Adipose tissue-derived nerve growth factor and brain-derived neurotrophic factor: results from experimental stress and diabetes

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Abstract. Recently, adipobiology (adiposcience) became a focus of numerous studies showing that the adipose tissue is the body's largest endocrine and paracrine organ producing multiple signaling proteins collectively designated adipokines; at present these include more than hundred proteins. However, studies on adipobiology of neurotrophins have recently emerged, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) being examples of adipose-derived neurotrophins. Here we present data showing that NGF and BDNF are expressed in both white and brown adipose tissue following experimental stress (in mice) and in type 1 diabetes (in rats). We suggest that both neurotrophic and metabotrophic potentials of NGF and BDNF may be involved in the molecular mechanism of stress and diabetes and consequently, in the pathogenesis of cardiometabolic diseases.

Key words: Adipose tissue — Adipokines — Neurotrophins — Diabetes — Stress

Introduction

There is a growing awareness that adipose tissue is an endocrine and paracrine organ producing multiple signaling proteins designated adipokines (Chaldakov et al. 2003; Bulcão et al. 2006; Sacks and Fain 2007; Töre et al. 2007; Chaldakov 2008; Trayhurn et al. 2008). There is evidence indicating that the neurotrophin nerve growth factor (NGF) is also produced by adipose tissue (Chaldakov et al. 2004; Ryan et al. 2008), whereas only two available publications exist about adipose tissue-derived brain-derived neurotrophic factor (BDNF) (Hausman et al. 2006; Sornelli et al. 2007), another important member of the protein family of neurotrophins. In addition to their stimulatory action on neuronal growth and survival, the neurotrophins also act on a number of other cell types including immune cells (Aloe et al. 2001) and pancreatic β cells (Yamanaka et al. 2006). Noteworthy, NGF and BDNF were recently designated metabokines (Sornelli et al. 2007), because i) exert metabotrophic effects on glucose, lipid and energy homeostasis (Töre et al. 2007; Unger et al. 2007), and ii) the levels of NGF and BDNF in coronary atherosclerosis (Chaldakov et al. 2004), acute coronary syndromes (Manni et al. 2005), metabolic syndrome (Chaldakov et al. 2004; Geroldi et al. 2006; Bulló et al. 2007) and obesity/diabetes (El-Gharbawy et al. 2006; Larrieta et al. 2006; Yamanaka et al. 2006; Bulló et al. 2007; Sposato et al. 2007; Unger et al. 2007) are significantly altered, also in stress condition (Aloe et al. 1986; Manni et al. 2007; Fiore et al. 2009).

Hence the question was asked whether the presence of NGF and BDNF might also be altered in both white and brown adipose tissues (WAT and BAT, respectively), in experimental stress and diabetes.

Materials and Methods

Stress was produced in adult male mice of Swiss CD-1 strain (n = 10; 90-day-old, body weight 35.03 ± 0.88 g) by social isolation for 8 weeks followed by housing with a fellow male mice of the same strain and age for 60 min, isolated for the same time period. This profile leads to aggressive behavior and fighting. Intact non-isolated male subjects (n = 10; body weight 34.08 ± 1.34 g) of the same strain and age were used as control group (see Aloe et al. 1986; Fiore et al. 2005). All

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experimental procedures have been carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), the Italian legislation (Decreto L.vo 116/92) and following the guidelines indicated by Intramural Committee and Institutional Guidelines in accordance with National and International law (EEC council directive 86/609, OJ L 358, 1, 12 December, 1987). All efforts were taken to prevent or reduce animal suffering and for limiting the number of experimental subjects.

Diabetes (type 1) was induced in adult Sprague Dawley male rats ($n = 10$; 120-day-old, body weight 358.76 ± 12.24 g) by a single intravenous injection of streptozotocin at a dose of 60 mg/kg dissolved in PBS (Larrieta et al. 2006; Sposato et al. 2007). Rats were considered diabetic and included in the study if they had a fasting plasma glucose level >350 mg/dl 8 weeks after administration of streptozotocin. Ketoacidosis was not observed following this experimental procedure. An equal number of adult male rats of the same age ($n = 10$; body weight 365.12 ± 18.44 g) received a physiological solution and served as controls. During the treatments, rats were housed three per cages.

Stressed and diabetic animals and their respective controls were sacrificed with an overdose of Nembutal, and WAT of the epicardial region, and BAT of the interscapular region were isolated and used for NGF and BDNF immunoenzymatic assay. As heart-associated adipose tissue plays a role in cardiovascular pathology (Chaldakov et al. 2003; Sacks and Fain 2007; Chaldakov 2008), we used epicardial WAT for our studies.

For NGF and BDNF determination, WAT and BAT samples were homogenated with ultrasonication in extraction buffer (in mmol/l: 20 Tris-acetate, pH 7.5, 150 NaCl, 1 EDTA, 1 EGTA, 2.5 sodium-pyrophosphate, 1 orthovanadate, 1 ß-glycerolphosphate, 100 NaF, 1 PMFS, and 1 µg/ml leupeptin) and centrifuged at 4°C for 20 min at 10,000 rpm. The resultant supernatants were recovered and used for analysis of NGF by an enzyme-linked immunosorbent assay (ELISA) kit, "NGF Emax immunoassay system number G7631" by Promega (Madison, WI, USA), following the manufacturer's instructions. BDNF was measured by an ELISA kit, "BDNF Emax immunoassay system number G7611" by Promega (Madison), following the manufacturer's instructions. The concentration of NGF and BDNF was expressed as pg/g of protein. ANOVA was performed by using the StatView software (Mac version). Data were expressed as mean ± SEM.

Results

NGF and BDNF were detected in both WAT and BAT. As shown in Fig. 1 the concentration of NGF in BAT is significantly increased in stressed mice as compared to control tissue ($p < 0.05$). In WAT the elevation was not fully significant

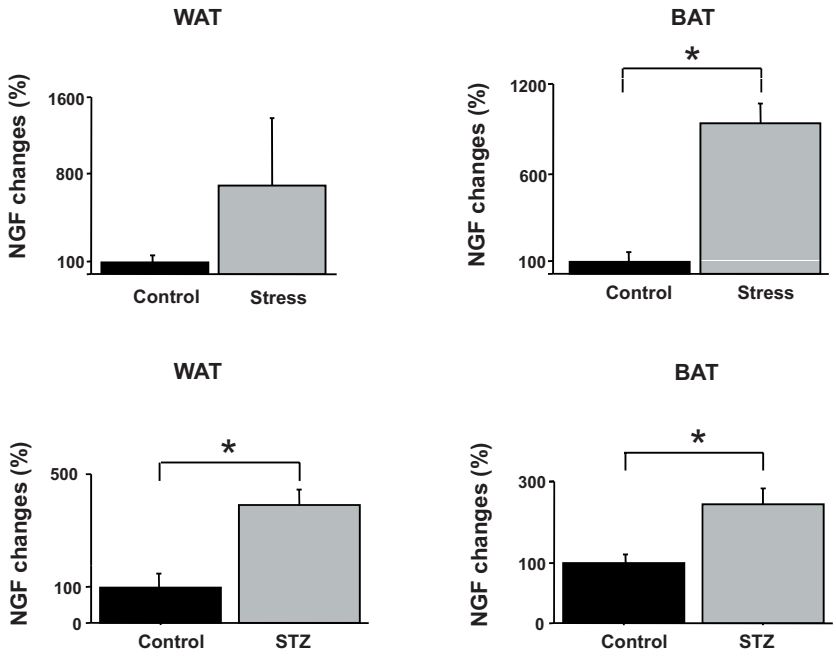

Figure 1. Changes in the amount of nerve growth factor (NGF) in white adipose tissue (WAT) and brown adipose tissue (BAT) of controls compared to the concentration of NGF in stressed mice (Stress) and streptozotocin-induced diabetic rats (STZ), expressed as percentage of controls. Note the enhanced presence of NGF in WAT and BAT in stressed mice as well as diabetic rats. The vertical lines in the figure indicate pooled S.E.M. derived from appropriate error mean square in the ANOVA. * significant differences between groups ($p < 0.05$).

($p < 0.075$). Likewise the presence of NGF in WAT and BAT is significantly enhanced in diabetic rats ($ps < 0.05$). Together these findings indicate that adipo-derived NGF is affected by stressful and diabetic conditions.

Changes were also found in the concentration of BDNF (Fig. 2). Diabetic rats displayed enhanced levels of BDNF in the adipose tissues analyzed ($p < 0.05$). Specifically, ANOVA revealed a mild increase in both WAT and BAT ($p = 0.08$ in post-hocs). However, BDNF presence was not altered in stressed mice.

The cellular source of NGF (see Ryan et al. 2008 for adipocytes) and BDNF in WAT and BAT is not known. Since the adipose tissue contains mast cells (Chaldakov et al. 2000), and these cells are known to be both source of and target for NGF and BDNF (Aloe et al. 1977; Aloe and Levi-Montalcini 2001), we investigated the number of mast cells in WAT and BAT. Our results demonstrate that these cells undergo numerical and degranulation changes under stress or diabetic conditions as previously shown (De Simone et al. 1990; Cirulli et al. 1998; Hristova et al. 2001), suggestive of NGF and/or BDNF secretion by adipose mast cells (data not shown).

Discussion

Since a number of emerging findings indicated that certain obese-related disorders are characterized by altered expression of systemic and/or local NGF and BDNF (Chaldakov et al. 2004; Manni et al. 2005; El-Gharbawy et al. 2006; Geroldi et al. 2006; Larrieta et al. 2006; Yamanaka et al. 2006; Bullò et al. 2007; Manni et al. 2007; Sposato et al. 2007; Unger et al. 2007), the aim of the present study was to evaluate the presence of these neurotrophins in both WAT and BAT of rodents in experimentally-induced stress and diabetic conditions.

We found that NGF and BDNF are present in the adipose tissues and that NGF levels are more significantly altered than BDNF levels in the experimental conditions studied. Further studies are required to prove the secretory activity of adipose tissue cells, as related to NGF and BDNF. Intriguingly, NGF exerts healing effect in diabetic skin ulcers (Generini et al. 2004), and BDNF is known to be a potent metabokine having antidiabetic and anorexigenic effects (Geroldi et al. 2006; Lebrun et al. 2006; Yamanaka et al. 2006; Unger et al. 2007). Further, our data for the first time reveal the presence of both NGF and BDNF in epicardial adipose tissue, which role in the pathogenesis of cardiovascular disease is increasingly studied recently (reviewed in Sacks and Fain 2007; Chaldakov 2008). Note that in addition to NGF and BDNF, other neurotrophic factors as well as various neuropeptides and their receptors are also expressed in adipose tissue (Kos et al. 2007 and references therein; Sacks and Fain 2007; Wang et al. 2008). This might be suggestive of a neuroendocrine potential

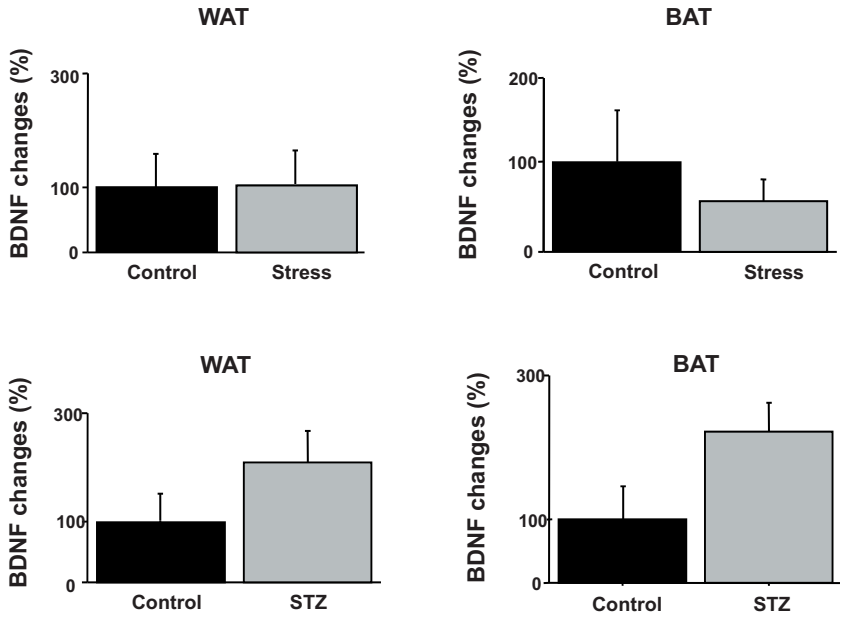

Figure 2. Changes in the amount of brain-derived neurotrophic factor (BDNF) in epicardial white adipose tissue (WAT) and brown adipose tissue (BAT) of controls compared to the concentration of BDNF in stressed mice (Stress) and streptozotocin-induced diabetic rats (STZ), expressed as percentage of controls. The vertical lines in the figure indicate pooled S.E.M. derived from appropriate error mean square in the ANOVA.

of adipose tissue. In line with Levi-Montalcini's general paradigm (Levi-Montalcini 1987), NGF produced and released by adipocytes and/or mast cells (see De Simone et al. 1990; Cirulli et al. 1998; Chaldakov et al. 2000, 2004; Hristova et al. 2001) may serve to regulate the sympathetic innervation of adipose tissue. Such an activity together with NGF/BDNF's effects on extraneuronal functions (Aloe et al. 2001; Chaldakov et al. 2003, 2004; Lebrun et al. 2006; Yamanaka et al. 2006; Unger et al. 2007; Ryan et al. 2008) may implicate adipose-derived neurotrophins to the pathogenesis of cardiometabolic diseases.

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