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Introduction

Oxytocin (OT) is a hormone synthesized in magnocellular neurons that are located in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. During parturition and lactation in the rat, OT neurons display periodic high frequency bursts of action potentials (AP) that are synchronized in the whole OT neuron population. This triggers a massive and pulsatile release of OT in the bloodstream which, in tum, promotes pup delivery and milk ejection. During parturition and lactation, neurosecretory oxytocin (OT) neurons in the hypothalamus achieve pulsatile hormone secretion by coordinated bursts of firing that occur throughout the neuronal population. This activity is partly controlled by somatodendritic release of OT, which facilitates the onset and recurrence of synchronized bursting. To further investigate the cellular mechanisms underlying the control exerted by OT on the activity of its own neurons, we studied the effects of the peptide on membrane potential and synaptic activity in OT neurons in hypothalamic organotypic slice cultures. Bath application of low concentrations of OT (<100 nM) facilitated GABA<sub>A</sub> receptor-mediated inhibitory transmission through a presynaptic mechanism without affecting membrane potential and excitatory glutamatergic synaptic activity. The facilitatory action of OT on GABAergic transmission was dose-dependent, starting at 25 nM and disappearing at concentrations >100 nM. As shown previously, higher concentrations of OT (>500 nM) had the opposite effect, inhibiting GABA<sub>A</sub> receptors via a postsynaptic mechanism. Surprisingly, OT-mediated facilitation of GABAergic transmission promoted action potential firing in 40% of the neurons. Each action potential occurred at the end of the repolarizing phase of an inhibitory potential. Pharmacological dissection revealed that this firing involved the activation of low-threshold activated calcium channels. Detailed statistical analysis showed that OT-mediated firing upregulated bursting activity in OT neurons. It is thus likely to optimize OT secretion and, as a consequence, facilitate delivery and milk ejection in mammals.

Key words: hypothalamus; supraoptic; GABA; calcium current; neuroendocrine; lactation

Oxytocin-Induced Postinhibitory Rebound Firing Facilitates Bursting Activity in Oxytocin Neurons

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During parturition and lactation, neurosecretory oxytocin (OT) neurons in the hypothalamus achieve pulsatile hormone secretion by coordinated bursts of firing that occur throughout the neuronal population. This activity is partly controlled by somatodendritic release of OT, which facilitates the onset and recurrence of synchronized bursting. To further investigate the cellular mechanisms underlying the control exerted by OT on the activity of its own neurons, we studied the effects of the peptide on membrane potential and synaptic activity in OT neurons in hypothalamic organotypic slice cultures. Bath application of low concentrations of OT (<100 nM) facilitated GABA<sub>A</sub> receptor-mediated inhibitory transmission through a presynaptic mechanism without affecting membrane potential and excitatory glutamatergic synaptic activity. The facilitatory action of OT on GABAergic transmission was dose-dependent, starting at 25 nM and disappearing at concentrations >100 nM. As shown previously, higher concentrations of OT (>500 nM) had the opposite effect, inhibiting GABA<sub>A</sub> receptors via a postsynaptic mechanism. Surprisingly, OT-mediated facilitation of GABAergic transmission promoted action potential firing in 40% of the neurons. Each action potential occurred at the end of the repolarizing phase of an inhibitory potential. Pharmacological dissection revealed that this firing involved the activation of low-threshold activated calcium channels. Detailed statistical analysis showed that OT-mediated firing upregulated bursting activity in OT neurons. It is thus likely to optimize OT secretion and, as a consequence, facilitate delivery and milk ejection in mammals.

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Materials and Methods

Slice preparation. Cultured slices were prepared using the roller tube method as described previously (Jourdain et al., 1996). Briefly, 4- to 6-d-old female Wistar rats were anesthetized with isoflurane (95% O₂ and 5% isoflurane) for 1 min and decapitated. Brains were removed, and tissue blocks that included the hypothalamus were quickly dissected and sectioned (400 μm). Frontal slices containing the supraoptic nucleus (SON) were cut into two parts along the third ventricle, and each part was placed on a glass coverslip coated with heparinized chicken plasma. Thrombin was then added to the coverslip to coagulate the plasma and permit adhesion of the slice to the coverslip. The coverslip was inserted into a plastic flat-bottomed tube (Nunc, Roskilde, Denmark) containing (in mM) 125 NaCl, 3 KCl, 1 MgSO₄, 1.25 KH₂PO₄, 5 NaHCO₃, 2 CaCl₂, 5 glucose, and 10 HEPES, pH 7.25, 290–295 mOsm/kg. Intracellular microelectrodes were filled with 1 M potassium acetate and 1% biocytin (Sigma, St. Louis, MO). Electrode resistance varied from 150 to 250 MΩ. The patch clamp technique was used in whole cell configuration (current or voltage clamp mode) using electrodes (4–8 MΩ) filled with a solution containing (in ms) 120 K-gluconate, 20 KCl, 10 HEPES, 1 EGTA, 1.3 MgCl₂, 0.1 CaCl₂, 2 Mg-ATP, and 0.3 GTP. For IPSC recording, electrodes were filled with (in mM) 141 CsCl, 10 HEPES, 5 QX-314-Cl, and 2 Mg-ATP. Series resistance (10–25 MΩ) was monitored on line and cells were excluded if >20% change occurred during the experiment.

Signals were filtered at 2 kHz, digitized at 5 Hz and analyzed using pClamp 9 (Molecular Devices, Union City, CA). Firing rate preceding high-frequency burst was estimated from frequency histograms calculated over 0.5 s integration periods and plotted versus time using pClamp 9. The preburst period was defined as the period occurring 20 s before burst incidence. A change in basal firing frequency was considered as significantly different when changes exceeded 10% of control values measured during the 200 s period preceding the preburst period (Gouze`nes et al., 1998). Detection of synaptic events was achieved offline using a sliding template whereas action potentials were detected using an amplitude threshold (AxoGraph Scientific, Kagi, Berkeley, CA). An action potential was considered triggered by an IPSP if occurring within 300 ms of IPSP onset. Values are expressed as means ± SD. Data obtained were compared statistically with the nonparametric Kolmogorov–Smirnov test or the paired Student’s test.

Drugs. The following were added to the bath medium when required: synthetic OT (Peninsula, Lakewood, CA), the OT-R agonist synthetic OT (Peninsula, Lakewood, CA), the OT-R agonist 

\[ \text{OT} \] 

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Drugs. The following were added to the bath medium when required: synthetic OT (Peninsula, Lakewood, CA), the OT-R agonist synthetic OT (Peninsula, Lakewood, CA), the OT-R agonist OT facilitates inhibitory transmission. A1, Example of a recording where OT was applied at two different concentrations (50 nM and 1 μM) in the presence of TTX, bicuculline, CNQX, and APS. The peptide did not affect membrane potential nor membrane resistance measured from negative current pulses of decreasing amplitude (from −150 to 0 pA by successive 15 pA steps). A2, Voltage–current relationship obtained from the experiment shown in A1. The presence of OT did not modify this relationship. A3, Histogram summarizing the lack of action of OT (10 nM to 1 μM) on membrane potential (Em) and membrane resistance (Rm) in OT-sensitive cells. The numbers of experiments are indicated in brackets. B, In the presence of CNQX, application of 50 nM OT reversibly increased IPSP activity in 72% of OT neurons as illustrated in the example on the left (OT responsive cell). In the remaining neurons (28%), GABAergic synaptic transmission was unaffected as shown on the example illustrated on the right (OT-nonresponsive cell).
nitroquinoxaline-2,3-dione (CNQX; RBI, Natick, MA), D(-) 6-cyano-7-phosphono- pentanoic acid (AP5), ZD 7288 (ZD; Tocris, Ellisville, MO), bicuculline, CsCl, picrotoxin and tetrodotoxin, and mibefradil (15 M), showing that synaptic events modulated by OT were mediated by GABA<sub>A</sub> receptors (data not shown). The effect of OT was mimicked by a specific OT-R agonist, D(-) OT (100 nM; n = 6) which increased the frequency (377 ± 82% of control; p < 0.05) and amplitude (283 ± 78% of control; p < 0.05) of spontaneous IPSPs (Fig. 2C). Conversely, the effect of 50 nM OT was blocked in the presence of 1 μM D-OVT, a specific OT-R antagonist (79 ± 31% and 91 ± 9% of control in frequency and amplitude, respectively; n = 4) (Fig. 2C).

To identify the locus of action of OT, TTX (1 μM) was added to the external solution to block AP-driven inhibitory synaptic events and thus make sure that monovalent synaptic responses (miniatures) were recorded. For these experiments we used the whole-cell patch-clamp technique in voltage-clamp configuration instead of intracellular sharp electrode recording. Under these conditions, OT (50 nM) significantly increased the frequency (198.3 ± 29.3% of control, n = 4; p < 0.05) but not the amplitude (103.5 ± 8.1% of control, n = 4; p > 0.05) of miniature IPSCs (Fig. 2D–F). Although this set of

Results

All of the results reported in this study have been obtained in 153 OT magnocellular neurons, which were identified according to two criteria: (1) their ability to display high-frequency bursts of action potentials which are a specific property of OT neurons (Jourdain et al., 1998; Israel et al., 2003), and (2) post hoc immunoidentification. Intracellular recordings (n = 122) obtained from these neurons revealed a mean resting membrane potential of −54.6 ± 5.0 mV (n = 50), a mean input resistance of 237.0 ± 40.9 MΩ (n = 50), and action potentials (APs) of 71.9 ± 10.9 mV (n = 250 from 50 cells).

OT upregulates GABAergic transmission

Intracellular recordings in current-clamp mode in the presence of TTX (1 μM), bicuculline (15 μM), CNQX (10 μM), and APV (40 μM) indicated that bath-applications of OT (10–1000 nM) did not affect the resting membrane potential nor the input resistance of OT neurons (Fig. 1A–A3). Bath-application of OT (100 nM) in normal medium did not alter the amplitude (98.4 ± 5.2% of control; n = 8, p > 0.05) or frequency (95.4 ± 3.6% of control, n = 8, p > 0.05) of EPSPs recorded at ~80 mV (data not shown). Under conditions where EPSPs were blocked with CNQX (10 μM) and AP5 (40 μM), low concentrations (25 to 100 nM) of OT significantly and reversibly increased IPSPs (Fig. 1B) and IPSCs (Fig. 2A) in 52 of 78 neurons (72%). In the remaining neurons (28%), OT did not affect GABAergic activity (Fig. 1B) and these cells were thus considered as nonresponsive to the neuropehysin peptide. In OT-responsive cells, the enhanced IPSP activity was associated with an increase in the frequency (257 ± 41% of control; p < 0.05; n = 7) and amplitude (211 ± 52% of control; p < 0.05; n = 7) of spontaneous events (Fig. 2B,C). OT-sensitive IPSPs/IPSCs were blocked by bicuculline (15 μM), showing that synaptic events modulated by OT were mediated by GABA<sub>A</sub> receptors (data not shown). The effect of OT was mimicked by a specific OT-R agonist, D(-) OT (100 nM; n = 6) which increased the frequency (377 ± 82% of control; p < 0.05) and amplitude (283 ± 78% of control; p < 0.05) of spontaneous IPSPs (Fig. 2C). Conversely, the effect of 50 nM OT was blocked in the presence of 1 μM D-OVT, a specific OT-R antagonist (79 ± 31% and 91 ± 9% of control in frequency and amplitude, respectively; n = 4) (Fig. 2C).

Identification of recorded neurons. At the end of the recording, neurons were filled with biocytin (1%) using hyperpolarizing current pulses. This was not necessary for patch clamp recording. Slices were then fixed in 4% paraformaldehyde and 0.15% picric acid for 2 h at room temperature and rinsed in 4% paraformaldehyde (2 × 20 min). Biocytin was visualized with streptavidin-conjugated Texas Red fluorescence (Biosys, Compiegne, France) with appropriate filters (Leitz DMR microscope; Leica, Rueil-Malmaison, France). Slices then underwent double immunofluorescence for OT or vasopressin, using a mixture of primary antibodies, one being a monoclonal mouse IgG raised against OT-related neurophysin (OT-NP; provided by Dr. H. Gainer, National Institutes of Health, Bethesda, MD), the other a polyclonal rabbit serum raised against vasopressin-associated neurophysin (VP-NP; provided by Dr. A. Robinson, University of California, Los Angeles, Los Angeles, CA).

Figure 3. OT has bimodal effects on GABAergic transmission. A. Histograms summarizing the changes in IPSP amplitude and frequency induced by different concentrations of OT. Insets are example obtained from a cell where OT was successively applied at 50 and 300 nM. OT triggered or facilitated IPSP activity at a threshold concentration of 25 nM, an effect attenuated totally IPSPs and almost completely the postsynaptic response.

OT-R antagonist desGly-NH<sub>2</sub>[D(CH<sub>2</sub>)<sub>5</sub>–D-Tyr<sub>2</sub>,Thr<sub>4</sub>–OVT (D-OVT; [4-threonine, 7-glycine]-oxytocin ([Thr<sub>4</sub>, Gly<sub>7</sub>]-OT ([4–7] OT), the OT-R agonist desGly-NH<sub>2</sub>[D(CH<sub>2</sub>)<sub>5</sub>–D-Tyr<sub>2</sub>,Thr<sub>4</sub>–OVT ([O–OVT; gifts from Dr. Manning, University of Toledo, Toledo, OH), 6-cyano-7-nitroquinolin oxide-2,3-dione (CNQX; RBI, Natick, MA), D(-)-2-amino-5-phosphono- pentoic acid (AP5), ZD 7288 (ZD; Tocris, Ellisville, MO), bicuculline, CsCl, picrotoxin and tetrodotoxin, and mibefradil (15 M) indicated that bath-applications of OT (10–1000 nM) did not affect the resting membrane potential nor the input resistance of OT neurons (Fig. 1A–A3). Bath-application of OT (100 nM) in normal medium did not alter the amplitude (98.4 ± 5.2% of control; n = 8, p > 0.05) or frequency (95.4 ± 3.6% of control, n = 8, p > 0.05) of EPSPs recorded at ~80 mV (data not shown). Under conditions where EPSPs were blocked with CNQX (10 μM) and AP5 (40 μM), low concentrations (25 to 100 nM) of OT significantly and reversibly increased IPSPs (Fig. 1B) and IPSCs (Fig. 2A) in 52 of 78 neurons (72%). In the remaining neurons (28%), OT did not affect GABAergic activity (Fig. 1B) and these cells were thus considered as nonresponsive to the neurophysin peptide. In OT-responsive cells, the enhanced IPSP activity was associated with an increase in the frequency (257 ± 41% of control; p < 0.05; n = 7) and amplitude (211 ± 52% of control; p < 0.05; n = 7) of spontaneous events (Fig. 2B,C). OT-sensitive IPSPs/IPSCs were blocked by bicuculline (15 μM), showing that synaptic events modulated by OT were mediated by GABA<sub>A</sub> receptors (data not shown). The effect of OT was mimicked by a specific OT-R agonist, D(-) OT (100 nM; n = 6) which increased the frequency (377 ± 82% of control; p < 0.05) and amplitude (283 ± 78% of control; p < 0.05) of spontaneous IPSPs (Fig. 2C). Conversely, the effect of 50 nM OT was blocked in the presence of 1 μM D-OVT, a specific OT-R antagonist (79 ± 31% and 91 ± 9% of control in frequency and amplitude, respectively; n = 4) (Fig. 2C).

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data implies that OT-R are located pre-synaptically on GABAergic neuron terminals impinging on OT cells, they do not rule out the possibility that OT-R are also located on GABA neuron somata or on other neurons contacting GABAergic cells, and that these receptors also contribute to facilitate inhibitory activity in OT neurons.

**Bimodal dose-dependent action of OT on inhibitory transmission**

The stimulatory effect of OT on GABAergic transmission was dose-dependent, with a threshold of 25 nM and a maximal facilitation at 50 nM (Fig. 3A). Higher concentrations of OT progressively inhibited GABAergic synaptic activity. The inhibition was almost total with 300 nM OT, a result in agreement with the postsynaptic inhibitory action of OT on GABA<sub>A</sub> receptors previously reported in the SON (Brussaard et al., 1996). The dynamics and dose-dependency of these two opposite effects of OT on GABAergic transmission were then compared by monitoring simultaneously synaptic currents and responses obtained with local puffs of GABA. Whereas low concentrations of OT (50 nM) triggered IPSP activity without affecting the amplitude of GABA-induced responses, IPSP amplitude and frequency gradually decreased when increasing OT concentrations, with a complete inhibition obtained at 1000 nM (Fig. 3B, D). In the same recordings, GABA-induced responses were slightly affected when OT concentration reached 500 nM and were completely inhibited with 2000 nM (Fig. 3C, D). These findings demonstrate that OT acts both at presynaptic and postsynaptic levels, depending on its concentration, to upregulate or downregulate GABAergic transmission.

**OT-mediated IPSPs facilitate firing**

In intracellular current-clamp recordings, low concentrations of OT (50–100 nM) increased the firing activity in 11 of 27 OT neurons (183 ± 17% of control) (Fig. 4A1, A2). To identify the cellular mechanism responsible for this increase in firing rate, and its possible relation to OT-mediated facilitation of inhibitory transmission, we investigated the action of OT on OT neuron electrical activity in the presence of CNQX to block EPSPs (Fig. 4B). Whereas CNQX inhibited AP firing, as reported previously (Jourdain et al., 1996), OT still triggered spiking activity at resting membrane potential in 15 of 36 neurons (41%). Examination of recordings at high resolution revealed that during such OT-triggered activity, most APs occurred at the end of the repolarizing phase of individual IPSPs (Fig. 4B3, B4). This was particularly clear during OT washout where IPSP frequency decreased to values <5 Hz, making it easier to reveal the link between IPSP and AP firing (Fig. 4B3, trace d). That APs were exclusively triggered by IPSPs was confirmed in a series of experiments where this OT-triggered firing occurring in the presence of CNQX was completely inhibited by the specific GABA<sub>A</sub> receptor antagonist picrotoxin (5 μM) (Fig. 5A) (n = 5). It is worth noting that we never observed an increased in AP firing without an increase in IPSP activity.

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**Figure 4.** OT-induced IPSP activity triggers AP firing. A1, Example of an OT neuron where exogenous application of OT (50 nM) increased firing activity in a reversible manner as illustrated by the change in the sequential frequency histogram. A2, Histogram summarizing the stimulatory effect of OT on firing rate. The number of cells is indicated in brackets. B1, Under conditions where CNQX totally abolished spontaneous firing (b), OT (50 nM) still triggered AP discharge (c). B2, Summary histogram showing the stimulatory effect of OT in the presence of CNQX. B3, Traces obtained from the recording shown in B1. In control conditions (a), APs were mainly triggered by EPSPs. CNQX totally abolished EPSP activity and APs firing (b). Subsequent addition of OT (50 nM) dramatically increased IPSP activity (arrowheads) and restored AP firing (c). During washout of OT, although IPSP frequency and AP firing decreased, spikes were still occurring at the end of IPSPs (d). B4, Magnification of the trace c shown in B3 revealed that APs (asterisks) occurred at the offset of IPSPs (arrowheads).
Ionic mechanisms underlying OT-induced firing

In presence of CNQX, each OT-triggered spike occurred at the end of the repolarizing phase of an individual IPSP (Fig. 4B4). Such process is reminiscent of PIR, as described in other structures (Bertrand and Cazalets, 1998; Angstadt et al., 2005; Sohal et al., 2006). PIR is defined as the depolarization that occurs at the offset of a hyperpolarizing period. At least, two nonexclusive mechanisms might account for PIR-induced spikes. One involves LVA Ca\(^{2+}\) channels that are first deinactivated by hyperpolarization and then activated during the repolarization period (Bertrand and Cazalets, 1998; Jahnsen and Linneas, 1984; Fan et al., 2000), thereby generating a depolarization known as a low-threshold spike (LTS). This LTS, if of a sufficient amplitude, can generate APs (Huguenard, 1996). A second possibility is the activation of a hyperpolarization-activated inward current (I\(_{\text{H}}\)) which underlies rebound responses in many neurons (Matsushima et al., 1993; Straub and Benjamin, 2001; Sekirnjak and du Lac, 2002).

Both LVA Ca\(^{2+}\) current and I\(_{\text{H}}\) have been described in SON and PVN neurons (Fisher and Bourque, 1995; Ghamari-Langroudi and Bourque, 2000; Luther and Tasker, 2000). Thus, we first checked for their presence in our cultured slices before studying their respective contribution to OT-mediated PIR-firing. Because the presence of LVA Ca\(^{2+}\) channels is associated with the generation of an LTS, we applied brief (50 ms) hyperpolarizing pulses in the presence of TTX to block Na\(^{+}\)-dependent APs. As illustrated in Figure 6A1, such pulses triggered a rebound depolarization typical of an LTS in 17 of 32 neurons, a process that was compromised when the amplitude of the negative step was reduced, as previously described (Erickson et al., 1993). In the absence of TTX, such hyperpolarizing pulses triggered rebound APs (Fig. 6A2). To study the contribution of LVA Ca\(^{2+}\) channels to this process, we bath-applied Ni\(^{2+}\) at 100 \(\mu\)M, a concentration that inhibits T-type Ca\(^{2+}\) currents (Fisher and Bourque, 1995) and blocks LTS (Erickson et al., 1993) in SON neurons. In agreement with a role for these channels in LTS generation, APs triggered by long (>100 ms; \(n = 5\)) (Figs. 6B1, 7B1) or brief (25 ms; \(n = 6\)) (Figs. 6B2, 7B2) hyperpolarizing pulses were completely abolished in the presence of this inhibitor. Because Ni\(^{2+}\) might also interact with other voltage-gated Ca\(^{2+}\) channels, we tested the action of mibebradil, a compound considered to be a specific T-type channel antagonist (Van der Vring et al., 1999). At a concentration of 40 \(\mu\)M, mibebradil also inhibited pulse-triggered APs (\(n = 5\)) (Fig. 6B3, 7B1). Together, these data suggest that LVA Ca\(^{2+}\) channels mediating LTS in these neurons are of the T-type.
We then examined the role of $I_{H}$ in this process. During 100 ms long hyperpolarizing pulses, we reliably observed the typical depolarizing sag in the voltage response (Fig. 6C1,D1) that reflects activation of $I_{H}$ (Ghamari-Langroudi and Bourque, 2000). As previously reported (Ghamari-Langroudi and Bourque, 2001), this sag was inhibited by 3 mM external $\text{Ca}^{2+}$ (n = 4) (Fig. 6C1) or by 50 $\mu$M ZD (n = 5) (Fig. 6D1), two well known blockers of $I_{H}$. Conversely, this sag was not affected in the presence of $\text{Ni}^{2+}$ or mibefradil (Fig. 6B1,B3). Interestingly, blockade of $I_{H}$ with Cs or ZD did not prevent pulse-triggered rebound spikes (Figs. 6C1,D1, 7B1) even when hyperpolarization was adjusted to that obtained in control conditions to compensate for changes in membrane resistance (Fig. 6C2,D2). The lack of effect of ZD on pulse-triggered APs was also observed with pulses of shorter duration (25 ms; n = 6) (Figs. 6D3, 7B2).

$\text{Ni}^{2+}$, mibefradil, Cs $^{+}$, and ZD were then used to assess the respective contribution of LVA Ca$^{2+}$ channels and $I_{H}$ to OT-mediated PIR firing. As illustrated in Figure 7A and summarized in C, $\text{Ni}^{2+}$ (n = 4) and mibefradil (n = 5) inhibited AP firing, but not IPSP activity (Fig. 7D), triggered by OT. However, neither Cs $^{+}$ (n = 4) nor ZD (n = 4) affected significantly OT-triggered firing activity (Fig. 7A,C) or IPSPs (Fig. 7D). These data suggest that IPSPs can trigger rebound firing through the recruitment of LVA Ca$^{2+}$ channels. If this is true, a rebound depolarization should be observed after individual IPSPs. In four cells where OT-triggered AP firing and IPSP activity were not intense enough to mask such a phenomenon, rebound excitations were clearly observed after inhibitory synaptic potentials (Fig. 8A1,A2; see also Fig. 4B3, trace d). These rebounds were not affected by the subsequent application of ZD (Fig. 8B1,B2) whereas they were completely abolished in the presence of $\text{Ni}^{2+}$ (Fig. 8C1,C2). As illustrated on the averaged traces in Figure 8D and from cumulative histograms in Figure 8E, inhibition of the rebound depolarization with $\text{Ni}^{2+}$ resulted in an increased IPSP width, an effect that was not observed with ZD. We thus used IPSP duration to assess the effect of the different blockers on IPSP-triggered postinhibitory rebound excitation. Whereas both $\text{Ni}^{2+}$ and mibefradil increased IPSP duration (126 $\pm$ 12% of control, n = 4 for $\text{Ni}^{2+}$; 121 $\pm$ 9%, n = 4, for mibefradil), neither ZD nor Cs $^{+}$ modified significantly this parameter (Fig. 8F). Together, these findings reveal the involvement of LVA Ca$^{2+}$ channels, but not of $I_{H}$ in IPSP-mediated postinhibitory rebound firing. These data also indicate that IPSPs have to be of sufficient amplitude to trigger AP firing, which may not be the case under control conditions.

**Physiological relevance**

Throughout this study, OT neurons recorded in the absence of glutamatergic and GABAergic blockers usually displayed a bursting activity, either spontaneously or in response to bath application of 100 nM OT (Fig. 9A) (Jourdain et al., 1998, Israel et al., 2003). This activity is characteristic of that recorded in vivo in lactating rats (Lincoln and Wakerley, 1975). Careful analysis of this activity in cultured slices revealed an increase in firing rate occurring just before burst onset in 40% of the neurons (Fig. 9A). Such increases in background firing rate immediately preceding the bursts have been already reported in vivo where they are directly and positively correlated to the magnitude of the bursting activity itself (Lincoln and Wakerley, 1975). This prompted us to investigate whether a similar type of correlation prevailed in OT neurons recorded from organotypic slice cultures, and to test whether OT-mediated PIR firing was playing a role in this process.

Within bursts, both the mean AP frequency and the peak frequency (over 0.5 s) were increased (175 $\pm$ 38% of control and 183 $\pm$ 40% of control, respectively; n = 25) in neurons showing an enhanced background firing activity before burst onset (Fig. 9B1,B2). To further analyze burst magnitude, we used the same index as described by Lincoln and Wakerley which corresponds to the number of spikes within the burst multiplied by the peak frequency (Lincoln and Wakerley, 1975). As illustrated in Figure 9C, the burst index was positively correlated (r = 0.61; n = 48 bursts from 15 cells) to the firing frequency measured 20 s before the incidence of each burst, a result in complete agreement with previous in vivo data (Lincoln and Wakerley, 1975; Brown et al., 2000).

Although the bursts are driven by glutamatergic inputs (Jourdain et al., 1998; Israel et al., 2003), the origin of the increase in background firing rate in OT cells is unknown. One possibility is
that this phenomenon is attributable to OT-mediated PIR firing. To test this hypothesis, we analyzed synaptic activity just before the occurrence of each burst. EPSP activity occurring during this period remained unchanged whether an increase in background firing rate occurred or not (Fig. 10A). On the contrary, in neurons that displayed an increase in firing rate before the bursts, a marked increase in both amplitude (155 ± 12% of control; n = 10; p < 0.05) and frequency (189 ± 39% of control; n = 10; p < 0.05) of IPSPs occurred (Fig. 10A,B). These findings strongly support a relationship between IPSP activity and increased background firing rate. Because IPSP-mediated PIR firing is related to OT, it is likely that the increased firing observed in these neurons resulted from the dendritic release of endogenous OT. If this is true, then activation or inhibition of OT-R should affect background firing and, consequently, the burst index. In agreement with this hypothesis, OT (50–100 nM) increased the mean firing rate before burst onset from 2.3 ± 0.7 Hz to 4.78 ± 1.24 Hz (211 ± 41% of control; n = 5) (Figs. 9C, 10C1,D) whereas the OT-R antagonist D-OVT decreased it from 2.98 ± 0.20 Hz to 0.98 ± 0.40 Hz (32 ± 14% of control; n = 4) (Figs. 9C, 10C2,D). In these neurons, OT and D-OVT respectively augmented (163 ± 26%, n = 5) and diminished (36 ± 19% of control) the burst index as expected (Fig. 10D). It is noteworthy that in the presence of OT, a concomitant increase in IPSP frequency (190 ± 26% of control, n = 5, p < 0.05) and amplitude (191 ± 35% of control, n = 5, p < 0.05) occurred (Fig. 10E), whereas D-OVT by itself induced an opposite effect (frequency, 73 ± 6% of control, n = 4, p < 0.05; amplitude, 56 ± 9% of control, n = 4, p < 0.05) (Fig. 10E). This suggests that endogenous ambient OT has a positive action on IPSP activity and, consequently, on burst magnitude.

Discussion

Action potential firing in neurons is usually obtained when the membrane potential is depolarized above spike threshold. This generally occurs through activation of ion channels or ligand-gated receptors or through the relief of tonic inhibition, a process known as disinhibition. Another mechanism promoting neuronal firing, although less usual, is PIR. In this phenomenon, one to several APs can be generated during the membrane repolarization that follows the offset of a hyperpolarizing event. PIR may involve \( I_{\text{M}} \), deactivation of voltage-gated \( Ca^{2+} \) currents or both. Such process is responsible for triggering activity in motoneurons (Bertrand and Cazalets, 1998) in thalamocortical neurons (Sohal et al., 2006) and in rat caudal hypothalamic neurons (Fan et al., 2000) for example. Here, we described a process in which a peptide, oxytocin, by facilitating the occurrence of hyperpolarizing GABAergic synaptic potentials, promotes AP discharge through PIR firing. This process potentiates bursting activity of OT neurons which is responsible for the massive and intermittent release of OT in the blood, and thus for pup delivery and milk ejection.

**Figure 8.** IPSP-mediated rebound depolarization. A1, Example of a recording obtained in the presence of CNQX and 50 nM OT showing the presence of rebound depolarizations (asterisks) occurring at the end of IPSPs. A2, Superimposition of eight consecutive IPSPs obtained from the recording in A1 clearly shows that a rebound depolarization follows several of these inhibitory potentials. B1, B2, In the same recording, application of ZD did not affect the occurrence of such IPSP-triggered rebound depolarizations. C1, C2, Subsequent addition of 100 \( \mu \)M Ni \(^{2+} \) in the bathing solution completely abolished AP firing and rebound depolarizations. D, Average IPSPs (n = 93–105) obtained from the recordings shown in A–C. This graph shows that rebound depolarization was unaffected by ZD (gray trace) whereas it was abolished in the presence of Ni \(^{2+} \). Note that IPSP duration was increased with Ni \(^{2+} \). E, Cumulative histograms representing the distribution of IPSP half-width obtained from the recording shown in A–C. This distribution was significantly shifted toward higher values in the presence of Ni \(^{2+} \). F, Summary histogram illustrating the percentage change in the half-width of OT-triggered IPSPs (n = 4 cells). Whereas IPSP duration was significantly increased by Ni \(^{2+} \) and mibefradil (Mib), it remained unchanged in the presence of Cs \(^{+} \) or ZD.

**OT modulation of firing activity in OT neurons**

The low concentrations of OT that we used here (25–100 nM) is more compatible with physiological concentration, as suggested by microdialysis experiments (Neumann et al., 1993). Interestingly, the facilitatory action of OT on IPSP activity was observed in 72% of OT neurons. That such low concentrations of OT accelerate firing in OT-responsive neurons through the generation of IPSPs is paradoxical because hyperpolarizing synaptic potentials are usually associated with inhibition rather than facilitation of firing activity. The action of OT was receptor-mediated because it was mimicked by an OT-R agonist and inhibited by an OT-R antagonist. Similar upregulation of GABAergic activity has been reported in CA1 hippocampal neurons (Zaninetti and Raggenbass, 2000) and in putative vasopressin hypothalamic neurons (Hermes et al., 2000) in response to OT and VP, respectively. Furthermore, our experiments revealed that OT-mediated GABAergic activation facilitated AP firing in ~40% of OT neurons. Although moderate, this increase in firing rate was in the range of that reported in lactating rat in vivo in response to local OT applications (Brown et al., 2000).

How can a GABAergic inhibitory synapse become excitatory? Several mechanisms may underlie IPSP-mediated PIR firing. One involves an LTS resulting from deactivation of LVA \( Ca^{2+} \) currents, as described previously in the SON (Fisher and Israel et al. • GABAergic Activity Facilitates Bursting Firing J. Neurosci., January 9, 2008 • 28(2):385–394 • 391
Bourque, 1995, Erickson et al., 1993, Dudek et al., 1989). This is likely to be the case here for several reasons. First, the percentage of neurons exhibiting an LTS is similar to that displaying OT-triggered firing. Second, rebound OT-triggered depolarizations and spikes were entirely blocked by Ni2+ and ZD 7288 inhibited its concentration and its targets, namely, GABA, glutamate and its concentration, OT has presynaptic effects, increasing then decreasing the probability of GABA release previously described (De Kock et al., 2001). Alternatively, if there is only one type of receptor mediated postsynaptic responses (Brussaard et al., 1996). Together, our results reveal, therefore, that depending on its concentration, OT has presynaptic effects, increasing then decreasing the probability of GABA release, and postsynaptic effects, inhibiting GABA receptors on OT neurons.

It is obvious from this and our previous studies (Jourdain et al., 1998; Israel et al., 2003) that OT acts differently, according to its concentration and its targets, namely, GABA, glutamate and OT neurons. Such heterogeneity of actions may reflect differences in OT-R mediating these responses. However, although there is evidence supporting the existence of different receptor subtypes, only one type of OT-R has been described so far (Gimpl and Fahrenholz, 2001). Alternatively, if there is only one type of OT-R, there may be a differential expression of this receptor in different cells and/or different OT-R-coupled second messenger pathways (Verbalis, 1999).

Heterogeneity of OT actions
It is clear from our observations that OT mediates distinct effects in the SON according to its concentration. At 50 nM, OT stimulated increasing the probability of GABA release, and postsynaptic effects, inhibiting GABA receptors on OT neurons.

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Physiological considerations

At parturition and during suckling, local release of OT from the somatodendritic compartment is necessary to trigger and facilitate the periodic activation of OT neurons (Freund-Mercier and Richard, 1984; Moos et al., 1984). We have shown previously that OT neuron bursting is controlled by an intrahypothalamic network in which bursting glutamate neurons govern OT neurons. In turn, OT somatodendritic release is essential to modulate the bursting pattern of glutamatergic neurons (Jourdain et al., 1998; Israel et al., 2003). The modulation of GABA transmission by OT as reported here may provide another mean of generating APs during background activity, in addition to those generated by EPSPs. Such a process may also explain previous in vivo data obtained in lactating rats showing that locally applied GABA unexpectedly facilitated bursting activity (Moos, 1995) whereas the same activity was impaired when GABA_A receptors were inhibited (Voisin et al., 1995).

We here showed a strong correlation between background firing activity before each burst and the magnitude of the bursts, a result similar to that reported in vivo (Lincoln and Wakerley, 1975; Brown and Moos, 1997). One likely explanation to account for this observation is that such an increase in firing rate facilitates the somatodendritic release of OT, thereby increasing its ambient concentration and range of action in the extracellular space. This, in turn, could positively modulate the intrahypothalamic pacemaker neurons responsible for the bursting activity of OT-secreting cells (Jourdain et al., 1998). In agreement with this hypothesis, we noticed that activation of OT-R with exogenous OT increased background firing rate and, consequently burst magnitude, as reported in vivo (Brown et al., 2000) whereas inhibiting OT-R with d-OVT had the opposite effect. In view of these data, it appears that OT-mediated PIR firing is an important process by which OT neurons could not only regulate their own activity but also influence the efficacy of the intrahypothalamic network generating the bursting behavior responsible for pup delivery and milk ejection. These results are reminiscent of those obtained in vivo where OT neurons showing an increase in their background firing rate before the bursts have been described as “leader” neurons whose task is to recruit “follower” neurons to optimize the activation of the entire OT network, thereby maximizing synchronized bursting activity (Moos et al., 2004).

References


