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Presynaptic actions of endocannabinoids mediate α-MSH-induced inhibition of oxytocin cells

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Sabatier, Nancy, and Gareth Leng. Presynaptic actions of endocannabinoids mediate α-MSH-induced inhibition of oxytocin cells. Am J Physiol Regul Integr Comp Physiol 290: R577–R584, 2006. First published November 3, 2005; doi:10.1152/ajpregu.00667.2005.—We recently showed that central injections of α-melanocyte-stimulating hormone (α-MSH) inhibits oxytocin cells and reduces peripheral release of oxytocin, but induces oxytocin release from dendrites. Dendritic oxytocin release can be triggered by agents that mobilize intracellular calcium. Oxytocin, like α-MSH, mobilizes intracellular calcium stores in oxytocin cells and triggers presynaptic inhibition of afferent inputs that is mediated by cannabinoids. We hypothesized that this mechanism might underlie the inhibitory effects of α-MSH. To test this, we recorded extracellularly from identified oxytocin and vasopressin cells in the anesthetized rat supraoptic nucleus (SON). Retrodialysis of a CB1 cannabinoid receptor antagonist to the SON blocked the inhibitory effects of intracerebroventricular injections of α-MSH on the spontaneous activity of oxytocin cells. We then monitored synaptically mediated responses of SON cells to stimulation of the organum vasculosum of the lamina terminalis (OVLT); this evoked a mixed response comprising an inhibitory component mediated by GABA and an excitatory component mediated by glutamate, as identified by the effects of bicuculline and 6-cyano-7-nitroquinoxaline-2,3-dione applied to the SON by retrodialysis. Application of CB1 receptor agonists to the SON attenuated the excitatory effects of OVLT stimulation in both oxytocin and vasopressin cells, whereas α-MSH attenuated the responses of oxytocin cells only. Thus α-MSH can act as a “switch”; it triggers oxytocin release centrally, but at the same time through initiating endocannabinoid production in oxytocin cells inhibits their electrical activity and hence, peripheral secretion.

The supraoptic nucleus (SON) contains magnocellular neurons that project to the posterior pituitary where they secrete oxytocin and vasopressin into the circulation. Vasopressin and oxytocin are also released centrally and are involved in several behaviors (47). Central release originates, in part, from parvo-cellar neurons of the paraventricular nucleus (PVN), but both peptides are also released from the dendrites of magnocellular neurons of the PVN and SON (20). Dendritically released oxytocin has autoregulatory actions on SON neurons; notably, these include facilitating the synchronized bursting that accompanies reflex milk ejection in lactating rats (26).

Dendritic oxytocin release can be triggered by agents that mobilize calcium from intracellular stores, including oxytocin itself (16) and can occur without an increase in electrical activity and without oxytocin secretion from nerve terminals (23). Thus, in some physiological conditions, including suckling, osmotic challenge, and, in response to some specific stressors (20), dendritic release occurs independently of secretion from the nerve terminals.

We recently studied the effect of α-melanocyte-stimulating hormone (α-MSH), a peptide produced by neurons in the arcuate nucleus (43), on SON oxytocin neurons. Centrally injected α-MSH and oxytocin have similar actions on many behaviors in the rat, and it seems possible that centrally released oxytocin mediates some of the behavioral effects of centrally administered α-MSH. The melanocortin 4 (MC4) receptor mRNA is expressed abundantly in the SON and PVN (28), and MC-containing neurons of the arcuate nucleus project directly to the SON (35).

We previously showed that α-MSH differentially regulates dendritic and systemic release of oxytocin (34). Central injections of α-MSH or MC4 agonists decrease the electrical activity of oxytocin cells and, consequently, reduce peripheral oxytocin secretion. However, α-MSH induces oxytocin release from dendrites in isolated SON, probably as a consequence of its stimulating effect on intracellular calcium stores. In the present study, we investigated the mechanisms by which α-MSH inhibits oxytocin cells. Oxytocin, like α-MSH, mobilizes intracellular calcium stores in oxytocin cells (16) and triggers presynaptic inhibition of afferent inputs that is mediated by cannabinoids (9). We therefore hypothesized that the same mechanism might underlie the inhibitory effects of α-MSH. To test this, we recorded from oxytocin cells in the SON in vivo, and showed that cannabinoid antagonists applied to the SON by retrodialysis could block the response of oxytocin cells to intracerebroventricular injection of α-MSH. We then went on to compare the effects of cannabinoids and α-MSH applied to the SON on the responses of SON neurons to electrical stimulation of an afferent pathway. The pathway chosen for these studies was the projection from the organum vasculosum of the lamina terminalis (OVLT). The OVLT...
projects to the SON both directly and indirectly via the nucleus medianus (18, 24); this projection has well been characterized in previous studies, and stimulation of the OVLT evokes a mixed response that has an inhibitory component mediated by GABA and an excitatory component mediated by glutamate (4).

MATERIALS AND METHODS

Electrophysiology. Experiments were performed on adult female Sprague-Dawley rats anesthetized with urethane (1.25 g/kg ip). A femoral vein and trachea were cannulated, and for intracerebroventricular injections, an injection cannula was implanted into the lateral ventricle (coordinates from bregma: 0.6 mm posterior; 1.6 mm lateral; 4 mm depth). The pituitary stalk, the left SON, and the OVLT were exposed transpharyngeally, and a microdialysis probe loop was placed onto the ventral surface of the SON. The extracellular activity of single neurons was recorded by a glass micropipette placed through the center of the probe loop. A bipolar side-by-side stimulating electrode (model SNEX-200; Clarke Electromedical) was placed on the pituitary stalk to deliver matched biphasic pulses (1 ms, <1 mA) to antidromically identify SON neurons. A bipolar concentric stimulating electrode (model SNEX-100, Clarke Electromedical) was placed in the OVLT to deliver single shocks (1 Hz, matched biphasic 1-ms pulses, 0.1–1 mA peak to peak) or trains of shocks (20 Hz for 200 ms every 5 s, 0.1–1 mA). Oxytocin cells were distinguished from vasopressin cells by their firing pattern and by their opposite response to intraventricular injection of [20 µg/kg, cholecystokinin-(26–33)-sulfated; Bachem; Refs. 31 and 32]. Artificial cerebrospinal fluid (aCSF; pH 7.2; composition in mM: 138 NaCl, 3.36 KCl, 9.52 NaHCO3, 0.49 NaHPO4, 2.16 urate, 0.49 NaH2PO4, 1.26 CaCl2, 1.18 MgCl2) was dialyzed at 3 µl/min, and changed to aCSF containing CNQX, bicuculline, WIN 55,212–2 mesylate (ACPA) (in Tocrisolve 100), AM 251 (all Tocris Cookson, Avonmouth, UK), or α-MSH (Calbiochem-Novabiochem, Nottingham, UK).

Data analysis. Responses evoked by OVLT stimulation were initially analyzed using Spike2 software (CED, Cambridge, UK). Stimulation-evoked spike activity was visualized with raster displays and peristimulus time histograms (PSTH) were constructed to quantify the responses. For each cell, the responses to trains were analyzed by constructing PSTHs for 1,000 s (200 trains, 250 bins of 20 ms) in control conditions and during and after drug application by retrodialysis to the SON. The PSTHs were first normalized to the ongoing spontaneous firing rate, measured as the average rate in the 2.5–s periods before each stimulus train. For PSTHs in control conditions, the stimulus-evoked response profile was then identified. Peristimulus inhibition was recognized when five or more consecutive bins each contained fewer spikes than expected from the prestimulation control, and peristimulus excitation when five or more consecutive bins contained more than the expected number of spikes. The response amplitude was defined as the number of additional spikes per stimulus train during the identified periods. To assess changes in responses, the same poststimulus periods were used to quantify response amplitudes as those identified in control PSTHs. For the experiment with intracerebroventricular injection of α-MSH, firing rate in 30-s bins in the 5 min before injection was compared with the firing rate between 10 and 15 min after injection, 15 min before injection. In all cases, the duration of the experimental procedures meant that only one or two cells could be fully tested in any one experiment.

RESULTS

Effect of cannabinoid antagonist on MSH-induced inhibition of oxytocin cells. Although intracerebroventricular injection of α-MSH inhibits oxytocin cells, α-MSH induces dendritic re-

lease of oxytocin (34). In vitro, oxytocin presynaptically inhibits excitatory inputs via release of endocannabinoids (9), so we investigated whether α-MSH inhibits oxytocin cells by a similar mechanism. The effect of α-MSH (300 ng icv) was compared before and during retrodialysis of the SON with the CB1 antagonist AM251 (10–1,000 µM). The response to α-MSH was measured as the difference in firing rates in the 5 min before and 10–15 min after α-MSH injection. In control conditions, α-MSH produced a mean change of −0.7 ± 0.2 spikes/s from an initial rate of 3.6 ± 0.3 spikes/s (P < 0.01). After 30- to 45-min retrodialysis with AM251, the inhibitory response to α-MSH was absent in six of seven cells (Fig. 1), including in all three during dialysis with 1 mM AM251, in two of three during 100 µM AM251, and in one during 10 µM AM251. For these seven cells, the response to α-MSH in the presence of AM251 was an increase of 0.4 ± 0.2 spikes/s from an initial rate of 3.3 ± 0.3 spikes/s (P < 0.01 vs. initial response).

We also analyzed the effect of AM251 on the background activity of 10 oxytocin cells. After 30 to 55 min of retrodialysis with AM251, the mean background firing rate increased by 0.4 ± 0.15 spikes/s from an initial rate of 4 ± 0.6 spikes/s (P < 0.05 Student’s paired t-test).

These experiments indicated that α-MSH-induced inhibition of oxytocin cells is mediated by endogenous cannabinoids. At many sites in the CNS, including in the SON, cannabinoids are known to act presynaptically. We therefore went on to compare the effects of α-MSH and cannabinoids on the responses of SON neurons to activation of an afferent pathway, the OVLT.

Effects of OVLT stimulation on oxytocin and vasopressin cells. Typically, SON neurons responded to single-shock stimulation of the OVLT with a brief inhibition followed by a more
prolonged excitation, as reported by others (46). Short-latency inhibitions (mean onset 15 ± 3 ms) were apparent in all five oxytocin cells tested and in four of six vasopressin cells. Two cells showed short-latency excitation only. In subsequent experiments, we applied brief trains of stimuli to the OVLT (20 Hz for 200 ms every 5 s). In these conditions, the excitatory effects summed, giving clearer overall excitation. We used raster displays to visualize the responses and PSTHs to quantify them.

In response to these trains (Fig. 2), 27 cells were excited, with an onset latency of 61 ± 9 ms and duration of 468 ± 28 ms. Two cells were inhibited only (not shown), and 13 were inhibited (latency 12 ± 9 ms, duration 183 ± 16 ms) and then excited (latency 230 ± 26 and 397 ± 40 ms). The mean onset latency, duration, time to peak response, and peak response amplitude of vasopressin cells were very similar to those of oxytocin cells (Fig. 3).

In cells that were excited only, the mean response for the 27 cells (16 vasopressin and 11 oxytocin cells) was an additional 1.8 ± 0.2 spikes/train, and the responses were maximal at 220 ± 9 ms (Fig. 3A). Among the 13 cells (4 vasopressin and 9 oxytocin cells) that were inhibited and then excited, the mean inhibition was −0.6 ± 0.09 spikes/train, maximal at 97 ± 17 ms, and the mean excitation was 1.4 ± 0.35 spikes/train, maximal at 358 ± 43 ms (Fig. 3B). In the two cells that were inhibited only (both vasopressin cells), the mean response was −0.6 ± 0.2 spikes/train, maximal at 170 ± 42 ms (not shown). Thus inhibitory responses were slightly more prominent among oxytocin cells than among vasopressin cells (9/20 vs. 6/22 cells). Otherwise, responses to OVLT stimulation were very similar between oxytocin cells and vasopressin cells.

To test whether the responses to OVLT stimulation were stable over time, we monitored responses during >90 min of microdialysis with aCSF (Fig. 4). For all seven cells tested (vasopressin cells and oxytocin cells), the response to stimulation remained apparently unchanged; initially, the mean excitatory response of these cells was an additional 2.8 ± 0.4 spikes/train, and after 90 min, the response was an additional 2.9 ± 0.4 spikes/train (Fig. 4C; mean change 0.05 ± 0.1 spikes/train, no significant difference).

Effects of glutamate and GABA<sub>A</sub> antagonists on responses to OVLT stimulation. To characterize the neurochemical nature of the responses to OVLT stimulation, we applied glutamate and GABA<sub>A</sub> receptor antagonists to the SON by microdialysis (retrodialysis). The concentrations quoted for all drugs are

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Fig. 2. Effects of organum vasculosum of the lamina terminalis (OVLT) stimulation on electrical activity of supraoptic nucleus (SON) neurons. Aa and Bc: firing rate of a vasopressin cell (Aa) and an oxytocin cell (Ba) during OVLT stimulation [200 trains of stimulation (1,000 s) at 20 Hz]. Ab and Bb: rasters of the responses of the cells shown in Aa and Ba; illustrating excitation (Ab) and inhibition followed by excitation (Bb); the onset of stimulus trains is indicated by the vertical line at t = 0. Ac and Bc: peristimulus time histograms (PSTH) averaging the responses to the 200 trains of stimulation in the neurons shown in Aa and Ba, illustrating excitation and inhibition followed by excitation. The period of train stimulation is indicated by the shaded bar.

Fig. 3. Characteristics of the responses of SON neurons to OVLT stimulation. Aa: mean response (± SE) to OVLT stimulation of SON neurons that displayed excitation (Excit) only (n = 27). Ab: mean amplitude (top, left), onset latency (top, right), duration (bottom, left), and peak time (bottom, right) of the responses of 16 vasopressin (VP) and 11 oxytocin (OT; closed and open bars, respectively) cells included in Aa. Bc: mean response (± SE) to OVLT stimulation of SON neurons that were inhibited then excited (n = 13). Bb: mean amplitude (top, left), onset latency (top, right), duration (bottom, left), and peak time (bottom, right) of the responses of 4 vasopressin and 9 oxytocin cells. Inhib, inhibition.

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Effects of glutamate and GABA<sub>A</sub> antagonists on responses to OVLT stimulation. To characterize the neurochemical nature of the responses to OVLT stimulation, we applied glutamate and GABA<sub>A</sub> receptor antagonists to the SON by microdialysis (retrodialysis). The concentrations quoted for all drugs are
those in the microdialysate. Resulting tissue concentrations in the SON are three to four orders of magnitude lower, as estimated previously (21). The glutamate antagonist 6-cyano-7-nitroquinazoline-2,3-dione (CNQX, 1 mM) was retrodialyzed for 15–25 min during recording from four cells excited by OVLT stimulation. Within 10 min, both the background electrical activity and the excitatory response to OVLT stimulation were abolished in all four cells (Fig. 5A); both background activity and excitatory response recovered within 30 min of returning to aCSF.

The GABA_A antagonist bicuculline (2 mM) was applied while recording from seven cells that, in response to OVLT stimulation, were either inhibited only or were inhibited and then excited. After 10–25 min, the inhibition was abolished in five of the seven cells (Fig. 5B) and markedly attenuated in the other two; the mean response reversed from $-0.33 \pm 0.085$ spikes/train to $+0.34 \pm 0.26$ spikes/train ($n = 7$; mean change $0.67 \pm 0.25$ spikes/train), apparently unmasking coincident excitation. Despite this dramatic effect on stimulation-evoked inhibition, bicuculline had little sustained effect on background activity (mean change after 20 min: $0.3 \pm 0.02$ spikes/s, from $3.4 \pm 1.1$ to $3.7 \pm 1.1$ spikes/s, $n = 7$), although an earlier transient excitation was generally observed. Recovery of responses after changing back to dialysis with aCSF was slow, (40 min or more), consistent with previous studies.

These results are consistent with the conclusion that the excitatory and inhibitory responses reflect glutamate and GABA inputs acting mainly at AMPA/kainate receptors and GABA_A receptors, respectively, in agreement with previous studies (4).

Effects of cannabinoids on background firing rate. The effects of the CB1 agonist WIN 55,212–2 on background firing rate were tested on 10 vasopressin cells (3 continuously firing, 7 phasic) and 8 oxytocin cells. The firing rate 5 min before retrodialysis with WIN 55,212 (100 µM for 35–45 min) was compared with the rate in the last 1,000 s of retrodialysis. WIN 55,212 produced a small (not significant) reduction in the firing rate of oxytocin cells (mean change, $-0.45 \pm 0.25$ spikes/s) and a small (not significant) increase in vasopressin cells (mean change, $0.3 \pm 0.47$ spikes/s) (Fig. 6A).

Effects of cannabinoid agonists on excitatory response of oxytocin and vasopressin cells. We then investigated whether cannabinoid agonists modulate the afferent inputs to the SON. The CB1 agonists WIN 55,212–2 (250 µM, 12 cells) and ACPA (1 mM, 5 cells) were applied to the SON by retrodialysis for 30–60 min during recordings of 12 vasopressin cells and five oxytocin cells that were excited by OVLT stimulation. Both agonists had similar effects on vasopressin and oxytocin cells, so results from the two types of neurons and the two agonists were pooled.

In 13 of 17 cells, the excitatory response to stimulation was reduced by >20% in the presence of a CB1 agonist; four cells were not affected. Overall, the mean excitatory response was reduced by $0.38 \pm 0.09$ spikes/train ($28 \pm 5\%$, $n = 17$; $P < 0.05$).

Fig. 4. Stability of the response to OVLT stimulation in SON neurons. A: example of a vasopressin cell recorded for 105 min during OVLT stimulation. B: PSTHs constructed at the beginning of the recording (left) and 90 min later (right). C: mean amplitude (±SE) of excitation in response to OVLT stimulation at $t = 0$ and $t = 90$ min ($n = 7$). Note that the response is very stable over time.

Fig. 5. Effect of glutamate and GABA_A antagonists on responses of SON neurons to OVLT stimulation. A: left: vasopressin cell recorded during retrodialysis with the glutamate antagonist CNQX (1 mM). The background firing was abolished shortly after starting the antagonist application and recovered after return to artificial cerebrospinal fluid (aCSF). A: right: raster display of the same recording showing the loss of the excitatory response to OVLT stimulation during retrodialysis with CNQX. B: firing rate of a vasopressin cell during retrodialysis with 2 mM bicuculline, showing little effect on background firing rate (top); raster display of the same recording showing the inhibition evoked by OVLT stimulation is abolished by bicuculline (middle); PSTHs constructed before and during bicuculline application showing total loss of the inhibitory response in the presence of bicuculline (bottom).
was reduced by 29% in this cell (mean 24% for all oxytocin cells). During retrodialysis with WIN 55,212, the excitatory response to stimulation partially recovered within 20 min.

Fig. 6. Effect of CB1 agonists on background firing rate of SON neurons and on their excitatory responses to OVLT stimulation. A: mean firing rate of 10 vasopressin and 8 oxytocin cells before and during retrodialysis with CB1 agonists WIN 55,212 and ACPA (250 μM-1 mM). The firing rates of neither vasopressin nor oxytocin cells were significantly affected. B: firing rate of an oxytocin cell during retrodialysis with CB1 agonist WIN 55,212 (1 mM) and PSTHs comparing the excitatory responses to OVLT stimulation before and during retrodialysis with WIN 55,212. The excitatory response to stimulation was reduced by 29% in this cell (mean 28% for all cells).

0.001, paired Student’s t-test, Fig. 6B). The 17 cells include three that initially were inhibited and then excited in response to OVLT stimulation. For these three cells, we tested the CB1 agonist in the presence of bicuculline. In this condition, the inhibitory component of the response was abolished, so we could ensure that the reduction of excitation by CB1 agonist was not the result of an increase in inhibition (Fig. 7). In these three cells, the response in the presence of bicuculline was 1.2 ± 0.7 spikes/train, and in the additional presence of CB1 agonist, this was reduced by 0.4 ± 0.08 spikes/train.

Effects of α-MSH on the excitatory response of oxytocin cells. We demonstrated (see Fig. 1) that α-MSH-induced inhibition of oxytocin cells is prevented by blocking endogenous cannabinoid actions, indicating that the inhibitory effect of α-MSH might be mediated by endocannabinoids. If so, the effects of α-MSH on the responses of oxytocin cells to OVLT stimulation should be similar to those of CB1 agonists. To test this, α-MSH (500 μM) was retrodialyzed onto the SON for 30–60 min. In the seven oxytocin cells tested, α-MSH attenuated the excitatory response to OVLT stimulation by 24 ± 3% (Fig. 8; initial response, 1.0 ± 0.3 spikes/train; during α-MSH, 0.7 ± 0.2 spikes/train; mean change −0.3 ± 0.01 spikes/train; P < 0.05). By contrast, α-MSH had no significant effect on the responses of four vasopressin cells tested (initial response 1.9 ± 0.3 spikes/train, during α-MSH, 2.2 ± 0.5 spikes/train; mean change 0.3 ± 0.3 spikes/s, significantly different from oxytocin cells P < 0.05).

DISCUSSION

OVLT stimulation evoked mixed excitatory and inhibitory responses in SON neurons that were sensitive to the AMPA/kainate receptor antagonist CNQX and the GABA_A receptor antagonist bicuculline, respectively. The excitatory responses of both oxytocin and vasopressin cells were attenuated by direct application of CB1 agonists to the SON, whereas direct application of α-MSH attenuated the responses of oxytocin cells only. Moreover, the inhibitory effects of intracerebroventricular α-MSH on the spontaneous activity of oxytocin cells were blocked by application of a CB1 antagonist to the SON. Together, these results suggest that α-MSH inhibits oxytocin cells by inducing the production of cannabinoids that presynaptically attenuate excitatory inputs to the SON.
The OVLT, adjacent to the anteroventral wall of the third ventricle, is prominently involved in body fluid and electrolyte homeostasis, and it projects to the SON both directly, and indirectly via the nucleus medianus (24). Consistent with previous reports (10, 17, 46), OVLT stimulation evoked two main types of responses in SON neurons: 1) excitation or 2) inhibition followed by excitation. The stimulation used here (trains at 20 Hz for 200 ms every 5 s) elicited moderate responses that were stable for long periods in control conditions. Both the background firing of SON neurons and excitatory responses to OVLT stimulation were abolished by the AMPA/kainate antagonist CNQX, and the inhibitory responses were abolished by the GABA_A antagonist bicuculline. This is consistent with in vitro electrophysiological experiments in hypothalamic explants, showing that OVLT stimulation evoked inhibitory postsynaptic potentials in SON neurons that were sensitive to bicuculline and excitatory postsynaptic potentials (EPSPs) that were mainly sensitive to CNQX. The residual component of EPSPs was blocked by the N-methyl-D-aspartate (NMDA) antagonist 2-amino-5-phosphonovalerate (APV, also APS) APV (46). However, recent recordings in hypothalamic explants showed that GABA_B receptors also modulate both excitatory and inhibitory postsynaptic currents evoked by OVLT stimulation in SON neurons (13). In the present experiments, although bicuculline blocked the inhibitory response of SON neurons to OVLT stimulation, it had little effect on background firing rate. This is surprising, because about half of the afferent input to SON neurons is thought to be mediated by GABA (39), but we have reported this relative lack of effect in previous experiments (22). It seems that SON neurons compensate for a progressive diminution of GABA input with an increase in intrinsic excitability. Similar perversely stable of spontaneous activity has been described in other circumstances (e.g., 6), possibly indicating that spontaneous firing rate is strongly buffered by multiple negative feedback mechanisms, including feedbacks mediated by nitric oxide (38), adenosine (29, 30), dynorphin (5), vasopressin (21, 33), oxytocin (14), and endocannabinoids (9), as well as intrinsic activity-dependent hyperpolarization (12).

In the present study, CB1 agonists inhibited the excitatory response to OVLT stimulation by 20–30% in both oxytocin cells and vasopressin cells, indicating that inputs to both cell types are sensitive to presynaptic inhibition by cannabinoids. The concentration of CB1 agonist used in these experiments was chosen to produce an estimated extracellular concentration of 0.25–1 μM, similar to the dose shown to be effective in the experiments of Hirasawa et al. (9). CB1 receptors have been localized by immunohistochemistry to both excitatory and inhibitory axon terminals innervating dendrites in the SON (9), and the cannabinoid agonist presynaptically inhibits spontaneous EPSCs and IPSCs in SON neurons recorded in slices (37). As with bicuculline, CB1 agonists had surprisingly little effect on spontaneous firing rate, but oxytocin cells were activated in the presence of a cannabinoid antagonist, indicating tonic activity of endogenous cannabinoids. Again, we suspect that agonist-induced changes in excitability are buffered by negative feedback, whereas interfering with these mechanisms has clearer consequences. Presynaptic actions of cannabinoids in the SON have been reported recently in vitro studies; the present study is the first to characterize actions on identified oxytocin and vasopressin cells, the first to show endogenous activity in vivo, and the first to identify actions on a characterized afferent projection. However, many studies on different neurons have shown that presynaptic inhibitory effects of cannabinoids are very widespread in the CNS (e.g., 15, 44).

α-MSH had an effect similar to CB1 agonists, as it reduced by 24% the excitatory response to OVLT stimulation. However, unlike CB1 agonists, its effects were selective for oxytocin cells; responses of vasopressin cells were unaffected. Recently, we showed that intracerebroventricular injection of α-MSH inhibits oxytocin cells but not vasopressin cells in vivo (34). In the present study, this α-MSH-induced inhibition was prevented by application of CB1 antagonist AM251 onto the SON. The simplest explanation is that α-MSH inhibits oxytocin cells by depressing glutamate afferents through the release of endocannabinoids from oxytocin cells. The production of endocannabinoids is regulated by calcium influx through voltage-dependent channels (19) and generally depends on elevation of intracellular Ca^{2+} concentration ([Ca^{2+}]_i) (8, 42). Like oxytocin (16), α-MSH triggers an increase in [Ca^{2+}]_i in oxytocin cells (34), which could lead to production of endocannabinoids.

In SON neurons, dendritic peptide release can either precede (26) or succeed systemic release (20), or it can occur without systemic release, such as during forced-swimming (45). We showed recently that α-MSH can also differentially regulate dendritic and systemic release of oxytocin as α-MSH inhibits the electrical activity of oxytocin cells in vivo and, as a consequence, systemic release of oxytocin, but induces dendritic release of oxytocin (34). Oxytocin and α-MSH have similar effects on several behaviors in rats; in particular, both suppress feeding (2, 41), and both stimulate female and male sexual behavior (1). There are many centrally projecting oxytocin neurons in the PVN, and these have generally been assumed to govern behavioral effects of oxytocin. However, although these neurons synthesize oxytocin, this is unlikely to be their major neurotransmitter product; most centrally projecting peptidergic neurons also synthesize a conventional neurotransmitter, which appears to be their main synaptic output. Indeed, large dense-cored vesicles that contain peptides are quite rare in synapses, but they can be released from all parts of neurons. Morris and Pow (27) estimated that half of the peptide release in the CNS is from extrasynaptic sites. The centrally projecting oxytocin neurons project mainly outside the hypothalamus, notably to the brainstem and spinal cord, where there are dense terminal projections. Within the hypothalamus itself and in adjacent structures, the innervation is more sparse, and mismatches have been noted between the distribution of oxytocin receptors and oxytocin innervation.

Large quantities of oxytocin are released from the dendrites of magnocellular neurons. The concentration in the extracellular fluid of the SON is 10–100 times higher than in the systemic circulation (20), and the basal release rate of oxytocin from the isolated SON is 1 pg oxytocin/min per SON (26). Oxytocin receptors expressed within the brain appear identical to these expressed peripherally, with affinities for oxytocin in the nanomolar range (3). Because the half-life of oxytocin in the brain is estimated to be 19 min (25), it is difficult to see how the magnocellular neuronal dendrites cannot be a major contributor to modulation of central oxytocin receptors, assuming no barrier to diffusion of oxytocin within the brain. Thus...
oxytocin released from SON dendrites is likely to be involved in the regulation of behaviors in rats.

It appears that α-MSH acts as a switch to turn off systemic release of oxytocin and switch on dendritic release, depending on physiological demands. Centrally, both oxytocin and α-MSH inhibit feeding and stimulate sexual behaviors, but peripheral secretion of oxytocin stimulates natriuresis in rats (40) and this appropriately accompanies food intake. Without food intake, natriuresis is inappropriate, hence sexual arousal accompanying inhibition of appetite may require stimulation of central oxytocin release and concurrent inhibition of peripheral release. Nevertheless, oxytocin is released into the circulation at orgasm (11). This release is pulsatile, a pattern ineffective for natriuresis but potently effective for smooth muscle contraction (36). Analogous pulsatile release during reflex milk ejection in lactating rats requires relative quiescence in the background activity of oxytocin cells (7), so another possibility is that the suppression of background activity by α-MSH predisposes oxytocin cells for burst firing during ejaculation.

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