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Research report

Analgesic, learning and memory and anxiolytic effects of insulin in mice

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ABSTRACT

Insulin is a polypeptide hormone that is present in mammals and its main function is the maintenance of adequate blood sugar level. Insulin receptors are widely but unevenly distributed in the brain. Insulin has been reported to be involved in the regulation of neurotransmitters release. It has also been linked to the pathogenesis of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Although there is abundant literature on the study of biochemical and molecular properties of insulin, there has been no literature on its central behavioural effects on anxiety and pain relief among other behavioural effects. This study therefore investigates whether insulin has any anxiolytic and other CNS effects.

This experiment was carried out in mice using animal behavioural models including a hot plate analgesic test, holeboard and elevated plus maze for anxiolytic test. A Y-maze was used for the locomotor activity and spontaneous alternation investigations. Mice were administered intraperitoneally with insulin at different doses of 0.5, 1.0 and 2.0 IU/kg.

The results obtained showed that insulin has no analgesic activity, however, it caused significant central inhibitory effects by decreasing both locomotor activity in both holeboard and Y-maze models and also decreased the exploratory behaviour in holeboard at doses administered dose-dependently indicating its sedative effects. In elevated plus maze, insulin had no effects on percentage of open arm entries at all doses but had a significant effect on percentage of open arm duration at the dose of 1.0 IU/kg only. Insulin administration at lower doses (0.5 and 1.0 IU/kg, i.p.) had no effect on spatial working memory, however, it had significant spatial working memory impairment at the dose of 2.0 IU/kg, i.p. in mice. The study showed that insulin has several neuropharmacological effects at doses used.

1. Introduction

Insulin is an acidic proteinous hormone having a molecular weight of approximately 5600. This hormone is composed of two polypeptides called A and B chains which are covalently joined by two intrachain disulfide bonds. A third intrachain disulfide bridge is present in the A chain. The chains are formed by proteolysis of pro-insulin, a larger single chain precursor, by removal of the intervening sequence of amino acids, referred to as the C-peptide. Insulin is essential for metabolic processes involving carbohydrates, fats and proteins. In most tissues, insulin stimulates the uptake of glucose, fatty acids and amino acids and aids their eventual conversion into storage forms. Naturally, higher animals produce their own endogenous insulin from the pancreas. Today there are various types of insulin, which are classed based on their onset of action, peak time and duration of action. Insulin is used in the management of diabetes mellitus, where there is either a lack of insulin production or non-sensitivity of the β-cells of the pancreas to produce insulin.

The existence of insulin receptors within the brain is less well known and the functions of these receptors are somewhat an enigma. It is important to note that brain cells are not fully reliant upon insulin for glucose supply, they have independent means of obtaining glucose. Also, brain insulin receptors (InsRb) differ somewhat from their peripheral counterparts [1]. The physiological role of insulin receptors in the brain appears to be twofolds: (1) the tight control of glucose transport in specific brain regions and (2) the yet incompletely understood function in the central nervous system (CNS) development and function. Aberrant function of the brain insulin receptors has been hypothesized to be involved in CNS dysfunction [29]. Even distribution of insulin receptors would be expected throughout the brain particularly if their only function is to mediate insulin-induced glucose transport into neurons as a source of energy. It has been shown that the highest brain-insulin receptor densities are found in the olfactory bulb, cerebral cortex, hypothalamus, cerebellum and choroids plexus [1,2,12,16,33]. Furthermore, high densities of brain insulin receptors are found in the thalamus, caudate putamen and some mesencephalic and

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brain stem nuclei. Insulin concentrations in extracts of whole brain averaged about 25-fold greater than the concentration in plasma [13,14,35].

The brain distribution of insulin receptor messenger RNA investigated using in situ hybridisation is consistent with the distribution of insulin receptors. Insulin brain receptor messenger RNA is most abundant in the cell layers of the olfactory bulb, in dentate gyrus of the pyriform cortex and hippocampus, in the choroid plexus and in the arcuate nucleus of the hypothalamus [1,23].

Surprisingly, there is some mismatch between receptor density and insulin concentration. Hypothalamus and cerebral cortex have similar levels of receptor content but have more than a 4-fold difference in insulin content. Such disparity suggests that the insulin receptors may have other functions than those related to glucose utilization only [13,14]. Recently, Hoyer et al. [17] demonstrated that the brain is sensitive to exogenous glucose metabolism because of the receptors in the brain. Furthermore, energy homeostasis, including control of food intake and energy expenditure, is affected by insulin levels [6,24,25]. Also, the presence of insulin in the brain has effects on behavioural functions such as food intake, motor activity and memory due to their classical role in metabolism [26]. These behavioural activities clearly support the hypothesis that insulin has central nervous system functions.

Insulin brain receptors and degenerative diseases have exhibited a certain relationship since the brain receptors are of higher densities on the hippocampus and parts of the cerebral cortex which are important in learning and memory in man. In disorders like Alzheimer's disease, Parkinsonism and Huntington's chorea, brain insulin concentrations, have been shown to decrease with age. It has been suggested that insulin should be administered in brain degenerative diseases to enhance memory in such patients [4,7,18,22]. It appears that insulin has been implicated in energy deficits pertaining to these diseased states [3]. From the literature on the role of insulin in the brain, it is revealed that there still remains a large disparity in the knowledge about insulin possible cognitive effects.

Therefore, even though some studies have been carried out on the relationship between insulin and its effects on the brain [29], there are still areas of insulin-brain activity, which have not been studied. No direct studies of a possible relationship between insulin or insulin receptor with anxiety have been found in literature. Furthermore, insulin has been reported to be involved in energy homeostasis and deficiency in insulin can cause neuropathic pain [26] and changes in energy status in the brain could impact many behavioural effects. Thus, this experiment was designed to study the effects of insulin in the brain and to establish any possible relationship with pain, learning and memory and anxiety in mice using various behavioural animal models such as hot plate (central analgesic effect), Y-maze (spatial working memory effect), elevated plus maze and hole board (exploratory behaviour).

2. Materials and methods

2.1. Animal

The animals used for the experiments were young albino Vom strain mice (19.4 ± 0.4) of both sexes that were purchased from the Institute of Medical Research Yaba, Lagos, Nigeria. Mice were housed in a plastic cage with a stainless steel wire covering the open top of the cage. Food (Ladokun feeds, Ibadan, Nigeria) and water were provided *ad libitum* and the cages kept clean at all times. The mice were housed in the Pharmacology Department of the Faculty of Pharmacy animal house and all rules applying to animal safety and care were observed. Each animal was only administered once.

2.2. Drugs

Insulin (Actrapid, Novo Nordisk)® a neutral insulin parenteral; 40 IU/ml (Novo Nordisk A/S, 2880 Bagsvaard, Denmark), a soluble insulin reported to have a rapid onset action (after 30–60 min) and a duration of action up to 8 h, was purchased from Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State, Nigeria and stored at 8 °C before use. Prepared insulin was administered to different groups of mice at doses (0.5, 1.0 and 2.0 IU/kg) intraperitoneally at a maximum volume of 10 μl/g body weight, respectively. Normal saline (0.9%, w/v) was administered at the same maximum volume of 10 μl/g body weight as vehicle to the control group. Each animal was used only once for each experimental model.

2.3. Analgesic test

The hot plate test of Eddy and Leimbach [10] was used to determine the analgesic effect of insulin in mice. Animals were intraperitoneally administered insulin at different doses (0.5, 1.0 or 2.0 IU/kg) and vehicle (saline) 30 min before being placed on the hot plate. The respective time(s) for paw licking thereafter or jumping on a hot plate at 55 °C was registered.

2.4. Spontaneous alternation test (Y-maze)

Activity in a Y-maze was used to measure spontaneous alternation performance (spatial working memory) and locomotor activity [15,21,28]. The Y-maze is composed of three equally spaced arms (120°; 41 cm long × 15 cm high). The floor of each arm consists of wood 5 cm wide. Each group of animals was tested 30 min after administration of normal saline (vehicle) and different doses of insulin (0.5, 1.0 and 2.0 IU/kg). Each mouse was placed in one of the arm compartments and was allowed to move freely for 6 min without reinforcers. Examined parameters were: (1) frequency of entry into each arm, (2) total arm entries, (3) spontaneous alternation and (4) 'Events". Spontaneous alternation percentage (SA%) was defined as a ratio of the arm choices that differed from the previous two choices ("successful choices") to total choices during the run ("total entry minus two"). An arm entry is when the body of the mouse, except for its tail, completely entered into an arm compartment. The sequence of the arm entries, which are alternations, was manually recorded. An alternation is defined as an entry into all three arms in consecutive choices. For instance, each alternation is followed by a comma in the following sequence of arm entries (each arm is labelled A, B, or C): ACBCABCACABCA. In this example, the mouse entered 13 arms, 8 of which are alternations. The number of maximum spontaneous alternation is then the total number of arms entered minus 2 and the percentage is calculated as

$$\frac{\text{actual alternations}}{\text{maximum alternations}} \times 100.$$

Therefore, the spontaneous alternation percentage in this case is 61.5%. The apparatus was cleaned with 70% ethyl alcohol and permitted to dry between sessions to remove the previous animal odour.

2.5. Anxiolytic tests

The present study was conducted to examine the anxiolytic effects of the hormone in animal's models using the hole board and elevated plus maze methods. In these two models, four groups of mice were tested: SAL ($n = 14$) and three different doses of insulin (0.5, 1.0 and 2.0 IU/kg) ($n = 13$, 14 and 7, respectively). The same set of animals in each group were tested in the holeboard (5 min) and elevated plus maze (6 min) in a sequential order.

2.5.1. Hole board test

In the holeboard test, a paradigm involving novelty and uncertainty is employed. Head dipping is generally considered to provide a measure of exploration that was distinct from motor activity The animals were placed on a board (40 cm × 40 cm) with 16 holes (symmetrically distributed in four rows) 10 min after intraperitoneal administration of vehicle (saline) or different doses of insulin (0.5, 1.0 or 2.0 IU/kg) and the frequency of head dips into the holes during 5 min was registered. The results are expressed as mean total number of head dips [5,19].

2.5.2. Elevated plus maze

The apparatus is made of wood and has two narrow enclosed arms which are bordered by high walls and has two open arms which are essentially unprotected boards, naïve mice will normally prefer to spend much of their allotted time in the enclosed arms. The elevated plus maze, a modification of the apparatus validated for mice by Lister [19,20], consisted of two open arms (30 cm × 5 cm × 0.25 cm) and two closed arms (30 cm × 5 cm × 15 cm) emanating from a common central platform (5 × 5). The two pairs of identical arms are opposite each other. The entire apparatus is elevated to a height of 50 cm above floor level. At the start of the session, the mouse was placed at the centre of the maze, with its head facing an open arm and allowed to explore the maze for 6 min. During a 6-min test period, the following measurements were recorded: the number of entries and the time spent in open and closed arms, and the exploratory behaviour (total number of arm entries). An entry with all four feet into one arm is defined as an arm entry. In this experiment, four groups of mice were tested: SAL ($n = 14$) and different doses of insulin (0.5, 1.0 and 2.0 IU/kg) ($n = 13$, 14 and 7, respectively). The plus maze was carefully wiped with a wet towel after each animal. The results were expressed as percentage of time spent in open arms relative to total time spent in both open and closed arms; and percentage of