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The role of apitoxin in alleviating propionic acid-induced neurobehavioral impairments in rat pups: The expression pattern of Reelin gene

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ABSTRACT

The efficacy of apitoxin (bee venom; BV) in ameliorating propionic acid (PPA) –induced neurobehavioral impacts was studied. Sixty rat pups were enrolled in a split litter design to six groups: a control group, a PPA-treated group, a BV-treated group, a BV/PPA protective group, a PPA/BV therapeutic group, and a BV/PPA/BV protective and therapeutic group. Exploratory, social, locomotor, and repetitive/stereotype-like activities were assessed and prosocial, empathy, and acquired behavior were evaluated. Levels of neurotransmitter including serotonin, dopamine, and gamma-aminobutyric acid (GABA) were determined and a quantitative analysis of Reelin gene expression was performed. PPA treatment induced several behavioral alterations, as reduced exploratory activity and social behaviors, increased repetitive/stereotypic behaviors, and hyperactivity. In addition, a marked decline of neurotransmitters and down-regulation of Reelin mRNA expression were observed. BV exhibited high efficiency in ameliorating the PPA-induced neurobehavioral alterations, particularly when applied both before and after PPA administration. Overall, the results implied that BV has merit as a candidate therapeutic treatment to alleviate PPA-induced neurobehavioral disorders.

1. Introduction

Approximately 3% of neurobehavioral disorders are directly induced by environmental exposures to toxicants and about 25% of these conditions are induced via interactions between inherited susceptibilities and environmental pollutants [1]. Propionic acid (PPA) is a short chain fatty acid and the main end product of numerous enteric gut bacteria [2,3]. PPA is commonly utilized as a food preservative due to its effective preservative activity, mold inhibition and bactericidal properties. Also, PPA is an additive used in dairy products, poultry litter, livestock rations, and drinking water [4].

By both active and passive means, PPA easily crosses both the gut–blood and blood–brain barriers [5]. Brain exposure to PPA may disturb various neurophysiological processes, including neurotransmitter release, gene expression, mitochondrial function, immune modulation, gap junction gating, and behavior [6,7]. In rat models, propionic acidemia resulted from either central (i.e., intraventricular) or peripheral (i.e., intraperitoneal or oral) administration of PPA have revealed diverse behaviors and brain markers including repetitive behaviors, impairments in cognition and social behavior, increases in oxidative stress markers, reductions in glutathione, alterations of brain phospholipids/acylcarnitines and neuroinflammation [8–13].

Therapies have been studied to repair or guard against various neurobehavioral disorders. Various vertebrate and arthropod venoms from snakes, toads, frogs, spiders, scorpions, bees, wasps, and ants have shown pharmacological applications [14]. In particular, apitoxin (bee venom; BV) is a very complex mixture of about 18 active components, including enzymes, active peptides, and amines containing melittin, histamine, polyamines,

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phospholipase A2, and catecholamines [15]. The radioprotective, antimutagenic, pain relieving, anti-inflammatory, antihyperalgesic, anticancer and antinociceptive effects of BV have been documented [16–20].

Recently, the BV role in combating neurodegenerative ailments of the central nervous system (CNS) has been documented in many clinical studies [20–22]. Furthermore, in a 1-methyl-4-phenyl-1,2,3,6-tetrahy dropyridine (MPTP)-induced mouse model, BV acupuncture displayed an obvious neuroprotective action [22]. Moreover, BV might exert a direct neuroprotective effect on SH-SY5Y human neuroblastoma cells against MPP+-induced apoptotic cell death [23]. Yet, there are no studies demonstrating the protective or therapeutic role of BV in PPA-induced neurobehavioral disorders. Our earlier study showed that BV provides beneficial effects by diminishing PPA-induced oxidative stress, neuronal death, and DNA damage in the brains of rat pups [11].

Reelin is a glycoprotein secreted in the cerebral cortex and hippocampus from Cajal-Retzius cells and it acts as a regulator of many phases of laminar formation [24,25]. Many studies have documented altered Reelin expression in rodents with cognitive dysfunction related to neuropsychiatric disorders [26,27]. Recent genome screening studies using both a case-control and a family-based design have provided supporting evidence for the linkage/association between Reelin gene variants and either maternal deprivation or autistic disorder [28,29]. Furthermore, post-mortem autistic brain biochemical analyses have implicated Reelin in the pathogenesis of autism [29,30].

The specific aims of the current study were to verify whether BV presents a potential protective or therapeutic treatment against PPA-induced neural markers and behavioral performance, and to evaluate the expression pattern of Reelin gene in PPA- intoxicated rat pup brain.

2. Material and methods

2.1. Tested compounds and chemicals

Egyptian honey bee venom (BV) (*Apis mellifera lamarckii*) was obtained in lyophilized pure form from the Department of Bee Research, Plant Protection Institute, Ministry of Agriculture, Egypt. It was stored, desiccated at 4 °C and dissolved in sterile phosphate buffer saline directly before use (PBS; pH 7.2). PPA was purchased from Alpha Chemika, Mumbai, India. Enzyme-linked immunosorbent assays (ELISA) using commercial kits were applied to estimate dopamine (CUSABIO, P.R. China, Catalogue No. CSB-E08660r), GABA (Wuhan EIAab Science Co., Ltd China, Catalogue No. E0900r), and serotonin (MyBioSource, Inc. USA. Catalogue No. MBS-166089). All other reagents and chemicals were obtained from Sigma-Aldrich Co. St. Louis, MO, USA.

2.2. Animal grouping and experimental design

Ten pregnant female Sprague-Dawley rats, obtained from the Laboratory Animal's farm of the Faculty of Veterinary Medicine, Zagazig University, were used in these experiments. The dams were housed separately in stainless steel cages under a 12-h light/12-h dark cycle, a minimum relative humidity of 50% and a room temperature of 22 ± 2 °C. Food (standard diet) and water were accessible *ad libitum*.

After birth, the litters were allowed to nurse from the dams. We carried out a placebo- controlled experimental animal study using sixty pups assigned on PND7 into 6 groups (ten pups/group) in a split litter design where equal numbers of pups from every litter were labeled and assigned randomly to each group. For the control group, the pups were administered daily oral doses of PBS for 31 days (7th–37th PND) as a vehicle. The BV-only treated group was subcutaneously injected daily with BV at a dose of 0.5 mg/kg/day for 31 days (7th–37th PND) [18,31]. The BV/PPA co-treated protective group received BV by subcutaneous injection daily at 0.5 mg/kg/day for 15 days (7th–21th PND), followed by oral administration of PPA at a dose of 250-mg/kg/day body weight for 3 days (21th–23th PND) [32]. The BV/PPA/BV co-treated protective/therapeutic group was administered BV for 15 days prior to and after PPA administration at the same doses and routes previously mentioned (Table 1). The PPA-only treated group was orally gavaged daily with PPA for 3 days (21th-23th PND). Lastly, PPA/BV co-treated therapeutic group received PPA, followed by BV administration in the same doses and routes previously mentioned (23th–37th PND).

The experimental procedures followed guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals in scientific investigations and approved by the Ethics of Animal Use in Research committee of Zagazig University, Egypt (Approval number is IBR-3675).

2.3. Behavioral testing

All behavioral tests were performed in the same experimental testing room. The experimenter was blind to the treatments of the animals. All testing was carried out between hours of 8:00 h–16:00 h. On each test day, rats were transferred to the testing room in their home cages, and remained about 30 min to accommodate prior to testing. Additionally, during handling prior to training, rats were individually acquainted with the testing room and arena for 5 min/training day. Rat behavior was recorded using a video camera positioned 2.5 m above the arena and covering the entire view of the arena. At the end of each test, the rat was returned to its home cage, and the test apparatus cleaned with a wet sponge to eliminate odor.

2.3.1. The hole-board test

This test is a widely accepted paradigm used to evaluate exploratory activity in rodents [33,34]. The test was conducted in a 60 × 60 cm open-field wooden square arena with four equally spaced holes (3 cm in diameter) in the floor. The number of head-dips (head of an animal inserted inside the hole) was measured during a 3-min time session. If both eyes vanished into the hole, a head-dip was scored.

2.3.2. Social contact test

For evaluating social contact behaviors, rats were individually separated into their home cages for 24 h before behavioral testing [35]. The social testing apparatus was a rectangular clear arena (100 cm W × 100 cm W × 35 cm H) with lighting conditions like those of their home cages. The test consisted of placing a tested rat and a "neutral" stimulus one for 15 min. For the duration of the habituation phase, tested rats were placed in the arena for a 10 min session.The stimulus rat was previously habituated to the arena

Table 1
Experimental groups and treatments.

Treatments Groups	PBS	PPA	BV
Control (PBS)	PND 7–37	–	–
BV	–	–	PND 7–37
PPA	–	PND 21–23	–
BV/PPA	–	PND 21–23	PND 7–21
PPA/BV	–	PND 21–23	PND 23–37
PPA/BV/PPA	–	PND 21–23	PND 7–21 + PND 23–37

PBS: physiological buffer saline; BV: bee venom (0.5 mg/kg b.wt, S/C); PPA: propionic acid (250 mg/kg b.wt, oral); S/C: subcutaneous; b.wt: body weight; PND: postnatal day; –: no treatment.