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Effects of *in utero* exposure to *Tityus bahiensis* scorpion venom in adult rats

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ABSTRACT

The toxicity of *Tityus bahiensis* scorpion venom is well known, but there are little data about the damage in offspring of dams that were exposed to the venom during pregnancy. The objective of this work was to determine the toxic effects of venom in adult offspring of Wistar rats exposed to venom *in utero*. Dams were divided into a control group, subcutaneously injected with saline solution on the 10th (GD10) and 16th (GD16) days, and two experimental groups, subcutaneously injected with venom (2.5 mg/kg) on GD10 or GD16, respectively. Adult offspring were evaluated according to behavioral development and neuronal integrity in the hippocampus. Tests performed in the activity box and in the enriched environment demonstrated that males from GD10 had motor decrease. Females from GD10 showed a depressive-like state and were more anxious, as demonstrated by the forced swimming test and social interaction. The plus-maze discriminative avoidance task demonstrated that GD16 males had lower levels of anxiety. The number of neuronal cells was decreased in CA1, CA3 and CA4 hippocampal areas of males and females from GD10 group and in CA1 of females and CA4 of males from GD16 group. Thus, we conclude that venom exposure in pregnant dams causes subtle alteration in the behavioral and neuronal development of offspring in adult life in a gender-dependent manner.

1. Introduction

The main effect of scorpion sting is local pain which is constant in human scorpion envenomatiom. It occurs immediately after the sting, its intensity can vary from discrete up to unbearable, and normally, it is followed by paresthesias. Serious systemic dysfunction such as considerable increase in body secretions, respiratory arrhythmias, hypertension followed by hypotension and shock, cardiac arrhythmia, congestive heart failure, thermoregulatory alterations, hyperglycemia and sometimes convulsions may occur, depending on the scorpion species [9,18,26].

Despite that scorpion accidents are a public health problem in many countries, few laboratory-controlled experimental studies are available in the scientific literature about the effects on offspring of mothers who were exposed to this venom during the prenatal period [2,3,5,10,27].

Some studies have demonstrated the ability of scorpion venom to induce alterations in fetuses when pregnant dams are stung. The venom of *Androctonus amoreuxi* scorpion species causes congenital abnormalities and fetal resorption when injected for some days in pregnant rats [27], and the venoms of *Buthus minax* and *Leiurus quinquestriatus* scorpion species are able to induce abortion in pregnant women [28,29,41]. The venom of the native Brazilian scorpions *Tityus serrulatus* and *Tityus bahiensis* causes alterations in fetuses of rats such as increased weight of some organs [3,10] and an increase in post-implantation losses [3]. Moreover, it was demonstrated that these venoms are able to cause alterations in the physical and behavioral development of newborns of dams that received them during pregnancy [2,13]. In previous studies, it was determined that the administration of 2.5 mg/kg of *T. bahiensis* venom during pregnancy, a dose that causes moderate envenoming without maternal toxicity, is able to elicit alterations in physical and reflexological development in the postnatal period [13]. Nevertheless, no data are yet available about the behavior of these pups in adulthood.

The present study aimed to examine specific alterations in behavior in adult offspring of dams exposed to *T. bahiensis* scorpion venom during two developmental time windows of pregnancy, the 10th day which is included in the organogenesis period, and the 16th day which is included in the period of fetal development [34].

2. Methods

2.1. Animals

Male ($n=21$) and female ($n=42$) Wistar rats weighing 250–300 g were used for the mating. Three hundred and thirty-six pups that were born from these matings were designated for the experiments in adult life. The animals were housed in standard cages ($40\times50\times20$ cm) under controlled conditions: 12:12 h photoperiod, 22 ± 2 °C, and water and

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food provided *ad libitum* during the study. All the experimental procedures were conducted with prior permission of the institution's Ethics Committee for Experiments on Animals.

2.2. Venom and solution

Saline at a salt concentration of 1.46% (necessary to dissolve the venom, based on our own studies) and crude venom of *T. bahiensis* (Butantan Institute) at a dose of 2.5 mg/kg dissolved in saline (1.46%) were used.

2.3. Animal mating and pregnancy diagnosis

Two females were kept in each cage. A male was placed in the cage approximately at 4 p.m. and taken out the morning after. The pregnancy was diagnosed by the detection of spermatozoa in a vaginal smear using light microscopy (gestation day zero — GD0). After this confirmation, the females were housed individually. Males and non-pregnant females were no longer used and returned to the general animal facility.

2.4. Treatment

Forty-two pregnant females were separated into three groups, each with fourteen females: the animals in the control group received 1 ml/kg NaCl (1.46%) subcutaneously on GD10 and GD16; the animals in the GD10 group received subcutaneous injections of *T. bahiensis* crude venom on GD10 and saline on GD16; and the animals in GD16 group received saline on GD10 and venom on GD16. The moderate-envenoming dose of 2.5 mg/kg was determined in previous experiments in our laboratory [13]. The number of rat per progeny and the sex ratio of the litters were not significantly different in the control and experimental groups ($p > 0.05$ data not shown). The offspring were standardized on the 2nd day of life with 8 rat pups from the same mother distributed with same number of males and female (4 males and 4 females per dam). At weaning, the littermates were separated and housed by sex and by experimental treatment until 2 months of age, when the animals were submitted to the experimental procedures. The litter was considered the experimental unit.

2.5. Behavioral experiments of adult male and female offspring

The same animals were employed for two subsequent experiments as described below. To avoid interferences on the animal behavior, the same group of rats underwent different kinds of tests.

2.5.1. Activity box

This equipment contains two groups of sensors, one on the horizontal line that measures the locomotion of the animal, and the other on the vertical line that measures vertical activity of the animal. Activities detected by sensors were analyzed. The scores for horizontal sensors were considered as locomotion and the scores for vertical sensors were considered as total activity, including locomotion. Twenty-eight adult rats (14 females and 14 males) of each group (at PN60) were placed individually in the box and observed for 5 min. After each observation, the box was cleaned with a 5% alcohol solution to prevent smell interferences with the next animal.

2.5.2. Forced swim

The same rats used in the activity box test performed this test in the next day. A plastic vessel (22 cm in diameter and 40 cm in height) with 19 cm of warm water (25 °C) was used. The animals were placed individually in this vessel for 10 min in the training session (PN61). Twenty-four hours later (PN62), they were placed again in the vessel for 5 min (test session), where the time was spent until the animal stopped swimming and the time of immobility, both in seconds, were determined. After each session, the animals were taken out, dried and warmed. The animals were constantly observed to avoid drowning. After each observation, the water in the vessel was changed to prevent smell interferences with the next animal.

2.5.3. Enriched environment

The activity box was enriched with an object able to induce an exploratory behavior in the rat. Twenty-eight rats (14 females and 14 males) of each group at PN60 were placed individually in the box, facing the wall to avoid other distractions, and were observed for 5 min. The total motor activity was automatically scored by the box, and the time of exploration of the new object was annotated by the observer.

After each observation the box was cleaned with a 5% alcohol solution to prevent smell interferences with the next animal.

2.5.4. Social interaction

The same rats used in the enriched environment were used to perform this test on the next day. All animals were individually submitted to an acclimation session of 5 min in the wooden box (PN61), 24 h before the test. In the test session (PN62), two animals of the same experimental group, sex, age and weight were placed in a wooden box ($72 \times 45 \times 55$ cm) to evaluate the social interaction between the two. The animals were observed for 5 min. The parameters of social interaction evaluated were: smelling, following, mounting, licking, and biting and/or pushing. Aggressive and passive behaviors were not considered. After each observation period, the box was cleaned with a 5% alcohol solution to prevent smell interferences with the next animal.

2.5.5. Plus-maze discriminative avoidance task

Twenty-eight rats (14 females and 14 males) of each group performed this test. The discriminative avoidance conditioning was performed in a modified elevated plus-maze, made of wood, containing two closed arms ($50 \times 15 \times 40$ cm) opposite to two open arms (50×15 cm). An aversive stimulus consisted of a 100-W lamp and noise produced by a hair dryer, placed exactly over the middle of one of the closed arms (aversive closed arm). Each rat was placed in the center of the apparatus, and, over a period of 10 min (training session PN60), an aversive situation was produced every time that it entered the aversive closed arm until the animal left it. On each side of the apparatus, there were different extra maze visual cues (door, window, clipboard and observer) that the rats could use to distinguish the location of the different arms of the maze. In the test session (PN61), performed 24 h after the training session, the rats were placed again in the apparatus for 3 min, without receiving the aversive stimuli. In both sessions, the time spent and the number of entries into each arm were recorded.

2.6. Histological evaluation of hippocampus

The same rats used in the plus-maze discriminative avoidance task were anesthetized immediately after this with CO_2 and perfused through the heart (left ventricle) with phosphate-buffered saline (PBS) followed by 10% formalin. The brains were removed, stored in formalin for at least one week and then embedded in Paraplast®.

Coronal brain sections of 10 μm were made. The slices were mounted on a glass slide and stained with cresyl violet. The cells were counted in a 100-μm^2 area of the pyramidal cell layers of the CA1, CA3 and CA4 regions of the hippocampus by light microscopy, using a 40× objective. Only cells showing normal morphological characteristics were counted. Neurons with picnotic nucleolus were considered damaged.

The number of neurons from each area was pooled and expressed as mean (±SEM) neurons per brain area.

2.7. Statistical analysis

Behavioral data were analyzed by Bartlett's test to determine the parametric distribution. All data showed a parametric distribution and