in planar crystalline form like the channel arrays in Fig. 1B facilitate electron microscopy (*9*). The random noise in electron images from such specimens can be filtered from the ordered, structure-related components by computer based Fourier- or correlation-averaging techniques. The more unit cells included in such averages, the lower the electron dose per molecule needed to obtain the averaged image and the more reliable its structural representation. Only a few membrane proteins have been found to occur as ordered arrays in nature. Several others have been crystallized in vitro by detergent solubilization, purification, and some degree of reconstitution with lipids. The technique of controlled phospholipase digestion presented here is another source of membrane protein crystals. The 30-kD pore protein typically represents 60 to 80 percent of the total protein mass in outer membrane fractions of *N. crassa* mitochondria. Other biological membranes naturally enriched in a particular protein component or protein complex (for example, Ca^{2+} transporter in sarcoplasmic reticulum and acetylcholine receptor in synaptosomes) may also be amenable to phospholipase-induced crystallization.

CARMEN A. MANNELLA
Center for Laboratories and Research, New York State Department of Health, Albany 12201

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4. A low-resolution three-dimensional reconstruction of the negatively stained mitochondrial array has been calculated by weighted back-projection of a tilt-series of Fourier-filtered, electron microscopic images [C.A. Mannella, M. Radermacher, J. Frank, *Proceedings of the 8th European Congress on Electron Microscopy, Budapest, 1984* (8th European Congress on Electron Microscopy Foundation, Leiden, Netherlands), in press]. This reconstruction indicates that the array is composed of regularly arranged, stain-filled, cylindrical channels, each approximately 2.5 nm in diameter.
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6. The surface density of the channels increases by about 10 percent as the array changes from oblique to rectangular. The different arrangements of the stain-filled channels are illustrated in C. A. Mannella, M. Colombini, J. Frank, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2243 (1983).
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8. Outer membranes isolated from *N. crassa* mitochondria contain 1.1 μg of phospholipid per microgram of protein [G. Hallermeyer and W. Neupert, *Hoppe-Seyler's Z. Physiol. Chem.* **355**, 279 (1974)]. Complete hydrolysis of the membrane phospholipids in these experiments would therefore release 10^{-5} to $10^{-6}M$ fatty acids and lysophospholipids. The range of critical micelle concentrations of the expected fatty acids is 10^{-2} to $10^{-4}M$ [P. Mukerjee and K. J. Mysels, *Critical Micelle Concentration in Aqueous Surfactant Systems* (National Bureau of Standards, Washington, D.C., 1971)]; that of the lysophospholipids is 10^{-3} to $10^{-5}M$ [A. Helenius, D. R. McCaslin, E. Fries, C. Tanford, *Methods Enzymol.* **56**, 734 (1979)].
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Immunohistochemistry of Adenosine Deaminase: Implications for Adenosine Neurotransmission

Abstract. *Immunohistochemical analysis of adenosine deaminase in rat brain revealed an extensive plexus of adenosine deaminase–containing neurons in the basal hypothalamus. These neurons converged on and were most numerous in three major centers, namely, the tuberal, caudal, and postmammillary caudal magnocellular nuclei. Most other brain regions were devoid of cells containing adenosine deaminase. Some adenosine deaminase–containing neurons were retrogradely labeled with the fluorescent dye fast blue when the dye was injected into the frontal cortex and striatum. Specific populations of neurons having high levels of adenosine deaminase may release adenosine as a neurotransmitter.*

Immunohistochemical studies of the central nervous system (CNS) have provided information about the location and organization of neurons defined by their putative neurotransmitter content. In addition, the visualization of neuroactive peptides or of enzymes responsible for the synthesis and degradation of the better known neurotransmitters within neurons has helped to elucidate the biochemical nature of diverse neuronal systems. A recent addition to the list of putative central neurotransmitters is the purine nucleoside adenosine (*1, 2*). Behavioral, electrophysiological, and biochemical evidence indicates that adenosine can function as a neurotransmitter (*1–3*). Biochemical and anatomical methods capable of identifying adenosine-releasing neurons are not yet available and, as a consequence, no candidate neurons or systems in the CNS for which adenosine is a neurotransmitter have been described. Our study was based on the premise that the metabolism of adenosine in presumptive adenosine-releasing neurons may exhibit features unique to these cells. A major metabolic pathway for the degradation of adenosine is through deamination by the enzyme adenosine deaminase (AD) (*4*). We therefore conducted immunohistochemical studies of the distribution of AD in the rat CNS. We found a network of neurons in the basal hypothalamus that are distinguished from other central neurons by their high AD content. We suggest that AD-rich neurons may occur in neural systems that release adenosine as a neurotransmitter.

Calf intestinal AD was purified to homogeneity, and an antiserum specific to this enzyme was prepared in rabbits (*5*). Antibodies to calf intestinal AD have been shown to cross-react with AD derived from rat tissues (*6*). For our immunohistochemical studies, adult rats were placed under deep anesthesia with chloral hydrate and perfused intracardially with a 0.9 percent saline wash followed either by 4 percent formaldehyde solution or 4 percent paraformaldehyde in 0.1*M* phosphate buffer, *p*H 7.4. Sections of brain (20 μm) were cut on a freezing microtome and incubated at 4°C for 48 hours with antiserum to AD diluted 1:500. The sections were subsequently processed by the peroxidase-antiperoxidase method (*7*).

Examination of many brain regions revealed a highly restricted distribution of neurons that stained for AD by our immunohistochemical procedures. The most intensely stained neurons were found in three distinct cell groups in the basal hypothalamus (Fig. 1, a to c). According to the anatomical descriptions of Bleier *et al.* (*8*), these neurons were located in the tuberal (TM), caudal (CM), and postmammillary caudal (PCM) magnocellular nuclei. In addition, many AD-containing neurons were found dispersed between these major cell groups, thus forming a contiguous network from the TM anteriorly to the PCM posteriorly. Immunohistochemical staining for AD was completely abolished in tissue sections incubated with antiserum to AD that had been adsorbed with purified AD (Fig. 1d).

Vincent *et al.* (*9*) demonstrated that neurons in the TM, CM, and PCM have widespread projections to the amygdala, striatum, and cortex. To determine whether AD-containing neurons in these nuclei have similar diffuse projection

sites, we injected fast blue, a retrogradely transported fluorescent dye, into the frontal cortex and striatum of a group of rats. After 5 days, the animals were perfused with formaldehyde solution. Brain sections were either examined for fast blue labeling in the basal hypothalamus or were processed for AD immunohistochemistry by the indirect immunofluorescence method with fluorescein isothiocyanate–conjugated goat antibody directed against rabbit antibody to AD (*10*). The results for the fast blue–labeled neurons in TM, CM, and PCM were essentially as described by Vincent *et al.* (*9*). In brain sections processed for both fast blue and AD immunofluorescence, neurons containing both fast blue and AD were found in each of the three magnocellular nuclei (Fig. 1, e and f), indicating that some AD-containing neurons innervate diverse areas of the brain.

This conclusion is supported by our immunohistochemical observation of AD-containing fiber systems (data not shown). Bundles of axons heavily stained for AD can be traced over long distances in the brain to numerous apparent termination areas where they have a varicose appearance. Frequently, these axons can be traced back to the hypothalamus and are found to originate from the AD-rich neurons therein. The ability to visualize the axons, and therefore the projection areas of AD-rich neurons, provides the opportunity to locate in detail the CNS structures that are responsive to the influence of AD–containing neural systems. This should be of value in electrophysiological and behavioral studies aimed at determining the function of such systems.

Although, among central neurons, the hypothalamic neurons described above exhibited by far the greatest AD immunoreactivity, we have observed weaker but consistent immunostaining of a small number of neurons in the septal nuclei and superior colliculus (data not shown). The apparent absence of immunoreactivity in neurons and glia in all of the other brain regions we examined does not necessarily indicate that AD is absent in these cells. The enzyme may occur in quantities that are not detectable by the present immunohistochemical procedure. Alternatively, tissue fixation may destroy the immunogenicity of AD to the extent that it may be undetected in all structures except those rich in the enzyme. We have investigated these possibilities with a number of fixation conditions and various methods of incubation and processing for immunohistochemistry, as well as with intracerebroventricular injections of colchicine. The results obtained after these manipulations were essentially as described above and further indicate the existence of a select population of neurons in the brain having relatively high levels of AD.

The nature of the relation between high levels of AD and the role of adenosine in neurons of the basal hypothalamus is still uncertain. However, several points favor the proposal that these neurons represent either the source of a major adenosinergic neural system innervating widespread regions of the brain or a system that interacts with adenosine-related neurotransmission at various projection sites. First, our immunohistochemical studies indicate a limited distribution of AD-rich neurons in the rat brain. Hypothalamic neurons were intensely stained for AD and, indeed, no prior treatment with colchicine to cause buildup of enzyme was required to visualize AD in these neurons. Thus, the uneven distribution AD-rich neurons in the brain suggests that in these neurons AD may have a role in addition to or distinct from its ordinary participation in the intracellular metabolic pathways governing the concentration of adenine derivatives. Second, the action of iontophoretically applied adenosine on neuronal electrical activity is typically inhibitory (*2, 11*). Specific and potent inhibitors of AD when applied to central neurons evoke depression of spontaneous electrical activity and potentiate the depressive effects of adenosine (*2, 11*). Moreover, treatment of animals with AD

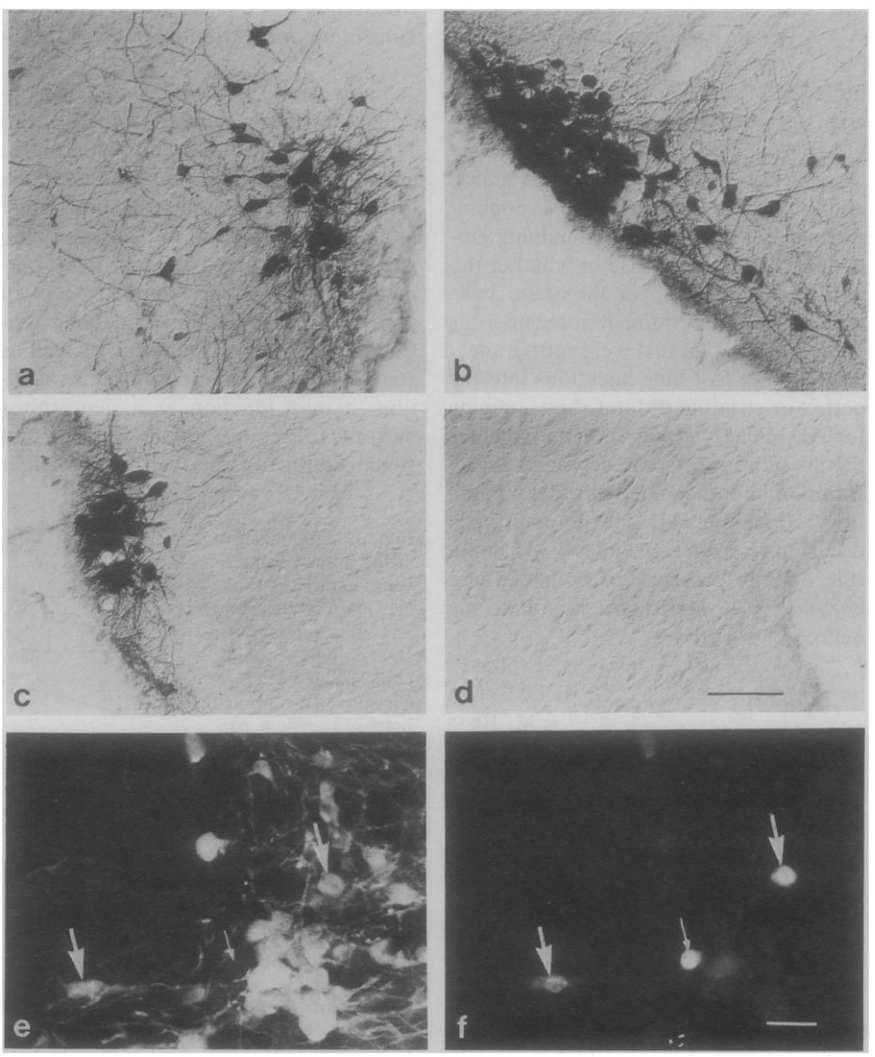

Fig. 1. (a to c) Interference contrast photomicrographs of neurons in (a) tuberal (TM), (b) caudal (CM) and (c) postmammillary caudal (PCM) magnocellular nuclei of the basal hypothalamus that have been stained immunohistochemically for adenosine deaminase (AD). (d) No AD-containing neurons were observed with antiserum to AD adsorbed with purified AD, as demonstrated for TM. (e and f) Fluorescence photomicrographs of (e) neurons in the CM stained for AD by immunofluorescence and (f) in the same section neurons retrogradely labeled with fast blue after injection of this dye into the cortex. Large arrows indicate neurons that are both AD-positive (e) and retrogradely labeled with fast blue (f). Small arrows indicate a neuron retrogradely labeled with fast blue (f) but devoid of AD-staining (e). Scale bar in (d), 100 μm, applies to (a) to (d); scale bar in (f), 50 μm, applies to (e) and (f).

inhibitors (*12*) produces behavioral effects similar to those of potent adenosine receptor agonists (*2*). These findings indicate that AD has a major role in regulating the presynaptic levels of adenosine during the process of nucleoside release and uptake. Alternatively, AD may participate in terminating the pre- or postsynaptic actions of adenosine, or both, by degrading this nucleoside to its relatively inactive metabolite, inosine (*2*). Such a putative role is consistent with the location and the high specific activity of AD in cortical synaptosomes (*4*). Finally, the application of adenosine has been shown to modify neuronal electrical activity in many brain areas including cortex and striatum (*2, 11, 13*), a finding compatible with the diverse axonal projections of AD-containing neurons in the TM, CM, and PCM nuclei.

A separate issue concerns the report by Vincent *et al.* (*9*) that many neurons in TM, CM, and PCM contain glutamic acid decarboxylase and may therefore utilize γ-aminobutyric acid as a neurotransmitter. Whether AD-containing neurons in these nuclei form a population separate from those containing glutamic acid decarboxylase or whether the two enzymes occur in the same cells remains to be determined. Some neurons in the TM and CM that were retrogradely labeled after fast blue injections into the cortex or striatum did not show staining for AD (Fig. 1, e and f). This indicates the possibility for some degree of segregation of hypothalamic neuronal populations containing AD and glutamic acid decarboxylase. Nevertheless we cannot rule out the presence of both of these enzymes in at least some hypothalamic neurons.

J. I. NAGY*
L. A. LABELLA
M. BUSS
Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba R3E OW3, Canada
P. E. DADDONA
Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, 48109

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* Address requests for reprints to J.I.N.

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Sequence of the Human Somatostatin I Gene

Abstract. *Two human genomic DNA fragments containing alleles for the gene coding for somatostatin I were isolated and sequenced. This gene contains a single intron that interrupts the coding sequence in the propeptide portion of the somatostatin moiety. The site of initiation of transcription of the gene was located by transcription experiments in HeLa cell extracts, and the putative regions for controlling the initiation of transcription were identified.*

Somatostatin is a 14–amino acid neuropeptide and hormone (somatostatin-14) found in the brain and spinal cord, and in the pancreas, stomach, and intestine. It suppresses the release of many pituitary, pancreatic, and gastrointestinal polypeptide and glycoprotein hormones and regulates some aspects of gastrointestinal function, including gastric acid and pepsin secretion as well as smooth muscle contractility (*1*). In addition, it may function as a neurotransmitter (*1*). Previous studies suggest that somatostatin-14 is derived from a larger polypeptide, prosomatostatin (*1*). The sequence of the somatostatin precursors, including their signal peptides, have been deduced from the nucleotide sequence of the cloned somatostatin complementary DNA's (cDNA's) from anglerfish (*2, 3*), human (*4*), catfish (*5–8*), and rat (*9*). The nucleotide sequences of two independently isolated cDNA's from the anglerfish endocrine pancreas (*2*) revealed the amino acid sequences of two different prosomatostatin molecules, each of which contained distinct 14– and 28–amino acid somatostatin moieties

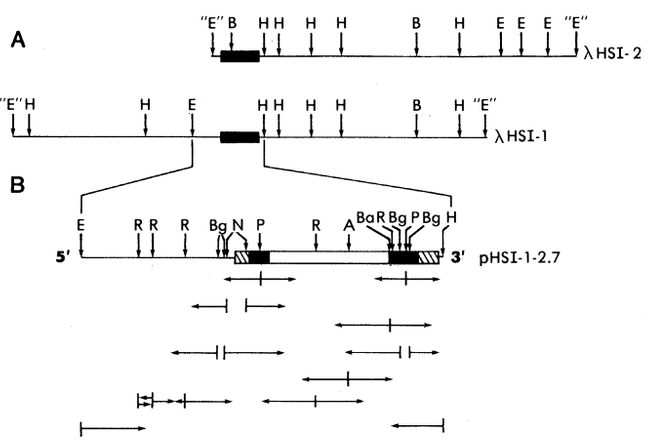

Fig. 1. Organization of the human somatostatin gene. (A) Linkage map of the gene and adjacent regions. One million recombinant phage of a λ Charon 4A library prepared from a partial Hae III–Alu I digest of human fetal liver DNA were screened with a nick-translated human preprosomatostatin cDNA probe (*22*). Phage containing somatostain gene sequences were plaque-purified, and then DNA was prepared from phage grown in liquid culture (*23*). The location of the somatostatin gene (indicated by the filled box) was determined by hybridization of the ^{32}P-labeled cDNA to Southern blots of single and double restriction endonuclease digests of DNA from λHSI-1 and λHSI-2. The restriction enzymes indicated are as follows: A, Acc I; B, Bam HI; Ba, Bal I; Bg, Bgl II; E, Eco RI; H, Hind III; N. Nar I; P, Pst I; and R, Rsa I. The ordering of the three Eco RI sites at the 3′ end of λHSI-2 has not been determined. The quotation marks indicate Eco RI sites produced by linker addition during the construction of the library. The Eco RI site of λHSI-1 on the right appears close to or identical to the natural site. (B) Organization of the gene. The 2.7-kbp Eco RI–Hind III fragment of λHSI-1 was cloned into plasmid pBR322 (pHSI-1-2.7). The box delimits the structural portion of the gene. The hatched section indicates the region encoding the untranslated portion of the mRNA, the filled region indicates the protein coding portion, and the open region represents the intervening sequence. The sequencing strategy and the restriction sites at which sequence determinations were initiated are shown.