Vasopressin and Neurophysin: High Concentrations in Monkey Hypophyseal Portal Blood

Abstract. *Vasopressin and its binding protein, neurophysin, were measured by radioimmunoassay in the hypophyseal portal blood of monkeys after cannulation of individual long portal veins. Mean vasopressin concentrations (13,800 picograms per milliliter) in portal blood were more than 300 times as high as those in the systemic circulation (42 picograms per milliliter). Neurophysin concentration was approximately 25 times as high in portal as in systemic blood. By immunoperoxidase techniques, high concentrations of neurophysin were demonstrated around portal capillaries of the median eminence. These studies indicate direct secretion of vasopressin and neurophysin into the portal circulation; the quantities secreted during stress may be sufficient to exert significant effects on secretion of anterior pituitary hormone.*

A possible physiological role of the posterior pituitary hormone, vasopressin, in anterior pituitary function has been debated for many years (*1–3*). Exogenous vasopressin can stimulate the release of growth hormone (*4, 5*) and adrenocorticotropic hormone (ACTH) in vivo (*6, 7*). However, brain peptides other than vasopressin which can release ACTH have been isolated (*2*). A potentiating effect of vasopressin on the action of ACTH releasing factor has also been demonstrated (*8*). Anatomical evidence for the secretion of posterior pituitary peptides into the hypophyseal portal system and thence to the anterior pituitary is inconclusive, although many investigators have suggested such a pathway (*1, 9*). We now report evidence for the secretion of both vasopressin and its binding protein, neurophysin, in high concentrations into hypophyseal portal blood (*10*).

Using an immunoperoxidase technique, we had found in monkeys that the neurophysin class of binding proteins for oxytocin and vasopressin was present in the supraoptic and paraventricular neurons of the hypothalamus and in the axons of these neurons (supraopticohypophyseal tract), which pass through the median eminence and the pituitary stalk to end in the posterior pituitary gland (*11*). In the present study, the three-layer immunoperoxidase bridge technique was applied to cross sections of the hypothalamus of two adult female rhesus monkeys. In experimental slides, rabbit antiserum against bovine neurophysin I was used as the first reactant, followed by sheep antiserum against rabbit serum, rabbit antiserum against peroxidase, and 3,3′-diaminobenzidine (*11, 12*). Controls in which the first reactant was replaced by saline, normal rabbit serum, or neurophysin antiserum absorbed with neurophysin were negative.

The immunoperoxidase method revealed a high concentration of immunoreactive neurophysin in the external layer of the median eminence (Fig. 1a). At higher magnification prominent neurophysin staining in the external layer (palisade layer) was seen as fine granular reaction products near portal capillaries (Fig. 1b). Neurophysin was also seen in axons of the supraopticohypophyseal tract as it passes ventromedially through the fascicular layer of the hypothalamus and the pituitary stalk. Some axons of the tract appear to enter the external layer, which indicates that at least some of the neurophysin in the palisade layer is derived directly from this tract. Since we previously demonstrated neurophysin (*12*)

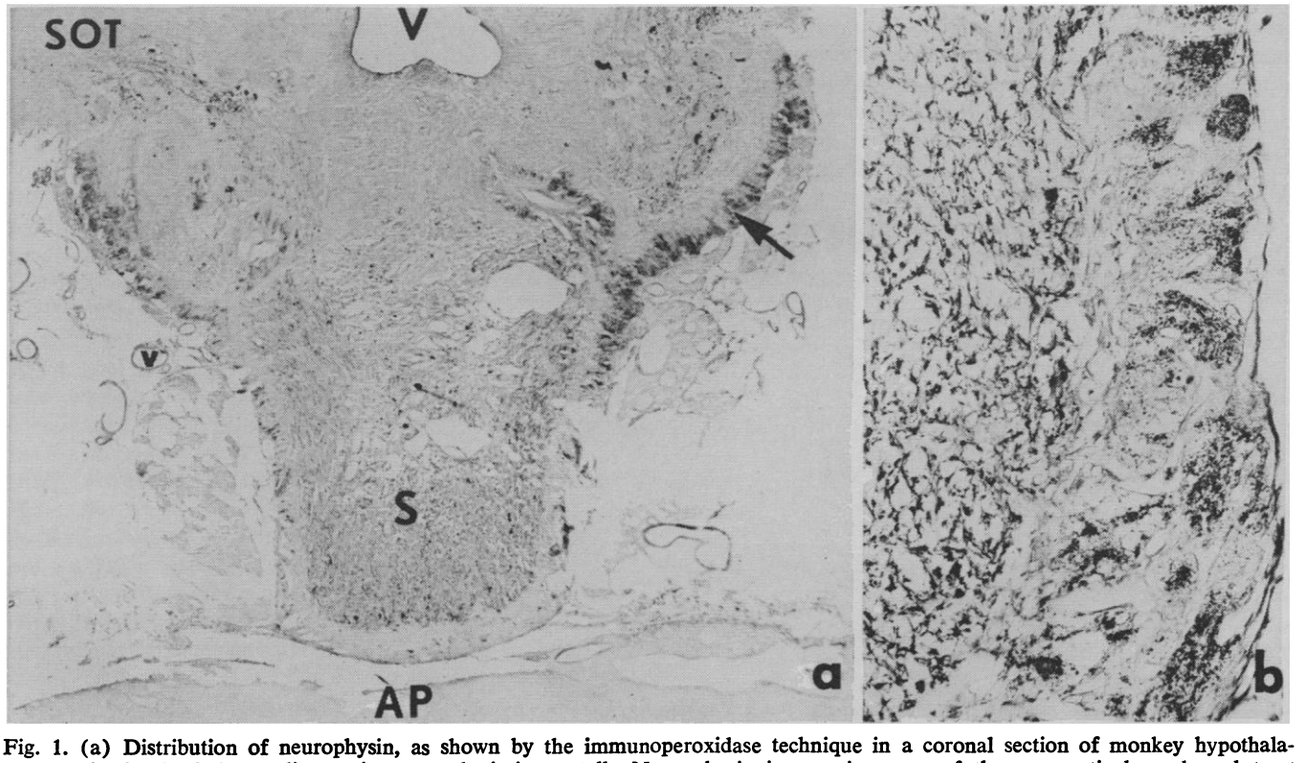

Fig. 1. (a) Distribution of neurophysin, as shown by the immunoperoxidase technique in a coronal section of monkey hypothalamus at the level of the median eminence and pituitary stalk. Neurophysin is seen in axons of the supraopticohypophyseal tract (*SOT*) as it passes ventromedially from the hypothalamus through the pituitary stalk (*S*) to the posterior pituitary. Intense neurophysin staining is seen in the external layer of the median eminence (arrow); *V*, third ventricle; *v*, cross section of a long portal vein; and *AP*, anterior pituitary (× 43). (b) A higher magnification of the same section shows neurophysin concentrated around portal capillaries in the external layer (right) and in axons of the tract (left) (× 504).

in tanycyte cell bodies and in their processes that end on portal capillaries in the external layer (*13*), neurophysin may arrive in this region by this route as well as by axons of the supraopticohypophyseal tract. Parry and Livett (*14*), using the fluorescent antibody technique, also found neurophysin in axons of supraoptic and paraventricular neurons ending in the external layer of the median eminence of sheep.

These anatomic results prompted the measurement of neurophysin and arginine vasopressin (AVP) in portal blood by means of recently developed specific radioimmunoassays for these peptides (*12, 15*). Neurophysin was measured by an immunoassay with neurophysin isolated from human posterior pituitary extracts; this assay was similar to that described in (*12*). Arginine vasopressin was determined by a radioimmunoassay system (*15, 16*). Because of extremely high plasma concentrations of AVP in portal plasma (Table 1) and the low concentrations of AVP that can be detected (0.1 to 0.2 pg/ml), portal plasma was assayed without extraction at dilutions ranging from 1 : 1,000 to 1 : 10,000; nonspecific interfering factors were thereby eliminated. Systemic venous plasma, however, was extracted before assay by a procedure consisting of protein precipitation from 1 ml or less of plasma with two volumes of acetone, fat removal with petroleum ether, and drying under air; dried extracts were assayed after reconstitution in buffer. Recovery of added vasopressin was 63 to 73 percent, although values reported here were not corrected for recovery.

The feasibility of cannulating individual portal veins in the rat was demonstrated by Porter *et al.* (*17*). In our experiments, each monkey was anesthetized with pentobarbital and fixed in a stereotaxic head holder; after removal of a portion of the calvarium, the pituitary stalk was approached through the right orbit. The right frontal lobe was elevated, the right optic nerve and chiasm were resected, and the arachnoid covering the stalk was divided. Animals were then given heparin, and single long portal veins viewed through an operating microscope (× 40) were punctured by a glass pipette (tip diameter, 30 μm) guided by a micromanipulator. Portal blood was collected at a withdrawal rate of 1 to 6 μl/min. Total amounts of portal blood collected ranged from 0.1 to 1.0 ml. Peripheral blood was obtained simultaneously from femoral veins at 5- to 10-minute intervals and matched to corresponding segments of collection tubing containing 10 to 30 μl of portal blood. Cerebrospinal fluid was also obtained from the third ventricle of monkey 2 by needle aspiration. At the end of the experiment, the pituitary glands were removed from monkeys 3 and 4, dissected into anterior and posterior portions, homogenized in 0.1*N* HCl, and assayed for vasopressin and neurophysin. Control specimens of frontal lobe were also homogenized. Acid-extractable protein was determined by the method of Lowry *et al.* (*18*).

Table 1. Neurophysin and vasopressin measured by radioimmunoassays in simultaneous samples of monkey hypophyseal portal blood (P) and systemic venous blood (S).

Monkey	Neurophysin (ng/ml)		Vasopressin (pg/ml)	
	S	P	S	P
1	2.1	88	*	*
2	1.5	48	35	11,000
3	4.4	100	50	16,000
4	2.0	10	40	14,500

* Not measured.

The portal plasma concentrations of both neurophysin and vasopressin greatly exceeded peripheral venous concentrations in all animals (Table 1). Small but measurable quantities of these peptides were found in acid extracts of anterior pituitary in monkeys 3 and 4 (neurophysin, 0.3 and 0.05 ng per microgram of protein; vasopressin, 44 and 28 pg/μg). As expected, large amounts were present in the posterior pituitary of monkeys 3 and 4 (neurophysin, 14.1 and 16 ng/μg; vasopressin, 4.3 and 23 ng/μg). In control homogenates of frontal lobe, neurophysin was undetectable, and vasopressin concentration was very low (0.024 pg/μg). The possibility that the high levels of AVP in portal plasma were due to an artifact of the radioimmunoassay was rendered unlikely by the following experiments: (i) The dose-response curve obtained by assaying varying concentrations of portal plasma was identical to that of standard AVP (Fig. 2), a result indicating no detectable immunologic difference. (ii) No significant "incubation damage" to ^{125}I-labeled AVP was caused by up to 2 μl of portal plasma after the usual incubation, as determined by total radioactivity bound to excess antibody. (iii) More than 95 percent of immunoreactivity in portal plasma was destroyed on treatment with plasma from pregnant women, which contains an enzyme known to degrade vasopressin (*19*). Identical degradation of the AVP standard was observed after exposure to this plasma under similar conditions.

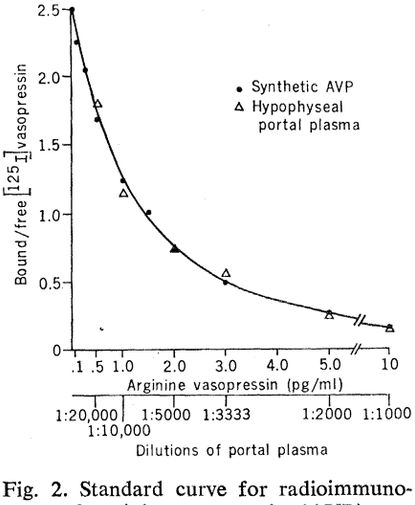

Fig. 2. Standard curve for radioimmunoassay of arginine vasopressin (AVP) employing synthetic AVP standard (Schwarz/Mann, 270 I.U./mg) and antiserum to AVP at a dilution of 1 : 500,000. Serial dilutions of portal plasma containing high concentrations of AVP result in a curve identical to that of the standard.

Axons of the supraopticohypophyseal tract which terminate in the median eminence may be the source of the large amounts of immunoreactive vasopressin and neurophysin in portal blood. That cerebrospinal fluid of the third ventricle is not the immediate source of these peptides in portal blood by rapid tanycyte transport is suggested by the low neuropeptide concentrations in the cerebrospinal fluid of monkey 2 (neurophysin, 2.0 ng/ml; vasopressin, 20 pg/ml). However, tanycytes might secrete significant amounts of these peptides into portal blood by concentrating them in their terminals. Vasopressin and neurophysin in the anterior lobe probably arrive via the long portal vessels. It is also possible that these neuropeptides could get to the anterior pituitary by short portal vessels (*1*).

Under conditions of stress, vasopressin may be normally secreted into portal blood to facilitate the release of tropic hormones. If one calculates the maximum concentration of vasopressin that might reach the anterior pituitary via its blood supply and cause growth hormone and ACTH release after intravenous injection of 5 units of vasopressin in man (*5, 7*), the result, about 4000 pg/ml (*20*), is similar to the concentrations actually measured in portal blood (mean, 13,800 pg/ml) in these experiments. It is not known whether

neurophysin has a role in anterior pituitary function. Its presence in high concentration in plasma from long portal vessels supports the concept that the contents of secretory granules containing neurophysin and vasopressin are secreted in the median eminence. Since oxytocin also has an intragranular association with neurophysin (*21*), it too may be secreted into the portal circulation.

These data provide evidence that vasopressin and neurophysin reach the anterior pituitary gland via the portal circulation. Collection of blood from individual portal vessels in primates and radioimmunoassay estimates of neurohypophyseal peptides in portal blood have not been previously reported. On the basis of our studies and those of others, we propose two distinct secretory pathways and two hormonal functions for vasopressin: One, the well-established secretion into the general circulation from the posterior pituitary gland for antidiuretic action in the kidney; and the other, secretion into the hypophyseal portal circulation to influence the secretion of tropic hormones.

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22. We thank J. C. Porter for advice on portal blood collection; K. C. Hsu for help in developing immunoperoxidase techniques; S. Glick for antiserum to AVP; M. Salcman for preparing glass pipettes, and S. Rosario for technical assistance. Supported in part by PHS grants NS 11,008, NS0561805, AM11294, AM5397, CA11704, and CA13696; and by grants from the John A. Hartford Foundation, Ford Foundation, Health Research and Services Foundation of Pittsburgh (0–62), and the Population Council of New York (M72.074C).

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Phosphorylation of Muscle Membranes: Identification of a Membrane-Bound Protein Kinase

Abstract. *A membrane-bound protein kinase occurs in membranes derived from rat skeletal muscle and appears limited to a surface membrane fraction. The enzyme is magnesium dependent, is only minimally stimulated by cyclic nucleotides, and phosphorylates serine and to a lesser extent threonine residues of three membrane proteins with molecular weights of less than 30,000.*

Soluble protein kinases in various eukaryotic tissues participate in the regulation of cellular metabolism (*1*). Isolated membranes from various tissues contain endogenous protein kinases that phosphorylate membrane proteins and may participate in the regulation of membrane ion-binding and permeability (*2–5*). While such membrane phosphorylation may be of significance in many excitable tissues, only the membrane-bound protein kinase of brain has been studied (*2, 3, 6, 7*). We now report on a particulate protein kinase not previously described in skeletal muscle.

Our study of muscle membrane protein kinase is a result of our demonstration (*8*) of altered activity of membrane-bound protein kinase of frozen aged erythrocytes from patients with myotonic dystrophy, a systemic disorder that involves the membranes of many excitable tissues including skeletal muscle. The membrane-bound protein kinase reported here in skeletal muscle phosphorylates three membrane proteins whose molecular weights are less than 30,000. This phosphorylation in vitro occurs only in a muscle membrane subfraction that has several characteristics of surface membrane.

Muscle membrane isolation and subfractionation were performed as described (*9, 10*). Rat hind limb muscles were homogenized; the nuclei and mitochondria were removed by centrifugation. The resulting supernatant was centrifuged at 100,000*g*, and the sediment was extracted sequentially with lithium bromide, potassium chloride, and deionized water and then centrifuged on continuous density sucrose gradients that ranged from 15 to 35 percent sucrose. The lighter density membranes which failed to penetrate 19 percent sucrose contained three markers for surface membranes. These were a Na,K-stimulated, Mg-dependent adenosine triphosphatase, sialic acid–containing macromolecules, and components of the intact tissue which could be iodinated by extracellular lactoperoxidase and ^{125}I. Higher density membranes which penetrated 19 percent sucrose contained peak specific activities of Ca- or Mg-dependent adenosine triphosphatase, the membrane marker for cytoplasmic membranes, or sarcoplasmic reticulum (*9*).