Chronic hyponatremia impairs memory in rats: effects of vasopressin antagonist tolvaptan

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Abstract

The effects of stable chronic hyponatremia on the central nervous system are largely unknown, clinically, or in experimental animals. The aim of this study was to identify and characterize these effects in rats. Tolvaptan, a vasopressin V₂ receptor antagonist, was used to correct hyponatremia and determine any potential benefits of such treatment in this condition. Stable chronic hyponatremia was induced by combination of the continuous vasopressin V₂ receptor stimulation and liquid food intake. The hyponatremic rats did not exhibit significant changes in general symptoms or neurological functions assessed by modified Irwin’s method, or in motor function assessed by the rotarod test. In passive avoidance test, however, rats with moderate and severe hyponatremia had significantly reduced step-through latency, indicating impairment in memory. This reduced step-through latency was improved by the treatment of tolvaptan (0.25–8 mg/kg daily doses), a vasopressin V₂ receptor antagonist. This improvement is associated with normalization of plasma sodium concentrations in hyponatremic rats. In conclusion, these data suggest that chronic hyponatremia may impair memory, and treatments that normalize sodium level, such as vasopressin V₂ receptor antagonists, may be beneficial to patients with hyponatremia.


Introduction

Hyponatremia is the most common disorder of body fluid and electrolyte balance and is associated with a variety of underlying disease states (Miller 2006). It has been reported that the symptoms of hyponatremia depend on the magnitude of low sodium level and the rapidity of its onset. Acute severe hyponatremia, which develops over <48 h, is often symptomatic and leads to neurological symptoms or death, in part because the resultant hypoosmolality produces brain edema (Soupart & Decaux 1996). On the other hand, stable chronic hyponatremia that develops over periods of more than 48 h is believed to be asymptomatic in many cases due to brain adaptation to hypoosmolality (Verbalis & Gullans 1991). Therefore, few studies have demonstrated the effects of chronic hyponatremia on the function of central nervous system (CNS) in either experimental animals or clinical studies. Several clinical studies have indicated that patients with mild to moderate chronic hyponatremia may experience attention deficits and gait disturbance, as associated with large bone fractures in the elderly (Renneboog et al. 2006, Sandhu et al. 2009). However, conclusive evidence is lacking to link chronic hyponatremia to neurological dysfunction.

Arginine vasopressin (AVP) plays a major role in body fluid regulation via V₂ receptors in the renal collecting ducts, and altered regulation of AVP is a major factor in developing hyponatremia (Miller 2006). Thus, V₂ receptor antagonism is effective to correct for hyponatremia (Schrier et al. 2006, Verbalis et al. 2008). Tolvaptan is a potent, highly selective, and orally effective nonpeptide V₂ receptor antagonist. It promotes aquarexis: an increase in urine volume and decrease in urine osmolality, increase in electrolyte-free water clearance, and increase in serum sodium concentration (Yamamura et al. 1998, Hirano et al. 2000). Recently, FDA approved tolvaptan for the treatment of hypervolemic and euvoletic hyponatremia including patients with heart failure, cirrhosis, and syndrome of inappropriate antidiuretic hormone (SIADH; FDA Labeling Information 2009). In clinical studies, tolvaptan treatment demonstrated a significant increase in serum sodium after 30 days of therapy in patients with euvoletic or hypervolemic hyponatremia (SALT-1 and SALT-2). Furthermore, there was a significant improvement in self-assessed mental health status (vitality, social functioning, emotionally limited accomplishment, calmness, and sadness) assessed by the Mental Component Summary of the SF-12 General Health Survey (Schrier et al. 2006). While these results suggest chronic hyponatremia may be associated with reduced quality of life (mental condition), more detailed studies are needed to fully understand the impact this condition on CNS function.

The aims of this study were to clarify these questions: 1) does chronic hyponatremia cause CNS-related changes in general symptoms and behavior (Irwin’s assessment test),
motor activity (rotarod treadmill test), and memory (passive avoidance test, PAV)? 2) does correction of hyponatremia using tolvaptan have any benefit in these evaluations?

**Materials and Methods**

**Animals**

The care and handling of animals were in accordance with the ‘Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co., Ltd (Tokushima, Japan); October 1, 1994’. Male Sprague–Dawley rats (6 weeks old) were purchased from Charles River Japan, Inc. (Yokohama, Japan), and housed under conditions of: humidity, 60 ± 10%; temperature, 23 ± 2 °C; 12 h light:12 h darkness cycle (0700 to 1900 h for the light period and 1900 to 0700 h for the darkness period) for 2 weeks before the experiments. They had free access to food and tap water before the experiments.

**Drugs**

[Deamino-Cys¹, d-Arg⁸]–Vasopressin (DDAVP) was purchased from Sigma–Aldrich Japan Co., Ltd. Hydroxypropyl methylcellulose (HPMC) was purchased from Shin-Etsu Chemical Co., Ltd (Tokyo, Japan). Tolvaptan ((±)-7-chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenzoyl-amino)benzoyl]-2,3,4,5-tetrahydro-1H-benzazepine) was prepared at Otsuka Pharmaceutical Co., Ltd, and was suspended in 1% HPMC solution.

**Study 1: Assessments of CNS-related changes caused by hyponatremia**

To determine the effects of hyponatremia on CNS-related function, three groups of rats were studied: normonatremia, moderate hyponatremia, and severe hyponatremia.

Hyponatremia was induced by a method described elsewhere (Miyazaki et al. 2005). In brief, rats (8 weeks old) were implanted with osmotic minipumps (ALZET model 2002, DURECT Corporation, Cupertino, CA, USA) containing DDAVP (0.3 and 1 ng/h to produce moderate and severe hyponatremia respectively) or saline in the back of each rat under ether anesthesia on day 0. Animals received 40 ml/day of modified liquid food containing 14% dextrose. The procedure for modified liquid food preparation was based on the ‘Preparation of Liquid Food’ (Oriental Yeast Co., Ltd, Osaka, Japan), but water was exchanged with a 14% dextrose solution. Blood (about 0.3 ml) was collected from the tail vein using a capillary tube on days 0, 5, 9, and 13 for plasma sodium measurement.

**Irwin’s test** General symptoms and neurological function were evaluated on day 5 using modified Irwin’s assessment test (Irwin 1968). All evaluations were performed by two researchers who were blinded to the treatments. The animals were individually placed in transparent plastic cages (27.3×49.1×27.3 (height) cm) containing the wire-mesh grid for observation. After the animals were acclimatized for at least 1 h, general symptoms and neurological functions were determined by 38 assessments (respiratory rate, stereotypy, grooming, vocalization, restlessness, Straub tail, tremor, twitch, convulsion, body posture, writhing, body tone, piloerection, urination, defecation, aggression, touch response, alertness, palpebral opening, ptosis, startle response, passivity, grip strength, catalepsy, spontaneous activity, abnormal gait, exophthalmos, skin color, lacrimation, salivation, pupil size, corneal reflex, pinna reflex, pain response, ipsilateral flexor reflex, limb tone, righting reflex, and hypothermia).

**Rotarod treadmill test** On day 10, motor function was evaluated in a rotarod treadmill apparatus (MK-660D, Muromachi Kikai Co., Ltd, Tokyo, Japan). The rotarod test was performed by a method described elsewhere (Yu et al. 2007) with some modification. Rats were placed on a rotating drum (6 r.p.m., trained until animals could remain on the apparatus at least 1 min at the training session, 6–60 r.p.m. accelerated for 3 min at the testing session, cut-off time was 180 s), and the time to maintain balance on the rotarod was measured.

**Passive avoidance test** PAV was performed by a method described elsewhere (Darbra et al. 2004) with some modification using an automatic-controlled, step-through type passive avoidance chamber (Yamashita–Giken, Tokushima, Japan) on days 12 and 13. The chamber consisted of two compartments made of acryl which separated a movable guillotine door; lighted room (15×20×20 (height) cm), and the dark room (20×25×20 (height) cm, the stainless steel grid floor which was supplied with an electric foot shock). In the training session (day 12), each rat was placed in the light compartment. After about 10 s, the guillotine door was automatically elevated, and when the rat entered the dark compartment, a foot shock was applied. Animals were trained with five reinforced trials. On day 13, latency time (LT) was observed and measured from the beginning of the avoidance test to the press of the foot shock in the training session, 0.5 s was added. Statistical analysis was performed using repeated measures ANOVA, followed by Dunnett’s test at each time point. ● denotes normonatremia, □ denotes moderate hyponatremia, ○ denotes severe hyponatremia.
moved into the dark compartment, the door was immediately closed, and 2-mA foot shock was applied through the grid floor for 3 s. Twenty-four hours after the training session, the rat was returned to the light compartment and the time for the rat to move into the dark compartment was measured up to the maximum cut-off time (180 s). Step-through latency was measured as an index of memory.

Study 2: Effect of tolvaptan treatment on reduced step-through latency of hyponatremic rats in PAV

Three groups of rats were studied, normonatremia, hyponatremia (vehicle), and hyponatremia (tolvaptan). On day 0, rats were implanted with osmotic minipumps containing DDAVP (1 ng/h, n=20) or saline (normonatremia, n=10). The rats in the hyponatremia group were further divided into the vehicle (n=10) and tolvaptan (n=10) groups by matching their individual body weight and plasma sodium concentrations measured on day 2. Tolvaptan or the vehicle (1% HPMC) was administered by dose titrations beginning on day 4. Administered doses of tolvaptan were as follows: 0.25 (day 4), 0.5 (day 5), 1.0 (day 6), 2.0 (day 7), 4.0 (day 8), and 8.0 (days 9–13) mg/kg. Vehicle was administered to the normonatremia group. Blood (about 0.3 ml) was collected from the tail vein using a capillary tube on days 0, 2, 4, 7, and 10 for plasma sodium measurement. PAV was performed on days 13 (training) and 14 (testing) using the method as described in Study 1. After PAV on day 14, blood samples were collected from the abdominal vein under anesthesia. All the animals were killed, and the brain was isolated for measuring brain water content. Isolated brain was weighed (wet weight), dried at 110 °C for at least 48 h and weighed again (dry weight), and brain water content was calculated by the formula shown below:

\[
\text{Water content} (%) = \left(\frac{\text{wet weight (g) - dry weight (g)}}{\text{wet weight (g)}}\right) \times 100.
\]

Study 3: Effect of tolvaptan itself on step-through latency of normal rats in PAV

To determine whether tolvaptan itself influences memory, step-through latency of normal rats in PAV with/without tolvaptan was measured. PAV was performed as described above; however, tolvaptan (10 mg/kg) or 1% HPMC solution was orally administered 1 h before PAV, and 0.8-mA foot shock was applied for 2 s.

Statistical analysis

Differences were considered to be statistically significant at P<0.05.

Differences in plasma sodium concentration were statistically analyzed using repeated measures of ANOVA, followed by two-tailed Dunnett’s test or two-tailed t-test. Differences in step-through latency were analyzed by two-tailed Steel’s test, Wilcoxon’s rank sum test, or Steel–Dwass’s test. Differences in other parameters were analyzed by two-tailed t-test.

Results

Study 1: Assessments of CNS-related changes caused by hyponatremia

Stable and chronic hyponatremia were created by combination of the continuous vasopressin V2 receptor stimulation and the excessive water loading using liquid food intake. By day 5, mean plasma sodium concentrations...
Hyponatremia tolvaptan 2.74 \times 10^8.

Comparison with normonatremia group. $$

Sodium levels in rats with moderate and severe hyponatremia whisker) are shown. (circle) and median with interquartile range (box and

were 124.5

Tests using a modified Irwin's assessment test on day 5 compared with the normonatremic group as assessed by the behavior in either moderate or severe hyponatremia groups

Step-through latency (s)

Figure 4 Effect of tolvaptan on step-through latency in passive avoidance test in hyponatremic rats. Step-through latency was measured up to the maximum cut-off time (180 s). The differences between the normonatremia and the hyponatremia vehicle, and between the hyponatremia vehicle and hyponatremia tolvaptan groups were analyzed by Wilcoxon's rank sum test. **P<0.01 compared with normonatremia group. $\bar{\bullet}$P<0.01 compared with hyponatremia vehicle group. The individual step-through latencies of ten animals (circle) and median with interquartile range (box and whisker) are shown.

decreased to 130.9 \pm 0.8 mEq/l in moderate hyponatremia (DDAVP: 0.3 ng/h) and to 116.6 \pm 1.3 mEq/l in severe hyponatremia (DDAVP: 1 ng/h), and were maintained from day 5 to the end of the experiment (Fig. 1). On day 13, plasma sodium levels in rats with moderate and severe hyponatremia were 124.5 \pm 1.0 and 117.4 \pm 0.5 mEq/l respectively.

There was no obvious general symptom or abnormal behavior in either moderate or severe hyponatremia groups compared with the normonatremic group as assessed by the 38 tests using a modified Irwin's assessment test on day 5 (data not shown).

The walking time on the rotarod test were 35.1 s (normonatremia), 41.3 \pm 5.4 s (moderate hyponatremia), and 31.4 \pm 3.5 s (severe hyponatremia) respectively, and there was no statistical significant difference among the groups.

However, the step-through latency of hyponatremic rats was significantly reduced compared with that of the normonatremic rats. Although all rats in the normonatremic group stayed in the light compartment for more than 180 s, almost all rats in moderate and severe hyponatremic groups moved into the dark compartment within 180 s (Fig. 2). The median value of step-through latency in the normonatremia group was >180 s, and reduced to 35.1 s in the moderate hyponatremia group and to 26.3 s in the severe hyponatremia group. The reductions were statistically significant (Fig. 2).

Results in Study 1 showed that only impaired memory was observed in our hyponatremic model, although there was no general symptom, abnormal behavior, or impaired motor activity.

Study 2: Effect of tolvaptan treatment on reduced step-through latency of hyponatremic rats in PAV

Using the severe hyponatremia model, the effects of tolvaptan were evaluated. Beginning on day 4, tolvaptan gradually and significantly elevated plasma sodium concentrations along with increasing tolvaptan doses (0.25, 0.5, 1, 2, 4, and 8 mg/kg; Fig. 3). On day 10, tolvaptan increased the plasma sodium concentration to 133.5 \pm 1.0 vs 115.0 \pm 0.9 mEq/l in the vehicle-treated group.

Similar to results in Study 1, hyponatremia significantly reduced the step-through latency (median: 11.0 s) compared with the normonatremic group (median: >180 s; not all animals stayed in the light compartment in the normonatremia group in this study compared with animals in Study 1 (Fig. 2), likely due to individual behavior differences). Tolvaptan treatment prolonged the step-through latency in the hyponatremic group (median: 99.3 s; Fig. 4).

Plasma osmolality, hematocrit, and plasma electrolyte concentrations were determined after PAV on day 14 in this study as shown in Table 1. Plasma osmolality, hematocrit, and electrolyte concentrations were significantly lowered in hyponatremic, and were improved by tolvaptan. These data are consistent with the ability of tolvaptan to correct hyponatremia.

To identify potential mechanisms of hyponatremia-mediated memory impairment, brain water content was determined. As shown in Fig. 5, hyponatremia significantly increased brain water content, indicating brain edema. Tolvaptan was able to partially normalize brain water content.

To further investigate the role of plasma sodium on memory, it was plotted against step-through latency for each animal in all the groups (Fig. 6). Rats in Study 1 and 2 were

Table 1 Effect of tolvaptan on plasma parameters in chronic hyponatremia after passive avoidance test on day 14. The differences between the normonatremia and the hyponatremia vehicle, and between the hyponatremia vehicle and hyponatremia tolvaptan groups were analyzed by two-tailed t-test

<table>
<thead>
<tr>
<th>Group</th>
<th>Osmolality (mOsm/kg)</th>
<th>Sodium (mEq/l)</th>
<th>Potassium (mEq/l)</th>
<th>Chloride (mEq/l)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normonatremia</td>
<td>304.6 \pm 1.4</td>
<td>144.9 \pm 0.3</td>
<td>5.19 \pm 0.11</td>
<td>109.0 \pm 0.4</td>
<td>42.4 \pm 0.3</td>
</tr>
<tr>
<td>Hyponatremia vehicle</td>
<td>242.0 \pm 2.8*</td>
<td>117.0 \pm 1.1*</td>
<td>4.57 \pm 0.08*</td>
<td>81.9 \pm 1.1*</td>
<td>37.6 \pm 0.7*</td>
</tr>
<tr>
<td>Hyponatremia tolvaptan</td>
<td>274.1 \pm 3.0†</td>
<td>132.6 \pm 1.4†</td>
<td>5.14 \pm 0.19†</td>
<td>97.7 \pm 1.4†</td>
<td>41.3 \pm 0.5†</td>
</tr>
</tbody>
</table>

*P<0.01 compared with normonatremia group. †P<0.05, ‡P<0.01 compared with hyponatremia vehicle group.


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shown in Fig. 6, step-through latency was significantly
concentrations on the days when PAV was conducted. As
tolvaptan, a vasopressin V2 receptor antagonist, and the
hyponatremic rats was improved by the treatment of
significant memory impairment.
135 mEq/l seems to be a critical level that can cause
shortened depending on the degree of hyponatremia, and
vehicle group.
not influence to the memory of normal rats in PAV.
suggesting that treatment with tolvaptan (10 mg/kg) itself did
analyzed by a two-tailed
normonatremia and hyponatremia, and between
hyponatremic model in rats. There was no obvious general
symptom or abnormal behavior (Irwin’s test), and impairment
of motor activity was not observed (rotarod test) in
hyponatremic rats. From these results, our hyponatremia
model seemed to be ‘asymptomatic’ even in the severe case.
However, the step-through latency of hyponatremic rats was
significantly reduced compared with the normonatremic rats
in the PAV. Thus, an impairment of memory (or learning) was
observed in rats with hyponatremia even in the moderate case.
Next, we performed PAV after plasma sodium correction
by specific vasopressin V2 receptor antagonist tolvaptan, to
examine whether impaired memory was caused by hypona-
tremia. When hyponatremia was partially normalized with
tolvaptan, it was associated with an improvement of reduced
step-through latency. In our data, vasopressin V2 receptor
stimulation by DDAVP without water overload (not
hyponatremic) did not impair PAV performance (data not
shown). Furthermore, tolvaptan did not cross the blood–brain
barrier; and tolvaptan itself did not influence the step-through
latency as shown in Study 3. These data suggest that the
improvement of step-through latency by tolvaptan treatment
is not direct effect of vasopressin receptor antagonism but
likely due to the normalization of plasma sodium.
In addition, our results indicate that a plasma sodium level
of 135 mEq/l is critical. Below this level, impairment of
memory occurs, and impairment of memory is observed in
almost all rats when plasma sodium falls below 120 mEq/l.
Although further studies will be required to clarify which
process of memory, such as memory acquisition, consolida-
tion, and/or retrieval, are impaired by chronic hyponatremia,
categorized in three groups (> 135 (n=24), 120–135
(n=20), and < 120 mEq/l (n=16)) by their plasma sodium
concentrations on the days when PAV was conducted. As
shown in Fig. 6, step-through latency was significantly
shortened depending on the degree of hyponatremia, and
135 mEq/l seems to be a critical level that can cause
significant memory impairment.
These results showed that reduced step-through latency in
hyponatremic rats was improved by the treatment of
tolvaptan, a vasopressin V2 receptor antagonist, and the
improvement might be associated with normalization of
plasma sodium concentrations.

Study 3: Effect of tolvaptan itself on step-through latency of
normal rats in PAV
To determine whether tolvaptan itself directly influences
memory, step-through latency of normonatremic rats in
PAV with/without tolvaptan treatment was measured. In Study 3, foot shock condition (0.8 mA for 2 s) of PAV
was weaker than those in Study 1 and 2 (2 mA for 3 s). In this
condition, all rats moved into the dark compartment
within 180 s; and extension (or reduction) of time that rats
stayed in the light compartment should be observed if the
memory was influenced by tolvaptan itself. However, there
was no difference in step-through latency between vehicle-
(63.6 ± 25.4 s) and tolvaptan- (57.8 ± 22.5 s) treated groups,
suggesting that treatment with tolvaptan (10 mg/kg) itself did
not influence to the memory of normal rats in PAV.

Discussion
At first, we tried to clarify whether ‘asymptomatic’
hyponatremia was really asymptomatic using a standard
hyponatremic model in rats. There was no obvious general

![Figure 5](image_url)

**Figure 5** Effects of tolvaptan on brain water content. Values are
expressed as the mean±S.E.M. of ten animals. The differences
between the normonatremia and hyponatremia, and between
hyponatremia vehicle and hyponatremia tolvaptan groups were
analyzed by a two-tailed t-test. **P<0.01 compared with
normonatremia group. §§P<0.01 compared with hyponatremia
vehicle group.

![Figure 6](image_url)

**Figure 6** Relationship between step-through latency and plasma
sodium concentration in hyponatremic rats. The individual values
of 60 animals (rats in Study 1 and 2) were categorized in three
groups (>135, 120–135, and <120 mEq groups) by their plasma
sodium concentrations on the days when PAV was performed. The
differences between the groups were analyzed by Steel–Dwass’s
test. **P<0.01 compared with >135 mEq group. The individual
step-through latencies of animals (circle) and median with
interquartile range (box and whisker) are shown.
our data suggest that the state of hyponatremia may have an influence for memory processes without affecting general symptoms, behavior, or motor activity.

The mechanisms of this phenomenon are still to be determined, but the abnormalities of brain electrolytes and/or organic osmolytes and neurotransmitters may play important roles. It has been reported that several neurotransmitters strongly influence tolvaptan, e.g. dopamine (Doyle & Regan 1993), glutamate (Dany et al 1988, Gandolfi et al 1990, Venable & Kelly 1990, Riekkinen et al 1996), and acetylcholine (Piercey et al 1987, Hiramatsu et al 1998, Anglade et al 1999).

In the chronic hyponatremia, brain cells export electrolytes and organic osmolytes including neurotransmitters to prevent excess swelling, resulting in decreased electrolytes and/or organic osmolytes and neurotransmitter (e.g. glutamine/glutamate, GABA, aspartic acid, and glycine) content (Thurston & Hauhart 1987, Lien et al 1991, Verbals & Gullans 1991, Bedford & Leader 1993, Massieu et al 2004). The decrease of the neurotransmitters especially glutamine/glutamate might strongly influence the memory. Furthermore, it has been reported that hypoosmolality inhibits glutamate and GABA uptake in rat brain synaptosome in vitro (Waseem et al 2004).

In the present study, brain swelling (increased water content) and hypoosmolality was still observed on the days when PAV was performed in hyponatremic rats. Both brain cell swelling (which might change the physical environment around the neurons) and abnormalities in organic osmolytes (especially neurotransmitters) may have led to an impaired memory.

The other possible mechanism is the participation of angiotensin II (Ang II). In the SIADH model rats, it has been reported that plasma renin activity is lowered due to the mild neurotensin (which might change the physical environment around the structures involved in consolidation of memory traces. Brain Research 272 1–9.


Declaration of interest

The authors are employees of Otsuka Pharmaceutical Co., Ltd which develops tolvaptan.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial, or non-for-profit sector.

Acknowledgements

The authors express their sincere thanks to Dr Y Liu for his critical reading of this manuscript. The authors also thank Dr E Hosoki, Dr K Aihara, Dr T Futamura, Mr Y Toba, Mr T Ibuki, and Ms S Nakajima for their contribution and their excellent technical assistance.

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Received in final form 1 April 2010
Accepted 14 April 2010
Made available online as an Accepted Preprint
14 April 2010