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Dissociation of adrenocorticotropin and corticosterone as well as aldosterone secretion during stress of hypoglycemia in vasopressin-deficient rats

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ABSTRACT

Aims: In vasopressin-deficient rat pups stressor-induced adrenocorticotropin (ACTH) and corticosterone elevations markedly dissociate. We have shown recently that during the postnatal period mineralocorticoid secretion is more sensitive to stressor exposure than that of glucocorticoids. We have therefore hypothesized that in vasopressin-deficient pups during hypoglycemia, a stressor triggering aldosterone release mainly via ACTH, aldosterone release will change in parallel with ACTH. An additional aim was to reveal at which stage of the development occurs the shift from aldosterone to corticosterone as primarily stressor-induced adrenocortical hormone.
Main methods: Vasopressin-deficient (di/di) and control Brattleboro rats were used both postnatally (10-day-old rats) and in adulthood.
Key findings: Hypoglycemia induced similar ACTH elevations in pups and adults with significantly lower levels in di/di rats. In contrast, vasopressin-deficiency resulted in elevated resting aldosterone and stressor-induced corticosterone levels in pups without genotype differences in adults. Thus, aldosterone levels also dissociated from ACTH secretion. During stress, pups showed only minimal corticosterone increase, with relatively high aldosterone elevation. Resting levels of gluco- and mineralocorticoid receptor mRNA were smaller, while corticosterone-deactivating enzyme (11β-HSD2) mRNA level were higher in the hippocampus of 10-day-old rats compared to adults.
Significance: AVP does not seem to substantially regulate the stressor-induced aldosterone production, but both hormones contribute to salt-water regulation. Postnatally higher stressor-induced aldosterone than corticosterone production was still detectable in 40-day-old rats, although to a lesser extent, supporting a shift in the balance between stressor-induced glucocorticoid and mineralocorticoid hormone release throughout the development occurring in rats after postnatal day 40.

1. Introduction

Hormonal changes induced by stressors throughout the life contribute to both adaptive processes and stress-related disease states. With respect to pathological alterations, hormones released during early developmental periods are of particular importance. So far, the main attention has been devoted to glucocorticoids (corticosterone in rodents), the executive endpoint of the hypothalamic–pituitary–adrenocortical (HPA) axis defined as the major stress component already by Hans Selye, the father of stress concept [37].

Stressors induce corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) elevation in the hypothalamic paraventricular nucleus. These hormones affect the corticotropic cells of the anterior pituitary producing adrenocorticotropic hormone (ACTH), which in turn elevates synthesis and release of glucocorticoids in the zona fasciculata of the adrenal gland [12]. Glucocorticoids act on the low affinity glucocorticoid receptor (GR) and the high affinity mineralocorticoid receptor (MR) which is the main target of aldosterone and other mineralocorticoid action in the peripheral tissues [31]. One of the main functions of glucocorticoids in the brain is the feedback inhibition of the HPA axis, which can be attributed to MR action in the hippocampus and GR action in the hypothalamus [42]. Recent studies indicate that also the mineralocorticoid hormone aldosterone may induce central effects [20,21,44].

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The enzymes 11-beta-hydroxysteroid dehydrogenase type 1 and 2 (11β-HSD1/2) regulate the balance between gluco- and mineralocorticoid actions via increasing/decreasing the glucocorticoid level near the receptors [45].

In adulthood, the main hypothalamic factor triggering ACTH release is CRH and AVP only potentiates its effect [5,23,29]. This is not the case in the early postnatal period. Using a genetic model, a pharmacological treatment and immunoneutralisation we have demonstrated that during the postnatal period, the release of ACTH is mainly under the control of AVP [26,46]. Moreover, we have recently demonstrated [44] that the mineralocorticoid hormone aldosterone rather than corticosterone is the main adrenocortical stress hormone during the postnatal period. Stressor-induced elevations in aldosterone concentration were significantly higher in 10-day-old pups compared both to corticosterone elevations in pups and to the rise in aldosterone in adult Wistar rats. In pups, corticosterone responses to stressors were significantly lower than in adults, which were the basis to define the existence of the so called stress hyporesponsive period (the first two weeks of life) [33]. Greater importance of mineralocorticoids compared to glucocorticoids in the postnatal period was further supported by changes in the gene expression of 11-beta-hydroxysteroid dehydrogenase 2 (11β-HSD2) [44], an enzyme enabling preferential effects of aldosterone on mineralocorticoid receptors [11].

In vasopressin-deficient rat pups stressor-induced ACTH and corticosterone elevations markedly dissociate with lagging corticosterone secretion [47]. As ACTH is able to stimulate aldosterone production [17] and aldosterone seems to be the main adrenocortical stress hormone during the postnatal period, we have hypothesized that in vasopressin-deficient pups aldosterone response will mirror changes in ACTH concentrations. This was tested by experiments performed in Brattleboro rats, originating from the Long Evans strain, that lack functional AVP due to a point mutation in the neurophysin region of the AVP gene [34]. We have chosen insulin-induced hypoglycemia as the stressor because during hypoglycemia aldosterone secretion was mainly regulated by ACTH [44]. Pups were compared to adults to reveal age-dependent alterations. An additional aim of the present studies was to reveal at which stage of the development occurs the shift from mineralocorticoid to glucocorticoid as primarily stressor-induced adrenocortical hormone. We compared the stressor-induced corticosterone and aldosterone levels in 20- and 40-day-old rats.

2. Materials and methods

2.1. Animals

Brattleboro rats were maintained in the Institute of Experimental Medicine (Budapest, Hungary) in a colony started from breeder rats from Harlan, Indianapolis, IN, USA. Adult (265–415 g, 10–12 weeks old) and postnatal (28–31 g, 10 days old) male vasopressin-deficient rats (di/di) were compared to their heterozygous littermates (di/+) to avoid any differences related to intrauterine or maternal environment [3,13,51]. Male 20- and 40-day-old homozygous normal Brattleboro rats (+/+) were also investigated. Rats were kept in controlled environment (23 ± 1 °C, 50–70% humidity, 12 h light starting at 07:00 h) and given commercial rat chow (Charles River, Budapest, Hungary) and tap water ad libitum. Due to extremely high level of urination in AVP-deficient rats sawdust bedding was changed daily.

Offspring were kept with their parents in the same environment till 21-day-old age. The genotype of the pups was tested by measuring the AVP level from their pituitary by radioimmunoassay (RIA). Adult rats were tested for water consumption at the age of 6 weeks to define the diabetes insipidus phenotype. The experiments were performed in accordance with the European Communities Council Directive (2010/63/EU), and were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine.

2.2. Experimental design

2.2.1. Experiment 1. Differences between adults and pups under stress conditions

The hypoglycemia was induced by intraperitoneal (i.p.) insulin injection (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) at the dose of 3 IU/1 ml/kg in saline in fasting di/+ and di/di rats [48]. Adult rats were fasted for 18 h, while 10-day-old pups for only 4 h to achieve comparable degree of starvation. Rats of control groups were injected with the same volume of vehicle (0.9% NaCl). Animals were decapitated at 60 min (adult [7]) or 90 min (pups [35]) after the i.p. injection to test the peak of the elevation in both cases [46]. Blood glucose levels were measured by commercially available analyser (D-Cont Personal, 77 Elektronika Kft., Budapest, Hungary) at the time of decapitation. Trunk blood was collected for ACTH, corticosterone and aldosterone measurements. Because of the low amount of plasma in pups, two separate series were used for different measurements.

2.2.2. Experiment 2. Differences between adults and pups under basal, non-stress conditions

Adults and 10-day-old pups of the di/+ and di/di genotypes were sacrificed at rest during the morning hours. Trunk blood was collected for aldosterone, plasma renin activity, osmolarity and ion measurements. Because of the low amount of plasma in pups, two separate series were used for different measurements. Selected brain regions (whole hypothalamus and both hippocampus) were taken out under RNA free conditions into sterile Eppendorf tubes and were kept at −70 °C till RNA isolation.

2.2.3. Experiment 3. Developmental changes

As previous results showed no significant role of AVP in stressor-induced aldosterone secretion for this aim we used only control animals. Homozygous normal (+/+) 20- and 40-day-old Brattleboro rats were exposed to hypoglycaemia after 15 h starvation. They were tested 90 min following the insulin injection as described under Experiment 2.

2.3. Hormone assays

Trunk blood was centrifuged at 3000 rpm/min for 20 min at 4 °C and the serum was stored at −20 °C until analyzed. Pups were genotyped by measuring the hypophyseal AVP content. Pituitaries were sampled on 100 μl 0.1 N HCl and were frozen to −20 °C. Samples from a particular experiment were always analyzed in the same RIA.

Serum ACTH concentrations were measured by RIA in 50 μl of unextracted serum as described earlier [49]. The intraassay coefficient of variation was 4.7%. Concentrations of serum corticosterone were measured in 10 μl of unextracted serum by RIA as described earlier [50]. The intraassay coefficient of variation was 12.3%. Serum aldosterone levels and renin activity were measured by RIA using commercially available kits (RIA Aldosterone kit and Angiotensin I RIA kit, Immunotech, France). The intraassay coefficient of variation was 9.5%. Pituitary AVP content were measured by RIA as described earlier [46]. The intraassay coefficients of variation was 10.7%.

2.4. Measurements of gene expression of selected receptors and enzymes

Total RNA was isolated from homogenates of the hypothalamus and hippocampus using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and then converted to cDNA by high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). cDNA samples were pooled for each group and measured as described previously [2,44]. Shortly, real-time PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) on ABI StepOne instrument according to the manufacturer's instructions. Primers used for the comparative C_T experiments were designed by the Primer Express 3.0 program. Melting curve analysis to confirm the identity of PCR products had been