Vasopressin Exhibits a Rhythmic Daily Pattern in Cerebrospinal Fluid But Not in Blood

Abstract. *Examination of vasopressin concentrations in cerebrospinal fluid continuously withdrawn from free-ranging cats revealed a large daily rhythm, with high concentrations occurring during daylight hours. A similar daily pattern of the peptide was not detected in the circulation. The rhythmic fluctuation of vasopressin in the cerebrospinal fluid further implicates this body fluid as a vehicle of neuroendocrine communication and provides additional evidence that vasopressin may act by means of the cerebrospinal fluid to modulate behavioral processes, such as memory.*

The posterior pituitary nonapeptide, arginine vasopressin, has a number of important functions within the brain other than the well-known peripheral effects on salt and water balance. These brain effects include facilitation of learning and memory processes (*1*). Immunoreactive vasopressin has been detected in cerebrospinal fluid (CSF) of the rat, dog, and human (*2*, *3*), but little is known about the dynamic patterns of the hormone in this body fluid. We now report that the concentrations of vasopressin in CSF, continuously withdrawn from unanesthetized cats, exhibit a highly organized pattern of secretion. Furthermore, the rhythm is limited to CSF and is not expressed in the circulation.

Cisternal CSF was collected from adult male cats by a system that allowed for continuous CSF withdrawal in the undisturbed animal (*4*). Vasopressin concentrations in CSF were measured in unextracted samples by radioimmunoassay (*5*). The immunoreactive material in CSF was analyzed by a high-performance liquid chromatographic (HPLC) technique that separates vasopressin from two related nonapeptides, oxytocin and arginine vasotocin. Radioimmunoassay of the HPLC elution fractions disclosed that virtually all of the immunoreactivity in CSF was present in the fractions corresponding to the elution profile of authentic vasopressin (data not shown).

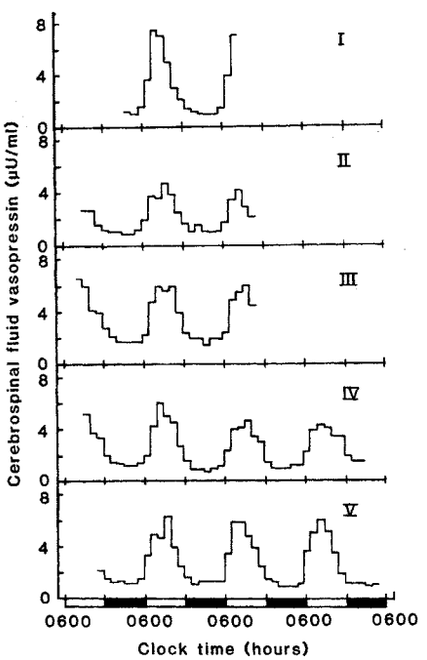

Fig. 1. Patterns of CSF vasopressin for five cats (I to V) studied in diurnal lighting (12 hours of light and 12 hours of darkness). The CSF was collected as 2-hour fractions.

Vasopressin concentrations in the CSF of each of five cats exhibited a large daily rhythm during a daily light-dark cycle (Fig. 1). The rhythm was characterized by low concentrations (1 to 2 μU/ml) of the hormone throughout the dark period. Within 2 to 4 hours after the lights were turned on in the morning, CSF vasopressin concentrations had markedly increased, reaching peak values (4 to 7.5 μU/ml) by the 6th to 8th hour in light. By the 10th hour in light, the amount of vasopressin began to decrease, reaching a minimum shortly after the onset of the dark period. Although peak values differed among the animals, the amplitude of the daily rhythm was remarkably consistent from day to day in individual animals. The CSF vasopressin rhythm persisted during 3 days of continuous light, without alterations in either amplitude or phase (data not shown). This finding suggests that the rhythm is endogenously generated. Preliminary evidence indicates a similar daily alteration of vasopressin in the CSF of rhesus monkeys (*6*) and of sheep (*7*).

To determine whether there is a vasopressin rhythm in the circulation, we examined plasma obtained from three cats during a 24-hour period in constant light (*8*). Although each cat exhibited a clear daily rhythm in CSF vasopressin concentrations during the 24-hour period of light, there was no corresponding daily rhythmic variation in plasma vasopressin concentrations (Fig. 2). This finding suggests that separate populations of neurons are responsible for secreting the peptide into the CSF and into the circulation, and that each is regulated by a different control mechanism. The dissociation between plasma and CSF vasopressin levels in the cat is similar to that found in humans (*3*).

The rhythmic character of CSF vasopressin concentrations suggests that the hormone in CSF is the direct result of organized neurosecretory activity from a discrete population of hypothalamic neurons. The magnocellular neurons of the supraoptic nuclei and paraventricular nuclei, as well as the parvocellular perikarya of the paraventricular nuclei, are all potential sites of vasopressin secretion into CSF (*9*). Another possible source of the peptide in CSF is the parvocellular neurons of the suprachiasmatic nuclei, which contain both vasopressin and vasopressin-neurophysin (*10*). The suprachiasmatic nuclei as a potential site of vasopressin secretion into the CSF is of special interest since this region of the hypothalamus functions as a circadian pacemaker or biological clock, generating a multitude of hormonal and behavioral rhythms in mammals (*11*). Thus, our finding of a large daily rhythm in vasopressin in the CSF suggests that the CSF rhythm might originate within this biological clock.

The rhythmic nature of CSF vasopressin lends support to the notion that CSF

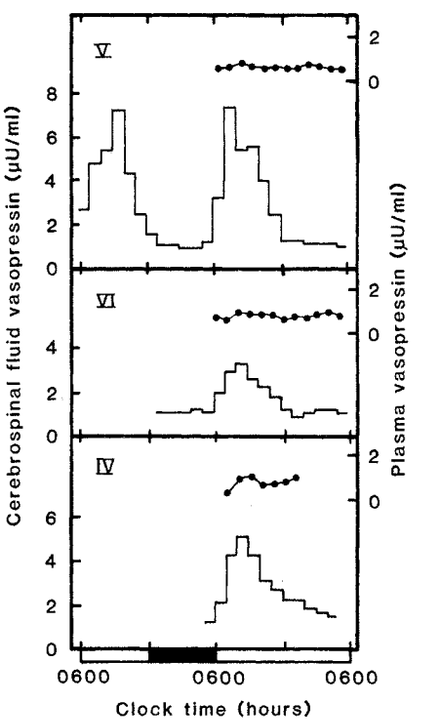

Fig. 2. Patterns of CSF (bars) and plasma (●) vasopressin for three cats studied over a 24-hour period in constant light. Samples of CSF and plasma were collected over the same 24-hour period for each animal. The CSF was collected as 2-hour fractions and plasma was obtained at the times indicated. Plasma vasopressin concentrations ranged from 0.6 to 0.9 μU/ml for animal V, 0.7 to 1.0 μU/ml for animal VI, and 0.6 to 1.2 μU/ml for animal IV.

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functions as a neuroendocrine conduit (*12*), transporting various peptides made in one brain region to sites of action in distant brain areas. A possible function of vasopressin in CSF is regulating the behavioral processes of learning and memory. This conjecture is based primarily on studies of the vasopressin-deficient Brattleboro rat, which has deficits in acquisition and retention of information necessary for success in active and passive avoidance training (*13*). The deficit in retention can be corrected by vasopressin administration, the intraventricular route of delivery being several hundred times more effective than the systemic route (*14*). Also, inactivation of endogenous CSF vasopressin by intraventricular administration of vasopressin antiserum to normal rats induces severe impairment of memory (*15*); systemic administration of the antiserum elicits no behavioral effects despite inducing profound alterations in water balance.

An interesting aspect of memory consolidation is evidence that the circadian timekeeping system plays an important role in that behavior (*16–18*). For example, rats manifest a repetitive daily variation in retention performance after one-trial passive-avoidance training (*17*). Also, disrupting circadian organization in the rat results in a long-term loss of memory (*18*). Thus, our finding of a daily vasopressin rhythm in mammalian CSF provides a potential link between the reported effects of CSF vasopressin on memory and the circadian character of memory processes and thus strengthens the argument that vasopressin in the CSF may function physiologically to modulate memory.

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4. Cats weighing 3.5 to 5.0 kg were individually housed in cages under an automated daily light-dark cycle (12:12), with lights on from 0600 to 1800; food and water were freely available, and the time of day that daily care was provided was randomized. An indwelling styleted metal cannula was inserted into the cisternal CSF space of anesthetized animals. The exposed end of the implanted cannula was sleeved with a hollow brass screw and both were anchored to the skull with dental cement. Two to six weeks after the operation, the stylet was removed, polyethylene tubing was attached to the cannula, threaded through a protective stainless steel spring, and connected to the inlet port of a single-channel cannula swivel (Alice King Chatham Medical Arts, Los Angeles) located at the top of the cage. A nut affixed to the proximal end of the steel spring was then threaded over the brass screw on the head, and the distal end was attached to the base of the cannula swivel. From the outlet port of the swivel, polyethylene tubing led to a peristaltic pump in an adjoining room, whereby CSF was continously withdrawn for periods up to 10 days at a constant rate of 600 μl/hour and collected as 2-hour fractions by an automated fraction collector. The CSF was collected for a 24-hour period preceding the start of an experiment. The collected CSF samples remained in the fraction collector for up to 8 hours at room temperature (20° to 22°C) and were then stored at −40°C until analysis. We determined that vasopressin is stable in CSF for at least 24 hours at room temperature.
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8. Continuous CSF samples and intermittent blood samples were withdrawn from free-ranging cats over a 24-hour period in constant light; the period of constant light was accomplished on the experimental day by leaving the lights on throughout the normal 12-hour dark period of diurnal lighting. Blood samples were collected from an indwelling venous catheter, surgically placed through the external jugular vein into the superior vena cava, and by use of a two-channel swivel incorporated into the tethering system described for continuous CSF withdrawal (*4*). Withdrawn blood samples (2 ml) were transferred to chilled tubes containing 10 μl of 15 percent K_2EDTA and centrifuged at 4°C. Vasopressin was extracted from 1 ml of plasma by use of the bentonite extraction technique [W. R. Skowsky, A. A. Rosenbloom, D. A. Fisher, *J. Clin. Endocrinol. Metab.* **38**, 278 (1978)]. For each plasma sample, vasopressin content of duplicate portions, each equivalent to 400 μl of plasma, of the resuspended extract was determined by radioimmunoassay (*5*). The lower limit of assay sensitivity for plasma vasopressin was 0.1 μU/ml.
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Toxic Injury to Isolated Hepatocytes Is Not Dependent on Extracellular Calcium

Abstract. *Freshly isolated hepatocytes from phenobarbital-treated rats were incubated in the presence or absence of extracellular calcium with three differently acting liver cell toxins, namely carbon tetrachloride, bromobenzene, and ethylmethanesulfonate. In the absence of extracellular calcium these three compounds were far more toxic to the cells than in its presence. This result is inconsistent with the hypothesis that an influx of extracellular calcium is required as the final step in toxic liver cell injury.*

Liver cell death and its morphological expression as necrosis have been the subject of many clinical and experimental studies. Since liver cell death may be the ultimate result of a number of different hazards in the cell's environment, including toxic chemicals, anoxia, and viruses, various experimental models have been designed to investigate the morphological and biochemical changes associated with liver cell injury and death. As a result, morphological and biochemical changes of increasing severity have been described and attempts have been made to define "a point of no return" at which the process becomes irreversible (*1–3*). Moreover, several hypotheses have been proposed to explain the underlying biochemical lesion [for example, see (*3*)]. One such hypothesis is that the plasma membrane is the primary target in the pathogenesis of liver cell death and that the functional consequence of this membrane injury is a lethal influx of a high concentration of Ca^{2+} ions into the liver cells (*4*). The influx of extracellular Ca^{2+} has therefore been proposed as the final common pathway in toxic liver cell death (*4*, *5*).

Although it has been known for many years that calcium accumulates in necrotic tissues, such accumulations of

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