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Research Report

Differential responses of the vasotocin 1a receptor (V1aR) and osmoreceptors to immobilization and osmotic stress in sensory circumventricular organs of the chicken (*Gallus gallus)* brain

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ABSTRACT

Past studies have shown that the avian vasotocin 1a receptor (V1aR) is involved in immobilization stress. It is not known whether the receptor functions in osmotic stress, and if sensory circumventricular organs may be involved. An experiment was designed with four treatment groups including a 1 h immobilization acute stress (AS) group, an unstressed acute control (AC), a third given an intraperitoneal (ip) hypertonic saline injection (HS) and isotonic saline controls (IC) administered ip. One set of chick brains was perfused for immunohistochemistry while a second was sampled for quantitative RT-PCR. Plasma corticosterone (CORT) and arginine vasotocin (AVT) concentrations were significantly increased in the immobilized and hypertonic saline groups ($p < 0.01$) compared to controls. Intense staining of the V1aR occurred throughout the organum vasculosum of the lamina terminalis (OVLT) and subseptal organ (SSO)/subfornical organ (SFO). The immunostaining allowed the boundaries of the two circumventricular organs (CVOs) to be described for the first time in avian species. Both treatment groups showed marked morphological changes in glia within the OVLT and SSO/SFO. The avian V1aR, angiotensin II type 1 receptor (AT1R), and transient receptor potential vanilloid receptor 1 (TRPV1) mRNA levels were increased in the SSO/SFO in hypertonic saline treated birds compared to isotonic controls. In contrast, the latter two genes (*AT1R* and *TRPV1*) were significantly decreased in the OVLT of birds subjected to hyperosmotic stress, while all three genes were significantly up-regulated after immobilization. Taken together, results show a possible differential function for the same receptors in two anatomically adjacent CVOs.

1. Introduction

In non-mammalian vertebrates, arginine vasotocin (AVT), and corticotropin-releasing hormone (CRH) are major secretagogues stimulating release of adrenocorticotropic hormone (ACTH) from the anterior pituitary (Castro et al., 1986) and shown to be effective in the release of corticosterone (CORT) from the adrenal gland of male and female birds (Madison et al., 2008). Two vasotocin receptors, type two (VT2R; Jurkevich et al., 2005,. 2008; Kuenzel and Jurkevich, 2010; Cornett et al., 2013) and type four (VT4R; Selvam et al., 2013; Kuenzel et al., 2013; Kang and Kuenzel, 2014) have been shown to be involved in the neuroendocrine hypothalamo-pituitary-adrenal (HPA) axis of chickens. Due to the genome sequence and anatomical and functional similarity of the two avian receptors to the mammalian vasopressin V1b and V1a receptors, it has been proposed that the VT2R and VT4R be termed the avian V1bR (Cornett et al., 2013) and avian V1aR (Kuenzel et al., 2016), respectively. We have utilized an acute psychogenic stressor, immobilization, and showed that both the avian V1bR and V1aR as well as the CRH receptor 1 (CRH-R1) and CRH-R2 are involved in acute and chronic stress along with the release of CORT (Kuenzel et al., 2013; Selvam et al., 2013; Kang and Kuenzel, 2014).

Studies performed in birds to date have shown that the avian V1bR has not been found in the chicken brain (Jurkevich et al., 2005), nor in the brain of the white-throated sparrow or zebra finch (Leung et al., 2011). In contrast the avian V1aR has been reported in both the white-throated sparrow and zebra finch brains (Leung et al., 2011) and throughout the chicken brain (Selvam et al., 2015). Curiously, the V1aR was found in glia in all

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ten circumventricular organs (CVOs) of the chick brain (Selvam et al., 2015). A typical vertebrate CVO usually displays specialized ependymal cells, an incomplete blood-brain barrier, cerebrospinal fluid (CSF)-contacting neurons, and is located adjacent to the ventricles of the brain (Vigh and Vigh-Teichmann, 1973). Using an antibody to the avian V1aR, immunoreactivity (V1aR-ir) was documented in glia located in two sensory CVOs, the organum vasculosum of the lamina terminalis (OVLT) and subseptal organ (SSO), homologous to the mammalian subfornical organ (SFO). The finding prompted us to perform a detailed anatomical study of the distribution of the V1aR in the two CVOs as it was reported earlier using classical stains that the OVLT of birds was extensive and appeared to span from the base of the preoptic region to the anterior commissure (Dellmann, 1964). This observation was later confirmed by immunocytochemistry showing terminal fields of gonadotropin releasing hormone type one (GnRH-1) along the entire lamina terminalis (Kuenzel and Golden, 2006). However, what was not clear was where the OVLT ended and the SSO/SFO began as data from the duck suggested that the SFO likewise began at the dorsal region of the anterior commissure (Schmidt, 1995). In the chick brain, it was not documented where the SSO/SFO first appeared in brain sections, and more importantly, where the lateral extent of the two CVOs occurred in this or other avian species.

In addition to clarifying anatomical data, the location and expression of the V1aR in glia of the two CVOs may determine whether both the OVLT and SSO/SFO play major roles in osmoregulation as demonstrated in mammals, rather than the SFO being the primary CVO as reported in the duck brain (Simon et al., 1992). Simpson and Routtenberg (1973) demonstrated in mammals that the SFO was the site where circulating angiotensin II (ANG II) mediated the onset of drinking behavior. Subsequent studies showed that the rodent SFO sends major projections to the OVLT and nucleus preopticus medianus (MnPO; Miselis et al., 1979, 1987). All three structures (OVLT, SFO, MnPO) are also involved in osmoregulation through the action of angiotensin II on vasopressin release in mammals (McKinley et al., 1998, 1999). High affinity binding sites for ANG II were identified in the mammalian brain (Mendelsohn et al., 1984) and subsequently characterized as AT_1 and AT_2 receptors (Wright and Harding, 1994; Aguilera et al., 1995). In birds, it likewise has been shown that avian species drank water after administration of ANG II (Wada et al., 1975; Evered and Fitzsimons, 1976; Takei, 1977a) and an anatomical site, the SFO, in Japanese quail showed sensitivity to ANG II (Takei, 1977b). Autoradiographic localization of ANG II receptors was found in the SFO and anteroventral third ventricle (AV3V) region, an area where the avian OVLT was also located (Gerstberger et al., 1987, 1992; Simon et al., 1992).

The SFO and OVLT in mammals have been proposed to not only affect water intake and conserve body water but also function in osmotic and body fluid homeostasis (Sunn et al., 2003). In particular, a family of transient receptor potential vanilloid (TRPV) receptors have been discovered and proposed as candidates for vertebrate osmoreceptors in the SFO and OVLT (Liedtke et al., 2000). In the duck brain, the rostral extension of the third ventricle where the lamina terminalis occurs appeared to be the comparable site for stimulating drinking and antidiuresis (Gerstberger et al., 1987; Simon-Oppermann et al., 1988). To the best of our knowledge, no study has been reported in birds where a particular member of the TRPV family, nor a particular member of the ANG receptor family, has been shown to play a role in osmoregulation within the avian SFO or OVLT. Our discovery that the avian V1aR occurs in glia located in those two CVOS suggests that perhaps the V1aR may also be involved in osmoregulation in birds. We therefore subjected a group of chicks to a physical stressor (intraperitoneal administration of hypertonic saline) and another treatment group subjected to a psychological stressor (immobilization) along with their respective control groups. Our hypotheses were 1) both the SFO and OVLT would be associated with sensing osmotic stress, and 2) the V1aR and one of the receptors in the TRPV and/or AT family may play a role in either one or both avian CVOs.

2. Results

2.1. Anatomy of the OVLT/SSO in the Avian Brain

The avian V1aR antibody was effective in identifying the location of circumventricular organs (CVOs) as glia are major components of CVOs and all 10 avian CVOs have glia containing the V1aR (Selvam et al., 2015). In particular, the avian V1aR has been shown to be abundant in both the OVLT and SSO and therefore the receptor has been an effective marker in defining the boundaries of the two CVOs in the chick brain. As can be seen in the following set of coronal images, the OVLT begins as a small triangular shape at the base of the preoptic area (Plate A8.8, Fig. 1, Fig. 2A, B) and lines the anterior most wall of the third ventricle (3 V) called the lamina terminalis. Caudally, the OVLT assumes a more dorsal position as it approaches the anterior commissure (CA, Fig. 2C-F), a prominent fiber tract that marks the dorsal region of the anterior hypothalamus. The OVLT passes in front of the CA and ends at its final location just dorsal to the CA (Fig. 2F). At approximately the mid-region of the CA and just dorsal to it, the SSO begins (Fig. 2G) and thereafter occupies the ventral part of the nucleus of the hippocampal commissure (NHpC), previously called the bed nucleus the pallial commissure. Glial cells within the SSO show ir for the V1aR along the roof of the 3 V and V1aRs are also seen surrounding blood vessels located along the midline of the SSO (Fig. 2H-J). Posteriorly, the SSO occupies a greater proportion of the NHpC (Fig. 2I-K). At the caudal end of the SSO, a transition occurs and the space previously occupied by blood vessels enlarges and becomes filled with cerebrospinal fluid (Fig. 2K-L). Immediately posterior, the enlarged, dorsal space at midline joins the 3 V below it and the two ventral arms of the lateral ventricles join the 3 V to form the interventricular foramina where the choroid plexus resides. A most characteristic part of the SSO is its caudal-most structure, a ventral projection into the 3 V showing dense V1aR-ir (Fig. 2K-L).

One objective of the study was to determine if any anatomical changes occurred to the cells immunoreactive for the V1aR in the OVLT or SSO following the two distinct stressors (immobilization or administration of hypertonic saline) compared to their respective control groups. The OVLT, had one site that displayed consistent anatomical changes in both stress treatment groups. It was located at the ventral region of the OVLT, just below the attachment of the OVLT to the brain parenchyma on each side of the base of the preoptic area (Fig. 2D). Note the triangular-shaped ring comprising the major structure of the OVLT and its two lower attachments to the brain. A narrow, vertical space (the cistern (CIS)) is evident just dorsal to each of the two attachment sites (Fig. 2D). The CIS most likely is filled with cerebrospinal fluid similar to the 3 V. The glial cells immunoreactive for the V1aR just lateral to the major ring structure of the OVLT, suggest that they too should be included as part of the OVLT structure, due to their clear and restricted immunostaining. Just ventral to the thin space (the CIS) on the right and left sides of the brain are where changes in glial structures were noted among the four treatment groups. Images of the isotonic saline controls (IS, Fig. 3A) and acute immobilization controls (AC, Fig. 3E) and their respective boxed-in images at higher magnification (Fig. 3C, G), respectively, show immunoreactive glial processes oriented in consistent, parallel, horizontal rows. In contrast, the boxed-in areas of the ventral portion