

Neonatal Treatment With Acetylcholinesterase Inhibitor Causes Retardation of Weight Gain and Somatic Growth in Rats

Tratamento Neonatal com Inibidor da Acetilcolinesterase Induz Retardo na Evolução Ponderal e no Crescimento Somático em Ratos

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RESUMO

Estudos prévios do nosso grupo observou alterações da maturação reflexa e crescimento somático de ratos expostos perinatalmente à antidepressivos que atuam sobre os sistemas serotoninérgico e noradrenérgico. O presente estudo examinou o sistema colinérgico, através da exposição de fármacos inibidores da acetilcolinesterase (rivastigmina). Foram avaliados seus efeitos durante o período lactacional sobre o desenvolvimento somático e sensório-motor de ratos Wistar. Ao 11^o dia de vida pós-natal, os filhotes tratados com a rivastigmina pesavam 4% menos que animais controle. Esse efeito poderá ser consequência de redução da ingestão energética, pois drogas que atuam sobre o sistema colinérgico exercem efeito inibitório sobre a ingestão alimentar atuando sobre o controle de ganho de peso. Observamos também que vários parâmetros de crescimento somático, como eixos do corpo e do crânio sofreram retardo por exposição à agente potencializador do sistema colinérgico.

PALAVRAS-CHAVE: rivastigmina, inibidor da acetilcolinesterase, sistema colinérgico.

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ABSTRACT

Previous studies in our group demonstrated alteration in the maturation reflex and somatic growth in rats exposed, during the perinatal period, to anti-depressives that act in the serotonergic and noradrenergic systems. This study examined the cholinergic system, through the exposition to acetylcholinesterase inhibitors (rivastigmine). Its effects on the somatic and motor-sensorial development in rats *Wistar* were evaluated during the lactation period. In the 11th day of life, the broods treated with rivastigmine weighted 4 per cent less than the animals in the control group. This effect may be a consequence of the reduction of energetic ingestion, since drugs that affect the cholinergic system inhibit the food ingestion, acting in the weight gain control. We also noticed that various parameters of somatic growth, such as body and skull axis suffered retardation caused by the exposition to the potentializer agent of the cholinergic system.

KEY WORDS: rivastigmine, acetylcholinesterase inhibitor, cholinergic system.

INTRODUCTION

The intense neuronal activity during periods of development such as pregnancy and lactation makes the organism vulnerable to any kind of aggression (1). So disturbs might occur in the activity of neurotransmitters and its receivers, which causes structural and functional repercussions in many systems (2) These sequels can occur in molecular, cellular and behavioral levels, and they are usually associated with nutritional and pharmacological perinatal aggressions in rats (3-6) and human beings (7-9). This concern has been pertinent in the last years because of the utilization of anti-depressives in the treatment of post-partum depression or in early stages of life (8, 9). In rats, it was observed temporal retardation in the maturation reflex and in the somatic growth due to perinatal exposure to anti-depressives that act in the serotonergic neurotransmission system (4, 5) or in the noradrenergic one (3).

In this study, we have a particular interest in another neurotransmitter vulnerable to perinatal aggressions, the acetylcholine (10). The cholinergic system is important in the ganglionar transmission in neuromuscular junctions (11, 12), in the cortical activation, memory, learning and muscular tonus control (13, 14). Environmental aggressions may affect this system by the utilization of pharmacologic

acetylcholinesterase inhibitors, that potentialises the action of the acetylcholine (15, 16), and even by pesticides frequently used in agriculture that cause the same reaction, i.e., they are acetylcholinesterase inhibitors (17). When the exposition occurs during the development of organisms, even in non-toxic doses, it compromises the development of neural cells, altering the synaptic function of acetylcholine in pubescence and in adult life (18), and alters also the development of other neurotransmitters, such as serotonin (19).

The present study evaluated the effects of the exposition, during the lactation period, of an acetylcholinesterase inhibitor on the somatic and motor-sensorial development of rats.

MATERIALS AND METHODS

The rats used were from the *Wistar* lineage, and proceeded from the bioterio's colony of the Federal University of Pernambuco's Nutrition Department. The animals were kept in a room with a $23 \pm 2^{\circ}\text{C}$ temperature, with illuminated and dark cycles of 12h each and *ad libitum*. One day after birth, the broods were adjusted to 6 males of the offspring, each weighting 6-7g. This was considered their first day of life. The experiments with these animals followed the "Ethical Animal Testing Principles" approved by the Brazilian School of

Animal Testing, and obeyed the rules about how to handle and take care of animals (103/03 protocol).

The experimental groups were formed: Control (C), animals (n=18) that received a saline solution (NaCl 0.9%, 1ml/kg, sc), and Treated (T), animals (n=18) treated with an acetylcholinesterase inhibitor (rivastigmine, 2mg/Kg pc., sc). The treatments occurred at 12h, during the nursing period (from the 1st to the 21st day post-natal). The experimental groups were evaluated daily, during lactation, about measurements of somatic growth, maturation of physical characteristics and orthogenesis of reflexes. As indicators of somatic growth, were considered the Body Weight (BW), the Mediolateral Head Axis (MLHA), Anteroposterior Head Axis (APHA), the Body Length (BL) and Tail Length (TL). The physical characteristics evaluated followed the criteria established by FOX (1965). For each animal, it was registered the maturation (day of appearance)

of physical characteristics: Opening of the Auditive Pavilion (OAP), Opening of the Auditive Conduct (OAC), Irruption of the Inferior Incisors (III) and Opening of the Eyes (OE). The reflexes' maturation analysis (Collocation by Vibrissae, Prone Recuperation, Acceleration Reaction, Precipice Aversion, Negative Geotaxis and Scare Reflex) was based on the descriptions of (20) (Table 1). The day of each reflexes' consolidation was registered after three consecutive days of its first appearance.

For statistic analysis, the "U" Mann-Whitney test was used to compare the date of the appearance of physical characteristics and reflexes between the groups. These results were represented as medians and minimum and maximum values. The data of body weight and growth measurements were analyzed by Two-ANOVA and followed by Bonferroni's test.

Tabela 1

Procedures for detection of reflexes maturation According to Smart and Dobbing (1971)

Reflex	Stimulus	Response
Righting	Rat placed on back on a flat surface	It turns over, to rest in ventral decubitus, with the four paws on the surface, in 10 s.
Vibrissa placing	Rat held by the tail, head facing an edge of bench, vibrissa just touching vertical surface.	Lifts head and extends forepaws in the direction of the bench, making oriented "walking" movements to go far from the edge, in 10 s.
Cliff avoidance	Rat put on edge of bench, with nose and forefeet just over edge.	Withdrawal of head and both forefeet from edge, moving away from "cliff", in 10 s.
Auditory startle	AS Sudden sound stimulus by percussion with a metallic stick in a metal surface	Body retraction, with a transitory immobility. The stimulus was given twice in each test, with a 1-min interval
Negative geotaxis	Rat placed with head downwards, on a 45- slope	Turns to face up the slope, at least 130°, in 10 s.
Free-fall righting	Rat held by the paws, back downwards, is dropped from 30 cm onto cotton- wool pad.	Turns body in mid-air, to land on all fours. All legs must be free of body on landing.

RESULTS

The treated animals (rivastigmine, 2mg/Kg pc) presented retardation in the somatic development, which were verified in the body weight gain and skull axis growth patterns. The statistical analysis identified effects of time and rivastigmine's treatment, as well as the interaction factor between these two variables in every measurement studied. In the 11th day of life, the treated young weighted four per cent less than the control animals ($C = 28.6 \pm 2.4$, $T = 25 \pm 3.2$), and this percentage increased ($p < 0.001$) to thirteen per cent after the weaning ($C = 53.9 \pm 4.5$; $T = 47.2 \pm 6.5$). During this

period, there was significant interaction age versus drug: $F(4.4, 270) = 120.1$, $p < 0.0001$ for the body weight (Fig. 1).

Skull axis presented drug effects by the 10th day of life: Control (MLHA 15.3 ± 0.3 , $n=18$; APHA 30.2 ± 0.6 , $n=18$, BL 90.7 ± 3.2 , $n=18$; TL 44.5 ± 1.9 , $n=18$), Rivastigmine (MLHA 14.6 ± 0.4 , $n=18$; APHA 29.6 ± 0.8 , $n=18$, BL 87.7 ± 3.4 , $n=18$; TL 42.5 ± 2.8 , $n=18$), $p < 0.0001$ (Fig. 2A, 2B, 3A and 3B).

The day of maturation of the analyzed reflexes and physical characteristics (Table 2) did not show differences between the control and the rivastigmine groups.

Figure 1

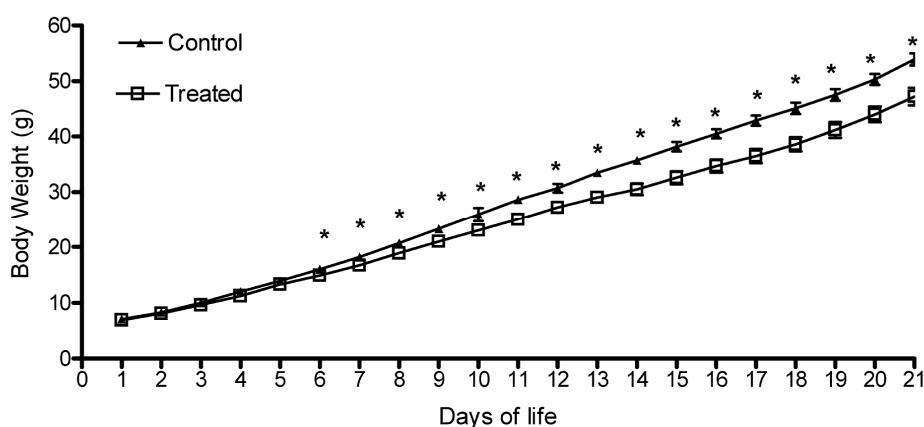


Fig. 1. Body weight of suckling rats from the 1st to 21st days of life, treated with saline solution ($n = 17$) or rivastigmine (2 mg/kg, $n = 17$). The results represent mean \pm S.D. * $P < .001$.

Figure 2A

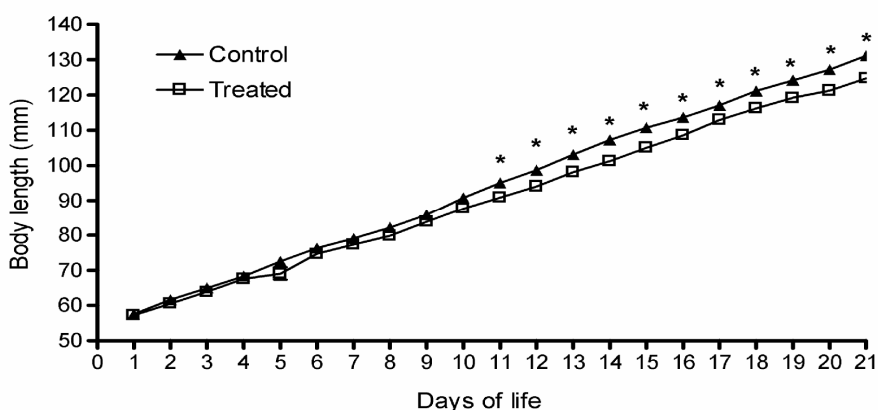


Figure 2B

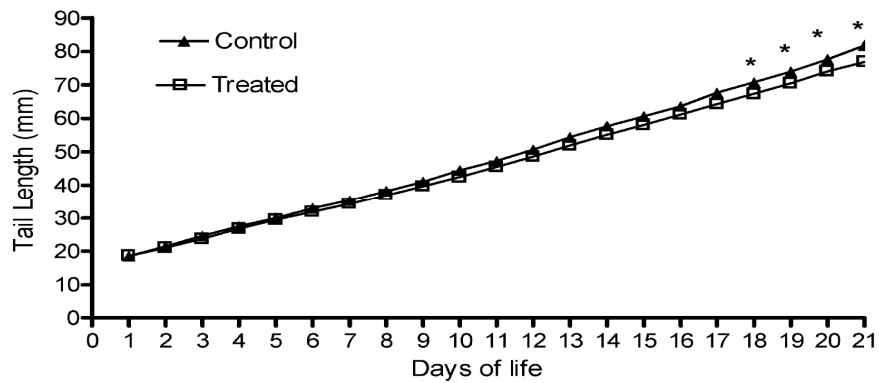


Fig. 2. Body length (A) and tail length (B) of suckling rats from the 1st to 21st days of life, treated with saline solution ($n = 17$) or rivastigmine (2mg/kg, $n = 17$). The results represent mean \pm S.D. * $P < .001$.

Figure 3A

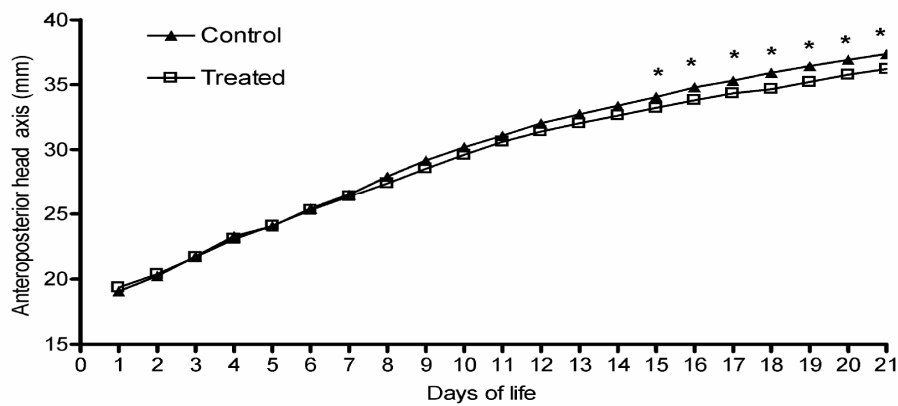


Figure 3B

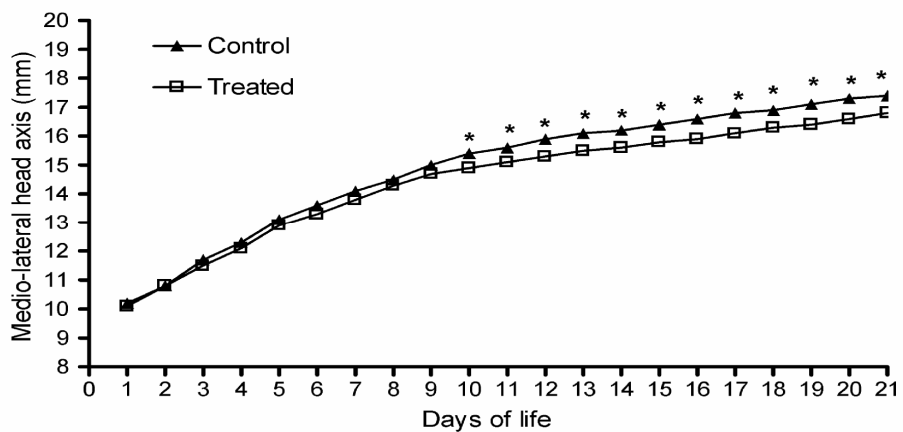


Fig. 3. Head axis length of suckling rats from the 1st to 21st days of life, treated with saline solution ($n = 17$) or rivastigmine (2 mg/kg, $n = 17$). (A) Antero– posterior axis (APA), (B) Médio– lateral axis (MLA). The results represent mean \pm S.D. * $P < .001$.

Tabela 2

Effect of neonatal rivastigmine administration on the development of physical characteristics and onset of behavioral reflexes in rats.

Appearance (righting, free-fall righting, negative geotaxis, cliff avoidance, auditory startle response and vibrissa placing) day in which the reflex and physical features matured (ear unfolding, auditory conduit opening, incisor eruption and eye opening) of suckling rats treated from the 1st to the 21st days of life with saline solution (n = 17) or rivastigmine (2 mg/kg, n = 17). The results represent median, maximum and minimum values.

INDEXES OF MATURATION GROUPS	CONTROL	TREATED
(A) Physical characteristics		
Ear unfolding	2.4 (3– 2)	2.6 (3– 2)
Auditory conduit opening	12.2 (13– 11)	12.2 (13– 12)
Eruption of the lower incisors	10.9 (12– 10)	10.3 (12– 9)
Eyes opening	14.1 (15– 13)	13.4 (15– 12)
(B) Reflexes		
Righting	4.5 (8– 3)	3.8 (6– 2)
Vibrissa placing	9.6 (11– 8)	8.8 (12– 6)
Cliff avoidance	9.3 (13– 8)	8.8 (12– 7)
Negative geotaxis	11.3 (13– 9)	11 (13– 9)
Auditory startle	12.1 (13– 11)	12 (12– 11)
Free-fall righting	12 (13– 11)	11.7 (13– 10)

DISCUSSION

Treatment with rivastigmine during the lactation period caused retardation in the somatic growth, which was evidenced by the reduction of body weight and measurements of skull and body axis of the animals. The deficit of the growth increased when leading to the weaning. However, no alterations in the development of physical characteristics or reflexes were observed. We identified, by these results, distinct reactions to the precocious exposure to the acetylcholinesterase inhibitor in factors concerning growth and development. The manipulation of other neurotransmission systems demonstrated a liaison between the type of neurotransmitter and the altered characteristic (3-5).

In the present study, we observed that the treatment with rivastigmine (2mg/Kg, p.c.) retarded the evolution of the animals' body weight. This effect

may be consequence of food ingestion reduction, since drugs that affect the cholinergic system inhibit the food ingestion, acting in the weight gain control (21-23). The acetylcholine is present in neurons of the arched hypothalamus' nucleus (24), one of the central nervous system's structures involved in the regulation of the eating behavior (25). The potentialization of acetylcholine, with acetylcholinesterase inhibitors, causes the liberation of pro-opiomelanocortin (POMC) and the transcript related to amphetamine-cocaine (CART) in the arched nucleus, inhibitor substances of food ingestion (26). We also observed that various somatic growth parameters such as body and skull axis suffered retardation caused by the exposition to the potential agent of the cholinergic system. Classic neurotransmitters such as dopamine (27), noradrenalin (3), serotonin (28), gamma-aminobutyric acid (29) and particularly acetylcholine (30) signalizes the growth in animal species. These

substances regulate the orthogenesis, including the early stages of the nervous system development (31, 32). In this phase, exposure to environmental aggressors may affect the cholinergic system, jeopardizing the cerebral development, with morphological, functional and neurochemical consequences (33). The reduction of cholinergic activity with bilateral lesion of the hippocampus in the adolescence caused retardation of the somatic growth (34). This result indicates stimulatory action of acetylcholine in the growth, whereas our study proposes inhibitory action. However, these studies promoted intervention in the cholinergic system in different phases of the development, which suggests different roles of this neurotransmitter in the growth process, depending on the phase of this phenomenon. Earlier researches in our laboratory showed that the potentialisation of the serotonergic system during lactation, with serotonin recapitulation inhibitors (citalopram e sertralina) in various doses, caused important effect on the development and growth in rats. In these studies, it was observed retard in the somatic growth, affecting the skull axis and the lengths of the tail and body (4, 5). In another study, utilizing citalopram (10mg/Kg p.c.), it was also observed reductions in the cranial measurements, which were related to weight reduction and smaller encephalon (28).

In the present study, the action of acetylcholine was different under parameters of motor-sensorial development and somatic growth. The skull and body axis, as well as the body weight, seem to be vulnerable to alterations of this neurotransmitter during the period of rapid growth, which does not occur with the reflexes maturation. Similar results were observed in neonatal exposure to clomipramine, which inhibits the recapitulation of serotonin and noradrenalin (3). The exposure to clomipramine during lactation caused problems in somatic growth parameters, reducing the skull and body axis, but, however, maintaining a normal motor-sensorial development, and thus, not interfering in the day of reflexes' maturation (3). On the other hand, selective potentializer of the

serotonin action also promoted developmental reflexes retardation (4, 5). A reflex is a behavior provoked by pre established and precise stimulation (20). The many reflexes overlay, causing the simultaneous occurrence of various events of the nervous system development (20, 35). Thus, comparing the exposition to agents that alter the neurotransmission and the study of somatic growth and reflex maturation, we are able to gather information about neurotransmitters' specific actions on these processes.

During certain vulnerable periods of their growth and development, organisms may be submitted to the exposure of substances that interfere in the neurotransmission via drugs, and, in acetylcholine's case, via alimentation. Acetylcholinesterase inhibitors are used in agriculture as pesticides. Recent researches show that the exposure to pesticides harms the nervous system development. However, studies show that the pernicious reaction of this exposure on the neural development may occur because of alterations in other neurotransmission systems, such as the serotonergic.

Summing up, the acetylcholinesterase inhibition during the encephalon's vulnerable period troubles the somatic growth; on the other hand, it does not interfere in the sensorial-motor development of such animals. This data may indicate distinct roles of the acetylcholine on the growth and development processes.

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