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Testosterone deprivation has neither additive nor synergistic effects with obesity on the cognitive impairment in orchiectomized and/or obese male rats

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ABSTRACT

Objective. Previous studies demonstrated a correlation between cognitive decline and either testosterone deprivation or obesity. However, the effect of obesity combined with testosterone deprivation on cognitive function has not been investigated. This study investigated the effects of obesity on brain insulin sensitivity, brain mitochondrial function, hippocampal synaptic plasticity and cognitive function in testosterone-deprived male rats.

Materials/Methods. Male Wistar rats were divided into sham-operated (control) and bilateral orchiectomized (ORX) groups. Rats in each group were further divided into two subgroups to receive either a normal diet (ND) or a high fat diet (HFD) for 4, 8 or 12 weeks. Blood samples were collected to determine metabolic parameters. Cognitive function was tested using the Morris Water Maze Test. At the end of the study, brains were removed to investigate brain insulin sensitivity, brain mitochondrial function and hippocampal synaptic plasticity.

Results. Both control-obese and ORX-obese rats developed peripheral insulin resistance at week eight, and brain insulin resistance as well as brain mitochondrial dysfunction at week 12. However, the ORX-obese rats developed cognitive impairment and decreased hippocampal synaptic plasticity beginning at week eight, whereas the control-obese rats developed these impairments later at week 12. Although both peripheral and brain insulin

Abbreviations: S, sham-operated; O, bilateral orchiectomized; ND, normal diet; HFD, high fat diet; NDS, control-lean rats; NDO, ORX-lean rats; HFS, control-obese rats; HFO, ORX-obese rats; T2DM, type-2 diabetes mellitus; LTD, insulin-induced long-term depression; CA1, cornus ammonis 1; DHT, dihydrotestosterone; MWM, Morris water maze test; OGTT, oral glucose tolerance test; LTP, hippocampal synaptic long-term potentiation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA, Homeostasis Model Assessment; MDA, malondialdehyde levels; ECLIA, the electrochemiluminescence immunoassay; fEPSPs, field excitatory postsynaptic potentials; aCSF, artificial cerebrospinal fluid; p-IR, insulin receptor phosphorylation; IR, insulin receptor; Akt/PKB, serine/threonine protein kinase/protein kinase B; BCA, bicinchoninic acid; ROS, reactive oxygen species, $\Delta\Psi m$, mitochondrial membrane potential change; DCFHDA, dichloro-hydrofluoresceindiacetate; JC-1, fluorescent dye 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl benzimidazolcarbocyanine iodide; HPLC, high performance liquid chromatography; OFT, open-field test.

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resistance were not observed in both the control-lean and ORX-lean rats, impaired cognition and decreased hippocampal synaptic plasticity were found in the ORX-lean rats beginning at week eight.

Conclusion. These findings suggest that testosterone deprivation has neither additive nor synergistic effects over obesity in the development of cognitive dysfunction in orchiectomized-obese male rats.

1. Introduction

Insulin resistance is a hallmark of metabolic syndrome, which can lead to the development of type-2 diabetes mellitus (T2DM) [1]. It has been shown that long-term high calorie diet consumption causes obesity, insulin resistance, and T2DM in animal models [2,3]. Our previous work has also demonstrated that male rats fed long-term on a high-fat diet (HFD) developed not only obesity and insulin resistance, but also brain insulin resistance, as indicated by an impairment in insulin-induced long-term depression (LTD), impaired insulin signaling in the brain and brain mitochondrial dysfunction as well as cognitive impairment [4–6]. Moreover, HFD also caused increased oxidative stress and finally decreased synaptic plasticity [7], and cognition [8,9].

In addition to obesity, the androgenic sex steroid hormone, testosterone, has been shown to play an important role in the regulation of glucose metabolism and insulin signaling [10], as well as the modulation of normal brain function, including improved synaptic plasticity, and is involved in cognitive formation [11,12]. In orchiectomized rodents, the impairment of learning and memory has been demonstrated, indicating that testosterone was involved in cognitive function [13–16]. Furthermore, the reduction of the number of dendritic spines and changes in dendritic spine morphology in hippocampal CA1 regions have been observed in male orchiectomized rodents [17], which were restored by testosterone or dihydrotestosterone (DHT) replacement [18,19]. Although obese-insulin resistant has been shown to be associated with testosterone deficiency in animal models [20,21] and human studies [22,23], several reports regarding castrated male rats demonstrated that testosterone deprivation did not induce obesity [24–27].

Despite some links reported among testosterone, obesity and cognition, the effects of testosterone deprivation with or without obesity on brain insulin receptor function, brain insulin signaling and brain mitochondrial functions have not been reported. In addition, the effects of testosterone deprivation with or without obesity on hippocampal synaptic plasticity as well as cognitive function have not been thoroughly determined In the present study, we hypothesized that testosterone deprivation alone can cause an impairment of brain insulin receptor function, decreased brain insulin signaling, impaired brain mitochondrial function, impaired hippocampal synaptic plasticity, resulting in cognitive dysfunction. We also hypothesized that obesity accelerates these impairments in the brain of testosterone-deprived male rats. Although several parameters have been determined in the present study, the first endpoint was recorded in line with the changes in cognitive function.

2. Materials and Methods

2.1. Animal Models and Experimental Protocols

Seventy-two male Wistar Rats, weighing 180–200 g (aged ~5–6 weeks old) obtained from the National Animal Center, Salaya Campus, Mahidol University, Bangkok, Thailand were used. All experiments were conducted in accordance with the approved protocol from the Faculty of Medicine, Chiang Mai University Institutional Animal Care and Use Committee, in compliance with NIH guidelines. All animals were housed in environmentally controlled conditions (25 ± 0.5 °C, 12 hour light/dark cycle) and allowed to acclimate for one week. Rats were then divided into two groups (n = 36/group) as either sham-operated (S) or orchiectomized (O) rats. One week after the surgery, each group was subdivided into two subgroups. Animals in each subgroup were fed either a normal diet (ND: 19.77% E fat) or a high-fat diet (HFD: 59.28% E fat) for 4, 8 or 12 weeks [6]. In each time period, animals were divided into four different treated groups, including NDS (control-lean rats), NDO (ORX-lean rats), HFS (control-obese rats) and HFO (ORX-obese rats). All animals were given free access to drinking water and their respective diets.

An open field test was performed to test locomotive function. The cognitive function was determined by the Morris Water Maze (MWM) Test. Both tests were determined at the end of each time period (4, 8 and 12 weeks). The body mass of each animal was recorded weekly. Blood samples were collected from a tail vein at week zero, (before dietary feeding) and at the end of each time period (4, 8 and 12 weeks) for further plasma analysis. The oral glucose tolerance test (OGTT) was performed at the end of weeks 4, 8 and 12, using the technique described previously [6,28]. At the end of each time period, animals were deeply anesthetized with isoflurane and sacrificed via decapitation. Each brain was rapidly removed and carefully sliced in preparation for extracellular recording (insulin-induced LTD and hippocampal synaptic LTP), immunoblot, brain oxidative stress and brain mitochondrial study as well as dendritic spine density determination.

2.2. Orchiectomized Procedure

Orchiectomy was performed using the method described previously [29]. Rats were anesthetized and maintained by 2–3% isoflurane in a dorsal recumbent position, and the skin of scrotal area was scrubbed utilizing sterile technique. A small incision was made at the tip of the scrotum. The tunica albuginea was incised and the testis, vas deferens and the spermatic blood vessels were exposed. Then, the blood vessels and vas deferens were ligated with 4–0 absorbable