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Histomorphometric Evaluation of Soleus and Lateral Gastrocnemius Muscles of Animals Submitted to Pharmacological Manipulation with Fluoxetine

Evaluación Histomorfométrica de los Músculos Sóleo y Gastrocnemio Lateral de Animales Sometidos a Manipulación Farmacológica con Fluoxetina

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SUMMARY: The pharmacological manipulation with selective inhibitor of serotonin reuptake (SSRIs) can modify the operation of the serotonergic system and may facilitate or inhibit the action of this system, inducing changes in the morphology of the skeletal muscle of rats. The objective of this study was to evaluate the action of the treatment with fluoxetine during the critical period of the animal´s life on development of the soleus and lateral gastrocnemius muscles, under the aspects of weight, the number of nuclei of myocyte cells and cross-sectional area of muscle fibers. Twenty four (30 and 90-day-old) male Wistar rats were used. They were treated with saline solution (NaCl 0.9%; 1 ml/100 g of body weight) or fluoxetine (10 mg; 1 ml/100g of body weight). The animals were divided in Saline Group (GS-30 and GS-90) and Fluoxetine Group (GF-30 and GF-90). The fluoxetine group showed a reduction on weight (g) of soleus ($p=0.046$) and lateral gastrocnemius ($p=0.02$) muscles in rats with 90 days. A lesser number of myonuclei was observed in fluoxetine group than saline group of 30 days (soleus, $p<0.001$; lateral gastrocnemius, $p\leq0.007$) and 90 days (soleus, $p=0.002$; lateral gastrocnemius, $p\leq0.038$). The cross section area of fluoxetine groups is also smaller than the saline groups with 30 days (soleus, $p=0.03$; lateral gastrocnemius, $p=0.041$) and 90 days (soleus, $p=0.042$; lateral gastrocnemius, $p=0.012$). The treatment of fluoxetine during the critical period of development of the nervous system of rats, causes early changes in the structure of muscle fibers that seem to be related to reducing the weight of the soleus and gastrocnemius muscles only in late stage of the animal's life. Thus, the dosage used ISRS, suggests an inhibitory effect of 5-HT in relation to variables on the development of the skeletal muscle tissue of rats.

KEY WORDS: Fluoxetine; Muscles Fiber; Skeletal Muscle; Myonuclei; Cross Section Area.

INTRODUCTION

The growth, development and the plasticity of the Nervous System are determinants to the morphofunctional organization of various tissues (Morgane *et al*., 1993). These properties can be modified by exogenous factors such as dietary changes and pharmacological manipulation of neurotransmitter systems (Borba *et al*., 2000).

The neurotransmission is a main function of nerve cells (Buznikov *et al*., 1993). However, neurotransmitter molecules have other functions, in particular, during the pre and postnatal development (Lidov & Molliver, 1982). Serotonin (5-hydroxytryptamine or 5-HT) stands out between neurotransmitters to be related to central and peripheral physiological effects including, for example, sensory and motor controls (Tecott *et al*., 1995). It also has the function of regulating factor on the growth of neural and non-neural tissues (Buznikov *et al*., 2001). In the latter case, the 5-HT has been linked to the development of muscle tissue (Sanfilippo *et al*., 1995). There is evidence that interference with the serotonergic system is able to block or change the important regulatory functions, emphasizing the role of 5-HT in the regulation of early stages of embryogenesis and

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morphogenesis (Buznikov *et al*., 2001). The pharmacological manipulation with drugs can be modified in the operation of the serotonergic system, among them the selective inhibitor of serotonin reuptake inhibitors (SSRIs), may facilitate or inhibit the action of this system, inducing changes in the morphology of the skeletal muscle of rats (Buznikov *et al*., 2001), mostly if occur during the critical period of the development of the nervous system that, in the rat, comprises the first 21 days of postnatal life (Morgane *et al*., 1978).

Thus, the objective of this study was to evaluate the action of the treatment with fluoxetine during the critical period of the animal life on development of the soleus and lateral gastrocnemius muscles, under the aspects of weight, the number of nuclei of myocyte cells and cross-sectional area of muscle fibers.

MATERIAL AND METHOD

In this study 24 male albino Wistar, rats were used aged 30 and 90 days, from the breeding colony of the Department of Nutrition of the Federal University of Pernambuco. The animals were confined in collective cages (maximum of 6 animals/cage) and maintained with food (Labina-Purina of Brasil S/A) and water ad libitum. They were maintained in an environment with a temperature of 23±1°C in a constant light cycle (06:00 to 18:00 h) and dark cycle (18:00 to 06:00 h).

The animals were divided in four groups following the age and the pharmacological manipulation: Saline Group (GS - 30) (control) - n = 06, 30 days, treated with physiological solution of sodium chloride at 0.9%, 1ml/100g body weight, subcutaneously; Saline Group (GS - 90) (control) - n = 06, 90 days, treated with physiological solution of sodium chloride at 0.9%, 1ml/100g body weight, subcutaneously; Fluoxetine Group (GF - 30) - n = 06, 30 days, treated with Fluoxetine in a dose of 10 mg, 1ml/100 g body weight, subcutaneously, Fluoxetine Group (GF - 90) - n = 06, 90 days, treated with Fluoxetine in a dose of 10mg, 1 ml/100g body weight, subcutaneously. Treatments were performed daily between 12 and 14 hours on 1st to 21th day of life of the animal.

In the 30th and 90th days of life, the animals were anesthetized intramuscularly with ketamine hydrochloride solution (Ketalar® - 0.25 ml/100 g weight) and xylazine (Rompum® - Bayer - 0.03 ml/100 g weight). Then an incision was made in the left paw of the animal, aiming to detach the lateral gastrocnemius and soleus muscles from its origin to insertion into the tendon of the triceps surae.

The muscles were weighed on a digital balance (AND brand, model HR-200, maximum capacity of 210g, density of 0.1 mg). The collected materials were fixed by immersion, first in buffered formaldehyde solution (10 ml of 37% formalin and 27 ml of 0.1M phosphate buffer - pH = 7.0) and then in 100% ethanol. Subsequently, the material collected from six animals randomly chosen from each group was referred for routine histology and finally subjected to histological cross sections (4 μm) and stained with hematoxylin and eosin.

The analyzed histological parameters were: a cross-sectional area of the muscle fiber and the number of myocyte cell nuclei. The cross-sectional areas of the 15 fibers and lateral gastrocnemius and soleus muscles from each animal were analyzed (considering only the fibers with preserved morphology) by means of an image pickup system constituted by a Power VCR II software, one Samsung video camera (Model SHC-410NAD) coupled to a Leica optical microscope integrated to a microcomputer. All images were captured at a final magnification of 1000X. The morphometric analysis was performed using Scion Image software for Windows (Beta 4.0.3.). The number of nucleus of myocyte cells was obtained in 20 microscopic fields per slide using a Leica optical microscope with magnification of 400X and with the aid of a millimeter grid of 441 points.

For statistical analysis of the data we applied the t Student and Mann-Whitney tests. Statistical significance was considered, assuming a critical level of 5% in all cases. This study was approved by the Ethics Committee on Animal Experimentation of the Center for Biological Sciences, Federal University of Pernambuco (ECAE-UFPE), letter No. 03/05, and is in accordance with current standards.

RESULTS

There was a reduction of the weight of the soleus muscle ($p = 0.046$) and lateral gastrocnemius ($p = 0.02$) of the animals treated with fluoxetine only those at the age of 90 days (Table I).

In animals treated with fluoxetine there was a reduction of the number of myonuclei of muscle fiber in soleous and lateral gastrocnemius muscles (Table II), in both ages of study (30 and 90 postnatal).

The cross section area of the muscles under study was also reduced in animals treated with fluoxetine both early stage of development (30 days) and in a later phase (90 days) (Table III).

Table I. Effect of pharmacological manipulation with fluoxetine on the weight of the soleus and lateral gastrocnemius muscles.

	Soleus M.	Lateral gastrocnemius M.
GS – 30	0.05±0.01	0.25±0.05
GF – 30	0.05±0.01	0.22±0.03
GS – 90	0.17±0.02	1.29±0.14
GF – 90	0.15±0.01*	1.09±0.11**

* Significant reduction in the soleus muscle weight, p = 0.046, t Student test. ** Significant reduction of the lateral gastrocnemius muscle weight, p = 0.02, *t* Student test.

Table II. Effect of pharmacological manipulation with Fluoxetine on myonucleus of muscle fibers Soleous and Lateral Gastrocnemius.

	Soleus M.	Lateral gastrocnemius M.
GS – 30	45.48±6.19	42.11±9.33
GF – 30	30.39±3.80*	28.58±2.97***
GS – 90	47.37±4.88	39.28±5.53
GF – 90	33.67±5.63**	29.97±7.80****

* Significant reduction in the number of myonuclei in the soleus muscle at 30 days, p <0.001, t Student test.** Significant reduction in the number of myonuclei in the soleus muscle at 90 days, p <0.002, Mann Whitney test. *** Significant reduction in the number of myonuclei in the lateral gastrocnemius muscle at 30 days, p ≤ 0.007, Student *t* test. **** Significant reduction in the number of myonuclei in the lateral gastrocnemius muscle at 90 days, p ≤ 0.038, Mann Whitney test.

Table III. Effect of pharmacological manipulation with Fluoxetine on the cross-sectional area (mm∑) of the muscle fiber of the Lateral Gastrocnemius and Soleus muscles.

	Soleus M.	Lateral gastrocnemius M.
GS – 30	593.38±89.81	512.09±156.23
GF – 30	414.33±72.54*	358.14±58.74***
GS – 90	844.94±101.60	733.12±83.78
GF – 90	730.06±65.61**	579.67±88.66****

* Significant reduction in cross-sectional area of the fiber of the soleus muscle, p = 0.03, Student t test. ** Significant reduction in the weight of the lateral gastrocnemius muscle, p=0.042, Student t test. *** Significant reduction in cross-sectional area of the lateral gastrocnemius muscle fiber, p=0.041, Student *t* test. **** Significant reduction in cross-sectional area of the lateral gastrocnemius muscle fiber, p=0.012, Student t test.

DISCUSSION

Drugs acting on serotonin receptors may enhance or inhibit the action of serotonin, during the neonatal period, both acting on the nervous system and on its target tissues (Manhães de Castro *et al*., 2001).

The present study has demonstrated that the administration of fluoxetine in doses of 10 mg/kg, during the critical period of the rat´s development, induced a reduction on the weight of soleus and lateral gastrocnemius muscle in a later phase (90 days) of the animal's life. Studies, utilizing the ISRS, also demonstrated changes in the weight of other organs such as brain, spleen, liver, lung, heart, kidney and testicles (Deiro *et al*., 2002). It is known that increasing the synaptic availability of serotonin promotes hypophagic effect (decrease of food intake) (Simansky, 1996). As a consequence of this effect there could have been a shortage of supply of necessary nutrients for the development of muscles, thereby resulting in a reduction in the weight of the muscles under study at a late stage of the animal's life.

The main factor that influences the growth potential of skeletal muscle is the number of myonuclei in muscle fiber, that is even more important than the number of fibers itself (Greenwood *et al*., 2000). For the growth and regeneration of skeletal muscle, studies suggest that the only source of new myonuclei are cells located between the plasma membrane and basal membrane of skeletal muscles, the satellite cells (Macfarland, 1999; Schafer *et al*., 2005).

The present study demonstrated a reduction in the number of nuclei of skeletal muscle fibers in both muscles studied, lateral gastrocnemius and Soleus muscles both an early phase (30 days) and in a late phase (90 days) postnatal development of the animals treated with fluoxetine. Buznikov *et al*. (2001), using mesenchymal cells of mice jaw have demonstrated that the use of fluoxetine blocked the mitogenic effects of 5-HT previously described by Buznikov *et al*. (1996), suggesting that high synaptic concentration of serotonin may lead to adverse effects in the development of several organs (Deiro *et al*., 2002, 2004) and the skeletal muscle tissue as described by Manhães de Castro *et al*. This suggests an inhibitory role of 5-hydroxytryptamine in the differentiation of satellite cells in myonuclei present in animals since the early stage of development, lasting until the later stages of his life.

The growth in thickness of muscle tissue is due to the increase in diameter of muscle fibers (hypertrophy), the number of muscle fibers (hyperplasia) (Jacquemin *et al*., 2004) and the number of nucleus of the muscle fiber. Hikida *et al*. (1998) and Eriksson *et al*. (2005) demonstrated a positive correlation between the cross section area of the muscle fiber and the total number of myonuclei in skeletal muscle, suggesting a key role of myonuclei in the mechanism of muscular hypertrophy. This same direct relationship was demonstrated in our study, since we observed a reduction in the number of myonuclei and the cross-sectional area of the muscle fiber in animals treated with ISRS at the same ages, 30 and 90 days. The reduction in the number of myonuclei implies a decrease in the activity of RNA transcription, essential for the process of protein synthesis (Adams *et al*.,

2002, 2006; Haddad *et al*., 2005), resulting in reduced production of actin and myosin, the primary mechanism for muscle hypertrophy (Haddad *et al*., 2003). In addition, the reduced uptake of nutrients may also have been caused by changes in the intestinal absorption surface as a consequence of the reduction of intestinal villous height in the animals treated with fluoxetine. Thus suggesting a reduction of protein synthesis. Thus, according to Greenwood *et al*., nutritional reduction can restrict the development of skeletal muscle. Therefore, our data suggest an inhibitory effect of 5-HT in the protein synthesis process by myonuclei, resulting in a lack of contractile proteins, which are responsible, in part, by hypertrophy of skeletal muscle.

From the results of this study, we conclude that treatment of fluoxetine during the critical period of development of the nervous system of rats, causes early changes in the structure of muscle fibers that seem to be related to reducing the weight of the soleus and gastrocnemius muscles only in late stage of the animal's life. Thus, the dosage used ISRS, suggests an inhibitory effect of 5-HT in relation to variables on the development of the skeletal muscle tissue of rats.

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RESUMEN: La manipulación farmacológica con inhibidores selectivos de la recaptación de la serotonina (ISRS) pueden cambiar el funcionamento del sistema serotoninérgico y facilitar o inhibir la acción de este sistema, induciendo cambios en la morfología del músculo esquelético de ratones. El objetivo fue analizar los efectos de la manipulación farmacológica neonatal con fluoxetina en el desarrollo de la masa muscular, número de núcleos y área de la sección transversa de las fibras de los músculos sóleo y gastrocnemio lateral. Se utilizaron 24 ratones Wistar machos, de 30 y 90 días de edad, tratados con solución salina (NaCl 0,9%, 1m/100 g de peso corporal) y fluoxetina (1 mg; 1 ml/100 g de peso corporal). Los animales fueron divididos en grupos con solución salina (GS-30 y GS-90) y fluoxetina (GF-30 y GF-90). El grupo tratado con fluoxetina mostró una reducción de peso (g) de los músculos sóleo (p=0,0046) y gastrocnemio lateral (p=0,02) en 90 días. Además, se observó en este mismo grupo una reducción de núcleos en 30 días (M. sóleo, p<0,001; M. gastrocnemio lateral, p≤0,007) así como en el período de 90 días (M. sóleo, p=0,002; M. gastrocnemio lateral, p≤0,038). También se observó reducción del área de la sección transversal en los animales tratados con fluoxetina durante el período de 30 días (M. sóleo, p=0,03; M. gastrocnemio lateral, p= 0,041;) y 90 días (M. sóleo, p=0,042; M. gastrocnemio lateral, p=0,012). El tratamiento con fluoxetina durante el período crítico del desarrollo del sistema nervioso de ratones, induce cambios prematuros en la estructura de la fibra muscular, los que parecen estar relacionados con la reducción de peso de los músculos sóleo y gastrocnemio en una fase tardía de vida del animal. En consecuencia, la dosis utilizada de ISRS, sugiere un efecto inhibidor de la 5-HT, en relación a las variables estudiadas sobre el desarrollo del tejido muscular esquelético de ratones.

PALABRAS CLAVE: Fluoxetina; Fibra muscular; Músculo esquelético; Mionúcleo; Área de sección transversal.

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