypertension 1995; 26: 1173–1176
49 *Therien AG, Blostein R.* Mechanisms of sodium pump regulation. Am J Physiol Cell Physiol 2000; 279: 541–566
50 *Bersohn MM.* Sodium pump inhibition in sarcolemma from ischemic hearts. J Mol Cell Cardiol 1995; 27: 1483–1489
51 *Golden WC, Brambrink AM, Traystman RJ, Martin LJ.* Failure to sustain recovery of Na,K-ATPase function is a possible mechanism for striatal neurodegeneration in hypoxic-ischemic newborn piglets. Brain Res Mol Brain Res 2001; 88: 94–102
52 *Jamme I, Trouvé P, Maixent JM, Gerbi A, Charlemagne D, Nouvelot A.* Regulation of Na+,K(+)-ATPase alpha subunit isoforms in mouse cortex during focal ischemia. Ann N Y Acad Sci 1997; 834: 658–660
53 *Jamme I, Petit E, Gerbi A, Maixent JM, MacKenzie ET, Nouvelot A.* Changes in ouabain affinity of Na^+, K^+-ATPase during focal cerebral ischaemia in the mouse. Brain Res 1997; 774: 123–130
54 *Sun H, Zhang L, Ren C, Chen C, Fan S, Xia JJ, Lin H, Hu C.* The expression of Na, K-ATPase in *Litopenaeus vannamei* under salinity stress. Mar Biol Res 2011; 7: 623–628
55 *Wang L, Wang WN, Liu Y, Cai DX, Li JZ, Wang AL.* Two types of ATPases from the Pacific white shrimp, *Litopenaeus vannamei* in response to environmental stress. Mol Biol Rep 2012; 39: 6427–6438
56 *Mynett-Johnson L, Murphy V, Mccormack J, Shields DC, Claffey E, Manley P, Mckeon P.* Evidence for an allelic association between bipolar disorder and a Na^+, K^+ adenosine triphosphatase α subunit gene (ATP1A3). Biol Psychiatry 1998; 44: 47–51
57 *Rose AM, Mellett BJ, Valdes R jr., Kleinman JE, Herman MM, Li R, El-Mallakh RS.* Alpha 2 isoform of the Na^+,K^+-adenosine triphosphatase is reduced in temporal cortex of bipolar individuals. Biol Psychiatry 1998; 44: 892–897
58 *Farman N, Bonvalet JP, Seckl JR.* Aldosterone selectively increases Na^+-K^+-ATPase α3-subunit mRNA expression in rat hippocampus. Am J Physiol 1994; 266: C423–C428
59 *Ewart HS, Klip A.* Hormonal regulation of the Na^+-K^+-ATPase: mechanisms underlying rapid and sustained changes in pump activity. Am J Physiol 1995; 269: C295–C311
60 *Müller LG, Salles LA, Sakamoto S, Stein AC, Cargnin ST, Cassel E, Vargas RF, Rates SMK, von Poser GL.* Effect of storage time and conditions on the diene valepotriates content of the extract of *Valeriana glechomifolia* obtained by supercritical carbon dioxide. Phytochem Anal 2012; 23: 222–227
61 *Cassel E, Vargas RMF, Brun GW, Almeida DE, Cogoi L, Ferraro G, Filip R.* Supercritical fluid extraction of alkaloids from *Ilex paraguariensis* St. Hill. J Food Eng 2010; 100: 656–661
62 *Brazil, Ministério Público.* Lei nº 11.794, de 8 de outubro de 2008, Brasília – DF, Brazil
63 *Brazil, Ministério da Ciência, Tecnologia e Inovação, CONCEA.* Diretriz brasileira para o cuidado e a utilização de animais para fins científicos e didáticos – DBCA. Portaria nº 465, de 23 de maio de 2013, Brasília – DF, Brazil
64 *Brazil, Ministério da Ciência, Tecnologia e Inovação, CONCEA.* Diretrizes da prática de eutanásia do CONCEA. Portaria n. 596, de 25 de junho de 2013, Brasília – DF, Brazil
65 *Porsolt RD, Bertin A, Jalfre M.* Behavioural despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977; 229: 327–336
66 *Viana AF, Do Rego JC, von Poser GL, Ferraz A, Heckler AP, Constantin J, Rates SMK.* The antidepressant-like effect of *Hypericum caprifoliatum* Cham & Schlecht (Guttiferae) on forced swimming test results from an inhibition of neuronal monoamine uptake. Neuropharmacology 2005; 49: 1042–1052
67 *Rezende DC, Pôças ES, Muzi-Filho H, Cunha VM, Caricati-Neto A, Jurkiewicz A, Noël F, Quintas LEM.* Mechanisms associated to impaired activity of cardiac P-type ATPases in endothelial nitric oxide synthase knockout mice. J Physiol Biochem 2013; 69: 207–214
68 *Schaffazick N, Amaral LS, Fonseca TF, Tomaz MA, Gaban GA, Borges PA, Calil-Elias S, Noël F, Melo PA, Quintas LEM, Cunha VMN.* Effect of heparin treatment on the expression and activity of different ion-motive P-type ATPase isoforms from mouse *extensor digitorum longus* muscle during degeneration and regeneration after *Bothrops jararacussu* venom injection. Toxicon 2010; 55: 52–60
69 *Quintas LEM, Noël F, Wibo M.* Na^+/K^+-ATPase α isoforms expression in stroke-prone spontaneously hypertensive rat heart ventricles: effect of salt loading and lacidipine treatment. Eur J Pharmacol 2007; 565: 151–157