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ORIGINAL PAPER

Effects of homeopathic *Anax imperator* on behavioural and pain models in mice

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Backgound: **Homeopathy is a medical theory and practice that asserts that disease can be cured by remedies that produce symptoms in a healthy person similar to those suffered by a patient with a malady.**
Methods: **The aim of this study was to investigate effects of homeopathic *Anax imperator* (dragonfly) (*Anax-i 30c* and *Anax-i 200c*) in the forced swim test (FST), elevated plus-maze (EPM) test, hot plate (HP) test and open field test and examined NPY1 receptor expression, in naive mice.**
Results: **In the FST, treatment with *Anax-i 30c* or *Anax-i 200c* significantly diminished immobility time while in EPM test, *Anax-i 200c* increased the percentage of time spent in open arms as well as the percentage of open arm/total arms. In the HP test, *Anax-i 30c* or *Anax-i 200c* decreased the total time mice spent licking their hind paws while in open field test, treatment with *Anax-i 200c* increased the total distance and speed mice traveled compared to the control group. Three weeks of daily injections with *Anax-i 30c* or *Anax-i 200c* caused significant weight loss in mice. *Anax-i 30c* or *Anax-i 200c* treatment significantly decreased NPY1 receptor expression, and *Anax-i 30c* also decreased NPY2 receptor expression.**
Conclusion: **These results suggest that the homeopathic *Anax-i* exerts antidepressant, anxiolytic and analgesic-like effects and causes hyperlocomotion and weight loss.**
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Keywords: Homeopathy; *Anax imperator*; Adipokinetic hormone; Behavioral models; Neuroreceptor; Mice

Introduction

Anax imperator, belonging to the order Odonata and the family Aeshnidaean, is an insect commonly known as the Emperor dragonfly or Blue Emperor. It is a large (averaging 78 mm in length) and powerful species of hawker dragonfly.[1]

The neurosecretory cells in the corpus cardiacum of insects synthesize a set of peptide hormones known as adipokinetic, hypertrehalosaemic or hyperprolinaemic, depending on the type of insect. These neuropeptides act as hormones (neurohormones) and are especially necessary when the oxidative metabolism of insects is high; for example, when flight muscles contract maximally or over long periods. Hence, insects need large amounts of energy that must be mobilized from stores in the fat body.[2–4]

Adipokinetic hormones (AKHs) are metabolic neuropeptides mediating mobilization of energy substrates from the fat body in many insects. Studies using transgenic manipulations of the dAKH gene have demonstrated that AKH induces both hypertrehalosemia and hyperlipemia. Similar to other neuropeptides, AKHs are multifunctional, functioning, for example, in cardioacceleration in cockroaches and in migration of tegumentary and retinal distal pigments in crustaceans. In addition, AKH peptides have

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excitatory effects on motor neurons, and evidence supports a central role for AKH in locomotion in some insects.[5]

Insect AKHs are a large family of peptide hormones involved in the mobilization of sugar and lipids from the fat body during energy-requiring activities, such as flight and locomotion, but they also contribute to the homeostasis of hemolymph sugar. Interestingly, the insect AKH receptors are structurally and evolutionarily related to the gonadotropin-releasing hormone receptors from vertebrates.[6]

Using a heterologous (locusts and cockroaches) and a homologous bioassay, the neuropeptide pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-NH_2 was isolated from extracts of the corpora cardiaca of the Emperor dragonfly, *A. imperator*. Low concentrations of this synthetic peptide injected into the Emperor dragonfly increased the hemolymph lipid concentration, suggesting a possible role for this peptide in lipid homeostasis during flight. Therefore, it was named Ani-AKH, to denote *A. imperator* adipokinetic hormone.[7] Results from a previous study suggested that adipokinetic hormone may contribute to neuronal function in the human central nervous system.[8]

Homeopathy is a medical theory and practice asserting that disease can be cured by remedies that produce symptoms in a healthy person similar to those suffered by a patient with the malady; the basic principle of homeopathy, known as the law of similars, is "*let like be cured by like*." The remedies are usually administered in low doses.[9] The aim of the present study was to investigate the effects of a homeopathic remedy prepared from dragonfly (*Anax-i*) on naive mice on depression, anxiety, analgesia and locomotion as examined in the forced swim test (FST), elevated plus-maze (EPM) test, hot plate (HP) test, and open field (OF) test. FST, EPM and HP tests are animal models of depression, anxiety and analgesia. The body weight of the animals was also evaluated during chronic injections lasting three weeks. Sections through the hippocampus of the mouse brain were probed with antibodies to evaluate c-fos, NMDAR (N-methyl-D-aspartate) 2B, NPY (Neuropeptide Y) 1R, and NPY (Neuropeptide Y) 2R activity.

Material and methods

Animals

Inbred male BALB/c ByJ mice (Uludag University, Bursa, Turkey) were 7−8 weeks old when they arrived at the laboratory. The mice were kept in the laboratory for two weeks before the onset of the experiments. Mice were maintained under standard laboratory conditions (12-h light:12-h dark cycle, lights on at 07:00 h, 21 ± 1°C). All animals received food and water ad libitum. All procedures described in this paper were conducted in accordance with the European Community Council's directive for the ethical treatment of animals (86/609/EEC) and with the approval of the Kocaeli University Medical Faculty (7/3/2013).

Experimental groups and drug administration

We directly used *A. imperator* 30C and 200C liquid homeopathic remedy (25% alcohol) purchased from Helios Homoeopathy, UK in test groups. We used vehicle (saline with 25% alcohol) in control groups. For the FST, EPM test, HP test and open field test (OP), the animals were treated with *Anax-i 30c* or *Anax-i 200c* (Helios Homoeopathic Pharmacy, London, England) or vehicle (saline with 25% alcohol) (n = 10 per group) for 10 days. Fluoxetine 15 mg/kg and diazepam 2 mg/kg were used as reference drugs in the FST and EPM tests. Fluoxetine and diazepam were purchased from Sigma Chemicals (St. Louis, Mo, USA). The drugs were administered intraperitoneally (i.p.) 60 min before each test in a volume of 0.05 ml/10 g body weight. For body weight evaluations, *Anax-i 30c* (n = 9), *Anax-i 200c* (n = 12), or vehicle (n = 10) were administered i.p. daily for three weeks at 17:00 in a volume of 0.1 ml/10 g body weight. Separate groups of animals were used in each test.

FST

The FST method employed in our study was similar to that previously described.[10] Briefly, the mice were individually placed into and remained for 6 min in Plexiglas cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water maintained at 23−25°C. Because mice cannot escape the cylinder, they rapidly become immobile, floating in an upright position and making only small movements to keep their heads above water. The duration of immobility was recorded during the last 4 min of the 6-min testing period.

EPM test

Anxiety-related behavior was measured by using the EPM test. Experiments were conducted in a dimly lit, semi-soundproof room illuminated with a table lamp (80 lux). The maze was constructed from wood and consisted of two open (29 cm long × 5 cm wide) and two closed arms (also 29 cm long × 5 cm wide, but with walls 15 cm in height) forming a square cross, with a 5 cm square-shaped center. To prevent falls, the open arms were surrounded by a short (1 cm) Plexiglas edge. The maze was elevated 40 cm above the floor. Each mouse was placed in the center of the maze facing one of the open arms and was allowed to explore the maze. The open-arm activity was evaluated as follows: 1) time spent in the open arms relative to the total time spent in the plus maze (300 s) expressed as a percentage; 2) the number of entries into the open arms relative to the total number of entries into both open and closed arms, also expressed as a percentage. These values were interpreted as indexes of anxiety in mice.

HP test

The HP test was used to measure the pain reaction latencies. Animals were placed into a square glass container maintained on a HP at 55 ± 0.1°C. The total time spent licking the hind paws or jumping was recorded and served as an index of pain reaction. An endpoint time of 120 s was used.