Amino Acids (2015) 47:2245–2253
DOI 10.1007/s00726-015-2026-x

REVIEW ARTICLE

Vasopressin signaling at brain level controls stress hormone release: the vasopressin-deficient Brattleboro rat as a model

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Received: 4 May 2015 / Accepted: 8 June 2015 / Published online: 23 June 2015

Abstract The nonapeptide arginine vasopressin (AVP) has long been suggested to play an important role as a secretagogue for triggering the activity of the endocrine stress response. Most recent studies employed mutant mice for analyzing the importance of AVP for endocrine regulation under stress. However, it is difficult to compare and draw overall conclusions from all these studies as mixing the genetic material from different mouse strains has consequences on the individual's stress response. Moreover, mice are not ideal subjects for several experimental procedures. Therefore, to get more insight, we used a rather old mutant rat model: the AVP-deficient Brattleboro rat. The present short review is aimed at providing the most interesting results of these studies within the last 8 years that allowed gaining new insights in the potential signal function of AVP in stress and endocrine regulation.

Keywords AVP · Vasopressin · Stress · Brattleboro rat · Hormone · HPA axis

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The role of vasopressin in stress regulation

Adaptations to the constantly changing environment and internal milieu are essential prerequisites for life. These adaptations and their underlying mechanisms become more and more sophisticated during phylogenesis and, thus, involve numerous regulatory systems in mammals that communicate at intercellular level within a given system and between the cells of different systems via chemical signals such as neurotransmitters, neuromodulators, and hormones including releasing and inhibiting ones. The hypothalamus–pituitary–adrenocortical (HPA, Fig. 1) axis is one of the most important neuroendocrine regulatory systems involved in the adaptive responses of the mammalian organism to external and internal threatening stimuli (Selye 1975). These stimuli—commonly called 'stressors'—activate the HPA axis that is reflected by high plasma adrenocorticotropin (ACTH) and glucocorticoid levels (Aguilera 1994). This (neuro)endocrine response is independent from the defined nature of the stressor and—therefore—belongs to the so-called general adaptive response. However, the mechanisms underlying the activation of the HPA axis at brain level are likely to vary between the stressors (Zelena et al. 2009a). Thus, it would be important to understand the relevance of defined intercellular signaling molecules for controlling HPA axis activity not only at the level of the pituitary and adrenals, but also within the hypothalamus.

Two neuropeptides—originating from the hypothalamus—act as the main signals to transfer information within the HPA: the corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP). Both are synthesized—together with, e.g., oxytocin (OT)—in cells of the paraventricular nucleus of the hypothalamus (PVN) and released into the extracellular fluid from parvocellular cells of the PVN into hypophyseal portal blood (Antoni 1993) (Fig. 1).

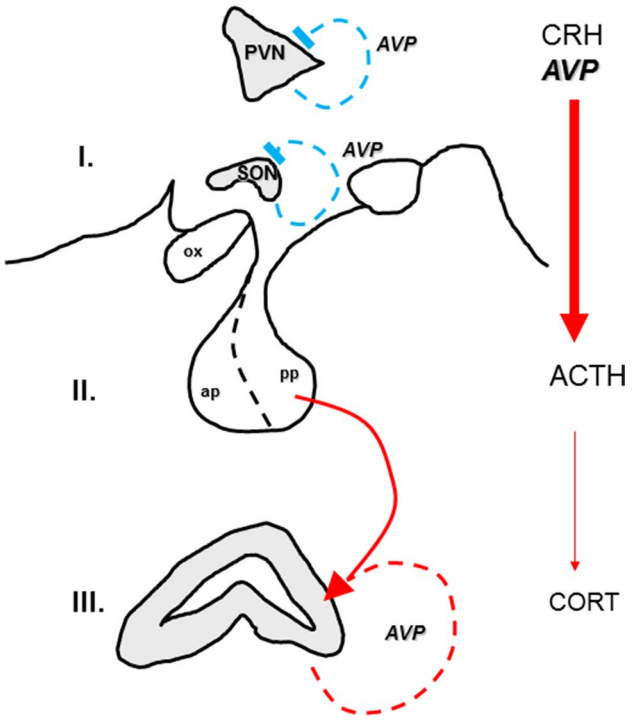

Fig. 1 Schematic drawing of the anatomical elements of the hypothalamus–pituitary–adrenal axis and selected neuroendocrine signals acting at different levels of the axis to control its activity in a virtual sagittal section of the mammalian brain. Focus is on the signal action of arginine vasopressin (AVP). The *arrow* illustrates stimulating, the *bar-headed line* illustrates inhibiting action on the respective element. *I* hypothalamus, *II* pituitary, *III* adrenal cortex. *PVN* paraventricular nucleus of the hypothalamus, *SON* supraoptic nucleus of the hypothalamus, *ox* optic chiasm, *ap* anterior pituitary, *pp* posterior pituitary *CRH* corticotropin-releasing hormone, *ACTH* adrenocorticotropin, *CORT* glucocorticoids, in rodents corticosterone

Further, CRH and AVP act synergistically on the anterior pituitary to stimulate the release of ACTH and its synthesis from the proopiomelanocortin (POMC) precursor (Lutz-Bucher et al. 1980; Rivier and Vale 1983). Through the circulating blood ACTH reaches the adrenal gland where it stimulates glucocorticoid release and synthesis. Glucocorticoids (including cortisol in humans and corticosterone (CORT) in rodents) are the effector molecules of the axis with permissive (expression of various proteins), stimulatory (carbohydrate metabolism, anti-inflammatory, immunosuppressive) and suppressive (negative feedback) actions (Sapolsky et al. 2000).

Originally AVP was considered to act as the main secretagogue for ACTH (Du Vigneaud 1954), but the discovery of CRH questioned its importance (Spiess et al. 1981; Vale et al. 1981). Nowadays, it is widely accepted that CRH is the main regulator, but AVP supports and—partially—also compensates for CRH effects at almost all levels of the HPA axis (Scott and Dinan 2002). The following observations made at different levels of the HPA axis (hypothalamus, pituitary, adrenals) contributed to this suggestion:

1. *At the level of the pituitary* When given alone at physiological concentrations (≤2 nm) AVP had little or no effect on ACTH secretion (Antoni et al. 2003). However, it markedly augmented ACTH release induced by CRH (Gillies et al. 1982) by amplifying the CRH-evoked cAMP response (Abou-Samra et al. 1987; Giguere and Labrie 1982). Thus, at this level, AVP supports and potentiates the stimulating action of CRH on HPA axis activity.
2. *At the level of the hypothalamus*
   2a. During chronic stress, an increase in the number of CRH neurons co-expressing AVP and AVP mRNA concentration was observed in the PVN (Aguilera 1994; Dallman 1993). Therefore, AVP was suggested to be the indispensable secretagogue for the hyperactivity of the HPA axis in response to chronic stressor exposure. Further, at rest higher AVP mRNA was detected in CRH knockout mice (Muglia et al. 2000). Genetic manipulations producing local absence of CRH receptors in a mouse model resulted in an increase in AVP-like immunoreactivity both in the PVN and (magnocellular) supraoptic nucleus of the hypothalamus [SON, (the other main source of hypothalamic AVP Preil et al. 2001)]. These observations imply that AVP may—at least partially—compensate the absence of CRH as secretagogue for ACTH.
   2b. AVP is released not only from axon terminals into the portal blood, but also from dendrites and somata of hypothalamic neurons into the extracellular fluid under stress conditions. The latter may affect the activity of the secretory neurons directly (by auto-feedback) or indirectly (e.g., via modulation of afferents and/or blood supply and/or via the action of other, co-expressed neuropeptides, e.g., Bundzikova et al. 2008). Depending on its release site (dendrites/somata versus axon terminals), AVP originating from hypothalamic neurons differently affects HPA axis activity: (1) if released from axon terminals into the portal blood from (parvocellular) PVN neurons AVP stimulates the HPA activity; (2) if released into the extracellular fluid of the PVN from dendrites/somata, AVP seems to inhibit the endocrine stress response (Wotjak et al. 1996) (Fig. 1). The role of AVP originating from the SON neurons is not entirely clear, yet. Interestingly, activation of the HPA axis may trigger a sustained somatodendritic release of AVP (and that of its sister

nonapeptide OT, the twin molecule to AVP) not only within the PVN, but also in the SON. AVP (and OT) may partially diffuse out of the PVN (and SON) to reach remote brain areas involved in the generation of emotions (Ebner et al. 1999, 2002), thereby has the potential of altering the interpretation of the stressor, thus the entire stress response (see below).

3. *The adrenal gland* is another source for circulating AVP (Gallo-Payet and Guillon 1998). Indeed, AVP is synthesized and secreted by chromaffin cells either present in the adrenal medulla or scattered throughout the cortex with a prominent concentration in the zona glomerulosa (Gallo-Payet and Guillon 1998). AVP receptors are also present on chromaffin cells and in the adrenal cortex, where they can stimulate the secretion of gluco- and mineralocorticoids (Gallo-Payet and Guillon 1998). These findings suggest that also at the level of the adrenals, AVP provides a positive input into the HPA axis (Fig. 1).

The Brattleboro rat: a model organism for stress research

Experimental neuroendocrine stress research is increasingly based on the use of mutant mouse models. However, genetically engineered manipulations inevitably result in a mixture of genetic material from different mouse strains or sub-strains. This seems to be particularly critical if we consider the well-known strain differences in the behavioral performance monitored in tasks thought to provide insight into the emotional evaluation of perceived stimuli (Crawley 1996; Crusio 1996, 2004; Gerlai 1996). Stress may be considered as the individual's "interpretation" of aversive, potentially life-threatening stimuli (stressors) finally leading to an activation of the HPA axis. This interpretation highly depends not only on the subject, life history and ontogenetic stage, but also on the genetic predisposition (Engelmann et al. 2004). Therefore, it is difficult to draw overall conclusions from all these studies as mixing the genetic material from different mouse strains has consequences on the individual's stress response through additional factors including imprinting (Davies et al. 2005; Gregg et al. 2010; Wilkins 2005) and maternal behavior (Bredy et al. 2003; Francis and Meaney 1999; Liu et al. 2000). Moreover, mice are not ideal subjects for some experimental procedures (Anonymus 2010) including those where repeated blood sampling from the unrestrained animal is required. Therefore, below we will concentrate on a single rat model.

A substantial part of today's knowledge about the involvement of AVP signaling in the regulatory mechanisms underlying the control of the endocrine stress response at the level of the hypothalamus results from the application of the microdialysis technique (Wotjak et al. 2008). One of the advantages of this technique involves its application in freely moving animals without the need of direct acute handling (Horn and Engelmann 2001). With respect to the progress in the field of genetics, a combination of genetics and microdialysis would provide new insight into the importance of AVP in the regulation of the mammalian endocrine responses. Unfortunately, the application of microdialysis and continuous blood sampling in vivo is limited in mice (Wotjak et al. 2008). Therefore, we looked for an alternative animal model in which microdialysis can easily be performed. We found it in the AVP-deficient Brattleboro rat strain where we combined microdialysis and other contemporary stress research techniques to gain new insight into the importance of AVP in HPA axis regulation.

In the early 1960s, a substrain of Long-Evans rats was identified with hereditary diabetes insipidus. Animals of this substrain were originally bred in Brattleboro (Vermont, U.S.A.) and, therefore, subsequently named "Brattleboro rats" (Sawyer et al. 1964; Valtin and Schroeder 1964). Later research revealed that the diabetes insipidus was caused by a point mutation leading to an inability to properly process the AVP precursor (Ivell et al. 1986; Schmale and Richter 1984). One of the interesting features of this strain is the fact that the AVP production is blocked at brain level, but not in other—peripheral—tissues (Friedmann et al. 1993) indicating that it is not a "general AVP knock-out" animal model. Among others, their adrenaIs also contain AVP (Nussey et al. 1984), which is—in contrast to their plasma AVP levels originating from the hypothalamus—reactive to hemorrhagic challenges (Somova et al. 1986). Initially, Brattleboro rats were extensively used in different lines of neuroendocrine, behavioral and stress research (Sokol and Valtin 1982). However, the use of Brattleboro rats in neuroendocrine research declined from the mid 1980s.

Within the past 10 years, we analyzed the consequences of the congenital absence of AVP on the endocrine stress response at both the hypothalamic and peripheral level. The results of these and additional experiments shed new light on the role AVP plays for HPA axis regulation.

Alterations of HPA axis regulation in Brattleboro rats

For a detailed review of HPA axis regulation in the Brattleboro rat see Makara et al. (2012). Here we focused on elevated plus maze (EPM) and forced swimming (FS)-induced changes, being the most widely used behavioral tests for anxiety- and depressive-like behavior, respectively. These acute stressors allow parallel estimation of endocrine and emotional state of the animals.

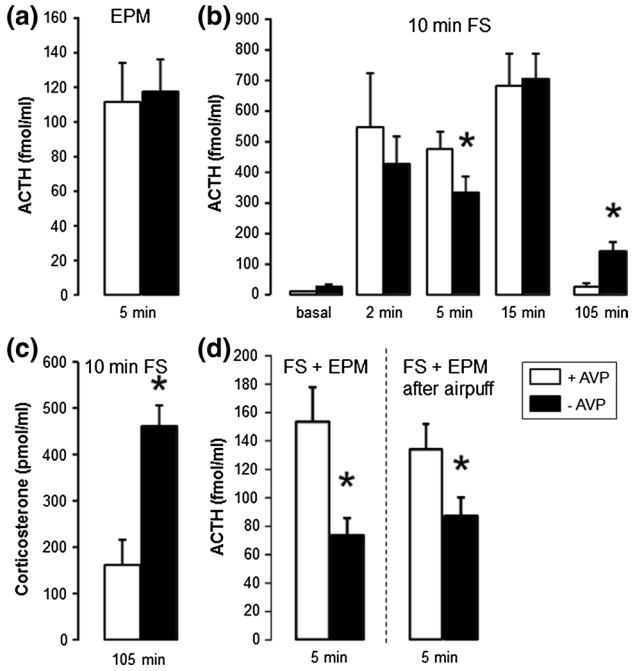

Fig. 2 Response of the hypothalamus–pituitary–adrenal axis as measured by plasma adrenocorticotropin (ACTH) (**a**, **b** and **d**) and corticosterone levels (**c**) in Brattleboro rats. Plasma ACTH at the end of a 5 min elevated plus maze (EPM) test (**a**) ($n = 11$, 15) and (**b**) before (basal) and—at different time points—after a 10 min forced swimming session (FS; $n = 4$, 6). **c** Shows corticosterone levels measured at 105 min after onset of a 10 min forced swimming. **d** Illustrates the effect of sub-chronic forced swimming (2 × 10 min on two consecutive days) on plasma ACTH levels at the end of 5 min elevated plus maze with ($n = 11$, 8) or without ($n = 10$, 11) previous airpuff in vasopressin-deficient (−AVP; *black*) and non-deficient (+AVP; *white*) Brattleboro rats. *$p < 0.05$ versus the other genotype for the same parameter

At the end of a 5 min EPM test, there was no difference between the genotypes in their ACTH and CORT levels as well as in their anxiety-like behavior (open arm time) (Fig. 2a) (Mlynarik et al. 2007). Similarly, when the blood was sampled 2 min and 15 min after the onset of a 10 min FS exposure, no differences in the hormone values were detectable between the genotypes (Fig. 2b) (Zelena et al. 2009a, b). However, at 5 min AVP-deficient animals had lower ACTH levels accompanied by lower depressive-like behavior (floating) (Mlynarik et al. 2007). Interestingly, the lack of AVP resulted in a slower recovery of ACTH and CORT levels (measured at 105 min after the onset of a 10 min FS; Fig. 2b, c). Moreover, intracerebroventricular injections of AVP (but not that of OT) in urethane anesthetized Brattleboro rats diminished the plasma CORT levels even when it was followed by a painful intraperitoneal injection of 3.5 M NaCl-solution (unpublished observations). In a more specific experiment, retrodialysis of AVP into the PVN area facilitated the return of plasma CORT to basal levels 105 min after 10 min FS (Zelena et al. 2009a, b).

Previous studies showed that—in response to repeated FS—AVP is increasingly released into the extracellular fluid of PVN, but not SON (Engelmann et al. 1998; Wotjak et al. 1998). After exposing the Brattleboro rats to a sub-chronic repeated FS (10 min daily for two days), the 5 min EPM resulted in reduced ACTH and CORT plasma levels in AVP-deficient animals (Fig. 2d), suggesting that the absence of central AVP results in a blunted ACTH reactivity to a second stress experience. Interestingly, when we exposed sub-chronically stressed animals to an airpuff, a stressor known to activate HPA axis and to alter defensive withdrawal behavior (Engelmann et al. 1996), the difference in the HPA axis response was still detectable for ACTH (Fig. 2d) but disappeared for CORT.

To further investigate the cause for the reduced ACTH response, we focused on two other secretagogues: CRH and OT. In the course of our experiments, we were surprised to find that under basal conditions, the number and intensity of CRH mRNA containing neurons in the PVN were lower in AVP-deficient Brattleboro rats when compared to control animals (Fig. 3a, b) (see also Sterrenburg et al. 2011); thus, most probably it can be excluded as a compensatory molecule for AVP deficiency. On the other hand, FS induced a reduction in CRH mRNA signal in the PVN of control animals only, suggesting that AVP is necessary for CRH mRNA regulation.

Further, we demonstrated for OT that in the PVN not the signal intensity, but the area of the signal (most probably the number of OT producing cells) is higher in AVP-deficient than in control animals (Fig. 3c) (Zelena et al. 2009b). A similar observation was made for the SON (Zelena et al. 2013) (Fig. 4a). Interestingly, in response to acute stressor exposure (FS), OT release into the SON was blunted in AVP-deficient rats (Fig. 4c), whereas both the OT mRNA level (not only in the SON but also in the PVN; Fig. 3c) and plasma OT levels (Fig. 4d) were shown to remain increased (Zelena et al. 2009a, b).

As previous studies (based mainly on molecular biological techniques: Aguilera 1994; Dallman 1993) suggested a prominent role of AVP in HPA axis regulation during chronic stressor exposure, Brattleboro rats were also used to study this phenomenon. Both two and four weeks of daily repeated restraint (chronic stress) failed to trigger significant differences between control and AVP-deficient Brattleboro rats in POMC mRNA of the anterior lobe of the pituitary (Fig. 5a) and adrenal gland weight (Fig. 5b) as well as plasma CORT levels (Fig. 5c) (Zelena et al. 2004, 2007). Subsequent studies, involving the application of other stressors in Brattleboro rats for two weeks (repeated morphine withdrawal: Domokos et al. 2008; streptozotocin-induced diabetes mellitus: Zelena et al. 2006) also failed to reveal a clear impact of the lack of AVP on the (endocrine) chronic stress response. This may imply congenitally

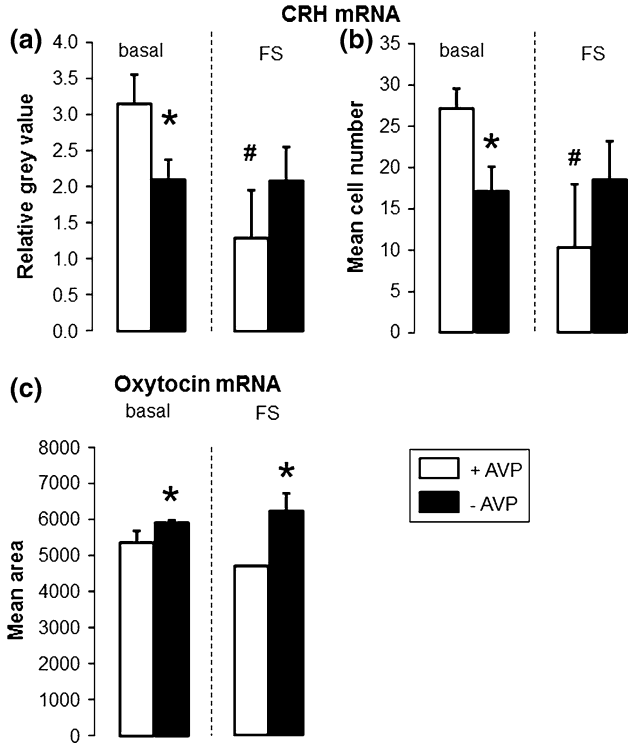

Fig. 3 **a**, **b** Corticotropin-releasing hormone (CRH) mRNA in the paraventricular nucleus of the hypothalamus (PVN) obtained from animals sampled under basal conditions (basal) or 4 h after 10 min forced swimming (FS) in vasopressin-deficient (−AVP; *black*) and non-deficient (+AVP; *white*) Brattleboro rats ($n = 4$–5); **a** shows the relative gray value and **b** the estimated number of cells showing CRH mRNA. **c** Shows oxytocin mRNA levels from the same animals. $^{*}p < 0.05$ versus the other genotype for the same parameter; $^{\#}p < 0.05$ versus same genotype under basal

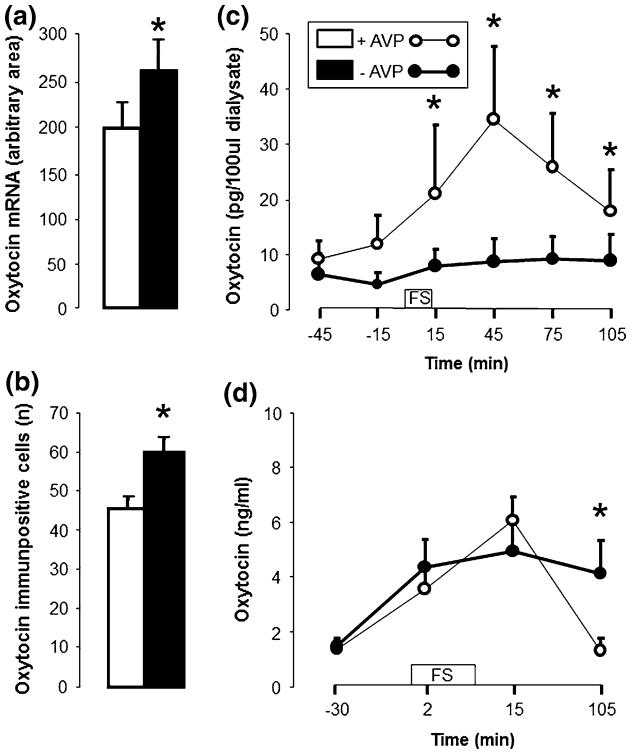

Fig. 4 **a**, **b** Show oxytocin mRNA (**a**, measured via in situ hybridization and expressed as arbitrary area) and immunoreactivity (**b**; measured by immunohistochemistry and expressed as number of immunopositive cells), respectively, as measured in the supraoptic nucleus of the hypothalamus (SON) area under basal conditions ($n = 5$). **c**, **d** Show oxytocin release profiles into the SON area (**c**; $n = 7, 9$) and plasma (**d**; $n = 7, 13$) in response to a 10 min forced swimming session (FS). Data were obtained in vasopressin-deficient (−AVP; *black*) and non-deficient (+AVP; *white*) Brattleboro rats. $^{*}p < 0.05$ versus the other genotype for the same parameter (and time point); modified from (Zelena et al. 2013)

absent AVP to be substituted by other secretagogues of ACTH (e.g., the aforementioned OT); however, other tools (e.g. V1b receptor antagonist treatment) (Chen et al. 2008) confirmed our conclusion that during chronic stressor exposure AVP did not became the main secretagogue of ACTH secretion.

Which role plays AVP in acute and chronic stress?

The use of the Brattleboro rat in stress research provided new insights into the consequences of the congenital absence of intracerebral AVP on HPA axis activity and regulation. First, we observed that the relevance of AVP signaling on HPA axis regulation cannot be easily attributed to the commonly used categories to describe the quality of the stressor (e.g., "weak" versus "strong", "psychological" versus "physical" versus "metabolic", "systemic" versus "neurogenic", "interoceptive" versus "exteroceptive" Zelena et al. 2009a, b). In this context, it is of interest to note that HPA axis activity is considered to be part of the general adaptive syndrome, and thus, the non-specific response of the organisms to (life) threatening stimuli. The present data suggest that the integrative part of this non-specific response resides within the hypothalamus and that AVP signaling at that level plays an important role. Although the theory of a predominant AVP regulation of the HPA axis predominantly during chronic stress (Aguilera 1994; Dallman 1993) is widely accepted, the data collected in recent studies do not support this hypothesis (Zelena et al. 2004, 2007; Fig. 5), but point towards a contribution of AVP for acute stressor exposure (Makara et al. 2012).

The observation of the reduced CRH mRNA level in AVP-deficient rats came as a surprise (Fig. 3). Originally, we thought that the reduced ACTH levels might be due to the missing permissive action of AVP on the corticotrope cells at the level of the adenohypophysis. However, the present data suggest that CRH release itself might be reduced. Nevertheless, previous data suggested that the sensitivity of the corticotropic cells in the adenohypophysis to CRH was

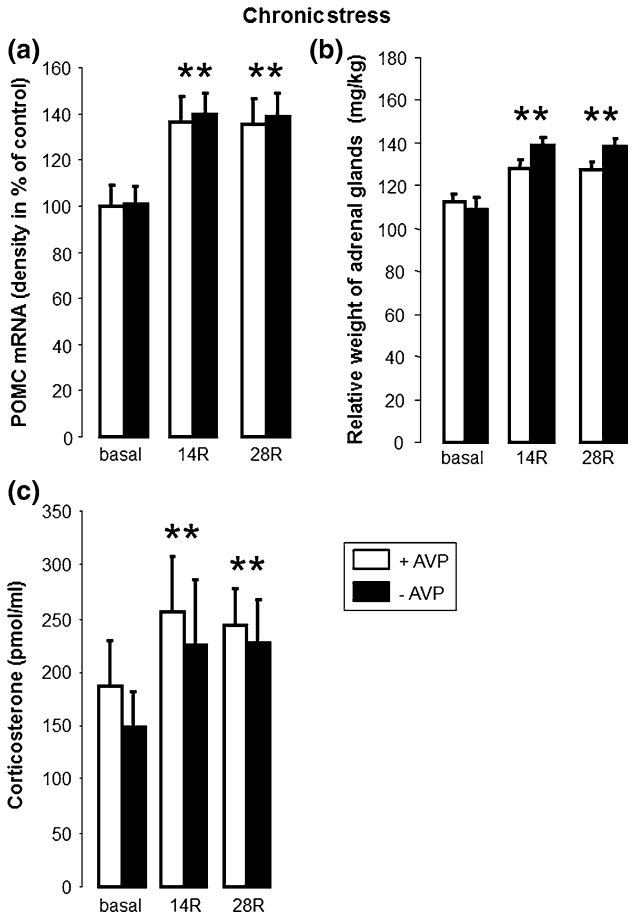

Fig. 5 Effect of chronic restraint stress [daily 1 h, 14 repetitions (14R); 28 repetitions (28R)] on **a** proopiomelanocortin (POMC) mRNA density in the adenohypophysis, **b** adrenal gland weight and **c** plasma corticosterone levels 24 h after the last stressor exposure in vasopressin-deficient (−AVP; *black*) and non-deficient (+AVP; *white*) Brattleboro rats. Basal values were obtained from resting animals without stressor exposure ($n = 9$–12). $^{**}p < 0.01$ both genotypes versus basal conditions

also reduced in Brattleboro rats (Zelena et al. 2011) and, thus, might have contributed to our findings. Whether this is indeed the case and the sole reason for the reduced ACTH response under sub-chronic stress conditions requires further investigations.

The results of the experiments focusing on the role centrally synthesized AVP may play in HPA axis regulation under acute and sub-chronic stress conditions led to at least two different hypotheses that individually or together may explain our observations:

First, a plausible explanation for the release of CORT virtually independent from changes in ACTH levels in Brattleboro rats is that the central lack of AVP released from hypothalamic axon terminals into the portal blood results in a blunted ACTH response in stressed rats. In contrast, CORT levels could remain unaffected by the blunted ACTH response for instance by the release of AVP at the level of the adrenals (Nussey et al. 1984) or by an increased activity of the sympatho-adrenal system (Kvetňanský et al. 1990); (for a detailed review on ACTH-independent CORT regulation see Bornstein et al. 2008; on the role of ACTH beyond CORT regulation see Zelena and Makara 2012). The latter is supported by the fact that retrodialysis of AVP into the PVN (as a regulatory center of the autonomous nervous system, thereby controlling the activity of the sympatho-adrenal axis) facilitated the return of stressor exposure-induced increased CORT levels to basal values (Zelena et al. 2009b).

Second, the reduced ACTH release in response to acute stressor exposure sheds also light on the function of brain AVP signaling in controlling the emotional evaluation of potential (life) threatening situations and thereby in the behavioral stress response. Because of the absence of AVP signaling in limbic brain areas (such as medio-lateral septum and medial amygdala) the stimulus exposure might have been "interpreted" by the experimental animals as a less severe one. This hypothesis may need additional information: (1) It is known that AVP acts as neuromodulator to affect acute behavioral stress coping strategies in an opposite manner in the medio-lateral septum (Ebner et al. 1999) versus medial amygdala (Ebner et al. 2002). Thus, the missing AVP signaling in these brain areas might have altered the emotional "interpretation" of the stressor, leading to a reduced ACTH release. Indeed, we found an increased struggling and reduced floating behavior in AVP-deficient Brattleboro rats when compared to controls (Balazsfi et al. 2015), indicating an altered coping strategy in these animals. Moreover, prior airpuff reduced the percentage of time the animals spent on (Fig. 6a) and the percentage of entries into the open arms (Fig. 6b) of an EPM in control animals only without inducing differences in the general locomotor activity between the genotypes (Fig. 6c). (2) In addition, it has been suggested that AVP released within the PVN (and thus originally aimed at controlling HPA axis activity) may reach other brain areas such as the medio-lateral septum or medial amygdala which also might have an effect on the interpretation of the situation, and via this way may moderate the stress-hormone release (Engelmann et al. 2004). The absence of AVP originating from PVN and SON neurons in the Brattleboro rats may result in the missing of local AVP signaling in the medial amygdala and medio-lateral septum.

A wider, unexpected conclusion of our experiments was related to the possible (mis)interpretation of mRNA and protein density/content on the signal intensity in neuropeptidergic signaling. In the course of our experiments, we investigated the importance of the SON for the regulation of HPA axis activity. To do that, we first analyzed the OT mRNA and peptide levels in Brattleboro rats. Then,

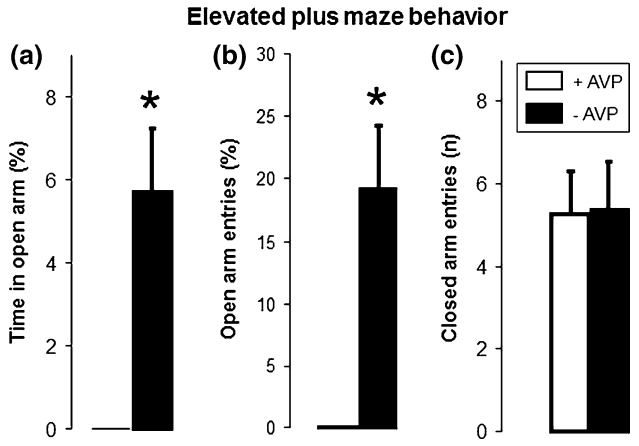

Fig. 6 Effect of combined forced swimming (2 × 10 min on two consecutive days preceding the test day) and airpuff administration immediately before the exposure to the elevated plus-maze for 5 min in vasopressin-deficient (−AVP; *black*) and non-deficient (+AVP; *white*) Brattleboro rats ($n = 11, 8$). **a** Shows the percentage of time spent on the open arms. **b** Shows the percentage of the number of entries into the open arms and **c** the total number of entries into the closed arms of the elevated plus-maze. *$p < 0.05$ versus the other genotype for the same parameter

microdialysis coupled with blood sampling via chronically implanted jugular venous catheters were used to elucidate the release activity of oxytocinergic SON neurons in response to defined stressor exposure (Zelena et al. 2013). The data of this study revealed that—despite the synthesis of OT was increased in this brain area (as measured by both immunohistochemistry and in situ hybridization; Fig. 4a, b)—central OT release was significantly decreased in AVP-deficient Brattleboro rats (Fig. 4c) without showing significant changes in peripheral OT release profile (Fig. 4d). This, again, illustrates that neither the increase nor the decrease of immuno-positively stained cell numbers allow an easy interpretation of the bioavailability of neuropeptides as hormones or signals in intercellular information transfer.

Taken together, a long sequence of stress studies on the Brattleboro rat suggests a multifaceted role for AVP in the regulation of the HPA axis to stress. We suggest that AVP release contributes significantly to the acute HPA axis activation following some but not all stressful stimuli. This contribution involves intrahypothalamic (PVN) release of AVP with intrahypothalamic site(s) of action to inhibit/restrain the hormonal response and modify the behavioral response to stress. Lifelong AVP deficiency also decreases hypothalamic CRH mRNA levels and the sensitivity of the anterior pituitary POMC cell to the ACTH releasing action of CRH. During chronic stress, the previously suggested special role of increased AVP in maintaining HPA responsiveness could not be confirmed. These studies also have shown situations where ACTH and CORT secretions markedly dissociated with unchanged CORT secretion in parallel with a blunted or missing ACTH response thus emphasizing the possibility that AVP and/or other possible mediators may have a stimulating role at the level of the adrenal gland.

In the course of our experiments, several questions were answered, but new questions emerged. These include the impact of AVP on CRH mRNA expression at the hypothalamic level. Future studies will address these new questions possibly also by local rescue of AVP synthesis by the intracerebral administration of viral vectors. We hope that this will shed new light on the interplay between the hypothalamic regulators of the HPA axis (e.g., AVP, CRH and OT) and may open new windows for therapeutic interventions aimed at normalizing the endocrine stress response under pathological conditions.

Acknowledgments This work was supported by OTKA Grants F 48783, IN 67249 and NN 71629, Hungary and and DFG (EN 366/3-1, 436 UNG 113/168/0-1 & 0-2).

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval We treated the animals according to guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and our work was supervised by the Animal Welfare Committee of the Institute of Experimental Medicine, Budapest, Hungary.

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