Evaluation of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity in the brain of young rats after acute administration of fenproporex

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Objectives: Fenproporex is an amphetamine-based anorectic which is rapidly converted into amphetamine in vivo. Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is a membrane-bound enzyme necessary to maintain neuronal excitability. Considering that the effects of fenproporex on brain metabolism are poorly known and that Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is essential for normal brain function, this study sought to evaluate the effect of this drug on Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity in the hippocampus, hypothalamus, prefrontal cortex, and striatum of young rats.

Methods: Young male Wistar rats received a single injection of fenproporex (6.25, 12.5, or 25 mg/kg intraperitoneally) or polysorbate 80 (control group). Two hours after the last injection, the rats were killed by decapitation and the brain was removed for evaluation of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity.

Results: Fenproporex decreased Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity in the striatum of young rats at doses of 6.25, 12.5, and 25 mg/kg and increased enzyme activity in the hypothalamus at the same doses. Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity was not affected in the hippocampus or prefrontal cortex.

Conclusion: Fenproporex administration decreased Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity in the striatum even in low doses. However, in the hypothalamus, Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity was increased. Changes in this enzyme might be the result of the effects of fenproporex on neuronal excitability.

Keywords: Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity; brain; fenproporex

Introduction

Fenproporex is the second most commonly consumed amphetamine-based anorectic worldwide\textsuperscript{1} and acts by releasing or blocking neuronal reuptake of norepinephrine and dopamine.\textsuperscript{2,3} This leads to some adverse effects similar to those of amphetamine (AMPH), such as behavioral alterations, addiction, and withdrawal syndrome.\textsuperscript{4} Fenproporex is rapidly converted in vivo into AMPH. Consumption of a low daily dose of 10 mg of fenproporex led to the detection of AMPH in urine within 3 hours, with peak urinary levels greater than 4.0 ng/mL.\textsuperscript{5} Previous studies have demonstrated that AMPH induces neurotoxicity by producing free radicals\textsuperscript{6,7} and activating the mitochondrial apoptotic pathway through cytochrome c release,\textsuperscript{8,9} accompanied by a decrease in mitochondrial potential.\textsuperscript{10}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Schematic representation of the experimental design.}
\end{figure}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
Drug & Concentration (mg/kg) \\
\hline
Fenproporex & 6.25, 12.5, 25 \\
\hline
Polysorbate 80 & Control \\
\hline
\end{tabular}
\caption{Drug concentrations used in the study.}
\end{table}

Na\textsuperscript{+}, K\textsuperscript{+}-ATPase (E.C 3.6.1.37) is an ion pump that maintains sodium and potassium gradients across the basolateral plasma membrane in all animal cells.\textsuperscript{11} Following the description of different Na\textsuperscript{+}, K\textsuperscript{+}-ATPase isoenzymes, three isoforms of the alpha (catalytic) Na\textsuperscript{+}, K\textsuperscript{+}-ATPase subunit have been disclosed in the central nervous system (CNS): α1, α2, and α3.\textsuperscript{12} Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is an enzyme concentrated at nerve ending membranes, responsible for generating and maintaining the ionic gradient necessary for neuronal excitability, consuming about 40-50% of the ATP produced in the brain.\textsuperscript{12,13} Several studies have shown that Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity is altered in various disorders affecting the brain, such as neurodegenerative diseases,\textsuperscript{14} neuropsychiatric disorders,\textsuperscript{15,16} and animal models of depression and mania.\textsuperscript{17,18}

A relationship between Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity and neurotransmitter release has been demonstrated, suggesting that this enzyme could play a role in neurotransmission modulation.\textsuperscript{19,20} Therefore, considering that Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity is critical for normal brain function and that few studies have evaluated the possible neurotoxic effect of fenproporex in the CNS, we investigated the effect of acute administration of fenproporex on...
Na⁺, K⁺-ATPase activity in the hippocampus, hypothalamus, prefrontal cortex, and striatum – cerebral structures known to involved in fenproporex action – in young rats.

Material and methods

Animals

Young male Wistar rats (100-150 g) were obtained from the Central Animal House of Universidade do Extremo Sul Catarinense (UNESC), Criciúma, state of Santa Catarina, Brazil. The animals were caged in groups of five with free access to food and water, maintained on a 12-hour light-dark cycle (lights on at 7:00 a.m.), at a temperature of 23±1 °C. All experimental procedures were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication no. 80-23, revised 1996) and the Brazilian Society for Neuroscience and Behavior recommendations for animal care, with the approval of the UNESCO Ethics Committee. Moreover, all efforts were made to minimize animal suffering and to use the smallest possible number of animals.

Acute administration of drugs

Animals (n=6) received a single intraperitoneal injection of fenproporex (6.25, 12.5, or 25 mg/kg in 2% polysorbate 80) at a volume of 1.0 mL/kg. Control animals received only vehicle (2% polysorbate 80, 1.0 mL/kg). The range of doses of fenproporex employed in this study was chosen on the basis of previous research conducted with a dose of 12.5 mg/kg. We chose to use half this dose and double this dose as cutoff points for the dose-response curve. Two hours after the injection, the rats were killed by decapitation. The administration schedule was chosen on the basis of experiments previously performed.

Tissue preparation

Hippocampus, hypothalamus, prefrontal cortex and striatum specimens were homogenized in 10 volumes (1:10, w/v) of 0.32 mM sucrose solution containing 5.0 mM HEPES and 1.0 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5. The homogenates were centrifuged at 1000 x g for 10 min, and the resulting supernatants used for Na⁺, K⁺-ATPase activity determination.

Na⁺, K⁺-ATPase activity assay

The reaction mixture for Na⁺, K⁺-ATPase assay contained 5.0 mM MgCl₂, 80.0 mM NaCl, 20.0 mM KCl, and 40.0 mM Tris-HCl, pH 7.4, in a final volume of 200 μL. After 10 min of pre-incubation at 37 °C, the reaction was initiated by addition of ATP to a final concentration of 3.0 mM, and was incubated for 20 min. Controls were carried out under the same conditions with the addition of 1.0 mM ouabain. Na⁺, K⁺-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured as described by Chan et al. Specific activity of the enzyme was expressed as nmol of Pi released per min per mg of protein.

Protein determination

Protein was measured as described by Bradford, using bovine serum albumin as standard.

Statistical analysis

Normally distributed data were analyzed with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Thus, data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test when F was significant and are expressed as mean ± standard deviation. All analyses were performed using SPSS version 17.0.

Results

In the present study, we evaluated the effects of acute administration of fenproporex on Na⁺, K⁺-ATPase activity in some brain regions (hippocampus, hypothalamus, prefrontal cortex, and striatum) of young rats. Our findings demonstrated that acute administration of fenproporex decreased Na⁺, K⁺-ATPase activity in the striatum at doses of 6.25, 12.5, and 25 mg/kg (F = 13.00, p < 0.05). Enzyme activity was increased in the hypothalamus at the same doses (F = 9.31, p < 0.05). However, no alterations were observed in Na⁺, K⁺-ATPase activity in the hippocampus or prefrontal cortex after acute administration of fenproporex (Figure 1).

Discussion

Na⁺, K⁺-ATPase is a protein responsible for maintaining resting cell membrane potential by pumping sodium and potassium against the electrochemical gradient across cell membrane for these ions. This ubiquitous protein is particularly abundant in brain areas such as the striatum, in which it plays a fundamental role in maintaining cellular ionic gradients that are required for neural activity, transport of amino acids and glucose, and regulation of cell pH and volume. Given its important role in regulating neural excitability, impairment of Na⁺, K⁺-ATPase activity might lead to neural dysfunction. Fenproporex is an amphetamine-derived anorectic used as an appetite suppressor which acts by increasing dopamine and norepinephrine levels in the synaptic cleft. It has been shown that catecholamines can modulate Na⁺, K⁺-ATPase activity. The stimulation of Na⁺, K⁺-ATPase activity by norepinephrine has been observed in CNS homogenates, synaptic membranes, and synaptosomes. Indeed, the relationship between Na⁺, K⁺-ATPase activity and neurotransmitter release works as a modulatory mechanism of neurotransmission. We demonstrated that acute administration of fenproporex is able to increase Na⁺, K⁺-ATPase activity in the hypothalamus even at low doses. In agreement, a study with desipramine, which inhibits norepinephrine uptake and possesses antidepressive action, showed an increase in Na⁺, K⁺-ATPase activity in the hypothalamus and mesencephalon of rats.
In this context, Mallick et al. suggested that norepinephrine stimulated \( \text{Na}^+\),\( \text{K}^+\)-ATPase activity in the rat brain through the \( \alpha 1 \)A adrenoceptor, by dephosphorylation of the enzyme. Considering that the expression of \( \alpha 1 \)A adrenoceptor mRNA was found to be widespread within the hypothalamus, we hypothesized that increased \( \text{Na}^+\),\( \text{K}^+\)-ATPase activity in the hypothalamus after administration of fenproporex may occur by modulation of the \( \alpha 1 \)A adrenoceptor.

Furthermore, measurement of the regional distribution of \([\text{H}]^+\)(-)-amphetamine binding sites in the brain revealed that the brainstem has the highest density of specific binding sites, followed by the hypothalamus and striatum. The induction of hypothalamic \([\text{H}]^+\)(-)-amphetamine binding sites is directly correlated with changes in neuronal \( \text{Na}^+\), \( \text{K}^+\)-ATPase, indirectly suggesting that AMPH (and related anorectic compounds) may influence energy utilization by stimulating \( \text{Na}^+\), \( \text{K}^+\)-ATPase in appetite regulation centers of the hypothalamus and/or brainstem. Indeed, both AMPH and fenfluramine selectively stimulate \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity in the hypothalamus.

On the other hand, administration of fenproporex decreased \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity in the striatum. Wistar rats that receive an acute dose of fencafmamine (FCF) – a CNS stimulant, which has the same pharmacological profile as amphetamine and cocaine – exhibit a strong reduction in \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity in the striatum and nucleus accumbens. FCF has both inhibitory action on dopamine uptake and facilitating action on dopamine release in the dopaminergic system. This observation corroborates evidence that the inhibitory action of dopamine on \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity is mediated by cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) in the striatum. Previous studies reported that dopamine is an important neuromodulator in the striatum and inhibits \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity. Although the exact mechanism through which fenproporex causes a decrease in \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity is still unknown, evidence from the literature showed that the structural properties and lipid composition of the synaptosomal membrane are essential for enzyme activity, and that even low concentrations of free radicals inhibit \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity in the brain. It has also been observed that \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity is decreased by lipid peroxidation and that the thiol (-SH) groups of cell proteins are highly susceptible to oxidative stress. In this scenario, considering that dopamine autoxidation can form reactive quinones that attack and potentially inhibit the function of intracellular proteins, we speculate that oxidative stress is a possible candidate for the inhibition of \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity, possibly by oxidation of essential thiol groups in the structure of the enzyme that are necessary to its function.

In summary, the present study demonstrated that fenproporex administration decreased \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity in the rat striatum even in low doses; however, there was increased \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity in the hypothalamus. The mechanisms by which fenproporex administration alters \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity were not clear. We suggest that these changes might be the result of the effects of fenproporex on neurotransmitters (norepinephrine and dopamine) in the brain and on neuronal excitability. Whereas the increase of norepinephrine in the synaptic cleft stimulated \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity in the hypothalamus through the \( \alpha 1 \)A adrenoceptor, the decrease in \( \text{Na}^+\), \( \text{K}^+\)-ATPase activity in striatum...
can be explain by the inhibitory action of dopamine especially in this area. Further investigations to elucidate the mechanisms underlying the effect of fenproporex on Na⁺,-K⁺-ATPase are warranted. Chronic administration of fenproporex may reveal more about the effects of this drug on the CNS.

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Disclosure
The authors report no conflicts of interest.

References