Alcohol preference in mice lacking the Avpr1a vasopressin receptor

Atsushi Sanbe,1 Norio Takagi,2* Yoko Fujiwara,1* Junji Yamauchi,1 Toshiya Endo,1 Reiko Mizutani,1 Satoshi Takeo,2 Gozoh Tsujimoto,3 and Akito Tanoue1

1Department of Pharmacology, National Research Institute for Child Health and Development, Tokyo, Japan; 2Department of Pharmacology, Tokyo University of Pharmacy and Life Science, Tokyo, Japan; 3Department of Genomic Drug Discovery Science, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

Submitted 2 October 2007; accepted in final form 20 February 2008

Sanbe A, Takagi N, Fujiwara Y, Yamauchi J, Endo T, Mizutani R, Takeo S, Tsujimoto G, Tanoue A. Alcohol preference in mice lacking the Avpr1a vasopressin receptor. Am J Physiol Regul Integr Comp Physiol 294: R1482–R1490, 2008. First published February 27, 2008; doi:10.1152/ajpregu.00708.2007.—[Arg8]-vasopressin (Avp), a nonapeptide hormone, is known to regulate blood pressure, water balance, and a variety of behaviors such as anxiety, aggression, and bonding. Although some evidence that Avp modifies ethanol consumption and some of the effects of ethanol on behavior have been reported, the role of Avp in alcohol consumption and preference is poorly understood. The Avp1a receptor (Avpr1a) is ubiquitously expressed in the central nervous system. To determine the role of Avp signaling on the behavioral effects of alcohol, we examined voluntary ethanol consumption in mice with targeted disruptions of the Avp1a knockout (Avpr1a KO) gene. Avp1a KO mice displayed both increased ethanol consumption and preference compared with wild-type (WT) mice. Enhanced ethanol consumption was dramatically and reversibly reduced by treatment with N-methyl-D-aspartic acid antagonists. Basal glutamate release was elevated around the striatum in Avp1a KO mice. Elevation of extracellular glutamate was also produced in WT mice by local application of an Avp1a antagonist though a dialysis probe, and this elevation was quickly reversed by stopping the perfusion. These results suggest that Avp can inhibit the release of glutamate from the presynaptic terminal via the Avp1a receptor and that elevation of glutamate levels owing to loss of the inhibitory effect via Avp-Avpr1a signaling may play an important role in the preference for ethanol.

[Arg8]-vasopressin

[ARG8]-VASOPRESSIN (Avp), a nonapeptide hormone, synthesized mainly in the paraventricular nuclei and supraoptic nuclei of the hypothalamus, regulates blood pressure, water balance, and the release of adrenocorticotropic hormone from the anterior pituitary gland (26, 43, 46). Recently, Avp has been shown to be reversibly reduced by treatment with n-methyl-D-aspartic acid antagonists. Basal glutamate release was observed in a previous study (40). The elevation of glutamate level in the extracellular space of the striatum region to reduced uptake of glutamate by astrocytes and an increased glutamate level in the extracellular space of the striatum region in Per2 mutant mice (40). These effects are accompanied by increased alcohol intake in this mutant mouse (40). Because Avp signals can inhibit glutamate release in the brain stem and modulate the non-NMDA receptor signal in the supraoptic nucleus (4, 21), it is possible to hypothesize that the Avp neuron has functional significance in NMDA receptor-dependent alcohol preference and excessive alcohol consumption.

We used a two-bottle choice test to analyze the behavioral responses of Avpr1a-null mutant mice in terms of voluntary ethanol consumption and preference. Avp1a KO mice displayed both increased ethanol consumption and preference due to activation of glutamate-NMDA receptor signaling. In addition, basal glutamate release was elevated around the striatum region of Avp1a KO mice, where elevation of the glutamate release was observed in a previous study (40). The elevation of

* N. Takagi and Y. Fujiwara contributed equally to this work.
Address for reprint requests and other correspondence: Atsushi Sanbe Dept. of Pharmacology, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan (e-mail: asanbe@nch.go.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
extracellular glutamate was reproduced in wild-type (WT) mice treated with an Avpr1a antagonist, and this elevation quickly returned to normal after treatment was stopped. These results suggest that Avp inhibits the release of glutamate from the presynaptic terminal via Avpr1a, and that elevation of glutamate levels due to the loss of an inhibitory effect via the Avp-Avpr1a signal plays an important role in ethanol preference behavior.

MATERIALS AND METHODS

Animals. The generation of Avpr1a-deficient (Avpr1a KO) mice has been described previously (22, 26). The genetic background of the animals used for all experiments was a mixture of 129Sv and C57BL/6Cr Slc (SLC, Shizuoka, Japan). Non-Avpr1a KO littermates (WT) were used as age-matched control subjects for Avpr1a KO mice. In some sets of experiments, we also examined the alcohol preference of Avpr1a KO mice with a genetic background of C57BL/6Cr Slc; the Avpr1a KO mice were generated by backcrossing with C57BL/6Cr Slc mice more than six times. Avpr1a KO mice were housed in microisolator cages in a pathogen-free barrier facility and placed on a 12:12-h light-dark cycle with ad libitum access to food and water unless otherwise specified. Animals were used at 8 to 10 wk of age. All data presented here were obtained from male mice, except when otherwise specified. All experiments were conducted in accordance with the guidelines for the care and use of animals approved by the ethics committee of the National Research Institute for Child Health and Development (approval no. 2002-006).

Ethanol intake test. Throughout the experiments, fluid intake, food intake, and body weight were recorded every morning. Male Avpr1a KO and WT mice were habituated in their home cages to drinking from two bottles containing water over a 1-wk period. Mice were then given 24-h access to two bottles, one containing water and the other containing 4% to 16% (vol/vol) ethanol in water. The positions of the bottles were changed every day to exclude position preferences. After 1 wk, we measured the ethanol and water consumption and body weight every other morning for 2 wk. After we had determined the ethanol consumption, with corrections for evaporation and handling loss of water and ethanol, the average ethanol consumption per day (24 h) for each ethanol concentration was calculated (g kg body wt−1·24 h−1). The average water consumption and total fluid (water and ethanol) intake per day was also calculated (g kg body wt−1·24 h−1). To estimate the relative ethanol preference, we calculated the ethanol preference ratio at each ethanol concentration by dividing the ethanol consumption by the total fluid consumption (ethanol plus water). We performed several ethanol uptake and preference experiments, including comparisons between WT and Avpr1a KO mice, between male and female WT and Avpr1a KO mice, and between WT and Avpr1a KO mice with or without drug treatments. In each experiment, we used different WT and Avpr1a KO mice and examined the WT and Avpr1a KO mice simultaneously in the same animal room to ensure uniform experimental conditions.

To address the taste preferences of the mice, sucrose- and quinine-preference tests were performed. As in the ethanol intake test, all mice were habituated in their home cages to drinking from two bottles containing water. The mice were then given plain water in one bottle and 0.5% or 2.5% sucrose or 5 mg/ml quinine in the other.

Plasma ethanol concentrations. To measure plasma ethanol concentrations after administration of a standard dose, mice were injected intraperitoneally with 4.0 g/kg of 20% (wt/vol) ethanol in isotonic saline and immediately returned to their home cages. One and three hours after ethanol injection, the mice were rapidly anaesthetized with ether, and blood samples were immediately taken from the tail vein. The plasma ethanol concentration was measured with a gas chromatograph (SRL, Tokyo, Japan).

Extracellular amino acids and determination of glutamate release. Extracellular amino acids were quantified by assessing the amino acid concentration in a supernatant of centrifuged brain homogenates from the brain of the WT and Avpr1a KO mice, as described previously (40). The amino acid concentration was determined by the Ninhydrin assay (40).

The level of glutamate released was measured by a microdialysis technique (42). Each mouse was anesthetized with chloral hydrate (400 mg/kg ip) and then placed in a stereotaxic frame for implantation of a microdialysis probe. The microdialysis probe (D-1-6-02; 0.22 mm outer diameter, 2 mm membrane length; Eicom, Japan) was inserted into the right striatum at the following coordinates: 0.5 mm anterior and 1.5 mm lateral to the bregma, and 3.5 mm below the dura according to the atlas of Paxinos and Franklin (35). To determine the extracellular concentration of glutamate, the microdialysis perfusate was collected at 20-min intervals for 100 min, derivatized with α-phthalaldehyde, and then injected into a HPLC with an electrochemical detector (42). The Avpr1a receptor antagonist d(CH2)5[Tyr(Me)2]Avp (200 ng/μl in Ringers solution) was perfused for 20 min (42).

In situ hybridization. The mouse Avpr1a receptor cDNA was subcloned into pGEM vectors (Promega, Madison, WI) as described previously (26). To analyze Avpr1a mRNA expression in the brain, a sense or antisense probe for the full length of the mouse Avpr1a receptor cDNA was prepared by an in vitro transcription method using digoxigenin RNA Labeling Mix (Roche Diagnostics, Basel, Switzerland). Mouse brain was perfused with 4% paraformaldehyde and postfixed in the same solution at 4°C; frozen sections were cut at a thickness of 40 μm. In situ hybridization was performed under a free-floating condition. The sections were treated with 1 μg/ml proteinase K and 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0) at room temperature for 10 min. Hybridization was carried out overnight at 55°C in a hybridization buffer (50% formamide, 3× SSC, 0.12 M phosphate buffer, 1× Denhardt solution, 0.125 mg/ml sonicated salmon sperm DNA, and 10% dextran sulfate). Signals were detected with nitro-blue tetrazolium chloride/5-bromo-4-chloro-3′-indolylphosphosphate p-toluidine salt solution (Roche Diagnostics) as described previously (20).

Drug treatment. After the habituation period described above, we treated WT and Avpr1a KO mice with the following drugs. MK801, ifenprodil, NMDA receptor antagonists, and naltrexone, a nonspecific opioid receptor antagonist, purchased from Sigma (St. Louis, MO) were dissolved in saline. We injected 0.3 or 1 mg/kg MK801 or 1 mg/kg ifenprodil or 1 mg/kg naltrexone intraperitoneally into the mice once a day for 3 wk. As a control, saline was injected into mice. After 1 wk of treatment, we measured the ethanol and water consumption of the mice for the following 2 wk and calculated the average ethanol consumption per 24 h for each ethanol concentration.

In some sets of experiments, we examined the alcohol preference in Avpr1a KO mice 48 h after discontinuing the MK801 treatment. After the habitation period described above, we measured water and ethanol consumption for 2 wk. Then, we injected 1 mg/kg MK801 intraperitoneally into the mice once a day for 3 wk. After 1 wk of MK801 treatment, we measured the ethanol and water consumption of the mice for the following 2 wk and calculated the average ethanol consumption. Forty-eight hours after discontinuing the MK801 treatment, we measured the ethanol and water consumption of the mice and calculated the average ethanol consumption.

Statistics. Data are expressed as means ± SE. Statistical analysis was performed by one-way, two-way, or repeated ANOVA, followed by a post hoc comparison with Fisher’s paired least significant difference (PLSD) using Statview software, version 5.0 (Concepts, Berkeley, CA). Unpaired t-test was performed for comparison between two groups. Differences between groups were considered statistically significant at the level of P < 0.05.
RESULTS

Ethanol consumption by Avpr1a KO mice. Voluntary ethanol consumption was examined in mice with targeted disruptions of the Avpr1a gene. No difference in food intake was observed in any of the groups (data not shown). Before measurement of ethanol consumption, Avpr1a KO and WT mice were habituated in their home cages to drinking from two water bottles. During the habituation period, the total water consumptions of the WT and Avpr1a KO mice were similar (WT mice, $15,192 \pm 27 \text{ g} \cdot \text{kg}^{-1} \cdot 24\text{ h}^{-1}$ and Avpr1a KO mice, $17,178 \pm 26 \text{ g} \cdot \text{kg}^{-1} \cdot 24\text{ h}^{-1}$, $P = 0.342$ by unpaired t-test). After the habituation period, water consumption ($P < 0.0001$ by one-way ANOVA), ethanol consumption at various ethanol concentrations ($P < 0.0001$ by one-way ANOVA), total fluid consumption ($P = 0.792$ by one-way ANOVA), and preference ($P < 0.0001$ by one-way ANOVA) were measured in the WT and Avpr1a KO mice. Because the water and ethanol consumptions of the WT and Avpr1a KO mice were stable and sustained at the same level during the experimental periods (2 wk) (data not shown), we estimated the average values for each group. At a 4% ethanol concentration, no difference in ethanol consumption or ethanol preference was observed in any of the groups (Fig. 1). Unlike with the consumption of ethanol at a low concentration (4%), Avpr1a KO mice consumed twice as much of the 8% and 16% ethanol solutions as did WT mice ($P < 0.01$ by Fisher’s PLSD) (Fig. 1B), whereas the total fluid consumption (water + ethanol) was similar at all ethanol concentrations among all groups (Fig. 1C). Avpr1a KO mice showed a higher preference for ethanol, as expressed by the consumption of ethanol relative to the total fluid consumption, during periods of access to the 8% ($P < 0.0001$ by Fisher’s PLSD) and 16% ($P < 0.0001$ by Fisher’s PLSD) ethanol solutions (Fig. 1D).

To exclude the effects of genetic background on the modification of ethanol consumption and preference, we measured ethanol consumption as well as ethanol preference in Avpr1aR KO mice with a C57B/6 Cr Slc genetic background. The Avpr1aR KO mice with a C57B/6 Cr Slc genetic background also consumed more of the 8% ethanol solution than did the WT mice (WT mice, $73 \pm 10 \text{ g} \cdot \text{kg}^{-1} \cdot 24\text{ h}^{-1}$ vs. Avpr1aR KO mice $195 \pm 26 \text{ g} \cdot \text{kg}^{-1} \cdot 24\text{ h}^{-1}$, $P < 0.01$ by unpaired t-test, $n = 6$) and showed a higher ethanol preference (WT mice, 38% ± 5% vs. Avpr1aR KO mice, 75% ± 4%, $P < 0.05$ by unpaired t-test, $n = 6$), whereas the total fluid consumption of the two groups was similar (WT mice, $182 \pm 19 \text{ ml} \cdot \text{kg}^{-1} \cdot 24\text{ h}^{-1}$, Avpr1aR KO mice $228 \pm 21 \text{ ml} \cdot \text{kg}^{-1} \cdot 24\text{ h}^{-1}$, $P = 0.140$ by unpaired t-test). Thus, these data clearly indicate that Avpr1a deficiency can alter ethanol consumption and alcohol preference.

Increased ethanol consumption in Avpr1aR KO mice could be the result of an altered sense of taste. Therefore, we estimated the preference for a bitter solution (quinine) and a sweet solution (sucrose) (40, 44). No difference was observed between Avpr1aR KO and WT mice in the quinine- and sucrose-preference tests (Fig. 2, A and B). Thus, an altered sense of taste is unlikely to account for the increased alcohol consumption and enhanced alcohol preference of Avpr1aR KO mice.

Because the Avpr1a gene is highly expressed in the liver (22), deficiency of the Avpr1a gene may modify ethanol

Fig. 1. Ethanol (EtOH) consumption and preference in vasopressin V1a receptor (Avpr1a) knockout (KO; $n = 17$) and wild-type (WT; $n = 15$) mice. All mice were subjected to a 2-bottle choice test. Mice were given 24-h access to 2 bottles, 1 containing water and the other containing 4%, 8%, or 16% (vol/vol) EtOH in water. The consumption of water (A), EtOH (B), and total fluid (water + EtOH; C) is shown. D: EtOH preference was estimated by EtOH consumption per total fluid consumption. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. WT mice.
metabolism in Avpr1a KO mice. Changes in alcohol metabolism due to Avpr1a deficiency in the liver offer another possible explanation for the ethanol consumption and preference of Avpr1a KO mice. To analyze alcohol metabolism in these mice, we examined ethanol elimination in the plasma after an ethanol injection (Fig. 2C). The plasma ethanol concentrations 1 h and 3 h after the ethanol injection were similar in Avpr1a KO and WT mice (Fig. 2C). This result indicates that altered alcohol metabolism is not responsible for the increased ethanol consumption and enhanced ethanol preference of Avpr1a KO mice.

Sex difference in Avpr1a KO mice. To analyze the possible involvement of a sex difference in ethanol consumption, we examined the ethanol preferences [P < 0.0001 (sex) and P < 0.0001 (strain) by two-way ANOVA] of female and male Avpr1a KO mice (Fig. 3). Although the trend was similar, the enhancement of ethanol preference observed in male Avpr1a KO mice was much less in female Avpr1a KO mice (Fig. 3). These results suggest that a sex difference is present in the ethanol preference of Avpr1a KO mice, as in anxiety-like behaviors (9).

NMDA receptor in Avpr1a KO mice. Previous studies have shown that blockade of neurotransmitter receptors, such as the NMDA and opioid receptors, can reduce alcohol preference (24). Thus, we examined the effects of a nonselective opioid receptor antagonist, naltrexone (Fig. 4), and two NMDA receptor antagonists, MK801 and ifenprodil (Fig. 5), on alcohol preference in Avpr1a KO mice. Ethanol consumption and preference were not altered in Avpr1a KO mice treated with naltrexone (Fig. 4). Avpr1a KO mice had significantly greater ethanol consumption (strain, P < 0.0001 and treatment, P <

---

Fig. 2. Characteristics of Avpr1a KO mice (V1a). A: taste preference test. Consumption of water containing sucrose (A) or quinine (B) is shown (n = 12–15 in each group). The mice were given plain water in one bottle and 0.5% or 2.5% sucrose (A) or 5 mg/ml quinine (B) in the other. C: plasma EtOH concentration (mg/ml) 1 or 3 h after EtOH injection (4.0 g/kg ip) (n = 6 in each group). The mice were injected intraperitoneally with 4.0 g/kg of 20% (wt/vol) EtOH in isotonic saline and 1 and 3 h after EtOH injection, EtOH concentration was determined.

Fig. 3. Sex differences in EtOH consumption and preference between Avpr1a KO (V1a KO) and WT mice. All male (M) and female (F) mice were subjected to a 2-bottle choice test with 8% EtOH, as described in MATERIALS AND METHODS (n = 15 in each group). EtOH preference was estimated by EtOH consumption per total fluid (EtOH + water) consumption. ***P < 0.001 vs. WT mice.
0.0001 by two-way ANOVA) and preference (strain, \( P < 0.0001 \) by two-way ANOVA) and lower water consumption (strain, \( P < 0.0001 \) and treatment, \( P < 0.0009 \) by two-way ANOVA) than WT mice (\( P < 0.001 \) by Fisher’s PLSD) in the absence of MK801. When Avpr1a KO mice were given MK801, their ethanol preference was significantly lower (\( P < 0.01 \) for 0.3 mg/kg MK801 and \( P < 0.001 \) for 1 mg/kg MK801 by Fisher’s PLSD) than when these mice were given saline alone (Fig. 5, A and B). Total fluid consumption was similar among the WT and Avpr1a KO mice.

Fig. 4. Effects of nonselective opioid receptor antagonist naltrexone on EtOH consumption and preference in Avpr1a KO (V1a KO) and WT mice (\( n = 15 \) in each group). The mice were treated with 1 mg/kg naltrexone once a day for 3 wk. After 1 wk of treatment, the EtOH and water consumption of the mice for the following 2 wk were measured. S: saline treatment.

Fig. 5. Effects of \( N \)-methyl-D-aspartic acid (NMDA) antagonists MK801 (A and B) and ifenprodil (C and D) on EtOH consumption and preference in Avpr1a KO (V1a KO) and WT mice. The NMDA antagonist treatment reduced EtOH consumption (A and C) and preference (B and D) relative to the saline (S) treatment. The mice were treated with 0.3 or 1 mg/kg MK801 or 10 mg/kg ifenprodil once a day for 3 wk. After 1 wk of treatment, the EtOH and water consumption of the mice for the following 2 wk were measured. \(* * * P < 0.001\) vs. WT mice (\( n = 15 \) in each group), \#P < 0.05, \#\#P < 0.01, and \#\#\#P < 0.001 vs. S treatment.
with or without MK801 treatment (Fig. 5A). Another NMDA antagonist, ifenprodil, also reduced ethanol consumption and preference (ethanol consumption: strain, $P < 0.0001$ and treatment, $P < 0.0001$; ethanol preference: strain, $P < 0.0001$ and treatment, $P < 0.0001$, by two-way ANOVA; $P < 0.05$ for ifenprodil-treated Avpr1a KO mice vs. saline-treated Avpr1a KO mice by Fisher’s PLSD). After measurement for $2 \text{ wk}$, we discontinued the MK801 treatment of Avpr1a KO mice. Forty-eight hours later, ethanol consumption was restored in Avpr1a KO mice, suggesting that the reduction of alcohol preference by the NMDA receptor antagonist was reversible (ethanol consumption: strain, $P < 0.0007$ and ethanol preference: $P < 0.0001$ by repeated ANOVA; $P < 0.05$ for treated Avpr1a KO mice vs. posttreated Avpr1a KO mice by Fisher’s PLSD) (Fig. 6). These data indicate that the NMDA receptor plays an important role in the ethanol consumption and preference caused by Avpr1a gene knockout.

Activation of the glutamate-NMDA receptor signal can lead to the enhancement of ethanol preference (33, 40). This evidence suggests that the Avp-Avpr1a signal can modify the glutamate-NMDA receptor signal. To address the possible underlying mechanisms of the interaction between the Avpr1a and NMDA receptors, we analyzed basal glutamate release in the Avpr1a KO mouse brain (Fig. 7). First, we measured the extracellular amino acid concentration in the brains of Avpr1a KO mice. The extracellular amino acid concentration in the brains of Avpr1a KO mice was significantly higher than that in the brains of WT mice ($P < 0.05$ by unpaired $t$-test) (Fig. 7A). To further identify the elevated amino acid in the Avpr1a KO mouse brain, a microdialysis analysis was performed. A microdialysis probe was positioned in the striatum region of Avpr1a KO and WT mice. The extracellular glutamate level around the striatum region was significantly higher in Avpr1a KO mice than in WT mice ($P < 0.05$ by unpaired $t$-test) (Fig. 7B). These results suggest that deficiency of the Avpr1a receptor results in the elevation of glutamate levels around the striatum region.

Because Avp can inhibit evoked glutamatergic synaptic currents in the brain stem (4) as well as the parabrachial nucleus (12), inhibition of the glutamatergic synapse by a signal via the Avpr1a around the striatum is a feasible mechanism for the elevation of glutamate. To examine this hypothesis, we infused a selective Avpr1a antagonist, d(CH2)$_5$[Tyr(Me)$_2$]Avp, into the striatum by using a microdialysis probe to inhibit Avpr1a in a distinct region around the striatum in WT mice (Fig. 7C). After a 20-min infusion of d(CH2)$_5$[Tyr(Me)$_2$]Avp, the glutamate level was significantly elevated around the striatum region in WT mice ($P < 0.05$ by repeated ANOVA), and the elevation was quickly reversed when the antagonist treatment was discontinued (Fig. 7C). Because Avpr1a is expressed around the striatum, where we positioned the microdialysis probe (Fig. 7D), all results strongly suggest that Avp can inhibit the release of glutamate at the glutamatergic synapse via Avpr1a and that inhibition of Avpr1a or disruption of the Avpr1a gene may increase basal release of glutamate.

**DISCUSSION**

*Regulation of alcohol preference by Avp.* Our findings suggest that Avp, which is known as a regulator of aggression (7), pair bonding (54), and anxiety-related behaviors (10, 16) can act as a regulator of alcohol preference via the Avpr1a receptor. The ability to metabolize blood ethanol and the taste preferences were similar in Avpr1a KO and WT mice. These results suggest that Avp regulates alcohol consumption and preference via the Avpr1a gene in the brain. The enhanced ethanol preference of Avpr1a KO mice was dramatically and reversibly attenuated by treatment with NMDA antagonists, such as MK801 and ifenprodil, whereas no significant difference was seen after treatment with naltrexone, a nonselective opioid receptor antagonist. The NMDA receptor is thought to be a target of drug therapy for alcoholics (17). Activation of the NMDA receptor by increased glutamate release and a decrease in glutamate reuptake, as well as increased NMDA receptor density, can lead to activation of Ca$^{2+}$ influx into the neurons and dynamic modulation of the actin cytoskeleton (25, 33, 38, 40, 55). Another study has shown that interaction between tyrosine kinase Fyn and NMDA receptors plays an important role in mediating acute tolerance to ethanol and regulating acute ethanol sensitivity (53). It is hypothesized that these alterations are associated with excessive alcohol consumption and enhanced alcohol preference (25, 33, 38, 40, 55). Thus, the inhibitory effect of Avp via the Avpr1a gene on alcohol consumption and preference is probably mediated by the glutamate-NMDA receptor signal.

![Fig. 6](http://ajpregu.physiology.org/)

**Fig. 6.** Inhibitory effect of MK801 on EtOH consumption and preference is reversible in Avpr1a KO (V1a KO) mice. EtOH consumption and preference were determined before (Pre), during treatment for 3 wk (Tre), and 48 h after (Post) MK801 treatment in WT and V1aR KO mice. Treatment with MK801 (1 mg/kg ip) reduced EtOH consumption (A) and preference (B) in V1aR KO mice. Forty-eight hours after discontinuing the MK801 treatment, EtOH consumption and preference were restored in the V1aR KO mice (Post). ***$P < 0.001$ vs. WT mice (WT) ($n = 6$ in each group). ##$P < 0.01$ vs. mice before treatment (Pre), and *$P < 0.05$ vs. mice treated with MK801 (Tre).
Is Avp an inhibitory neurotransmitter of NMDA receptor activation? The exact mechanism by which the Avpr1a signal inhibits the release of glutamate in the brain is unclear. One possible explanation is interaction between glutamatergic neurons and Avp-Avpr1a signals. Previous studies showed electrophysiological alterations in the glutamatergic neurons due to Avp treatment (21). Avp modulates the glutamate signal via the non-NMDA receptor in the supraoptic nucleus (21). In the parabrachial nucleus, Avp can reversibly decrease the amplitude of the evoked glutamate-mediated, excitatory postsynaptic current (12). Recently, it was shown that Avp modifies afferent synaptic transmission by two distinct mechanisms: presynaptic inhibition of terminal glutamate release and extraterminal blockade of conducted excitation in the medial nucleus tractus solitarius (4). Here, we examined basal glutamate release in Avpr1a KO and WT mice around the striatum region, where the elevation of glutamate release was associated with ethanol consumption and preference (40). The basal release of glutamate was higher in Avpr1a KO mice than in WT mice. In addition, reversible enhancement of basal glutamate release by local infusion of an Avpr1a antagonist, d(CH₂)₅[Tyr(Me)₂]Avp (200 ng/μl in Ringer’s solution), was perfused for 20 min via the microdialysis probe. D: in situ hybridization indicates that expression of Avpr1a mRNA is present around the striatum in WT mice. St, striatum; Cor, cortex; Hip, hippocampus.

---

**Fig. 7.** Glutamate release in Avpr1a KO (V1a KO) and WT mice. A: extracellular amino acid concentration. B: basal glutamate release. Glutamate release was determined using the microdialysis method described in MATERIALS AND METHODS (n = 6 in each group). C: Avpr1a receptor antagonist, d(CH₂)₅[Tyr(Me)₂]Avp (200 ng/μl in Ringer’s solution), was perfused for 20 min via the microdialysis probe. D: in situ hybridization indicates that expression of Avpr1a mRNA is present around the striatum in WT mice. St, striatum; Cor, cortex; Hip, hippocampus.
In contrast, higher Avp production and a higher number of Avp-positive cells were seen in the medial amygdala and bed nucleus of the stria terminalis in male than female brains (14, 31, 50). Thus, all sex differences in alcohol consumption and preference of Avpr1a KO mice may result from different numbers of Avp fibers in the extrahypothalamic vasopressin system (9, 30). Further study will be necessary to address sex differences in alcohol consumption and preference.

**Perspectives and Significance**

To analyze the role of Avpr1a in alcohol preference, we examined voluntary ethanol consumption in Avpr1a KO and WT mice. The Avpr1a KO mice displayed both increased ethanol consumption and preference. The enhanced ethanol consumption was dramatically reduced by treatment with NMDA antagonists, and this effect was reversible. Glutamate release was elevated around the striatum in Avpr1a KO mice, as well as in WT mice treated with an Avpr1a antagonist. These results suggest that Avp can inhibit the release of glutamate from the presynaptic terminal via Avpr1a, and that the elevation of glutamate due to loss of the inhibitory effect of Avp-Avpr1a signaling may play an important role in ethanol preference behavior. Further study is needed to analyze alcohol-related behaviors, such as ethanol tolerance. These data raise a possible concern about the adverse effects of Avpr1a KO mice exhibit impairment of spatial memory in an eight-arm radial maze. 

**GRANTS**

This work was supported, in part, by research grants from the Scientific Fund of the Ministry of Education, Science, and Culture of Japan, the Ministry of Human Health and Welfare of Japan, the Japan Heart Foundation, the Novartis Foundation, the Suzuken Memorial Foundation, and the Japan Heart Foundation/Novartis Grant for Research Award on Molecular and Cellular Cardiology, the Takeda Science Foundation, and the Mochida Memorial Foundation for Medical and Pharmaceutical Research.

**REFERENCES**


