Glutamate Microstimulation of Local Inhibitory Circuits in the Supraoptic Nucleus From Rat Hypothalamus Slices

JEAN-PIERRE WUARIN
Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, Colorado 80523

Wuarin, Jean-Pierre. Glutamate microstimulation of local inhibitory circuits in the supraoptic nucleus from rat hypothalamus slices. J. Neurophysiol. 78: 3180–3186, 1997. The hypothesis of a local inhibitory input to the hypothalamic supraoptic nucleus was tested with combined glutamate microstimulation and whole cell patch-clamp recordings in slices from rat hypothalamus. Synaptic activity in supraoptic magnocellular neuroendocrine cells (MNCs) was monitored and glutamate microdrops were applied in the perinuclear region of the supraoptic nucleus to evoke firing of action potentials in putative presynaptic inhibitory cells. The effect of glutamate microdrops applied in the perinuclear region was tested on 57 supraoptic MNCs. In control conditions, spontaneous excitatory (EPSCs) and inhibitory (IPSCs) postsynaptic currents were observed at resting membrane potential in all MNCs tested. Glutamate microstimulation evoked an abrupt increase in the frequency and size of spontaneous IPSCs in eight MNCs. Forty-nine MNCs did not show any change in the inhibitory synaptic input. Microapplication of glutamate in the periphery of the supraoptic nucleus did not modify the amplitude or the frequency of spontaneous EPSCs in any of the 57 MNCs tested. In the group of eight MNCs that responded to glutamate microstimulation by an increase in inhibitory input, two types of responses were observed. Four MNCs showed an increase in both size and frequency of spontaneous IPSCs through the entire range of amplitude. In the other four MNCs, local glutamate stimulation produced a dramatic increase in the size of IPSCs and a lesser increase in the frequency of the smaller IPSCs. The potential effect of the glutamate-evoked increase in inhibitory input on the firing activity of MNCs was tested in current-clamp conditions. Intracellular current injection was applied to evoke firing of action potentials in six MNCs that had responded to local glutamate microstimulation by an increase in inhibitory input. Glutamate microdrop applications inhibited the evoked action potential firing in all six cells. These results suggest 1) that local inhibitory interneurons are present in the periphery of the supraoptic nucleus, 2) that they contain functional glutamate receptors, 3) that they form inhibitory synapses with supraoptic MNCs, and 4) that activation of these interneurons inhibits firing in MNCs. These results support the hypothesis that local inhibitory interneurons play an important role in the firing activity of supraoptic MNCs.

INTRODUCTION

γ-Aminobutyric acid (GABA) is considered to be the dominant inhibitory neurotransmitter in the hypothalamus (Decavel and van den Pol 1990; van den Pol 1985). Approximately one-half of the synaptic boutons in the supraoptic nucleus are immunoreactive for GABA (Decavel and van den Pol 1990). A large body of literature shows that GABA inhibits firing in magnocellular neuroendocrine cells (MNCs) (for a review, see Renaud and Bourque 1991), in particular the bursting activity, which has been shown to facilitate hormonal release (Poulain and Wakerley 1982). MNCs that synthesize and secrete vasopressin and oxytocin are the final effectors for neuronal inputs involved in blood pressure regulation, as well as during parturition and lactation. Therefore a GABAergic synaptic input to MNCs could influence directly the amount of vasopressin and oxytocin in the blood circulation by modulating firing activity.

Several lines of evidence suggest that synaptic inhibition mediated by GABA may regulate the secretion of both oxytocin and vasopressin by depressing the activity of MNCs. Intracerebroventricular injections, as well as microinfusions in the supraoptic nucleus, of the GABA_A agonist muscimol and of the antagonist bicuculline, inhibited the milk ejection reflex (Voisin et al. 1995). Both muscimol and bicuculline also inhibited the background firing of identified oxytocinergic and vasopressinergic cells and delayed the high-frequency bursts in oxytocinergic cells (Voisin et al. 1995). In contrast, another in vivo study showed that GABA and the GABA_A agonist isoguvacine injected in the supraoptic nucleus evoked or facilitated the occurrence of high-frequency bursts in oxytocinergic cells, whereas GABA_A antagonists inhibited the suckling-induced bursting activity (Moos 1995). Taken together, these data strongly suggest that GABA inputs to the supraoptic nucleus are involved in the regulation of bursting activity of oxytocinergic neurons during lactation. However, the nature of the mechanism of action of GABA on the milk ejection reflex is still unclear.

The notion that the supraoptic nucleus may be surrounded by a discrete perinuclear zone was first proposed by anatomic studies using autoradiography and immunohistochemical-tracer injections showing that projections from limbic structures terminate in the periphery of the supraoptic nucleus forming a “halo” around this nucleus (Sawchenko and Swanson 1983; Silverman and Oldfield 1984). Immunohistochemical studies have shown neurons immunopositive for GABA and glutamic acid decarboxylase in and around the supraoptic nucleus (Herbison 1994; Roland and Sawchenko 1993; Theodosis et al. 1986; van den Pol 1985). These results suggest that a substantial portion of the inhibitory input to supraoptic MNCs may originate from local GABAergic interneurons. The general hypothesis tested here is that an important mechanism for synaptic regulation of hormonal release involves “feed-forward” inhibition of supraoptic MNCs by local GABAergic neurons. With combined patch-clamp recordings and glutamate microstimulation, the following specific hypotheses were tested: 1) glutamate-evoked action potential firing in GABAergic cells located in the periphery of the supraoptic nucleus should produce or increase inhibitory synaptic input in supraoptic MNCs and 2)
an increase in the level of the local inhibitory synaptic input to supraoptic nucleus should inhibit firing activity in supraoptic MNCs.

METHODS

Slice preparation

Male Sprague-Dawley rats (70–120 g) were decapitated and their brains dissected and placed for ~1 min in oxygenated, ice-cold perfusion solution containing (in mM) 124 NaCl, 26 NaHCO₃, 3 KCl, 1.3 MgSO₄, 1.4 NaH₂PO₄, 1.3 CaCl₂, and 10 glucose, pH 7.4. A block of tissue containing the hypothalamus was then cut and glued on the stage of a Vibratome (Campden Instruments). One or two slices 400–600 µm thick, containing the supraoptic nucleus, were cut frontally and transferred to a recording chamber (Haas et al. 1979). In the recording chamber, the slices were thermoregulated (~34°C), oxygenated (95% O₂-5% CO₂), and perfused (1.5 ml/min) with a solution identical to the one used for the dissection.

Recording methods

Whole cell patch-clamp recordings were obtained in hypothalamic slices as described by Blanton et al. (1989). Pipettes were pulled from borosilicate glass capillaries of 1.7 mm diam and 0.5-mm wall thickness. Open resistances ranged from 2 to 5 MΩ and seal resistances from 1 to 10 GΩ. Series resistance ranged from 4 to 23 MΩ with a mean of 12 ± 0.5 (SE) MΩ (n = 54). No series compensation was applied. Patch pipettes were filled with a solution containing (in mM) 140 K-gluconate, 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 1 NaCl, 1 CaCl₂, 1 MgCl₂, 5 bis-(o-aminophenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA), and 4 ATP, pH 7.2. ATP was added to prevent the rundown of inhibitory postsynaptic currents (IPSCs) (Stelzer et al. 1988). Hypothalamic slices were transilluminated and the electrodes were positioned into the supraoptic nucleus under visual control. Electrical signals were recorded with an Axopatch-1D amplifier, low-pass filtered at 2 kHz, and digitized at 44 kHz with a Neuro-Corder (Neurodata) for storage on video tapes. The amplitude and frequency of spontaneous synaptic currents were measured on a personal computer with pClamp 6 (Axon Instruments). Data were analyzed with sampling rates ranging from 5 to 20 kHz.

Glutamate microapplication

Glutamate microdrops were used to evoke action potential firing selectively in the soma and dendrites of putative inhibitory cells located in the periphery of the supraoptic nucleus, without activation of firing activity in passing axons (Christian and Dudek 1988), and in axons from distal sources such as the diagonal band of Broca (Randle et al. 1986). Patch pipettes were used to apply glutamate microdrops on the surface of the slices. Their tip (2–4 µm OD) was coated with Sylgard (Dow Corning) to prevent the microdrops from being pulled back from the tip by capillary forces. The pipettes were then filled with perfusion solution containing glutamate (20 mM, L-glutamic acid, Sigma). Pressure was applied to the back of the pipettes with a Picospritzer (General Valve). Microdrops (50–150 µm diam) were formed at the tip of the pipette and stayed attached. They were then lowered on the surface of the slice under visual control. A stereomicroscope equipped with a reticle (20-µm resolution) was used to measure microdrop diameters and to place them in the selected areas. The entire periphery of the supraoptic nucleus was systematically stimulated. The relative position of the sites of application of the microdrop was described as medial, dorsal, and lateral to the supraoptic nucleus.

RESULTS

Whole cell recordings were obtained from 57 supraoptic MNCs. At a holding potential corresponding to the resting membrane potential, spontaneous IPSCs and excitatory postsynaptic currents (EPSCs) were observed in all the cells. In MNCs, inhibitory postsynaptic potentials (IPSPs) are hyperpolarizing at resting membrane potential (Randle et al. 1986). Therefore spontaneous inward postsynaptic currents were considered as IPSCs and outward postsynaptic currents as IPSCs. This was confirmed in a previous study, by using selective blockers for GABA and glutamate receptors (Wuarin and Dudek 1993).

Effect of local glutamate microstimulation on IPSCs

Fifty-seven supraoptic MNCs were recorded in 38 slices and tested with microapplication of glutamate in the periphery of the supraoptic nucleus. Mean resting membrane potential was −62 ± 0.9 mV (n = 54) and mean input resistance, measured from resting membrane potential with a 10-mV negative voltage step, was 985 ± 44 MΩ (n = 51). Eight supraoptic MNCs (14%) responded to local stimulation by an abrupt increase in the frequency and amplitude of IPSCs (Fig. 2). In this group of cells, the frequency of spontaneous IPSCs in control ranged from 0.5 to 3 Hz. The change in IPSC frequency evoked by glutamate microstimulation varied considerably between cells. The frequency of IPSCs increased from <1 Hz in control to 3–5 Hz (n = 2), e.g., Fig. 2A), 6–10 Hz (n = 2), and 15–25 Hz (n = 2), e.g., Fig. 2B) during the stimulation. In two cells, the IPSC frequency increased from 1–3 to 5–10 Hz. Forty-nine supraoptic MNCs did not show any change in the frequency and the amplitude of the spontaneous IPSCs. If no positive response could be evoked, microdrops were then applied into the supraoptic nucleus close to, or directly onto, the recording electrode to evoke a direct effect on the recorded MNC, to verify that the stimulation was effective. All the nonresponding cells could be activated directly by glutamate microdrops, suggesting that the stimulation was effective (Fig. 3).
FIG. 3. Example of direct activation of a supraoptic magnocellular neuroendocrine cell (MNC) by glutamate microdrops. First stimulation (A) was done 50 μm away from point of entry of the recording electrode in the slice; 2nd microdrop application (B) touched the recording electrode. Diameter of the glutamate microdrop was 50 μm. Recorded MNC was located 150 μm deep into the slice. Arrows, artifact produced by the microdrop touching the slice. interruptions labeled a and b are 20 s.

IPSCs in the recorded MNCs were stimulated repetitively to evaluate if a progressive loss of the increase in the inhibitory input could be detected. All the positive cells were stimulated from 5 to 11 times. Repeated stimulations produced similar increases in IPSCs (Fig. 4) and no tachyphylaxis of the effect was detected, even after repeated stimulations over a period a several tens of minutes.

The effect of local glutamate stimulation on the frequency and the amplitude of spontaneous EPSCs was evaluated in all supraoptic MNCs tested. In the group of 49 cells that did not show any change in the inhibitory input to glutamate microstimulation, the spontaneous EPSCs were not modified either. In the group of cells that showed an increase in the frequency of IPSCs to <10 Hz, no significant effect on the frequency and size of spontaneous EPSCs was detected (Fig. 5). In MNCs that showed an increase in the IPSC frequency to more than 10 Hz (n = 2), a small decrease in the frequency of EPSCs was detected during the peak of the microstimulation effect (e.g., Fig. 2B). This effect was more pronounced in MNCs showing the highest increase in IPSC frequency. Therefore this apparent decrease in the inhibitory input was probably the result of an increased shunting of

For each responding cell, glutamate microdrop application in only one of the three sites tested resulted in an increased inhibitory input. Stimulation of the other two sites were without effect. Glutamate microstimulation of the dorsal site evoked an increase in IPSCs in four MNCs, the lateral site in two MNCs, and the medial site in two MNCs. This result suggests that inhibitory GABAergic interneurons located throughout the periphery of the supraoptic nucleus send projections to supraoptic MNCs.

The potential neurotoxicity of glutamate, when used at a high concentration (20 mM), might have damaged the local inhibitory neurons and therefore resulted in loss of inhibitory input. To investigate this possibility, all the sites in which application of glutamate microdrops evoked an increase in

FIG. 2. Two examples of the effect of local glutamate microstimulation in supraoptic MNCs. A and B: whole cell patch-clamp recordings of 2 supraoptic cells obtained at resting membrane potential: −67 and −70 mV, respectively. In both, microdrops were applied dorsally to the supraoptic nucleus. Input resistance was 1 GΩ in both. Traces are continuous in A and B. A1 and B1 are frequency histograms of the inhibitory postsynaptic currents (IPSCs) in the cells shown in A and B. Arrows, time of microdrop application.

FIG. 4. Effect of repeated glutamate microdrop applications on frequency of IPSCs. Frequency histogram showing repetitive increase in IPSCs frequency in response to 3 glutamate microstimulations applied in the lateral region of the supraoptic nucleus. Distance between recording electrode and site of application was 200 μm. All 3 drops were applied in the same spot. Cell input resistance was 1.6 GΩ. Resting membrane potential was −63 mV.
FIG. 5. Glutamate microstimulation did not change frequency of excitatory postsynaptic currents (EPSCs). A: synaptic activity during the effect of a microdrop of glutamate applied in dorsal area. Time refers to frequency histogram (B); traces are consecutive. B: amplitude histogram for both EPSCs and IPSCs. Arrow, time of application of the glutamate microdrop. Cell input resistance was 1 GΩ and holding potential was resting membrane potential (∼68 mV).

the IPSCs on the EPSCs and not to a decrease in the excitatory input.

Characteristics of glutamate-evoked IPSCs

Glutamate microstimulation produced two qualitatively different types of increase in inhibitory input to MNCs. Although glutamate microdrops increased both frequency and amplitude of IPSCs in all the responsive MNCs, one group of cells was characterized mostly by an abrupt increase in the frequency of IPSCs (e.g., Fig. 2B). The other group showed a dramatic increase of the amplitude of IPSCs (Fig. 6). Cumulative probability plots were built to quantify the effect of the glutamate microstimulation on the amplitude and frequency of IPSCs. Glutamate microstimulation produced a constant shift to the right of the cumulative probability distribution in the four supraoptic MNCs that showed an abrupt increase in the frequency of IPSCs (Fig. 7A). In contrast, in the four MNCs that showed particularly large IPSCs during the effect of glutamate microstimulation, the cumulative probability plots revealed a bimodality characterized by a break in the distribution of the IPSCs (Fig. 7B). Repploting the distributions as the frequency for a given amplitude revealed that, in the MNCs that showed a high frequency of IPSCs in response to glutamate microstimulation, the frequency of the IPSCs was increased throughout the entire range of amplitudes (Fig. 7C). In the group of MNCs characterized by an increase in the size of the IPSCs, repploting the amplitude distributions showed a population of large IPSCs that were not present in control and only modest increase of the frequency of the smaller events (Fig. 7D). These results suggest that increased action potential firing in local interneurons sending terminals into
membrane potential was

MNCs, spontaneous IPSCs and EPSCs were clearly detected of inhibiting firing activity in the majority of supraoptic

Glutamate has been shown to evoke action-potential firing of the supraoptic nucleus and to record with high resolution

Lesions including, but probably not limited to, the perinuclear zone (Herbison 1994; Roland and Sawchenko 1993; Theodosis et al. 1986), the projections identified as originating from this region seem sparse (Roland and Sawchenko 1993). These authors did not find many projections, in particular GABAergic projections, from the perinuclear zone into the supraoptic nucleus and concluded that the contribution of this area to the innervation of the supraoptic nucleus appears to be weak. Therefore this study corroborates the present results suggesting that only a fraction of the supraoptic MNCs receives inhibitory input from local GABAergic cells.

Several studies using in vivo electrophysiological recordings indicate that local inhibition may produce a robust and consistent inhibitory input to supraoptic nucleus, at least to vasopressinergic MNCs. Increase in blood pressure, as well as direct electrical stimulation of the diagonal band of Broca, selectively inhibited firing in supraoptic vasopressinergic MNCs (Jhamandas and Renaud 1986a,b). Neurochemical lesions of the diagonal band of Broca have been shown to disrupt the baroreflex-evoked inhibition of supraoptic MNCs (Cunningham et al. 1992), pointing to the diagonal band as a major relay structure in the baroreceptor reflex. However, although the diagonal band contains GABAergic neurons, very few diagonal band GABAergic neurons seem to project directly into the supraoptic nucleus; their terminals seem to be concentrated mostly in the perinuclear area of the supraoptic nucleus (Jhamandas et al. 1989). Both activation of peripheral baroreceptors with intravenous injections of a vasoconstrictor and direct electrical stimulation of the diagonal band consistently induced an inhibition of the firing in supraoptic vasopressinergic MNCs (Nissen et al. 1993). Lesions including, but probably not limited to, the perinuclear area with ibotenic acid led to a loss of the decrease in firing activity of vasopressinergic MNCs produced by baroreceptor activation, as well as a loss of the firing inhibition produced by electrical stimulation of the diagonal band (Nissen et al. 1993). These results suggest that GABAergic neurons located in the periphery of the supraoptic nucleus relay information from the diagonal band and are capable of inhibiting firing activity in the majority of supraoptic vasopressinergic MNCs. However, immunocytochemical

Effect of local glutamate microstimulation on firing activity

Finally, the hypothesis that an increased inhibitory input could influence the firing activity in supraoptic MNCs was tested. In six MNCs that previously responded to glutamate microstimulation by an increase in IPSC frequency, depolarizing current was injected intracellularly in current-clamp mode to evoke firing. Glutamate microdrops were then applied in the site where previous applications had evoked an increase in IPSCs. In all of the six MNCs tested, glutamate microstimulation of the periphery of the supraoptic nucleus decreased or blocked action potential firing evoked by current injection (Fig. 8). In four of the six MNCs tested in current clamp, bursts of IPSPs could be detected in the recorded MNCs when firing was blocked by local glutamate microstimulation (e.g., Fig. 8). This result suggests that local inhibitory input to the supraoptic nucleus can modulate firing activity in MNCs.

DISCUSSION

The combination of glutamate microstimulation and whole cell voltage-clamp was used to obtain selective stimulation of somata and dendrites in cells located in the periphery of the supraoptic nucleus and to record with high resolution any change in the synaptic input of supraoptic MNCs. Glutamate has been shown to evoke action-potential firing in neuron somata without evoking firing in passing axons (Christian and Dudek 1988; Tasker and Dudek 1993). In a previous study also using whole cell recording of supraoptic MNCs, spontaneous IPSCs and EPSCs were clearly detected in the presence of tetrodotoxin (Wuarin and Dudek 1993).

This result suggests that all synaptic activity (i.e., action-potential–dependent synaptic activity and synaptic activity due to spontaneous release) can probably be detected with whole cell patch-clamp configuration in MNCs. Also, when recorded at resting membrane potential, all outward spontaneous synaptic currents in supraoptic MNCs could be blocked by bicuculline, indicating that they are GABA-mediated IPSCs (Wuarin and Dudek 1993). The main result from the present experiments is that glutamate microstimulation of the periphery of the supraoptic nucleus can evoke an increase in the inhibitory input to supraoptic MNCs and that this local inhibitory input is capable of depressing firing activity in supraoptic MNCs. Only a relatively small fraction of MNCs responded to local glutamate microstimulation by an increase of the inhibitory input, suggesting that few local GABAergic cells send projections into the supraoptic nucleus.

Although a substantial population of cell bodies immunoreactive for GABA and glutamic acid decarboxylase (GAD) have been found in the region immediately dorsal and lateral to the supraoptic nucleus, the perinuclear zone (Herbison 1994; Roland and Sawchenko 1993; Theodosis et al. 1986), the projections identified as originating from this region seem sparse (Roland and Sawchenko 1993). These authors did not find many projections, in particular GABAergic projections, from the perinuclear zone into the supraoptic nucleus and concluded that the contribution of this area to the innervation of the supraoptic nucleus appears to be weak. Therefore this study corroborates the present results suggesting that only a fraction of the supraoptic MNCs receives inhibitory input from local GABAergic cells.
identification of the peptidergic content of the cells receiving local inhibition remains to be done.

Glutamate microstimulation of the periphery of the supraoptic nucleus produced an increase in inhibitory input to only a relatively small fraction of the cells tested (14%). Because the supraoptic nucleus is composed mostly of oxytocinergic and vasopressinergic MNCs in approximately equal proportions, even if the local inhibitory input is targeted to vasopressinergic MNCs, glutamate microstimulation should have increased the inhibitory input of >14% of the cells. Technical problems associated with glutamate microdrop applications could be invoked to explain the relatively low percentage of responsive cells: poor penetration of the glutamate solution into the tissue or rapid diffusion at the surface of the slice resulting in glutamate concentrations within the tissue that are insufficient to depolarize the cells above threshold for action potential. Extensive use of this technique in several brain-slice preparations did not reveal that diffusion or poor penetration in the tissue were significant problems (Christian and Dudek 1988; Tasker and Dudek 1993; Wuarin and Dudek 1996). Alternatively, the area stimulated with glutamate microdrops may have been too restricted (i.e., GABAergic cells projecting into the supraoptic nucleus may exist beyond the immediate periphery of the nucleus). The perinuclear region defined in the present study corresponds to the area where cells immunoreactive for GAD and GABA-transaminase were found (Herbison 1994; Iijima and Kojima 1986; Roland and Sawcheko 1993; Theodosis et al. 1986). It also corresponds well to the area in which retrogradely labeled cells were found after injections of rhodamine-labeled latex microspheres in the supraoptic nucleus (Jhamandas et al. 1989; Raby and Renaud 1989). However, retrograde tracers injected iontophoretically in the supraoptic nucleus revealed a population of cells located in an area dorsal to the supraoptic nucleus and extending medially beyond the limits of the perinuclear region (Thellier et al. 1994a,b). This area overlaps with the perinuclear region, but its most medial aspect was not included in the stimulation protocol. Although it is not known if the retrogradely labeled cells in this dorsochiasmatic area are GABAergic, these results raise the possibility that local inhibition to the supraoptic nucleus may be provided by GABAergic cells located not only in the immediate periphery of the nucleus but also more medially.

Glutamate microstimulation of the periphery of the supraoptic nucleus evoked two qualitatively different types of changes in the inhibitory input: increases in the frequency or size of IPSCs. Spontaneous IPSCs recorded in supraoptic MNCs in a slice preparation can originate from inhibitory neurons present in the slice or from cut axons. Both action-potential–dependent IPSCs and miniature IPSCs can be generated by cut axons. Cut axons can generate action potentials and presynaptic terminals isolated from the soma have been shown to produce spontaneous release (Drewe et al. 1988). Glutamate microstimulation does not influence firing in cut axons; therefore it will not modify the IPSCs that are the result of action potentials in cut axons or to spontaneous release that occurs at presynaptic terminals from cut axons. Glutamate microstimulation will increase only the fraction of IPSCs that are mediated by action potentials generated in the soma of GABAergic cells present in the slice. These action-potential–dependent IPSCs are presumably larger than the IPSCs mediated by spontaneous release. Therefore one possible interpretation for the occurrence of very large IPSCs in response to application of glutamate is that glutamate microstimulation evoked action-potential firing in inhibitory cells that were silent, thus resulting in the appearance of a new population of synaptic currents much larger than the currents evoked by spontaneous release.

Both anatomic and electrophysiological data indicate that the supraoptic nucleus receives an extensive inhibitory input: one-half of the synaptic boutons in the supraoptic nucleus are immunoreactive for GABA (Decavel and van den Pol 1990) and spontaneous IPSCs can be observed in the vast majority of supraoptic MNCs (Wuarin and Dudek 1993). The present study shows that local glutamate microstimulation could increase the inhibitory input in only a small fraction of supraoptic MNCs. It is therefore reasonable to conclude that the perinuclear area included in the stimulation protocol provides only a relatively small fraction of the inhibitory input to supraoptic nucleus. Most of the inhibitory input probably originates from distant areas. Glutamate microstimulation also revealed a relatively sparse local inhibitory input to both MNCs and parvcellular neurons in the paraventricular nucleus (Boudaba et al. 1996; Tasker and Dudek 1993), supporting the conclusion that local GABAergic cells provide probably only a modest fraction of the inhibitory input to the magnocellular system. Together, these results tend to suggest that local inhibitory circuits play only a minor role in the regulation of the secretion of oxytocin and vasopressin. However, the axonal trajectory of neurons in the perinuclear zone is unknown and connections may have been lost during preparation of the slices. Thus, it is possible that the present results underestimate the strength of local inhibitory projections to the supraoptic nucleus in intact animals.

The synaptic architecture of the magnocellular neuroendocrine system has been shown to be remarkably dynamic in conditions of increased hormonal secretion and the existence of recurrent excitatory connections between oxytocinergic MNCs during lactation was recently suggested (McKenzie et al. 1995). Therefore the hypothesis can be proposed that in physiological conditions demanding high circulating levels of oxytocin or vasopressin, the normally weak inhibitory input from the perinuclear zone could become very important for the firing-pattern formation of oxytocinergic and vasopressinergic cells.

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