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Short communication

Plasma vasopressin concentrations positively predict cerebrospinal fluid vasopressin concentrations in human neonates

Dean S. Carson[a,*], Christopher L. Howerton[b], Joseph P. Garner[a,b], Shellie A. Hyde[a], Catherine L. Clark[c], Antonio Y. Hardan[a], Anna A. Penn[c,d], Karen J. Parker[a,**]

[a] Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305, USA
[b] Department of Comparative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
[c] Department of Pediatrics, Division of Neonatal and Developmental Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
[d] Departments of Fetal and Transitional Medicine, Neonatology, and the Center for Neuroscience Research, Children's National Medical Center, Washington, DC 20010, USA

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ABSTRACT

Central arginine vasopressin (AVP) plays a critical role in mammalian social behavior and has been hypothesized to be a biomarker of certain human neurodevelopmental disorders, including autism. However, opportunities to collect post-mortem brain tissue or cerebrospinal fluid (CSF) from children are extremely limited, and the use of less invasive peripheral assessments (e.g., blood, urine, or saliva) of AVP as a proxy for more invasive central measures has not been well validated. Further, almost nothing is known about AVP biology in very young infants. Therefore in the present study we concomitantly collected basal CSF and plasma samples from $N = 20$ neonates undergoing clinical sepsis evaluation (all were sepsis negative) and quantified AVP concentrations via well-validated enzyme-immunoassay methodology. Plasma AVP concentrations significantly and positively predicted CSF AVP concentrations ($r = 0.73$, $p = 0.0021$), and this relationship persisted when variance attributed to sex, gestational age, and sample collection time was controlled for in the statistical model ($r = 0.75$, $p = 0.0047$). These findings provide preliminary support for the use of basal plasma AVP measurement as a proxy for basal brain AVP activity in pediatric populations. Future studies are now required to determine the relationship between behavioral measures and AVP concentrations in both central and peripheral compartments in young infants and older children.

Introduction

The neuropeptide arginine vasopressin (AVP) has been linked to a wide variety of physiological processes (e.g., water balance and cardiac function) and more recently to complex psychological processes including human social cognition and behavior [15]. Several studies have outlined the potential for utilizing plasma AVP concentrations as a biomarker of disease status in neuropsychiatric disorders, including autism and schizophrenia [17,20]. Indeed, our group recently found that plasma AVP concentrations positively predict social cognition performance in children with autism, but not in siblings of probands or in matched neurotypical controls (D.S. Carson et al., manuscript submitted).

Plasma AVP concentrations as a biomarker of social functioning are most meaningful if they are associated with brain AVP activity. The relationship between central and peripheral nervous system AVP activity, however, is not well understood. AVP is primarily produced in the paraventricular and supraoptic nuclei of the hypothalamus. It is transported via axonal projections from hypothalamic magnocellular neurons to the posterior pituitary for storage and release into peripheral circulation where it regulates water balance, glucose, sodium, and potassium concentrations [19]. Importantly, AVP is also released centrally from the perikaryon of hypothalamic parvocellular neurons, as well as from their dendritic and axonal projections, and is delivered to a diverse range of brain regions via volume transmission. AVP is additionally released from neurons in the bed nucleus of the stria terminalis, medial amygdala, medial preoptic area, and suprachiasmatic nucleus [6]. Given that the extracellular fluid of the brain interconnects freely with

* Corresponding author at: Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, 1201 Welch Road, Stanford, CA 94305-5485, USA. Tel.: +1 650 498 5187; fax: +1 650 498 7761.
** Corresponding author at: Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, 1201 Welch Road, Stanford, CA 94305-5485, USA Tel.: +1 650 736 9863; fax: +1 650 498 7761.
E-mail addresses: dcarson@stanford.edu (D.S. Carson), kjparker@stanford.edu (K.J. Parker).

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the cerebrospinal fluid (CSF), it is generally accepted that the neuropeptide contents of CSF are a good, albeit non-specific, measure of their activity in the brain [11]. In adults, CSF circulates within the ventricular system of the brain and is thought to be reabsorbed in to the vascular system by entering the dural venous sinus via the arachnoid granulations. There is some evidence, particularly in neonates in which arachnoidal granulations are sparse, that suggests CSF largely flows along cranial nerves and spinal nerve roots where it then enters into lymphatic channels and subsequently in to circulating venous blood [27]. Further, differences in the anatomy of the blood–brain barrier (BBB) in neonates and adults, whereby the endothelial junctions of the brain's venous system are not as tightly formed during the early stages of life compared with adulthood, potentially allows for larger molecules (including neuropeptides) to flow more freely between the brain and body [22]. Thus, although large molecules such as proteins and neuropeptides are known to be sequestered by the BBB, the mechanisms of potential shared central and peripheral circulation of large molecules in neonates, or during certain disease states, may differ meaningfully from healthy adults [1].

There are some data from preclinical research that shows stimulation of AVP release from the neurohypophysis inhibits the release of AVP from the magnocellular perikaryon and dendrites, which in turn reduces further release from the neurohypophysis. These studies have largely measured concomitantly collected central microdialysates and peripheral blood samples following both psychological and physiological stressors in adult rodents [5,12]. Thus, how these findings inform our understanding of synchronized central and peripheral release patterns under basal conditions across the lifespan largely remains unclear. Further, previous studies investigating the relationship between CSF and plasma AVP concentrations in adult animals and humans have reported mixed findings [10,24,25], likely due to state specific factors (e.g., the stress of forced swimming) in animals, or disease and physiological imbalance (including osmolality and natremia status) inherent in the clinical indications that require invasive lumbar CSF sampling in humans. This collective information indicates that it is essential to consider all such variables in studies of AVP concentrations in both central and peripheral compartments and further highlights the importance of assessing neuropeptide activity during the early stages of life.

Given the growing interest in the role of AVP in neurodevelopmental disorders, there is an urgent need to clarify the relationship between basal CSF and plasma AVP concentrations in young humans. Due largely to the invasive nature of assessment, there has been only one prior pediatric study that investigated the relationship between AVP concentrations in concomitantly collected CSF and plasma samples. Bartrons et al. [2] provided evidence for a positive relationship between CSF and plasma AVP concentrations in neonatal humans with hypoxic-ischemic encephalopathy. Our research group was in a unique position to analyze basal AVP concentrations in concomitantly collected CSF and plasma samples from a small cohort of neonatal humans undergoing clinical sepsis evaluation. Only a small portion (< 5%) of these patients is generally found to be sepsis positive, which provides the rare opportunity to assess AVP concentrations in disease/syndrome free human newborns.

Methods

Participants

The study was approved by the Stanford University Institutional Review Board. Twenty human neonates (11 males, 9 females) undergoing clinically indicated sepsis evaluation for standard risk factors (e.g., maternal fever, prolonged rupture of membranes, infant respiratory distress) were recruited to the study. All subjects received <48 h of antibiotic treatment. All participants were found to be sepsis negative. Gestational age ranged from 31 to 40 weeks at birth ($M = 36.1$, $SD = 3.2$). Demographic information and maternal-infant medical condition necessitating neonatal CSF collection are presented in Table 1. Exclusion criteria consisted of known chromosomal anomalies and major malformations on the basis of historical evidence. A subset of these participants was included in a previously published study from our group [4].

Sample collection and processing procedures

Parental consent was obtained prior to initiation of the study procedures. Within 72 h of birth, and at the time of clinically indicated lumbar puncture, additional CSF (up to 0.5 mL) was obtained for research purposes using standard sterile procedures, and whole blood (up to 3 mL) was collected into chilled, aprotinin-treated EDTA vacutainer tubes from a central or arterial line, or via heel prick, if a line was not placed. CSF was immediately aliquoted and snap frozen on dry ice, and stored at −80 °C until the time of the assay. Blood samples were transported on wet ice, centrifuged at 4 °C for 10 min at 1300 × *g*, and then the plasma supernatant was aliquoted and snap frozen on dry ice, and stored at −80 °C until the samples were assayed in the Parker Laboratory.

Sample preparation and vasopressin quantification

CSF and plasma AVP concentrations were quantified using a commercially available enzyme immunoassay kit (Enzo Life Sciences, Inc., Farmingdale, NY). This kit has been validated for use in humans and is highly specific and selectively recognizes AVP and not related peptides (i.e., cross reactivity with oxytocin is <0.001%). Our laboratory has optimized procedures for determination of AVP concentrations in a variety of biological matrices (i.e., plasma, saliva, CSF) and in several species (i.e., adult, child, and infant humans as well as in juvenile and adult rhesus monkeys) using the methodology outlined here. Data obtained from an optimization experiment using neonatal CSF and plasma samples, run in duplicate, provided evidence that 300 μL of unextracted and hyperconcentrated CSF, and 400 μL of extracted and hyperconcentrated plasma, was sufficient for measuring AVP concentrations above the limit of detection. The minimum assay sensitivity where the standard curve is still linear is 3.39 pg/mL. A trained technician without knowledge of the experimental conditions performed sample preparation and AVP quantification. All sample preparation procedures were initiated by thawing samples in an ice bath. CSF samples were then mixed with an equal volume of ice-cold acetone, briefly vortexed, and centrifuged at 1 °C for 15 min at 4,000 × *g* prior to hyperconcentration (see below). Based on our optimization experiment, the small volumes of CSF available for research purposes, and evidence that there is minimal matrix interference in CSF, we followed the methods outlined in the previously published literature and did not perform extraction on these CSF samples [9,24]. However, there is a growing body of evidence that suggests extracting plasma samples is necessary in order to accurately determine concentrations of neuropeptides [14,23]. Thus, plasma samples were extracted using the solvent method recommended by the manufacturer. Briefly, equal volumes of 40:60 butanol:diisopropyl ether were added to samples prior to centrifugation at RT for 5 min at 8000 × *g*. The top organic layer was discarded and the aqueous solution transferred to a new mircocentrifuge tube. A 2:1 volume of ice cold acetone was then added to all samples prior to centrifugation at 4 °C for 20 min at 12,000 × *g*. Supernatent was then transferred to 15 mL Falcon tubes and a volume of 5:1 ice cold petroleum ether was added. Samples were