Vasopressin Increases GABAergic Inhibition of Rat Hypothalamic Paraventricular Nucleus Neurons In Vitro


INTRODUCTION

Magnocellular neurons of the hypothalamic supraoptic (SON) and paraventricular nuclei (PVN) secrete the peptideergic hormones vasopressin (VP) and oxytocin (OT) into the bloodstream from axon terminals in the neurohypophysis (Brownstein et al. 1980). The magnitude of this neurosecretory process can be correlated with both the frequency and the pattern of action potentials generated at the cell somata, features that are dependent on intrinsic membrane properties and synaptic input (Armstrong 1995; Poulain and Wakerley 1982; Renaud and Bourque 1991). Interestingly, the neurohypophysial seal peptides are believed to regulate their own secretion by acting as neurotransmitters on magnocellular neurons either following synaptic release from magnocellular axon collaterals or nonsynaptically from somatodendritic regions of the magnocellular cells (Landgraf 1995; Ludwig 1998; Morris et al. 1993).

Data from in vitro studies support the notion that OT enhances the excitability of putative OT-synthesizing magnocellular cells (Inenaga and Yamashita 1986). Although some of these observations may represent a direct effect of the peptide (Yamashita et al. 1987), OT also has been noted to alter cell excitability through a reduction of GABAergic inhibition (Brussaard et al. 1996) and by an influence on excitatory afferents (Kombian et al. 1997). In vivo, OT appears to facilitate bursting activity in OT-synthesizing neurons during suckling (Fenoul-Mercier and Richard 1984). By contrast, reports of the effects of VP on VP-synthesizing magnocellular cells vary with data from extracellular studies in vivo or in vitro implying facilitatory, depressant, or no influence on cell excitability (Abe et al. 1983; Carette and Poulain 1989; Inenaga and Yamashita 1986; Ludwig and Leng 1997). However, a recent analysis suggests that these combined actions of VP augment its hormonal release by promoting the expression of phasic firing among putative VP-synthesizing neurons (Gouzénes et al. 1998).

To investigate the membrane actions of VP, we obtained whole cell and perforated-patch recordings from magnocellular PVN neurons in hypothalamic slice preparations. We here report that a subset of the magnocellular cells demonstrates an increase in bicuculline-sensitive inhibitory postsynaptic events in response to bath applications of VP, mediated by V1a-type VP receptors presumably located on GABAergic neurons synaptically to the PVN target cells. These effects were selective for neurons that displayed a rebound depolarization after transient membrane hyperpolarization, reminiscent of a feature seen in identified OT-synthesizing neurons in SON (Stern and Armstrong 1997). A portion of these observations has been reported briefly (Hermes et al. 1996b).

METHODS

Slice preparation

Hypothalamic slices were prepared from male Long-Evans rats 30–70 days of age. In accordance with national guidelines, animals were decapitated without anesthesia to minimize unknown persisting actions of anesthetics on neural tissue. Brains were removed rapidly from the cranial cavity, cooled in ice-cold oxygenated (95% O2-5%
CO$_2$ artificial cerebrospinal fluid (ACSF), and sectioned with a vibratome. Slices (coronal plane, 400–500 μm thickness) were maintained for ≥1 h in oxygenated ACSF at room temperature (20–22°C) or at 36°C before recording.

**Solutions and drugs**

ACSF contained (in mM) 119 NaCl, 3.2 KCl, 2.4 CaCl$_2$, 1.3 MgCl$_2$, 26.2 NaHCO$_3$, 1 NaH$_2$PO$_4$, and 10 glucose and had an osmolality of 295–300 mOsm/kg, and a pH of 7.35–7.40. The patch pipette contained a gluconate-based recording solution of the following composition (in mM): 140 potassium gluconate, 10 KCl, 10 HEPES, and 1 EGTA. With these solutions, the chloride reversal potential was −68.0 mV (at 35°C), approximating the values measured for spontaneous or evoked bicuculline-sensitive inhibitory postsynaptic potentials (IPSPs) recorded in SON or PVN magnocellular neurons using potassium acetate-containing sharp electrodes (Hermes et al. 1996a; Renaud and Bourque 1991). For voltage-clamp recording of reversed inhibitory postsynaptic currents (IPSCs), the recording solution contained (in mM) 100 KCl, 35 potassium gluconate, 10 HEPES, 1 EGTA, and 2 lidocaine N-ethyl bromide (QX-314). Without ATP in the recording solution no rundown of reversed IPSCs was observed for recording periods exceeding 2 h (cf. Staley and Mody 1992). The recording solutions had an osmolality of 285–300 mOsm/kg and a pH of 7.30–7.40. For perforated-patch recording, both amphotericin B (250 μg/ml) and gramicidin (5 μg/ml) were added to the gluconate-based recording solution (Kyrozis and Reichling 1995; Rae et al. 1991). In some cases, only gramicidin (2 μg/ml), which forms pores in the plasma membrane that are not permeable to chloride, was added for an additional verification of the hyperpolarizing direction of the bicuculline-sensitive IPSPs (Hermes et al. 1996a; Renaud and Bourque 1991).

Drugs used in the study included bicuculline methochloride (BMC), d(-)-2-amino-5-phosphonopentanoic acid (d-APV), d-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (d-CPP), and 6-nitro-7-sulfoamoylbenzofuro[2,3-d]quinoxaline-2,3-dione (NBQX) from Tocris Cookson, Bristol, UK; bicuculline methiodide (BMI), VP, OT, and [β-Mercapto-β,β-cyclopentamethylenepropionyl]⁻O-Me-Tyr²⁻Arg⁶⁻VP or d(CH$_2$)$_3$[Tyr(Me)]⁴AVP (Manning compound) from Sigma; [deamino-Cys¹⁷,Val¹⁰,D-Arg⁶⁻VP (dVDAVP) from Bachem, Switzerland; vasotocin [Phe¹, Orn⁶]-)VP (PO-VT) from American Peptide, Sunnyvale, CA; and tetrodotoxin (TTX) from Research Biochemicals Int., Natick, MA. Some VP analogs were kindly provided by Dr. M. Manning (Toledo, Ohio).

Drugs were applied from reservoirs connected to the ACSF perfusion line by manually operable three-way valves. For agonists, mentioned concentrations were those in the reservoirs, although radioimmunoassay measurements revealed that the levels in the recording chamber rose to maximally 50% of these values at the end of 1-min applications. Antagonists were bath-applied for 5–10 min, and their concentrations in the recording chamber were similar to those in the reservoirs.

**Recording and stimulation**

Whole cell and perforated-patch recordings were obtained from submerged slices that were superfused continuously with gravity-fed oxygenated ACSF flowing at 5–8 ml/min, either at room temperature or at 33 ± 1°C. The “blind” recording technique was used (Blanton et al. 1989). Patch pipettes were pulled from thin-walled borosilicate glass capillaries (4–7 MΩ when filled with a gluconate-based solution) and connected to an Axopatch-1D amplifier (Axon Instruments, Foster City, CA). In current-clamp experiments, the series resistance was estimated in brief voltage-clamp sessions from the whole cell capacitive current in response to a voltage pulse (Marty and Neher 1995). With whole cell recording, the estimated series resistance was generally <20 MΩ, whereas with perforated-patch recording (using the combination of amphotericin B and gramicidin), this value stabilized at 25–35 MΩ. In whole cell voltage-clamp recording, cells were clamped at potentials between −45 and −60 mV. The series resistance ranged between 8 and 23 MΩ and usually was compensated for 60%; current signals were filtered at 1 kHz (4-pole Bessel filter). All data were stored on videotape for later analysis off-line.

To evoke postsynaptic responses, electrical stimulation was applied between monopolar tungsten electrodes (tip diameter, 20 μm) placed in the medial and lateral parts of the subparaventricular zone (sub-PVN), using an isolated stimulation unit (Digitimer, Welwyn Garden City, UK) that provided pulses of constant voltage (7–25 V) or current (10–300 μA; duration, 0.2 ms).

**Cell identification**

Previous investigators have recognized at least three categories of neurons in PVN based on action potential waveform and responses to transient positive and negative current injections (e.g., Hermes et al. 1996a; Tasker and Duke 1991). Analogous with recordings in SON, magnocellular neurons can be recognized by a characteristic shoulder on the repolarizing phase of their action potentials, which contributes to activity-dependent action potential broadening, and a depolarizing sag (i.e., time-dependent inward rectification) and a delayed return to original membrane potential (i.e., transient outward rectification) during and after transient membrane hyperpolarization, respectively (Erickson et al. 1993; Renaud and Bourque 1991).

**Data analysis and statistics**

All numbers are expressed as mean ± SE. To assess possible postsynaptic influences of VP, several membrane properties were evaluated with perforated-patch recordings at 33 ± 1°C in ACSF containing BMC and NBQX to block synaptic potentials. Resting membrane potentials (not corrected for the occurrence of a junction potential) were calculated from the mean potential over representative 1-min periods (sampled at 200 Hz). Input resistances were measured from the instantaneous voltage deflections induced by negative current injections (10–20 pA; 1-s duration). Because the firing frequency in this preparation was low (Hatton 1990), the number of action potentials evoked by 2-s depolarizing current injections (5–60 pA) was taken as an index of cell excitability. Statistical significant changes after VP application were assessed using the Wilcoxon matched-pairs test.

To assess the influence of VP on inhibitory synaptic input, 3- to 4-min records (sampled at 5 kHz) of reversed IPSCs recorded in ACSF containing NBQX and d-CPP (d-APV), were analyzed according to frequency, amplitude, time to peak, and time constant of decay using software written in Unix environment. Similar to methods described previously (Bergles et al. 1996), detection of events was accomplished by setting a threshold of five times the standard deviation of the noise in the derivative of the original data filtered at 300 Hz (Gaussian filter). As judged by eye, this method reliably detected synaptic events, including those located on the falling phase of previous events. Artifacts in the recording were removed manually.

For each record, the frequency was calculated and a cumulative amplitude distribution was constructed. The Wilcoxon matched-pairs test was used to determine the presence of statistical significant (i.e., $P < 0.05$) differences in the mean frequency of reversed IPSCs between two dependent experimental groups. Comparing more than two dependent experimental conditions, the Friedman test, followed by a multiple comparison of groups, was applied. To assess changes in the amplitude distribution of reversed IPSCs after a certain treatment, Kolmogorov-Smirnov (K-S) statistics were used to test significant (i.e., $P < 0.01$) differences between two cumulative amplitude distributions. To evaluate the influence over a certain number of cells, $P$ values of each K-S test were combined to one value ($P$ combined K-S) using the method described by Koziol and Perlman (1978).
RESULTS

VP increases GABA_A-receptor-mediated synaptic input

Observations are based on data, using conventional whole cell (at room temperature; \( n = 15 \)) or perforated-patch (at 33 ± 1 °C, \( n = 23 \)) recording methods, from PVN neurons that displayed properties attributable to magnocellular neurons. In 22/38 neurons a 1-min bath application of VP (100–500 nM) was followed by an increase in the frequency of IPSPs or IPSCs (Fig. 1A). The effect usually started within 2 min, persisted for a variable amount of time independent of the temperature of recording (0.5–18 min; mean of 9.50 ± 1.09 min; \( n = 22 \)), and was repeatable after washout (6/6 cells tested). In spontaneously active cells recorded with the perforated-patch method, VP-induced increases in IPSPs were not associated with significant decreases in firing frequency (control, 1.01 ± 0.28 Hz; VP, 0.83 ± 0.20; \( n = 6 \), \( P = 0.22 \)).

VP-induced increases in IPSP(C) frequency were dependent on functional GABA_A receptors because the effects of a second application were blocked by preperfusion with BMC or BMI (10–20 \( \mu M \); \( n = 9 \); Fig. 1B). In the latter circumstances, there was no detectable influence on the occurrence of excitatory postsynaptic events (Fig. 1B). In 5/5 cells tested, VP-induced increases in the frequency of inhibitory postsynaptic events were equally large after the addition of d-CPP (or D-APV) and NBQX, applied in concentrations (10–20 and 2–5 \( \mu M \), respectively) known to block fast glutamatergic synaptic transmission in this preparation (a 2.24 ± 0.16-fold increase in control vs. a 2.21 ± 0.86-fold increase in d-CPP/D-APV and NBQX; \( n = 5 \), \( P = 0.89 \); not illustrated) (cf. Hermes et al. 1996a).

Because cytoplasmic dialysis of second-messenger pathways consequent to whole cell recordings might obscure other direct effects of VP on the neurons, the influence of VP also was studied with perforated-patch recordings, in ACSF containing BMC (10–20 \( \mu M \)) and NBQX (2–5 \( \mu M \)). Table 1 compares observations on seven cells where VP (500 nM) induced an increase in IPSP frequency in control ACSF, and 10 cells where VP had no such effect. Little or no significant influence was noted on resting membrane potential, input resistance or cell excitability (as defined by the number of current evoked action potentials) in either group.

Responsiveness to VP is selective to a subpopulation of magnocellular neurons

Whereas whole cell recordings did not reveal any distinguishing property, current-clamp data from 23 cells recorded in perforated-patch recording methods illustrate time-dependent inward rectification to hyperpolarizing pulses and spike frequency adaptation during a depolarizing current pulse (pulse protocol: +10 to −70 pA, in 10-pA steps, duration 1 s). i: traces from a cell that displays an increase in IPSP frequency after bath application of VP reveal a rebound depolarization (*) after return from a transient current-induced membrane hyperpolarization. By contrast, traces (right) from a nonresponsive magnocellular cell show a delayed return to basal membrane potential (**) after transient membrane hyperpolarization (current pulse protocol: 0 and −30 pA; duration, 1 s).
The frequency or amplitude distribution of miniature IPSCs. This indicates that the effect requires neurotransmission in GABAergic neurons and that VP has little or no influence on the presynaptic release mechanisms for, or postsynaptic responsiveness to, GABA. Third, the effects are mimicked by a selective rebound depolarization noted in OT-immunoreactive neurons in SON, where its demonstration requires that the cells be held at depolarized membrane potentials (Stern and Armstrong 1997). Because in PVN the underlying ionic mechanism seems partly dissimilar (because the rebound is detectable at resting membrane potentials), it remains to be verified whether the rebound depolarization is also a property unique to OT-synthesizing magnocellular neurons in this nucleus.

**Location and type of VP receptors**

There are several reasons to propose that the targets for VP are V1as-type receptors located directly on GABAergic neurons that innervate a subpopulation of PVN magnocellular neurons. First, responses persist in the presence of glutamate receptor antagonists, indicating that VP is not acting through a glutamatergic innervation of GABAergic neurons. Second, the VP-induced responses are abolished completely in TTX-containing media with no remaining influence of the peptide on the frequency or amplitude distribution of miniature IPSCs. This indicates that the effect requires neurotransmission in GABAergic neurons and that VP has little or no influence on the presynaptic release mechanisms for, or postsynaptic responsiveness to, GABA. Third, the effects are mimicked by a selective V1a receptor agonist and blocked by a V1a/OT receptor antagonist. And last, the duration of the VP effect is mostly prolonged as might be anticipated given the long depolarizing action of this peptide and of V1as-type receptor activation on other central neurons (e.g., Kolaj and Renaud 1998; Raggenbass et al. 1989).

**Location of GABAergic neurons**

The location of the GABAergic neurons mediating these responses remains to be defined. Although many hypothalamic

**TABLE 1. Changes in membrane properties of electrophysiologically identified magnocellular PVN neurons by vasopressin**

<table>
<thead>
<tr>
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<th>VP-Responsive Neurons</th>
<th>VP-Unresponsive Neurons</th>
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<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>−56.6 ± 12 (7)</td>
<td>−54.8 ± 0.9 (10)</td>
</tr>
<tr>
<td>VP</td>
<td>−55.5 ± 1.5 (7)</td>
<td>−54.2 ± 1.1 (10)</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1130 ± 96 (7)</td>
<td>1089 ± 52 (10)</td>
</tr>
<tr>
<td>VP</td>
<td>1068 ± 105 (7)</td>
<td>1027 ± 51 (10)†</td>
</tr>
<tr>
<td>No. of evoked spikes</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.5 ± 0.3 (4)</td>
<td>9.5 ± 0.6 (6)</td>
</tr>
<tr>
<td>VP</td>
<td>8.5 ± 1.0 (4)</td>
<td>10.2 ± 1.0 (6)</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE with the number of cells in parentheses. Membrane properties of electrophysiologically identified magnocellular paraventricular nucleus (PVN) neurons, segregated according to the presence or absence of a response (increase in inhibitory synaptic events) to vasopressin (VP), in control media [containing 2–5 μM 6-nitro-7-sulphamoylbenzo(f)quinoxaline-2,3-dione (NBQX) and 10–20 μM bicuculline methoiodide (BMC) to block excitatory and inhibitory postsynaptic potentials, respectively], and after a 1-min bath application of VP (500 nM). Statistical significant differences (*P < 0.05) were assessed using the Wilcoxon matched-pairs test.

with perforated-patch techniques showed a feature unique to magnocellular cells that displayed a response to VP. In all of nine cells where VP induced an increase in IPSP frequency, the return from a transient membrane hyperpolarization (from resting levels) was characterized by a rebound depolarization (Fig. 1C, i and ii). By contrast, in 12/14 neurons that lacked the VP-induced effect, the return from hyperpolarized levels to resting membrane potential was delayed (Fig. 1Cii).

**VP response is mediated via V1a-type receptors located on GABAergic interneurons**

To localize (pre- or postsynaptic) and characterize the receptor that mediates the VP influence, voltage-clamp recordings using chloride-loaded electrodes were obtained from magnocellular neurons (recognized from their action potential waveform before inactivation by QX-314). In responding cells, VP (500 nM) induced a significant mean threefold increase in the frequency of reversed IPSCs (from 2.63 ± 0.48 to 7.69 ± 1.86 Hz, n = 17; P < 0.01), but no change in the mean amplitude of IPSCs (from −61.09 ± 6.84 to −68.16 ± 6.47 pA, n = 17; P = 0.36). However, significant (P < 0.01) rightward (toward larger amplitudes: in 10 cells) or leftward (toward smaller amplitudes: in 5 cells) shifts in cumulative amplitude distributions were observed in 15/17 cells when applications of a selective V2 receptor agonist (dVDAVP) as well as of OT (both at 500 nM) were ineffective (Fig. 3, Table 2). In addition, VP (500 nM) was without effect in four cells in the presence of the V1a/OT receptor antagonist Manning compound (500 nM; VP increased the frequency of reversed IPSC 2.46-fold in control conditions and 1.06-fold in the presence of Manning compound).

**DISCUSSION**

The present study reveals that application of VP to hypothalamic slices in vitro induces an increase in the frequency of bicuculline-sensitive IPSP(C)s recorded in a subpopulation of magnocellular neurons in PVN. Interestingly, the magnocellular neurons that demonstrate this response display a characteristic rebound depolarization after transient membrane hyperpolarization from rest. This feature is reminiscent of a rebound depolarization noted in OT-immunoreactive neurons in SON, where its demonstration requires that the cells be held at depolarized membrane potentials (Stern and Armstrong 1997). Although the selective presence in OT-immunoreactive neurons of a depolarization-activated sustained outward rectification may be responsible for this, differences in the magnitude of transient outward rectification also may contribute to the rebound depolarization (Fisher et al. 1998; Stern and Armstrong 1996). Because in PVN the underlying ionic mechanism seems partly dissimilar (because the rebound is detectable at resting membrane potentials), it remains to be verified whether the rebound depolarization is also a property unique to OT-synthesizing magnocellular neurons in this nucleus.

Selective VP agonists and antagonists were administered in random order. In four cells where VP (500 nM) induced an increase in the frequency of IPSCs, application of a selective V1a receptor agonist (PO-VT: 500 nM) was ≥50% as effective; this is in accordance with the reduced potency of PO-VT as compared with VP (Barberis and Tribollet 1996). However, applications of a selective V2 receptor agonist (dVDAVP) as well as of OT (both at 500 nM) were ineffective (Fig. 3, Table 2). In addition, VP (500 nM) was without effect in four cells in the presence of the V1a/OT receptor antagonist Manning compound (500 nM; VP increased the frequency of reversed IPSC 2.46-fold in control conditions and 1.06-fold in the presence of Manning compound).
areas innervate PVN, candidate regions in this slice preparation would include the perinuclear region of PVN, including sub-PVN, and suprachiasmatic nucleus (SCN). These are areas that contain many GABA- or glutamic acid decarboxylase (GAD)-synthesizing neurons (Moore and Speh 1993; Okamura et al. 1990; Roland and Sawchenko 1993). Moreover, local stimulation in these regions evokes GABAergic inputs in PVN magnocellular neurons (Boudaba et al. 1996; Hermes et al. 1996a; Tasker and Dudek 1993). Interestingly, VP has been shown to excite neurons in SCN as well as in perinuclear regions of PVN, an influence that probably is mediated by V1a-type VP receptors (Carette and Poulain 1989; Shibata and Moore 1988).

### Table 2. Frequency and amplitude distribution changes of reversed inhibitory postsynaptic currents by vasopressin

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vasopressin</th>
<th>TTX</th>
<th>TTX-Vasopressin</th>
<th>PO-VT</th>
<th>dVDAVP</th>
<th>Oxytocin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPSC frequency, Hz</td>
<td>3.07 ± 0.88 (6)</td>
<td>9.46 ± 2.42 (6)*</td>
<td>2.00 ± 0.50 (6)</td>
<td>1.77 ± 0.41 (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-S statistics on IPSC amplitude distribution</td>
<td></td>
<td></td>
<td>*P &lt; 0.01 (5/6 cells)</td>
<td></td>
<td>*P &lt; 0.25 (1/6 cells)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ IPSC frequency (% of control value)</td>
<td>395 (4)†</td>
<td></td>
<td></td>
<td>234 (4)†</td>
<td>110 (4)</td>
<td>96 (4)</td>
<td></td>
</tr>
</tbody>
</table>

Changes in frequency and amplitude distribution of reversed inhibitory postsynaptic currents (IPSCs) in responsive magnocellular PVN neurons by vasopressin and vasopressin receptor analogs, and by vasopressin in tetrodotoxin (TTX)-containing media. Values are expressed as means ± SE with the number of cells in parentheses. Note that significant changes (as compared with control values) in the frequency (*P < 0.05, Wilcoxon matched-pairs test) and amplitude distribution [P combined Kolmogorov-Smirnov (K-S) statistics < 0.01] of IPSCs by vasopressin in control media are absent in TTX-containing media. Vasopressin, the V1a receptor agonist vasotocin [Phe², Orn⁸] (PO-VT), the V2 receptor agonist [deamino-Cys¹, Val⁴, D-Arg⁸]-vasopressin (dVDAVP), and oxytocin have significantly different influences on the frequency of reversed IPSCs (as compared with their respective control values; Friedman test; P < 0.01). Multiple comparison of groups indicates that the influence of vasopressin was significantly different from that of PO-VT ([‡]) and that both of these treatments differed significantly from those of dVDAVP and oxytocin ([†]) (which were not significantly different from each other).
However, GABAergic neurons in SCN presumably do not mediate the observations reported here because we detected similar VP-induced changes in IPSP(C)s in transverse hypothalamic slices that did not contain SCN. Therefore we would suggest that it is the GABAergic neuronal populations situated in perinuclear regions of PVN (i.e., subPVN) that are the most likely origins of the VP-induced responses in PVN magnocellular neurons.

Possible endogenous sources of VP

The SCN, a site responsible for generation of circadian rhythmicity in mammalian brain, is an obvious source for a VP innervation of GABAergic neurons located in the area cited in the preceding text. VP-immunoreactive neurons in SCN project their axons into the perinuclear region of PVN (Buijs et al. 1993). Moreover, a pronounced circadian rhythm in plasma and pituitary levels of OT and VP (Windle et al. 1992) suggests that SCN neurons indeed may be capable of regulating the activity of PVN magnocellular neurons either directly, as implied from recent in vitro electrophysiological data (Hermes et al. 1996a), or indirectly, as might occur through GABAergic interneurons. Whereas a role of VP in SCN efferents requires further clarification, the present observations suggest that an action via the GABAergic interneuron pathway may be one of the possibilities.

Other possibilities are suggested by recent evidence that sources of neurohypophyseal peptides also release them locally in brain (reviewed in Landgraf 1995; Ludwig 1998). Should this occur via TTX-sensitive synaptic release from terminals of axon collaterals of magnocellular neurons, the present observations suggest that VP-synthesizing magnocellular neurons could be engaged in a feedback inhibitory mechanism to other magnocellular neurons, operating through GABAergic neurons. In addition, VP and/or OT are likely released into the extracellular space via TTX-insensitive exocytosis from somatodendritic regions of the magnocellular cells (Landgraf 1995; Ludwig 1998; Morris et al. 1993). Somatodendritic release is believed to be responsible for many of the described peptidergic influences on magnocellular neurons or on synaptic input to these neurons (Brussaard et al. 1996; Freund-Mercier and Richard 1984; Gouzé `nes et al. 1998; Kombian et al. 1997). Most of these influences result from a peptidergic action close to the site of (somatodendritic) release, i.e., on the neuron itself or on synaptic terminals innervating the neuron, because extensive diffusion of active peptide probably is limited (Kombian et al. 1997). Because our observations suggest that
the GABAergic interneurons are probably located at a distance from the magnocellular cells, a distant dendritic source of the peptide seems less likely. Further investigations should clarify some of these possibilities.

The authors thank Dr. C.M.A. Pennartz for contributing to the development of the software for analysis of postsynaptic currents.

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REFERENCES


