Original Article

Chronic Reboxetine Treatment does not Delay Somatic and Reflex Development of Rats

Oliveira, L.S.[1], Oliveira-de-Lira, L.[1], Galvão, B.H.A.[1], Silva, A.A.M.[1], Souza, S.L.[2], Azmitia, E.C.[3], Nogueira, M.I.[4], Manhães-de-Castro, R.[1].*

RESUMO

O período de aleitamento é considerado crítico para o desenvolvimento do sistema nervoso. Insultos durante este período pode resultar em alterações no crescimento e desenvolvimento, bem como as observadas com inibidor da recaptação serotonina ou serotonina/noradrenalina. O objetivo desse trabalho foi estudar o efeito da manipulação farmacológica com reboxetina, um inibidor seletivo da recaptação de noradrenalina, durante o período crítico de desenvolvimento do sistema nervoso, sobre o crescimento e desenvolvimento. Ratos Wistar neonatos foram tratados com solução salina (NaCl 0,9%, s.c.), ou reboxetina (20mg/Kg p.c., s.c) durante os primeiros 21 dias de vida. Durante esse período foi avaliado as medidas de crescimento somático e a ontogênese de reflexos. O inibidor seletivo da recaptação de noradrenalina não alterou o crescimento somático, mas antecipou o reflexo de aversão ao precipício. Esses dados sugerem um efeito neuroprotetor da noradrenalina sobre o crescimento e desenvolvimento somático.

PALAVRAS-CHAVE: crescimento, desenvolvimento, período crítico, ontogênese de reflexos, noradrenalina, reboxetina.

ABSTRACT

The suckling period is considered critical to the development of nervous system. Injury during in this period can to result in alterations in growth and development, as well as those observed with serotonin or serotonin/noradrenaline selective reuptake inhibitor. The objective of this work was to study the effect of the pharmacological manipulation with reboxetine, a noradrenaline selective reuptake inhibitor, during the critical period of development of nervous system, on the growth and development. Neonates Wistar rats were treated with saline solution (NaCl 0,9%, s.c.), or reboxetine (20mg/Kg p.c., s.c) during the first 21 days of life. During this period it was evaluated the measures of somatic growth and reflex ontogeny. The noradrenaline selective reuptake inhibitor does not alter somatic growth, but anticipates the cliff avoidance reflex. This data suggests a neuroprotective effect of the noradrenaline on the somatic growth and development.

KEY WORDS: growth, development, critical period, reflexes ontogeny, noradrenaline, reboxetine.

[1]Departamento de Nutrição, Universidade Federal de Pernambuco, Cidade Universitária, CEP 50670-901, Recife, PE, Brazil.
[2]Departamento de Anatomia, Universidade Federal de Pernambuco, Cidade Universitária, CEP 50670-901, Recife, PE, Brazil.
[3]Department of Biology, New York University, New York, NY 10003, USA.
[4]Instituto de Ciências Biomédicas, Departamento de Anatomia, laboratório de Neurociências da Universidade de São Paulo, CEP 5508900, São Paulo, SP, Brazil.
[*]Corresponding author: Lisiane dos Santos Oliveira, Departamento de Nutrição, Centro de Ciências da Saúde, UFPE, Av. Morais Rego, 1235, Cidade Universitária, CEP 50670-901, Recife, PE, Brasil. E-mail: lisianenutricao@yahoo.com.br

1. INTRODUCTION

Neurotransmitters are present during the early developmental stages of mammals (1, 2) and in primitive organisms like the sea urchin (3,4). During such stages, neurotransmitters may act as hormones or as growth factors. Noradrenaline (NA) is one of such compounds that, besides playing a relevant role as a neurotransmitter in adult life (5), participates in the processes of cellular organization, regulating neuronal migration, differentiation and synaptogenesis, during embryogenesis, thus contributing functionally to neural plasticity (6, 7, 8, 9). Such functions have been demonstrated by the expression of metabolic enzymes, and the density and sensitivity of noradrenalin receptors (6, 10, 11).

Injury to the nervous system during its critical maturation period can produce long-term changes in its structure and organic function (12). Increased catecholamine levels during the perinatal phase result in stress and morphofunctional damage as a consequence of the substances involved (9). By increasing the synaptic availability of neurotransmitters selective reuptake inhibitor antidepressants (12, 13), induce the above mentioned alterations in physical growth and developmental parameters (14, 15). Treatment of neonatal rats by daily injection of the serotonin-noradrenaline reuptake inhibitor (SNRI), Clomipramine (14), or injection of the specific serotonin reuptake inhibitor (SSRI) Citalopram or Sertraline (15, 16), produces somatic developmental changes. The use of SSRI, differently from the SNRI, produces a more pronounced delay in somatic development, and also alters the maturation of reflexes characteristic of this phase, indicators of sensory-motor development.

Thus, both the noradrenergic and serotonergic systems play important roles during ontogenesis. This finding may constitute to a public health issue since the prescription of monoaminergic selective reuptake inhibitors has increased lately (17, 18). Our aim is to investigate these interactions, and to explore the hypothesis of a protective noradrenergic role during development.

2. MATERIAL AND METHODS

2.1. Animals

Rattus norvergicus, Wistar strain, from the colony of the Department of Nutrition, Federal University of Pernambuco, Brazil, were coupled to obtain litters. The male pups, conceived by different mothers, received a small signal of methyl violet solution on their skin, and were distributed into litters of six neonates each, 24 h after birth. From each litter, three pups were used as controls and three were assigned to the experimental groups. Each pup was suckled by one of the dams until the postnatal 21 day (PND; day of birth was considered as day 0). The animals were maintained at a room temperature of 23 °C, on a light–dark cycle of 12:12 h (lights on at 0600 h) with free access to food (Labina, Purina, Brazil) and water.

These animals were used in a behavioral study during adult life. The experimental procedures followed the *International Guiding Principles for Biomedical Research Involving Animals,* and approved for ethic committee in animal experimentation from Universidade Federal de Pernambuco.

2.2. Pharmacological treatment and experimental groups

The experimental (n=25) and control groups (n=25) received a single, daily, subcutaneous injection of Reboxetine (20 mg/kg, 1 ml/kg) or saline vehicle, respectively, at 1400 h during the suckling period (from the PND 1 to 21). Reboxetine, obtained in the salt form (Reboxetine metanosulfonate, Pfizer, USA), was dissolved and diluted in saline. The dose used was in accordance with the literature (19) and was non-lethal or toxic.

2.3. Observations and measurements

The progress of each animal was accompanied longitudinally during the experiment; on a daily schedule between 1300 and 1400 h. Records of physical maturation were taken according to the criteria of Fox (20). The examiner was unaware of the animals' treatment category (control or experimental group). The growth indicator parameters appraised were: body weight; horizontal axis of the

head (least distance between ear holes); rostro-caudal axis of the head (least distance between the snout and head articulation of the head with the neck); antero-posterior axis of the body; and tail length.

The same schedule was adopted for the observing onset of the following physical characteristics: ear unfolding – unfolding of both the external ears to the fully erect position; auditory conduit opening – opening of the internal auditory conduit of both ears; incisor eruption – eruption of the lower incisors (which always follows that of the upper incisors), and eye opening – any visible rupture in the membrane covering an eye.

2.4. Ontogeny of reflexes

Tests for reflexes were performed between 1200 and 1300 h and were carried out according to a modification of Smarting & Dobbing (21): righting, free-fall righting (acceleration), negative geotaxis, cliff avoidance, auditory startle response and vibrissa placing. The day of consolidation of each approaching reflex was considered as day 1, in a sequence of three consecutive days, of the complete emergence of the expected reflex response.

2.5. Statistical Analysis

Statistical analysis was performed using Student's t-test. The Mann-Whitney test was used to analyze differences in reflexes and physical characteristics.

3. RESULTS

3.1. Physical development

Comparison of somatic development between rats treated with the NARI, Reboxetine, and the control group revealed no significant difference from the post natal days 1 to 21 for all analyzed parameters ($p>0,05$; Student's t-test).

The median weight gain was 733% in the control rats and in the experimental animals 695% (Figure 1). Median head growth was 200% in the horizontal axis in the control group and 176% in the experimental group; growth in the anterior-posterior axis was 194% for both groups (Figures 2 A, B and 3).

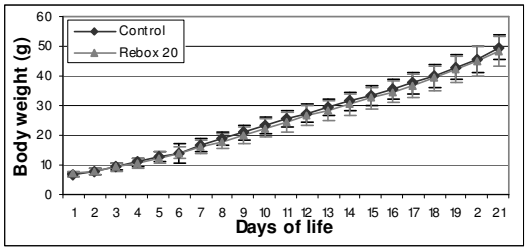

Fig. 1. Body weight of suckling rats from days 1 to 21 of life, treated with saline solution (n=25) or Reboxetine (20 mg/kg, n=25). Data are the mean ± SD

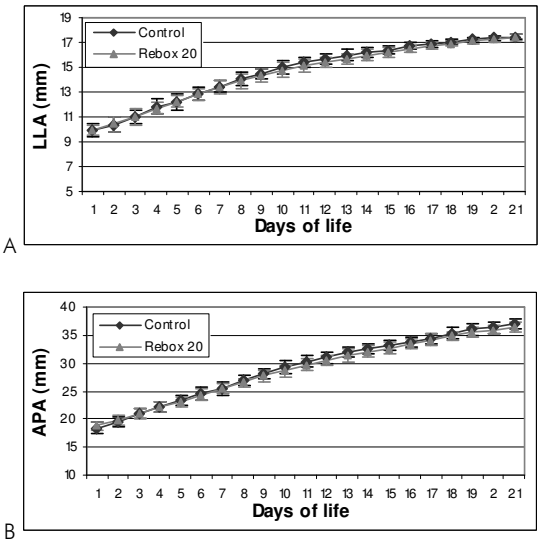

Fig. 2. Head axis length of suckling rats days 1 to 21 of life, treated with saline solution (n=25) or Reboxetine (20 mg/kg, n=25). (A) Horizontal axis (LLA), (B) Antero-posterior (APA). The data are the mean ± SD

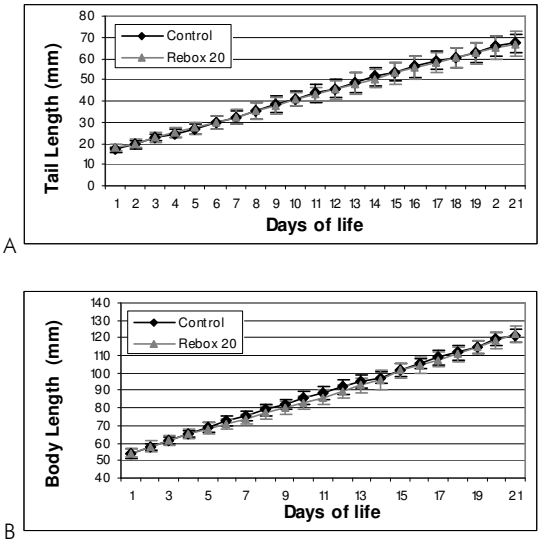

Fig. 3. (A) Body length and (B) Tail length of suckling rats from days 1 to 21 of life, treated with saline solution (n=25) or Reboxetine (20 mg/kg, n=25). The data are mean ± SD

Both the control and experimental groups presented an increase of 225% in body length in the longitudinal axis, although tail growth is considerably greater, increasing 395% in the control group and 370% in the experimental animals.

These experiments also demonstrated no difference in maturation of the physical characteristics in the group receiving the NARI compared to that receiving saline during the early postnatal period ($p>0.05$; Mann-Whitney) (Table 1A).

Table 1. Effect of Reboxetin treatment on the development of physical characteristics and on the onset of behavioral reflexes in neonatal rats.

Maturation Indexes	Groups	
	Control	Reboxetine
(A) Physical characteristics		
Ear unfolding	3 (2-5)	3 (2-5)
Auditory conduit opening	12 (10-13)	12 (10-14)
Eruption of the upper incisors	9 (7-10)	9 (8-10)
Eruption of the lower incisors	11 (10-13)	11 (10-13)
Eye opening	15 (13-16)	14 (12-16)
(B) Reflexes		
Righting	4 (1-8)	4 (2-8)
Vibrissa placing	10 (7-12)	9 (5-11)
Cliff avoidance	8 (6-12)	7 (4-10)*
Negative geotaxis	14 (12-17)	14 (10-17)
Auditory startle	12 (10-13)	12 (11-13)
Free-fall righting	15 (13-17)	15 (13-17)

Day of appearance (righting, free-fall righting, negative geotaxis, cliff avoidance, auditory startle response and vibrissa placing) on which the reflex and physical features matured (ear unfolding, auditory conduit opening, incisor eruption and eye opening) in suckling rats treated from days 1 to 21 of life with saline solution (n=25) or Reboxetine (20 mg/kg, n=25). The data are the median, maximum and minimum values. (*) $P<0.05$, Mann-Whitney test.

3.2. Ontogeny of reflexes

The treatment significantly influenced the time of onset of the cliff avoidance reflex, which was anticipated in the Reboxetine treated group compared to the control group. However, the other reflexes acquired during the suckling period were similar in both groups (Table 1B).

4. DISCUSSION

Different from other monoaminergic reuptake inhibitors, the NARI, administered chronically to male rats during the suckling period, did not affect the normal pattern of weight gain, or growth of the body, head and tail, or the maturation of physical characteristics. Furthermore, during the ontogenesis of developmental reflexes, except for the cliff avoidance reflex that was anticipated, no other reflex analyzed was altered.

The chemical environment of the developing nervous system has been explored under altered nutritional and/or pharmacological states. Such approaches aim to provide a better understanding of the influence of neuroactive substances during this critical period of life on the central nervous system itself, on growth parameters, and on the maturation of physical characteristics (14, 16, 22). Neonatal treatment with Clomipramine, SNaRI, caused delay in the somatic growth (14). However, the use of SSRIs such as Citalopram and Sertraline produces a more pronounced effect, since, in addition to delaying somatic growth, they also delay sensory-motor development (15,16). These findings reveal a differential effect related to the nature of the reuptake inhibitor used; use of a double inhibitor for both noradrenalin and serotonin, produced less effect than when a serotonin reuptake inhibitor alone was used. Given their action as selective inhibitors that increase the neurotransmitter availability, this data suggest that serotonin inhibition alone produced the above mentioned affects when used coupled with noradrenalin in the treatment with Clomipramine (14). This hypothesis is corroborated by the present study, since the effect of a specific noradrenaline reuptake inhibitor, Reboxetine, on the same parameters analyzed in previous research was unchanged. Indeed, noradrenalin seems to exert a protective influence during physical development and at the level of the nervous system. The question remains: what mechanisms might underlie such findings? Research in humans suggests that the release of growth hormone increases with neonatal administration of NARI (23) while the rate of somatic

growth is decreased in children and teenagers treated with SSRI, probably through a secondary action suppressing growth hormone (24).

Differently from the administration of SSRI and SNRI, respectively (14, 15,16), which causes growth and maturational delays, the use of NARI, which anticipated only the cliff avoidance reflex, corroborates the above mentioned hypothesis. The cliff avoidance reflex, in being related to an individual's survival instinct when exposed to a risk situation, also comes under management of the noradrenergic system (21). Further, the central noradrenalin regulates development of the Cajal-Retzius cells development; these are the early neurons found in the developing cortex, which are responsible for the neuronal migration and the laminar formation of the cortex (25).

The absence of a delay in the maturation of most reflexes seen in this study with NARI, is similar to other findings obtained using NARI (14), but in disagreement with studies using only SSRI alone (15). Such findings are supported by data showing effect of noradrenalin on muscle-nerve interactions. Research has revealed a trophic noradrenergic role in the development of spinal cord motoneurons (26,27), also related to spinal reflex facilitation followed by locus coeruleus stimulation (28), and to increased mono- and poly-synaptic reflexes, which appear after elevated doses of L-DOPA (26).

In conclusion, the present findings unequivocally reveal differential action of neurotransmitters during ontogeny, particularly selective serotonin and noradrenalin reuptake inhibitors, the most common drugs prescribed as antidepressants. We confirm the neuroprotective effect of noradrenalin on somatic growth and development, and we emphasize the need for careful consideration when prescribing drugs which increase serotonin in pregnant women or young individuals, despite beneficial effects on depressive disturbances.

Acknowledgements: This study was financed by FAPESP, CAPES, CNPq and ECA (MH55250), Pfizer provided the Reboxetine compound.

5. REFERENCES

1. Lauder JM and Krebs H. Serotonin as a differentiation signal in early neurogenesis. Dev Neurosci 1978;1:15-30.

2. Weiss ER; Maness P; Lauder JM. Why do neurotransmitters act like growth factors? Perspect Dev Neurobiol 1998;5:323-335.

3. Wallace JA. Monoamines in the early chick embryo; Demonstration of serotonin synthesis and the regional distribution of serotonin-concentrating cells during morphogenesis. Am, J. Anat 1982;165:261-276.

4. Busnikov GA; Lambert HW; Lauder JM. Serotonin and serotonin-like substances as regulators of early embryogenesis and morphogenesis. Cell Tissue Rep 2001;305:177-186.

5. Moore RY; Bloom FE. Central catecolamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. Annu Rev Neurosci 1979;2:113-168.

6. Lauder JM. Neurotransmitters as growth regulatory signals: role of receptors and second messengers. Trends Neurosci 1993;16:233-240.

7. Rowe SJ; Messenger NJ; Warner AE. The role of noradrenaline in the differentiation of amphibian embryonic neurons. Development 1993;119:1343-1357.

8. Pendleton RG; Rasheed A; Roychowdhury R; Hillman R. A new role for catecholamines: ontogenesis. Trends Pharmacol Sci 1998;19:248-251.

9. Herlenius E and Lagercrantz H. Development of neurotransmitter systems during critical periods. Experimental Neurology 2004;190:8-21.

10. Meier E; Hertz L; Schousboe A. Neurotransmitters as developmental signals. Neurochem Int 1991;19:1-15.

11. Whitaker-Azmitia PM. Role of serotonin and other neurotransmitter receptors in brain development: basis for developmental pharmacology. Pharmacol Rev 1991;43:553-561.

12. Sayer AA, Cooper C, Barker DJ. Is lifespan determined in utero? Arch. Dis. Child. Fetal Neonatal Ed 1997;77:162-164.

13. Carrasco JL, Sandner C. Clinical effects of pharmacological variations in selective serotonin reuptake inhibitors: an overview. Int J Clin Pract 2005;59:1428-1434.

14. Souza SL; Nogueira MI; Deiró TCBJ; Castro FMM; Silva CM; Silva MC; Lira LO; Azmitia EC; Manhães-de-Castro R. Differential effects on somatic and reflex development by chronic clomipramine treatment. Physiol Behav 2004;82:375-379.

15. Deiro TC,Manhaes-de-Castro R,Cabral-Filho JE,Barreto-Medeiros JM,Souza SL,Marinho SM,Castro FM,Toscano AE,Jesus-Deiro RA,Barros KM. Sertraline delays the somatic growth and reflex ontogeny in neonate rats. Physiol Behav 2006;87:338-344.

16. Deiró TCBJ; Manhães-de-Castro R; Cabral-Filho JE; Souza SL; Freitas-Silva SR; Ferreira LMP; Guedes RCA; Câmara CRV; Barros KMFT. Neonatal administration of citalopram delays somatic maturation in rats. Braz J Med Biol Res 2004;37:1503-1509.

17. Boscarino BT, Parfitt DB. Chronic oral administration of clomipramine decreases sexual behavior in the male Syrian hamster (Mesoocricetus auratus).Physiol Behav 2002;75:361-366.

18. Sanders JD; Happe HK; Bylund DB; Murrin LC. Development of the norepinephrine tranporters in the rat CNS. Neuroscience 2005,130:107-117.

19. Cryan JF, Page, ME, Lucki I. Noradrenergic lesions differentially alter the antidepressant-like effects of reboxetine in a modified forced swim test. Eur J Pharmacol 2002;436:197-205.

20. Fox MW. Reflex-ontogeny and behavioral development of the mouse. Animal Behavior 1965;13:234-241.

21. Smart JL, Dobbing J. Vulnerability of development brain: VI. Relative effects of fetal and early postnatal undernutrition on reflex ontogeny and development of behavior in the rat. Brain Res 1971;33:303-314.

22. Barros KM, Manhaes-De-Castro R, Lopes-De-Souza S,Matos RJ, Deiro TC, Cabral-Filho JE, Canon F. Related Articles. A regional model Northeastern Brazil of induced mal-nutrition delays ontogeny of reflexes and locomotor activity in rats. Nutr Neurosci 2006;9:99-104.

23. Schule C, Baghai T, Laakmann G. Mirtazapine decreases stimulatory effects of reboxetine on cortisol, adrenocorticotropin and prolactin secretion in healthy male subjects. Neuroendocrinology 2004;79:54-62.

24. Weintrob N, Cohen D, Klipper-Aurbach Y, Zadik Z, Dickerman Z. Decreased growth during therapy with selective serotonin reuptake inhibitors. Arch Pediatr Adolesc Med 2002;156:696-701.

25. Naqui SZ, Harris BS, Thomaidou D, Parnavelas JG. The noradrenergic system influences the fate of Cajal-Retzius cells in the developing cerebral cortex. Brain Res Dev Brain Res 1999;113:75-82.

26. Ono H, Fukuda H. Pharmacology of descending noradrenergic systems in relation to motor function. Pharmacol Ther 1995;68:105-112.

27. Tanaka H, Takahashi S, Miyamoto A, Oki J, Cho K, Okuno A. Developmental changes in the noradrenergic innervations of spinal motoneurons in neonatal rats. Pediatr Neurol 1996;14:21-27.

28. Ono H, Hasebe Y, Satoh M, Nagao T, Ohta S, Hirobe M, Fuduka H. Amphetamine-antagonistic properties of 4-phenyl-1,2,3,4- tetrahydroisoquinoline: inhibition of spinal reflex-enhancing effects of methamphetamine, phenylethylamine and nomifensine. Brain Res 1991;564:319-322.