

Full Length Research Paper

Indomethacin during early perinatal age is associated with low levels of anxiety and stress at pre- and post-pubertal age

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It has been observed that indomethacin has undesirable effects on CNS like reduced white matter, cognitive dysfunction, depersonalization, hallucination and psychosis. So, the aim of the present work was to investigate if neonatal administration of indomethacin causes behavioral changes related with anxiety and stress. Thirty-two male Sprague Dawley rats were randomly divided into four groups. Three groups received indomethacin administration at different postnatal day. The first group received indomethacin at P2-P3 day; the second group was administrated at P4-P6 day; the third group received the administration at P7-P9 day, and the control animals (group 4) were injected with an equal volume of the vehicle. It was analyzed the effect of indomethacin administration on the behavioral tests (traveled distance, social contact, and immobility response) at day 36 and 57 postnatal. It was found that indomethacin reduced the traveled distance and ambulatory time at pre and post-pubertal age, meanwhile indomethacin administration at P7-P9 caused an increase of traveled distance. Also, indomethacin increased the contact number at pre-pubertal age with a reduction at post-pubertal age. Finally, the indomethacin administration modified immobility responses. We conclude that indomethacin administration during post-natal age causes stress or anxiety related behavior because open field test, social interaction test, and immobility responses presented differences.

Keywords: Indomethacin, behavioral test, anxiety, stress, perinatal age.

INTRODUCTION

Stress is characterized by physiological changes that occur in response to novel or threatening stimuli. Stressful events can activate hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA axis increase the release of corticotrophin-releasing hormone (CRH) from the hypothalamic paraventricular nucleus, which in turn stimulate the secretion of adrenocorticotropin (ACTH) from the anterior pituitary concluding in the secretion of glucocorticoids (Herman and Cullinan, 1997). Also, glucocorticoids affect limbic system that modulates

behavior. For example, high levels of glucocorticoids in mygdale and hippocampus are implicated in psychiatric disorders as autism, attention-deficit hyperactivity disorders and anxiety (Shin and Liberzon, 2010). These hormones affect the neurocircuitry into limbic structures, and when it occurs during neonatal age, they modulate behavioral responses like fear, stress and anxiety. It has been observed that dopaminergic and serotonergic systems are critical during postnatal CNS development and prematurity (Galineau et al., 2004).

Prematurity is associated with adverse neonatal outcome including intraventricular hemorrhage, respiratory distress syndrome, periventricular leukomalacia and patent ductus arteriosus. The arterial duct is an essential fetal structure and only becomes

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abnormal if it remains open after the neonatal period. Persistence of the arterial duct has been defined as continued patency in term infants older than 3 months. Patent ductus arteriosus is the most frequent congenital heart abnormality in Mexico (Luis-Miranda et al., 1996) and the drugs widely used for its treatment are the cyclooxygenase inhibitor of prostaglandin synthesis like ibuprofen and indomethacin (Chorne et al., 2007). However, it has been observed that indomethacin has undesirable effects on CNS like reduced white matter (Miller et al., 2006), cognitive dysfunction, depersonalization, hallucination and psychosis (Tharumaratnam et al., 2000).

So, the aim of the present work was to investigate if neonatal administration of indomethacin causes behavioral changes related with anxiety and stress.

MATERIAL AND METHODS

Animals

All animal procedures were carried out in accordance with the guidelines of the Laws and Codes of Mexico in The Seventh Title of the Regulations of the General Law of Health Regarding Health Research. Thirty-two male Sprague Dawley rats were randomly divided into four groups.

The day of birth was taken as postnatal day 0 (P0) and litters with a maximum of 8 male neonatal rats were formed. The day after birth, male pups were assigned to either, a treatment group or a control group. The treated animals received 50 mg/Kg indomethacin (Sigma, Co.) once daily at 0900, whereas the control animals were injected with an equal volume of the vehicle (5% NaHCO₃). Four groups of animals were formed. Rats received indomethacin from day P2 to P3, P4 to P6, and P7 to P9 with their corresponding control groups. On P21, animals were weaned and housed in acrylic cage per group.

Behavioral Procedure

All behavioral testing was done between 900 and 1200 and they were recorded on videotape using a VHS video camera (NV-N3000PN, Panasonic).

At day 35 and 56 the rats were brought, in an individual plastic cage to the testing area which, it was in the same building and floor as the colony room. Two animals from the same experimental group were placed on the testing area and 10 min of their social interaction was videotaped and automatically analyzed by the Videomex V apparatus (Columbus Instruments, Columbus, OH). After each trial, the rats were returned to the colony room and the area was cleaned with a neutral detergent. The test time of day was balanced across

experimental groups.

At day 36 and 57 each rat was brought in an individual plastic cage to the testing area. The animal was placed on the open field for 6 min and locomotor behavior was recorded every 3 min. After this, the animal was tested for the immobility induced by clamping the neck and dorsal immobility. Each test was separated about 3 minutes. When all behavioral testing were completed, the rat was returned to the colony room and the open-field area was cleaned with a neutral detergent. The test time of day was balanced across experimental groups.

The behavioral test was analyzed by a Student's t-test. P less than 0.05 was considered statistically significant.

Behavioral testing

Social Contact Test

It was used the test as previously we described (Pineda-Reynoso et al., 2010). Twenty-four hours previous to test, animals were singly housed in a metallic cage (20 x 30 x 18 cm) with food and water ad libitum. The cages were located together in racks for maintaining auditory and olfactory contact. The test was performed in an open arena (60 x 60 x 30 cm). The floor was made of smooth black wood. The light conditions were comparable to the light intensity in the housing room; two 39-W overhead fluorescent bulbs were suspended 180 cm above the center of the field and provided 210 Lx at floor. The social behavior was measured using a video image analyzer (Videomex-V) during 10 min. The social contact program provides a simple method for recording the interaction between two animals. So, it gives the total number of contacts during the experiment and the total amount of time the animals have been in contact.

Open-field test in an unknown environment

this test has been regularly used to assess emotionality in rodents (Walsh and Cummins, 1976) and it was made as previously we described (Blas-Valdivia et al., 2009). The animal was transferred to the testing room and immediately placed on the middle of the open field (black-painted wooden box, 60 x 60 x 30 cm). Light condition was comparable to the social contact test. The spontaneous locomotor activity in unfamiliar environment was measured using a video image analyzer in two sessions of three min each one. With this test it has recorded the distance of the animal travels, the amount of time spent traveling, the amount of time spent in a non ambulatory activity, and the amount of time resting. It also displays the path tracings during the session.

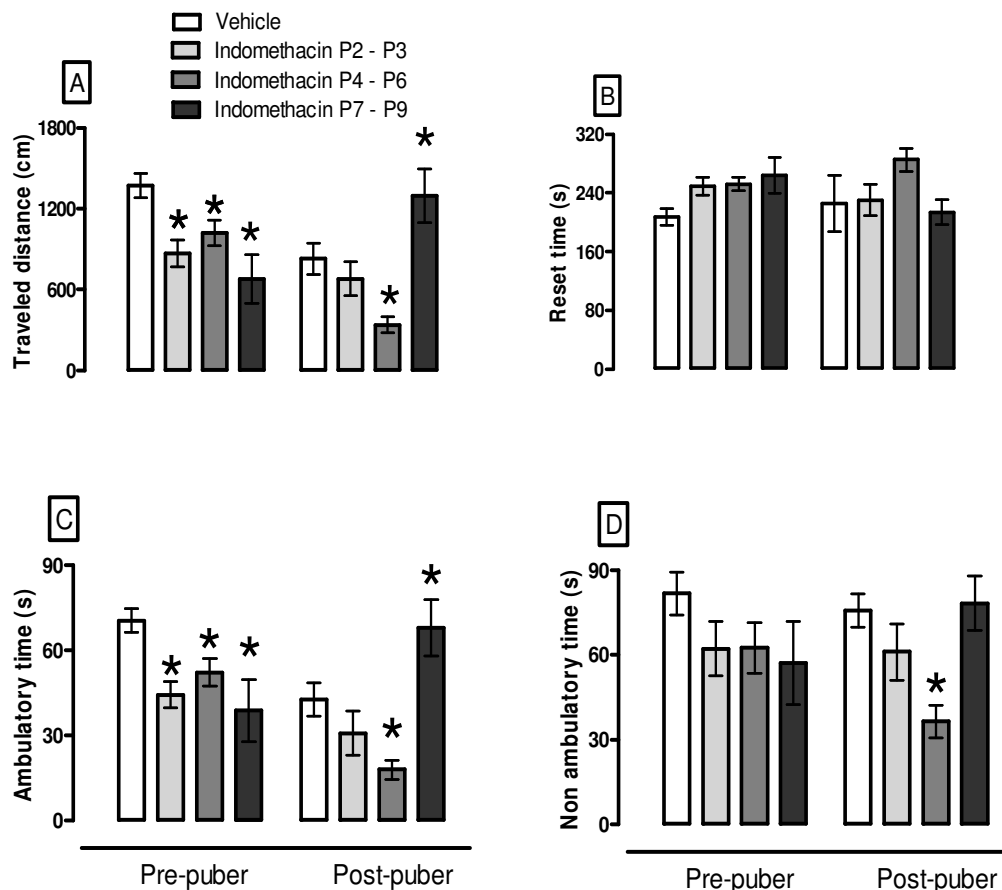


Figure 1. Effect of indomethacin post-natal administration on traveled distance (A), amount of time resting (B), as well as amount of ambulatory (C) and non ambulatory (D) time during six minutes of open field test in an unknown environment. * $P < 0.05$ vs. vehicle at same age.

Immobility induced by clamping the neck

The immobility response was made by applying a clamp (a 5-cm alligator clip with its tips covered with masking tape to avoid any injury to the rat's skin) between the base of the skull and the back of ears with the pressure being sufficient to lift the whole animal by the clamp. A second clamp was applied to the ventral part of the neck to increase the pressure in this area. The animal was then inverted to a supine position. The duration of immobility was measured from the time the animal was placed on its back until it recovered the prone position or until 180 s had elapsed (Blas-Valdivia et al., 2009; Pineda-Reynoso et al., 2010).

Dorsal immobility

The animal was gently grasped by the skin at the back of the neck and lifted off its feet 30–40 cm over the experimental table, avoiding disturbing the animal's breathing and applying only enough pressure to lift the whole rat body. When animal stopped moving, the time of

immobility was recorded until the animal made escape-like movements directed at the experimenter's hand or until 180 s had elapsed (Blas-Valdivia et al., 2009; Pineda-Reynoso et al., 2010).

RESULTS

Open-field test

Figure 1 shows how indomethacin affects the spontaneous locomotor activity measure in the open-field test as traveled distance (between age $F_{1,56} = 5.90$, $P < 0.05$; between treatment $F_{1,56} = 6.12$, $P < 0.001$; and interaction age \times treatment $F_{1,56} = 11.40$, $P < 0.001$), reset time (between age $F_{1,56} = 0.11$, $P = 0.74$; between treatment $F_{1,56} = 3.09$, $P < 0.05$; and interaction age \times treatment $F_{1,56} = 1.96$, $P = 0.13$), ambulatory time (between age $F_{1,56} = 7.32$, $P < 0.01$; between treatment $F_{1,56} = 6.83$, $P < 0.001$; and interaction age \times treatment $F_{1,56} = 9.73$, $P < 0.001$) and non ambulatory time (between age $F_{1,56} = 0.20$, $P = 0.66$; between treatment $F_{1,56} = 4.04$, $P < 0.05$; and interaction age \times treatment

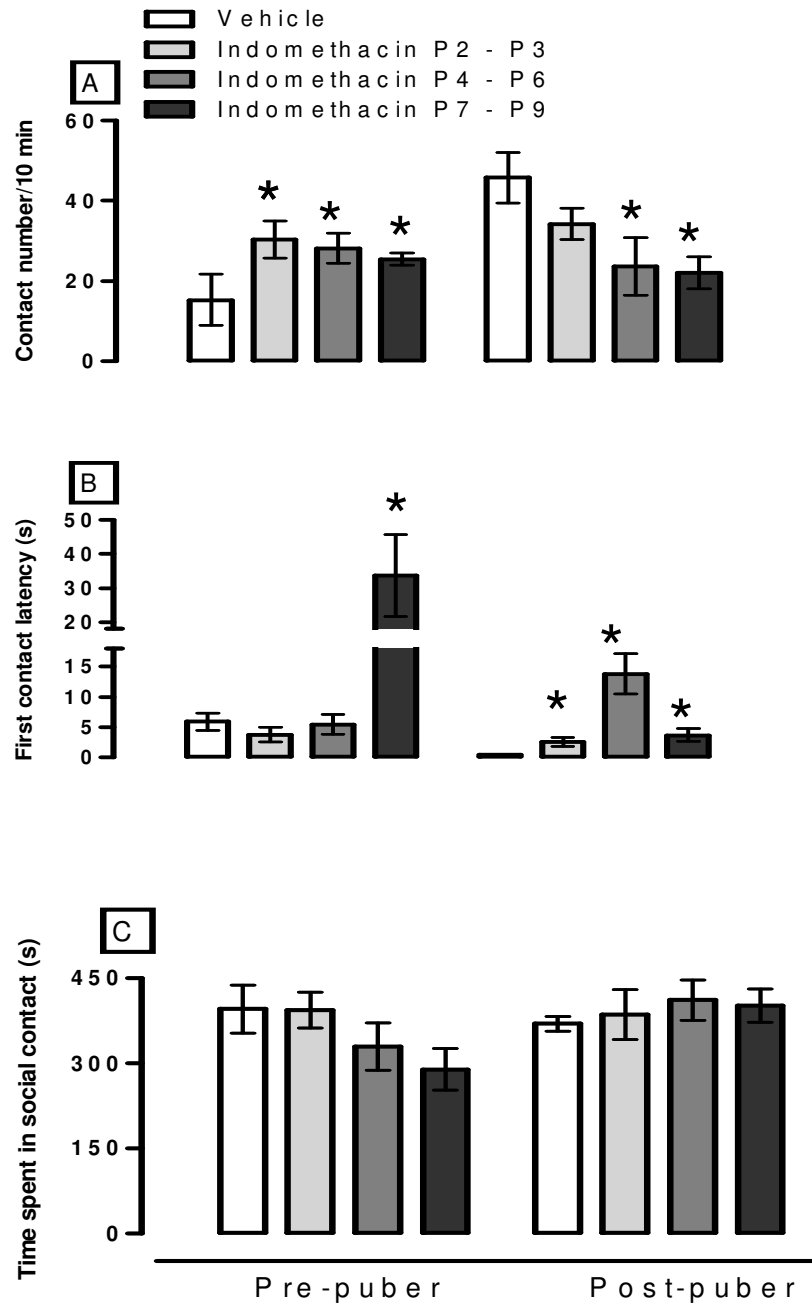


Figure 2. Effect of indomethacin post-natal administration on social contact number (A), first contact latency (B), and time spent in social contact number (C) in 10 minutes of behavioral test. * $P < 0.05$ vs. vehicle group at same age.

$F_{1,56} = 2.22$, $P = 0.1$). Panel A and C shows that indomethacin reduced the traveled distance (panel A) and ambulatory time (panel B) at pre and post-pubertal age when the administration were between P2 and P4, meanwhile indomethacin administration on P7-P9 caused a traveled distance increase. Also, it was shown that indomethacin did not affect the reset time (panel B) and the non ambulatory time (panel D).

Social contact test

Figure 2 reveals that animals which received indomethacin increased the contact number (panel A) at pre-pubertal age with a reduction on post-pubertal age (between age $F_{1,28} = 11.20$, $P < 0.05$; between treatment $F_{1,28} = 59.05$, $P < 0.0001$; and interaction age \times treatment $F_{1,28} = 0.04$, $P = 0.85$). Also, in panel B it was shown a delay in the first

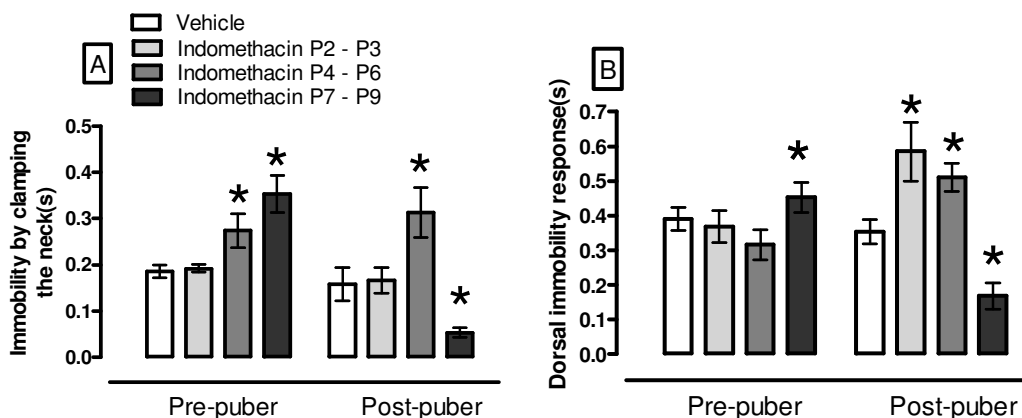


Figure 3. Effect of indomethacin post-natal administration on durations of immobility induced by clamping the neck (A) and dorsal immobility (B). * $P < 0.05$ vs. vehicle group at same age.

contact on pre-pubertal age when indomethacin was administered on P7-P9 and on post-pubertal age, in all treatments (between age $F_{1,28} = 1.43$, $P = 0.25$; between treatment $F_{1,28} = 5.03$, $P < 0.05$; and interaction age \times treatment $F_{1,28} = 3.01$, $P < 0.05$). The time spent in social contact was not affected by indomethacin treatment (panel C).

Immobility responses

Indomethacin administration modified immobility responses. Thus, panel A of figure 3 shows that indomethacin administration on P4-P6 and P7-P9 caused a higher duration of dorsal immobility at two ages. However, the P7-P9 group reduced dorsal immobility time on post-pubertal age (between age $F_{1,56} = 3.11$, $P = 0.08$; between treatment $F_{1,56} = 4.41$, $P < 0.01$; and interaction age \times treatment $F_{1,56} = 3.01$, $P < 0.05$). Also, panel B of figure 3 shows that only P7-P9 group increased immobility by clamping the neck on pre-pubertal age, meanwhile it was reduced at post-pubertal age (between age $F_{1,56} = 0.24$, $P = 0.62$; between treatment $F_{1,56} = 3.66$, $P < 0.05$; and interaction age \times treatment $F_{1,56} = 10.35$, $P < 0.001$).

DISCUSSION

Indomethacin is a drug widely used for obstetric prematurity illness. However, it has not considered its effect on long-term behavior. We observed that neonatal administration of indomethacin on P2 to P6 caused a reduce response to stress, meanwhile indomethacin administration on P7-P9 caused anxiety. This differential response is probably due the alteration on neurochemistry system, particularly dopaminergic and serotonergic (Galineau et al., 2004; Shin and Liberzon, 2010). Indomethacin could disturbance the ontogeny of

these neurocircuitries changing the behavior. For example, the expression of receptors could be modified, in particular, if indomethacin is enhanced dopamine levels in striatum in immature rat brains. This state could induce a reduction in the amount of dopamine receptors (down regulation) altering inhibition responses (Ogasawara et al., 1999).

With these results we conclude that indomethacin administration during post-natal age caused stress or anxiety related behavior because open field test, social interaction test, and immobility responses presented differences.

The social interaction test is a simple protocol that has been used in rats, to test for anxiety in a novelty environment. We proposed that indomethacin alters neuronal pathways related with hippocampus, due its neuronal structure participates in sequences of social behavior (starting, stopping or continuous social contact) (Maaswinkel et al., 1997). The indomethacin administration during neonatal period increases the number of contact, but these occur with a short duration. This is related with low-stress states. Also, the open-field test in an unknown environment confirms it. It is possible that indomethacin during CNS development deplete the serotonin content as well as p-chlorophenylalanine (Dringenberg et al., 1995). In this way, in the adult steady state the rats treated with indomethacin during neurodevelopment, produces high levels of serotonin reducing the exploratory activity in an unknown environment which caused neophobia in rodents (Mallo et al., 2007).

In the other way, in nature, the immobility response is part of antipredatory behavior and the last resources is used by prey to reduced the probably of predator attacks. Experimentally, immobility responses can be induced in laboratory and they are controlled by fear, and the use of a dopamine antagonist (as haloperidol). It is possible that activation of dopaminergic mesolimbic pathway which arrives to accumbens nucleus from ventral tegmental

area (VTA) modify the immobility duration response, since the animals with low locomotor response to novel environment, reduce their basal firing and the bursting activity of dopaminergic neurons in VTA (Marinelli and White, 2000). In addition, the immobility responses are easy behavioral test which shows a general idea of dopaminergic system state (Blas-Valdivia et al., 2009; Zamudio et al., 2005).

In general, indomethacin administration during neonatal period is related with low levels anxiety and stress. However, it would fail to clarify whether these behavioral events may be beneficial or detrimental respect to the mental health.

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