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Testosterone deficiency impairs glucose oxidation through defective insulin and its receptor gene expression in target tissues of adult male rats

Thirupathi Muthusamy [a], Sivakumar Dhevika [a], Palaniappan Murugesan [b], Karundevi Balasubramanian [a,*]

[a] Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai-600 113, Tamil Nadu, India
[b] Department of Obstetrics and Gynaecology, University of Michigan Medical School, Ann Arbor, Michigan-48109, USA

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Abstract

Testosterone and insulin interact in their actions on target tissues. Most of the studies that address this issue have focused on the physiological concentration of testosterone, which maintains normal insulin sensitivity but has deleterious effects on the same when the concentration of testosterone is out of this range. However, molecular basis of the action of testosterone in the early step of insulin action is not known. The present study has been designed to assess the impact of testosterone on insulin receptor gene expression and glucose oxidation in target tissues of adult male rat. Adult male albino rats were orchidectomized and supplemented with testosterone (100 μg/100 g b. wt., twice daily) for 15 days from the 11th day of post orchidectomy. On the day after the last treatment, animals were euthanized and blood was collected for the assay of plasma glucose, serum testosterone and insulin. Skeletal muscles, such as gracilis and quadriceps, liver and adipose tissue were dissected out and used for the assay of various parameters such as insulin receptor concentration, insulin receptor mRNA level and glucose oxidation. Testosterone deprivation due to orchidectomy decreased serum insulin concentration. In addition to this, insulin receptor number and its mRNA level and glucose oxidation in target tissues were significantly decreased ($p<0.05$) when compared to control. However, testosterone replacement in orchidectomized rats restored all these parameters to control level. It is concluded from this study that testosterone deficiency-induced defective glucose oxidation in skeletal muscles, liver and adipose tissue is mediated through impaired expression of insulin and its receptor gene.
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Keywords: Adipose tissue; Glucose oxidation; Insulin receptor; Liver; Orchidectomy; Skeletal muscles; Testosterone

Introduction

Low levels of testosterone predict the development of type-2 diabetes in men (Haffner et al., 1996; Tibblin et al., 1996; Stellato et al., 2000; Oh et al., 2002; Laaksonen et al., 2004; Svartberg et al., 2004) and, in addition, aging is accompanied by insulin resistance and decline in the levels of testosterone (Morley et al., 1997; Harman et al., 2001; Feldman et al., 2002). The pathogenesis of type-2 diabetes involves impairment of insulin secretion in pancreatic β-cell and insulin resistance in the skeletal muscles, liver, and adipose tissue (Ashcroft and Rorsman, 2004; Panunti et al., 2004). In men, low level of plasma testosterone is associated with obesity, upper body fat distribution, increased levels of glucose and insulin (Haffner et al., 1994), and strongly associated with insulin resistance and metabolic syndrome (Kapoor et al., 2005). Holmang and Bjorntorp (1992) have shown that insulin resistance is associated with the castration of male rats and the condition is reversed with subsequent testosterone replacement. Therefore, there appears to be a link between testosterone deficiency and diabetes (Barrett-Connor, 1992; Andersson et al., 1994). Physiological level of testosterone maintains normal insulin sensitivity, whereas both excess and deficiency of testosterone promote insulin resistance (Holmang and Bjorntorp, 1992). However, the molecular mechanisms involved in such actions of testosterone on target tissues have not yet been identified.

* Corresponding author. Professor, Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai-600113, India. Tel.: +91 44 24540784; fax: +91 44 24540709.
E-mail address: kbala82@rediffmail.com (K. Balasubramanian).

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The effects of insulin are mediated by efficient signal transduction process, which is initiated by its binding to the extracellular domain of the insulin receptor (Taha and Klip, 1999). The binding of insulin to the α-subunits induces a transmembrane conformational change that activates the β-subunit tyrosine kinase domain. Subsequently, the β-subunits undergo a series of autophosphorylation reactions at specific tyrosine residues (Franco, 1992). The insulin receptor phosphorylates many proximal intracellular target molecules that serve as docking sites for effector proteins (White, 1998). Tyrosine-phosphorylated insulin receptor substrate provides SH2-domain binding sites for the regulatory subunit of phosphotidylinositol-3-kinase (PI3-kinase) (Shepherd et al., 1998). Two classes of serine/threonine kinases are known to act downstream of PI3-kinase, namely, PKB and a typical PKC whereby their activation is brought about by phosphoinositide-dependent protein kinase (PDK-1), which leads to glucose uptake by the cell through activation and translocation of GLUT4 from cytosol to plasma membrane (Ishiki et al., 2005). In this event, the initial interaction between insulin and its receptor on target cell surface is followed by a series of surface and intracellular steps that participate in the control of insulin action such as glucose clearance from circulation. Abnormalities in any of these steps could result in defective modulation of receptor number on the cell surface and thus inappropriate cell sensitivity to the hormone (Carpentier, 1994).

Skeletal muscles, liver and adipose tissue are insulin responsive target organs, which have specific receptors for testosterone (Eagon et al., 2001; Carson et al., 2002). There is a wealth of clinical and experimental data, demonstrating that the testosterone and insulin interact in their actions on target tissues (Livingstone and Collison, 2002). However, specific effects of testosterone on insulin receptor gene expression and glucose oxidation in target tissues of insulin are unknown. On the basis of the available information, it is hypothesized that testosterone may be an important regulator for insulin receptor expression and glucose oxidation in target tissues such as skeletal muscles, liver and adipose tissue. This study is designed to test the above hypothesis using adult male rats.

Materials and methods

Chemicals

All chemicals and reagents used in the present study were of molecular biology and analytical grade, and they were purchased from Sigma Chemical Company (St. Louis, MO, USA) and Amersham Biosciences Ltd. (UK). Glucose estimation kit was supplied by CPC diagnostics (Spain). ^{14}C-glucose and [^{125}I] were purchased from Board of Radiation and Isotope Technology (BRIT, Mumbai, India). Radioimmunoassay kits for the assay of insulin and testosterone were obtained from Diasorin (Italy). Total RNA isolation reagent (TRIR), one-step reverse transcriptase-polymerase chain reaction (RT-PCR) kit and primers were purchased from ABgene (UK), Qiagen (Germany) and Integrated DNA technologies, Inc. (Coralville, IA), respectively.

Animals

Animals were maintained as per the National Guidelines and Protocols, approved by the Institutional Animal Ethical Committee (IAEC No. 03/016/05). Healthy adult male albino rats of Wistar strain (*Rattus norvegicus*) weighing 180–200 g (90 days old) were used in the present study. Animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house with constant 12 h light and 12 h dark schedule. They were fed with standard rat pellet diet (Lipton India Ltd., Mumbai, India) and clean drinking water was made available ad libitum.

Experimental design

Rats were divided into three groups. Each group consists of six animals. Group-I: Intact adult; Group-II: Orchidectomized (ORD); and Group-III: Orchidectomized and treated with testosterone (ORD + T). Orchidectomy was performed under ether anesthesia. Group-III rats were treated with testosterone (dissolved in propylene glycol) 10 days after orchidectomy at a dose of 100 μg /100 g body weight (~200 μl per rat), subcutaneously, twice daily (at 8.00 a.m. and 6 p.m.) for 15 days. The dose was selected based on the previous report (Holmang and Bjorntorp, 1992). At the end of treatment, animals were killed by decapitation, blood was collected, sera separated and stored at −80 °C until use. Skeletal muscles, such as quadriceps and gracilis, liver and subcutaneous adipose tissue were dissected out and used for the measurements of glucose oxidation and insulin receptor. Quadriceps and Gracilis muscles are anaerobic and glycolytic type. These muscles have high glucose utilization capacity (Holloszy and Coyle, 1984; Thayer et al., 1993). Therefore, quadriceps and gracilis muscles were considered for the present study. Subcutaneous white adipose tissue is abundant in the abdomen and excessive central adiposity (especially intraabdominal) has been linked to the metabolic syndrome, which includes insulin resistance, dyslypidemia and increased risk of cardiovascular diseases (Collins et al., 2005). Therefore, in the present study subcutaneous adipose tissue was considered.

Collection of blood samples

One day prior to the last injection, blood samples were collected after overnight fasting in microfuge tubes containing EDTA by puncturing the orbital sinus with the help of heparinized microhematocrit capillary tubes (Riley, 1960).

Plasma glucose

Plasma was separated from blood by centrifugation for 10 min at 800 ×*g* at 4 °C within 30 min to prevent autoglycolysis by leukocytes. Plasma glucose was estimated by glucose oxidase–peroxidase method (CPC diagnostics, Spain). The coefficient of variations was 1.8%. Results are expressed as mg/dl.

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