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VASOPRESSIN INDIRECTLY EXCITES DORSAL RAPHE SEROTONIN NEURONS THROUGH ACTIVATION OF THE VASOPRESSIN1A RECEPTOR

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Abstract—The neuropeptide vasopressin (AVP; arginine-vasopressin) is produced in a handful of brain nuclei located in the hypothalamus and extended amygdala and is released both peripherally as a hormone and within the central nervous system as a neurotransmitter. Central projections have been associated with a number of functions including regulation of physiological homeostasis, control of circadian rhythms, and modulation of social behavior. The AVP neurons located in the bed nucleus of the stria terminalis and medial amygdala (i.e., extended amygdala) in particular have been associated with affiliative social behavior in multiple species. It was recently demonstrated that in the mouse AVP projections emanating from extended amygdala neurons innervate a number of forebrain and midbrain brain regions including the dorsal raphe nucleus (DR), the site of origin of most forebrain-projecting serotonin neurons. Based on the presence of AVP fibers in the DR, we hypothesized that AVP would alter the physiology of serotonin neurons via AVP 1A receptor ($V_{1A}R$) activation. Using whole-cell electrophysiology techniques, we found that AVP increased the frequency and amplitude of excitatory post-synaptic currents (EPSCs) in serotonin neurons of male mice. The indirect stimulation of serotonin neurons was AMPA/kainate receptor dependent and blocked by the sodium channel blocker tetrodotoxin, suggesting an effect of AVP on glutamate neurons. Further, the increase in EPSC frequency induced by AVP was blocked by selective $V_{1A}R$ antagonists. Our data suggest that AVP had an excitatory influence on serotonin neurons. This work highlights a new target (i.e., $V_{1A}R$) for manipulating serotonin neuron excitability. In light of our data, we propose that some of the diverse effects of AVP on physiology and behavior, including social behavior, may be due to activation of the DR serotonin system.

Key words: BNST, vasopressin, serotonin, electrophysiology, V1A receptor, glutamate.

INTRODUCTION

Common features of a number of mental disorders such as social anxiety, autism, and schizophrenia include disrupted social behavior, altered responses to stress, and increased predisposition to anxiety (www.nimh.nih.gov/health/topics). The vasopressin (AVP; arginine-vasopressin) and serotonin neurotransmitter systems are major foci of research to elucidate the neural underpinnings of disorders such as those listed above that affect emotion and behavior. Both AVP and serotonin have been implicated in the regulation of social behavior, stress, and anxiety (Ferris et al., 1997; Lucki, 1998; Egashira et al., 2007; Caldwell et al., 2008; Veenema, 2009). A recent study detailing AVP-immunoreactivity (ir) throughout the mouse brain indicated a moderate-to-dense innervation of several midbrain structures including the dorsal and median raphe (Rood and De Vries, 2011), the main source of all forebrain-projecting serotonin neurons (Azmitia and Segal, 1978). Our data indicated two important features of AVP innervation to the dorsal raphe (DR): it is gonadal steroid dependent (i.e., eliminated by gonadectomy) and sexually dimorphic (Rood et al., 2012). These features strongly suggest that AVP innervation of the DR comes from the bed nucleus of the stria terminalis and or the medial amygdala (i.e., extended amygdala, which includes both the bed nucleus of the stria terminalis (BNST) and the amygdala) as these AVP-producing nuclei are the only ones in which AVP expression is both dependent on circulating gonadal steroid hormones and sexually dimorphic (De Vries and Panzica, 2006; Rood et al., 2008, 2012).

AVP projections from the extended amygdala are thought to facilitate a number of affiliative and aggressive social behaviors in species ranging from rodents to primates (reviewed in Caldwell et al., 2008; Goodson, 2013). Critical brain regions and relevant receptors have been identified for a select few behaviors, including social memory in rats and mice

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Abbreviations: aCSF, artificial cerebrospinal fluid; AHP, after-hyperpolarization; ANOVA, analysis of variance; AP, action potential; AVP, arginine-vasopressin; BNST, bed nucleus of the stria terminalis; BSA, bovine serum albumin; dm, dorsomedial; DIC, differential interference contrast; DNQX, 6,7-dinitroquinoxaline-2,3-dione; DR, dorsal raphe; EGTA, ethylene glycol tetraacetic acid; EPSC, excitatory post-synaptic current; HEPES, hydroxyethyl piperazineethanesulfonic acid; IPSC, inhibitory post-synaptic current; Ir, immunoreactive/immunoreactivity; PB, phosphate buffer; PBS, phosphate buffered saline; PBST, PBS with Triton X-100; TPH, tryptophan hydroxylase; TTX, tetrodotoxin; $V_{1A}R$, vasopressin1A receptor; vm, ventromedial; YFP, yellow fluorescent protein.

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(Dantzer et al., 1988; Landgraf et al., 1988; Bielsky et al., 2005) and pair-bond formation in prairie voles (Insel et al., 1994; Young et al., 1999; Lim and Young, 2004). However, the exact contribution of the extended amygdala vs. other AVP producing regions to social behavior remains to be elucidated.

Interestingly, serotonin is known to influence many of the same behaviors and physiological functions as AVP, including social affiliation and aggression and response to stress (Insel and Winslow, 1998; Lucki, 1998; Veenema and Neumann, 2007). One reason for the functional similarities between these two neurotransmitter systems may be that they are interacting with each other. There are a few examples of interactions between the AVP and serotonin systems in the literature (Insel and Winslow, 1998; Veenema and Neumann, 2007). For example, intracerebroventricular injections of selective serotonin agonists increases peripheral AVP release (Jorgensen et al., 2003) and blocks AVP-induced territorial aggression (Ferris et al., 1997; Albers et al., 2002). Conversely, bath application of AVP augments synthesis and release of serotonin from dentate gyrus slice culture (Auerbach and Lipton, 1982). In addition, serotonin and AVP systems have been discussed in parallel regarding both aggressive and affiliative behaviors (Veenema, 2009). However, there are currently no reports of AVP action on serotonin neuron activity in the DR. Based on the dense AVP innervation of the DR, we hypothesized that AVP must have some effect on serotonin neurons.

In brain regions where a direct effect of AVP has been observed, AVP acting through the AVP 1A receptor ($V_{1A}R$) induces depolarization of the membrane by activation of non-specific cation channels (Stephens and Logan, 1986; Shewey and Dorsa, 1988; Raggenbass, 2008). However, in some systems multi-synaptic responses have been observed as well. For example, increases in post-synaptic currents (EPSCs) in spinal motor neurons and increased inhibitory post-synaptic currents (IPSCs) in lateral septal neurons have also been observed (Liu et al., 2003; Allaman-Exertier et al., 2007). Given the dense AVP innervation of the DR and current evidence regarding the action of AVP on neurons, we predicted that AVP exposure would alter the physiology of DR serotonin neurons. Our data indicated that AVP increased the frequency and amplitude of glutamatergic EPSCs in a subset of DR serotonin neurons.

EXPERIMENTAL PROCEDURES

Animals

Adult male Pet-1::YFP transgenic mice, 63 total, were used for all experiments. Heterozygous and homozygous Pet-1::YFP mice express yellow fluorescent protein (YFP) driven by a Pet-1 promoter, a transcription factor unique to serotonin neurons, enabling visualization of serotonin neurons prior to recording (Scott et al., 2005; Crawford et al., 2010, 2011). This transgenic line has been backcrossed to the C57BL6 strain for more than 10 generations. Animals were derived from breeding pairs housed in our AAALAC accredited facility at the Children's Hospital of Philadelphia. All animals were used in accordance with the National Institutes of Health guide for the care and use of laboratory animals, and all experiments were approved by the institutional IACUC committee.

Immunohistochemistry

Tissue preparation. Animals (n = 10) were perfused with 0.9% NaCl followed by 30 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB) under isoflurane anesthesia. Following perfusion, brains were removed and placed in 4% paraformaldehyde overnight and then placed in 30% sucrose in 0.1 M PB. Brains were sectioned at 40 μm on a cryostat; sections were stored in cryoprotectant (30% sucrose and 30% ethylene glycol in 0.1 M PB) at −20 °C until processing.

Immunohistochemistry. Brain sections were washed in phosphate buffer solution (PBS) (0.9% NaCl in PB) 3 × 10 min, incubated in blocking solution (PBST–BSA, PBS with 0.5% Triton X-100 and 0.04% bovine serum albumin) for 30 min, and then incubated overnight at room temperature in PBST–BSA containing mouse anti-tryptophan hydroxylase (TPH; 1:500, Sigma–Aldrich, St. Louis, MO, USA) and guinea-pig anti-AVP (1:8000; Bachem, Bubendorf, Switzerland). After overnight incubation, sections were washed with PBST–BSA 3 × 10 min and then incubated for 2 h in donkey anti-mouse Alexa Fluor 488 (1:250, Invitrogen, Carlsbad, CA, USA) and biotinylated goat anti-guinea pig (1:250; Vector Labs, Burlingame, CA, USA) in PBST–BSA. Sections were again washed 3 × 10 min with PBST–BSA, incubated for 2 h in streptavidin Alexa Fluor 647 (1:500, Invitrogen) in PBST–BSA, and then washed a final 3 × 10 min in PBS. Immuno-labeled sections were mounted to Colorfrost Plus slides (Fisher Scientific, Pittsburgh, PA, USA) and coverslipped using Fluoromount-G mounting medium (Electron Microscopy Sciences, Hatfield, PA, USA). Slides were sealed with generic clear nail polish.

Electrophysiology

Number of animals. A total of 53 mice were used for electrophysiological experiments. From these 53 mice, 35 AVP-responsive cells from 31 different mice were identified and included in various experiments. Thus the number (n) of cells reported in the results is roughly the same as the number of animals for each experimental condition. In no case were all data for a given condition derived from a single animal, and in no case were more than two cells from single animal included in a given experiment.

Brain slice preparation. Mice were rapidly decapitated, and the head was immediately placed in carbogen (95% O_2, 5% CO_2) bubbled ice-cold sucrose artificial