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The role of vasopressin in diabetes mellitus-induced hypothalamo-pituitary-adrenal axis activation: Studies in Brattleboro rats

Dóra Zelena *, Zsuzsa Mergl, Gábor B. Makara

Institute of Experimental Medicine, Hungarian Academy of Sciences, 1083 Budapest, Szigony 43, Hungary

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Abstract

Chronic diabetes mellitus (DM) induces hyperactivity of the hypothalamo-pituitary-adrenal axis (HPA). Our present study addresses the role of vasopressin (AVP) in maintaining adrenocortical responsiveness during DM.

AVP-deficient mutant Brattleboro rats were used with heterozygous controls and the V2 agonist, desmopressin was infused to replace peripheral AVP. To induce DM the rats were injected by streptozotocin (STZ, 60 mg/ml/kg i.v.) and studied 2 weeks later. The acute stress stimulus was 60 min restraint.

The signs of DM (the increase in water consumption and in blood glucose levels) were discovered in all rats. The diuretic effect of the lack of AVP was additional to the DM-induced osmotic diuresis. DM induced significant, chronic stress-like somatic changes on which AVP-deficiency had no effect and although desmopressin infusion normalized the water consumption and the body weight gain in AVP-deficient rats, it had no effect on DM-induced changes. The acute stress-induced plasma ACTH elevation was smaller in AVP-deficient or DM rats but these effects were not additive. Desmopressin did not normalize the decreased ACTH-elevation of AVP-deficient animals. The resting morning plasma corticosterone level was elevated both in DM and AVP-deficient rats without interaction. The restraint-induced corticosterone rise was influenced neither by the lack of AVP nor by DM and the basal and stress-induced prolactin levels were smaller in DM rats without any effect of AVP-deficiency.

In conclusion, our data suggest that AVP does not play a crucial role in HPA axis regulation during DM-induced chronic stress. In contrast, the role of AVP seems to be more important during acute stress, however, it is restricted to the ACTH regulation. According to the water consumption data diabetes insipidus seems to be an additional risk factor for DM.

Keywords: Stress; Restraint; Adrenocorticotropin; Corticosterone; Prolactin; Oxytocin

1. Introduction

Many chronic diseases subject the body to constant stress. Stressful stimuli activate the hypothalamo-pituitary-adrenal (HPA) system. Corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are the main neuropeptide-hormones of the hypothalamic paraventricular nucleus (PVN) that regulate the HPA axis [3,40]. Inhibitory feedback is provided by adrenal glucocorticoids. It has been proposed that during chronic stress there is a shift in favor of a preferential activation of AVP rather than CRH, and the relative resistance to feedback inhibition of adrenocorticotropin (ACTH) release stimulated by AVP may explain how chronic stressful stimulation maintains high adrenocortical hormone levels [1,15,32,40].

A tight connection between HPA axis activity and blood glucose regulation is well known [11,42]. Glucocorticoid hormones participate in maintaining normal blood glucose level that is especially important for the brain. Hypoglycemia is a strong stimulus for HPA axis activation, which produces glucocorticoids [20]. Glucocorticoids increase hepatic gluconeogenesis, inhibit glucose uptake in adipocytes and fibroblasts, sensitize the liver to glucagon and epinephrine and decrease the hepatic sensitivity to insulin [12,14] resulting overall in an increase in blood glucose levels. Diabetes mellitus (DM) is a common endocrine disorder and, left untreated, may also induce a dysfunction of the HPA axis due to its role as a stressor [14,41]. Hyperactivity

* Corresponding author. Tel.: +36 1 210 9950; fax: +36 1 210 9951.
E-mail address: zelena@koki.hu (D. Zelena).

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of the HPA axis in experimental streptozotocin-induced diabetes mellitus (STZ-DM) was found already in 1976 [18]. It is also known that patients with both types of DM can have increased plasma cortisol level [14]. Acute increase in plasma glucocorticoid levels is useful during stress condition [21,22,38]. However, chronic increase in plasma glucocorticoid concentration can be harmful in general [37] and also for diabetic patients, because these hormones may aggravate the disorder through further elevation of blood glucose levels.

As a part of the changes in neuroendocrine function occurring in diabetic animals reduced prolactin levels and response was also shown [4,36]. We measured prolactin as an additional, non-habituating indicator of stress-responsiveness. The plasma prolactin level is known to be elevated after different acute stresses [26]; however, its function in male rats is not fully understood. One can hypothesize that during acute stress, prolactin may have some influence on the immune system [19]. Prolactin is mainly secreted under the inhibitory effect of dopamine [7], however, vasopressin may also have some kind of influence [33] and prolactin secretion may be regulated simultaneously by angiotensin II [25].

As AVP is supposed to have a preferential role during chronic stress situation, it was reasonable to propose the contribution of AVP in hyperactivation of HPA during STZ-induced DM [1,15]. The present study tests the hypothesis that the presence of AVP is necessary for maintaining high levels of adrenocortical activity during DM-induced chronic stress in the AVP deficient mutant Brattleboro rat [46]. AVP acts on vasoconstriction and diuresis through two different types of receptors (V1 and V2, respectively) but modifies pituitary hormone release primarily through V1b (pressor-like) receptors [2,10]. To eliminate the possible influence of the peripheral AVP deficiency a portion of the diabetes insipidus animals were also subjected to peripheral AVP replacement with a V2 agonist (desmopressin). The hormonal reaction to an acute stress stimulus (restraint) was recorded.

2. Materials and methods

2.1. Animals

Male Brattleboro rats were maintained in our Institute in a colony using of breeder rats from Harlan, Indianapolis, IN, USA. We compared the AVP deficient homozygous (di/di) rats with diabetes insipidus to heterozygous (di/+) control rats from the same litters. Part of the animals came from di/+ and others from di/di mother rats [49]. Rats were kept in controlled environment (23 ± 1 °C, 50–70% humidity, 12 h light starting at 06:00) and given commercial rat chow (Charles River, Hungary) and tap water ad libitum. The animals were kept one per cage from the beginning of the experiment. The experiments were performed in accordance with regulations set by the Hungarian Council for Animal Care and were supervised by the Institutional Animal Care and Use Committee.

2.2. Experiment

The Brattleboro animals had two different genotypes: heterozygous (di/+) and diabetes insipidus rats (di/di). After 3 days water measurement half of the di/di rats were implanted with Alzet osmotic minipump (1 μl/h, 7 days) filled with 200 μl of 1 μg/ml desmopressinum (V2 receptor agonist; Ferring Léciva, Prague, Czech Republic) under ether anaesthesia, while other animals went through sham operation (the same procedure except using minipump implantation). On day 14, the minipump was changed. On day 7, all rats were injected intravenously either with streptozotocin (STZ; 60 mg/kg, Sigma–Aldrich, Budapest, Hungary) or the citrate buffer vehicle (1 ml/kg; pH 5; control) through the tail vein under short restraint (max. 2 min) and left largely undisturbed (except weekly body weight measurement and daily water measurement and bedding change). Rats were decapitated on day 22. Half of the animals underwent an acute challenge (60 min restraint in transparent plastic tubes) [48] and decapitated at the end of restraint. Altogether 12 groups were studied according to Table 1.

2.3. Blood glucose

Appearance of diabetes was monitored by daily measurement of water consumption and confirmed by measuring the blood glucose concentration from trunk blood using an analyser (D-Cont Personal, 77 Elektronika Kft., Budapest).

2.4. Hormone analysis

Plasma was collected at the end of 2 weeks from trunk blood in K_2-EDTA containing tubes on ice. After centrifugation the plasma was stored at a temperature of −20 °C until hormone measurement. Plasma ACTH was measured with radioimmunoassay (RIA) in 50 μl unextracted plasma as described earlier [47]. The intra- and inter-assay coefficients of variation were 6.4 and 17.7%, respectively. Plasma corticosterone was measured from 10 μl unextracted plasma with a RIA using a specific antiserum developed in our institute [48]. The intra- and inter-assay coefficient of variation was 9.6 and 16.6%, respectively. Materials used for prolactin RIA were donated by the Pituitary Program (National Institute of Diabetes, Digestive and Kidney diseases, NIH, Bethesda, MD). The intra- and inter-assay coefficients of variation were 15.8 and 24.5%, respectively. The neurointermediate lobe of the pituitary (NIL) was homogenized in 100 μl 0.1 N HCl and after centrifugation the supernatant was kept at −20 °C until AVP and oxytocin (OT) content was measured by specific RIAs. Anti-AVP and -OT antisera were produced in rabbit and obtained from Dr. M. Vecsernyés (Szent-Györgyi Medical University, Szeged, Hungary). ^{125}I-labelled tracers were produced by the Chloramine-T method. Bound and free fractions were separated by charcoal in the AVP-RIA and by a second antibody in the OT-RIA. The intra- and inter-assay coefficient of variation for AVP-RIA were 5.42 and 19% and for OT-RIA 5.12 and 14.28%, respectively.

2.5. Statistical analysis

Values are presented as mean ± S.E.M. Hormonal data was analysed after logarithmic transformation. Data was analysed by two or three-way analysis of

Table 1
Experimental design and genotypes

Genotype	di/+				di/di							
From day 0	Water measurement				Water measurement							
On day 4, minipump	Sh				Sh				D			
On day 7, i.v. injection	C		DM		C		DM		C		DM	
On day 14, minipump	Sh		Sh		Sh		Sh		D		D	
On day 22, decapitation	nR	R	nR	R	nR	R	nR	R	nR	R	nR	R

Sh: sham operation; D: desmopressin; C: control, citrate buffer vehicle; DM: diabetes mellitus induced by i.v. 60 mg/kg streptozotocin; nR: non-restraint; R: restraint.