Developmental Regulation of a Local Positive Autocontrol of Supraoptic Neurons

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Mature oxytocin (OT) and vasopressin (AVP) magnocellular neurons of the hypothalamic supraoptic nuclei (SON) autocontrol their electrical activity via somatodendritic release of their respective peptides. Because OT and AVP are synthesized early in development and could play an important role in the maturation of these neurons, we checked whether the peptides are released within the SON and act on their secreting neurons during 3 weeks of postnatal development. We used patch-clamp recordings from SON neurons in rat hypothalamic horizontal slices to show that the spontaneous electrical activity of immature SON neurons is blocked by OT or AVP receptor antagonists, demonstrating a basal somatodendritic release of the peptides. Application of OT or AVP depolarizes SON neurons and stimulates activity typical of the corresponding mature neurons. This effect is directly on SON neurons because it is recorded in dissociated

neurons. Radioimmunoassays from isolated SON were used to show that each peptide facilitates its own release at a somatodendritic level, exhibiting a self-sustaining positive feedback loop. This autocontrol is not uniform during development because the proportion of neurons depolarized by the peptides, the amplitude of the depolarization, and the propensity of the peptides to facilitate their own release are maximal during the second postnatal week and decrease thereafter. These data are consistent with a role of autocontrol in the maturation of SON neurons because it is maximal during the delimited period of postnatal development that corresponds to the period of major synapse formation.

Key words: release; oxytocin; vasopressin; hypothalamus; electrical activity; neuropeptides; receptors

Hypothalamic supraoptic nuclei (SON) contain two populations of magnocellular neurons that project to the neurohypophysis and secrete either oxytocin (OT) or vasopressin (AVP) into the blood circulation. The peptides are very similar, and the neurons are morphologically indistinguishable. However, the two types of neurons belong to specific networks, respond to distinct physiological stimuli (Leng et al., 1999) and display highly characteristic electrical activities (Poulain and Wakerley, 1982). In addition to being secreted as hormones by axon terminals in the neurohypophysis, OT and AVP are released at the somatodendritic level (Di Scala-Guenot et al., 1987; Pow and Morris, 1989) and optimize the electrical activity of their secreting neurons (Freund-Mercier and Richard, 1984; Gouzenes et al., 1998) via an autocrine–paracrine mechanism. AVP acts by favoring a phasic activity in AVP-secreting neurons (Gouzenes et al., 1998), i.e., a succession of active and silent periods being best suited for maximal AVP secretion (Cazalis et al., 1985). OT is known to enhance the amplitude and frequency of the high-frequency bursts of action potentials that occur simultaneously on all OT neurons during parturition and lactation and induce the pulsatile release necessary for delivery of the fetus and milk ejection (Moos et al., 1998). Both effects are complex and cannot be described as simple excitation or inhibition. The membrane mechanisms supporting OT and AVP actions on electrical activity of SON neurons are not elucidated, although it is known that they involve specific receptors and that both peptides act specifically on their secreting neurons to increase intracellular calcium (Dayanithi et al., 1996).

Both peptides are synthesized at birth (Lazcano et al., 1990), but neither OT nor AVP exerts its main adult physiological role yet (reproduction and regulation of water balance) (Rajerson et al.,

1976). In view of their early expression and putative role as developmental factors on both central and peripheral structures (Carter et al., 1993), OT and AVP released locally and acting specifically on their secreting neurons could thus contribute to the maturation of SON neurons. This study was designed to test whether postnatal SON neurons are autocontrolled by somatodendritic release of their peptide. Our results show that OT and AVP depolarize immature SON neurons and that endogenous peptide release is involved in the spontaneous activity of these neurons. Moreover, each peptide has a facilitatory effect on its own somatodendritic release, and all of these effects are maximal during the second postnatal week, suggesting important roles of this autocontrol in the maturation of SON neurons.

MATERIALS AND METHODS

Slice preparation. Male Wistar rats aged from postnatal day 0 to 20 (P0–P20) were killed by decapitation, and the brain was rapidly removed and bathed in cold (4°C) and oxygenated (95% O₂ + 5% CO₂) sucrose solution containing (in mM): sucrose 220, KCl 2.3, NaHCO₃ 26, CaCl₂ 2.5, glucose 10, KH₂PO₄ 1.2, MgSO₄ 1.2, pH 7.4; 300 mOsm/l. A block of hypothalamus containing both SON was dissected, and horizontal slices (250–300 μm thick) were obtained using a Campden Instruments Vibratome. Before recordings, slices were allowed to recover for 1–2 hr at room temperature in oxygenated (95% O₂ + 5% CO₂) artificial CSF (ACSF) containing (in mM): NaCl 110, KCl 1.2, NaHCO₃ 26, CaCl₂ 2, glucose 10, KH₂PO₄ 1.2, MgCl₂ 2, pH 7.4; 300 mOsm/l.

Preparation of dissociated neurons. Male Wistar rats (P8) were killed by decapitation, the brain was rapidly removed, and two narrow tissue pieces lateral to the optic chiasma were dissected. The tissue pieces were incubated for 25 min in oxygenated ACSF (95% O₂ + 5% CO₂) supplemented with protease X (1 mg/ml) at room temperature, rinsed, and incubated for 25 min in oxygenated ACSF supplemented with protease XIV (1 mg/ml) and deoxyribonuclease I (650 U/ml). The tissue pieces were then rinsed and mechanically dissociated by trituration, and the cell suspension was plated in 35 mm culture dishes. The recordings began as soon as the cells were attached to the bottom of the dish (15–20 min).

Electrophysiological recordings. Slices or dissociated neurons were placed in a recording chamber under a Leica microscope (40×) and perfused with ACSF (34°C) at a rate of 2 ml/min. Current-clamp recordings were obtained with an Axoclamp 2A amplifier, filtered at 6 kHz, and digitized at 2 kHz. Data were stored and analyzed using the P-Clamp software (Axon Instruments). Pipettes (4–6 MΩ) were pulled with a horizontal puller (Flaming/Brown, Sutter Instruments) and filled with solution containing (in mM): KMeSO₃ 135, KCl 5, CaCl₂ 1, EGTA-Na 5, ATP-Mg 4, HEPES-Na 10, pH 7.2; 290 mOsm/l for whole-cell recordings and with

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solution containing (in mM): KCl 132.5, HEPES-Na 10, CaCl₂ 5, gramicidin 80 μ g/ml for perforated-patch recordings (to preserve the Cl[−] equilibrium, which is known to change during development). Series resistance and cell capacitance and resistance were measured using a 10 mV hyperpolarizing square pulse from −70 mV. The resting membrane potential was measured after establishment of the whole-cell configuration in neurons displaying stable membrane potential and no activity. The membrane potential values recorded in whole-cell configuration are corrected for a 10 mV junction potential, measured as described by Neher (1992). Neurons located in the SON and freshly dissociated neurons were selected on morphological [cell size and presence of a large dendrite corresponding to the α dendrite described by Hatton (1990)] and electrophysiological (high cell capacitance > 15 pF) criteria. Such large cells were shown to synthesize either OT or AVP at all stages of postnatal development (Hussy et al., 1997). OT, V_{1a}, and V₂ antagonists were applied through the general perfusion. OT and AVP were applied using a microperfusion system in close proximity to the recorded neurons. In the case of dissociated neurons, the general perfusion was switched off during the application of the peptides. The Ca²⁺-free solution was prepared as ACSF in which 2 mM CaCl₂ was substituted by 2 mM MgCl₂.

Measurement of peptide release from SON. Male Wistar rats (P2–P21) were killed by decapitation. After dissection (see Preparation of dissociated neurons), the SON were transferred into Locke buffer (maintained at 34°C) containing (in mM): NaCl 140, KCl 5, MgCl₂ 1.2, CaCl₂ 2.2, glucose 10, HEPES 10, pH 7.25; 298–300 mOsm/l. Three SON were incubated for AVP and OT release measurements. The tissues were preincubated with the Locke buffer at 34°C for ~30–45 min during which they were rinsed every 5 min before collection of the samples. Five minute samples were collected; OT and AVP agonists were applied for 15 min. The released AVP and OT were assayed by radioimmunoassay (Cazalis et al., 1985) using specific antibodies (kindly supplied by Dr. R. J. Bicknell, Babraham Institute, Cambridge, UK). AVP and OT antisera were raised in sheep (the AVP antiserum was raised by Dr. Bicknell; the OT antisera were raised by Drs. E. L. Sheldrick and A. P. Flint, Babraham Institute, Cambridge, UK). The final antibody dilution was 1:400,000 for AVP and 1:30,000 for OT. The cross-reactivity of the OT antiserum with AVP was 0.015%, whereas that of the AVP antiserum with OT was 0.001%. The sensitivities of the assays were 0.5 pg (AVP) and 1 pg (OT). The interassay and intra-assay coefficients of variation were 5–7% for AVP and 7–9% for OT.

Pharmacological compounds. OT antagonist (dOVT or Manning compound: D(CH₂)₅[Tyr(Me)², Thr⁴, Tyr-NH₂⁹]) was kindly provided by Dr. C. Barberis (Institut National de la Santé et de la Recherche Médicale U469, Montpellier, France). AVP antagonists [V_{1a} antagonist: SR 49059; (2S) 1-(2R,3S)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl))-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrro-lidine-2-carboxamide and V₂ antagonist: SR 121463A: 1-[4-(N-tert-butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4(2-morpholino ethoxy) cyclohexane]-indol-2-one, furamate; equatorial isomer] were provided by Sanofi Synthelabo Recherche (Toulouse, France). AVP V_{1a} agonist [F-180: Hmp-Phe-Ile-Hgn-Asn-Cys-Pro-Dab(Abu)-Gly-NH₂; Ferring France, Gentilly, France] and OT agonist [(Thr⁴, Gly⁷)OT] were kindly provided by Dr. C. Barberis. AVP V₂ agonist (dDAVP: 1-deamino-8-D-AVP) was kindly supplied by Dr J. L. Junien (Ferring France). OT and AVP were purchased from Calbiochem–Novabiochem (La Jolla, CA). ¹²⁵I-labeled AVP and OT were purchased from Amersham (Orsay, France). All salts were from Sigma (St. Quentin Fallavier, France). Results are expressed as mean $\pm$ SEM. Statistical analyses were performed with ANOVA test.

RESULTS

The electrical activity of SON neurons in acute horizontal hypothalamic slices of 0- to 20-d-old rats was recorded in current-clamp using whole-cell and perforated patch-clamp techniques. The resting potential hyperpolarized during development, and the mean value between P0 and P20 was -45 ± 1 mV. During the first postnatal week, an unstable membrane potential led to erratic activity. From the second postnatal week onward, the membrane potential stabilized, and we observed an activity that progressively differentiated to become typical of mature SON neurons (Poulain and Wakerley, 1982), i.e., tonic for OT neurons and phasic for AVP neurons. The maturation of passive and active membrane properties of SON neurons during postnatal development will be described in detail elsewhere (V. Chevalayre and M. G. Desarménien, unpublished observations).

Endogenous peptide release influences the electrical activity of maturing SON neurons

To test the involvement of somatodendritic release of endogenous peptides on cell electrical activity, OT and AVP receptor antagonists were added to the perfusion solution while we recorded from spontaneously active neurons. For AVP receptor blockade, V_{1a} and V₂ receptor antagonists were used because mature dissociated

Figure 1. The spontaneous electrical activity of SON neurons is dependent on endogenous peptides release. *a*, Application of the OT antagonist (dOVT; 100 nM) on a tonic neuron (P16) reversibly hyperpolarized the membrane and suppressed the electrical activity. *b*, Application of V_{1a} and V₂ antagonists (SR 49059 and SR 121463A; 10 nM) on a phasic neuron (P15) also hyperpolarized the membrane and suppressed reversibly the electrical activity.

AVP neurons have been shown to respond to both V_{1a} and V₂ agonists (Gouzenes et al., 1999). Antagonists were applied on nine neurons that could be classified as putative OT or AVP neurons on the basis of their pattern of activity. The phasic pattern was not as sharp as in adult; however, some neurons could maintain a sustained activity for minutes, whereas other could not. Accordingly, neurons were considered as tonic if they displayed periods of activity for >3 min without interruption longer than 10 sec; neurons were stated as phasic if they displayed successive periods of activity and silence. Some neurons displayed irregular activities, without distinguishable pattern, and were not classified as tonic or phasic. Perfusion of an OT receptor antagonist (dOVT, 100 nM) led to a hyperpolarization (4–10 mV) and decreased the mean spike frequency (by 90%; $n = 1$) or suppressed ($n = 4$) the spontaneous activity of tonic neurons (Fig. 1*a*). A combination of V_{1a} and V₂ receptor antagonists (SR 49059 and SR 121463A, respectively, each at 10 nM) induced a hyperpolarization (2–8 mV) and reduced the mean spike frequency (by 60 and 80%; $n = 2$) or abolished ($n = 2$) the spontaneous activity of phasic neurons (Fig. 1*b*). The effects of OT and AVP receptor antagonists were reversible in eight of nine neurons. These results show that OT and AVP are released under basal conditions within the SON and sustain the spontaneous electrical activity of SON neurons.

OT and AVP depolarize supraoptic neurons

The effects of OT and AVP (100 nM) were further characterized by local application of the peptides to the recorded neurons. Neurons were tested at the resting or slightly hyperpolarized voltages (to suppress spontaneous electrical activity masking small membrane potential changes). Among 74 neurons tested, 49 responded to only one peptide, 3 were sensitive to both, and 22 were unaffected by either peptide. OT (27 of 27 cells) and AVP (21 of 22 cells) induced depolarizations of similar amplitude (6 ± 1 mV) (Fig. 2). The depolarization was accompanied by random or tonic firing of action potentials for OT-sensitive neurons (Fig. 2*a*) and random or phasic activity for AVP-sensitive neurons (Fig. 2*b*). In 1 of 22 neurons, AVP induced a hyperpolarization. Significantly, application of OT

Figure 2. Application of OT and AVP led to a depolarization and an increased activity in supraoptic neurons. *a*, Example of a neuron (P9) responding to OT application (100 nM) by a sustained depolarization and a tonic activity. AVP had no effect in this neuron. *b*, Application of AVP (100 nM) on another neuron (P12) that was insensitive to OT led to a sustained depolarization and a phasic activity. These neurons have been slightly hyperpolarized before application of agonists to suppress the spontaneous activity.

and AVP evoked a sustained depolarization lasting several minutes after the end of peptide application [application of 53 ± 6 sec; mean ratio (duration of the depolarization/duration of the application) of 3.66 ± 0.32 , $n = 44$]. Similar long-lasting responses were observed using the perforated-patch technique (application of 47 ± 8 sec, mean ratio of 3.25 ± 0.65 ; $n = 9$), excluding the possibility that dialysis of a cytoplasmic component by the whole-cell procedure was responsible for a delayed recovery. In the three neurons sensitive to both OT and AVP, the depolarization induced by each peptide was of similar amplitude and duration. Interestingly, in one of these neurons, OT led to tonic activity, whereas the activity induced by AVP was phasic (data not shown). In the two other neurons, the duration of the response to each peptide was not sufficient enough to allow a characterization of the firing type induced. Besides, in some initially tonic neurons insensitive to OT, application of AVP induced phasic activity. This shows that each peptide depolarizes SON neurons and can trigger an electrical activity similar to the typical activity recorded in the mature responsive neurons.

To determine whether the site of action of the peptides is presynaptic or postsynaptic, OT and AVP were applied in the absence of extracellular Ca^{2+} . The depolarizing effect of both peptides was persistent in the absence of Ca^{2+} ($n = 3$ for OT and $n = 4$ for AVP) (Fig. 3*a,b*). However, the duration was significantly shorter than that observed in the presence of Ca^{2+} (application of 59 ± 4 sec; ratio of 1.75 ± 0.87 ; $n = 15$; $p < 0.01$). Because some synaptic activity can persist in Ca^{2+} -free solution in the SON (Inenaga et al., 1998), this result suggests but does not demonstrate a postsynaptic action. To further address this question, OT and AVP were applied to acutely dissociated SON neurons. Both peptides induced depolarization ($n = 4$ for OT and $n = 5$ for AVP) (Fig. 4*a,b*), confirming a postsynaptic mode of action. Again, briefer responses as compared with those observed in slices were induced by the peptides (application of 30 ± 2 sec, ratio of $2.35 \pm$

Figure 3. OT- and AVP-induced depolarization persisted in Ca^{2+} -free solution. Shown is an example of depolarization induced by OT (*a*) or AVP (*b*) in Ca^{2+} -free/(4 mM) Mg^{2+} solution (P15 and P8, respectively). The absence of Ca^{2+} did not prevent the depolarization but accelerated the repolarization on washout of the peptide (compare with Fig. 2).

Figure 4. OT and AVP depolarize SON dissociated neurons. Application of OT (*a*) and AVP (*b*) led to a depolarization and an increased activity in dissociated supraoptic neurons (P8), showing that they act directly on these neurons. Note that the depolarizations were of shorter duration than those obtained in slices.

0.22 , $n = 14$; $p < 0.05$). However, these responses were of longer duration than those obtained in Ca^{2+} -free solution ($p < 0.05$).

The effects of OT and AVP are maximum during a transitory period of development

We studied the incidence of the depolarization induced by OT and AVP from the day of birth to the last day before weaning (P0–P20).

Figure 5. Maturation of the autocontrol during postnatal development. *a*, Percentage of neurons responding to either OT or AVP (or both) versus postnatal age (in days). *b*, Amplitude of the depolarization induced by OT or AVP in responding neurons versus the postnatal age (in weeks). Means are significantly different between the first 2 weeks and the third postnatal week ($p < 0.05$). The number of neurons is represented in each bar.

Neurons responded neither to OT nor to AVP before P4 (Fig. 5). The first responding neurons were seen at P4. The incidence of the response increased to reach 100% by P7, and the incidence stayed maximal until P16. Then it dropped to 50% at P19–P20. No difference between the proportion of OT- and AVP-responding neurons was seen throughout the maturation.

As shown in Figure 5*b*, the amplitude of the depolarization induced by OT or AVP was similar during the first two postnatal weeks (6.5 ± 1 mV at P4–P6, $n = 10$, and 6 ± 0.5 mV at P7–P13, $n = 24$) and was significantly lower during the third postnatal week (4.5 ± 0.5 mV at P14–P20, $n = 17$; $p < 0.05$). This decrease in amplitude of the depolarization did not result from a decrease in membrane resistance (data not shown).

These results show that the depolarizing effect of the peptides is maximal during a transitory period of postnatal development. The peptides are released locally and lead to sustained depolarization of briefer duration in Ca^{2+} -free solution or in dissociated neurons. This suggests a regenerative mechanism by a somatodendritic release of the peptides (which is also a Ca^{2+} -dependent mechanism). We therefore determined whether the positive feedback loop could be closed by a facilitatory effect of the peptides on their own release.

OT and AVP selectively increase their own release from supraoptic nuclei

The release of OT and AVP from isolated SON was measured by radioimmunoassay. The specificity of the antibodies was checked by the absence of cross-reactivity between OT or AVP and their agonists (Fig. 6*a,b*). At all ages studied, application of V_{1a} agonist (F-180, $1 \mu\text{M}$) and V_2 agonist (DPAVP, $1 \mu\text{M}$) increased the release of AVP (Fig. 6*c*). OT agonist [(Thr⁴, Gly⁷)OT, $1 \mu\text{M}$] had no effect on AVP release (Fig. 6*c*). Conversely, the release of OT was

increased by OT agonist but unaffected by both V_{1a} and V_2 agonist (Fig. 6*d*).

Because the electrophysiological effects of the peptides displayed a transitory increase during development, we investigated whether the facilitatory effect on the release behaved similarly. As shown in Figure 7, the magnitude of the facilitation by the agonists increased between P2–P3 and P12–P13 (from $\sim 400\%$ of basal release at P2–P3 to ~ 700 – 800% at P12–P13). Then the evoked release decreased at P21 (200–300% of basal release) toward values in the range of those observed in adults (Moos et al., 1984; Wotjak et al., 1994). These data demonstrate that each peptide selectively increases its own release, and this effect is maximal during a transitory period of postnatal development.

DISCUSSION

This study demonstrates that hypothalamic magnocellular neurons in the developing rat are positively autocontrolled by a local positive feedback using the peptide they synthesize. OT and AVP are released under basal conditions within the SON and sustain electrical activity of SON neurons. Application of each peptide depolarizes specifically one population of SON neurons as early as P4, showing that the neurons express functional receptors early in development. The proportion of neurons responding to the peptides reaches 100% at P7 and decreases, along with the amplitude of the depolarization, during the third postnatal week. This suggests that the positive feedback is progressively replaced by more complex actions of the peptides as described in adults (excitatory and inhibitory effects). Interestingly, the peptides have a facilitatory effect on their own release, and this action is also maximal during the second postnatal week. However, the facilitatory effects on the release are observed as soon as P2–P3, when the neurons do not yet display depolarization during OT or AVP stimulation. It is possible that the proportion of responding neurons is too small before P4. However, this apparent discrepancy may also reflect the fact that the depolarizing effect of the peptides and their action on the release rely on distinct mechanisms.

Characteristics of autocontrol

The depolarization induced by the peptides persisted in Ca^{2+} -free solution and could be recorded on dissociated neurons, demonstrating a postsynaptic site of action. However, a presynaptic action as described in mature SON neurons (Kombian et al., 1997) is not excluded. The fact that brief depolarizations were observed in Ca^{2+} -free solution suggests that a Ca^{2+} -dependent process is involved in the maintenance of the response. Because the facilitatory effect of OT on its own release is a Ca^{2+} -dependent mechanism in adult (Moos et al., 1984), it is probable that a somatodendritic release of endogenous peptide, induced by the initial depolarization or the applied peptide, prolongs the response. Consistent with this view, responses were also brief in dissociated neurons that have lost a large part of their dendritic tree, but they were longer than those observed in Ca^{2+} -free solution, probably because the somatodendritic release has not been blocked.

In addition, our evidence indicates that OT and AVP act specifically on their secreting neurons. Indeed, each peptide increases selectively the electrical activity of one population of SON neurons and generally leads to the typical activity patterns of adult OT and AVP neurons (Poulain and Wakerley, 1982). Moreover, our release experiments show that each peptide increases only its own release, confirming that it acts selectively on neurons that secrete it. This specificity of action is in accordance with what is known in adult SON neurons. Electrophysiological recordings and calcium imaging in dissociated neurons, in slices or *in vivo*, have shown that most SON neurons respond solely to the peptide that they synthesize (Moos et al., 1984; Kawarabayashi et al., 1993; Wotjak et al., 1994; Dayanithi et al., 1996; Moos et al., 1998) and express only their corresponding receptors (Freund-Mercier et al., 1994; Hurbin et al., 1998). However, in this and in previous studies (Dayanithi et al., 1996), we found some neurons responding to both peptides, a fact that may be related to the existence of mixed electrophysiological

Figure 6. OT and AVP increase selectively their own release from isolated SON. *a*, Radioimmunoassay displacement curve for vasopressin (●), F180 (Ago V1a, ■), and DDAVP (Ago V2, ▲) shows that the agonists do not cross-react with the AVP antiserum. Points are means of triplicate assays. *b*, Same as *a* with OT and (Thr⁴, Gly⁷)OT (Ago OT). *c*, *d*, Examples of experiments (P16 and P13, respectively) showing spontaneous and evoked AVP (*c*) and OT (*d*) release. Note the specific effect of each class of agonist on the release of its corresponding peptide.

Figure 7. Maturation of the evoked release of OT and AVP. The AVP release evoked by a V₂ (black) or a V_{1a} (gray) agonist and the OT release evoked by the OT agonist (white) are represented as the percentage of the basal release of AVP and OT, respectively. The number of experiments is represented above each bar. For V₂ agonist, means are significantly different between P12–P13 and P2–P3 ($p < 0.01$) or P21 ($p < 0.001$). For V_{1a} agonist, means are significantly different between P12–P13 and P2–P3 ($p < 0.05$) or P21 ($p < 0.01$). For OT agonist, means are significantly different between P12–P13 and P2–P3 ($p < 0.05$) or P21 ($p < 0.01$).

phenotype neurons *in vivo* (Moos and Ingram, 1995) and of neurons synthesizing both peptides or both mRNAs (for review, see Gainer 1998).

In adult rats, the actions of the peptides are complex: excitatory and inhibitory effects have been described for both peptides. AVP has been reported to have no effect (Carette and Poulain, 1989), to

increase (Inenaga and Yamashita, 1986), to decrease (Leng and Mason, 1982), or to have either effect on the firing of AVP neurons (Gouzenes et al., 1998). Various actions have also been reported for OT. It stimulates OT neurons in male rats and in ovariectomized, lactating, or parturient females (Kawarabayashi et al., 1993; Kuriyama et al., 1993); in cycling females, OT leads to an inhibition of OT neurons (Murai et al., 1998). Although the mechanisms of action of the peptides on the electrical activity are not fully understood, their principal role seems to maximize their systemic release via an appropriate patterning of the electrical activity (Moos et al., 1998). Our data show that in postnatal male rats, OT and AVP are almost exclusively excitatory. Both peptides depolarize SON neurons, and application of their receptor antagonists decreased electrical activity in all neurons tested. This positive feedback on the electrical activity and on the somatodendritic release indicates that an increased release in a single or few neurons can activate neighboring neurons expressing the same receptors and induce a propagating wave of excitation throughout the nucleus. Moreover, we show that this positive autocontrol is maximal in incidence and in amplitude during a limited period of postnatal development, suggesting that it could play a different role than favoring the systemic release as in adult rats.

Possible roles of autocontrol during development

Electrical activity regulates several developmental processes in the nervous system. One mechanism is by tuning electrical activity and $[Ca^{2+}]_i$ fluctuations, which may influence various intracellular functions (Berridge, 1998). In the embryonic *Xenopus* spinal cord, the frequency of spontaneous calcium transients has been shown to regulate the number of neurons expressing GABA (Gu and Spitzer, 1995) and the rate of axon extension (Gomez and Spitzer, 1999). Hence, autocontrol of SON neurons is a way to induce or increase the electrical activity at a time when the neurons are poorly activated by afferents. This increased activity could also be important in increasing axonal release of the peptides. Indeed, even if OT and AVP are not involved in their main adult physio-

logical roles, they are thought to act as developmental factors on peripheral organs (Carter et al., 1993). A neurotrophic action (by collaterals of paraventricular neurons, also containing OT and AVP) has also been suggested. A transient postnatal expression of OT and AVP binding sites has been described in several rat brain areas (Tribollet et al., 1991), and AVP promotes neurite outgrowth of embryonic *Xenopus* neurons (Brinton and Gruener, 1987). Therefore, this autocontrol, by increasing electrical activity and hence axonal release, could be important not only for OT and AVP neurons themselves but also for the maturation of other structures.

This autocontrol could also be involved in the selection of appropriate synaptic connections. In several brain areas, growing axons form initially imprecise connections during development, and increasing evidence suggests that coordination of neuronal activity is involved in the refinement of synaptic connections (Cramer and Sur, 1995). In the visual cortex or spinal cord, activities of related neurons are strongly coupled during the period when inappropriate connections are eliminated (Walton and Navarrete, 1991; Peinado et al., 1993; Kandler and Katz, 1998). In these examples, the coordination of the activity involves communication via gap junctions, transmitting either an electrotonic message in the case of motoneurons (Walton and Navarrete, 1991) or an intracellular diffusible messenger in cortical neurons (Kandler and Katz, 1998). Our results show that an extracellular messenger could also ensure the coordination of the activity of a specific population of SON neurons and hence participate in the specific innervation of OT and AVP neurons. This hypothesis is in accordance with the observation that monoaminergic afferents, even if they reach the hypothalamus during the late embryonic period, increase in number and only start to form connections during the second postnatal week (Ugrumov, 1992). This is also in accordance with a study showing that the connections between vagal afferents and OT neurons become functional between P2 and P10 (Nelson et al., 1998). Moreover, at the same time there is a transient increase in the density of the current supported by NMDA receptors (Hussy et al., 1997), which are known to be involved in shaping axonal arbors during development (Scheetz and Constantine-Paton, 1994). Finally, the neurons show important and transient increases in their dendritic shafts during the second postnatal week (V. Chevalayre and M. G. Desarménien, unpublished results). Because autocontrol is maximal during this period, it could have important implications in the selection of specific OT or AVP afferents.

Conclusion

Whatever the exact role of autocontrol described here, it presents ideal characteristics for involvement in the maturation of SON neurons. OT and AVP increase electrical activity, and this action is amplified by a facilitatory effect on their own somatodendritic release. Each peptide acts selectively on its respective neuron in most cases, and maximal effects are concomitant with important developmental processes such as synapse formation or topographical segregation between OT and AVP neurons (Lazcano et al., 1990). Our data and those of previous investigators (Hussy et al., 1997; Ugrumov, 1992) show that the second postnatal week is a key period in the maturation of SON neurons, in which positive autocontrol could play important roles.

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