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NEONATAL TREATMENT WITH FLUOXETINE REDUCES DEPRESSIVE BEHAVIOR INDUCED BY FORCED SWIM IN ADULT RATS

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ABSTRACT - Serotonin plays a role at the pathophysiology of depression in humans and in experimental models. The present study investigated the depressive behavior and the weigh evolution in adult rats (60 days) treated from the 1st to the 21st postnatal day with fluoxetine, a selective serotonin reuptake inhibitor (10 mg/kg, sc, daily). The depressive behavior was induced by the forced swim test (FST). The animals were submitted to two sessions of FST: 1st session for 15 min and the 2nd session 24h later, for 5 min. During the 2nd session the Latency of the Attempt of Escape (LAE) and Behavioral Immobility (BI) were appraised. The Fluoxetine group when compared to the Control group, showed an increase in LAE and a decrease in BI. The neonatal administration of fluoxetine reduced the depressive behavior in adult rats, possibly by increase in the brain serotonergic activity. This alteration can be associated to process of neuroadaptation.

KEY WORDS: depression, serotonin, selective serotonin reuptake inhibitor, neurogenesis

Tratamento neonatal com fluoxetina reduz o comportameto depressivo induzido pelo nado forçado em ratos adultos

RESUMO - Estudos em humanos e em modelos experimentais demonstram que a serotonina (5-HT) participa da fisiopatologia da depressão. O presente estudo investigou o comportamento depressivo e a evolução ponderal de ratos adultos jovens (60 dias) tratados do 1º ao 21º dia pós-natal com fluoxetina, um inibidor seletivo de recaptação da serotonina, (10 mg/kg, sc, diariamente). A depressão experimental foi induzida através do teste de nado forçado (NF). Os animais foram submetidos a duas sessões de NF, a primeira por 15 min e a segunda após 24 h, por 5 min. Durante os 5 min de NF a latência da tentativa de fuga (LTF) e o tempo de imobilidade (TI) foram avaliados. O grupo tratado com fluoxetina apresentou aumento da LTF e redução do TI comparado ao controle. A administração neonatal de fluoxetina reduziu o comportamento depressivo em ratos adultos, possivelmente em função do aumento da atividade serotoninérgica cerebral. Esta alteração poderá estar relacionada a processos neuroadaptativos.

PALAVRAS-CHAVE: depressão, serotonina, inibidor de recaptação da 5-HT, neurogênese.

Studies in animals and humans have demonstrated the role of serotonin (5-hidroxytryptamine, 5-HT) in psychiatric depressions[1], through the use of pharmacological tools[2]. Experimental evidences of serotonin receptors involvement in the pathophysiology of depression and in the action mechanisms of antidepressant drugs, come from various biochemical, electrophysiological and behavioral approaches[3]. Animal models have largely contributed to the understanding of the 5HT receptors and depressive behavior relations[4]. Adult rats treated with antidepressants such as the selective serotonin reuptake inhibitor (SSRIs) presented behavioral changes in the forced swim test (FST), a recognized experimental model for depression studies[5].

The SSRIs increase the synaptic availability of 5-HT accentuating or facilitating its action[6,7]. According to some researches the chronic administration of

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SSRIs during the neonatal stage (days 1 to 21, suckling period) induces several behavioral changes in adult life[8,9]. Other studies demonstrated that chronic use of the SSRIs increases the expression of brain-derived neurotrophic factor (BDNF), one target gene of antidepressant treatment, in rat limbic structures, most notably the hippocampus[10]. In addition, the BDNF plays an important role in development, synapse remodeling[11] and it has trophic effects on serotonergic neurons in the central nervous system[12].

Furthermore, experimental works indicates that itself 5-HT can influence the embryogenesis and the growth[13,14] presumably by acting as a developmental sign[15] or as a neurotrophic factor[16,17]. Moreover, it is well known that very fast growth and development of the nervous system occur during pregnancy and suckling, and that pharmacological or nutritional manipulations at this phase, can induce drastic morphological and functional changes in these processes[15,18,19]. Drastic consequences have been also observed in behavior[9]. These alterations can become irreversible depending on the magnitude of the aggression[19]. Thus, there is a possibility that the use of SSRIs, in the initial phase of the life, could present long-lasting effects on behaviors related to the serotonergic function[9], such as the emotional behaviors[20]. Therefore, since there are no data concerning this point, the investigation of the possible long-lasting effects caused by early manipulations of the serotonergic system is highly desirable. This study proposed to test the hypothesis that the administration of a selective serotonin reuptake inhibitor - fluoxetine - to suckling rats, promotes changes in depressive behavior induced by forced swim in adult rats.

METHOD

Animals and treatments

Wistar rats were maintained at a room temperature of 23 ± 1 °C, on a light-dark cycle of 12:12 hours (light on at 7:00 a.m.), with free access to water and food. The animals were assigned randomly to two groups (6 pups per litter) 24 h after birth. One group (Fluoxetine group, 26 rats) received fluoxetine (10 mg/kg, *sc*, dissolved in saline solution, 1 ml/kg), and the other (Control group, 26 rats) received an equivalent volume of saline (NaCl 0.9%). The treatments were applied every day from the 1st to the 21st postnatal day (suckling period). Body weights were determined at 1st to the 21st (weaning) and 60th day.

Behavioral evaluation

The animals aged around 60 days, weighing 220-240g, were evaluated with regard to depressive behavior induced by forced swim (Forced Swim Test), modified method of Porsolt et al.[21]. This procedure consists of exposing an animal to a situation of inescapable stress, in which the rat is forced to swim. After an initial period of vigorous swimming activity in the direction to the tank border (denominated Latency of the Attempt of Escape), the animal reduces the intensity of the movements, just producing the necessary movements to maintain its head out of the water. This answer was classified as behavioral immobility, indicating a possible state of despair of the animal when it realizes that there is no escape.

The rats were placed individually in a tank (height, 42 cm; diameter, 104.5 cm), whose level of water do not allow the animal to lean on the floor, nor arise by the border. The temperature of the water was maintained in 25 °C. The animals were submitted to the forced swimming during 15 minutes (Pre-test). After the 15 min of forced swim each animal was led to dry in the Camera of Heating (CH; 32 °C /15min), an then returned to their cages. Twenty-four hours after the Pre-test, all the appraised animals were put back inside of the tank. At this time, the individual behavioral evaluation was accomplished and quantified during 5 minutes of swim (Test); soon after they were again led for CH. The behavioral parameters as Latency of the Attempt of Escape (LAE) and Behavioral Immobility (BI) were quantified in seconds (s) with aid of digital chronometers.

Statistical analysis

The corporal weight evolution (expresses mean ± SEM) was analyzed by Student's "t" test. The behavioral parameters (expressed as median and percentiles 25-75) were appraised for Mann-Whitney two-tailed test. The significance level adopted for all the used statistical tests was $p < 0.05$ (Statgraphics Statistical Graphics System v.6.0, Manugistics, Inc. and Statistical Graphics).

RESULTS

Compared to the Control group, the Fluoxetine group presented a reduction in the corporal weight gain ($p < 0.05$) starting from the 9 th day of life and continuing to 21st day (Fig 1). At the 60th day of age none difference was observed among the corporal weights of the groups (Table 1).

The behavioral parameters were appraised during the FST. LAE of the Fluoxetine group was significantly larger ($p < 0.01$) while BI was smaller ($p < 0.01$) when compared with the Control group (Tables 2 and 3).

DISCUSSION

The present study demonstrated that chronic administration of fluoxetine, during the critical period of the nervous system development, besides harming the evolution of the corporal weight, in adult rats, also reduced the depressive behaviors induced by FST. These effects can be correlated with the reported developmental alterations of the serotonergic system, as suggested by Palén et al.[13]. These authors

Table 1. Body weight comparisons between the Control and Treated Groups at three different days.

Experimental groups	Weight (g)		
	1st day	21st day	60th day
Control group	7.49 ± 0.73 (26)	47.45 ± 7.73 (26)	214.67± 19.97 (13)
Fluoxetine group	7.60 ± 0.71 (26)	35.32 ± 8.07* (26)	209.62 ± 27.31 (13)

The treated group received fluoxetine (10mg/kg, sc; Fluoxetine group). The Control group received saline (0.9% NaCl, 1ml/kg, sc) from the 1st to the 21st day of age. The weight of the 1st, 21st and 60th day of age are compared and reported as mean ± SEM. * $p < 0.05$ (unpaired two-tailed Student *t* test). Number of animals (n) are presented below the weight columns.

Table 2. Latency of the attempt of escape of rats treated with fluoxetine or saline during the suckling period.

Experimental groups	Latency of the attempt of escape (s)	
	Md	PE_{25-75}
Control group (n=26)	154.5	117-222
Fluoxetine group (n=26)	97.5	49-161

Latency of the attempt of escape (LAE) of rats treated with fluoxetine or saline during the suckling period. All the animals were submitted to experimental depression at the 60 day of age. For each group (26 rats/group), the LAE is represented as median (Md) and 25 and 75 percentiles (PE_{25-75}). * $p< 0,01$ compared to the Control group (Mann-Whitney two-tailed U-test).

Table 3. Behavioral Immobility (BI) of rats treated with fluoxetine or saline during the suckling period.

Experimental groups	Behavioral Immobility (s)	
	Md	PE_{25-75}
Control group (n=26)	9	2-13
Fluoxetine group (n=26)	24.5	4-66

Behavioral Immobility (BI) of rats treated with fluoxetine or saline during the suckling period. All the animals were submitted to the experimental depression at the 60 day of age. For each group (26 rats/group), the BI is represented as median (Md) and 25 and 75 percentiles (PE_{25-75}). * $P< 0.01$ compared to the Control group (Mann-Whitney two-tailed U-test).

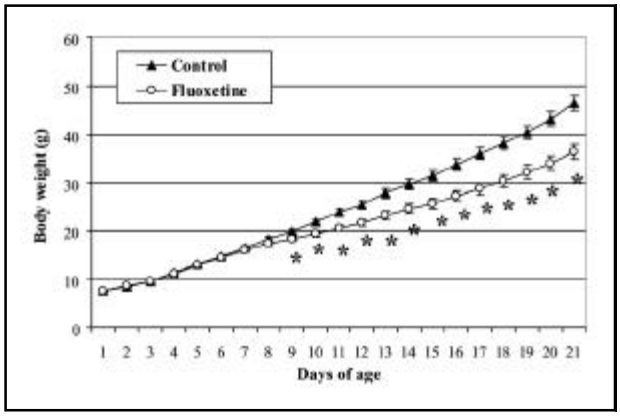

Fig 1 . Weight evolution of rats treated with fluoxetine or saline during the suckling period. Rats received fluoxetine (10mg/kg, sc; Fluoxetine group, n=26) or saline (0.9% NaCl, 1ml/kg, sc; Control group, n=26) from the 1st to the 21st day of age. The animals were weighed daily. The data are reported as mean ± SEM. * $P < 0,05$ compared to the Control group at the same age (unpaired two-tailed Student t test).

observed that the administration of drugs acting on the synthesis and serotonin liberation, or on the activation of the serotonergic receptors, during the embriogenesis, could result in disturbances of the growth and development of several tissues, including the nervous one. These data were confirmed by Lauder et al.[22], that demonstrated a delay in the neuronal differentiation, through inhibition in the embryonic synthetic pathway of 5-HT, after maternal administration of p-chlorophenylalanine (pCPA). Decrease on the levels of 5-HT in the neonatal period, also induced reduction of the dendritic spines density in the developing nervous system. This constitute morphologic alterations of specific and permanent character, possibly resulting in damages on the synapses establishment[15]. In contrast, cultured embryonic serotonergic neurons of the mesencephalon of rats presented an increase of its density and survival[19].

Other hypotesis can be relationship to the BDNF, we believe which chronic administration of SSRIs in the early life may to affect this neurotrophin, consequently leading the possible process of the neuroadaptation. Accordingly with recent study a infusion of BDNF into the of hippocampus produced antidepressant effect in the FST[23]. In spite of that, it is not totally been clarified that these alterations may persist until the adult life promoting functional damages, our data corroborate this possibility.

Our observations about the reduced corporal weight gain of fluoxetine treated rats can be attributed to the inhibitory action of serotonin, controlling the food ingestion[24]. Although, the stress provided by chronic treatment with fluoxetine can induce decreased body weight, possibly by affect the hipothalamic-pituitary-adrenal axis resulting disturbances in the control of corticoid function, this fact was not considered in this study. Since, saline controls animals also were treated chronically. Besides, the chronic fluoxetine treatment normalize the corticosterone secretion in depressed pacients or experimental models[25]. Fluoxetine is an antidepressant drug that selectively inhibits the neuronal 5-HT uptake, conse-

quently increasing its synaptic availability[26], and so reduces the hunger and the alimentary ingestion in humans[27] and produces hipofagia in rats[28].

The pharmacological manipulation of the serotonergic system during the development, might have caused the post-treatment decrease of the depressant behaviors induced by the FST, that persisted until the adult age, confirming the well known relationship between depression and functional alteration of the serotonergic system[29]. In this context, the studies accomplished at our laboratory already demonstrated alterations of the aggressive behavior, in adult rats submitted to neonatal treatment with selective serotonin reuptake inhibitor[9]. The 5-HT is involved in the neurobiology of depression, as well as in the action mechanisms of antidepressant agents[30]. The presence of multiple types of serotonergic receptors corroborates the hypothesis that drugs with selective action in some of them, can have specific properties in emotional disorders[2].

The decrease in the concentrations of brain serotonin can precipitate the recurrence of the depression in depressed patients[31] while the manipulation of serotonergic receptors by pharmacological tools has evidenced antidepressant properties in some animal models[32]. The FST has been used to evaluate the effectiveness of several antidepressant treatments[5]. It was realized after evaluation of the several activity of the animals (dates not published), yet no alteration was observed. The reduction of depressant behavior in the FST, in the Fluoxetine group, seems to be related to the function of the serotonergic system[5]. The inhibition of the 5-HT uptake process by fluoxetine results possibly in its increased availability in the synaptic cleft, accentuating or facilitating its action[6]. The decreased depressant behavior evaluated in adult life after neonatal treatment, in the FST observed in the present study, seems to be associated with neuroadaptive mechanisms developed at the time of treatment, that persists until adult life.

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