vestigation for the examination of the physical properties of water and its interactions with macromolecules during the complex biochemical and physiological processes of cell growth and division.

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Vasoactive Intestinal Polypeptide: Abundant Immunoreactivity in Neural Cell Lines and Normal Nervous Tissue

Abstract. *Vasoactive intestinal polypeptide immunoreactivity is present in high concentrations in clonal lines of neuronal and glial origin. The central nervous system and sympathetic ganglia are also rich in the peptide. The findings suggest that this peptide, hitherto thought limited to the gastrointestinal tract, is widely distributed in neural tissue and may have broad physiological significance.*

Originally isolated from porcine duodenum (*1*), the vasoactive intestinal polypeptide (VIP) is a 28-residue peptide that is structurally and biologically related to secretin and glucagon (*2*), and is found throughout the gastrointestinal tract of mammals and birds (*3*). The peptide may also be secreted by a variety of tumors (*4*), including some of neurogenic and neuroendocrine origin (*5*). The latter finding prompted us to search for VIP in cloned tumor cell lines of neural origin and in normal nervous tissue. We found high levels of immunoassayable peptide in clonal neuroblastoma and astrocytoma cell lines, of neuronal and glial origin, respectively. Vasoactive intestinal polypeptide, or a peptide that cross reacts with it, was also present in normal brain tissue, with the highest concentrations in cerebral cortex, and the lowest in cerebellum and brainstem.

Neuroblastoma cell lines, derived from the transplantable, C 1300 mouse neuroblastoma (*6*), comprised three clones: NE115, which is adrenergic; S20, which is cholinergic; and C46, which is neither adrenergic nor cholinergic (gifts of Dr. Marshall W. Nirenberg, National Institutes of Health, Bethesda, Maryland). The glial cell line was the C6 rat astrocytoma clone (*7*) (gift of Dr. Gordon Sato, University of California, San Diego). Cell monolayers were grown in Dulbecco's modified Eagle's medium, containing 10 percent fetal calf serum plus 200 μg of kanamycin per milliliter and 125 μg of spectinomycin per milliliter. Cultures were grown in Falcon flasks or tissue culture dishes at 37°C in an atmosphere of 10 percent CO_2 in air, at 100 percent humidity. Cells from exponentially growing cultures of each line were inoculated into a series of 100-mm tissue culture dishes. At specified intervals, one plate from each line was scraped off and the cells were counted (Coulter counter), suspended in 2 ml of buffer (0.05*M* KH_2PO_4, 0.001*M* ethylenediaminetetraacetate, adjusted to *p*H 7.3 with KOH), and sonicated before assay of the peptide.

Samples of normal neural tissue were taken from different parts of the brain, peripheral sympathetic chain, and vagus nerve. These samples were removed from dogs within 1 hour after exsanguination, and were extracted in dilute acetic acid or acid alcohol. Peptides were concentrated from these extracts by adsorption to alginic acid, followed by elution with 0.2*M* HCl and salting out (*1*), or by precipitation with ether.

Vasoactive intestinal polypeptide immunoreactivity was measured by a highly specific radioimmunoassay (*4*), which has been improved to detect 50 pg of the porcine peptide per milliliter. All samples were assayed in duplicate, and the assay was performed at least twice. In this assay, antibodies to VIP showed minimal ($< 1 : 1000$) or no cross-reaction with secretin (GIH Laboratory, Karolinska Institute, Stockholm), glucagon (Eli Lilly), cholecystokinin-pancreozymin (GIH Laboratory), bradykinin (synthetic, Sandoz), substance P (synthetic bovine, Beckman), or somatostatin (synthetic ovine, Beckman).

Cells from all three neuroblastoma lines were rich in VIP (Table 1), with a concentration ranging from 0.6 ng per million cells (or 2.2 ng per milligram of protein) to 0.9 ng per million cells (or 3.6 ng per milligram of protein). In each case, as the cell counts increased between the second and fifth days, total VIP levels also increased, although the

Table 1. Concentrations of VIP in neuroblastoma and astrocytoma cell cultures.

Days from inoculation	Neuroblastoma						Astrocytoma	
	Clone NE115		Clone S20		Clone C46		Clone C6	
	Cells ($\times 10^6$/plate)	VIP (ng/plate)	Cells ($\times 10^6$/plate)	VIP (ng/plate)	Cells ($\times 10^6$/plate)	VIP (ng/plate)	Cells ($\times 10^6$/plate)	VIP (ng/plate)
2	5.5	6.2	4.5	5.8	4.9	5.7	5.6	5.6
5	27.4	16.0	15.6	13.2	26.6	17.4	30.6	6.5

concentration per million cells decreased. Astrocytoma cells were also rich in VIP, but the peptide concentration in these cells was less than half that in neuroblastoma cells. Buffer solution in which cells were suspended, and the medium in which they were grown, contained nondetectable levels of the peptide. After incubation for at least 1 day, however, immunoassayable VIP was demonstrable in cell-free medium, in concentrations of approximately 200 pg/ml.

Vasoactive intestinal polypeptide immunoreactivity was also present in normal neural tissue (Table 2), being highest in cortex from frontal and occipital lobes, and hypothalamus, moderately high in hippocampus and white matter from frontal lobe, and lowest in cerebellum, brainstem, and vagus nerve. Peptide concentrations per gram wet weight were lower in duodenum, ileum, and colon than in the richest nervous tissues, raising the possibility that the VIP content in gastrointestinal organs may be partly due to the innervation of these organs. Skeletal muscle and liver contained traces or nondetectable levels of the peptide. This distribution of VIP resembles in some respects (for example, its paucity in cerebellum and liver) the tissue distribution of norepinephrine (*8*).

Extracts of frontal lobe cortex and of neuroblastoma cells (C46 clone) were assayed for VIP-like biological activity, based on their ability to relax isolated, superfused rat stomach strip and guinea pig gallbladder (*4*). The bioassay confirmed the presence of biologically active peptide. The high levels of VIP or a related peptide in both gray and white brain matter correlate with its presence in tumor cell lines of both neuronal and glial origin. These findings, and the selective distribution of the peptide in the central and autonomic nervous systems, suggest a possible function for this peptide, or one that is similar to it, in the nervous system. Until additional data are available, including the possible effects of the peptide on neural function, its physiologic role remains speculative. Such a role could include a modulator, trophic, growth-promoting (*9*), or transmitter action (*10*). An endothelial proliferative factor, elaborated by clonal cell lines of neural origin (*11*), is probably distinct from VIP, since this factor is reported to be destroyed by heating to 56°C for 10 minutes, while VIP resists boiling for that period (*1*). Pertinent to our results are the recent findings that substance P, another vasoactive peptide that was originally discovered in intestine (*12*), also occurs in the central nervous system (*13*) and may have a modulator or transmitter function (*14*); that gastrin immunoreactivity occurs in brain (*15*); and that somatostatin (growth hormone release–inhibiting hormone) (*16*) is found both in the central nervous system and the gastrointestinal tract (*17*).

Table 2. Distribution of VIP immunoreactivity in nervous, gastrointestinal, and other tissues from dogs. Values are in nanograms per gram, wet weight.

Tissue	VIP
Nervous tissues	
Frontal lobe cortex	61
Frontal lobe white matter	35
Temporal lobe cortex	24
Occipital cortex	66
Cerebellar cortex	2
Hippocampus	39
Thalamus	2
Hypothalamus	65
Pons	1.3
Medulla	2.5
Midbrain	3.3
Sympathetic nerve	6.4
Vagus	0.6
Gastrointestinal tissues	
Duodenum	13.2
Ileum	14.0
Ascending colon	10.6
Liver	<0.06
Other tissues	
Skeletal muscle	0.1

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Human Handedness: A Partial Cross-Fostering Study

Abstract. *The hand preference of college students correlated significantly with the writing hand of their biological parents but not that of their stepparents. The results are consistent with a genetic theory of the origin of human handedness.*

In contrast with nonhuman mammals, which show no preference (*1*), at least 90 percent of the humans in those cultures studied prefer to use the right hand for most skilled activities (*2*). There is indirect evidence of right-handedness in prehistoric man (*3*) and even in Australopithecus (*4*). The left cerebral hemisphere controls the right hand and language in most right-handers (*5*). In addition, the two cerebral hemispheres are anatomically asymmetrical (*6*), and some of the observed asymmetries correlate with preferred handedness (*7*). Handedness and cerebral organization are also related in that left-handers, as compared to right-handers, have less clear-cut laterality of virtually all cognitive functions (*5*, *8*).

The genesis of handedness in humans is unclear. Collins (*9*) and Provins (*10*) have hypothesized that learning is primarily responsible. Nagylaki and Levy (*11*) have refuted much of Collins's (*9*) evidence that genetic factors are not involved, although studies of twins have never provided clear evidence for a genetic factor (*11*). They report that the frequency of left-handedness in twins, both dizygotic and monozygotic, is significantly higher than in singletons; that finding calls into question the results of heritabil-