tase (Sigma). The release of phosphate from *p*-nitrophenyl phosphate, due to the antibody-conjugated alkaline phosphatase, was monitored spectrophotometrically in a special reader (Microelisa Auto-Reader, Dynatech) and was proportional to TMV concentration in a log linear manner for about two logs (< 2 ng to > 200 ng).

The 2-5A used throughout this work was a core preparation of the trimer (A2′p5′A2′p5′A) (*16*). This 2-5A core was checked by thin-layer chromatography (polyethyleneimine plates developed with 0.1*M* LiCl) along with reference 2-5A samples (obtained from P-L Biochemicals) treated with alkaline phosphatase to remove their 5′-triphosphate. Phosphorylated 2-5A requires the calcium phosphate coprecipitation technique to penetrate into animal cells (*5*), whereas the 2-5A core penetrates if introduced in a regular medium for cell growth (*10*). Since there was no such experience with plant tissues, the 2-5A core in this series of experiments was applied with the calcium phosphate coprecipitation buffer (*5*).

Because tobacco leaf disks do not contain identical cells, and it is impossible to control the number of cells in each disk that are actually infected on initial inoculation, the level of TMV multiplication varied from one experiment to another. Nevertheless, TMV multiplication was invariably greatly inhibited in 2-5A–treated leaf disks in a concentration-dependent manner (Fig. 1). The kinetics of TMV multiplication in the absence and presence of 2-5A is shown in Fig. 2. Usually, concentrations of 100 to 200 n*M* 2-5A were sufficient to cause near-total inhibition. The 2-5A must be applied to the tissue early in infection to obtain an antiviral effect. A short treatment with 2-5A at any time earlier than 6 hours after inoculation was most effective, but thereafter the antiviral effect gradually diminished with time (Fig. 3).

The above results were corroborated by infectivity tests. Homogenates of buffer-treated and of 2-5A–treated disks were applied to opposite halves of the same *Datura stramonium* L. leaves. The control half-leaves inoculated with buffer-treated disks developed 81 local lesions, whereas the half-leaves inoculated with homogenates of disks treated with 200 n*M* 2-5A core developed only one local lesion.

We assume that 2-5A penetrates the plant cells, in analogy to the animal system. However, there is no indication that this is indeed the case and the 2-5A may exert its effect on plants by affecting cell membranes. The structure of the AVF-induced polymerized ATP has not yet been fully established, although many indications, such as thin-layer chromatography and sensitivity of various enzymes, point to a possible 2-5A nature (*17*). The biological activity of 2-5A in plants suggests a role for this compound in the plant's resistance mechanism.

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16. The 2-5A trimer was chemically synthesized by S. Rapoport and Y. Lapidot, and obtained through A. Kimchi.
17. Y. Devash, in preparation.
18. Supported by a grant from the Volkswagen Foundation.

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Oxytocin, Vasopressin, and Estrogen-Stimulated Neurophysin: Daily Patterns of Concentration in Cerebrospinal Fluid

Abstract. *The concentrations of oxytocin, arginine vasopressin, and estrogen-stimulated neurophysin in cerebrospinal fluid of monkeys showed a daily fluctuation with high concentrations occurring during the light period. The patterns of oxytocin and estrogen-stimulated neurophysin in the cerebrospinal fluid were not observed in the plasma nor were they altered after the administration of a dose of estradiol that increased concentrations of estrogen-stimulated neurophysin in plasma. The disassociation between these cerebrospinal fluid and plasma patterns and values suggests that the secretory activity of neurons that release estrogen-stimulated neurophysin and oxytocin into the cerebrospinal fluid is controlled by mechanisms different from those that control their release into the plasma.*

Oxytocin and arginine vasopressin (AVP) originate in neuron cell bodies in the hypothalamus (*1*, *2*). Projections from these neurons extend to many areas of the nervous system, including the posterior pituitary, brainstem, hypothalamus, and spinal cord (*3*). Anatomical and electrophysiological observations suggest that these several projections function independently (*4*). Our data support this concept of independence by demonstrating that the release of oxytocin and its carrier protein estrogen-stimulated neurophysin (ESN) into the cerebrospinal fluid (CSF) of rhesus monkeys is not linked to the release of oxytocin and ESN into plasma. We report here that the concentration of oxytocin and ESN in the CSF, continuously withdrawn from unanesthetized rhesus mon-

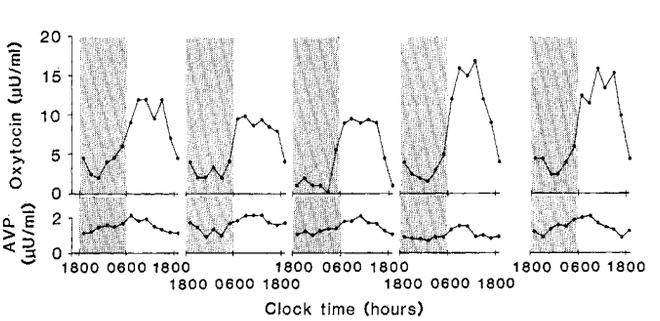

Fig. 1. Patterns of oxytocin and AVP concentrations in the CSF of five male rhesus monkeys studied during diurnal lighting (LD 12:12). Samples of CSF were collected in 2-hour fractions. Shaded areas indicate the time of day when lights were off, and unshaded areas indicate when lights were on.

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keys (*Macaca mulatta*), fluctuates over the course of the day in a highly organized pattern that is not observed in the plasma. Stimulation of the peripheral (plasma) release of ESN by administration of estradiol does not alter the pattern or change the concentration of ESN or oxytocin in the CSF. The concentration of AVP in the CSF also fluctuates over the course of the day.

Hormones were continuously monitored in partially restrained rhesus monkeys by measuring the amounts of hormone in cisternal CSF and plasma (*5*). The concentrations of AVP and oxytocin described in Fig. 1 were measured in one laboratory by means of a radioimmunoassay (*6*); the oxytocin and ESN described in Figs. 2 and 3 were measured in another laboratory by other radioimmunoassays (*7, 8*).

A daily rhythm characterized the concentration of oxytocin in the CSF (Figs. 1, 2, and 3B), with the daytime values being 3- to 12-fold higher than the nighttime values. Values were lowest in the middle of the dark period, increased sharply so that peak concentrations occurred early in the light period, and declined gradually until middarkness. The pattern and the magnitude of change of oxytocin were characteristic for each animal, and did not vary during the periods of observation. In one of the 14 animals studied, oxytocin was not measurable in CSF or plasma. The 24-hour mean oxytocin concentration for the five animals in Fig. 1 was 7.6 μU/ml (range 5.2 to 10.2). When oxytocin was measured in another set of similarly obtained samples of CSF by a different radioimmunoassay (*7, 8*), the pattern of changes in concentration was the same; the 24-hour mean oxytocin concentration was 7.1 μU/ml (range 5.6 to 9.3) (not illustrated).

Like oxytocin, AVP concentrations in the CSF of the rhesus monkey exhibited a daily rhythm, with daytime values approximately twofold higher than nighttime values (Fig. 1). This is similar, but smaller, than the daily rhythm recently described in the cisternal fluid of the cat (*9*). The 24-hour mean concentration for the five animals in Fig. 1 for AVP was 1.3 μU/ml (range 0.9 to 1.4).

In four additional animals, samples of CSF and plasma were obtained over the same 24-hour period. A daily rhythm in the CSF concentration of oxytocin was again observed, with the time courses identical to those described in Fig. 1 (see Fig. 2); no daily rhythm was apparent for the 24-hour pattern of plasma oxytocin. The CSF concentration of ESN also varied over the 24-hour day, with high concentrations occurring during the daytime and low concentrations at night. However, the magnitude of the variation was less than that for oxytocin and a diurnal rhythm was not invariably present. In some animals the curve describing the time course of the daily ESN fluctuation appeared to be shifted to the right, with the high and low concentrations occurring several hours after the corresponding high and low concentrations of CSF oxytocin [for individual animals in Fig. 2 (not illustrated) and Fig. 3B]. Mean data showed a similarity of peaks, with ESN having a broader curve (Fig. 2). Plasma ESN concentrations did not systematically vary over the 24-hour day, and they did not correspond to the simultaneously obtained CSF values (Fig. 2). In Fig. 2, when CSF and plasma samples were obtained simultaneously, the mean concentrations of oxytocin, in microunits per milliliter, in CSF and plasma were 10.0 (range 5.8 to 18.6) and 1.3 (range 1.0 to 2.4), respectively; the mean concentrations of ESN, in nanograms per milliliter, in the CSF and plasma samples were 3.6 (range 2.2 to 5.3) and 1.9 (1.5 to 2.8), respectively. The mean ratio of the CSF concentration to the plasma concentration was 8.0 (range 3.2 to 19.0) for oxytocin and is 1.9 (range 0.8 to 3.2) for ESN (Fig. 2).

Administration of estradiol benzoate (330 μg in sesame oil, subcutaneously) dramatically increased the plasma concentration of ESN for at least 4 to 5 days (*10*) (Fig. 3A), but did not systematically alter the concentration or pattern of change in CSF oxytocin and ESN levels, or plasma oxytocin (Fig. 3, A and B).

Most of the oxytocin, AVP, and ESN present in CSF (*11, 12*) appears to originate from the central nervous system rather than from blood, since large intravenous infusions of AVP, oxytocin, or neurophysin do not increase CSF con-

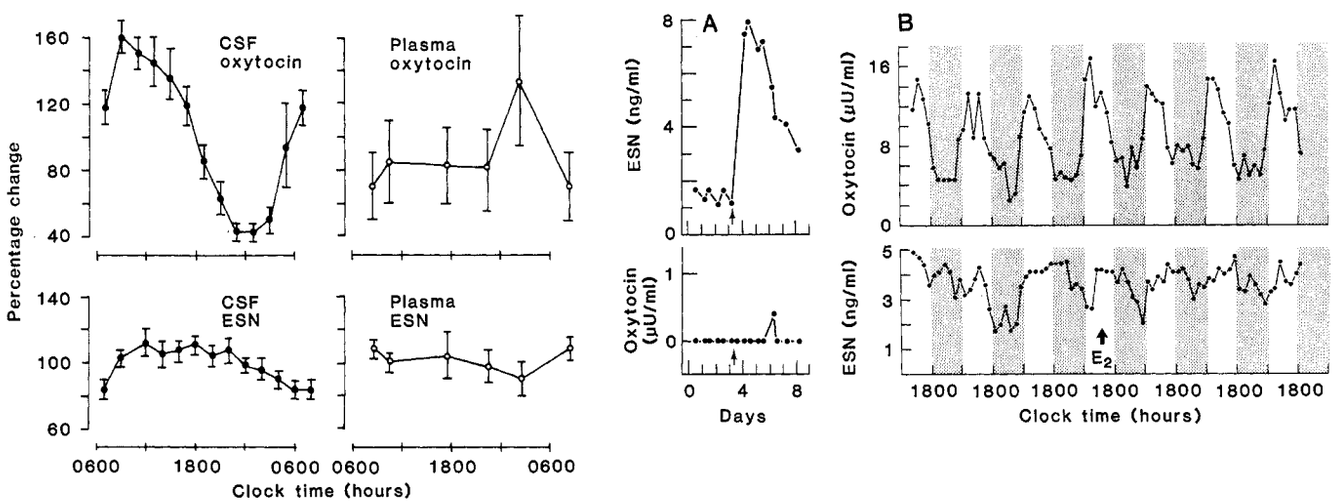

Fig. 2 (left). Patterns of oxytocin and ESN concentrations in the CSF and plasma of male rhesus monkeys (N = 4). Samples were obtained over a 24-hour period during diurnal lighting (LD 12:12). The CSF and plasma were collected over the same 24-hour period for each animal. The CSF was collected as 2-hour fractions; plasma was collected at the times indicated. Results are plotted as the percentage change from the daily mean (base line) concentration during the 24-hour day, with each point representing the mean of four samples ± standard error (see text for mean concentrations). Fig. 3 (right). (A) Patterns of plasma oxytocin and ESN concentrations in a representative male rhesus monkey that received estradiol benzoate (330 μg in sesame oil) (arrow). Plasma was obtained at the times indicated. (B) Patterns of oxytocin and ESN concentrations in the CSF of the male rhesus monkey described in (A). The arrow indicates the time of administration of estradiol benzoate (E_2). The CSF was collected in 2-hour fractions.

centrations (*13*). While one or more of these compounds may originate from the pituitary and travel back to the brain and to the CSF via retrograde vascular transport or hypothalamic tanycytes, as has been suggested for other pituitary hormones (*14*), the dissociation between oxytocin and ESN concentrations in plasma and CSF does not support this mechanism (Figs. 2 and 3). It seems more likely that these peptides reach the CSF either by diffusion from the central nervous system extracellular space or by direct secretion into the ventricles (*12*).

Hypothalamic efferent neuronal projections containing oxytocin, AVP, and their neurophysins extend to many areas of the central nervous system, including the walls of the third ventricle, the limbic brain, the brainstem, and the spinal cord (*3*). These axonal projections appear to be derived from neurons different from those that project to the posterior pituitary (*4*). One would suspect, therefore, that the release of oxytocin and AVP by the hypothalamic neurons into the blood or into different portions of the brain would vary, depending on the activity of the projecting neurons.

Until the present study only limited evidence has supported the view that neurons containing oxytocin act independently of one another. Using quantitative immunohistologic and radioimmunoassay techniques Rhodes *et al.* (*15*) and George (*16*) showed that estrogen treatment and water deprivation, respectively, altered the oxytocin content of some hypothalmic nuclei and not others. The results of the experiment described here suggest that the mechanisms controlling the release of oxytocin and ESN into blood differ from the mechanisms, and probably the neurons, controlling ESN and oxytocin release into CSF.

Oxytocin and its carrier protein ESN are present in a set of neurons distinct from those of AVP and its carrier protein nicotine-stimulated neurophysin (NSN) (*17, 18*). It is currently thought that ESN and NSN are released with their corresponding peptides (*19*). For this reason it is difficult to explain the lack of parallelism between oxytocin and ESN concentrations in either the CSF or the plasma in the monkey, and the delay in timing of the CSF-ESN curve with respect to the CSF oxytocin curve. These incongruities may result from differences in the half-lives of the small peptide oxytocin and the larger ESN, or may be due to the same process that permits oxytocin and ESN to be secreted into the plasma in less than equivalent molar amounts (*8, 18*).

The apparent separate control of oxytocin and ESN secretion into CSF compared to secretion into peripheral blood strengthens the concept that the posterior pituitary hormones have a function in the brain distinct from their better known peripheral effects (*1, 2, 20, 21*). Our results also support the notion that the CSF may function as a neuroendocrine conduit, transporting some peptide hormones made in one brain region to sites of action in distant brain areas (*22*). In the present study we demonstrate in a primate that both AVP and oxytocin concentrations in the CSF change over the course of the day.

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5. Male rhesus monkeys (5.5 to 6.5 kg) were placed in primate restraining chairs; each chair was housed in a separate, well-ventilated, sound-attenuated chamber. Each animal was given food and water daily between 0930 and 1200 hours. Lighting was automatically controlled; the intensity of illumination (15-watt Cool White fluorescent light, Westinghouse Electric) was 450 lux at the level of the animal's head. The animals were allowed 4 to 5 weeks to become adapted to restraint, after which time a polyethylene catheter was inserted into the lumbar subarachnoid space and advanced cephalad so that the tip terminated in the cisternal-cervical subarachnoid space. Cerebrospinal fluid was withdrawn continuously by a peristaltic pump at a constant rate of approximately 1 ml per hour, and collected in polypropylene tubes as 2-hour fractions. The CSF remained at room temperature (20° to 24°C) in collecting tubes for approximately 1.5 hours until it was refrigerated (4°C). Fractions from a 24-hour period were collected each morning and frozen (−40°C) until assayed [M. J. Perlow, S. M. Reppert, R. M. Boyar, D. C. Klein, *Neuroendocrinology* **32**, 136 (1981)]. Blood samples were collected from indwelling polyethylene catheters percutaneously inserted into a saphenous vein [S. M. Reppert, M. J. Perlow, L. Tamarkin, D. C. Klein, *Endocrinology* **104**, 295 (1979)]. The catheter was advanced so that the tip terminated in the vena cava. Blood samples (2 ml) were withdrawn intermittently into heparinized syringes, transferred to polypropylene tubes, and centrifuged. The plasma was stored in a refrigerator (4°C) for less than 4 hours and then frozen (−40°C) until assayed. We determined that AVP, oxytocin, and ESN are stable in the CSF and plasma for at least 24 hours at 4°C.
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