Differential effects of osmotic and SSR149415 challenges in maternally separated and control rats: The role of vasopressin on spatial learning

Vito S. Hernandez\textsuperscript{a}, Silvia Ruíz-Velazco\textsuperscript{b}, Limei Zhang\textsuperscript{a,∗}

\textsuperscript{a} Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México 04510, D.F., Mexico
\textsuperscript{b} Instituto de Investigaciones en Matemáticas Aplicadas y Sistemas, Universidad Nacional Autónoma de México, México 04510, D.F., Mexico

HIGHLIGHTS

\begin{itemize}
  \item Maternal separation (MS) up-regulates the hypothalamic vasopressin (VP) system.
  \item i.p hypertonic saline or SSR149415 was used to up or down regulate the VP system.
  \item The Morris water maze (MWM), was used to test learning, after the i.p. injections.
  \item MS rats had impairment after hypertonic saline, and control rats after V1b antagonism.
  \item This data support a role for VP in learning, dependent on the individual background.
\end{itemize}

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ABSTRACT

Maternal separation (MS) has been demonstrated to up-regulate the hypothalamic vasopressin (VP) system. Intracerebroventricularly released VP has been demonstrated to affect various types of animal behaviour, such as active/passive avoidance, social recognition, and learning and memory. However, the role of VP in spatial learning remains unclear. In the present study, we investigated the effects of an osmotic challenge and a V1b receptor-specific (V1bR) antagonist, SSR149415, on spatial learning of maternally separated and animal facility reared (AFR) adult male Wistar rats. The osmotic challenge was applied by injecting a hypertonic saline solution, 1 h before the Morris water maze test (MWM). V1bR antagonist SSR149415 (5 mg/kg) was injected i.p. twice (1 h and 30 min) previous to the MWM. A combined treatment with both osmotic challenge and the SSR149415 was applied to the third group whereas rats for basal condition were injected with isotonic saline. Under basal condition no differences between AFR and MS groups were observed. MS rats showed severe impairment during the MWM after the osmotic challenge, but not after the administration of SSR149415. For AFR rats, the opposite phenomenon was observed. The joint application of SSR149415 and osmotic challenge restored the spatial learning ability for both groups. The differential impairment produced by osmotic stress-induced-up-regulation and SSR149415 induced V1bR blockage in MS and control rats suggested that VP involvement in spatial learning depends on the individual intrinsic ligand-receptor functional state.

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1. Introduction

The neuropeptide vasopressin (VP) is synthesized primarily in the paraventricular and supraoptic nuclei of the hypothalamus, secreted from the neural lobe of the pituitary into the circulation, and serves various hormonal actions on peripheral tissues: regulation of water–electrolyte balance, hepatic glucose metabolism, and cardiovascular functions [1,19]. VP secretion is regulated principally by blood osmolality and volume [14]. VP neurons also project intracerebrally to several brain regions, particularly to limbic brain areas including the septo-hippocampal system [2,6]. It has been shown that VP and its metabolites can modulate the hippocampal theta rhythm [27] and facilitate memory processes [10,11] in intact animals. Ex vivo electrophysiological studies showed that nanomolar concentration of [Arg\textsuperscript{8}]-vasopressin (AVP) induced a prolonged increase in the amplitude and slope of the evoked population response in the presence of 1.5 mM calcium [8]. This AVP induced potentiation of the excitatory postsynaptic potential (EPSP) persisted following removal of AVP from the perfusion medium. The AVP induced sustained increase of EPSP is known as long-term vasopressin potentiation (LTVP) [8].

VP exerts its effects through three subtypes of receptors: V1a and V1b receptors are associated with phosphoinositide turnover, while the V2 receptor activates adenylate cyclase [19]. In the brain,
VP exerts its effects mainly by binding to V1a and V1b receptors. While V1a are widely distributed in the CNS [4,5,22,26], V1b receptors are much more specifically distributed [17,20,25]. Young et al. performed in situ hybridization histochemistry using a highly specific vasopressin V1b receptor riboprobe and found that in mice, vasopressin V1b receptors were prominently expressed in hippocampal pyramidal neurons located in the CA2 field [29]. Interestingly, the short-term effects of VP mentioned before were blocked by a V1a receptor antagonist whereas the long-term facilitatory effects remained despite this antagonism [27]. Moreover, Engelmann et al., administered either vasopressin or a V1a receptor antagonist via microdialysis into the rat septum, and found a lack of effect of the antagonist, but an impairment in the Morris water maze (MWM) performance of rats treated with exogenous vasopressin [16]. This evidence implied a possible role played by the V1b receptors. However, some studies showed that V1b receptor-knockout mice had no impairment on spatial learning performance [7,15], although VP system differences between rats and mice have been reported [23].

In the present study, we hypothesized that VP could exert a fine-tuning effect of spatial learning through the V1b receptor in rats and these effects could be uncovered with up- or down-regulation of the VP system in rats.

In order to demonstrate this hypothesis, we used male adult rats reared in two neonatal conditions: animal facility reared (AFR) and neonatal maternal separation (MS). These latter ones were demonstrated to have a persistent potentiated vasopressinergic system [21,28,30]. MS increase significantly the expression of AVP mRNA and peptide in the hypothalamus and produced a faster and higher release of vasopressin to plasma when MS rats are subjected to water deprivation [30]. By using salt load which is well known to up-regulate the VP release [14] and to increase its intracerebral release [18], and a recently characterized non-peptide vasopressinergic V1b receptor antagonist SSR149415 [24] to down-regulate the VP transmission, we examined VP modulatory effects on spatial learning in both animal facility reared (AFR) and MS male rats. The results of this study show that spatial learning in MS animals is vulnerable to an osmotic challenge, while AFR animals show disrupted learning only in presence of a high dose of vasopressinergic V1b antagonist SSR149415, compared with the literature [24].

2. Materials and methods

2.1. Animals and treatment

Wistar rats from the local animal facility were used in this study. All animal procedures were approved by the local bioethical and research committees in accordance with the principles exposed in the Handbook for the Use of Animals in Neuroscience Research (Society for Neuroscience, Washington DC, 1991). Rats were housed four per cage, maintained on an inverted 12 h light schedule in a room with controlled temperature between 20 °C and 24 °C with ventilation and given access to standard rat chow and water ad libitum.

Maternal separation (3 h daily, MS3h) procedure was described elsewhere [30]. Briefly, female and male adult rats were mated for two days. During the last week of gestation, female rats were single-housed in standard rat Plexiglas cages and maintained under standard laboratory conditions. On the day after parturition, postnatal day (PND) 2, each litter was culled to 7–8 pups, in which 5–6 were males. During the period from PND2–PND16, the pups were separated daily from their dams between 0900 h and 1200 h, placed into an incubator at 29 °C ± 1 °C. After this period rats were returned to their home cages. After ending the maternal separation protocol, animals were left undisturbed until the weaning at PND28, when male and female rats were separated. Four littersmates were put in one cage and each one was assigned to one of the four different treatment groups. Animals were then left undisturbed until PND90 when spatial learning assessment was performed during their activity period. Animal facility reared (AFR) rats were treated in the same conditions as above mentioned except that these animals were left undisturbed in their cages during the period when MS3h rats were separated from their dam. All the cages were cleaned twice a week with minimum disturbance to the rats.

Ninety-six young adult male rats (PND90, body weight 350 ± 10 g) from 12 AFR and 12 MS3h litters, were designated to 4 treatment groups: (A) isotonic (treatment 1, T1): rats received only a 2% b.w., i.p. injection of NaCl 0.9% 1 h previous to the MWM; (B) hypertonic (T2): rats received a 2% b.w., i.p. injection of 900 mM NaCl 1 h previous to the test; (C) SSR149415 (T3): rats received a 5 mg/kg i.p. injection of SSR149415 (Axon 1114, Axon Medchem BV Amsterdam, Netherlands, diluted in dimethyl sulfoxide (DMSO) first and then diluted in 0.9% saline, 1:20 respectively), twice at 1 h and 30 min time-points before the MWM test; (D) hypertonic + SSR149415 (T4): rats received combined “hypertonic” and “SSR149415” treatments as above described. Water bottles were removed at the moment of first injection for all groups until 10 min before the MWM.

2.2. Spatial learning assessment

The modified Morris water maze (MWM) procedure has been described elsewhere [31]. Briefly, a black circular pool (diameter 156 cm, height 80 cm) filled with 30 cm of water (25 ± 1 °C) with distant visual cues, was used for this cognitive test. A circular black escape platform (diameter 12 cm) was submerged 1 cm below the water surface. Rats were habituated to this swimming task (without the presence of the platform) a week before the MWM. On the day of the test, rats were allowed up to 60 s to locate the escape platform. If the allowed time ended and the experimental subjects had not found the platform, they were guided to it. Once on the platform, rats were permitted to stay for 10 s and allowed to observe their location. Each rat underwent 8 sequential trials on the same day, with an inter-trial interval of approximately 5 min. The time required to locate the hidden platform in each trial was recorded.

2.3. Statistical analyses

Quantitative results were expressed as mean ± standard error of the mean (SEM). Groups were tested for differences by performing three and two way mixed models analysis of variance followed by Bonferroni post hoc test using STATA 11. Differences were considered statistically significant at a value of *p < 0.05; **p < 0.01; ***p < 0.001.

3. Results

The three way mixed model analysis of variance of the MWM test showed a significant effect of treatment (F2,704 = 17.37 p < 0.0001) and trial (F1,704 = 382.15, p < 0.0001), whereas no significant effect is showed of group (AFR and MS), but the three factors and all the two factors interactions are statistically significant (F2,704 = 3.25 p = 0.01, F2,704 = 8.21 p < 0.001, F1,704 = 3.40 p = 0.05, F3,704 = 22.32 p = 0.001), therefore we used two way mixed models analysis of variance for each level of (AFR & MS) and each level of treatment. For the two groups we found that the interaction between treatment and trial is statistically significant whereas for each treatment only in SSR149415 treatment (T3) (Fig. 1C) this interaction is statistically significant (Table 1).

No significant difference in spatial learning performance was observed between the AFR and the MS3h groups when isotonic
treatment (T1) was administered (Fig. 1A). In the hypertonic treatment (T2), the MS3h group showed markedly increased escape latencies compared to AFR group at II, III, VII and VIII trials (Fig. 1B). The MS3h hypertonic group also showed significant impairment in spatial learning at the III, VII and VIII trials when compared to the MS3h isotonic group (Fig. 1F, blue line (T2) vs. black line (T1) respectively and Table 1, right panel, intersection of T2 and T1 for trial III, VII and VIII). The hypertonic treatment (T2) had no significant effect on MWM performance in the AFR group (Fig. 1E, blue line (T2) vs. black line (T1) respectively and Table 1, left panel, intersection of T2 and T1 for each trial). In the AFR group (Fig 1E), the V1b receptor antagonist SSR149415 treatment (T3) produced significant differences on learning performance when compared to isotonic treatment (T1) with significant differences at trials II, III, IV, V, VI and VII (Fig. 1E, red line (T3) vs. black line (T1), Table 1, left panel, intersection of T3 and T1 for those trial), whereas in MS3h animals, the SSR149415 (T3) produced significant differences on learning performance only at trial II compared to isotonic treatment (T1) (Fig. 1F, red line (T3) vs. black line (T1)). There were also significant differences at trials III, IV and V if the comparison was made between AFR and MS3h groups treated with SSR149415 (T3) (Fig. 1C). Interestingly, an apparent initial improvement (significant shorter escape time latencies) of the water maze performance was observed at the trial II in both AFR and MS3h rats treated with SSR149415 (T3) (Fig. 1E and F, red lines (T3) vs. black lines (T1)). When combined treatment (T4) was applied, both hypertonic and SSR149415, deleterious effects on spatial learning performance were effectively cancelled and performance reversed to the isotonic levels compared with the same rearing condition groups (Fig. 1E and 1F, green line (T4) vs. black line (T1)).

4. Discussion

This is the first study in the literature reporting the modulatory effects by up- and down-regulating the vasopressinergic system on spatial learning. Our data showed that the application of an osmotic challenge, which is known to strongly up-regulate the VP system [14], disrupted spatial learning only in the MS3h subjects, whereas a high dose of the V1b receptor-specific antagonist, SSR149415, markedly impaired the water maze performance in AFR subjects, while little effect was observed on the MS3h performance. Both impairments were effectively reversed by a combined treatment of both challenges.

The hippocampus seems to be the site of AVP action on memory processes [3]. Landgraf et al. showed that an hypertonic saline challenge caused a significant rise in plasma VP and an increase in extracellular VP content within the hippocampus, at both 30 and 60 min after intraperitoneal injection [18]. VP metabolite AVP4-9 enhanced learning and memory during a radial maze test and hippocampal lesions blocked this enhancing effect [12]. The
Table 1
Significance values table: Left panel shows statistically significant differences between treatments for different trials in the AFR group. *, **, *** represent 0.05, 0.01 and 0.001 significance differences. #, ##, ###, represent 0.05, 0.01, 0.001 significance differences between groups (MS3h and AFR) for different treatments and trials. Right panel shows statistically significant differences between treatments for different trials in the AFR group. T1: isotonic treatment; T2: hypertonic treatment; T3: SSR149415 treatment; T4 hypertonic + SSR149415 treatment. AFR: animal facility reared, MS3h: maternal separation 3 h.

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intracerebrovascular administration of vasopressin enhanced LTP in dentate gyrus [13] and long-lasting enhancement of synaptic excitability of CA1/subiculum neurons of the rat ventral hippocampus by vasopressin was demonstrated with in vitro preparation [9]. Moreover, Engelmann et al. showed that vasopressin administration via microdialysis into the septum, interfered with the spatial learning and memory during the Morris water maze task (MWM) [16]. Our data obtained under situations where vasopressinergic neurotransmission was up-regulated by osmotic stressor or down-regulated by V1br antagonists blockage suggested that VP involvement in spatial learning depends on the individual intrinsic ligand-receptor functional state and are in concordance with the previous data.

Several previous studies have shown that MS produces long-lasting up-regulation of the vasopressin system [21] but little is known about the effects of MS on spatial memory in rats. Our results showed that MS exerted no effect on spatial learning under basal conditions (T1). However, when rats were subjected to an osmotic challenge, impairment in the acquisition of spatial learning was displayed. This leads to speculate that the enhanced vasopressinergic system of MS offspring under basal conditions is well regulated by homeostatic mechanisms during water maze task, but not when the osmotic stressor is present — the further increase of VP content in the hippocampus would disrupt this cognitive task. In this study we show that this rearing-generated differences in AVP system anatomo-physiology can be exploited as an instrument to uncover the subtle role of VP played in spatial learning processes.

There are few and controversial studies assessing whether or not the V1b receptor has influence in hippocampal dependent learning. For example, Egashira et al. found that KO mice for V1b receptor displayed preserved spatial learning in an 8-arm radial maze test [15], while Murgatroyd et al. [21] showed that mice that underwent MS3h, presented impairment in the hippocampus dependent step-down avoidance learning test and treatment with V1b receptor antagonist, SSR149415, partially reversed the learning impairment. The obtained results of this study using SSR149415 support the idea that in rats, V1b receptor is involved in the modulation of spatial learning.

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