Short-Term Potentiation of Miniature Excitatory Synaptic Currents Causes Excitation of Supraoptic Neurons

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1Faculty of Pharmacy, Kuwait University, Safat 13110, Kuwait; 2Neuroscience Research Group and Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta T2N 4N1; and 3CHUL Research Centre, Laval University, Sainte-Foy, Quebec G1V 4G2, Canada

Kombian, Samuel B., Michiru Hirasawa, Didier Mouginot, Xihua Chen, and Quentin J. Pittman. Short-term potentiation of miniature excitatory synaptic currents causes excitation of supraoptic neurons. J. Neurophysiol. 83: 2542–2553, 2000. Magnocellular neurons (MCNs) of the hypothalamic supraoptic nucleus (SON) secrete vasopressin and oxytocin. With the use of whole-cell and nystatin-perforated patch recordings of MCNs in current- and voltage-clamp modes, we show that high-frequency stimulation (HFS, 10–200 Hz) of excitatory afferents increases in the frequency and amplitude of 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo(f)quinoxaline-7-sulfonamide (NBQX)-sensitive miniature excitatory postsynaptic currents (mEPSCs) lasting up to 20 min. This synaptic enhancement, referred to as short-term potentiation (STP), could be induced repeatedly; required tetrodotoxin (TTX)-dependent action potentials to initiate, but not to maintain; and was independent of postsynaptic membrane potential, N-methyl-D-aspartate (NMDA) receptors, or retrograde neurohypophyseal neuropeptide release. STP was not accompanied by changes in the conductance of the MCNs or in the responsiveness of the postsynaptic non-NMDA receptors, as revealed by brief application of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate. mEPSCs showed similar rise times before and after HFS and analysis of amplitude distributions of mEPSCs revealed one or more peaks pre-HFS and the appearance of additional peaks post-HFS, which were equidistant from the first peak. STP of mEPSCs was not associated with enhanced evoked responses, but was associated with an NBQX-sensitive increase in spontaneous activity of MCNs. Thus we have identified a particularly long-lasting potentiation of excitatory synapses in the SON, which has a presynaptic locus, is dissociated from changes in evoked release, and which regulates postsynaptic cell excitability.

INTRODUCTION

Magnocellular neurons (MCNs) of the supraoptic nucleus (SON) synthesize the peptides vasopressin (AVP) and oxytocin (OXT) that are released into the peripheral circulation, where they regulate important functions such as salt-water balance, blood pressure, parturition, and milk ejection. In response to increased demand for their products, MCNs not only display increased frequency of firing, but also switch to a bursting or phasic activity pattern that facilitates release (Armstrong 1995; Bourque and Renaud 1990). Both intrinsic conductances and synaptic mechanisms have been proposed to underlie this change in activity pattern. Although it has been shown that both inhibitory and excitatory synaptic inputs onto MCNs can modulate their excitability (Hu and Bourque 1991; Kabashima et al. 1997; Kombian et al. 1996; Mouginot et al. 1998; Wuarin and Dudek 1993), it is not clear what role these afferent inputs play in the switching and shaping of activity patterns that optimize peptide release. The majority of the afferents to the MCNs have been shown to be GABAergic and glutamatergic in nature. Manipulation of the strength of these inputs can markedly influence the output of the SON; in particular, the unique phasic activity patterns displayed by the AVP-containing neurons of the SON are dependent on the glutamatergic inputs to the nucleus (Nissen et al. 1995). Similarly, the ability of the OXT-containing cells to respond appropriately to the peripheral inputs associated with the milk ejection reflex requiresafferent inputs.

In a hypothalamic slice containing the SON, it is possible to record both action potential–dependent and –independent events (miniature excitatory or inhibitory postsynaptic currents; mEPSCs or mIPSCs). Electrophysiological studies of these miniature events have provided information concerning the mode of action of various presynaptic transmitters and pharmacological compounds on MCN activity. However, the possible role of miniature events in the synaptic function of the SON or indeed in any central neuron is poorly understood (Stuley 1999). In the present study, we have monitored miniature excitatory postsynaptic potentials/currents (mEPSPs/mEPSCs) and action potential firing in MCNs of the SON, and have observed an activity-dependent change in the properties of excitatory afferent terminals that may trigger and help maintain firing patterns that optimize peptide release from MCNs.

METHODS

Coronal (400 μm) hypothalamic slices from adult male Sprague-Dawley rats were perfused (submerged, 27–29°C) with artificial cerebrospinal fluid (ACSF) as previously described (Kombian et al. 1997). Recordings were done by the use of both whole-cell recording (WCR) and the nystatin-perforated patch technique by the use of electrodes with tip resistance of 4–10 MΩ and a series/access resistance of 10–40 MΩ. The internal recording solution for WCR contained (in mM) K-gluconate (130); KCl (10); NaCl (10); MgCl₂ (1); N2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES, 10), ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N”-tetraacetic acid (EGTA, 10); Mg-ATP (2); and (GTP, 0.3), whereas the nystatin patch solution contained (in mM): K-Acetate (120); MgCl₂ (5), EGTA (10), and HEPES (40). Nystatin was dissolved in dimethyl sulfoxide (DMSO) with Pluronic F127 and added to the internal solution to yield a final concentration of 450 μg/ml. The pH of both solutions was

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adjusted to between 7.2 and 7.4. Tungsten-stimulating electrodes were placed in the hypothalamic region dorsal-medial to the SON to evoke synaptic responses and to apply the high-frequency stimulation (HFS; 10 to 200 Hz for 1 s, applied twice in a 10-s interval). Unless indicated otherwise, all illustrated data used the 100-Hz frequency.

“Blind” patch recordings were made in the SON by the use of an Axopatch 1D amplifier. MCNs were identified on the basis of the delayed onset to action potential generation in response to depolarizing current injection, as originally reported for paraventricular neurons (Tasker and Dudek 1991), and now thought to be characteristic also of SON neurons (Armstrong 1995); however, no attempts were made to assign a phenotype to the neurons on the basis of their current–voltage relationships (see Stern and Armstrong 1995). Most experiments were done on cells voltage-clamped at −80 mV. Input resistance (\(R_{\text{input}}\)) and access resistance (\(R_a\)) of all cells were monitored regularly throughout each experiment by applying a 20-mV hyperpolarizing pulse for 75–100 ms. \(R_{\text{input}}\) was calculated from the steadystate current obtained during the pulse. The decay rate (\(\tau\)) of the capacitance transient was taken as a measure of \(R_a\). Data from cells that showed >15% (estimated inherent variability in evoked responses) change in these parameters were excluded from further analysis. Pharmacologically isolated spontaneous and evoked EPSCs (sEPSCs and eEPSCs) were recorded in the presence of 50 \(\mu\)M picrotoxin. All cells had a graded evoked synaptic response to increasing stimulation intensity; and an intensity giving 50–60% of the maximum evoked EPSC was used to elicit HFS or evoked responses. All data were acquired with the use of pClamp Software (Clampex 5.5 and 7; Axon Instruments). sEPSCs were acquired at a 2- to 5-kHz sampling rate, filtered at 500–1,000 Hz, digitized at 10 kHz, and stored for off-line analysis. Hard-copy chart records were also captured on a Gould Recorder.

Frequency–time plots were generated by taking the mean number of events in 16-s epochs. All values are stated as means ± SE. One-way ANOVA and post hoc tests, as indicated, were used to compare different curves and points. 

For experiments that involved changing holding potential, control frequencies were taken at −80 mV. Cells were then held at the appropriate test potential for about 1 min. HFS was applied at the test potential and the cell membrane returned to −80 mV and data collection resumed. Steady-state current–voltage relationships (I–V curves) were generated by applying slow-voltage ramps from −120 to −40 mV at a rate of 4.5 mV/s. The resulting steady-state current was then captured and stored for off-line analysis.

sEPSCs were detected and counted visually (in Clampfit) and by a commercially available Mini Analysis software (SynaptoSoft, Inc.) and counted if amplitude > 3 pA with fast rise times (\(Tr\); 1–4 ms measured from baseline to peak) and exponential decay. For the measurement of amplitude, \(Tr\), and decay constant (\(\tau\)), only events with a clearly defined baseline (>5 ms) and that did not have shoulders on the rising and falling phases, on visual inspection on an expanded scale, were used. Cumulative probability plots, frequency distribution histogram, graphs, and statistical analysis were done with the use of Mini Analysis, Sigmaplot, and GraphPad software. Amplitude–distribution histograms of sEPSCs were fitted with either one or the sum of several Gaussian curves, by Simplex nonlinear least-squares algorithm (Graphpad Prism 3.0). The number of Gaussian curves fitted to each distribution was determined by eye. For multimodal distribution, mean modal separation \(q\) was calculated from the peak value of each Gaussian curve according to \(q = \exp\left(-x/R_\text{peak}\right)\), where \(x\) refers to the mean value of each Gaussian function numbered \(k = 1, \ldots, n\), and \(n\) is the total number of curves fitted (Edwards et al. 1990). The quantal coefficient of variation (c.v.) of sEPSC amplitude was calculated as (variance of amplitudes in mode 1 − noise variance) \(q \times 100\), assuming that noise variance stays constant throughout peaks, independent of the quantal variance and added linearly (Edwards et al. 1990). The noise variance was calculated as the standard deviation of current recorded in between sEPSCs, for a total of more than 200 ms per sample.

The firing rate of the cells was monitored under current clamp at the resting membrane potentials. Those cells that had no spontaneous activity were injected with constant positive currents (±50 pA) to obtain baseline activity.

All drugs were bath-perfused at final concentrations indicated by dissolving aliquots of stock in the ACSF. All drugs and chemicals were from Sigma, except for 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzox(7)quinoxaline-7-sulfonamide (NBQX) and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA; RBD); Phuronic F127 (BASF), and the oxytocin receptor antagonist ([des-glycinamide\(^{9}\), d(CH\(^{2}\))\(_{5}\), O-Me-Tyr\(^{2}\), Thr\(^{4}\), Orn\(^{8}\)J]-vasotocin) and Manning Compound (Bachem).

R E S U L T S

In this study, 136 MCNs were recorded by the use of either nystatin patch or whole-cell recording (WCR) modes. Recordings made by the use of either technique gave similar access and input resistance (Kombian et al. 1996); however, in our hands, MCNs recorded in WCR mode were less stable, did not last long enough (<30 min from break-in) to perform prolonged experiments, and the evoked EPSCs routinely ran down. To avoid the possibility of washout of essential cellular substances [shown to be important in many plasticity studies (Malenka and Nicoll 1993)], the majority (132 cells) of experiments in this study were done with the use of the nystatin patch-recording technique (Kombian et al. 1996,1997; Mouginot et al. 1998).

Picrotoxin-resistant and NBQX-sensitive sEPSCs or sEPSPs were routinely recorded in MCNs as has been reported (Boudaba et al. 1997; Gribkoff and Dudek 1990; Kabashima et al. 1997; Schrader and Tasker 1997). The recorded sEPSCs had amplitudes, frequencies, and kinetics (decay time) similar to those reported (Kabashima et al. 1997). Because the recorded sEPSCs could arise either from action potentials in local interneurons or from action potential–independent events in the presynaptic terminal, we determined the nature of the sEPSCs by the use of tetrodotoxin (TTX, 1 \(\mu\)M). In six cells tested, TTX completely abolished the evoked EPSCs, but the frequency of the sEPSCs was not altered by TTX (1.95 ± 0.66 Hz in control vs. 2.38 ± 0.99 Hz in TTX, \(n = 6\); \(P > 0.05\); paired Student’s t-test). Thus sEPSCs recorded in MCNs represent mEPSCs, as has been reported before (Kabashima et al. 1997).

After high-frequency stimulation (HFS; 100 Hz) of afferents to the SON, 94% of MCNs tested responded with a marked increase in the frequency of mEPSCs (Fig. 1, A1, A2, and B). These mEPSCs remained sensitive to NBQX after HFS. Similar responses were obtained when MCNs were recorded by the use of WCR (\(n = 4\)). Analysis of the increase in mEPSC frequency after HFS showed that all cells responded with an initially large increase in events (3.141 ± 61.5% of control after two 100-Hz stimuli, \(n = 15\)) that then declined exponentially back to near baseline frequency in 5–20 min (Fig. 1C). The magnitude and duration of the potentiation were not correlated to the basal mEPSC frequency (\(r = 0.17\) and 0.4, respectively; \(P > 0.05\); \(n = 18\); Spearman rank-order correlation). To test the ability of these cells to consistently respond to repeated HFS, we determined whether this short-term potentiation (STP) in mEPSCs could be induced repeatedly. Repeated HFS (two times at 100 Hz, 1 s) consistently induced robust STP with no significant difference between the peak response and...
the time course of the two STPs (Fig. 1D, n = 7, P > 0.05, Kruskal-Wallis one-way ANOVA). In some cells, similar response patterns could be evoked with up to five repetitions. As the previous experiments were carried out with picrotoxin in the bath, it was possible that STP occurred only in situations of blocked GABAergic transmission. Therefore in five neurons, spontaneous events were recorded without picrotoxin in the bath and we observed that HFS induced increases in the frequency of these currents similar to those previously observed after HFS with GABAergic transmission blocked (552.2 ± 263% without picrotoxin and 475 ± 114% in picrotoxin; P > 0.05; data from 2 min post-HFS). Thus excitatory inputs onto MCNs are capable of undergoing STP, even in the presence of opposing inhibitory transmission.

Because potentiation of mEPSCs after HFS of this magnitude and duration has not previously been reported in central neurons, we asked whether it could be seen at more physiological frequencies. As seen in Fig. 2, HFS at frequencies as low as 10 Hz was capable of eliciting a short-lasting STP, which increased in magnitude and duration as HFS frequency increased. Above 100 Hz, STP magnitude and duration began to decrease. We then carried out further studies to identify the nature of the potentiation. As would be expected, HFS in the presence of TTX did not produce any STP (Fig. 3A, n = 6, P < 0.05 vs. control; Kruskal-Wallis one-way ANOVA), indicating that depolarization of afferent axons is necessary to generate STP of mEPSCs. Because action potentials are required to produce STP, it is possible that the observed enhanced synaptic activity arose from both mEPSCs and sEPSCs because of invasion of the terminals by action potentials arising from interneurons that may participate in afferent excitation onto SON MCNs. To investigate this possibility, TTX was applied immediately after HFS and both the evoked and mEPSCs were monitored. Within 2 min of 1 μM TTX application, the evoked EPSC was completely abolished, whereas the mEPSC frequency remained enhanced and followed a similar time course as in control experiments (Fig. 3B, n = 5, P > 0.05, Kruskal-Wallis one-way ANOVA). These TTX experiments indicate that sodium-dependent action potentials are not required for maintenance of STP and point to the synapse as the site for the effect. Furthermore, they indicate that the induction and maintenance of STP are two separate processes that, although causally related, may be independently regulated.

Our experiments would appear to have established the synapse as the locus for these changes. The next question we addressed was to determine whether it is the pre- or postsynaptic side of the activated synapses that is critical for the initiation of STP? We used several approaches to examine the contribution of MCNs to the generation of STP. First, we tested for the participation of postsynaptic N-methyl-D-aspartate (NMDA) receptors in STP, because these receptors are known to be involved in several forms of synaptic plasticity in other brain regions (Bliss and Collingridge 1993; Malenka and Nicoll 1993). Furthermore, they are voltage dependent and any
Next, we asked whether a brief depolarization of the postsynaptic cell alone is sufficient to induce STP. Therefore, in 16 MCNs, we briefly exited from voltage clamp to inject two or three depolarizing currents, by the use of the same duration and interval as the HFS, to initiate action potentials. This treatment did not affect the frequency of mEPSCs recorded when we immediately returned to voltage clamp (Fig. 4C); nonetheless, the synapses onto these neurons were amenable to STP as subsequent HFS applied to five of these cells induced robust STP. The results from these postsynaptic manipulations strongly suggest that it is the presynaptic terminal and not the MCN that is the site of induction of STP.

In addition to the marked increase in the frequency of mEPSCs, HFS appears to produce an increase in the mean mEPSC amplitude and this was substantiated by analysis (11.3 ± 1.23 pA pre-HFS and 14.6 ± 0.95 pA post-HFS, P < 0.05, n = 9; see Fig. 1A). Because this effect is often associated with a postsynaptic change, we performed a series of experiments and post hoc analysis of mEPSCs to determine whether postsynaptic cell properties were altered during STP. First of all, we asked whether there was a post-HFS change in the conductance of the recorded MCN in a voltage range that may contribute to the changes in mEPSCs, but that may not have been detected by the previous postsynaptic manipulations. In five MCNs, we applied slow-voltage ramps before and 2 min post-HFS and the steady-state currents were recorded. The two I-V curves were superimposable over the voltage range −100 to −40 mV, indicating that there was no conductance change in the physiologically relevant voltage range after HFS (Fig. 5A). Furthermore, neither the input resistance R_in, measured around resting membrane potential (766 ± 56 MΩ before, 736 ± 56 MΩ after, n = 7, P > 0.05 by paired t-test), nor the access resistance R_access both monitored regularly throughout each experiment, changed after the HFS (n = 5; Fig. 5B).

Next, we asked whether the properties of the postsynaptic non-NMDA receptors underlying the mEPSCs changed after STP. Repeated brief (5–15 s) bath applications of AMPA (5 μM) or kainate (5 μM) at intervals of 2 to 5 min produced consistent steady-state currents (3.8 ± 2.7% between application variation, n = 7; P > 0.3 for AMPA and 11.1 ± 8.0, n = 6; P > 0.4 for kainate; Fig. 5C). The AMPA-induced inward currents before and 30 s to 2 min post-HFS were identical (15 ± 10.2% change; n = 5, P > 0.05, paired t-test; Fig. 5C). Even though we detected an increase in the amplitude of the mEPSCs, the lack of a detectable increase in postsynaptic steady-state AMPA response suggests that HFS does not affect steady-state properties of postsynaptic AMPA receptors.

Despite this lack of available evidence in support of postsynaptic changes in AMPA receptors on MCNs during STP, others have reported possible activation of previously silent kainate receptors after HFS of hippocampal mossy fibers (Castillo et al. 1997). We could not discount this possibility, because kainate will activate postsynaptic receptors on magnocellular neurons (Hu and Bourque 1991) and the blockade of mEPSCs by NBQX after HFS would not differentiate between AMPA and kainate receptors (Wilding and Huettner 1996). However, application of kainate before and after HFS resulted in similar currents (−4.0 ± 11.4% change; n = 4, P > 0.05), indicating
that postsynaptic kainate receptors are unlikely to be responsible for the appearance of the new and big events.

Given the similarity of both electrical and pharmacological properties of the MCNs before and after HFS, we believe that the most likely reason for the increase in large amplitude events is the increased probability of multiquantal mEPSCs; thus we next performed an analysis of the amplitude distribution of mEPSCs before and after HFS in a typical cell (Fig. 6A). As described in METHODS, only those that could be distinguished as individual events were used for this amplitude analysis. When the amplitude distribution histograms of the events before and after HFS were plotted, the latter showed a much greater skewing to the right in all seven cells tested. In control condition, mEPSC amplitude distribution was best fitted by one to three Gaussian curves with mean mode separation \( q \) of 8.17 ± 0.66 pA, c.v. = 16.1 ± 3.0% (\( n = 7 \)). One to 2 min post-HFS, three to four Gaussian curves could be best fitted to mEPSC amplitude distribution. Mean mode separation \( q \) was 7.82 ± 0.71 pA (\( P = 0.14 \) vs. control), c.v. = 13.6 ± 1.0% (\( P = 0.45 \) vs. control). Mean standard deviation of \( q \), calculated from each modal peak \( (x_k/k) \) in these cells, was 6.51 ± 2.29% of \( q \) in control and 6.79 ± 0.93% after HFS (\( P = 0.78 \)).

Relatively low variance of the mean mode separation supports the equidistance of the modes, and suggests that mode 1 represents the quantal unit and the other modes are made up of summed quanta, released synchronously or near synchronously. The mean mode separation \( q \) (i.e., quantal unit) was the same before and after HFS, indicating the STP of mEPSCs did not result from an increase in the amplitude of small, previously undetected events because of changes in postsynaptic cell properties, or preferential recruitment of large vesicles after HFS. The putative multiquantal events used in the preceding analysis had a smooth rising phase, and thus from their waveform no indication of summation of small events could be ascertained (Fig. 6B, top trace). However, in some other putative multiquantal events, shoulders could be observed on their rising phase (Fig. 6B, middle and bottom traces), supporting the idea that the big events are indeed composed of smaller units. Thus the evidence from the preceding analysis suggests that the appearance of big events is the result of multiquantal release of transmitter.

The possibility remains that the spatial distribution of synapses activated after HFS is different. For example, under basal conditions, distally located synapses may predominate, whereas after HFS, the synapses closer to the recording site in the cell body could be preferentially activated, thereby giving bigger mEPSCs. We tested for this possibility by examining the relationship among the amplitude, rise time, and decay constant of mEPSCs before and after HFS. We considered that mEPSCs arising from locations closer to the soma would have a larger amplitude and faster rise and decay times than more distant mEPSCs because of less dendritic filtering (Bekkers and Stevens 1996). We calculated the rise time and decay constants in a typical cell that showed a clear shift in both frequency and amplitude distributions. As shown in Table 1 and Fig. 6C, despite the de novo appearance of a population of large events after HFS, the rise times and decay constants of both large- and small-amplitude events after HFS were iden-
plasticity studies to induce long-lasting changes of evoked synaptic responses. In this study, because HFS induces increases in mEPSC frequency and amplitude, it is important to determine whether there is also a parallel change in the evoked EPSC. To carry out these experiments, we perfused the slice with a vasopressin/oxytocin antagonist, Manning compound (10 μM), to block the actions of dendritically released peptides that could influence synaptic inputs (Kombian et al. 1997). When HFS was applied, robust STP of mEPSC could be induced in all eight cells tested (Fig. 7A). However, analysis of the simultaneously collected evoked EPSC amplitude revealed that there was no significant change in the evoked EPSC amplitude (71.5 ± 21.3% of control; P > 0.05; Fig. 7, B and C; value from 2 min post-HFS). This result indicates that HFS has two different effects on evoked EPSC and mEPSCs.

Finally, to investigate a functional significance of this phenomenon, we asked whether there was a change in the excitability of MCNs after the induction of STP. We therefore measured the action potential firing rate, under current clamp, as an index of MCN excitability. In four spontaneously firing cells, HFS caused an increase in the firing rate (1,200 ± 214%; Fig. 8, A and B). This increase in frequency followed a time course that was similar to that of STP of the mEPSCs (Fig. 8C). Similar increases in frequency (1,036 ± 411% of control; n = 12; P < 0.001) were observed for cells that were held near spike threshold by constant positive current injection. In all cells included in this analysis, we initially or subsequent to action-potential measurements (current clamp), voltage-clamped the cells and showed that they exhibited STP of mEPSCs after HFS (n = 16; Fig. 8, A and C).

Because HFS causes both STP of mEPSCs and increases in spike frequency, we asked whether these two events were causally related. Because oxytocin is known to have postsynaptic excitatory effects within this nucleus resulting from dendritic release (Ludwig 1998), it is possible that the increased activity of MCNs was subsequent to depolarization caused by released oxytocin. When we repeated these experiments during perfusion of an oxytocin receptor antagonist ([des-glycinamide<sub>9</sub>,d(CH<sub>2</sub>)<sub>5</sub>, O-Me-Tyr<sup>2</sup>, Thr<sup>3</sup>, Orn<sup>8</sup>]-vasotocin; 10 μM) (Elands et al. 1988; Kombian et al. 1997), the HFS was equally effective in increasing action-potential frequency (1,471 ± 616% of control, n = 4; P < 0.05, compared with control). We also considered the possibility that the postsynaptic depolarization itself elicited a long-lasting change in the postsynaptic cell properties, but injection of depolarizing current alone into the MCN did not alter subsequent spontaneous activity (140 ± 27% of control; n = 4; P = 0.1).

The similar time course of STP of mEPSC and action potential firing frequency suggests that the former may be responsible for the latter. If this is the case, then blockade of non-NMDA receptors that mediate the mEPSCs should also block any change in firing frequency. In five cells that were initially shown to respond to HFS with STP, bath application of NBQX (1 μM) eliminated all the mEPSCs. When these cells were subsequently subjected to HFS (under continuing NBQX blockade) in current clamp, there was no change in the spike frequency (78.7 ± 24.2%, P < 0.05 vs. no NBQX). Thus changes in mEPSC frequency and possible temporal and spatial summation of spontaneous events can lead to sustained changes in the excitability of MCNs and thus their firing pattern.
DISCUSSION

We have shown that excitatory afferents to MCNs of the SON can undergo a type of plasticity that may contribute to the generation of increased spontaneous activity. We have observed that physiologically relevant high-frequency afferent stimulations lead to short-term (5–20 min) enhancement in frequency of TTX-resistant spontaneous excitatory postsynaptic responses. To the best of our knowledge, enhancement of this magnitude and duration has not previously been described.

Salient features of this phenomenon include:

1) its induction is independent of postsynaptic NMDA receptors, membrane potential, or input resistance;

2) it is independent of 

3) its expression is action potential independent;

4) it is not affected by retrograde endogenous neurohypophyseal peptides; and

5) it causes an increase in the spontaneous activity of the postsynaptic MCN. Synapses that undergo such an excitability change will be predicted to contribute to or influence the pattern of firing that optimizes peptide release.

Posttetanic potentiation of mEPSC frequency was reported at the neuromuscular junction over 40 yr ago (Brooks 1956). Since then, potentiation of both mEPSC and mIPSC frequencies has been reported intermittently in central synapses (Atluri and Regehr 1998; Cummings et al. 1996; Goda and Stevens 1994; Jensen et al. 1999a,b; Mennerick and Zorumski 1995; Oliet et al. 1996). There is general agreement that posttetanic potentiation is related to increased levels of intracellular calcium that persist beyond the time of the stimulus train. What differentiates previously reported posttetanic potentiation from the phenomenon we report here is the magnitude and duration of the response. To the best of our understanding, the time course reported previously lasts for milliseconds to a few seconds, whereas the STP in the SON is of at least several minutes’ duration. Because of this radically different time course, we have carried out a number of experiments to define its site of induction and to rule out possible postsynaptic contributions.

Locus of STP induction is presynaptic

The locus of induction and maintenance of long-term synaptic enhancement in other brain regions has been intensively investigated. Evidence exists supporting both pre- and postsynaptic loci (Bliss and Collingridge 1993; Nicoll and Malenka
1995). Our experiments suggest that the induction of STP of mEPSCs in the SON is presynaptic to the MCNs. First, the ability to generate STP was independent of the voltage at which the postsynaptic cell was held during HFS. In fact, STP could be induced while the postsynaptic cell was voltage-clamped at a very negative potential (−80 mV). Second, positive current injected into the postsynaptic cell, to cause it to fire a burst of action potentials, did not induce STP.

In some types of synaptic plasticity, the phenomenon is dependent on NMDA-receptor activation in the postsynaptic cell (Malenka and Nicoll 1993). Because functional NMDA receptors are present in this nucleus (Hu and Bourque 1991; Nissen et al. 1995), perhaps colocalized with AMPA receptors (Stern et al. 1999), we considered their participation in the induction of STP. However, blockade of these receptors with D-APV did not affect the magnitude or duration of the STP. Furthermore, changes in postsynaptic holding potential, which will affect the contribution of NMDA receptors to STP, did not affect its induction. NMDA-receptor–independent induction of synaptic plasticity has been reported in other selected brain regions (Nicoll and Malenka 1995) but the accompanying change in mEPSC frequency has not been reported.

**HFS does not alter postsynaptic cell properties**

HFS would be expected to cause massive activation of the SON, resulting in alterations in extracellular ion concentrations and dendritic release of neurohypophyseal peptides. We could detect no alteration in postsynaptic cell properties that might result from these events that could account for the expression of STP.

Quantal analyses of amplitude–frequency distributions of miniature events have been used as indicators of pre- and postsynaptic loci of synaptic changes (Bekkers and Stevens 1990; Malinow and Tsien 1990). In this study, mEPSCs showed clear frequency and amplitude increases suggesting both pre- and postsynaptic changes may be involved. However, the steady-state and rapid kinetic properties and apparent distribution of the non-NMDA receptors that underlie the STP appeared unchanged.

First of all, the AMPA-induced steady-state whole-cell cur-

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**TABLE 1. Amplitude and kinetic parameters of mEPSCs before and after HFS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mEPSC Amplitude, pA</th>
<th>Rise Time, ms</th>
<th>Decay Constant, ms</th>
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<tbody>
<tr>
<td>Pre-HFS</td>
<td>8.59 ± 1.44</td>
<td>2.85 ± 0.25</td>
<td>3.58 ± 0.66</td>
</tr>
<tr>
<td>Post-HFS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>9.5 ± 0.90</td>
<td>2.78 ± 0.22</td>
<td>3.28 ± 0.27</td>
</tr>
<tr>
<td>Big</td>
<td>27.4 ± 1.07*</td>
<td>2.63 ± 0.21</td>
<td>3.67 ± 0.33</td>
</tr>
</tbody>
</table>

Values are means ± SE, representing a summary of amplitude and rapid kinetic parameters of miniature excitatory postsynaptic currents (mEPSCs) in a supraoptic nucleus (SON) magnocellular neuron that exhibited robust increases in the frequency and amplitude of mEPSCs after high-frequency stimulation (HFS). mEPSCs before HFS were chosen on the basis of Gaussian distributions as those between 5 and 12 pA in amplitude; there were too few larger events in this cell to obtain mean data. mEPSCs after HFS were classified into 2 broad groups (on the basis of Gaussian distributions) as small events (5–12 pA) and big events (18–41 pA); n = 18–26 events. * P < 0.05 compared to small events (pre- and post-HFS).
FIG. 7. STP of mEPSC is not accompanied by an increase in evoked EPSC amplitude. A: chart record of the membrane current of an MCN in the presence of 10 μM Manning compound. HFS induces a robust STP of mEPSCs. Regular upward and downward deflections represent, respectively, stimulus artifacts during evoking EPSCs and membrane responses to voltage pulses applied to monitor \( R_{\text{input}} \) and \( R_{\text{e}} \). B: sample averaged evoked EPSCs from the cell in A taken at the times indicated by letters. Note that these traces were filtered at 200 Hz before averaging at the end of the experiment. C: normalized averaged time–effect plot of evoked EPSC amplitude (n = 8) before and after HFS.

The ability to induce STP in the presence of opposing GABAergic transmission makes it novel, because GABAergic transmission is known to strongly influence the generation of other forms of synaptic plasticities (Davies et al. 1991; Kano et al. 1993). However, there was no qualitative or quantitative difference between the STP in the presence and absence of picrotoxin, a GABA\( _A \) receptor channel blocker. Nonetheless, because functional presynaptic \( \text{GABA}_B \) receptors are present on excitatory terminals in this nucleus (Kabashima et al. 1997; Kombian et al. 1996), it is possible that a presynaptic action of GABA on \( \text{GABA}_B \) receptors will limit the duration of an otherwise long-term change to a short-term change. This possibility remains to be explored.

STP is not a property of a network

The SON receives afferent fibers not only from a number of distant nuclei (Tribollet et al. 1985) but also from local inter-
neurons. The focal stimulation used in our experiments could activate either or both inputs. Previous studies (Boudaba et al. 1997) have shown that repetitive stimulation of the excitatory interneurons can elicit afterdischarges in MCNs, possibly resulting from metabotropic glutamate receptor activation. However, those afterdischarges were abolished by TTX, indicating...
that they were the result of increased spike generation in presynaptic cells. The STP reported in the present experiments appears to be quite different. Although initiation of STP requires action potentials, presumably to invade the presynaptic terminal after stimulation of the afferent axons, maintenance of the STP is not sensitive to TTX; thus it appears to be the axon terminal or some process limited to the terminal area that is altered after HFS to cause STP.

**HFS differentially modulates mEPSCs and evoked EPSCs**

We have earlier reported that HFS, such as that used in this study, depresses evoked EPSCs as a consequence of dendritically released oxytocin and vasopressin (Kombian et al. 1997). Despite this effect on evoked EPSCs, HFS causes a massive increase in miniature events, that is, STP of mEPSCs. Thus dendritically released oxytocin and vasopressin appear to have different effects on the two events. We next examined whether, in the absence of these peptides’ effect, HFS differentially affected evoked and mEPSCs. In the presence of Manning compound, an oxytocin and vasopressin receptor antagonist (Kombian et al. 1997), the evoked EPSC remained unchanged, whereas we could still induce robust STP of mEPSCs with HFS. These findings suggest that spontaneous and evoked release of glutamate in the SON can be differentially regulated. Similar observations concerning the differential modulation of evoked and spontaneous synapatic responses have been reached in several other systems (Auger et al. 1998; Bao et al. 1998; Cummings et al. 1996). Although the mechanism for this differential regulation is still obscure, it could have its basis in different calcium requirements of evoked and mEPSCs in the SON; that is, evoked EPSCs require calcium entry through voltage-gated calcium channels (unpublished observations), whereas mEPSCs are thought to be more dependent on intracellular stores of calcium (Inenaga et al. 1998). Alternately, the mEPSCs seen after STP may involve active zones/synapses that are not utilized in evoked transmitter action or release at the 0.1 Hz stimulation frequency we used. It remains to be seen whether evoked EPSCs elicited at higher frequencies would be increased.

**Physiological relevance**

The physiological relevance of the massive, short-term increase in mEPSCs is underscored by our observation that the postsynaptic MCNs increase their firing rate during this period of enhanced presynaptic activity. Because individual mEPSCs in the SON have been reported to be capable of eliciting action potentials (Inenaga et al. 1998), the increase in the excitability of MCNs during STP can result from big, multiquantal events. This is further underscored by the observation that elimination of the mEPSC with a specific antagonist of the non-NMDA receptors prevents the change in excitability of the MCN. Such an increase in clustered events could arise from afferents activated by changes in osmolality (Richard and Bourque 1995) and lead to changes in firing patterns to optimize peptide release (Nissen et al. 1995).

There is little doubt that the spectacular phasic activity of vasopressinergic neurons is generated by intrinsic conductances (Armstrong 1995). Nonetheless, these cells also must attain a threshold firing rate before these regenerative mechanisms come into play (Poulain et al. 1988). This may be accomplished through a glutamate-induced depolarization, because glutamate application to MCNs can activate phasic bursts (Wakerley and Noble 1982) and maintenance of spontaneous phasic firing requires tonic synaptic activation involving glutamate receptors (Jourdain et al. 1998; Nissen et al. 1995). We suggest that the STP seen in response to strong synaptic stimulation could provide the background glutamate activity reported to be necessary for phasic activity. Whereas the STP is most evident at higher frequencies, most likely above the physiological range, it can also be seen at more physiological frequencies (i.e., <0.5 Hz). The persistence of the enhanced synaptic activity seen after HFS could also provide the ideal condition for temporal summation of EPSCs, whereby afferent input from different activated pathways could summate, or indeed predispose, the neuron to other types of plasticity (cf. Abraham and Bear 1996). Although the molecular mechanisms underlying the STP are not yet known, the present data indicate that a unique type of synaptic potentiation may be found in a nucleus in which such changes can be correlated with known physiological functions. It has recently been proposed that miniature events may encode information in postsynaptic cells (Staley 1999). We have shown in this study that, in the SON, changes in the occurrence of miniature events remarkably influence the excitability of MCNs.

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**REFERENCES**


