Physiology & Behavior 158 (2016) 100–111

Contents lists available at ScienceDirect

Physiology & Behavior

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

Depressive- and anxiety-like behaviors and stress-related neuronal activation in vasopressin-deficient female Brattleboro rats

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HIGHLIGHTS

- AVP aggravates anxiety- and depression-like behavior in females.
- The regulatory role of AVP might be more important in depression than in anxiety.
- The impact of AVP-deficiency was sex dependent on social and memory function.
- In females behavioral regulation correlated significantly with stress-hormones.
- In females AVP-deficiency led to blunted stress-activation of some brain areas.

ARTICLE INFO

Article history:
Received 11 November 2015
Received in revised form 24 February 2016
Accepted 27 February 2016
Available online 3 March 2016

Keywords:
Anxiety
Depression
Behavior
Stress-hormones
c-Fos
Central amygdala

ABSTRACT

Vasopressin can contribute to the development of stress-related psychiatric disorders, anxiety and depression. Although these disturbances are more common in females, most of the preclinical studies have been done in males.

We compared female vasopressin-deficient and +/+ Brattleboro rats. To test anxiety we used open-field, elevated plus maze (EPM), marble burying, novelty-induced hypophagia, and social avoidance tests. Object and social recognition were used to assess short term memory. To test depression-like behavior consumption of sweet solutions (sucrose and saccharin) and forced swim test (FST) were studied. The stress–hormone levels were followed by radioimmunoassay and underlying brain areas were studied by c-Fos immunohistochemistry.

In the EPM the vasopressin-deficient females showed more entries towards the open arms and less stretch attend posture, drank more sweet fluids and struggled more (in FST) than the +/+ rats. The EPM-induced stress-hormone elevations were smaller in vasopressin-deficient females without basal as well as open-field and FST-induced genotype-differences. On most studied brain areas the resting c-Fos levels were higher in vasopressin-deficient rats, but the FST-induced elevations were smaller than in the +/+ ones.

Similarly to males, female vasopressin-deficient animals presented diminished depression- and partly anxiety-like behavior with significant contribution of stress-hormones. In contrast to males, vasopressin deficiency in females had no effect on object and social memory, and stressor-induced c-Fos elevations were diminished only in females. Thus, vasopressin has similar effect on anxiety- and depression-like behavior in males and females, while only in females behavioral alterations are associated with reduced neuronal reactivity in several brain areas.

1. Introduction

The 75–90% of adult visits to primary care physicians are for stress-related problems [1]. Thus, stress is an important pathogenetic factor, especially for psychiatric disturbances like anxiety and depression. Today, depression is already the 2nd cause of DALYs (disability-adjusted life year) in the age category 15–44 years for both sexes combined, but by the year 2020, depression is projected to reach 2nd place of the ranking of DALYs calculated for all ages, both sexes [2].

Vasopressin (AVP) is one of the well-known neuropeptides that has a confirmed role in fluid metabolism and in regulation of the main stress-system, the hypothalamic-pituitary-adrenocortical (HPA) axis [3]. The center of the HPA axis is the paraventricular nucleus (PVN)

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http://dx.doi.org/10.1016/j.physbeh.2016.02.041
0031-9384/

[4]. The key areas for osmotic regulation are the magnocellular cells (mPVN, and the supraoptic nucleus, SON) [5], but under stress the ventral parvocellular part of the PVN (vpPVN) also releases AVP, which — synergistically with corticotropin-releasing hormone — triggers the secretion of adrenocorticotropin (ACTH) from the anterior pituitary and finally glucocorticoids from the adrenal glands [6]. During stress the dorsal parvocellular part of the PVN (dpPVN) — containing also AVP [7] — ensures autonomic connections with other areas of the brain.

The regulatory role of AVP on the HPA axis suggests potential uses for AVP receptor modulators in various central nervous system indications, including depression and anxiety [3,8]. Although these disturbances are more common in females [9], most of the preclinical studies have been done in males [10,11]. That could be one reason why 80% of the drugs withdrawn from the market are due to side effects on women [12].

Therefore we aimed to clarify if AVP has a similar depression-like behavior inducing role in females as it was shown in males using AVP-deficient Brattleboro (di/di) rats as a model. This is a spontaneous mutant strain of the Long Evans colony, due to a single base deletion of exon B of the AVP gene [13]. Previous studies using this strain confirmed that AVP-deficient male subjects represented reduced depressive-like behavior with reduced floating during forced swim test (FST) and enhanced sweet preference in an anhedonia test [14,15], while anxiety-like behavior on the elevated plus maze (EPM) was decreased in some, but not changed in other studies compared AVP-deficient to appropriate control animals (Table 1). Cognitive disturbances [16] and social dysfunction [17] are prominent sources of distress and disability in patients with depression, but are commonly omitted from current studies. In line with the role of AVP in learning-memory and social interactions [3,8], male Brattleboro rats were shown to have impaired discriminative abilities both among subjects and social partners [18] and showed reduced social avoidance as well [19]. As anxiety and depression are stress-related disorders, we examined AVP-related alterations in basal and stressor-induced HPA axis parameters (pituitary level: adrenocorticotropin (ACTH), adrenal level: the main rodent glucocorticoid, corticosterone) as well. The possible underlying brain areas were studied by c-Fos immunohistochemistry both among basal conditions and after a well-known depression test, FST, which acted as a stressor. Beside the above mentioned PVN and SON we studied lateral septum (LS) regulating mood and motivation [20], as well as the HPA axis [21,22] being in close vicinity to PVN. Because of anatomical proximity of SON and medial nucleus of the amygdala (MeA) previous studies concentrated on MeA as an important switch site between stressor-induced somatic-dendritic AVP release and behavior [15]. However, central nucleus of the amygdala (CeA) might be also important for antidepressant actions [23], and its activation (measured by c-Fos) was also though to contribute to altered FST behavior after lipopolysaccharide-injection in mice [24]. Nucleus accumbens (Acc) plays an important role in reward and anhedonia, the diminished interest to pleasure, which is a core symptom of depression [25]. Prefrontal cortex (PFC), as a part of a network, has an important role in the stress-axis regulation [22,26], while paraventricular thalamic nucleus (PVA) is also sensitive to a wide range of stressors [27]. The ventromedial hypothalamic nucleus (VMH) and arcuate hypothalamic nucleus (ARC) are important regulators of energy homeostasis and are highly dependent on sexual steroids [28].

Table 1
Summary of the genotype differences in female and male adult Brattleboro rats.

Tests	Female	Male
Anxiety-like behavior		
Open-field (OF)	=	=
Elevated plus maze (EPM)	↓	(↓)*
Marble burying (MB)	=	↓
Novelty-induced hypophagia (NIH)	=	ND
Memory and social behavior		
Novel object recognition (NOR)	=	↑
Social recognition	=	↑
Social avoidance	=	↑
Depression-like behavior		
Anhedonia	↓	↓
Forced swim (FST)	↓	↓
Stressed-hormone levels (*ACTH/corticosterone*)		
Basal	=/=	=/=
Open-field (OF)	=/=	↓/=
Elevated plus maze (EPM)	↓/↓	↓/=
Forced swim (FST)	=/=	↓/=
c-Fos immunohistochemistry (*basal/stressed*)		
MeA, VMH, PVA	=/=	
dpPVN, vpPVN, CeA, ARC	=/↓	
PFC, SON	↑/=	
Acc, LS, mPVN	↑/↓	
PVN, BNST		=/= (ether)
LS,CeA, BLA		ND/=
CeA		=/↑ (ether)
MeA		ND/↑
SON, circumventricular organs		↑/ND

Male data are based on our previous results [14,29,76,80,18,15,35,19] as well as Guldenaar et al. [77]. Stress for c-Fos was FST, if not otherwise stated.
BLA: basolateral amygdala; BNST: bed nucleus of stria terminalis; =: no difference between genotypes; ↓: AVP-deficient animal showed lower levels or improved psychopathology-related behavior; ↑: AVP-deficient animal showed higher levels or enhanced psychopathology-related behavior; ND: no data; *: in one out of three studies.

2. Materials and methods

2.1. Animals

Female virgin Brattleboro rats (8–12 week old) were maintained in the Hungarian Academy of Sciences, Institute of Experimental Medicine in a colony started from breeder rats from Harlan, Indianapolis, USA. Rats were kept in controlled environment (21 ± 1 °C, 50–70% humidity, 12 h light starting at 07.00) and given commercial rat chow (Charles River, Hungary) and tap water ad libitum. We compared the AVP-deficient homozygous (di/di) rats with diabetes insipidus to homozygous (+/+) control rats [29]. To avoid disturbance of presently untested animals, all experiments were performed on single housed rats. Although we are aware that the phase of the estrous cycle may influence the behavior, we strongly think, that checking it is an additional manipulation per se, which might also influence the outcome of the behavioral tests e.g. vaginal lavage was showed to induce conditioned place preference [30,31]. Moreover, there are no currently data in favor that c-Fos activation induced by stress in females is different in the different phases of the estrous cycle [32]. The mixed/unknown phases of the estrous cycle may increase the variability within the groups, but most probably did not influence the effect of the AVP-deficiency, which was in agreement with previous studies in male and lactating female Brattleboro rats, as well as with most of the literature. Body weight, food and water consumptions were measured at the beginning of the studies and all experiments were done during the light phase of the day, between 9 and 13 h. Five sets of animals were used, and different behavioral tests were done in each case. Tests were conducted on each cohort at least 4 days apart (Table 2.). The experiments were performed in accordance with the regulations set by the European Communities Council Directive (2010/63/EU) and were supervised by the Institutional Animal Care and Use Committee.

If not otherwise stated behavior was video-recorded and later analyzed by an experimenter blind to the treatment by means of a computer-based event recorder (H77, Budapest, Hungary).

2.2. Assessing anxiety-like behavior

2.2.1. Locomotor activity (open-field, OF)

Locomotor activity was measured in an open-field test (round arena: 100 cm, wall: 50 cm) and analyzed by EthovisionXT (Version