Vasopressin (AVP) magnocellular neurons of hypothalamic nuclei express specific phasic firing (successive periods of activity and silence), which conditions the mode of neurohypophyseal vasopressin release. In situations favoring plasmatic secretion of AVP, the hormone is also released at the somatodendritic level, at which it is believed to modulate the activity of AVP neurons. We investigated the nature of this autocontrol by testing the effects of juxtamembrane applications of AVP on the extracellular activity of presumed AVP neurons in paraventricular and supraoptic nuclei of anesthetized rats. AVP had three effects depending on the initial firing pattern: (1) excitation of faintly active neurons (periods of activity of <10 sec), which acquired or reinforced their phasic pattern; (2) inhibition of quasi-continuously active neurons (periods of silences of <10 sec), which became clearly phasic; and (3) no effect on neurons already showing an intermediate phasic pattern (active and silent periods of 10–30 sec). Consequently, AVP application resulted in a narrower range of activity patterns of the population of AVP neurons, with a Gaussian distribution centered around a mode of 57% of time in activity, indicating a homogenization of the firing pattern. The resulting phasic pattern had characteristics close to those established previously for optimal release of AVP from neurohypophyseal endings. These results suggest a new role for AVP as an optimizing factor that would foster the population of AVP neurons to discharge with a phasic pattern known to be most efficient for hormone release.

**Key words:** vasopressin; autocontrol; phasic activity; optimization; supraoptic and paraventricular nuclei; extracellular electrical activity; hypothalamic magnocellular neurons

Most vasopressin (AVP) neurons in rat supraoptic nucleus (SON) and paraventricular nucleus (PVN) express a phasic activity characterized by a succession of periods of activity and silence. This specific pattern depends on intrinsic mechanisms as well as extrinsic factors such as plasma osmolality, blood volume, and pressure (for review, see Poulin and Wakerley, 1982; Armstrong, 1995). During hyposmotic stimulus or hemorrhage, AVP neurons either acquire or reinforce a phasic pattern (Brimble and Dyball, 1977; Poulin et al., 1977; Wakerley et al., 1978), which consequently increases systemic AVP release (for review, see Dyball, 1988). Hypo-osmotic stimulation has opposite effects, silencing AVP neurons in vitro (Oliet and Bourque, 1993a,b) and in vivo (Hussy et al., 1997). A supplementary control could be exerted by the peptide itself, similar to that described for oxytocin (OT) neurons, the stereotyped bursting activity of which depends on locally released OT during suckling (Moos and Richard, 1989; Lambert et al., 1993). Indeed, somatodendritic release of AVP has been demonstrated in both SON and PVN by morphological and pharmacological studies (Di Scala-Guenot et al., 1987; Pow and Morris, 1989; Ludwig et al., 1995). This local AVP release increases during osmotic stimulation and hemorrhage (Neuman et al., 1993; Ota et al., 1994), two physiological situations that favor the expression of phasic pattern in AVP neurons. Moreover, local application of AVP has been shown to modulate the activity of AVP neurons, although this effect is controversial. AVP has been reported to decrease (Leng and Mason, 1982; Abe et al., 1983), increase (Inenaga and Yamashita, 1986), or have no effect on the spontaneous firing (Carette and Poulain, 1989).

As an attempt to clarify the role of intranuclear AVP release on the firing rate and firing pattern of AVP neurons, in vivo extracellular recordings of these neurons were performed during juxtamembrane application of various concentrations of AVP, in male or lactating rats. We report that AVP favors the expression of phasic activity by presumed AVP neurons and homogenizes the characteristics of this pattern. A preliminary report of these results has appeared (Dayanithi et al., 1995).

**MATERIALS AND METHODS**

Experiments were performed on male or female lactating Wistar rats (Depré; 250–350 gm body weight). In the evening before the experiments, dams (8–12 d postpartum) were separated from all of their pups except one (~14 hr of separation). Rats were anesthetized with urethane (ethyl carbamate, 1.2 gm/kg; Sigma) applied by a single intra-peritoneal injection. A cannula was inserted into the jugular vein for intravenous injection of Brielat (9 mg/kg; Lilly) to supplement anesthesia when necessary. In lactating rats, a thoracic mammary gland was cannulated and connected to an electromagnetic pressure transducer (Vigo-Spectramed, Oxnard, CA) to measure intramammary pressure peaks as an index of OT release. Suckling with 10 pups was applied 3 hr after anesthesia to trigger the milk ejection reflex (Lincoln et al., 1973). The rats were placed in a stereotaxic frame. Xylocaine (hydrocaine, 1% solution; Rhône-Poulenc, Rohrer, France) was injected subcutaneously to all surgical sites and at the points of contact with the stereotaxic frame. After trepanning, a bipolar stimulating electrode was inserted into the pituitary stalk at the limit of the neurohypophysis (Anteriority, (A), 4.7; Laterality, (L), 0; Height, (H), 0.5; stereotaxic atlas of Albe-Fessard et al., 1966) for antidromic
identification of magnocellular neurons (SON or PVN neurons). The exact position of the electrode was attested (1) in lactating rats by a peak of intramammary pressure evoked by repetitive electrical stimulation of the pituitary stalk (40 Hz, 0.3–0.5 mA, 0.5 msec for 4 sec), and (2) in male rats by a posteriori observation of the site of lesion provoked by electrolytic current (10 mA during a few seconds). Recording glass micropipettes filled with 0.5 M sodium acetate solution (8–20 MΩ impedance) were placed in the SON (A, 7–8; L, 1–2.3; H, 2.5–2) or PVN (A, 6.5–7; L, 0.1–0.7; H, 4–3.5) and connected to conventional electrophysiological apparatus. Extracellular electrical activities were displayed on a chart recorder (Astromed SNC, Trappes, France) and simultaneously stored on computer by means of a Cambridge Electronic Design (Cambridge, UK) 1401 interface card. Juxtacellular injections of AVP close to the recorded neuron were performed using a glass micropipette glued 20–40 μm above the recording micropipette and connected to a pneumatic picopump (WPI Inc., Sarasota, FL). Nitrogen pressure was adjusted to 0.7–1.4 bars according to the tip diameter of the pressure micropipette (10–20 μm), and drugs were delivered by applying pressure pulses of 10–30 msec with a square waveform repeated every 1–10 sec over periods of 2–15 min. Calibration of ejection pulses performed under microscopic observation revealed that the total volume ejected for each application varied between 20 and 100 nl. Because the pipette tip could be partly occluded by the tissue while it was lowered through the brain, the volume applied was possibly less than estimated. The outflow from the pressure pipette was checked under binocular observation before and after each recording.

AVP (Boehringer Mannheim, Meylan, France) was used at three concentrations (0.1, 1, and 10 μM) in artificial CSF (aCSF, in mM: NaCl, 126.5; NaHCO3, 27.5; KCl, 2.4; KH2PO4, 0.5; CaCl2, 1.1; MgCl2, 0.83; and Na2SO4, 0.5 with glucose, 5.9 g/l). The pH was adjusted to 7.4, and osmolarity was 300 ± 2 mOsm/l. Because of variability of the injection parameters used in each experiment, the drug concentration seen by each neuron tested can differ.

Identification of neurons and data analysis. The magnocellular neurons were identified by their antidromic response to electrical stimulation of the neurohypophysis (the response had a constant latency, followed high frequency stimulation, and collided with an orthodromic spike). Presumed AVP neurons, in male or lactating rats, were in most cases characterized by their phasic activity comprising distinct periods of activity separated by periods of silence (Fig. 1A,B), were also considered putative AVP neurons, those that fired quasi-continuously (long periods of activity separated by silences of <10 sec; Fig. 1C, also see Fig. 3A), or that were quasi-silent (periods of activity of <10 sec; Fig. 2A) but that responded to AVP by the appearance of a phasic activity. Because (1) phasic activity is a major characteristic of AVP neurons (for review, see Armstrong, 1995), (2) only AVP neurons have been shown to possess AVP receptors (Berlove and Pickut, 1990; Dayanithi et al., 1995), (3) all OT neurons identified by milk ejection-related activity were found insensitive to AVP, and (4) none of the 21 AVP-sensitive phasic neurons recorded from lactating rats during milk ejection bursts showed milk ejection bursts, we will refer to the phasic neurons recorded in this study as AVP neurons. OT neurons displayed a random continuous basal activity, over which, in lactating rats during the milk ejection reflex, a characteristic periodic bursting activity developed. The latter consisted of an intense and brief activation 12–18 sec before each milk ejection (attested by a peak of intramammary pressure or by a stretch reaction of the pups). On some occasions, OT (1 ng, i.e., 1 μl of 1 μM solution in 0.9% NaCl; Sandoz, Basel, Switzerland) was injected into the third ventricle (A, 8; L, 0; H, 3) to induce or facilitate bursting activity (Freund-Mercier and Richard, 1984). In other cases, a hyperosmotic stimulation was applied (2 ml of 1.5 M NaCl solution, i.p.) to induce specific tonic activation of OT neurons (Wakerley et al., 1978).

Analysis of the electrophysiological recordings was performed using both in-house programs and Spike2 analysis software (Cambridge Electronic Design). The firing pattern of AVP neurons was analyzed for 15–20 min before (control period) and during the entire application of AVP. This pattern was characterized by the following parameters: (1) f, the mean firing rate during periods of activity called “intraburst frequency” (in spikes/sec); (2) d, the duration of active periods (in sec); (3) D, the duration of silent periods (in sec); and (4) Q, the proportion of time spent by a phasic cell during burst (calculated by exclusion of silent periods of >2 sec). This latter parameter is equivalent to the activity quotient described by Wakerley et al. (1975). For OT neurons recorded during suckling, the parameters considered were (1) the background activity (in spikes/sec) and (2) the burst amplitude (total number of spikes per burst). AVP was considered to affect the firing pattern of AVP or OT neurons when changes in parameters exceeded 10% of control values. Indeed, during 5–10 min applications of aCSF, the changes that occurred in the firing characteristics of eight AVP (Fig. 1A–C) and five OT neurons (data not shown; see Moos et al., 1997) were always <10%. This is illustrated for AVP neurons in Figure 1D, in which the Q value measured during aCSF microinjection (and expressed as a percentage of the initial Q, Q0) is plotted as a function of Q0. For OT bursting neurons, the mean firing rate and burst amplitude were similar during control period and aCSF application (3.0 ± 0.6 spikes/sec; 69 ± 12 and 69 ± 12 spikes, respectively). All values are expressed as mean ± SEM. Statistical significance was assessed by different tests indicated in Results. Curve fitting was performed with Origin software (Microcal Software).
RESULTS

Effect of AVP on AVP neurons

Recordings were obtained from 39 AVP neurons in either the SON (n = 26) or PVN (n = 13) from 14 rats. Three concentrations of AVP were applied, 0.1, 1, and 10 μM on, respectively, 9, 8, and 22 AVP neurons that displayed different patterns of discharge (phasic, quasi-continuously active, or quasi-silent neurons). Three different effects of AVP on the firing pattern were observed: excitatory (Fig. 2), inhibitory (Fig. 3), or no effect (Fig. 4), and these were observed on both PVN and SON neurons. Changes induced by AVP were sustained and reversible with a return to control parameter values within 5–10 min after the end of the application, independently of the dose of AVP or the duration of the application. Because no difference could be detected related to the concentration of AVP applied (see below), all results were pooled.

An excitatory effect was observed for 17 neurons. Nine were quasi-silent before AVP application and acquired a phasic pattern (A). AVP induced an increase in burst duration and a decrease in the duration of silences (B, C). This effect was accompanied with a slight decrease (B), an increase (A), or no effect in the intraburst frequency (C).

An inhibitory effect on the activity of 15 neurons. Eight were initially quasi-continuously active neurons and acquired a phasic pattern (Fig. 3). AVP inhibited the phasic pattern by decreasing the duration of active periods (B) or increasing the duration of silences (C). In C, this effect outlasted the duration of application, was more pronounced during the second test, and was accompanied by an increase in intraburst frequency.
Figure 4. AVP did not affect the firing pattern of some clearly phasic AVP neurons. AVP applied on clearly phasic neurons did not affect their firing pattern (A, B). Note that in B, AVP slightly increased the intraburst frequency, an effect more pronounced during the second application.

Figure 5. Relationships between the effects of AVP and the initial firing pattern. The duration of active periods, the duration of silent periods, and the activity quotient measured during the application of AVP (\(d_{AVP}, ds_{AVP}, \text{ and } Q_{AVP}\), respectively) are expressed as a percentage of their respective initial values (\(d_i, ds_i, \text{ and } Q_i\)) and plotted as a function of \(d_i, ds_i, \text{ and } Q_i\) on semilogarithmic scales. In A and C, lines are apparent linear regressions fitted with 38/39 data points initially plotted on a log/log scale. These fits indicate significant relationships between \(ds_{AVP}\) and \(d_i\), and \(Q_{AVP}\) and \(Q_i\). Fits cross the 100% line at 18 sec (\(d_i\)) and 0.50 (\(Q_i\)).

during the application (Fig. 3A), with \(d_i = 23 \pm 7\) sec and \(ds_i = 9 \pm 2\) sec. For seven initially clearly phasic neurons, inhibition consisted in a decrease in \(d_i\) (from 44 ± 8 to 32 ± 5 sec) and an increase in \(ds_i\) (from 19 ± 4 to 30 ± 6 sec) (Fig. 3B,C). These inhibitory effects resulted in a decreased \(Q_i\) (from 0.81 ± 0.03 to 0.61 ± 0.04; \(n = 15\)). AVP had no effect on seven neurons, all showing clear phasic activity (Fig. 4). AVP affected neither \(d_i\) nor \(ds_i\) (\(d_i, 19 \pm 2\) vs 20 ± 4 sec; \(ds_i, 23 \pm 4\) vs 21 ± 4 sec), and consequently, \(Q_i\) was equivalent before and during AVP application (0.47 ± 0.05 vs 0.49 ± 0.05). Strikingly, it appeared from these observations that the nature of the AVP effects was related to the characteristics of the initial pattern of activity of the neurons. Indeed, AVP seemed to excite mostly neurons with low initial activity, to inhibit mostly neurons with a high initial activity, and not to affect those displaying an intermediate phasic activity.

To characterize this dependence on the initial pattern of activity, we expressed the values of \(d_i, ds_i, \text{ and } Q_i\) in the presence of AVP (\(d_{AVP}, ds_{AVP}, \text{ and } Q_{AVP}\)) as a function of their initial value (\(d_i, ds_i, \text{ and } Q_i\); Fig. 5). Changes in \(d_i, ds_i, \text{ and } Q_i\) were clearly inversely related to \(d_{AVP}, ds_{AVP}, \text{ and } Q_{AVP}\) (Fig. 5). For \(d_i\) and \(Q_i\), an apparent linear regression could be fitted when the data were plotted on a double logarithmic scale, which indicated that the values of \(d_i\) and \(Q_i\) for which AVP had no effect were 18 sec (Fig. 5A) and 0.56 (Fig. 5C), respectively. The relationship between \(ds_{AVP}\) and \(d_i\) was evident, but the distribution prevented a satisfying fit of the data. However, it clearly appeared that \(ds_i\) values >30 sec tended to be decreased, whereas those <10 sec tended to be increased. We then compared the mean \(Q_i\) of neurons excited by AVP (\(Q_i = 0.11 \pm 0.02; n = 17\)) and of those inhibited (\(Q_i = 0.81 \pm 0.03; n = 15\)). Student’s \(t\) tests revealed a significant difference at \(p < 0.001\), indicating that these neurons belonged to different populations. Furthermore, both \(Q_i\) values differed significantly from 0.50 (\(p < 0.001\)), a value corresponding to the mean \(Q_i\) of neurons that were unaffected (0.49 ± 0.05; \(n = 7\)). We also compared the effect of AVP on neurons now categorized in three equivalents groups according to their \(Q_i\) (\(Q_i < 0.33\); \(0.33 < Q_i < 0.66\); \(Q_i > 0.66\); Fig. 6A). Low \(Q_i\) (0.11 ± 0.02; \(n = 17\)) were significantly increased by AVP to a mean value of 0.54 ± 0.04 (\(p < 0.001\), Student’s paired \(t\) test). High \(Q_i\) (0.82 ± 0.03; \(n = 15\)) was decreased to 0.63 ± 0.04 (\(p < 0.01\), and intermediate \(Q_i\) (0.50 ± 0.04; \(n = 7\) ) was unaffected (0.54 ± 0.06). Last, we looked at the effect of AVP on the distribution of \(Q_i\) values. Whereas there was no significant difference between the averaged values of
In the present work, vasopressin was applied onto magnocellular presumed AVP neurons to clarify its role in the control of their electrical activity. The responses observed were attributable to AVP and not to nonspecific mechanical perturbations, as attested by the lack of effect of aCSF application. This suggests that our local pressure microinjections allow the drug to diffuse gently around the neurons without creating deformation of brain tissue. Furthermore, the ineffectiveness of AVP to affect OT neurons, which like AVP neurons possess mechanoreceptors (Oliet and Bourque, 1993a,b), attests to the specificity of the effects of AVP on AVP neurons.

AVP had different effects on the activity pattern of AVP neurons: excitatory, inhibitory, or no effect. Qualitatively similar responses were obtained with the three doses of AVP tested, and no correlation could be found between the magnitude of the effect and the AVP concentration. The absence of dose dependency may result from an already high level of activation of AVP receptors by the lowest concentration used (0.1 μM; see Dayanithi et al., 1996). On the other hand, these effects remarkably depended on the initial activity pattern. Indeed, AVP excited neurons displaying a low activity quotient, triggering or enhancing phasic activity, and inhibited highly active neurons; phasic neurons initially displaying intermediate Q were not affected. Consequently, by reducing the proportion of neurons displaying very low and very high activity, AVP narrowed the distribution of Q, which became normal, at a mode of ~0.57. Therefore, application of AVP resulted in the homogenization of the firing pattern of the whole population of AVP neurons. This mean intermediate phasic pattern was characterized by a duration of active periods (da) of ~20 sec and duration of silences (ds) of 10–30 sec. With regard to the intraburst frequency, f, AVP also had different effects, but they were unrelated to the initial pattern of activity. So, AVP regulated the phasic pattern and firing rate differently and independently.

**Inhibitory and excitatory role of AVP**

As far back as 1981, Leng had already speculated that AVP could modulate the phasic pattern of AVP neurons in rats. Thereafter, using extracellular recordings from rat hypothalamic slices, AVP has been shown to modify the firing pattern of SON or PVN neurons, but different reports are contradictory; according to Inenaga and Yamashita (1986), AVP had an excitatory effect on the firing rate of unidentified PVN neurons, whereas Leng and Mason (1982) reported that AVP inhibited the phasic pattern of AVP neurons in SON from Brattleboro rats. By intracellular recordings on guinea pig hypothalamic slices, Carette and Poullain (1989) observed that application of AVP did not affect AVP phasic neurons but depolarized neighboring interneurons projecting onto AVP neurons. Finally, Abe et al. (1983) reported that AVP depolarized the membrane but decreased the spontaneous firing rate in most neurons tested (~69%), the others being either excited (~5%) or unaffected (~26%). However, when AVP was applied to a previously hyperpolarized neuron, the inhibitory effect on firing was reversed to excitatory in both spontaneously active and silent neurons. In agreement with this observation, dissociated SON neurons, which display no or little spontaneous activity (M.G. Desarménien, personal observation), respond to AVP by an influx of calcium through voltage-dependent calcium channels, attesting for an excitatory effect of AVP (Dayanithi et al., 1996; Sabatier et al., 1997). The resulting increase in intracellular calcium concentration could moderate the phasic pattern of...
AVP favors a phasic activity optimizing the neurohypophyseal AVP release

Previous in vitro experiments using freshly isolated rat neurohypophyses impaled onto a stimulating electrode have established a relationship between the activity pattern, the firing rate, and the release of AVP. Dutton and Dyball (1979) have demonstrated that stimulating the neurosecretory tissue with a phasic pattern optimizes AVP release from nerve endings. Thereafter, it was shown that most of the release of AVP from the neurohypophysis occurs during the first 20 sec of activity (Bicknell et al., 1984; Shaw et al., 1984), and interruption of firing of >20 sec dramatically enhances systemic release of AVP (Cazalis et al., 1985). These values are close to the values of $da$ and $ds$ found in this study. With regard to $f$, the values measured during AVP application remain in the range of frequency known to be most efficient for the release of AVP from neurohypophysis in vitro, i.e., frequency of $\geq 6$ pulses/sec (Dutton and Dyball, 1979). Consequently, the changes in $f$ induced by AVP are likely to have less influence on the systemic release of AVP than those in the activity pattern, as suggested already by Poullain et al. (1988). Therefore, AVP released intranuclearly in cases of high hormonal need may be considered as optimizer of the phasic activity pattern of AVP neurons, which is a major determinant of the amount of AVP released from the neurohypophysis. Interestingly, although different in nature, the role of intranuclear OT is also to facilitate a neuronal behavior optimum for hormonal function, i.e., the suckling-induced synchronous bursting activity (Moos and Richard, 1989; Lambert et al., 1993). This control is specific to OT, because AVP is ineffective (Freund-Mercier and Richard, 1984; present data). Thus, the two populations of magnocellular neurons display specific rhythmic patterns of discharge, which are controlled and fostered by their respective peptide.

Physiological meaning

Using a dual immunocytochemical labeling procedure, it has been shown that AVP receptor sites are associated with AVP-containing neurons in SON and PVN (Berische and Pickut, 1990). Through these autoreceptors, the activity of AVP neurons could be modulated by AVP released somatodendritically (Neumann et al., 1993) or by the connections between AVP neurons within the SON (Leng and Wiersma, 1981). These effects could be complemented by a presynaptic action of AVP on afferent terminals, as recently suggested by Kombian et al. (1997), or a recurrent action via neighboring interneurons projecting onto magnocellular neurons (Leng and Dyball, 1983). This modulatory role would be particularly prominent in physiological situations in which local AVP release is increased, e.g., during hyperosmotic stimulation (Neumann et al., 1993) and hemorrhage (Ota et al., 1994). The intranuclear release of AVP in response to these stimuli has been shown to be maintained for several hours (Ludwig et al., 1994). One would then expect sustained modulatory effects of AVP on the firing of AVP neurons. This should allow the expression of a phasic pattern of discharge that would be optimal for a sustained systemic AVP release, in accordance to the physiological demand. Interestingly, a former study has reported that 6–18 hr dehydration induced 80–100% of AVP neurons to fire phasically with mean burst duration between 20 and 24 sec and mean silence duration between 13 and 17 sec (Wakerley et al., 1978).

In conclusion, the present work shows that local AVP favors the expression by AVP neurons of a specific phasic activity known to optimize systemic AVP release. This reveals a new concept of modulation of neuronal activity by centrally released peptides: the optimization of a neuronal discharge in accordance with the physiological demand.

REFERENCES

Andrew RD, Dudek FE (1984) Analysis of intracellularly recorded pha-