Brazilian Journal of Medical and Biological Research (2001) 34: 1265-1269
ISSN 0100-879X Short Communication

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

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Abstract

The effects of *in vivo* chronic treatment and *in vitro* addition of imipramine, a tricyclic antidepressant, or fluoxetine, a selective serotonin reuptake inhibitor, on the cortical membrane-bound Na^+,K^+-ATPase activity were studied. Adult Wistar rats received daily intraperitoneal injections of 10 mg/kg of imipramine or fluoxetine for 14 days. Twelve hours after the last injection rats were decapitated and synaptic plasma membranes (SPM) from cerebral cortex were prepared to determine Na^+,K^+-ATPase activity. There was a significant decrease (10%) in enzyme activity after imipramine but fluoxetine treatment caused a significant increase (27%) in Na^+,K^+-ATPase activity compared to control ($P<0.05$, ANOVA; $N = 7$ for each group). When assayed *in vitro*, the addition of both drugs to SPM of naive rats caused a dose-dependent decrease in enzyme activity, with the maximal inhibition (60-80%) occurring at 0.5 mM. We suggest that a) imipramine might decrease Na^+,K^+-ATPase activity by altering membrane fluidity, as previously proposed, and b) stimulation of this enzyme might contribute to the therapeutic efficacy of fluoxetine, since brain Na^+,K^+-ATPase activity is decreased in bipolar patients.

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Research supported by CNPq, FAPERGS and PRONEX II-FINEP.

Received September 19, 2000
Accepted July 16, 2001

Key words
- Imipramine
- Fluoxetine
- Na^+,K^+-ATPase
- Cerebral cortex

Na^+,K^+-ATPase is the enzyme responsible for the active transport of sodium and potassium ions in the nervous system, maintaining the ionic gradient necessary for neuronal excitability and regulation of neuronal cell volume. It is present in high concentrations in brain cellular membranes, consuming about 40-50% of the ATP generated in this tissue (1) and its activity is decreased in patients with bipolar affective disorder and other psychiatric disorders (2).

Serotonin (5-HT) and noradrenaline, which have been implicated in the pathophysiology of affective disorders (3), are removed from the synaptic cleft by sodium-dependent uptake through transporters located in the plasma membrane of presynaptic cells, in close association with Na^+,K^+-ATPase activity (4). Interestingly, tricyclic antidepressants such as imipramine inhibit

noradrenaline and 5-HT reuptake in the central nervous system (5), whereas fluoxetine selectively inhibits 5-HT reuptake (6).

Changes in Na^+,K^+-ATPase activity by imipramine *in vivo* and *in vitro* have been reported. A decrease in enzyme activity was demonstrated in synaptosomes from cerebral cortex of guinea pigs after *in vitro*, but not *in vivo*, treatment (7). On the other hand, Nag and Ghosh (8) showed an increase in the activity of cerebral Na^+,K^+-ATPase and Mg^{2+}-ATPase in synaptosomes from cerebral cortex of albino rats induced by imipramine *in vivo* but not *in vitro*. However, there are no reports on the enzyme activity after administration of fluoxetine.

Since a) imipramine and fluoxetine are antidepressant drugs with similar efficacy and with an important role in the therapeutic arsenal for bipolar affective disorder, and b) Na^+,K^+-ATPase activity seems to be important for noradrenaline and 5-HT reuptake and its activity is decreased in patients with bipolar affective disorder, in the present study we investigated Na^+,K^+-ATPase activity in the synaptic plasma membrane from cerebral cortex of adult rats submitted to chronic administration of imipramine and fluoxetine. We also studied the *in vitro* effect of these drugs on enzyme activity.

Sixty-day-old Wistar rats were obtained from the Central Animal House of Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. The animals were housed in groups of eight with their mothers on the day of birth and maintained on a 12:12-h light/dark cycle (lights on from 7:00 to 19:00 h), in a constant temperature ($22^o \pm 1^oC$) colony room, with free access to water and 20% (w/w) protein commercial chow (Germani, Porto Alegre, RS, Brazil).

In chronic studies, animals received daily intraperitoneal injections of 10 mg/kg of imipramine or fluoxetine for 14 days. The selection of this regimen was based on previous studies showing important neurochemical effects for both drugs (9,10). The volume injected was 1 ml/100 g of body weight. Equivalent volumes of saline were given to controls. Rats were killed 12 h after the last injection, since the half-life of the drugs is 1 to 3 days (fluoxetine) and 18 h (imipramine) (11).

To avoid litter effects, drugs were administered to only three animals in each home cage, one receiving imipramine, one receiving fluoxetine and the other saline. Rats were sacrificed at the same time and the biological material was prepared simultaneously.

For *in vitro* studies, synaptic plasma membranes from cerebral cortex of 75-day-old untreated rats were used as the membrane enzyme preparation. Imipramine or fluoxetine was dissolved in Tris-HCl buffer, pH 7.4, and added to the incubation medium to a final concentration ranging from 0.1 to 0.5 mM. Control assays did not contain the drugs in the incubation medium. The concentration of the drugs used in the present investigation was chosen on the basis of previous studies using imipramine *in vitro* (12) and the solubility of fluoxetine in the buffer.

Rats were killed by decapitation without anesthesia. Brains were rapidly removed and the cerebral cortex was dissected out and homogenized in 10 volumes of 0.32 mM sucrose solution containing 5 mM HEPES and 1 mM EDTA. Membranes were prepared as previously described (13) using a discontinuous sucrose density gradient consisting of successive layers of 0.3, 0.8 and 1 mM. After centrifugation at 69,000 *g* for 2 h, the fraction at the 0.8-1 mM sucrose interface was taken to be the membrane enzyme preparation.

The reaction mixture for the Na^+,K^+-ATPase assay contained 5.0 mM $MgCl_2$, 80.0 mM NaCl, and 20.0 mM KCl in 40.0 mM Tris-HCl buffer, pH 7.4, in a final volume of 200 µl. The reaction was started by the addition of ATP (disodium salt, vanadium free) to a final concentration of 3.0

mM. Control assays 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 (13). All assays were carried out in duplicate. Enzyme specific activity is reported as nmol Pi min^{-1} mg $protein^{-1}$, unless otherwise stated.

Protein was measured by the method of Bradford (14) with bovine serum albumin used as standard. All chemicals were purchased from Sigma (St. Louis, MO, USA).

Data were analyzed by ANOVA followed by the Duncan multiple range test when the F test was significant. The dose-dependent effect was analyzed by multiple regression. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software. Data are reported as means for seven independent experiments (each rat contributed just one sample) performed in duplicate.

Rats chronically treated with imipramine or fluoxetine did not differ significantly from saline-treated rats with respect to body weight (control rats: 252 ± 28.2 g, fluoxetine-treated: 247 ± 22.36 g, imipramine-treated: 246 ± 9.08 g) [$F(2,21) = 0.18$, $P = 0.83$] or cerebral cortex weight (control rats: 534 ± 65.31 mg, fluoxetine-treated: 516 ± 51.83 mg, imipramine-treated: 498 ± 42.40 mg) [$F(2,21) = 1.22$, $P = 0.75$].

Figure 1 shows that Na^+,K^+-ATPase specific activity was significantly decreased by 10% in animals subjected to chronic treatment with imipramine. However, chronic fluoxetine administration caused a significant 27% increase in enzyme activity [$F(2,23) = 26.95$, $P<0.01$] when compared to control.

Next we determined the *in vitro* effect of imipramine and fluoxetine on enzyme activity. In agreement with a previous study (7), our results showed that imipramine significantly inhibited Na^+,K^+-ATPase activity in a dose-dependent manner ($r = 0.91$, $P<0.05$), with maximal inhibition (60%) occurring at 0.5 mM (control: 1877.50 ± 192.72, imipramine (0.1 to 0.5 mM): 1548.40 ± 184.38 to 726.83 ± 93.84) [$F(5,29) = 45.34$, $P<0.01$]. Figure 2 shows that fluoxetine significantly inhibited Na^+,K^+-ATPase activity in a dose-dependent way ($r = 0.89$, $P<0.05$), with maximal inhibition (80%) occurring at 0.5 mM [$F(5,29) = 61.28$, $P<0.01$].

In addition, it has been demonstrated that imipramine alters cortical membrane fluidity in *in vitro* and *in vivo* studies, and that chronic imipramine treatment affects brain membrane architecture in the rat (15).

We have shown here that chronic administration of fluoxetine significantly increased Na^+,K^+-ATPase activity by 27%. On the other hand, *in vitro* addition of fluoxetine to the enzyme assay provoked a significant inhibition of enzyme activity. These data agree with results obtained by other investigators showing an inhibition of Na^+,K^+-ATPase activity from other sources such as the basolateral membrane of the enterocyte and corneal endothelial cells (16,17). The *in vitro* effect of fluoxetine could be explained by its

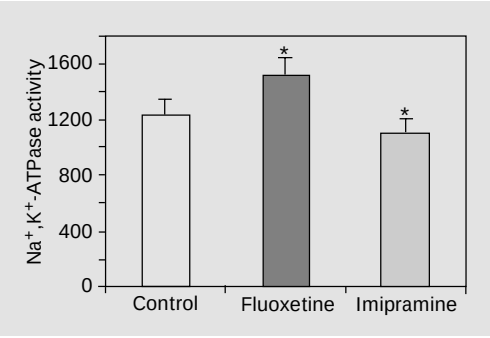

Figure 1. Effect of chronic administration (daily injections of 10 mg/kg for 14 days) of imipramine and fluoxetine on Na^+,K^+-ATPase activity in synaptic plasma membrane from rat cerebral cortex. Enzyme specific activity is reported as nmol Pi min^{-1} mg $protein^{-1}$. Data are reported as means ± SD for seven animals in each group. *P<0.05 compared to control (Duncan multiple range test).

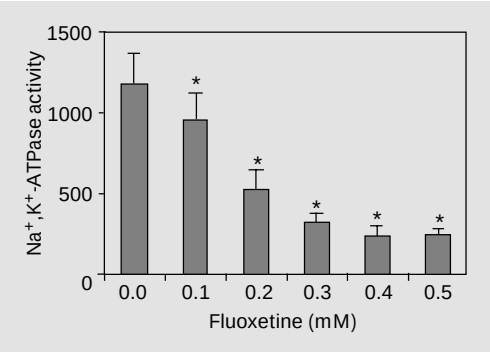

Figure 2. *In vitro* effect of fluoxetine on Na^+,K^+-ATPase activity in synaptic plasma membrane from rat cerebral cortex. Enzyme specific activity is reported as nmol Pi min^{-1} mg $protein^{-1}$. Data are reported as means ± SD for seven animals in each group. *P<0.01 compared to control (Duncan multiple range test).

hydrophobicity and this agrees with our previous results (13) and those reported by other investigators, emphasizing the importance of drug hydrophobicity in Na^+,K^+-ATPase inhibition (7). However, since fluoxetine inhibits Na^+,K^+-ATPase activity *in vitro* and that *in vivo* administration of fluoxetine provokes a significant increase of this enzyme, it is difficult to attribute a direct action of fluoxetine on Na^+,K^+-ATPase activity in our *in vivo* studies.

Although regulation of Na^+,K^+-ATPase activity is a very complex matter, it can be divided for convenience into a "short-term" control responsible for quick changes in pump activity, which occur in minutes, and a "long-term" control involving alterations in protein synthesis and/or degradation rate, occurring in hours or days. The rapid regulation of Na^+,K^+-ATPase activity involves complex networks of receptor-mediated signals activated in response to various stimuli in which altered phosphorylation with activation or inhibition of protein kinases and phosphatases seems to play a crucial role (18). Therefore, although the exact underlying mechanisms responsible for our results are not known, it is possible that the "short-term" regulation of Na^+,K^+-ATPase activity may be involved in the *in vitro* assays, whereas an increase in the number of pumps brought about by an increase in protein synthesis or a decrease in protein degradation may have occurred during the chronic administration of fluoxetine to the animals. These possibilities should be tested in future studies.

Although the precise mechanisms involved in the unexpected increase of Na^+,K^+-ATPase activity in rats receiving fluoxetine observed in this study are not known, it has been reported that serotonin and serotonin precursors increase this enzyme activity in serotoninergic-rich terminal regions of the brain such as cortex, striatum and hippocampus (19). However, it is important to note that chronic administration of other psychoactive drugs used for treatment of bipolar illness such as haloperidol, carbamazepine and lithium also increase membrane Na^+,K^+-ATPase activity in the rat brain (20).

In conclusion, it is possible that a) imipramine might decrease Na^+,K^+-ATPase activity by altering membrane fluidity, and b) stimulation of this enzyme activity might contribute to the therapeutic efficacy of fluoxetine, since brain Na^+,K^+-ATPase activity is decreased in bipolar patients.

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