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Glucocorticoid receptor activation selectively influence performance of Wistar rats in Y-maze

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ABSTRACT

Glucocorticoid receptors (GR) are ubiquitously expressed in metazoans. Different and contrasting phenotypes have been reported upon their activation. This study investigated the behavioral phenotypes characteristic of GR stimulation in male Wistar rats. Rats in each of the four groups of rats received one of the following treatments: distilled water (control) or one of three doses of dexamethasone (treatment) injected intraperitoneally for 7 days. The Rats were afterwards subjected to the Y maze, the elevated plus maze (EPM), the Morris water maze (MWM), and the novel object recognition (NOR) test. At the end of the study, the animals were anesthetized and neural activity from the prefrontal cortex recorded. Blood was collected via cardiac puncture to evaluate the levels of plasma insulin and glucose, and the prefrontal cortexes excised to determine the levels of insulin, markers of oxidative stress, and calcium in the homogenate.

This study showed that treatment with dexamethasone significantly reduced the total and percentage alternation in the Y maze, but had no significant effect on object recognition in the NOR test, long-term and short-term spatial memory in the MWM, or anxiety-like behavior in the EPM. Plasma and brain insulin and calcium levels were elevated moderately following treatment with the lowest dose of dexamethasone. All doses of dexamethasone decreased brain superoxide dismutase and increased lactate dehydrogenase levels. No significant change in neural activity was observed.

This study shows that activation of glucocorticoid receptors differentially affects different behavioral paradigms and provides evidence for a role for glucocorticoids in mediating insulin function in the brain.

1. Introduction

Glucocorticoids are steroids secreted by the adrenal cortex, most well known for their release in response to increase in circulating adrenocorticotropic hormones, resulting from stress. They are also known to stimulate gluconeogenesis and inhibit peripheral glucose uptake, promote protein catabolism, mobilize and redistribute fats, and contribute to other pleiotropic effects in the physiology of metazoans. These multiple functions are related to the presence of glucocorticoid receptors in almost all tissues in the body [1]. The known physiological glucocorticoid is cortisol in most mammalian species and corticosterone in rodents. The physiological mineralocorticoid is aldosterone.

Glucocorticoids and mineralocorticoids bind with differential affinity to glucocorticoid (GR) and mineralocorticoid receptors (MR). Corticosterone and aldosterone bind with higher affinity to the MR relative to the low affinity binding of dexamethasone to the same receptor. Dexamethasone on the other binds with the highest affinity to the GR, also referred to as dexamethasone-binding receptors. The MRs and GRs are widely distributed in the brain and can also be found co-expressed in the same neuron.

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The brain is a major target for glucocorticoids and their physiological effects are mediated through a nearly ubiquitously expressed low affinity glucocorticoid receptor (GR) and a regionally specific high affinity mineralocorticoid receptor (MR) [2,3]. MR are highly expressed in the hippocampus and other limbic areas and have a very high affinity for corticosterone [4,5]. MR expressed in renal, heart, and intestinal tissue have a high affinity for mineralocorticoids such as aldosterone [4]. On the other hand, GR are expressed in virtually all tissues of the body and are widely distributed in the brain [6,7]. The very high affinity of GRs for dexamethasone makes it a good agonist in understanding the physiology and pathophysiology of GRs [6]. Dexamethasone also readily crosses the blood brain barrier and has a terminal half-life of approximately 90 min [8].

The presence of multiple binding sites for corticosteroids as well as their differing levels in different cell types and neurons form the basis for their differential actions in the brain [9]. Glucocorticoid receptors are involved in different behavioral changes and cognitive processes. Glucocorticoids mediate and modulate memory consolidation by activating the glucocorticoid receptors that are highly expressed in brain regions such as the prefrontal cortex, hippocampus, and amygdala [10,11]. The activation of mineralocorticoid receptors enhances and prolongs long-term potentiation (LTP), whereas the activation of glucocorticoid receptors suppresses LTP in the dentate gyrus and hippocampus [12].

The prefrontal cortex, especially the medial prefrontal cortex, expresses glucocorticoid and mineralocorticoid receptors and thus may play a role in modulating various cognitive processes [13,14]. Glucocorticoids have contrasting effects on the medial prefrontal cortex. They act through their receptors to enhance long-term potentiation/memory consolidation, but impair short-term or working memory [15]. This contrasting effect points to the fact that the activation of GR is contextual in nature.

Insulin has emerged as a major regulatory substance within the central nervous system. Studies have shown that its source may be endogenous as it seems to occur in pyramidal neurons (e.g., from hippocampus, prefrontal cortex, entorhinal cortex, and olfactory bulb) [16]. Also, central insulin could be peripheral in origin, suggesting that insulin may first enter the cerebrospinal fluid (CSF) from plasma via the choroid plexus, then pass through the ependymal lining, to act on insulin receptors on adjacent neurons [17], or it may pass from the blood into the CNS (CSF and brain) via an insulin receptor-mediated, saturable pathway in brain capillary endothelial cells [18].

Glucocorticoids play a vital role in energy metabolism. Cortisol, for example, stimulates gluconeogenesis in the liver. Antagonistically, central injection of insulin reduces hepatic glucose production due to autonomic nervous system inhibition of gluconeogenesis [19]. Hence there may be a relationship between glucocorticoid receptor stimulation and central insulin levels.

Results from studies on the effects of GR stimulation on cognition have been variable owing to the biology of the GR. This study aims to determine the dose-dependent effects of GR potentiation on different behavioral paradigms and a probable role for glucocorticoids in mediating insulin action in the brain.

2. Materials and methods

2.1. Animals

Twenty-four male Wistar rats with an average weight of 175 ± 25.5 g were used for this study. They were selected at random and housed in separate cages (6 animals per cage) at the Faculty of Basic Medical Sciences' animal quarters, University of Ilorin. They were allowed free access to food and water ad libitum. The animal quarters were maintained at room temperature with good ventilation. All experimental protocols were approved by the University of Ilorin ethical review committee (UIERC). These protocols were also in accord with international guidelines for the care and use of laboratory animals.

2.2. Drug and reagents

A pharmaceutical grade dexamethasone injection (4 mg/mL) (Wuhan Grand Pharmaceutical group, Wuhan, China), Insulin ELISA kit (AccuBind, #5825-300), LDH kit (abcam, #ab102526), calcium kit (abcam, #ab102505), sodium carbonate, and norepinephrine were used for this study.

2.3. Grouping

The animals were randomly divided into four groups (n = 6) as follows:

- Group 1: received 2 mL/kg normal saline
- Group 2: received 2 mg/kg dexamethasone
- Group 3: received 4 mg/kg dexamethasone
- Group 4: received 8 mg/kg dexamethasone.

All treatments were administered intraperitoneally once daily for 7 days.

2.4. Y maze

Experiments with the Y maze were carried out on the 5th day of dexamethasone administration. This maze is used to measure the willingness of rodents to explore a new environment. To evaluate spontaneous alternation in the animals, a Y maze of 75 cm × 25 cm × 15 cm was constructed. It involved placing an animal in the middle of the maze and allowing the rat to make arm decisions afterwards. The animals were allowed 5 min each in the maze during which they alternated the different arms. An animal's decision was considered to be right if it visited the three arms consecutively, whereas visiting any individual arm more than once in three alternations was considered wrong [20,21]. The percentage alternation was calculated as shown below:

$$\%\text{alternation} = \frac{\text{No .of right decisions}}{\text{No .of total arm entries} - 2} \times 100$$

2.5. Elevated plus maze

The elevated plus maze test is an experimental model used to test for anxiety-related behaviors in rodents. This was carried out on day 5, four hours after completing the Y-maze test. The test setting was adapted from that of Handley and Mithani [22]. It consists of a plus-shaped apparatus which is 55 cm high with two open and two enclosed arms. The two open arms lie across each other and are perpendicular to the closed arms. Each closed arm has a high wall of 15 cm that encloses it while the open arms have no side walls. Each rat was placed at the junction of the four arms facing an open arm with its back to the other open arm. The duration of entries was recorded by a video-tracking system for 5 min. Other parameters such as rears were also observed. The open arm and closed arm entry entries were also recorded. The percentage time spent in open arms or closed arms was calculated as shown below:

$$\%\text{time spent in either arm} = \frac{\text{time spent in either arm}}{\text{time spent in both arms}}$$

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