European Journal of Pharmacology 602 (2009) 455–461

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Endocrine Pharmacology

Altered lipid metabolism in vasopressin V_{1B} receptor-deficient mice

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ARTICLE INFO

Article history:
Received 10 July 2008
Received in revised form 12 November 2008
Accepted 24 November 2008
Available online 3 December 2008

Keywords:
Vasopressin
Metabolism
Lipid

ABSTRACT

We previously reported that insulin sensitivity was increased in vasopressin V_{1B} receptor-deficient ($V_{1B}R^{-/-}$) mice. Here, we investigate the lipid metabolism in $V_{1B}R^{-/-}$ mice. Despite having lower body weight, $V_{1B}R^{-/-}$ mice had significantly greater fat weight of the epididymal white adipose tissue than $V_{1B}R^{+/+}$ mice. Glycerol production and β-oxidation were suppressed in $V_{1B}R^{-/-}$ mice under a fasting condition, and isoproterenol-stimulated lipolysis in differentiated adipocytes was significantly decreased in $V_{1B}R^{-/-}$ mice. These results indicated that lipolysis was inhibited in $V_{1B}R^{-/-}$ mice. On the other hand, lipogenesis was promoted by the increased metabolism from glucose to lipid. Furthermore, our *in vivo* and *in vitro* analyses showed that the secretion of adiponectin was increased in $V_{1B}R^{-/-}$ mice, while the serum leptin level was lower in $V_{1B}R^{-/-}$ mice. These findings indicated that the insulin sensitivity and lipid metabolism were altered in $V_{1B}R^{-/-}$ mice and that the increased insulin sensitivity could contribute to the suppressed lipolysis and enhanced lipogenesis, which consequently resulted in the increased fat weight in $V_{1B}R^{-/-}$ mice.

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1. Introduction

The neurohypophyseal peptide [Arg^8]-vasopressin (AVP) is involved in diverse functions, such as the regulation of body fluid homeostasis, vasoconstriction, and adrenocorticotrophic hormone (ACTH) release (Michell et al., 1979). These physiological effects are mediated by three subtypes of AVP receptors designated V_{1A}, V_{1B}, and V_2, all of which belong to G protein-coupled receptors (Birnbaumer, 2000). The vasopressin V_{1A} receptor is expressed ubiquitously, while the vasopressin V_{1B} receptor is specifically expressed in pituitary corticotrophs and pancreatic islets (Oshikawa et al., 2004). Both of them bring about phosphatidylinositol hydrolysis, leading to the mobilization of intercellular Ca^{2+}. The vasopressin V_2 receptor is primarily found in the kidney and is linked to adenylate cyclase and the production of cAMP, in association with antidiuresis (Thibonnier, 1988).

AVP plays a crucial role in the regulation of the blood levels of glucose and free fatty acid. AVP infusions induce an increase in circulating glucose levels (Rofe and Williamson, 1983; Spruce et al., 1985). This effect is supposed to be due to two distinct actions. AVP stimulates the glucagon and insulin releases from pancreatic islet cells *in vitro* (Oshikawa et al., 2004; Yibchok-anun and Hsu, 1998) via the vasopressin V_{1B} receptor (Fujiwara et al., 2007b; Oshikawa et al., 2004; Richardson et al., 1995). In addition, AVP can act directly in the liver to stimulate glucose production. In hepatocytes, AVP interacts with specific vasopressin V_1 receptor sites (Keppens and de Wulf, 1979) and promotes glycogenolysis and gluconeogenesis (Hems, 1977). These actions of AVP in the liver are distinct from those of glucagon and are mediated by a calcium-dependent pathway (Garrison and Wagner, 1982) via the vasopressin V_{1A} receptor. With regard to the lipid metabolism, AVP is known to decrease circulating ketone bodies (Rofe and Williamson, 1983) and to suppress isoproterenol-induced lipolysis depending on the existence of calcium ion (Tebar et al., 1996) via the vasopressin V_{1A} receptor. This finding indicates that AVP stimulates the lipid metabolism via the vasopressin V_{1A} receptor. Consisting with the finding, vasopressin V_{1A} receptor-deficient ($V_{1A}R^{-/-}$) mice exhibit a phenotype with the hypermetabolism of fat (Hiroyama et al., 2007a).

We generated $V_{1A}R^{-/-}$ and V_{1B} receptor-deficient ($V_{1B}R^{-/-}$) mice, which are not lethal and have no apparent anatomical anomalies. $V_{1A}R^{-/-}$ mice exhibit the hypermetabolism of fat and muscular protein, and insulin resistance (Aoyagi et al., 2007; Hiroyama et al., 2007a,b). These characteristics are in part due to an interference of insulin signaling by a deficiency of the vasopressin V_{1A} receptor, which could inhibit the activation of Gs signaling to hormone-sensitive lipase (Hiroyama et al., 2007a). On the other hand, $V_{1B}R^{-/-}$ mice exhibit the impairment of ACTH, corticosterone, and insulin secretion (Oshikawa et al., 2004; Tanoue et al., 2004). Recently, we have reported that $V_{1B}R^{-/-}$ mice have enhanced insulin sensitivity (Fujiwara et al., 2007a), which is a contrastive phenotype compared to $V_{1A}R^{-/-}$ mice. Here, we investigated the lipid metabolism in $V_{1B}R^{-/-}$ mice and found suppressed lipolysis and enhanced lipogenesis due to increased insulin sensitivity, leading to increased fat weight.

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doi:10.1016/j.ejphar.2008.11.043

2. Materials and methods

2.1. Animals

The generation of vasopressin $\mathbf{V_{1B}}$ receptor-deficient ($\mathbf{V_{1B}}R^{-/-}$) mice was described previously (Tanoue et al., 2004). Briefly, by homologous recombination, we disrupted exon 1, which contains the translation initiation codon. The generated mutant mice were of a mixed genetic background of 129 Sv and C57BL/6. Non-$\mathbf{V_{1B}}$ receptor-deficient littermates ($\mathbf{V_{1B}}R^{+/+}$) were used as age-matched control subjects for $\mathbf{V_{1B}}R^{-/-}$ mice and maintained on the 129 Sv and C57BL/6. Animals were housed in micro-isolator cages in a pathogen-free barrier facility. $\mathbf{V_{1B}}R^{+/+}$ and $\mathbf{V_{1B}}R^{-/-}$ mice were housed on a 12 h light/dark cycle with *ad libitum* access to food and water except when the experimental protocol specified otherwise. Male mice were used for this study under a feeding condition with a normal chow diet. Male $\mathbf{V_{1B}}R^{+/+}$ and $\mathbf{V_{1B}}R^{-/-}$ mice were weighed once weekly from weaning at 3 weeks of age. The body weights were measured using a microbalance. The epididymal white adipose tissue deposits were dissected immediately after mice were killed by cervical dislocation under anesthesia with diethyl ether. All experimentation was performed under the guidelines for the Care and Use of Laboratory Animals of the National Research Institute for Child Health and Development.

2.2. Biochemical analysis

The serum free fatty acid, triglyceride, and cholesterol obtained from the inferior vena cava of mice at 8–10 weeks of age under the feeding or 24 h-fasting condition were measured using the NEFA *C*-test Wako, triglyceride *E*-test Wako, and cholesterol *E*-test Wako (Wako, Tokyo, Japan), respectively. Serum adiponectin and leptin were measured using an Adiponectin EIA kit (SPI-BIO, Montigny Le Bretonneux, France), and a Leptin EIA kit (SPI-BIO, Montigny Le Bretonneux, France).

2.3. Electrospray tandem mass spectrometry

Electrospray tandem mass spectrometry for the analysis of carnitine and acylcarnitines in dried blood specimens collected from the tail vein of mice at 8–10 weeks of age under the feeding or 24 h-fasting condition was carried out as described previously (Hiroyama et al., 2007a).

2.4. Isoproterenol-induced lipolysis assay in differentiated white fat precursor cells

White fat precursor cells were isolated from $\mathbf{V_{1B}}R^{+/+}$ and $\mathbf{V_{1B}}R^{-/-}$ mice at 3–4 weeks of age by collagenase digestion as described previously (Hiroyama et al., 2007a). The precursor cells were seeded on a 96-well plate at $1.2–2.0\times10^4$/well, and the medium was changed to a fresh medium 20 h later. Adipocyte differentiation was induced by treating confluent cells in an induction medium (10% FBS-DMEM containing 0.5 mM isobutylmethylxanthine, 0.5 μM dexamethazone, 0.125 mM indomethacine, 20 nM insulin (Sigma, MO, USA), and 1 nM T3 for 3 days, and the lipolysis assay was then carried out in a phenol red free-DMEM medium. Isoproterenol was added at the indicated concentrations for 5 h, and the glycerol content in the culture medium was measured with a lipolysis assay kit (Zen-Bio Inc., NC, USA).

For oil-red O staining, cells fixed using 10% formalin for 1 h were soaked in 3 mg/ml oil-red O in 60% isopropanol for 1 h and then washed with 60% isopropanol for 2 min. For the quantification of intracellular triglyceride, the living cells were washed with PBS once, and then 200 μl of PBS was added into each well. Then, 5 μl of AdipoRed (BioWhittaker Inc., MD, USA) was added into each well, and the cells were incubated for 10 min at room temperature. After 10 min, the fluorescence was measured with excitation at 485 nm and emission at 535 nm. To determine the protein concentration, separate wells were prepared, and the protein concentration was quantified using the BCA protein assay (Pierce, IL, USA).

2.5. Insulin-induced lipogenesis and 2-deoxyglucose uptake assay in differentiated white fat precursor cells

For the glucose incorporation experiment, the precursor cells were seeded on a 6-well plate, and the medium was changed to a fresh medium 20 h later. Adipocyte differentiation was induced by treating confluent cells with an induction medium for 3 days for the glucose incorporation experiment. After a preincubation period of 30 min at 37 °C in Krebs–Ringer buffer containing 0.55 mM D-glucose, 15 mM sodium bicarbonate, 10 mM Hepes, and 1% BSA (pH 7.4), the cells were incubated with the indicated concentration of insulin for 5 min at 37 °C. The adipocytes were then incubated with 0.12 μCi D-[U–^{14}C] glucose/ml (Perkin Elmer, MA, USA) for 60 min. The supernatant was removed, and then the reaction was terminated by the addition of 1 ml chloroform:methanol (1:2). The extracts were transferred into 15 ml tubes. Three hundred μl chloroform was added, and then 600 μl of 1 N HCl was added to separate the layers by centrifugation (1000 *g*, 5 min). The lower phase was measured. Glucose incorporation into lipids is expressed as nano-moles of glucose incorporated per milligram of lipid.

For the 2-deoxyglucose uptake experiment, the precursor cells were seeded on a 6-well plate and differentiated for 3 days. The differentiated cells were then used for the 2-deoxyglucose uptake experiment. After a preincubation period of 30 min at 37 °C in Krebs–Ringer buffer containing 15 mM sodium bicarbonate, 10 mM Hepes, and 1% BSA (pH 7.4), the cells were incubated with the indicated concentration of insulin for 25 min at 37 °C. The adipocytes were then incubated with 50 μM 2-deoxy-D-[^{3}H] glucose (0.5 μCi/ml) (Perkin Elmer, MA, USA) for 30 min, and the reaction was terminated by the addition of 10 μm cytochalasin B (Sigma, MO, USA). Cells were washed three times with ice-cold PBS and lysed with 0.5 ml Solvable (Perkin Elmer, MA, USA). The radioactivity taken up by the cells was measured using a liquid scintillation counter.

2.6. Tissue triglyceride, free fatty acid, and cholesterol contents

The liver and skeletal muscle of mice at 8–10 weeks of age under the feeding or 24 h-fasting condition were dissected, and the exact mass of the sample was then determined using a microbalance. The tissues were homogenated in 100 μl of H_2O, and 600 μl of chloroform: methanol (1:2) was then added to the homogenates. The homogenates were mixed and placed overnight at room temperature to extract the total lipids. Two hundred ml chloroform was added, and then 200 μl of 1 N HCl was added to separate the layers by centrifugation. The lower phase was dried and resolved in the appropriate volume of isopropanol. The solutions were used for triglyceride, free fatty acid, and cholesterol measurements using the triglyceride *E*-test Wako (Wako, Tokyo, Japan), NEFA *C*-test Wako, cholesterol *E*-test Wako, respectively.

2.7. Adipokine secretion from adipocytes

For this experiment, the precursor cells were seeded on a 6-well plate, and the medium was changed to a fresh medium 20 h later. Adipocyte differentiation was induced by treating confluent cells with an induction medium for 5 days. After the differentiation, the culture medium was replaced with 0.5% BSA-DMEM. The cells were then incubated for 4 h. The level of adiponectin was measured using the Adiponectin EIA kit (SPI-BIO, Montigny Le Bretonneux, France).

2.8. Adipokine expression during the differentiation of adipocytes derived from $\mathbf{V_{1B}}R^{+/+}$ and $\mathbf{V_{1B}}R^{-/-}$ mice

For this experiment, the precursor cells were seeded on 10-cm dishes, and the medium was changed to a fresh medium 20 h later. Adipocyte differentiation was induced by treating confluent cells in an induction

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