tions. Smaller probes composed of internal fragments from the SLT structural genes are being characterized for use in additional hybridization experiments with *Shigella*, low-toxin producing *E. coli*, and other bacteria (*11*) that produce varying amounts of SLT. From the data presented we conclude that (i) the structural genes for SLT are present in the genome of phage 933J; (ii) the structural gene for the B subunit of SLT is encoded by the insert in pJN26, and a portion of the structural gene for the A subunit of SLT is encoded by the insert in pJN28; and (iii) the SLT genes from phage 933J appear to be homologous to structural genes for Shiga toxin in *Shigella*.

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Distinct Monoamine Oxidase A and B Populations in Primate Brain

Abstract. *Monoclonal antibodies specific for monoamine oxidase (MAO) A and MAO B, respectively, were used to localize these enzymes in primate brain. The reagents recognized different populations of neurons: those that recognized MAO A were located in cell groups containing catecholamines, including the substantia nigra, nucleus locus coeruleus, nucleus subcoeruleus, and the periventricular region of the hypothalamus, whereas those that recognized MAO B were observed in serotonin regions, including the nucleus raphe dorsalis and nucleus centralis superior. These data illustrate the physiological independence of MAO A and B and show that neurons may be specialized for their degradative as well as their synthetic functions.*

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Monoamine oxidase (E.C. 1.4.3.4) occurs in two forms, designated MAO A and MAO B, which differ in substrate preferences and inhibitor specificities (*1*), apparent molecular weights (*2*), and immunological properties (*3–5*). Two highly specific mouse monoclonal antibodies, one elicited in response to human placental MAO A and one to human platelet MAO B (*4–6*), were used to identify MAO A– and MAO B–containing structures in the primate central nervous system.

Three *Macaca fascicularis* monkey brains were stained to recognize MAO B and three others MAO A. Monkeys were prepared by serial cardiac perfusion with physiological saline, 4 percent buffered paraformaldehyde, and 20 percent buffered sucrose. Tissue sections (30 μm) were incubated overnight at room temperature with mouse monoclonal MAO A-3C9 or MAO B-1C2, followed by anti-mouse immunoglobulin G (diluted 1:50) for 30 minutes, then mouse peroxidase antiperoxidase (diluted 1:100) for 30 minutes, and finally diaminobenzidine-peroxide (0.25 percent/0.01 percent). Immunocytochemical controls included omission of one of the reagents in the peroxidase-antiperoxidase protocol, dilution of the antibody, incubation with ascites fluid containing nonreactive antibody, and inhibition of staining with antigen. Specific staining was not seen with the control stains or when specific antibody was adsorbed first with antigen.

Neurons positive for MAO A were observed in amine-containing regions systematically mapped in rat brain by Dahlström and Fuxe (*7*). Neurons containing MAO A were localized in the ventrolateral medulla (A1 cell group of Dahlström and Fuxe), in the dorsal medulla in the vicinity of the solitary and dorsal motor nuclei (A2), and in cells scattered tangentially between these two groups through the medullary tegmentum. In the caudal pons, cells were positively labeled in the dorsal and lateral walls of the fourth ventricle (A4) and in the ventrolateral pontine tegmentum (A5). The most densely populated sites were the nucleus locus coeruleus (A6) (Fig. 1A) and the subcoeruleus complex in the lateral pontine tegmentum (A7), including the Kölliker-Fuse nucleus and the medial and lateral parabrachial nuclei. Preliminary double-label staining studies indicate that MAO A is localized in cells of the locus coeruleus which in human brain contain the nonadrenergic synthetic enzyme, dopamine-β-hydrolyase. The MAO A–positive neurons were also observed in the lateral tegmentum at the junction of the pons and the midbrain (A8), in the substantia nigra (A9), especially in rostral portions of the nucleus, and sparsely in the midline throughout the midbrain (A10). Under the conditions used, the number of cells containing detectable stain for MAO A in the substantia nigra did not represent all dopaminergic cells normally observed in this region (*7, 8*).

Diencephalic cells labeled for MAO A were scattered dorsally around the ventral perimeter of the mammillothalamic tract in tuberal hypothalamic regions (A13), along the entire dorsal to ventral extent of the periventricular hypothalamic region (A11), and in the arcuate nucleus at the floor of the third ventricle (A12). A few stained neurons were located anteriorly in the periventricular region of the hypothalamus (A14). Some stained fibers were also observed, particularly near catecholamine cell groups, in ascending catecholamine pathways (Fig. 1C) and in some blood vessels, suggesting that this enzyme is probably distributed throughout the projection system in which it has been localized. Another large population of MAO A–positive neurons, which could not be ascribed to any previously identified catecholamine

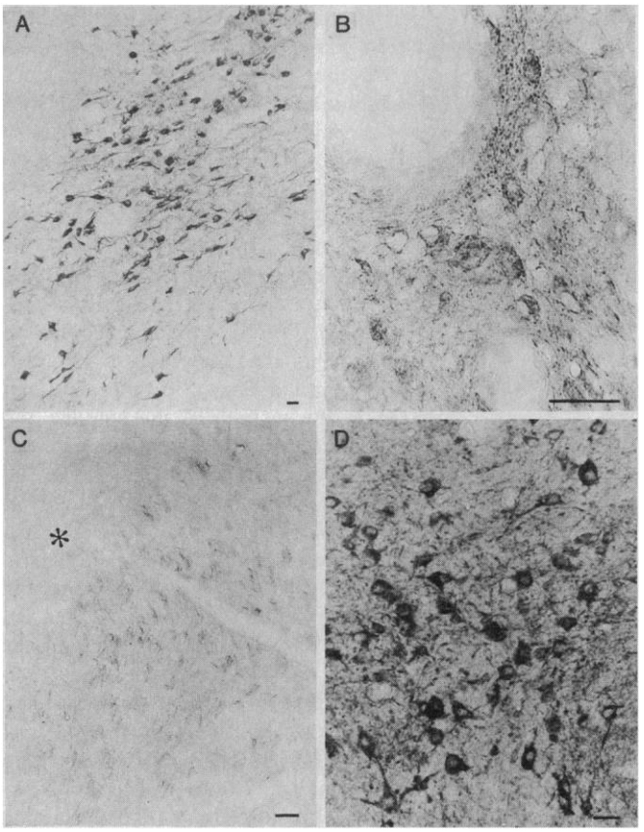

Fig. 1. Two pairs of matching fields stained for MAO A and MAO B in the pons (A and B) and the midbrain (C and D). (A) The nucleus locus coeruleus viewed at low power, containing many neurons stained positively for MAO A. (B) In a matching field of the locus coeruleus viewed at higher power, beaded neuronal varicosities are stained for MAO B. (C) In the dorsal ascending bundle in the midbrain tegmentum, just ventral to the periaqueductal gray (*), axons are stained for MAO A. (D) In the dorsal raphe in the midbrain periaqueductal gray, cells and varicose fibers stain positively for MAO B. Scale bar, 30 μm.

cell group, was localized in the posterior and lateral hypothalamic regions.

The MAO B–positive neurons were localized primarily in midline structures known to contain serotonin neurons, including all the raphe nuclei (B1–B9). Double-label staining studies indicate that cells in the dorsal raphe nucleus which stain for MAO B (Fig. 1D) also stain for serotonin. Cells staining for MAO B were also found in the lateral and posterior hypothalamic regions. Astrocytes in many brain regions stained for MAO B, particularly around large blood vessels and ventricles. The MAO B-stained fibers were observed in regions of dense serotonergic innervation, including the nucleus locus coeruleus (Fig. 1B) and the interpeduncular nucleus, which suggests that MAO B, like MAO A, probably occurs through the extent of a specific neuronal projection.

The distribution of neuronal cell bodies containing MAO A and MAO B was primarily in previously identified amine-containing regions (*7, 8*) (Fig. 2). The catecholamine cell groups, previously designated A1–A14 (*7*), stain positively for MAO A, whereas the indoleamine cell groups, B1–B9 (*7*), stain for MAO B.

Before the development and use of the Falck-Hillarp histofluorescence technique (*7*), enzyme histochemical techniques localizing MAO were used to identify cells of the monoamine system (*9*). Biochemical assays of homogenized tissue from dissected brain structures, sometimes preceded by specific inhibitor

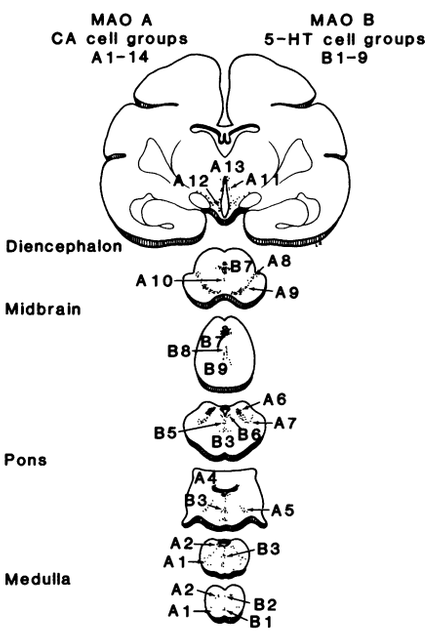

Fig. 2. The distribution of neurons positive for MAO A and MAO B throughout the brainstem of monkey, schematically illustrated in coronal tissue sections. Amine-containing cell groups of Dahlström and Fuxe (*7*) are indicated. Catecholamine (CA) cell groups are denoted A 1–14 and serotonin (5-HT) cell groups are denoted B 1–9.

treatments or denervation procedures, have also been used to localize MAO A and MAO B in various mammalian brain regions (*10*). Only immunocytochemical techniques (*11*), however, have allowed MAO type-specific localization on the cellular level. The distribution of MAO B in the primate agrees with the distribution of this enzyme in neurons and astrocytes in the rat reported by Levitt *et al.* (*11*). Our data are also consistent with the results of our localization studies of MAO A and MAO B in human brain.

In addition to localizing MAO A and B in known monoamine cell groups of the brainstem, our study has revealed the presence of both enzymes in a large population of diencephalic neurons not described by Dahlström and Fuxe (*7*). Cells matching the descriptions given for dopamine neurons, which stained for MAO A, and also two distinct groups of larger multipolar neurons localized in the posterior and lateral hypothalamic regions were stained positively for MAO A or MAO B. These findings suggest that other amines may be localized in the diencephalon. Some of the MAO B–positive cells may be the serotonin cells identified by Steinbusch and Niewenkuys and Frankfurt *et al.* after treatment of rats with the MAO inhibitors, nialamide or pargyline, and L-tryptophan (*12*); those in the basal hypothalamus may be the histamine cells described recently in the rat by Panula *et al.* (*12*).

The clear pattern of distribution observed for MAO A and MAO B in relation to amine neuronal groups should help to clarify the function of these enzymes in the brain, including their hypothesized role in controlling neurotransmitter levels. Serotonin is preferentially deaminated by MAO A and not MAO B (*13*), yet we have observed only MAO B in serotonin-containing cells. Since MAO A is capable of deaminating norepinephrine and dopamine (*13*), it might help regulate levels of these transmitters in catecholamine-containing cells; however, indirect evidence suggests that dopamine may be metabolized in humans primarily by MAO B (*14*). Levitt *et al.* (*11*) and others (*15*) have proposed that MAO A and B may protect their respective neurons by degrading extraneous amines, neurotransmitters, or potential false neurotransmitters. Monoamine oxidase A appears to serve a protective function in peripheral tissues (*16*).

Monoamine oxidase B activity has been studied in patients with psychiatric and neurological disorders that may be linked to monoamine abnormalities, including alcoholism, affective illness, and

schizophrenia (*17*). With knowledge of the localization of MAO A and B in specific neuronal tracts, retrospective examination of past basic and clinical studies may clarify the patterns of altered neurotransmitter function and metabolism that are thought to occur in some of these disorders.

Our localization studies may also contribute to explaining the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which produces parkinsonian symptoms in humans and monkeys (*18, 19*). This substance is thought to be oxidized by MAO B (*20*) to the active metabolites 1-methyl-4-phenyl-2,3-dihydropyridine ($MPDP^+$) and 1-methyl-4-phenyl-pyridine (MPP^+) (*21*), yet we find that MAO B is localized primarily in serotonin-containing neurons, which are not affected by MPTP (*19, 22*). Thus MAO B is not observed in the dopamine-containing neurons in the substantia nigra, which are selectively destroyed by the drug (*19, 22*). However, $MPDP^+$ or MPP^+ might be selectively taken up by these neurons (*23*) after release from serotonin-containing neurons that project onto the substantia nigra or the striatum (for example, cells of the nucleus raphe dorsalis) or from MAO B–containing astrocytes. Localization of MAO A and MAO B and clarification of their physiological roles in the brain may help resolve the molecular mechanisms underlying a variety of monoamine-related neurological and psychiatric disorders.

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Externalization of β-Adrenergic Receptors Promoted by Myocardial Ischemia

Abstract. *β-Adrenergic receptors were identified in two fractions of guinea pig myocardium: a purified sarcolemmal fraction and a light vesicle (presumably intracellular) fraction. In the light vesicle fraction, which contained approximately 25 percent of the myocardial receptors under control conditions, the receptors appeared to be segregated from the stimulatory guanine nucleotide binding and catalytic components of adenylate cyclase. During myocardial ischemia, β-adrenergic receptors were redistributed from the intracellular vesicles to the sarcolemmal fraction, where isoproterenol-stimulated adenylate cyclase activity was increased. These findings should facilitate further studies on cellular and molecular mechanisms that regulate adrenergic receptor traffic in the myocardium and may explain the rapid enhancement in adrenergic receptor expression that occurs with myocardial ischemia.*

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Cell surface receptors for hormones and neurotransmitters are actively regulated by processes such as synthesis, internalization, recycling, and degradation. Because the population of receptors on target cell surfaces is under dynamic control, steady-state changes in receptor number reflect an alteration in one or more aspects of the receptor life cycle (*1, 2*). Ischemia is one example of an altered cellular environment with important physiological and pathological ramifications. Earlier studies have shown that myocardial ischemia is associated with a rapid (<1 hour) increase in the number of β-adrenergic receptors in crude cardiac membranes, and this increase in receptors may account for the enhanced effects of catecholamines in the ischemic heart (*3*). However, the mechanism mediating this up-regulation of receptors is unknown. Not all β-ad-