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*Mini-Paper*

# Molecular neuroanatomy of monoamine oxidases in human brainstem

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**Abstract.** Specific, high-resolution techniques (quantitative enzyme radioautography and in situ hybridisation histochemistry) have revealed distribution, abundance and cellular localisation of the isoenzymes MAO–A and MAO–B and their mRNAs in human post-mortem brainstem. Whereas MAO–A protein and mRNA are expressed by noradrenergic neurons of the locus coeruleus, MAO–B protein and mRNA are expressed by serotoninergic neurons of the raphé nuclei. In the substantia nigra, MAO–B was more abundant than MAO–A; the former was localised in the reticular zone and the latter in the compact zone (where melanin-containing dopaminergic neurons are found). To date, it has not been possible to detect mRNA for either MAO–A or MAO–B in the substantia nigra or in glial cells of the brain regions investigated, suggesting either that the technique has limited sensitivity, or the possible existence of MAO–A and MAO–B subtypes.

**Key words:** Neuroanatomy – Monoamine oxidase

Monoamine oxidases (MAO) oxidatively deaminate neurotransmitter and xenobiotic amines in the CNS and peripheral organs. Isoenzymes (MAO–A and MAO–B) have been identified by their substrate selectivity and their inhibitor sensitivity: in rodent brain, serotonin, dopamine, and noradrenaline are preferentially metabolised by MAO–A, the trace amines phenethylamine and methylhistamine by MAO–B, and both, tyramine and octopamine by both enzymes (in human brain, a similar selectivity prevails except that dopamine is a substrate for both enzymes). The isoenzymes are integral flavoproteins of outer mitochondrial membranes in neurons, glia, and other cells.

The physiological role of MAO is probably two-fold: firstly, to maintain a low cytosolic amine concentration in monoaminergic neurons and other cells (e.g. glia) in order to further the uptake of monoamines by the respective transporters (i.e. to facilitate, indirectly, the inactivation of released neurotransmitter amines), and secondly, to prevent various natural substrates from accumulating in monoaminergic neurons, where they could interfere with the storage, release, uptake, and receptor function of physiological neurotransmitter amines.

Since the cellular compartmentalisation of MAO–A and MAO–B in the CNS and peripheral organs determines to a large extent which substrate has access to which isoenzyme, knowledge of the tissue distribution and cellular localisation of the enzymes is essential. Previously, this has been studied by enzyme histochemical and immunohistochemical techniques. Recently, however, two major advances in research on MAO, the development of novel, reversible and selective inhibitors and the molecular cloning of the human liver enzymes, have provided new research tools to study the molecular neuroanatomy of the enzymes in a quantitative and high-resolution manner (Saura Marti et al. 1990). Firstly, Ro 41–1049 and Ro 19–6327 (reversible, selective inhibitors of MAO–A and MAO–B, respectively) have been used, as tritiated radioligands, to map the distribution and abundance of the enzymes in microscopic regions of the rat CNS and peripheral organs, and human brain by quantitative enzyme radioautography. Secondly, $^{35}$S-labelled oligonucleotide probes (least conserved sequences specific for human liver MAO–A and MAO–B) have been used for transcript mapping to reveal, by in situ hybridisation histochemistry, cellular mRNAs coding for the enzymes in human brain.

## Materials and methods

*Quantitative enzyme radioautography.* Human brainstem was obtained at autopsy (~6 h post-mortem) and quickly frozen in dry ice. The individuals (four males, one female; average age 75 years) died without clinical or histopathological evidence of neurological disease. Cryostat sections (12 μm) of the brainstem were prepared at

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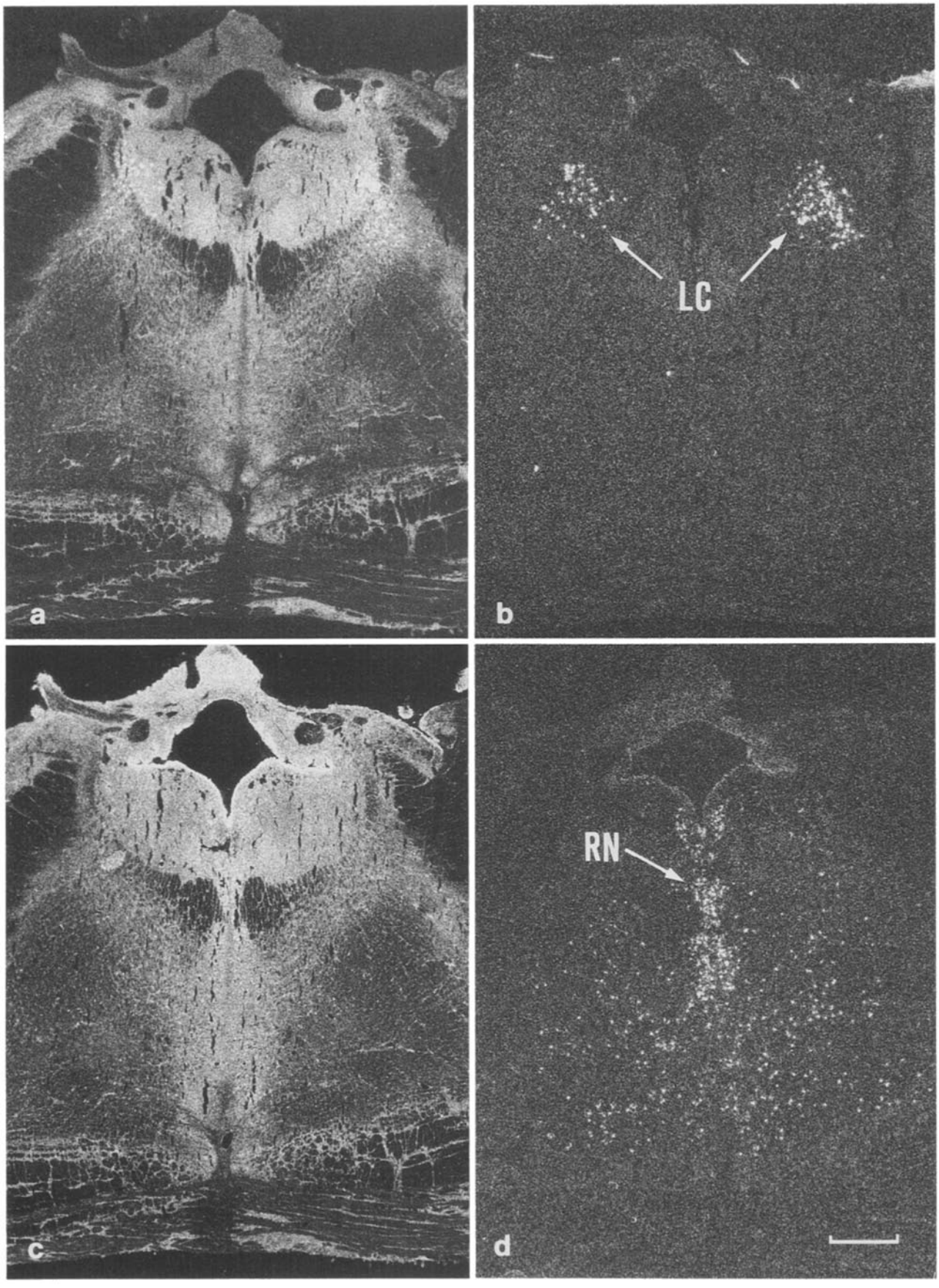


**Fig. 1a–d.** Distribution of MAO–A and MAO–B proteins in sections of human brainstem (**a, c**) revealed by $^{3}$H–Ro 41–1049 and $^{3}$H–Ro 19–6327 binding, respectively. *White areas* indicate high concentrations of the enzymes. The cellular localisation of MAO–A and MAO–B mRNAs in adjacent sections (**b, d**), revealed by in situ hybridisation histochemistry, indicates the presence of enzyme transcripts in distinct populations of cells. *LC*, locus coeruleus; *RN*, raphé nuclei. Bar represents 2 mm

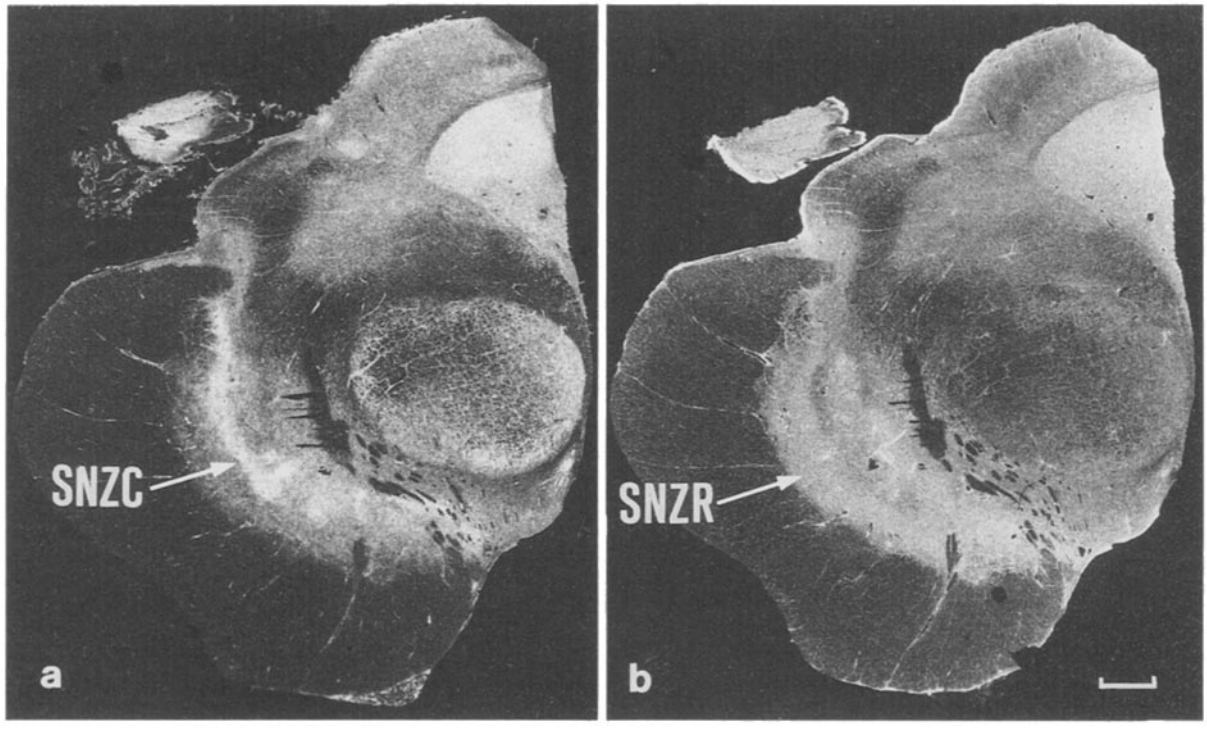


**Fig. 2a, b.** Distribution of MAO–A and MAO–B proteins (**a, b,** respectively) in human substantia nigra revealed by the high-affinity binding of $^3$H–Ro 41–1049 and $^3$H–Ro 19–6327. Note the distinct differences in the distribution of the enzymes. *SNZC,* substantia nigra, compact zone; *SNZR,* substantia nigra, reticular zone. Bar represents 2 mm

the levels of the substantia nigra, raphé nuclei and locus coeruleus, then stored at −20° C until used. The in vitro binding characteristics of the radioligands ($^3$H–Ro 41–1049 [s.a. 19.1 Ci/mmol] and $^3$H–Ro 19–6327 [s.a. 19.9 Ci/mmol], synthesised by Dr. H. Harder, Central Research Units, Roche) were determined in saturation and competition binding experiments at steady state (60 min at 37° C for $^3$H–Ro 41–1049 and 90 min at 20° C for $^3$H–Ro 19–6327). Non-specific binding was determined by incubating adjacent sections under identical conditions with 1 μM clorgyline or *l*-deprenyl (for detection of MAO–A and MAO–B, respectively). Radiolabelled sections, together with calibration standards, were exposed to tritium-sensitive film for approximately 3 weeks. The developed radioautogram was used to illustrate the distribution and measure the density of binding sites (i.e. MAO) in human brainstem.

*In situ hybridisation histochemistry.* After prior fixation with 4% paraformaldehyde, cryostat sections (adjacent to those used for binding experiments) were radiolabelled with 60-mer $^{35}$S-oligonucleotide sequences specific and complementary to human liver MAO–A and MAO–B mRNAs. Sections were exposed for approximately 2–3 weeks (to β-max film, Amersham) to reveal the cellular localisation of hybridisation signal.

## Results and discussion

The regional distribution of MAO–A and MAO–B in one frontal plane of the brainstem, revealed by enzyme radioautography and in situ hybridisation histochemistry, is illustrated in Fig. 1. At the protein level, MAO–A can be visualised in cells of the locus coeruleus and neighbouring tissues by the high density of binding sites for $^3$H–Ro 41–1049 (Fig. 1a). MAO–A mRNA has a correlative but not identical distribution (Fig. 1b). In contrast, MAO–B protein (revealed by $^3$H–Ro 19–6327 binding) is located in small diameter cells in the raphé nuclei but not in the locus coeruleus cells (Fig. 1c). MAO–B mRNA, again, has a correlative but not identical distribution to its translation product, i.e. MAO–B protein (Fig. 1d).

In the substantia nigra, the distributions of MAO–A and MAO–B differ markedly (Fig. 2a, b). Whereas the latter is more abundant and distributed in the reticular zone, the former is restricted to the compact zone, where melanin-containing dopaminergic neurons are to be found. No hybridisation signal for either MAO–A or MAO–B mRNAs could be detected in the substantia nigra or in glia cells of the brainstem regions investigated.

We conclude from this study that the cellular compartmentation of the two isoenzymes in human brainstem differs markedly and does not reflect the distribution of the presumed natural substrates (e.g. absence of MAO–A in serotoninergic neurons). Current investigations aim to provide evidence of receptor down-regulation following selective inhibition of MAO–A or MAO–B (leading to increased concentrations of synaptic neurotransmitter amines). This would indicate the substrate selectivity of the isoenzymes in situ and thereby fill one gap in our knowledge of the physiological role of MAO.

## References

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