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Enhanced glucose tolerance in the Brattleboro rat

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ABSTRACT

[Arg^8]-vasopressin (AVP) plays a crucial role in regulating body fluid retention, which is mediated through the vasopressin V_2 receptor in the kidney. In addition, AVP is involved in the regulation of glucose homeostasis via vasopressin V_{1A} and vasopressin V_{1B} receptors. Our previous studies demonstrated that vasopressin V_{1A} receptor-deficient ($V_{1A}R-/-$) and V_{1B} receptor-deficient ($V_{1B}R-/-$) mice exhibited hyperglycemia and hypoglycemia with hypoinsulinemia, respectively. These findings indicate that vasopressin V_{1A} receptor deficiency results in decreased insulin sensitivity whereas vasopressin V_{1B} receptor deficiency results in increased insulin sensitivity. In addition, vasopressin V_{1A} and vasopressin V_{1B} receptor double-deficient ($V_{1AB}R-/-$) mice exhibited impaired glucose tolerance, suggesting that the effects of vasopressin V_{1B} receptor deficiency do not influence the development of hyperglycemia promoted by vasopressin V_{1A} receptor deficiency, and that the blockage of both receptors could lead to impaired glucose tolerance. However, the contributions of the entire AVP/vasopressin receptors system to the regulation of blood glucose have not yet been clarified. In this study, to further understand the role of AVP/vasopressin receptors signaling in blood glucose regulation, we assessed the glucose tolerance of AVP-deficient homozygous Brattleboro (*di*/*di*) rats using an oral glucose tolerance test (GTT). Plasma glucose and insulin levels were consistently lower in homozygous *di*/*di* rats than in heterozygous *di*/+ rats during the GTT, suggesting that the blockage of all AVP/vasopressin receptors resulting from the AVP deficiency could lead to enhanced glucose tolerance.

1. Introduction

The neurohypophyseal peptide [Arg^8]-vasopressin (AVP) is involved in diverse functions, including the regulation of body fluid homeostasis, vasoconstriction, and adrenocorticotropic hormone (ACTH) release [1]. These physiological effects are mediated by three subtypes of vasopressin receptors, V_{1A}, V_{1B}, and V_2, all of which belong to the G protein-coupled receptor family [2]. The vasopressin V_{1A} receptor is expressed ubiquitously whereas the vasopressin V_{1B} receptor is specifically expressed in pituitary corticotrophs, pancreatic islets, and white adipose tissue in peripheral tissues [3–5]. Both of the vasopressin V_1 receptors bring about phosphatidylinositol hydrolysis, which leads 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 cyclic adenosine monophosphate (cAMP), in association with antidiuresis [6].

In our previous studies, we examined vasopressin V_{1A} receptor-deficient ($V_{1A}R-/-$), vasopressin V_{1B} receptor-deficient ($V_{1B}R-/-$), and V_{1A} and V_{1B} receptor double-deficient ($V_{1AB}R-/-$) mice. In both $V_{1A}R-/-$ and $V_{1B}R-/-$ mice, hormone secretion and glucose and fat metabolisms were altered [3–5,7–10]. Interestingly, $V_{1A}R-/-$ mice exhibit decreased insulin sensitivity [11] whereas $V_{1B}R-/-$ mice exhibit increased insulin sensitivity [3]. $V_{1A}R-/-$ mice exhibit hyperglycemia with insulin resistance, which is at least in part due to the decreased plasma volume, increased glycogenolysis, and/or decreased insulin signal in the adipocytes [11]. $V_{1B}R-/-$ mice, on the other hand, exhibit hypoglycemia with hypoinsulinemia, which is probably in part due to the enhanced insulin signal in the adipocytes [3]. In addition, glucose tolerance was impaired in $V_{1AB}R-/-$ mice, suggesting that the effects of vasopressin V_{1B} receptor deficiency do not influence the development of hyperglycemia promoted by vasopressin V_{1A} receptor deficiency [12]. These findings indicate that AVP regulates glucose homeostasis via both vasopressin V_{1A} and V_{1B} receptors, and that the blockage of both receptors could lead to impaired glucose tolerance. However the details of AVP's mechanisms on glucose homeostasis remain unclear. In this study, we investigate glucose tolerance in AVP-deficient Brattleboro (*di*/*di*) rats to further clarify the role of AVP/vasopressin receptors signaling in regulating glucose homeostasis.

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2. Materials and methods

2.1. Animals

Male Brattleboro rats from our institute were used for all experiments. We analyzed the plasma glucose and insulin levels of AVP-deficient homozygous Brattleboro (*di*/*di*) rats with diabetes insipidus using a glucose tolerance test (GTT). We compared the results to those of heterozygous Brattleboro (*di*/+) control rats from the same litters. The animals were housed in microisolator cages in a pathogen-free barrier facility. The rats had a 12 h light/dark cycle and *ad libitum* access to food and water. 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. Glucose tolerance test (GTT)

The GTT was given at 11 weeks of age ($n = 8$). Rats were fasted for 16 h before receiving an oral administration with 1.5 g glucose/kg body weight in water. Blood samples were taken from the tail vein in heparinized microcapillary tubes at 0, 10, 30, 60, and 120 min after glucose administration, and the plasma glucose and insulin levels were measured. To measure plasma glucose levels, blood samples (10 μl) were immediately mixed with 10 volumes of 0.33 M $HClO_4$ to inhibit the utilization of glucose before measurement. The mixtures were centrifuged at 3000 rpm for 5 min, the supernatants (10 μl) were taken up, and the glucose concentration was determined using the Glucose Test Wako (Wako Pure Chemical Industries, Osaka, Japan) with an identically treated standard. Plasma insulin was measured using an ELISA kit (Mercodia, Uppsala, Sweden).

2.3. Statistical analysis

All values are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using the unpaired Student's *t*-test or two-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) as a post hoc test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. GTT in Brattleboro rats

To evaluate the glucose metabolism in *di*/*di* rats, we first assessed the fasting plasma glucose and insulin levels. After 16 h fasting, the plasma glucose level of *di*/*di* rats was not significantly different from that of *di*/+ rats (101.1 ± 2.8 mg/dl in *di*/*di* rats, $n = 8$ vs. 108.5 ± 2.4 mg/dl in *di*/+ rats, $n = 8$, $p = 0.06$, Fig. 1A). This finding indicates that the fasting glucose level of *di*/*di* rats is not altered, as reported in previous studies [13,14]. In contrast, the plasma insulin level of *di*/*di* rats was significantly lower than that of *di*/+ rats (1.56 ± 0.33 mg/dl in *di*/*di* rats, $n = 8$, vs. 0.67 ± 0.07 mg/dl in *di*/+ rats, $n = 8$, $p < 0.05$, Fig. 1B). Next, we assessed the glucose tolerance of *di*/*di* rats using the oral GTT. The plasma glucose levels observed during the GTT were consistently lower in *di*/*di* rats than in *di*/+ rats (Fig. 2A). The area under the curve (AUC) for plasma glucose levels was significantly lower in *di*/*di* rats than in *di*/+ rats (Fig. 2B). The plasma insulin levels were also lower in *di*/*di* rats than in *di*/+ rats (Fig. 3A). The AUC for plasma insulin levels was lower in *di*/*di* rats than in *di*/+ rats (Fig. 3B). These results indicate that *di*/*di* rats have enhanced glucose tolerance, which is similar to the finding in $V_{1B}R-/-$ mice [3], but not $V_{1A}R-/-$ or $V_{1AB}R-/-$ mice [11,12].

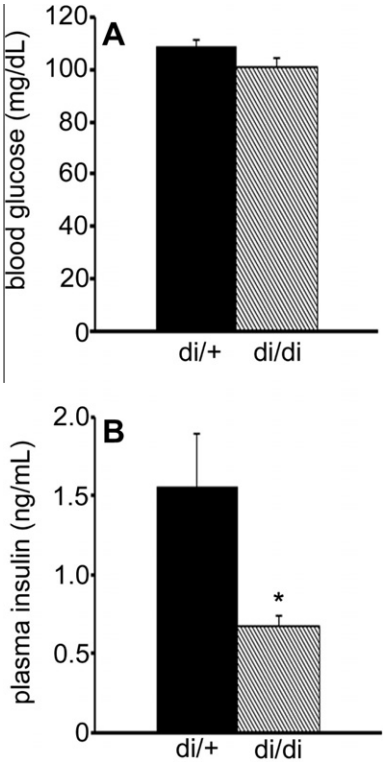

Fig. 1. Basal plasma glucose and insulin levels in *di*/*di* rats. Basal plasma glucose (A) and insulin (B) levels in *di*/+ ($n = 8$) and *di*/*di* ($n = 8$) rats after a 16 h fast. Values are means ± SEM. Significance: $^{*}p < 0.05$ vs. *di*/+ rats by post hoc comparison with Fisher's PLSD.

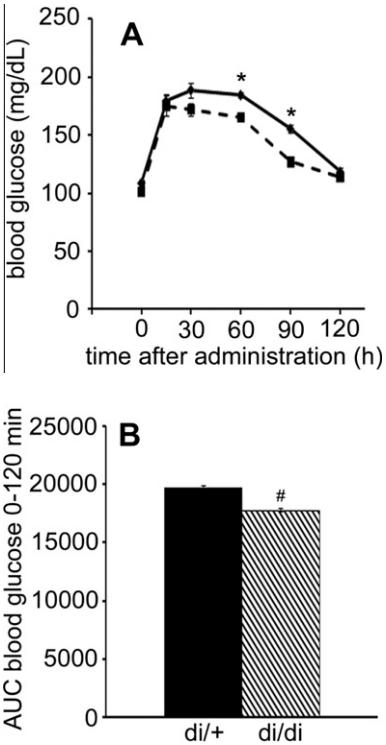

Fig. 2. Plasma glucose levels during the glucose tolerance test in *di*/*di* rats. Plasma glucose levels (A) and AUC (B) in *di*/+ (♦) and *di*/*di* (■) rats during the GTT. The GTT was administered to *di*/+ ($n = 8$) and *di*/*di* ($n = 8$) rats at 11 weeks of age. Values are means ± SEM. Significance: $^{*}p < 0.001$ vs. *di*/+ rats by post hoc comparison with Fisher's PLSD. $^{\#}p < 0.001$ vs. *di*/+ rats by the unpaired Student's *t*-test.

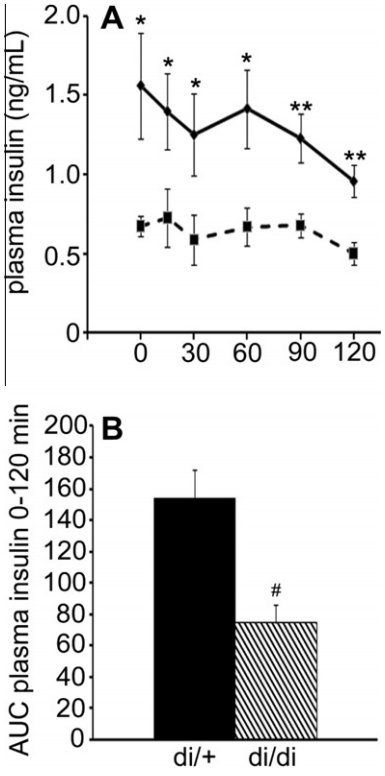

Fig. 3. Plasma insulin levels during the glucose tolerance test in *di*/*di* rats. Plasma insulin levels (A) and AUC (B) in *di*/+ (♦) and *di*/*di* (■) rat during the glucose tolerance test. The glucose tolerance test was administered to *di*/+ ($n = 8$) and *di*/*di* ($n = 8$) rats at 11 weeks of age. Values are means ± SEM. Significance: $^{*}p < 0.05$; $^{**}p < 0.01$ vs. *di*/+ rats by post hoc comparison with Fisher's PLSD. $^{\#}p < 0.01$ vs. *di*/+ rats by the unpaired Student's *t*-test.

4. Discussion

In our previous study, we evaluated the glucose tolerance in double mutant mice lacking both vasopressin V_{1A} and vasopressin V_{1B} receptors ($V_{1AB}R-/-$) using the GTT. Although the basal glucose level did not differ between $V_{1AB}R-/-$ and wild-type (WT) mice, the basal insulin level was significantly higher in $V_{1AB}R-/-$ mice than in WT mice. In addition, plasma glucose levels were higher and plasma insulin levels tended to be higher in $V_{1AB}R-/-$ mice than in WT mice during the GTT [12]. These findings indicated that the glucose tolerance of $V_{1AB}R-/-$ mice was impaired. Furthermore, this altered glucose tolerance observed in $V_{1AB}R-/-$ mice was more pronounced under a high-fat diet condition [12]. In this study, we investigated the plasma glucose and insulin levels in *di*/+ and *di*/*di* rats during the GTT and found that both the plasma glucose and insulin levels were lower in *di*/*di* rats than in *di*/+ rats, indicating that the glucose tolerance was enhanced in *di*/*di* rats. These results suggest that AVP deficiency, which may result in the blockage of all AVP receptor signals, leads to enhanced glucose tolerance, whereas the blockage of the vasopressin signal through both V_{1A} and V_{1B} receptors leads to impaired glucose tolerance. Signals through receptors other than V_1 receptors such as the V_2 receptor may be involved in regulating the blood glucose level as a result of the effects of AVP.

Numerous reports argue that AVP and AVP receptors are involved in regulating glucose homeostasis and that altered signals of AVP/AVP receptors lead to impaired glucose tolerance [3,10–12,15,16]. However, the mechanisms underlying the regulation of glucose homeostasis by AVP have not yet been clarified. Vasopressin V_{1B} receptor deficiency leads to increased insulin sensitivity whereas vasopressin V_{1A} receptor deficiency leads to decreased insulin sensitivity, even when it is accompanied by vasopressin V_{1B} receptor deficiency. Thus, the *in vivo* effect of AVP on glucose metabolism seems greater via the vasopressin V_{1A} receptor than via the vasopressin V_{1B} receptor, when comparing both receptors. Because the $V_{1AB}R-/-$ mouse exhibited impaired glucose tolerance, we expected impaired glucose tolerance in the AVP-deficient *di*/*di* rats, whose AVP deficiency could mimic deficiency for all AVP receptors [17]. However, we found that the AVP-deficient *di*/*di* rats exhibited enhanced glucose tolerance in this study. These findings suggest that the AVP signals through the V_2 receptors as well as V_{1A} and V_{1B} receptors may also be involved in regulating glucose homeostasis *in vivo*, although it remains to be clarified whether or not the AVP/V_2 receptor signals affect glucose homeostasis directly or indirectly. The vasopressin V_2 receptors are primarily present in the kidney where AVP plays a crucial role in regulating body fluid retention though the vasopressin V_2 receptor [18,19]. The AVP/V_2 receptor signal may affect glucose homeostasis by altering the regulation of water homeostasis. Vasopressin V_2 receptors were also found to be expressed in the heart, liver, muscle, white adipose tissue, and brown adipose tissue, all of which are insulin-sensitive tissues [3]. This suggests the possibility that the AVP/V_2 receptor signal influences insulin sensitivity in these insulin-sensitive tissues. Although there are few reports regarding the underlying mechanism(s) involved in the insulin sensitivity via the AVP/V_2 receptor signal, AVP may contribute to the insulin sensitivity via the V_2 receptor, leading to the activation of an adenosine 3′,5′-cyclic monophosphate (cAMP) responsive element binding protein (CREB) [20]. The adenylate cyclase-coupled V_2 receptor is activated by the AVP stimulation, which leads to the induction of the phosphorylation of CREB [20]. The phosphorylated CREB promotes insulin resistance by triggering the expression of the transcriptional repressor ATF3, thereby down-regulating the expression of the insulin-sensitive glucose transporter 4 (GLUT4) as well as the adipokine hormone adiponectin in adipose cells, which express the V_2 receptors [21]. Thus, AVP may play a role in regulating the insulin sensitivity by affecting the CREB signals via the V_2 receptor. In the AVP-deficient Brattleboro rats, the AVP/V_2 signal is blunted, and the CREB signal via the V_2 receptor is suppressed, which may lead to enhanced insulin sensitivity as a consequence.

In addition to the altered signal(s) via the AVP receptors, oxytocin (OXT) signals may also be involved in the altered glucose tolerance. OXT and AVP, which are nonapeptides, belong to a family of highly related peptides found throughout the animal kingdom [22]. The peptide sequences of AVP and OXT only differ in 2 amino acids in positions 3 and 8. The different AVP and OXT receptors cloned so far are classified into three AVP receptors ($V_{1A}R$, $V_{1B}R$, and V_2R) and one OXT receptor (OTR), on the basis of their binding and signal transduction properties [22]. These receptors are closely related, as their overall similarity varies from 40% to 85%. Owing to this conservation in both peptides and receptors, we can presume that these peptides interact with the different receptors in a common manner [22]. The best known biological effects of circulating oxytocin are those on female reproductive organs, such as the stimulation of uterine contraction and milk ejection. The effects of circulating oxytocin have also been documented in the organs of the male genital tract, such as penile erection and ejaculation [23,24]. There is also substantial evidence from animal research indicating that OXT plays a key role in the regulation of complex social cognition and behavior [25]. In addition to these physiological functions, recent studies with knockout mice revealed that OXT was involved in regulating glucose homeostasis. OXT-deficient mice exhibit an obese phenotype with decreased insulin sensitivity and glucose tolerance [26]. OTR-deficient mice exhibit an obese phenotype with increased abdominal fat pads and fasting

plasma triglycerides [27]. These findings indicate that OXT plays a crucial role in regulating the glucose tolerance as well as energy homeostasis. In addition, it was also reported that, in addition to plasma OXT level, the expression of OTR was elevated in the AVP-deficient Brattleboro rat [28,29], suggesting that the OXT signals were promoted *in vivo* in this rat. Taken together, these results indicate that the enhanced OXT signals in the AVP-deficient Brattleboro rat may contribute to the enhanced glucose tolerance.

In summary, the enhanced glucose tolerance observed in the AVP-deficient Brattleboro rat may be due to unknown complex mechanism(s), including altered insulin sensitivity due to V_{1A}, V_{1B}, and V_2 receptor signal deficiency as well as altered body fluid retention due to V_{1A} and V_2 receptor signal deficiency and/or enhanced OXT signals.

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