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Heterogeneous distribution of cytochrome oxidase activity in the human substantia nigra pars compacta

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SUMMARY

The activity of the enzyme cytochrome oxidase has been analyzed in the human substantia nigra at light microscope level. The distribution of this activity is relatively uniform in the substantia nigra, except in the caudomedial region of the pars compacta, where a reduction was observed. This nigral zone matched a region characterized by its relatively high content of acetylcholinesterase activity. Our results are analyzed with reference to data in the literature, showing the existence of a neuroanatomical and functional subdivision within the pars compacta. Our findings add new proof of the known neurochemical heterogeneity of the nigral complex.

Key Words: Substantia nigra pars compacta – Cytochrome oxidase – Acetylcholinesterase – Cytoarchitecture – Human – Basal ganglia

INTRODUCTION

From a cytoarchitectonic point of view, the adult human substantia nigra has been divided into a pars compacta (SNc) and a narrow pars reticulata (SNr), with the fibrillar stratum intermedium between them (Foix and Nicolesco, 1925). The SNc contains dopaminergic neurons projecting to the dorsal striatum and is implicated in the pathophysiology of Parkinson's disease.

The SNc has been subdivided by Hassler (1937) who delimited 21 cytoarchitectonic cell groups. The pars compacta has in turn been subdivided into alpha, beta and gamma portions (Olszewski and Baxter, 1954), the first being the most ventral, and the last, which appears as the red nucleus becomes more obvious, the most dorsal. More recently, and always in humans, the neuromelanin content of most neurons of the SNc and/or the immunoreactivity against the enzyme responsible for catecholaminergic synthesis tyrosine hydroxylase, which characterizes the dopaminergic neurons constituting the A9 group described initially by Dahlström and Fuxe (1964) in the rat (and equivalent to the SNc), have been used equally to establish a series of additional divisions in the SNc (Bogerts, 1981; Saper and Petito, 1982; Braak and Braak, 1986; Pearson et al., 1990; Weiss-Wunder and Chesselet, 1990; Paxinos and Huang, 1995). At the same time, using immunohistochemical techniques, the unequal distribution of a series of neuropeptides and related substances has been detected in the human mesencephalon, in both normal and pathologic brains (Gaspar et al., 1983; Mai et al., 1986; Palacios et al., 1989; Pearson et al., 1990; Yamada et al., 1991; Hirsch et al., 1992; McRitchie and Halliday, 1995).

Cytochrome c oxidase (COase) is a mitochondrial enzyme that takes part in oxidative phosphorylation, whose result is the production of ATP. A rise in neuronal activity in the encephalon causes an increase in cell respiration, which in turn raises COase activity. Thus, different levels of COase activity could be used to detect

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the relative functional activity of neuronal groups in different sites of the central nervous system. A non-uniform distribution of COase activity has been observed in the substantia nigra of the rat (Palacios et al., 1989) and macaque (Ma, 1989).

Material and Methods

We proposed to investigate the COase activity in the substantia nigra of the human adult, using the encephalons of three individuals who died without known neurological deficits (patient 1, a 65-year old male; patient 2, a 70-year old female; patient 3, a 67-year old male). Observations were made on immersion-fixed postmortem human mesencephalic tissue. The time elapsed between death and tissue fixation was 12-15 hours. Brainstems were cut into 2 blocks and exposed successively to (i) fixative solution (4% (wt/vol) paraformaldehyde in 0.1M sodium phosphate buffer, pH 7.4) for 24 h, and to (ii) a series of wash solutions (0.1M sodium phosphate buffer containing, consecutively, 0, 5, 10, 15, and then 20% (wt/vol) sucrose, each for 12 h.). Frozen sections were cut at 40 µm on a sliding microtome and collected successively in a compartmentalized box, to obtain 12 equivalent series of sections.

Successive compartments were processed to detect Nissl substance, COase activity, and acetylcholinesterase activity (Geneser-Jensen and Blackstad, 1971) or were treated with the Heidenhain-Woelcke myelin stain (Gruber, 1981). The incubation solution for the COase procedure contained 50 mg diaminobenzidine, 15 mg cytochrome c (Sigma, Type III) and 4 g sucrose per 90 ml of 0.1M sodium phosphate buffer solution. The sections were reacted on a rotator in a 37°C oven in the dark.

Results

At rostral levels of the substantia nigra, the distribution of COase activity is quite uniform, interrupted only by zones with abundant fibers of the stratum intermedium, in which the enzymatic activity is reduced, above all in the lateral portion of the substantia nigra. In the caudal portion, in contrast, a zone less stained for COase activity can be clearly distinguished (Fig. 1A). This zone is bordered medially by the roots of the III cranial pair, and extends laterally as a sort of wing, bordered more dorsally by a region (of the pigmented parabrachial nucleus) that also shows relatively weak COase activity. In addition, the region of the SNc considered shows a relatively high content in acetylcholinesterase activity (Fig. 1C). On comparing the distribution of COase activity with that of Nissl substance (Fig. 1B), it can be seen that this region is one where the neurons are not densely grouped, although many of them show an intense neuromelanin pigmentation. This zone is mostly of the medial portion of the SNc (presumably the pars beta of Olszewski and Baxter, 1954), although it is bordered at its medial end by the most lateral and densely grouped pigmented neurons of the paranigral nucleus. The neurons are medium-sized and fusiform, and from their eccentric distribution of Nissl substance and neuromelanin, which sometimes extends into the first portion of the dendrites, seem to correspond to the type I described by Braak and Braak (1986). Ventrally to this neuronal group can be seen an ovoid region densely stained by Nissl substance, with abundant small neurons generally lacking neuromelanin. This region is crossed by some fibrillar fascicles (Fig. 1D), and also shows a relatively low acetylcholinesterase activity (Fig. 1C).

Discussion

Our findings on the distribution of COase activity in the human substantia nigra do not coincide with those of other works carried out in other animal species. Thus, in the rat, the oxidative metabolic activity of COase is reduced or absent in the SNc, while in the SNr, where the enzymatic activity is more obvious, it is found distributed non-uniformly (Weiis-Wunder and Chesselet, 1990). In the macaque, the distribution of COase activity, together with the fibrillar staining of Gallyas, enables the SNr and the pars lateralis of the substantia nigra to be identified, while no differential histochemical subdivision is detected within the SNc (Ma, 1989).

Different works, using different techniques, have shown that the substantia nigra pars compacta, in both different animal species and in humans, is not a uniform structure. In the cat, a caudomedial portion of the SNc has been described, characterized by its relatively low acetylcholinesterase content, and further characterized by its preferential mesostriatal projections to the striosomal compartment of the dorsal striatum (Jiménez-Castellanos and Graybiel, 1987), which is also the preferential receiving zone of striatonigral projections emanating from striosomal neurons (Jiménez-Castellanos and Graybiel, 1989a). In the SNc of the squirrel monkey interdigitated zones of neurons have also been observed to project to either the caudate nucleus or to the putamen (Parent et al., 1983). A non-uniform distribution of acetylcholinesterase activity has also been observed in the SNc of this

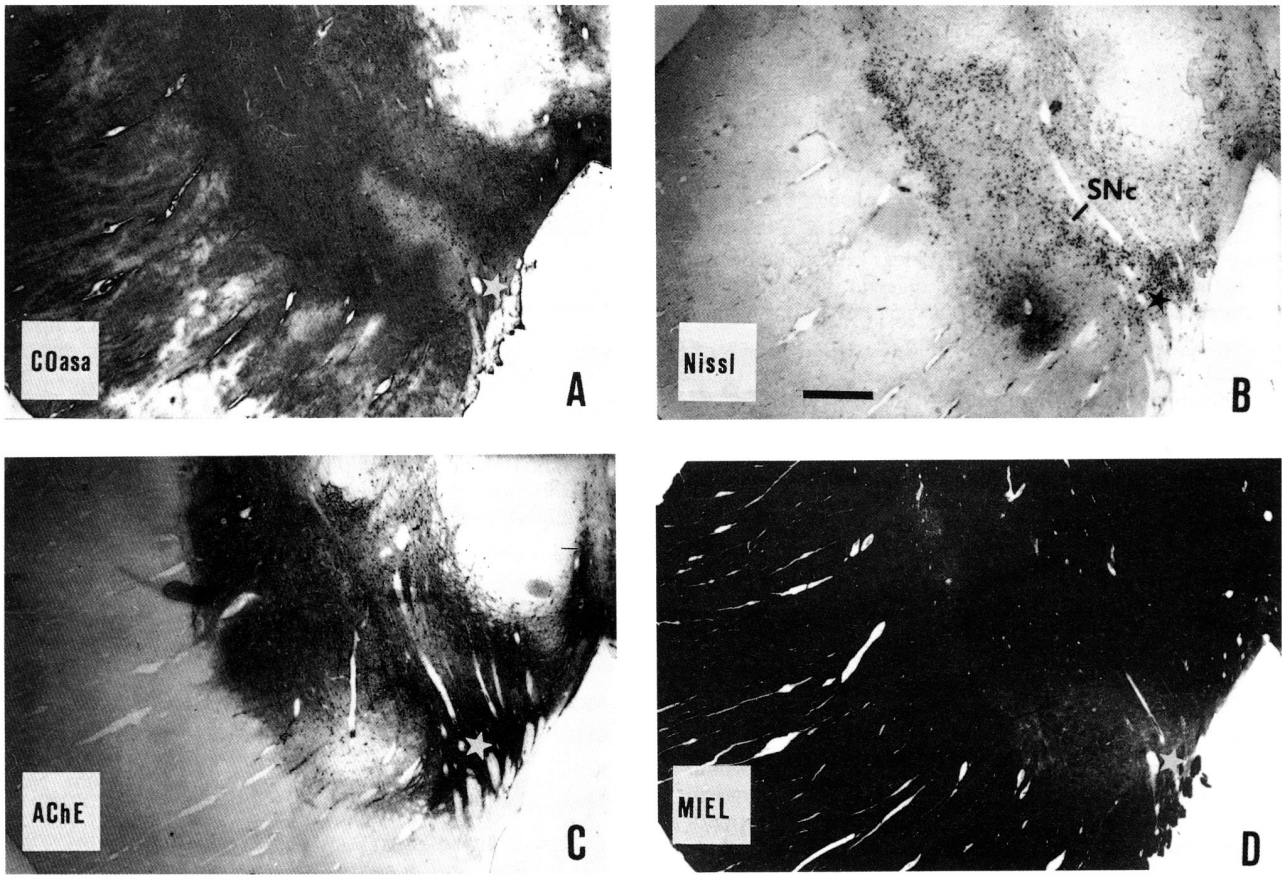

Fig. 1.- Low-power bright-field photomicrographs of sets of serially adjacent transversal sections of the human substantia nigra. **A**: COase stain. **B**: Nissl stain. **C**: Acetylcholinesterase stain. **D**: Myelin stain. Stars indicate the position of the same blood vessel. Scale bar = 2 mm.

species, and some of these neurochemical compartments are correlated with neuronal groups characterized by their preferential projections to the striosomes or to the extrastriosomal matrix (Feigenbaum and Graybiel, 1989; Jiménez-Castellanos and Graybiel, 1989b). In the rat, high levels of COase activity have been shown to characterize the extrastriosomal matrix in comparison with the relative low reaction product observed in the striosomal compartment (Augood et al., 1989). In addition, electrophysiological studies performed in the rat have reported different basal firing rates in dopaminergic neurons projecting to the striatum or to the cortex (Deniau et al., 1978).

The region of the human SNc described here, and characterized by its relatively low COase activity, does not correspond to a differential localization of immunoreactivity against tyrosine hydroxylase (Gaspar et al., 1983; Pearson et al., 1983; Pearson et al., 1990) nor against different neuropeptides, such as substance P (Pearson et al., 1983; Mai et al., 1986), met-enkephalin (Gaspar et al., 1983), calbindin D_{28k} (Yamada et al., 1991; Hirsch et al., 1992) or cholecystokinin (Palacios et al., 1989), all of which show, however, a heterogeneous distribution within the nigral complex. However, and in accord once with our findings, it does seem to correspond to a zone that is detectable in adjacent sections processed for acetylcholinesterase activity (Carmona et al., 1998), where enzymatic activity is relatively strong at both neuronal and neuropil level.

Our work contributes new evidence of compartmentalization in the nigral complex of humans. We do not know the functional significance of this finding, or its potential relationship with the mesostriatal projections, and it would be interesting to test other techniques and, above all, see the distribution of histochemical activity in parkinsonian encephalons, since it is known that the different neuronal groups of the nigral complex are not equally vulnerable (Deutch et al., 1986; German et al., 1988; Yamada et al., 1991; Hirsch et al., 1992). Such data, together with experimental models in animals, could throw light on the functional role of the differential compartmentalization of the neuronal groups of the mesencephalic nigral complex and of the mesostriatal projections.

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