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12. The LLC-PK$_1$ line, like other transporting epithelial cell lines, is characterized by the appearance of domes—fluid-filled, blister-like structures believed to result from vectorial transport of solute and water. Dome formation is characteristic of differentiated cells and is rarely observed in subconfluent cultures. Microscopic examination of living, newly confluent LLC-PK$_1$ cultures grown in 60-mm petri dishes placed directly on trays occasionally revealed no domes over hole areas, while many were observed over metal areas.

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The Carboxy Terminus of the Precursor to Vasopressin and Neurophysin: Immunocytochemistry in Rat Brain

Abstract. *A pituitary glycopeptide whose amino acid sequence was previously identified has now been recognized as the final portion of the precursor to arginine vasopressin and its associated neurophysin. Immunocytochemical techniques with antiserums against this 39 amino acid peptide and vasopressin were used to study their distribution in the rat central nervous system. The peptide is located in vasopressin-synthesizing cells in the neurosecretory magnocellular nuclei. Positively stained fibers project from the magnocellular nuclei through the median eminence to the posterior pituitary. Studies of the homozygous Brattleboro rat, which is known to be deficient in the production of vasopressin and its related neurophysin, also show the absence of immunoreactivity to this peptide. These immunocytochemical data strongly indicate that the peptide is synthesized with vasopressin.*

Until recently, the full structure of the precursor of vasopressin remained unknown, although it was hypothesized that neurophysin was contained within it. A 39 amino acid glycopeptide isolated from human pituitary by Seidah *et al.* (*1*) was a likely candidate for the missing portion of the vasopressin and neurophysin precursors. The peptide was isolated from the posterior pituitary and generally was found in the appropriate sites (*1*, *2*), its molecular weight was consistent with that predicted from precursor labeling experiments (*3*), and it was also glycoprotein (*3*). Furthermore, a computer search revealed that similar molecules had been isolated from pig, sheep, and ox pituitaries (*4*), indicating phylogenetic conservation and potential biological importance.

We now report that this glycopeptide and vasopressin are indeed present in the magnocellular vasopressin pathways projecting to the posterior pituitary. These results are consistent with the findings of Land *et al.* (*5*) on the sequence of complementary DNA to the messenger RNA of bovine arginine vasopressin (AVP) and neurophysin. Their report shows that the glycopeptide is located at the extreme carboxy terminus of the precursor, with neurophysin occupying the middle domain and vasopressin being adjacent to the leader sequence at the amino terminus. The production of vasopressin and neurophysin involves cleavage at pairs of basic residues, whereas cleavage of the carboxy terminal glycopeptide involves cleavage at a single arginine residue (*5*). This is known to occur for a large number of precursor molecules, including the pro-opiomelanocortin molecule (*6*). We therefore propose the name "carboxy terminus of propressophysin" (CPP) for this glycopeptide, which previously was termed "human pituitary glycopeptide."

Human CPP was extracted as described by Seidah *et al.* (*1*), coupled by carbodiimide to egg albumin, and injected into rabbits. This produced high-titer, specific antiserum. Immunocytochemistry was then carried out in rat brain and pituitary (*7*). Tissue sections were incubated with antiserum to CPP or vasopressin diluted 1:1000 in 0.3 percent Triton in phosphate-buffered saline. The tissue was prepared for immunocytochemistry by a modification of the peroxidase-antiperoxidase technique (*7*, *8*). The specificity of the immunoreactivity was systematically evaluated by performing blocking experiments with the following peptides (50 μM): AVP, oxytocin, β-endorphin, dynorphin, methionine-enkephalin, leucine-enkephalin, and adrenocorticotropic hormone. None of these peptides was effective in inhibiting the immunocytochemical reactions with antiserum to CPP. CPP itself effectively blocked CPP antiserum staining in concentrations as low as 10 nM. The antiserum to AVP is specific for AVP and is blocked by 20 nM AVP, but it is not blocked by oxytocin at concentrations up to 50 μM (*9*). Normal male Sprague-Dawley rats, male homozygous Brattleboro rats, and colchicine-treated (50 μg in 50 μl, intracerebroventricularly) animals of both types were studied.

In both normal and colchicine-treated Sprague-Dawley rats, CPP immunoreactivity was detected in the supraoptic nucleus (Figs. 1A and 2A), nucleus circularis, paraventricular nucleus (Fig. 1B), suprachiasmatic nucleus (Fig. 1D), median eminence (Fig. 1C), and posterior pituitary (Fig. 2C). A subset of cells in these nuclei was stained. Evaluation of the general anatomic staining patterns in the supraoptic (Figs. 1A and 2A) and paraventricular (Fig. 1B) nuclei strongly indicates that the CPP antiserum staining was similar to that seen with vasopressin (Fig. 2B). Indeed, using a set of serial 5-μm sections stained with antiserum to CPP or AVP, we found CPP- and AVP-like immunoreactivities in the same neurons in the supraoptic (Fig. 2, A and B) and paraventricular nuclei. Furthermore, antiserum to CPP stained many of the parvocellular elements in the suprachiasmatic nucleus (Fig. 1D). This nucleus is not known to contain oxytocin but does contain vasopressin and its related neurophysin (*10*). Fibers were seen coursing from the supraoptic and paraventricular nuclei to the median eminence (Fig. 1C), where they formed heavy bundles in the internal layer. Analysis of the posterior pituitary (the projection target for the internal layer of the median eminence) showed heavy staining (Fig. 2C); no staining was found in the intermediate or anterior lobes. Thus we conclude that the antiserums to CPP and AVP stained the same cells in the hypothalamus and the same fibers in the hypothalamus and pituitary.

The common location of two peptides does not necessarily imply a common precursor. Other substances have been identified in AVP cells of the magnocellular system, yet they are not related to the biosynthesis of AVP and its related neurophysin (*9*, *11*). One such substance, dynorphin, stains exactly the same cells as vasopressin (*9*). Yet homo-

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zygous Brattleboro rats, which do not synthesize vasopressin and its related neurophysin (*12*), show dynorphin in the magnocellular nuclei, suggesting separate genetic and biosynthetic controls for dynorphin and vasopressin (*9*). We therefore investigated the immunoreactivity of CPP in homozygous Brattleboro rats, both normal and colchicine-treated, in order to address the question of common biosynthesis. None of the rats showed any magnocellular staining with antiserum to CPP (Fig. 2D) or vasopressin. Yet, as reported elsewhere (*9*), serial sections from the same animals were stained with oxytocin. Thus the deletion of vasopressin, its neurophysin, and CPP can be found in the same genetic mutant, suggesting similar biosynthesis or at least a common genetic deletion.

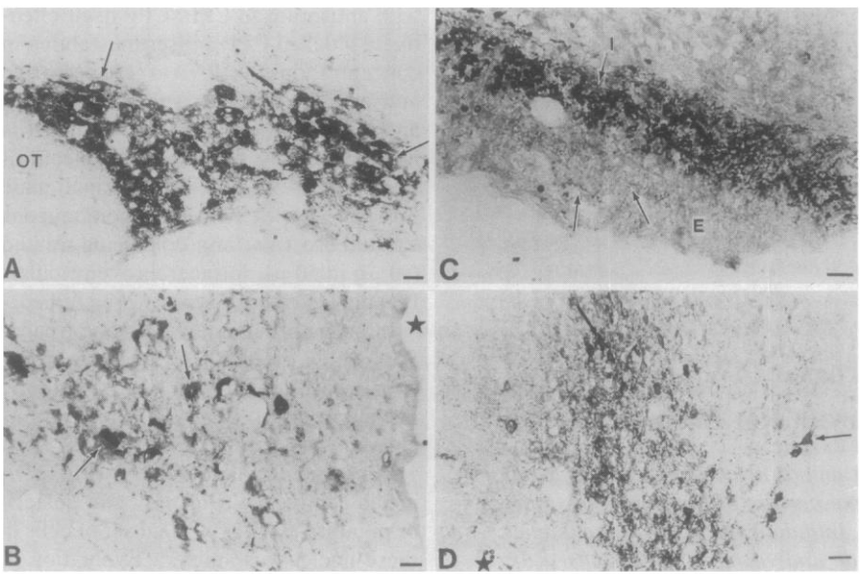

Fig. 1. Patterns of CPP staining in a colchicine-treated Sprague-Dawley rat. The staining is similar to that seen with vasopressin. (A) Typical stained cells (arrows) in the supraoptic nucleus of the hypothalamus. Other cells in the supraoptic nucleus are unstained (*OT*, optic tract). (B) Typical stained cells (arrows) in the paraventricular nucleus of the hypothalamus. The star indicates the third ventricle. (C) Staining in the median eminence of an untreated Sprague-Dawley rat. The heavily stained fibers in the internal layer (*I*) and lightly stained fibers (arrows) in the external layer (*E*) resemble those seen with antiserum to vasopressin. (D) Small parvocellular neurons (arrows) in the suprachiasmatic nucleus, stained with antiserum to CPP. The star indicates the third ventricle. This nucleus contains vasopressin cells and not oxytocin cells. Scale bars, 20 μm.

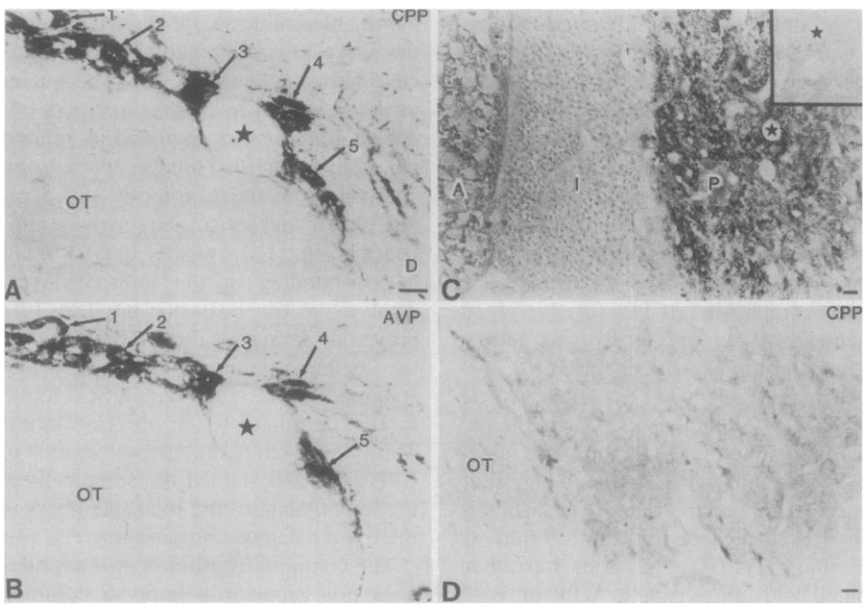

Fig. 2. (A and B) Common CPP (A) and AVP (B) staining of several cells in serial 5-μm sections of supraoptic nucleus from a colchicine-treated rat. The numbered arrows show correspondence. The star indicates a blood vessel. (C) CPP staining in the posterior lobe of the pituitary (*P*) but not in the anterior (*A*) or intermediate (*I*) lobes. Inset: adjacent section blocked with 5 μ*M* excess CPP. The stars in (C) and in the inset indicate the same vessel. (D) Absence of staining by antiserum to CPP in the supraoptic nucleus of a homozygous Brattleboro rat. As reported elsewhere (*9*), serial sections from the same rat stain with dynorphin and oxytocin. Scale bars, 20 μm.

To summarize, CPP-like immunoreactivity can be found in the magnocellular neurosecretory nuclei of the hypothalamus and in the posterior lobe of the pituitary. The staining detected for this glycopeptide occurs in the subset of magnocellular neurons containing AVP. Sections from the homozygous Brattleboro rat which do not stain for vasopressin or its neurophysin also do not stain for antiserum to CPP. Thus, the anatomical data presented here are in complete agreement with the newly reported messenger RNA structure for the vasopressin precursor (*5*). As noted above, the sequence of CPP is highly conserved across species. Furthermore, the cross-reactivity of antiserum against human CPP with the rat peptide suggests a similarity between human and rat CPP structures. Such conservation implies that the molecule has some biological importance. One portion of the propressophysin structure, AVP, has a critical endocrine function, whereas another part, neurophysin, has no known endocrine activity but has carrier functions. It is interesting to speculate on the role of the highly conserved sequence of CPP. In other neuropeptide neurons (such as those containing pro-opiomelanocortin), several substances derived from a common precursor are biologically active and often interact on release (*13*). Further studies of CPP are needed to address this issue.

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Amphetamine, Haloperidol, and Experience Interact to Affect Rate of Recovery After Motor Cortex Injury

Abstract. *Rats subjected to unilateral ablation of the motor cortex and placed on a narrow beam displayed transient contralateral paresis. An immediate and enduring acceleration of recovery was produced by a single dose of* d-*amphetamine given 24 hours after injury. This effect was blocked by haloperidol or by restraining the animals for 8 hours beginning immediately after amphetamine administration. A single dose of haloperidol given 24 hours after injury markedly slowed recovery. This effect was also blocked by restraining the animals.*

Despite major advances in the understanding of brain function, no medical treatments have been developed to promote recovery from brain injury; only secondary events, such as bleeding or edema, are treated to prevent further neuronal destruction. However, with time there may be marked spontaneous recovery of function in brain-injured animals. For example, after unilateral damage to the motor cortex there is a contralateral paralysis and loss of locomotor ability which may, depending on the species, be reversed over time. In humans these deficits can persist indefinitely, whereas recovery occurs within months in the cat (*1*) and within 2 weeks in the rat (*2*). The initial loss of function and subsequent recovery may be manifestations of a transient depression of neural functions in intact areas remote from but connected to the area of injury (*3*).

The concentration of catecholamines reportedly is reduced in rat and cat brainstem and in human cerebrospinal fluid following cerebral infarction (*4*). If depression of catecholamine levels contributes to the behavioral syndrome seen after cerebral injury, then it should be possible to reverse some of the deficits by pharmacological manipulation of catecholaminergic systems. The drugs *d*-amphetamine and haloperidol, which have potent opposing actions on catecholamines and neuronal activity (*5*), were used to test this hypothesis.

The subjects were 111 male albino rats (300 to 350 g) trained to run along a narrow beam to escape white noise and bright light (*6*). For surgery each animal was given ketamine hydrochloride (60 mg/kg, intramuscularly) as a preanesthetic; 5 minutes later, the animals were anesthetized with sodium pentobarbital (21 mg/kg, intraperitoneally). A wide craniotomy was performed over one hemisphere and the motor cortex was removed unilaterally by suction (*7*). Twenty-four hours after surgery the ability of each animal to negotiate the beam was evaluated in a single trial. Immediately thereafter the animals were given intraperitoneal injections of saline (N = 16); amphetamine at doses of 0.5 mg/kg (N = 8), 1 mg/kg (N = 10), 2 mg/kg (N = 13), or 4 mg/kg (N = 8); amphetamine (2 mg/kg) followed 2 minutes later by haloperidol (0.4 mg/kg) (N = 6); or haloperidol alone (0.4 mg/kg) (N = 6).

Each animal underwent one trial on the beam every hour for 6 hours after drug administration and at 12 and 24 hours. These trials were continued every other day for at least 15 days or until the animals recovered their agility. Locomotion was evaluated by two observers, one of whom did not know which drug treatment had been given to the animal on the beam (*8*).

To determine whether practice on the beam during amphetamine or haloperidol intoxication facilitated recovery, an additional 44 animals were treated as described above except that for 8 hours beginning immediately after drug administration the animals were confined to cages whose small size (7 by 17 by 15 cm) prevented locomotion. These animals received saline (N = 20), amphetamine (2 mg/kg) (N = 19), or haloperidol (0.4 mg/kg) (N = 5).

The trials held 24 hours after motor cortex ablation but before drug administration demonstrated a complete inability of all the animals to walk or run on the beam. After 1 hour, the rats given amphetamine at 2 or 4 mg/kg and given hourly tests while intoxicated showed significant improvements ($P < .01$) compared to their baseline performance and to the performance of the control group (Fig. 1A) (*9*). A dose of 0.5 mg/kg had no effect, and 1 mg/kg did not significantly improve performance. The performance of subjects given 2 mg/kg continued to improve for 3 to 6 hours ($P < .01$). Animals that had been unable to stand on the beam before drug administration could traverse the beam 6 hours after the 2 or 4 mg/kg dose of amphetamine. The control subjects showed no significant improvement during this period. Movies shown in slow motion indicated that, after 24 hours, the improvement of animals given amphetamine and practice on the beam was similar to that achieved by the control subjects after 1 or 2 weeks. They displayed an increased ability to use the affected limbs and to accurately place them on the horizontal surface of the beam. Improvement was most notable in the hind limb.

The animals given amphetamine and practice maintained their improved motor performance over the weeks of testing. The performance of the group receiving 2 mg/kg was significantly better ($P < .05$) than that of the control group for 5 days. In a similar experiment, Hovda and Feeney (*1*) found that beam-walking ability was restored more rapidly in cats given amphetamine 10 days after unilateral removal of the motor cortex than in control cats.

Confinement to prevent locomotion blocked the facilitation of recovery produced by amphetamine. The rate of recovery in these animals was the same as that in restrained controls (Fig. 1B). Therefore, a dose of amphetamine accelerates recovery of locomotion after motor cortex injury only if the animal is given practice during the period of drug

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