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Autofeedback effects of progressively rising oxytocin concentrations on supraoptic oxytocin neuronal activity in slices from lactating rats

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Wang, Yu-Feng, Todd A. Ponzio, and Glenn I. Hatton. Autofeedback effects of progressively rising oxytocin concentrations on supraoptic oxytocin neuronal activity in slices from lactating rats. Am J Physiol Regul Integr Comp Physiol 290: R1191–R1198, 2006. First published December 1, 2005; doi:10.1152/ajpregu.00725.2005.—Suckling stimuli induce somatodendritic oxytocin (OT) release from supraoptic nucleus (SON) neurons, which raises intranuclear OT concentrations and contributes to the effectiveness of the milk-ejection reflex. To clarify how such changes in OT concentrations modulate the activity of OT neurons, we examined OT effects using whole cell patch-clamp recordings from SON neurons in slices from lactating rats. Progressive increases from extremely low OT concentrations (0.1–10 fM) to high concentrations (0.1–10 nM) induced excitation and subsequent spike frequency reduction (SFR) in OT neurons. Significant effects of OT on firing rates were observed starting at 1 fM, reached peak level from 1 nM to 1 pM before SFR occurred in most neurons. The buildup of OT concentrations progressively promoted depolarization of membrane potential, spike broadening, decreases in spike amplitude, and increases in the rise time of spike afterhyperpolarizations, which were unrelated to firing rate. However, intermittent application of OT (1 fM, 1 pM, and 1 nM, each for 5 min) evoked dose-dependent excitation but not the SFR. Application of 1 pM OT for 40 min simulated the effects of progressively increasing OT concentrations. Vasopressin neurons were also activated by OT but did not show SFR. Consistent with presynaptic loci of OT action, ionotropic glutamate receptor antagonists reduced OT effects on firing rate, whereas bicuculline did not change the excitatory effects. These results suggest that the specific autoregulatory effects of OT, and perhaps other neuropeptides as well, are time and concentration dependent.

integration; lactation; neuropeptide; spike frequency reduction; synaptic transmission

THE MAGNOCELLULAR HYPOTHALAMO-NEUROHYPOPHYSEAL system, of which oxytocin (OT) secretion is an integral functional part, serves as a model for neurosecretion. During suckling, extracellular OT concentrations in the maternal supraoptic nucleus (SON) increase significantly (20, 21). This facilitates activation of OT neurons (27). Intracerebroventricular injection of OT also facilitates the activation of OT neurons, while injection of OT antagonists inhibits ongoing bursts of OT neurons (10, 20). Myriad in vitro studies revealed that OT induced its own release (19), resulting in excitation of OT neurons (4, 28). Moreover, OT reduces the amplitude of inhibitory postsynaptic currents (IPSCs) (3). In contrast to this evidence supporting its facilitatory effects, OT has also been found to reduce the amplitude of evoked postsynaptic currents (EPSCs) at presynaptic terminals on SON neurons (14), which possibly reduces excitatory drive. These results raise questions about the integrative actions of OT. As previous studies are mostly based on brief applications of single doses of OT, they did not address questions of the effects of gradual increases in OT concentrations, as occur in vivo, over longer periods of suckling. Because these increasing levels of OT may cause secondary and hitherto unknown responses in OT neurons, it was important to determine the effects elicited by slow, progressive increases in OT concentration.

To acquire these data relating to the mechanisms underlying OT autoregulation, we examined the effects of gradually increasing OT concentrations on the electrical activity of OT neurons using whole cell patch-clamp recordings in brain slices from lactating rats. In pilot experiments, in which extremely low OT concentrations were tested, we determined that OT in vitro could exert excitatory effects at concentrations that were much lower than the previously measured physiological range. We found that OT did cause decreased responsiveness, akin to desensitization, after initial excitatory effects. These actions are achieved via OT’s actions at both local neural circuits and on membrane electrical characteristics.

MATERIALS AND METHODS

All procedures in the animal experiments were in accordance with the guidelines on the use and care of laboratory animals set by National Institutes of Health (NIH) and approved by the Institutional Animal Care and Use Committee of the University of California, Riverside.

Electrophysiology. Sprague-Dawley (Holtzman strain) rats lactating for 8–13 days were used for the experiments. Rats were decapitated with a guillotine. Brains were quickly removed and put in oxygenated, ice-cold artificial cerebrospinal fluid (aCSF) for 1 min. The aCSF contained (in mM): 126 NaCl, 2.5 KCl, 1.3 MgSO4, 2.4 CaCl2, 1.3 NaH2PO4, 26 NaHCO3, 10 glucose, 0.2 ascorbic acid, and pH 7.4 adjusted with MOPS (~2 mM). The osmolality was adjusted to 300 mosmol/kgH2O. The aCSF was filtered (0.22 μm) and maintained with 95% O2-5% CO2 gas mixture. Hypothalami were dissected from the brain and cut coronally into cold medium on a Vibratome into 300-μm-thick slices. After preincubation at room temperature for 30 min, slices were returned to oxygenated aCSF with 95% O2-5% CO2 gas mixture and maintained for 80 min before recording.

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OXYTOCIN MODULATES OXYTOCIN NEURONAL ACTIVITY

Effects of progressively increasing OT concentrations on firing activity of OT neurons. It should be noted at the outset that the neurons recorded in this configuration tend to be superficial in the slice and, thus, well perfused by medium. This likely keeps concentrations of endogenously released substances from accumulating, as they might at sites deeper in the slice. OT is a crucial neuroactive substance in the SON during milk ejection (27). To examine the effects of OT on the electrical activity of OT neurons, we simulated the physiological changes in OT by bath application in progressively increasing concentrations (0.1 fM–10 nM in nine steps, each for 5 min). OT exerted an excitatory effect on nearly all OT neurons tested by increasing the firing rate by more than 20% of control level in a 5-min period (Fig. 1A). This excitatory effect started at the low concentrations (8/9 at 1 fM), then became less excitatory, and even reversed to silence at higher concentrations in all nine neurons, appearing to cause spike frequency reduction (SFR). The firing rate changes (excitation and ensuing SFR) through the nine doses were statistically significant ($P < 0.01$ by ANOVA) after square root transformation of the original data. Accompanying the firing rate changes, $E_m$ depolarized significantly, which appeared to be time and dose dependent.

Dose- and time-related effects of OT on electrical activity of OT neurons. The effect of progressively increasing OT concentrations may reflect both time and dose in OT actions. We first tested the dose effects by applying OT in three concentrations (1 fM, 1 pM, and 1 nM, each for 5 min, interrupted by 10-min washout). Different from the effect of progressively increasing concentrations, the intermittently applied OT caused dose-dependent increases in firing rate without SFR (Fig. 1B). This result indicates that a recovery time to restore the receptor to its prebound state appears to be necessary for restored activation of OT neurons and is supported by the next set of experiments. Prolonged application of a single OT dose (1 pM, 40 min, $n = 5$) did cause initial excitation (4/5) and ensuing SFR (Fig. 1C). Thus the effects of progressively increasing OT concentrations on the firing rate are both time and dose dependent.

Corresponding to the responses from OT neurons, the progressively increasing OT concentrations also caused excitation of phasically firing, putative VP SON neurons (for examples of phasically firing cells recorded in vitro, see Refs. 11 and 12). In contrast to the effects on OT neurons, the excitation in VP neurons occurred with longer latency and only at higher OT concentrations: the initial excitation appeared during 1–10 pM range (5/6), and was not followed by SFR in four of the six neurons recorded (data not shown).

Effects of progressively increasing OT concentrations on spike and other membrane electrical characteristics. The firing rates of OT neurons are also influenced by other membrane electrical activity (e.g., spiking threshold, $E_m$ level, spike AHPs, and others). Therefore, we further analyzed the effect of dynamic changes in OT concentrations on these electrical activities. Besides evoking the dual firing rate changes and $E_m$ depolarization, OT also caused a reduction in spike amplitude, increase in spike duration, prolongation of spike rise and decay time courses, elevation of spike threshold (from $-61.0 \pm 3.2$ to $-54.6 \pm 2.6$ mV at 1 pM, $n = 9, P < 0.05$), and an increase in rise time of the spike AHP (Fig. 2A). Despite dramatic effects of OT on other electrical properties of OT neurons,
membrane conductance \( n = 9 \) did not change significantly. Different from the effects on firing rate, the effects of OT on the above parameters were dose and time dependent, suggesting different underlying regulatory mechanisms. However, increases in the number of spike clusters were similar to effects of OT on the firing rate. Time course of the subthreshold \( E_m \) depolarization (STD) was extended significantly around 1 pM OT, then returned to basal level at higher concentrations (Fig. 2A).

It is noteworthy that the beginning of recovery (usually within 10 min) was significantly faster for firing rates than for spike amplitudes, spike width, \( E_m \), AHP amplitude, or rise and

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**Fig. 1.** Progressively increasing oxytocin (OT) concentrations induced excitation and subsequent depression in firing activity of OT neurons. A–C: voltage recordings of OT neuronal activity at resting membrane potential. A: effect of progressively increasing OT concentrations (from 0.1 fM to 10 nM, 5 min for each dose) on firing activity of an OT neuron (A1). At left is the initial membrane potential \( E_m \), corrected for liquid junction potential. Washout partially reinstated control levels of \( E_m \) and firing. The short solid lines above the recordings in A1, B1, and C1 indicate control levels of spike amplitude. The long dotted line below the traces corresponds to the resting \( E_m \) in the first trace. Note, after the initial excitation, the neuron became less active, accompanied by dramatic \( E_m \) depolarization and obvious spike amplitude reduction before becoming silent at 10 nM OT concentration. These responses were typical of recorded neurons. A2, left: scattergram of frequency (basal firing rate, FR, vs. FR during OT application). A2, middle: summary graphs. A2, right: firing rate (Freq.) and resting \( E_m \) (RMP), respectively. B: three OT concentrations applied with interapplication intervals of 10 min (1 fM, 1 pM, and 1 nM, each for 5 min). B1: example of one neuron. B2: summary graphs. C: effects of prolonged single OT concentration (1 pM, 40 min) on the activity of OT neurons. C1: example of one neuron. C2: summary graphs. *\( P < 0.05 \); **\( P < 0.01 \) compared with control by paired Student’s t-test after ANOVA evaluation; \( n \) = the number of neurons.
decay time courses of AHPs. This provides additional evidence for the existence of different regulatory processes involved in firing rate vs. most of the other electrical characteristics (summarized in Fig. 2B).

**Fast synaptic inputs and OT effects.** The autoregulation of OT neuronal activity may result from both direct and indirect effects of OT by modulation of other neuroactive substances, that is, those coexisting with OT in the extracellular environment (7, 27). Because glutamate and GABA inputs are the main sources of direct synaptic activity on OT neurons and are known to be modulated by OT, we examined the effects of blocking fast glutamatergic and GABAergic synaptic transmission on OT neurons. Application of 10 μM CNQX and 20 μM MK 801, and 20 μM bicuculline produced a brief increase in firing activity, then silence in 7 of 9 neurons, during which time the Em depolarized by 3–10 mV (6.5 ± 1.7 mV). The presence of these blockers reduced and delayed the excitatory effect of OT. Three neurons excited by OT showed no SFR. These results suggest that the excitatory effects of OT and the SFR are closely related to synaptic inputs, besides confirming a dominant GABAergic innervation of OT neurons in the SON.

To distinguish these presynaptic actions, we further examined effects of OT after blocking ionotropic glutamate receptors. In the presence of 10 μM CNQX and 20 μM MK 801 (Fig. 3A), spontaneous firing activity was reduced in a majority of OT neurons (6/9), addition of OT did not significantly increase the excitability in general, and did not cause SFR. However, other effects exerted by OT in normal aCSF were maintained, for example, Em depolarization (from -60.7 ± 2.4 mV at 1 pM, n = 9, P < 0.05), spike amplitude reduction, and spike broadening were observed, although they were not as dramatic as those seen in the normal aCSF (Fig. 3A2 and 3A3). The effects on spike amplitude and spike width were blocked by intracellularly loading 2 mM GDPβS, an inhibitor of G-proteins (Fig. 3C).

By contrast, the presence of bicuculline (20 μM) to block GABA<sub>A</sub>-mediated synaptic transmission (n = 9), depolarized Em in all neurons (>3 mV in 6/9) and increased firing rates in most of them (Fig. 4). Addition of OT caused qualitatively similar changes in the membrane electrical features, to those seen in the absence of bicuculline. However, the SFR phenomena were still present during GABAA receptor blockade. These results suggest that the excitatory effects of OT are associated with increased glutamate release and decreased GABA release and that the SFR phenomena are related to increased excitatory actions rather than to reduced inhibitory synaptic input.
DISCUSSION

Progressively increasing OT concentrations caused excitation and SFR, as well as a series of membrane electrical changes. Glutamatergic inputs promoted the excitatory action of OT and the subsequent SFR, while GABA played antagonistic roles in excitatory response of OT neurons. In addition, postsynaptic actions of OT determined most other electrical responses.

Excitatory effect of OT on the firing activity of OT neurons. High sensitivity of neurons to neuropeptides is commonly observed; however, few if any electrophysiological studies have reported femtomolar effects of neuropeptides. In the present work, OT triggered or increased firing activity of OT neurons, starting at 1 fM concentration. These are even lower than basal OT concentrations in the SON estimated by microdialysis (16, 21, 22). The nature of the perifusion of the tissue in vitro studies may remove endogenous OT from the surface of the slices, allowing higher accessibility and sensitivity to OT than the neurons recorded at sites deeper in the slice in extracellular or intracellular recordings. This, together with the relatively long time of OT application (providing enough time...
for temporal summation), using normal aCSF in observations but not low Ca\(^{2+}\) to elevate neuronal excitability artificially, carefully avoiding remaining effects of high-dose OT by thoroughly cleaning the perfusion system between tests, may account for the biological effect of lower OT concentration on OT neurons. It has been reported that 0.2 nM OT could excite hypothalamic neurons (15); and the natural cellular environment (the presence of Mg\(^{2+}\) and cholesterol) may also be a favorable condition for the functioning of saturable high-affinity OT receptors (13). Finally, what we measured was the electrophysiological output of the OT receptor in neurons, which may have different characteristics (affinities, KDs, etc.) from those in peripheral tissue where these measurements have previously been made. Thus it is reasonable to find the effect of this low concentration of OT in cellular function.

Because OT can activate both OT receptors and V\(_1\) receptors, it is also important to distinguish specific from nonspecific actions of OT. Recently, we have done the following experiment (Wang and Hatton, unpublished observations) to identify the receptors mediating OT functions in SON neurons. In the presence of an OT receptor antagonist, [β-Mercapto-β,β-cyclopentamethylene-propionyl\(^1\), O-Me-Tyr\(^2\), Orn\(^3\)]-oxytocin, the excitatory effects of on 6/6 OT neurons were blocked. However, a selective V\(_1\) receptor antagonist, [deamino-Pen\(^1\), O-Me-Tyr\(^2\), Arg\(^8\)]-vasopressin did not significantly influence the excitatory effects of OT on any of the six OT neurons. Moreover, we have also tested the effect of a specific OT receptor antagonist (kindly provided by Dr. M. Manning) in one OT neuron, which also blocked OT effects. These results indicate that the excitatory effect of OT is achieved via activation of OT receptors.

Spike frequency reduction. Faced with progressively increasing OT concentrations and the resulting maintained membrane depolarization, SFR characterized the tonic firing activity of OT neurons. This is consistent with the actions of many other excitatory neuroactive substances (17). Distinct from spike frequency adaptation (17), the two major components leading to SFR in OT neurons, the firing rate decrease and \(E_m\) depolarization, are not regulated simultaneously. OT-evoked SFR could occur on the basis of dramatic depolarization (most active neurons) or subtle \(E_m\) changes (some less active neurons).

SFR was related to the resting \(E_m\) and the basal firing activity in most cases. In response to OT administration, \(E_m\) depolarized progressively, accompanied by firing rate increases. Firing rates, however, declined while changes in other parameters kept in the same direction as OT concentration rose to a certain level, suggesting that two separate mechanisms were at work, one primarily affecting firing activity, the other influencing spike features and \(E_m\).
That OT receptors undergo rapid (within seconds to minutes) homologous desensitization after persistent agonist stimulation (9) may account for the SFR. Moreover, SFR may be related to activation of PKC and its negative feedback on the activation of phospholipase C. This may be explained by an activation of PKC that causes inhibition of subsequent responses of intracellular Ca^{2+} to activation of the diacylglycerol-PKC pathway (26). Other possible reasons are Ca^{2+} oscillations in the local neural circuits can begin. As a result, the firing rate increases gradually, and a new cycle of oscillations in the local neural circuits can begin.

Local neural circuits and OT effects. In considering the role of neural circuits, glutamatergic inputs are undoubtedly involved. Besides reducing the depolarizing effect of OT, blocking ionotropic glutamate receptors also blocked excitatory responses of OT neurons to low doses of OT, and delayed or removed SFR. This is in agreement with in vivo experiments investigating the functions of glutamate (23). On the other hand, inhibiting the effects of inhibitory neurotransmitters also modulated OT actions, that is, bicuculline increased OT excitatory effect. The balance of glutamatergic and GABAergic actions is an important factor. Although OT reduced EPSCs (14), it also decreased IPSCs (3). Because GABAergic inhibitory inputs were dominant, the inhibition of presynaptic currents by OT shifted the excitatory-inhibitory balance, permitting a dominant excitatory innervation by glutamatergic neurons. Thus, through activating cationic currents (AMPA receptors) or Ca^{2+} currents (NMDA receptors), glutamate could cause depolarization and excitation.

Physiological implications of OT autoregulation. Under in vivo conditions, the basal firing rate of OT neurons in lactating rats is low, and OT fails to facilitate OT neuronal activity in the absence of suckling stimulation (18). Possibly, basal firing activity of OT neurons may have already adapted to basal OT levels in that situation, as the higher concentrations of exogenous OT produced only permissive effects for the actions of other neuroactive substances during suckling stimulation (5, 6). That OT actions were weakened by blocking ionotropic glutamate receptors and were facilitated during fast GABAergic blockade suggests the OT actions are under intense modulation of the neurochemical environment, and suckling may allow reestablishment of time- and concentration-dependent actions. Upon nursing, suckling-associated neural pathways (e.g., noradrenergic, glutamatergic, and OTergic) are mobilized (24). Such inputs result in somatodendritic release of OT without causing significant changes in basal firing rates (27, 16) or adjusting the activity of OT neurons at moderate firing rates (2) by SFR. In our results for prolonged application of 1 pM OT, one highly active neuron did not increase its firing frequency but rather decreased its firing rate in response to OT application, supporting a dual modulatory role of OT in OT neuronal activity. Increased OT release inhibits GABAergic input, while sensitizing responses to glutamate and noradrenaline, lead to the initial activation of OT neurons. This activation further increases local OT concentrations (8). OT will activate extra- cellular proteases (14), accelerating OT decomposition. Astroglia in the SON will also help in the removal of excessive OT. In addition, nitric oxide and adenosine together with GABA will suppress the firing activity and aid in repolarization of E_m, reenabling OT neurons to excitatory inputs. As a result, the firing rate increases gradually, and a new cycle of oscillations in the local neural circuits can begin.

In conclusion, progressively increasing OT concentration causes excitation and subsequent SFR, accompanied by a series of changes in other membrane electrical features. Glutamatergic, but not GABAergic, inputs are influential in the firing rate changes, whereas other membrane electrical changes are modulated mainly through postsynaptic processes. These effects may well reflect the actions of OT during suckling stimulation and represent a common working model of neuroptides on their secretory neurons.

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REFERENCES


