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The influence of n-6 fatty acid supplemented diet on the effect of imipramine in an animal model of depression

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Abstract

Recent data have shown an association between polyunsaturated fatty acid and depression. This study examined the effect of the supplementation with n-6 fatty acid on the behavior of rats treated with imipramine and submitted to the Forced Swimming Test (FST). Non-supplemented imipramine-treated rats presented a significant reduction of immobility time in the FST whereas n-6 fatty acid-supplemented rats showed a significantly higher immobility time. Imipramine significantly increased norepinephrine plasma concentrations in the two groups. These results show that the diet supplemented with n-6 fatty acid altered the behavior of the animals in the FST, inhibiting the imipramine effect.

Keywords: Animal model; Depression; Imipramine; Polyunsaturated fatty acid; Primrose oil

1. Introduction

Most of membrane polyunsaturated fatty acids (PUFAs) are derived from dietary linoleic acid (18:2n-6, LA) and alpha-linolenic acid (18:3n-3, ALA), synthesized via a series of desaturation and elongation reactions. LA and ALA are considered essential fatty acids, i.e. they must be supplied by diet (Spector, 1999; Yehuda, 2003). The nervous tissue contains high amounts of PUFA, mainly arachidonic acid (20:4n-6, AA) and docosahexaenoic acid (22:6n-3, DHA), the latter being the most abundant (Bourre et al., 1993; Youdim et al., 2000). They control the composition of membranes and hence their fluidity and, as a result, their enzymatic activity, binding of molecules to their receptors, cellular interactions and the transport of nutrients (Bourre et al., 1993).

Studies have shown low blood levels of n-3 fatty acid in some medical conditions associated with depression, such as cardiovascular disease (Horrobin and Bennett, 1999a). A strong inverse relationship between n-3 fatty acid intake and prevalence of major depression has been reported (Hibbeln, 1998). Furthermore, depressed patients have decreased n-3 fatty acid or increased n-6/n-3 fatty acid ratio in blood and in phospholipid membrane (Adams et al., 1996; Peet et al., 1998; Maes et al., 1996, 1999).

There are but a few reports on the effects of dietary fatty acids in animal models of depression, and these studies use animals fed during pregnancy and lactation, but not in adulthood (Raygada et al., 1998; Naliwaiko et al., 2004). According to current results, fish oil supplementation, which contains high levels of n-3 fatty acid or diet containing high levels of n-6 fatty acid, decreases immobility time in the forced swimming test (FST) (Naliwaiko et al., 2004; Raygada et al., 1998). Apart from these studies, we are unaware of any other that investigated the effects of n-6 fatty acid supplementation on animal models of depression or about the association between PUFAs and antidepressant administration.

Clinically, the effects of antidepressant drugs do not appear earlier than two or three weeks after the onset of treatment (Blier, 2003). One of us (Galduróz JFC), noticed that patients treated with antidepressant drug plus n-6 fatty acid presented a faster therapeutic response (unpublished results). Other study report that irritability, depression, headache, breast pain and other aspects of premenstrual syndrome are improved with the use of n-6 fatty acid (Horrobin, 1983). Based on these findings, our hypothesis is that animals supplemented with n-6 fatty acid

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would present reduced immobility time or potentialization of the imipramine effect.

Therefore, the purpose of this study was to investigate the effect of n-6 fatty acid supplementation on the effect of imipramine in an animal model of depression, the Forced Swimming Test. Catecholamines and serotonin levels were measured in plasma, as an indirect index of imipramine action on these systems (Vlachakis et al., 1981; Sarrias et al., 1990).

2. Material and methods

2.1. Subjects and diets

Two months old *Wistar* male rats, weighing 214 g at the beginning of the study were used. They were maintained under a 12:12 h light dark cycle (lights on at 7:00 h) in a temperature-controlled room (23 °C). Food and water were available *ad libitum*. Animals were fed for six weeks with control diet (Nuvilab® rat chow — fat source: soy oil) or with n-6 fatty acid supplemented diet. All procedures were carried out in accordance with the guidelines on original care of the National Institute of Health (NIH) and were approved by the Animal Care and Use Committee of UNIFESP (CEP#1118/02).

The diet was supplemented by adding 11% of primrose oil (SP Farma, Sao Paulo, Brazil) to the control diet. The primrose oil contains high levels of n-6 fatty acid (85%), according to the analysis carried out by Adolfo Lutz Laboratory, in São Paulo, in addition to other biologically active compounds, such as gamma linoleic acid (10%). The diets were similar in protein, differing only in regards to fat content (control diet with 5% fat and n-6 enriched diet with 16% fat). Vitamin E (0.2%) was also added. The supplemented diet was prepared twice a month and stored in freezer at −13 °C (Guimarães et al., 1990).

2.2. Drug

Imipramine (Cristalia®, Itapira/SP, Brazil) was dissolved in distilled water at a concentration of 10 mg/kg of body weight and administered intraperitoneally (i.p.). Control animals received only vehicle.

2.3. Forced swimming test (FST)

The test was performed using a version adapted from that described by Porsolt et al. (1978). Following a six week-period of diet, rats were individually placed into a container 50 cm high and 30 cm in diameter, containing 30 cm of water at 25 °C. The animals remained in the water for 15 min before being removed, dried and returned to their home cage. They received a vehicle or imipramine injection 15 min after the first FST exposure. The final two vehicle or imipramine injections were given 5 h and 1 h prior to the second FST exposure, which took place 24 h after the first one. In the second FST exposure, rats were allowed to swim for 5 min, and immobility time was recorded during the test session. The rat was considered immobile when making only movements necessary to keep its head above the water.

2.4. HPLC-ED assay of catecholamines and serotonin

Plasma catecholamines [norepinephrine (NE), epinephrine, L-dopa and dopamine (DA)] and serotonin (5-HT) were measured by ion-pair reverse phase chromatography coupled with electrochemical detection (0.5 V) as described by Naffah-Mazzacoratti et al. (1992). All the concentrations are expressed as pg/mg.

2.5. Data analysis

Data were analyzed by a one-way ANOVA followed by Duncan test when appropriate. The non-normal data were normalized using logarithmic, but means and standard errors (SEM) are reported in the original units. Differences were considered significant when $p \leq 0.05$.

3. Results

3.1. Body weight

There were no differences in body weight among the groups $F(3,55) = = 0.87$, $p \equiv 0.46$.

3.2. Forced swimming test

ANOVA showed differences among groups $F(3,55) = = 3.40$, $p \equiv 0.02$. Non-supplemented imipramine-treated rats displayed a significantly lower immobility time than the control group ($p \equiv 0.03$). The n-6 fatty acid-supplemented rats treated with imipramine showed a significantly higher immobility time than the non-supplemented imipramine-treated rats ($p \equiv 0.01$),

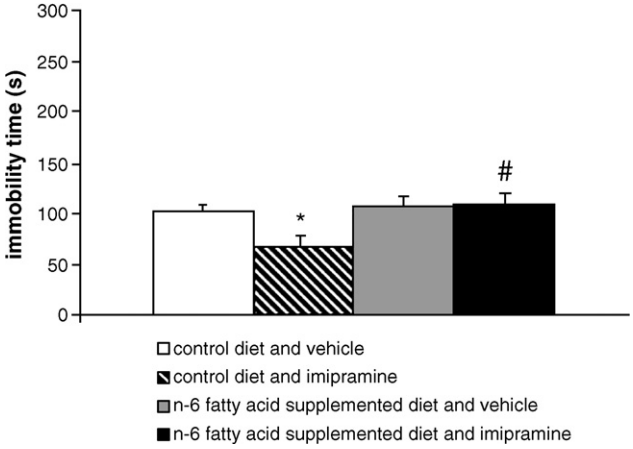

Fig. 1. Immobility time of rats (mean ± SEM; N= 14 –15) fed with a control or a n-6 fatty acid supplemented diets and treated with imipramine or vehicle, submitted to the FST. Duncan test: *$p < 0.05$, non-supplemented imipramine-treated rats vs vehicle; # $p<0.05$, non-supplemented vs n-6 fatty acid supplemented rats treated with imipramine.

Table 1
Catecholamines plasma concentration in rats under n-6 fatty acid supplemented or control diets and treated with imipramine. Monoamines plasma concentration (mean ± SEM) in rats groups (N=7–12) evaluated at the FST. Duncan test, *$p < 0.05$, represent vehicle x IMI in the corresponding diet. IMI=Imipramine

Groups	Norepinephrine	Epinephrine	L-dopa	Dopamine	Serotonin
Vehicle+control diet	1543.5±211.2	1737.8±281.7	169.9±18.2	80.6±17.8	1357.2±111.4
IMI+control diet	2678.6±412.8 *	2074.5±418.1	223.8±48.2	68.4±9.7	1236.9±126.9
Vehicle+n-6 fatty acid supplemented diet	1485.1±194.5	1711.5±257.5	206.8±22.6	81.7±15.6	1412.2±148.0
IMI+n-6 fatty acid supplemented diet	2405.4±438.3 *	1876.9±404.8	323.8±77.9	83.9±18.5	1061.6±153.2

indicating that n-6 fatty acid supplemented diet inhibited the imipramine effect (Fig. 1).

3.3. Plasma concentration of catecholamines

ANOVA of NE plasma concentration showed differences among the groups $F(3,36) = = 4.03$, $p \equiv 0.01$. Non-supplemented and n-6 fatty acid-supplemented rats treated imipramine showed higher of NE concentration compared to vehicle, ($p \equiv 0.01$) and ($p \equiv 0.05$) respectively. There were no significant differences in 5-HT plasma concentration, epinephrine, L-dopa and dopamine among groups (Table 1).

4. Discussion

According to the present results, n-6 fatty acid-supplemented rats did not show reduction of the immobility time in the FST following imipramine treatment when compared with the non-supplemented imipramine-treated group. It is interesting to note that there was an inhibition of the imipramine effect, in spite of a significant increase in norepinephrine plasma concentration in the group supplemented with n-6 fatty acid.

It is important to emphasize that by adding n-6 PUFA, we altered the proportion of macronutrients. So, in order to maintain at least part of this proportion, we also added protein to the diet. Although the supplemented diet became more caloric, it is important to emphasize that rats did not put on more weight than control-fed rats, indicating that this source of fat was used for other functions different from fat stores (such as membrane composition).

It has been reported that the adult offspring of n-6 fatty acid fed mothers display altered affective-like behaviors reflected by decreased immobility time in the FST, increased aggressiveness in the resident-intruder test and increased locomotor activity (Raygada et al., 1998). These results were obtained with soy oil, which contains high levels of n-6 and n-3 fatty acids and with corn oil which contains high levels of n-6 fatty acid and regular levels of n-3 fatty acid, suggesting that the responses appear to be mostly due to n-6 fatty acid. The reason why the present results are not in accordance with the Raygada et al. (1998) study is likely due to the fact that we used primrose oil which contains high levels of n-6 fatty acid and insignificant levels of n-3 fatty acid. Furthermore, methodological differences such as animal species (rats vs mice) and especially the type and percentage of oil added to the diet might have caused the observed discrepancies.

Few studies have shown the effect of the supplementation with n-6 fatty acid on animal model of depression, but the results seem to be a function of the imbalance between the levels of n-6 and n-3 fatty acids (Raygada et al., 1998). The primrose oil used in our study is a source of n-6 fatty acid being deficient in n-3 fatty acid. Nonetheless, there was no difference in the amount of n-3 fatty acid between control and n-6 fatty acid supplemented diet, indicating that blockade of imipramine effect might have been the consequence of excessive n-6 fatty acid. However, the oil used was very low in n-3 PUFAs, reflecting an unbalance between n-6/n-3 fatty acid. Therefore, chronic exposure to this unbalanced diet could result in increased depressive-like behavior. The response of n-6-treated rats to imipramine indicates that an imbalance between these two PUFAs influence the response to drugs. Francès et al. (2000) showed that mice treated with n-3 fatty acid deficient diet and submitted to the sucrose preference test exhibited a decreased sensitivity to the sweet solution, being less responsive to morphine in the conditioned place preference (4 mg/kg).

Animals fed with diet containing primrose oil showed decreased n-3 fatty acid and increased n-6 fatty acid concentration in the pituitary, while diet containing fish oil (rich in n-3 fatty acid) produces the opposite changes in this gland, indicating that the type of oil added to the diet alters the composition of PUFA present in the rat brain's phospholipids. According to Marteinsdottir et al. (1998), changes in membrane structure, as those described above, could be relevant to physiological and pathological processes in the brain, being extremely important in mood disorders.

Diets supplemented with n-6 fatty acid, especially arachidonic acid (AA) increase prostaglandin E_2 and corticosterone secretion, and induce anxiety-like behavior, whilst diets supplemented with n-3 fatty acid significantly reverse anxiety-like behavior, corticosterone secretion and inflammatory responses induced by central administration of the cytokine IL-1β(Song et al., 2003). Additionally, excess of n-6 fatty acid could also be responsible for changes observed in depressive patients serving as substrate for production of inflammatory mediators, as mentioned previously, which are responsible for sickness behavior observed in animals and humans (Leonard, 2001).

Our results showed that the effect of imipramine was influenced by diet, as animals became unresponsive to the drug. Although an increase in norepinephrine plasma concentration was observed, the antidepressant-like effect in the FST did not occur. Being a tricyclic antidepressant imipramine inhibits norepinephrine and serotonin reuptake, however in the present study we only observed a change in norepinephrine concentration. There is some evidence of alterations of brain phospholipid composition and membrane fluidity affecting processes such as

neurotransmitter-receptor binding and signal transduction, which may be involved in the pathophysiology of mood disorders (Hibbeln and Salem, 1995; Horrobin and Bennett, 1999a,b). In this case, the diet could change the concentration of n-6 fatty acid in the neuronal membrane and modify the action of drug or its interaction with receptor systems.

A main function of the PUFAs in the nervous system would be related with determination of the physical state of the neural membranes which requires a great state of fluidity to allow ionic interchange. The ideal composition of PUFA in the diet has not been fully determined, but it has been suggested that a n-6/n-3 fatty acid ratio of 4:1 improves cerebral functions such as memory, learning, cognition and mood. Thus, the ideal composition of PUFAs in the diet can alter brain function being extremely important to maintain a general state of mental health (Horrocks and Yeo, 1999).

The forced swimming test is a behavioral paradigm used to screen drugs with antidepressant activity in rodents (Porsolt et al., 1978). Rodents are sensitive acute administration of drug whereas symptoms of depression in humans are only ameliorated after chronic drug treatment. In this study, n-6 fatty acid-supplemented rats treated with imipramine did not present a behavioral response, despite the increase in norepinephrine concentration. One possible explanation is that animals supplemented with n-6 PUFA became resistant to acute administration of imipramine, thus requiring a prolonged treatment with the drug to express the non-depressive behavior. However, this idea is still speculative and deserves further investigation, including new models of depressive-like behavior and different classes of antidepressant drugs.

In conclusion, we reported that an n-6 fatty acid supplemented diet inhibited the imipramine effect in the FST. Animals supplemented with n-6 fatty acid and treated with imipramine showed increased norepinephrine plasma concentration but its antidepressant profile in the FST was not observed, suggesting that the imipramine effects appear to depend on additional mechanisms.

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