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Vasopressin and oxytocin excite MCH neurons, but not other lateral hypothalamic GABA neurons

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Vasopressin and oxytocin excite MCH neurons, but not other lateral hypothalamic GABA neurons. Am J Physiol Regul Integr Comp Physiol 302: R815–R824, 2012. First published January 18, 2012; doi:10.1152/ajpregu.00452.2011.—Neurons that synthesize melanin-concentrating hormone (MCH) colocalize GABA, regulate energy homeostasis, modulate water intake, and influence anxiety, stress, and social interaction. Similarly, vasopressin and oxytocin can influence the same behaviors and states, suggesting that these neuropeptides may exert part of their effect by modulating MCH neurons. Using whole cell recording in MCH-green fluorescent protein (GFP) transgenic mouse hypothalamic brain slices, we found that both vasopressin and oxytocin evoked a substantial excitatory effect. Both peptides reversibly increased spike frequency and depolarized the membrane potential in a concentration-dependent and tetrodotoxin-resistant manner, indicating a direct effect. Substitution of lithium for extracellular sodium, Na+/Ca2+ exchanger blockers KB-R7943 and SN-6, and intracellular calcium chelator BAPTA, all substantially reduced the vasopressin-mediated depolarization, suggesting activation of the Na+/Ca2+ exchanger. Vasopressin reduced input resistance, and the vasopressin-mediated depolarization was attenuated by SKF-96265, suggesting a second mechanism based on opening nonsynaptic cation channels. Neither vasopressin nor oxytocin showed substantial excitatory actions on lateral hypothalamic inhibitory neurons identified in a glutamate decarboxylase 67 (GAD67)-GFP mouse. The primary vasopressin receptor was vasopressin receptor 1a (V1aR), as suggested by the excitation by V1aR agonist [Arg8]vasotocin, the selective V1aR antagonist [Phe2]OVT and by the presence of V1aR mRNA in MCH cells, but not in other nearby GABA cells, as detected with single-cell RT-PCR. Oxytocin receptor mRNA was also detected in MCH neurons. Together, these data suggest that vasopressin or oxytocin exert a minimal effect on most GABA neurons in the lateral hypothalamus but exert a robust excitatory effect on presumptive GABA cells that contain MCH. Thus, some of the central actions of vasopressin and oxytocin may be mediated through MCH cells.

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MATERIALS AND METHODS

Experiments were performed on hypothalamic slices obtained from two different lines of transgenic mice. These lines selectively express enhanced GFP in MCH-containing neurons (55), or in GABA [(GAD67)-positive]-synthesizing neurons (1). All of the experimental procedures were approved by the Yale University Committee on Animal Care and Use.

Hypothalamic slices (250–300 μm) were cut from 2- to 6-wk-old mice maintained on a 12:12-h light-dark cycle that were given an overdose of pentobarbital sodium (100 mg/kg) during the light part of the cycle (11:00 AM to 4:00 PM). The brains were then removed rapidly and placed in an ice-cold, oxygenated (95% O2:5% CO2) high-sucrose solution that contained (in mM): 220 sucrose, 2.5 KCl, 6 MgCl2, 1 CaCl2, 1.23 NaH2PO4, 26 NaHCO3, and 10 glucose, pH 7.4 (with an osmolarity of 295–305 mOsm). A block of tissue containing the hypothalamus was isolated, and coronal slices were cut on a vibratome. After a 1- to 2-h recovery period, slices were moved to a recording chamber mounted on a BX51WI upright microscope (Olympus, Tokyo, Japan) equipped with video-enhanced infrared-differential interference contrast and fluorescence capability. Slices were perfused with a continuous flow of gassed artificial cerebrospinal fluid (ACSF) (95% O2:5% CO2) that contained the following (in mM): 126 NaCl, 2.5 KCl, 2 MgCl2, 2 CaCl2, 1.23 NaH2PO4, 26 NaHCO3, and 10 glucose, pH 7.4. Bath temperature in the recording chamber was maintained at 35 ± 1°C using a dual-channel heat controller (Warner Instruments, Hamden, CT). Neurons were visualized with an Olympus Optical 40X water-immersion lens.

Patch-clamp recording. Whole-cell current- and voltage-clamp recordings were performed using pipettes with 4- to 6-MΩ resistance after being filled with pipette solution. The pipettes were made of borosilicate glass (World Precision Instruments, Sarasota, FL) using a PP-83 vertical puller (Narishige, Tokyo, Japan). For most recordings, the composition of the pipette solution was as follows (in mM): 145 KMeSO4 (or KCl for IPSCs), 1 MgCl2, 10 HEPES, 1.1 EGTA, 2 PP-83 vertical puller (Narishige, Tokyo, Japan). For most recordings, after being filled with pipette solution. The pipettes were made of glass and sealed using a PP-83 vertical puller (Narishige, Tokyo, Japan).

For most recordings, access resistance was stable (changes <10%) were included in the analysis. An EPC10 amplifier and Patchmaster software were used for data acquisition (HEKA Elektronik). PulseFit (HEKA Elektronik), Axograph (Molecular Devices, Union City, CA), and Igor Pro (WaveMetrics, Lake Oswego, OR) software were used for analysis. Both excitatory and inhibitory spontaneous postsynaptic currents (sIPSCs) were detected and measured with an algorithm in Axograph, and only those events with an amplitude >5 pA were used. The frequency of action potentials was measured using Axograph as well.

RT-PCR. The single-cell RT-PCR method used is similar to that reported previously (14) with minor modifications. To prevent degradation of the mRNA, cells harvested for RT-PCR were not used for recording. All PCRs were performed using an iCycler thermocycler (Bio-Rad, Hercules, CA) and the Expand High Fidelity PCR kit (Roche Diagnostics, Mannheim, Germany). Gene-specific primer pairs were designed to amplify mouse β-actin, AVP receptor 1A (V1a), and the OXTR cDNA sequences based on GenBank accession numbers NM_001081147 and NM_001081148, respectively, using Oligo Primer Analysis Software version 6.89 (Molecular Biology Insights, Cascade, CO). Because of the relatively low abundance of G protein-coupled receptors, we used a nested RT-PCR protocol, with the first RT-PCR product amplified by a second amplification round using different forward and reverse primers. Digital photographs of the gels were made, and the contrast and brightness of the entire gel were corrected with Adobe Photoshop. The following list details the target gene, annealing temperature, amplicon length, and primer sequences for each PCR: β-actin, 56.7°C, 523 bp, forward 5′-GCC AAC CGT GAA AAG ATG AC-3′ and reverse 5′-CAA CGC CAT ACT TGA TG-3′; V1a (initial), 53.9°C, 401 bp, forward 5′-CAG TGA AGA CCT TTG TG-3′ and reverse 5′-ATA TGG GCC TCA AGT AGA AG-3′; V1a (second), 55.1°C, 216 bp, forward 5′-TCG TCC AGA CCT TGT CAG-3′ and reverse 5′-TGG TAT CCC AGT CCG TCC TTG T-3′; and OXRT (initial), 56.5°C, 241 bp, forward 5′-CTT CAT CGT CTG CTG GAC-3′ and reverse 5′-GCT AAT GCT CCT TTC AGC AG-3′.

Drugs and drug application. Arginine vasopressin (AVP), bicuculline (BIC), AVP, and CNQX were purchased from Sigma (St. Louis, MO). TTX was obtained from Alomone Laboratories (Jerusalem, Israel). AVP and V1a receptor agonist [Arg8]-vasotocin (AVT) were obtained from Phoenix Pharmaceuticals ( Burlingame, CA). OXT was obtained from Bachem Bioscience (King of Prussia, PA). V1aR agonist [Phe]2, [Orn]8-vasotocin ([Phe]2-OVT) and OXTR agonist [Thr]4, [Gly]7-oxytocin (TGOT) were generous gifts of Dr. M. Manning. KB-R7943 and SN-6 were obtained from Tocris Bioscience (Ellisville, MO). The relative receptor selectivity for the peptides used in the study is shown in Table 1. SKF-96365 was obtained from Calbiochem (La Jolla, CA). All drugs were given by large-diameter (500 μm) flow pipette, directed at the recorded cell, unless otherwise noted. When a drug was not being administered, normal ACSF continuously flowed from the flow pipette. Drug solutions were prepared by diluting the appropriate stock solution with ACSF.

Data analysis. Data are expressed as means ± SE. Group statistical significance was assessed using a paired Student’s t-test for comparison of two groups, and one-way ANOVA followed by a Bonferroni post hoc test for three or more groups. P < 0.05 was considered statistically significant.

RESULTS

Vasopressin activates MCH neurons. Using transgenic MCH-GFP mice, we first studied the effects of AVP on MCH neurons with whole cell patch-clamp recording. Under current-clamp conditions, AVP excited MCH neurons. Application of AVP depolarized the membrane potential and increased the spike frequency in a concentration-dependent and reversible manner (Fig. 1, A–F). AVP (100 nM) increased firing frequency from 0.0 ± 0.0 Hz to 0.6 ± 0.1 Hz (P < 0.05; t-test; n = 7) (Fig. 1, B and E) and depolarized the membrane potential by 5.5 ± 0.9 mV (P < 0.05; t-test; n = 7) (Fig. 1F).

Using transgenic mice that express GFP in GABA neurons under the control of the GAD67 promoter, we also examined the effect of AVP on GABA neurons in the LH. In contrast to the excitatory effect of AVP on MCH neurons, the same concentration of AVP (100 nM) had no effect on GABA-GFP neurons (membrane potential changed by 0.3 ± 0.6 mV; P > 0.05; t-test; n = 6) (Fig. 1G). This suggests that the response

<table>
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<th>Agonist</th>
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<td>TGOT</td>
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AVP, V1α receptor agonist [Arg8]-vasotocin; OVT, [Orn]8-vasotocin; OXT, oxytocin; TGOT, [Thr]4,[Gly]7-oxytocin; V1αR, vasopressin receptor 1a; V2R, vasopressin 2 receptor; OXTR, oxytocin receptor. [Modified from Manning et al. (35)].
to AVP is selective for a subset of presumptive GABA cells in the LH that synthesize MCH.

**Mechanism of excitation.** To determine whether the excitatory actions of AVP on MCH neurons were accompanied by changes in the input resistance, we delivered a series of negative current steps from −40 pA to 0 pA (increments of 10 pA; duration: 200 ms) through the recording pipette in the presence of TTX and evaluated the changes in membrane potential before and after AVP application. Since the cells were depolarized by AVP, before the current steps were given, the membrane potential was shifted to the control baseline by injecting negative current. In the presence of AVP (100 nM), the hyperpolarizing shifts of the membrane potential in response to the injection of negative currents were reduced (Fig. 2A). A linear function was fitted to the current-voltage relationship, and the input resistance was calculated; AVP decreased the input resistance from 575.4 ± 64.9 mΩ to 425.0 ± 50.9 mΩ (P < 0.05; t-test; n = 11) (Fig. 2, B and C), suggesting AVP opens ion channels.

We next addressed the question as to which ion mechanisms were responsible for the AVP-mediated depolarization. Under voltage-clamp at a holding potential of −60 mV, AVP (1 μM) evoked a 73.0 ± 13.0 pA inward current in the presence of TTX (Fig. 2D). To determine the reversal potential of the current, slow voltage ramp protocols (from −100 to 0 mV for 3 s) were delivered to the MCH neurons. These experiments were done in the presence of TTX (1 μM), TEA (40 mM), and CdCl₂ (200 μM) in the bath and using Cs-based pipette solution to block the voltage-dependent Na⁺, K⁺, and Ca²⁺ currents that could be activated by these depolarizing protocols. AVP consistently evoked an inward current that showed a mean reversal potential of 2.4 ± 2.3 mV (n = 10); reversal potential range, −4.7 mV to 18.4 mV (Fig. 2E).

In normal ACSF with TTX (1.0 μM), AVP (1 μM) depolarized the membrane potential by 7.2 ± 1.9 mV (P < 0.05, t-test, n = 8). To determine the Na⁺ contribution to the depolarizing effect of AVP on MCH neurons, 80% of the NaCl was replaced by equimolar concentrations of Tris·HCl or choline chloride in the extracellular solution in the presence of TTX. When Na⁺ was replaced by Tris, the AVP-induced depolarization was reduced to 2.9 ± 0.4 mV (P < 0.05 vs. normal ACSF; t-test; n = 14) (Fig. 2, F and G). Similarly, replacement of Na⁺ with choline significantly reduced the depolarization of AVP (1.7 ± 0.2 mV; P < 0.05 vs. normal ACSF; n = 17; t-test) (Fig. 2G). These results suggest that the depolarization evoked by AVP was, to a large degree, dependent on extracellular Na⁺.

To test for the involvement of the Na⁺/Ca²⁺ exchanger, the effect of Ni³⁺, a nonselective blocker of the Na⁺/Ca²⁺ exchanger (18, 31), was used. NiCl₂ (3 mM) attenuated the depolarization of MCH neurons by 45.4 ± 5.6 mV (P < 0.05 vs. control; t-test). Fig. 1. Vasopressin excites melanin concentrating hormone (MCH) neurons. A–C: typical traces showing that application of AVP 10 nM (A), 100 nM (B), and 1 μM (C) increased firing and depolarized the membrane of MCH neurons. D: dose-dependent effect of AVP on the spike frequency of MCH neurons (*P < 0.05 vs. Control, t-test). E: bar graph showing the reversible effect of AVP (100 nM) on spike frequency in MCH neurons (*P < 0.05 vs. control; #P < 0.05 vs. AVP; t-test). F: dose-dependent depolarizing effect of AVP on MCH neurons (*P < 0.05 vs. control; t-test). G: AVP (100 nM) did not change the membrane potential and firing status of a glutamate decarboxylase 67-green fluorescent protein (GAD67-GFP) neuron. Error bars indicate means ± SE.

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AVP. With NiCl₂ in the bath, the depolarization induced by AVP was 2.5 ± 0.5 mV (P < 0.05 vs. AVP in normal ACSF; n = 14; t-test) (Fig. 2G).

Next, the fast-acting Ca²⁺ chelator BAPTA (10 mM) was added to the pipette solution to buffer internal Ca²⁺. Infusion of BAPTA into the cells significantly reduced the depolarization by AVP to 2.5 ± 0.4 mV (P < 0.05 vs. normal ACSF; n = 16; t-test) (Fig. 2G), which is consistent with the involvement of the Na⁺/Ca²⁺ exchanger. We next tested KB-R7943, a nonselective Na⁺/Ca²⁺ exchanger blocker (29), on AVP-induced depolarization. KB-R7943 (60 μM) significantly attenuated the depolarization of AVP to 2.8 ± 0.4 mV (P < 0.05 vs. normal ACSF; n = 16; t-test) (Fig. 2G). As KB-R7943 may also antagonize some nonselective cation channels, we also used a more selective Na⁺/Ca²⁺ exchanger blocker, SN-6 (39). The addition of SN-6 to the ACSF reduced the AVP-mediated depolarization to 4.0 ± 1.2 mV, a partial but statistically significant (n = 5; P < 0.05) reduction compared with the effect of AVP in the absence of SN-6.

The reduction in input resistance induced by AVP suggests an additional mechanism based on opening ion channels that may underlie the depolarization. Therefore, we tested SKF-96365, a blocker of nonselective cation (transient receptor potential) channels (23), on AVP-induced depolarization. SKF-96365 (30 μM) significantly attenuated the depolarization of AVP to 2.9 ± 0.4 mV (P < 0.05 vs. normal ACSF; n = 14; t-test) (Fig. 2G).

Finally, the effect of AVP on MCH cells was studied in nominal Ca²⁺-free ACSF. Under this condition, the AVP-mediated depolarization was 10.1 ± 1.8 mV (n = 14; vs. normal ACSF; P > 0.05; t-test) (Fig. 2G). The reduction in input resistance, the blockade of depolarization by SKF-96365, together with the modest increase in depolarization in the absence of extracellular Ca²⁺, all suggest a second mechanism of AVP excitation based on the opening of nonselective cation channels.

MCH neurons express AVP V1a receptors. To determine the possible receptor subtypes involved in the action of AVP, we
used two V1aR agonists, [Arg8]-vasotocin (AVT) and [Phe2]-OVT (35). In the presence of TTX (1 μM), the mean depolarization by AVT (1 μM) was 4.0 ± 0.2 mV (P < 0.05; n = 7; t-test) (Fig. 3E). AVT acts primarily via the V1a receptor, but it is not very selective and can also activate related receptors (28, 37). Therefore, we used a more selective V1a receptor agonist, [Phe2]-OVT (35). The mean depolarization by [Phe2]-OVT (1 μM) in the presence of TTX (1 μM) was 4.0 ± 0.6 mV (P < 0.05; n = 5; t-test) (Fig. 3E). When compared with the depolarization of 7.2 ± 0.7 mV induced by AVP (1 μM) (n = 8), no significant difference was noted between AVP, AVT, and [Phe2]-OVT (P > 0.05; n = 20; ANOVA).

To test the identity of the receptor expressed by MCH cells that responded to AVP, we performed single-cell RT-PCR studies in MCH-GFP cells. All six MCH-GFP neurons tested were positive for AVP V1a receptor mRNA (Fig. 5A), with a band at the expected size. In contrast, none of the four GABA neurons from the LH of the GAD67-GFP mouse tested positive for AVP receptor mRNA.

**Oxytocin response and oxytocin receptors.** We next tested the effect of OXT on MCH neurons. OXT (1 μM) increased the spike frequency from 0.5 ± 0.2 Hz to 1.6 ± 0.6 Hz, a 320% increase (P < 0.05; n = 4; t-test) (Fig. 3A). OXT had a concentration-dependent action in depolarizing the membrane potential (Fig. 4C). In the presence of TTX (1 μM), OXT (1 μM) depolarized the membrane potential by 4.7 ± 1.3 mV (P < 0.05; n = 5; t-test, Fig. 3, C and E). To determine whether the OXT response was due to activation of the OXTR or to activation of AVP receptors for which OXT has a low affinity, we used the highly selective OXTR agonist, TGOT, which has a 16,000-fold greater affinity for the OXTR than for AVP receptors (11, 27, 35, 50). In the presence of TTX (1 μM), TGOT (1 μM) evoked a depolarization of 4.2 ± 2.0 mV (P < 0.05; n = 6; t-test, Fig. 3, D and E), suggesting that OXT activates OXTR in MCH cells. We also tested the effect of OXT on neighboring GAD67-GFP neurons. No obvious effect of OXT was observed on GAD67-GFP neuron excitability (membrane potential changed by 0.4 ± 0.6 mV; P > 0.05; n = 7; t-test, Fig. 3F).

To test whether the Na+/Ca2+ exchanger is involved in OXT excitation of MCH neurons, we examined the effect of Ni2+ (3 mM), KB-R7943 (60 μM), and SKF-96365 (30 μM) on OXT-induced depolarization. In the presence of NiCl2 in the bath, the depolarization induced by OXT was significantly attenuated to 2.3 ± 0.8 mV (P < 0.05 vs. normal ACSF; n = 8; t-test) (Fig. 4B). Similarly, KB-R7943 attenuated the depolarization of OXT to 2.5 ± 0.9 mV (P < 0.05 vs. normal ACSF; n = 9; t-test) (Fig. 4B). The OXT-induced depolarization was 6.5 ± 1.3 mV in the presence of SKF-96365 (30 μM) (P > 0.05 vs. control; n = 8; t-test). Together, these data are consistent with the activation of a Na+/Ca2+ exchanger.

We then tested for the expression of OXTR mRNA. Ten of eleven MCH-GFP neurons were positive for OXTR mRNA (Fig. 5B), with a band at the expected size. In contrast, only one out of the six GABA neurons from the LH of the GAD67-GFP mouse tested positive for OXTR mRNA (Fig. 5B). All 27 of the cells tested for OXT or AVP receptor showed β-actin mRNA. These results are consistent with the view that AVP activated MCH neurons through the V1a receptor, and OXT acted through the OXTR.

**Vasopressin and oxytocin enhance synaptic transmission.** To investigate further the effect of AVP on synaptic transmission in MCH neurons, we first tested the effect of AVP on excitatory postsynaptic currents (EPSCs). In these experiments, BIC (30 μM) was added to the bath to block GABA-
A-mediated synaptic currents. AVP (1 μM) significantly increased the frequency of spontaneous EPSCs (sEPSCs) (to $135.9 \pm 3.5\%$ of control; $n=7$; $P<0.05$, t-test). The glutamate receptor antagonists CNQX (10 μM) and APV (50 μM) completely suppressed these synaptic currents, indicating they were mediated by glutamate release. Next, we investigated the effect of AVP on inhibitory postsynaptic currents (IPSCs) in the presence of the ionotropic glutamate receptor blockers APV (50 μM) and CNQX (10 μM). AVP (1 μM) significantly increased the frequency of sIPSCs (to $140.0 \pm 1.7\%$, $n=7$; $P<0.05$, t-test). The GABA_A receptor antagonist BIC completely suppressed these synaptic currents, indicating the IPSCs were generated by GABA release (Fig. 6, A and C). We next tested the effect of OXT on synaptic transmission in MCH neurons. OXT (1 μM) significantly increased sIPSC frequency to $158 \pm 28\%$ of control ($n=7$; $P<0.05$, t-test) in the presence of glutamate receptor antagonists. In contrast, we found no substantive effect of OXT on the frequency of sEPSCs ($105 \pm 8\%$ of control, $n=7$; $P>0.05$, t-test, Fig. 7, A and C) in the presence of the GABA-A receptor antagonist BIC.

**DISCUSSION**

In the present study, we found that both AVP and OXT excite MCH neurons, but not GAD-GFP neurons in the same area. In addition, both AVPR and OXTR mRNA were consistently expressed in MCH neurons but only rarely in GAD-GFP neurons. These results together suggest that MCH neurons in the LH may mediate or modulate some of the central actions of AVP and OXT.

AVP, OXT, and MCH neurons each can alter feeding, enhance stress, social interaction, and anxiety (16, 45), and may be involved in water-seeking during dehydration (38, 48), suggesting that some of the central actions of AVP or OXT...
may be mediated by MCH neurons. Whether MCH cells respond to AVP or OXT has not been addressed previously. AVP exerted robust direct and indirect excitatory actions on lateral hypothalamic MCH neurons. The direct excitation appeared to be based on two underlying mechanisms, activation of a Na+/Ca2+ exchanger and opening of a nonselective cation channel. MCH cells were also excited by OXT via the OXTR. In contrast to the robust excitatory action on MCH neurons, other inhibitory neurons of the lateral hypothalamus showed no response to AVP or OXT and displayed minimal expression of the V1aR or OXTR, underlining the selectivity of the effects in MCH cells.

Underlying mechanisms. AVP evoked direct excitatory effects on MCH neurons via multiple mechanisms: the AVP-induced depolarization was strongly depressed by replacement of extracellular Na+ with Tris or choline, consistent with the involvement of extracellular Na+. The reversal potential of the AVP-induced current is consistent with the activation of the Na+/Ca2+ exchanger (17, 31) or a nonselective cation current (34). The depolarization was greatly reduced by inclusion of the high-affinity Ca2+ chelator BAPTA in the pipette solution, additional evidence that supports these two mechanisms (19, 32). The heavy metal nickel, which has little effect on the Na+-dependent nonselective cation current, significantly reduced the depolarization by AVP, suggesting involvement of the Na+/Ca2+ exchanger. The depolarization was substantially blocked by the Na+/Ca2+ exchanger blocker KB-R7943 and by the more selective Na+/Ca2+ exchanger blocker SN-6, but not by TTX, also suggesting that part of the underlying mechanism was activation of the Na+/Ca2+ exchanger. That the Gq subclass of G protein-coupled receptors may activate the Na+/Ca2+ exchanger has been suggested in other hypothalamic

Fig. 6. Vasopressin enhances synaptic transmission in MCH cells. A: spontaneous excitatory postsynaptic currents [(s)EPSC] traces before, during the application of AVP (1 μM), and washout, showing AVP increases EPSC frequency. B: sIPSC traces before the application, during the application of AVP (1 μM), and washout. C: mean effects of AVP on the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs) (*P < 0.05 vs. control; n = 7; t-test). Error bars indicate means ± SE.

Fig. 7. Oxytocin enhances inhibitory synaptic transmission of MCH cells. A: representative traces showing sEPSCs before and during the application of OXT (1 μM), and washout. B: representative traces showing sIPSCs before, during the application of OXT (1 μM), and washout. C: mean effects of OXT on the frequency of sEPSCs and sIPSCs (*P < 0.05 vs. control; n = 7; t-test). Error bars indicate SE.
systems; hypocretin activation of GABA neurons in the arcuate nucleus and thyrotropin-releasing hormone excitation of histaminergic tuberomamillary neurons have been reported to be due to activation of a Na+/Ca2+ exchanger (9, 40). Reduced extracellular Ca2+ increased the amplitude of the response, and AVP reduced input resistance, both consistent with activation of nonselective cation channels. This was complemented by the reduced depolarization in the presence of the nonselective channel blocker SKF-96365, suggesting that activation of nonselective cation channels may constitute a second mechanism of AVP action on MCH cells.

The AVP receptor V1aR, which couples to the Gq subclass of G protein that increases phosphatidylinositol hydrolysis to mobilize intracellular calcium, has been identified in the hypothalamus (4). Two independent lines of evidence are consistent with the primary AVP receptor in MCH cells being V1aR. AVP, AVT, and the selective V1aR agonist [Phe2]-OVT all depolarized MCH neurons, suggesting activation of V1aR (6). Parallel single-cell RT-PCR experiments showed V1aR mRNA in all MCH cells tested, but not in nearby non-MCH GABA cells, further substantiating the physiological interpretation. MCH cells were also directly excited by OXT and the selective OXTR agonist TGOT, and single-cell RT-PCR confirmed OXTR expression. Similar to AVP responses, OXT responses appeared to be mediated by the Na+/Ca2+ exchanger. As almost all cells tested expressed AVP or OXT receptors, single MCH cells probably express both receptors. This contrasts to some other regions of the brain where different cells express AVP or OXT (27, 43).

In addition to the direct actions, an indirect synaptic action was also found. AVP increased the frequency of spontaneous EPSCs, suggesting AVP had excitatory actions on glutamate cells, leading to an increased release of glutamate onto MCH neurons. AVP and OXT also increased the frequency of IPSCs.

The excitatory action of AVP and OXT on MCH cells, but not on other GABA cells in the lateral hypothalamus, suggests MCH neurons are a selective target for AVP and OXT actions.

Functional significance. Dehydration increases AVP levels in plasma and cerebrospinal fluid (13, 30, 49). In the periphery, AVP increases water reuptake in the kidney in times of dehydration and increases locomotor activity (53), possibly related to a search for water. As MCH can enhance a positive water balance, some of the actions of AVP in restoring water balance may, therefore, be mediated by excitation of MCH neurons. MCH neurons play a key role in energy homeostasis (33, 52) and respond to nutritional signals, including fasting and leptin deficiency (42). AVP is involved in complex behavioral and cognitive functions, including pair-bond formation and social recognition (15, 25). Some of the anabolic effects of AVP and the shared actions in increasing stress and anxiety of both AVP and MCH may, in part, be due to an AVP-mediated excitation of the MCH neurons. On the other hand, it is unlikely that MCH cells would directly excite AVP cells, as both MCH and colocalized GABA would inhibit the AVP cells.

Another neuron in the LH area that also responds to AVP is the hypocretin neuron. Hypocretin is an excitatory neuromodulator and may colocalize with glutamate (44). Similar to MCH neurons, hypocretin neurons are excited by AVP through a mechanism involving activation of nonselective cation channels (53). It is interesting that both MCH and hypocretin neurons are excited by AVP, whereas other (GABA) cells tested in the same area do not respond to the peptide. This suggests that AVP is not involved in general excitation or setting a general tone in the lateral hypothalamus, but rather selectively activates the MCH and hypocretin cells, both with long projection axons that terminate throughout the brain and spinal cord (7, 41, 54). Because glutamatergic hypocretin cells innervate and excite MCH neurons (22, 55) and since AVP also activates the hypocretin cell (53), it is possible that at least part of the increase in excitatory synaptic activity in MCH cells that we find here may be due to AVP activation of the hypocretin cell. The hypocretin cells also play a key role in the vasopressin-mediated increase in locomotion (53).

That AVP also activates the hypocretin system is consistent with a possible increase in attention or arousal related to potentially anxiogenic stimuli. Another possibility that cannot be discounted is that AVP may be released from axons of other brain regions (37) or from the hypothalamic suprachiasmatic nucleus (40) that orchestrates circadian rhythms of behavior and may modulate MCH neuron output dependent on circadian time.

MCH neurons innervate preoptic/septal neurons that synthesize gonadotropin-releasing hormone (GnRH), and MCH exerts a profound inhibitory effect on these neurons (57). During lactation, the reproductive potential of nursing mothers is attenuated. One can speculate that part of the mechanism underlying this action may be the nursing-induced release of OXT, leading to excitation of MCH neurons as described here; increased MCH release would secondarily reduce GnRH, and potentially reduce reproductive potential.

Perspectives and Significance

Here, we show that both AVP and OXT directly excite hypothalamic MCH neurons but not other LH GABAergic neurons. Thus, the MCH neuron may play a role in mediating or modulating some of the multiple central actions of AVP and OXT. These results suggest that the response of presumptive GABAergic MCH neurons reflects a very selective AVP and OXT action on a single type of LH inhibitory neuron and that AVP and OXT are not involved in a general enhancement of LH tone.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: Y.Y., L.-Y.F., and X.Z. performed experiments; Y.Y., L.-Y.F., and X.Z. prepared figures; Y.Y. interpreted results of experiments; Y.Y., L.-Y.F., and X.Z. drafted manuscript; Y.Y. and A.v.d.P. drafted manuscript; A.v.d.P. conceived and designed research; A.v.d.P. approved final version of manuscript.


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