Histol Histopath (1993) 8: 501-504

Histology and Histopathology

Ultrastructural relations between nigrostriatal dopaminergic neurons and cholinergic nerve endings in the human brain

P. Anglade[1], S. Tsuji[2], E.C. Hirsch[1], F. Javoy-Agid[1] and Y. Agid[1]

[1]INSERM U289, Hospital of the Salpêtrière, and [2]Department of Cytology, Institut of Neurosciences, P. et M. Curie University, Paris, France

Summary. The connections between cholinergic nerve endings and nigrostriatal dopaminergic neurons were studied in the substantia nigra pars compacta of the human brain. Immunocytochemistry of tyrosine hydroxylase followed by ionic fixation of acetylcholine-like cation in synaptic vesicles allowed dopaminergic neurons and cholinergic nerve endings to be visualized on the same ultrathin section. Numerous contacts, some of them with synaptic structures, were observed between nerve endings, with or without precipitates of acetylcholine-like cation and dendrites or cell bodies of tyrosine hydroxylase-immunoreactive neurons. These results, which agree with previous studies performed in the rat, suggest that cholinergic nerve endings control the activity of the nigrostriatal neurons at the level of the dendrites and cell bodies in the substantia nigra.

Key words: Human, Substantia nigra, Tyrosine hydroxylase, Acetylcholine, Ultrastructure

Introduction

The dopaminergic (DA) neurons of the substantia nigra are one of the major components of the modulatory input acting on the circuit of the basal ganglia (Graybiel, 1990). Their degeneration, resulting in a dramatic decrease in dopamine release in the projection area, namely the striatum, is the most striking phenomenon to occur in Parkinson's disease (Ehringer and Hornykiewicz, 1960). Afferent nerve endings to these DA neurons might be involved in both the alteration and compensation phenomena that take place during the course of the disease. In particular, an important cholinergic pathway originating in the pedunculopontine and laterodorsal tegmental nuclei and terminating on the DA neurons of the substantia nigra pars compacta has been described in mammals (Woolf and Butcher, 1986; Beninato and Spencer, 1987; Clarke et al., 1987; Gould et al., 1989). In order to study the cholinergic input received by the nigrostriatal DA neurons of the human brain we examined the ultrastructural relations between cholinergic nerve endings and nigrostriatal DA neurons in the substantia nigra, where the dendrites and cell bodies of these neurons are to be found. Both DA neurons and cholinergic nerve endings were visualized on the same section, using tyrosine hydroxylase (TH) detection by immunocytochemistry followed by ionic fixation of acetylcholine-like cation in the synaptic vesicles (Tsuji et al., 1983; Ohoka and Tsuji, 1988; Tsuji and Anglade, 1989).

Offprint requests to: Dr. Philippe Anglade, INSERM U289, Hôpital de la Salpêtrière, 47 Bd de l'Hôpital, 75013 Paris, France

Materials and methods

The brain of three patients (one 88-year-old woman, and two men aged 82 and 85), who died without any characterized neurological or psychiatric disease, were studied. The post-mortem delays before tissue fixation were respectively 6 h, 11 h and 10 h.

After autopsy, the substantia nigra was removed from the brain, cut into slabs 5-6 mm thick and fixed in a mixture of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer for 72 h at 4 °C and kept in 0.2M phosphate buffer including 0.1% sodium azide at 4 °C. Sections 40-50 µm thick, cut on a vibratome, were soaked in normal goat serum (5% in 0.01M phosphate buffer saline (PBS)) for 1h and transferred successively into a solution of a mouse monoclonal antibody raised against TH (1/500 in PBS), (Sorin Biomedica, Brussels) for one night at 4 °C, a goat anti-mouse IgG antiserum (1/100 in PBS, Nordic) and a mouse peroxidase-antiperoxidase complex (1/100 in PBS, Nordic). Tissues were rinsed three times in PBS after incubation in each antibody solution. The sections were then immersed in diaminobenzidine tetrahydrochloride (DAB) dissolved in PBS (4 mg/10 ml) to which hydrogen peroxide was added to a final

concentration of 0.03% after 5 min. The reaction was stopped in PBS and small areas of the substantia nigra pars compacta were selected and removed under binocular lens. Tissues were then processed for ionic fixation of acetylcholine-like cation by immersion in a 5% aqueous solution of silicotungstic acid for 30 min followed by immersion in a solution containing 2.5% silicontungstic acid and 2% osmium tetroxide for 30 min. After three rinses in distilled water, the tissues were dehydrated in graded series of ethanol solutions, impregnated and embedded in araldite. Ultrathin sections were cut and observed under a JEOL 1200 EX electron microscope at 70 KV without counterstaining.

Results

TH-immunoreactive nerve elements were detected by the presence of dark precipitates of DAB. Acetylcholine-like cations were visualized as punctiform precipitates in synaptic vesicles but not in axons or neuronal cell bodies. Omission of anti-TH antibody resulted in an absence of immunostaining. When silicotungstic acid was omitted no punctiform precipitate was detected in synaptic vesicles of nerve terminals. The preservation of ultrastructure, although mediocre due to post-mortem fixation, was sufficient to characterize the nerve elements. Tissues fixed 6 h after death appeared to be better preserved than those fixed 10 h or 11 h after death. Moreover, although the intensity of TH immunoreaction was the same in the three cases, the precipitates of acetylcholine-like cation appeared less intense in the tissues fixed 10 h and 11 h after death than in those fixed 6 h after death.

TH immunoreactivity was found in cell bodies and dendritic elements (Fig. 1A-1D). The intensity of immunoreaction was generally higher in dendrites than in cell bodies. Most of the immunostained cell bodies contained neuromelanin granules (Fig. 1A). No TH-immunoreactive nerve endings were found. Numerous TH-immunonegative nerve endings were observed in contact with TH-immunopositive dendrites and, more rarely, with TH-immunopositive cell bodies containing neuromelanin granules (Fig. 1A-1D). Some nerve endings devoid of TH immunoreactivity displayed punctiform precipitates of acetylcholine-like cation in small agranular synaptic vesicles, whereas other non TH-immunoreactive nerve terminals were devoid of precipitates (Fig. 1B-1D). Synapses, characterized by synaptic vesicles in the vicinity of the presynaptic membrane and postsynaptic thickening, were frequently observed between nerve endings with or without punctiform precipitates and TH-immunoreactive dendritic processes (Fig. 1B-1D). Almost all the nerve endings containing punctiform precipitates were observed to be in contact with TH-immunoreactive dendrites or cell bodies. Very unfrequently, nerve terminals containing punctiform precipitates were seen in contact with non TH-immunoreactive nerve elements.

Discussion

Ionic fixation by silicotungstic acid permitted acetylcholine-like cation to be precipitated only in synaptic vesicles of nerve terminals, where the highest concentrations of neurotransmitters are encountered (Tsuji et al., 1983; Ohoka and Tsuji, 1988). Silicotungstic acid has been shown to precipitate acetylcholine preferentially to the other neurotransmitters (Ohoka and Tsuji, 1988; Tsuji and Anglade, 1989). Therefore, nerve endings devoid of punctiform precipitates are believed to correspond to the non-cholinergic terminals. However, the absence of precipitates might occasionally be due to a local lack of penetration of silicotungstic acid. A progressive leak or a chemical modification of neurotransmitters occurring in the first hours after death might explain why the intensity of acetylcholine-like cation precipitates was weaker in the case of the longest post-mortem delays before fixation.

Numerous cholinergic nerve endings were observed to be in direct contact with the dendrites and, more rarely, with the cell bodies of the DA neurons. Many of these contacts were characterized by synaptic structures. These results suggest the presence of a substantial direct cholinergic input on the DA neurons in the substantia nigra, as previously described in the rat (Beninato and Spencer, 1988; Henderson and Greenfield, 1987; Gould et al., 1989; Martínez-Murillo et al., 1989; Bolam et al., 1991). Although very occasional cholinergic nerve endings were observed in contact with non TH-immunoreactive structures, this cholinergic input seemed to be concentrated on the DA neurons. These cholinergic nerve endings might modulate the DA metabolism of the nigrostriatal neurons, as previously suggested in the rat (Javoy et al., 1974; Lichtensteiger et al., 1976, 1982; Clarke et al., 1985). The cholinergic afferences of the DA nigrostriatal neurons probably originate in the pedunculopontine and laterodorsal tegmental nuclei, as is the case in the rat (Woolf and Butcher, 1986; Beninato and Spencer, 1987; Clarke et al., 1987; Gould et al., 1989).

In the vicinity of the cholinergic nerve endings, numerous non-cholinergic nerve terminals were also observed in direct contact with dendrites of DA neurons. Synaptic structures were frequently associated with these contacts. This study did not allow the chemical nature of the neurotransmitters released by the non-cholinergic nerve endings to be identified. However, it is probable, as shown in the rat, that most of these endings originate from striatal GABAergic neurons as well as from many other classes of neurons including those projecting from the globus pallidus and the dorsal raphe (Bolam and Smith, 1990; Smith and Bolam, 1991).

In summary, on the basis of the above morphological data, it could be suggested that a substantial direct cholinergic control of DA striatonigral neurons might take place at the level of the dendritic shaft in the substantia nigra. This cholinergic input could modulate

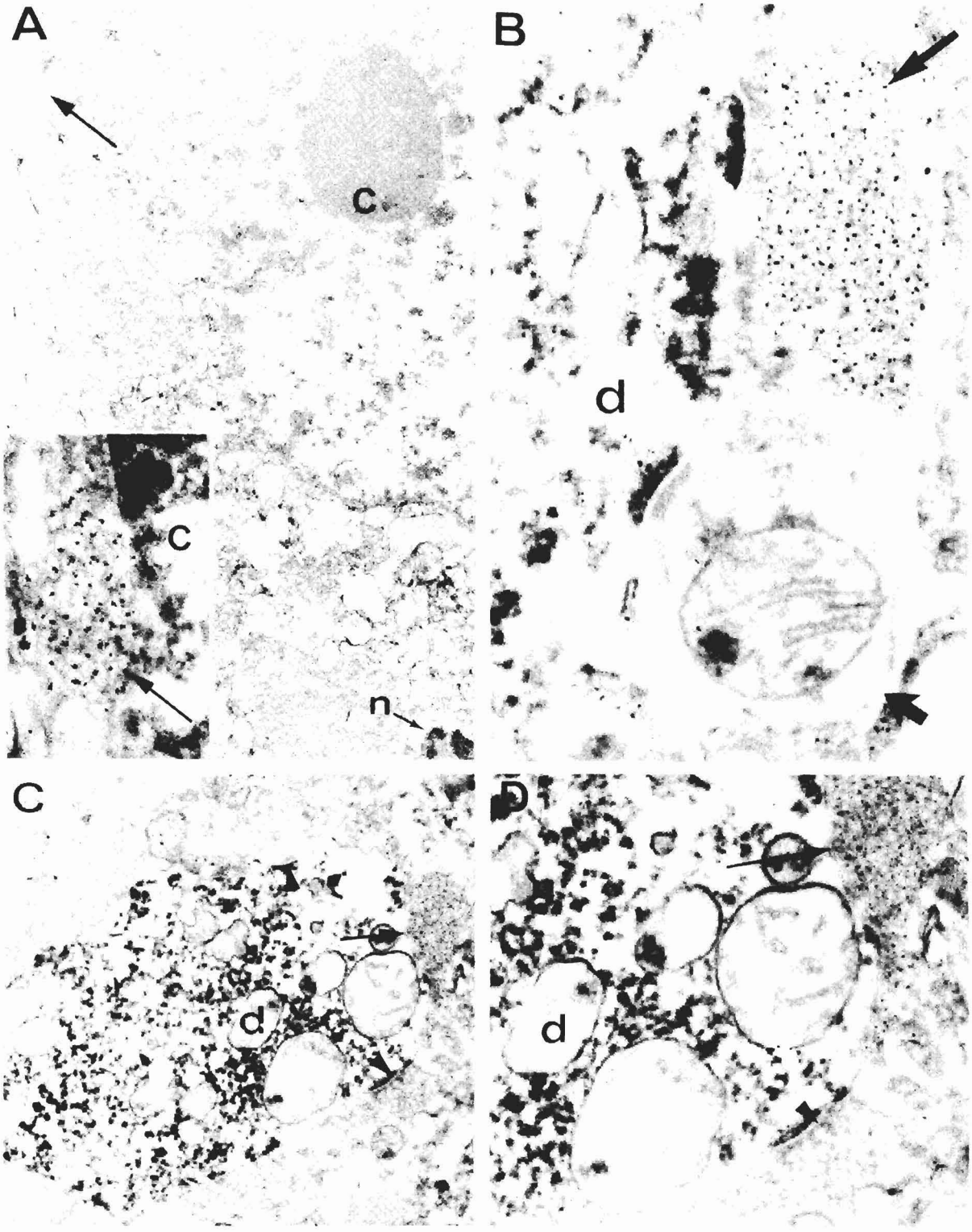

Fig. 1. Localization of TH immunoreactivity and acetylcholine-like cation in the substantia nigra. **A**. A TH-immunoreactive cell body (c) with neuromelanin granules (n). x 11,000. Inserted box: enlargement of a small area indicated in A by an arrow. A nerve ending with precipitates of acetylcholine-like cation in clear vesicles (arrow) is in contact with the TH-immunoreactive cell body (c). x 80,000. **B**. Two nerve endings in synaptic contact with a TH-immunoreactive dendrite-like process (d). One of the nerve endings contains vesicles with precipitates of acetylcholine-like cation (long arrow), the other one does not contain precipitates (short arrow). x 75,000. **C.** Nerve terminals (arrow and arrowheads) in contact with a TH-immunoreactive dendrite-like process (d). x 24,000. **D**. Detail of C. One nerve terminal displays synaptic vesicles with punctiform precipitates (arrow). Another nerve ending in synaptic contact with the TH-immunoreactive dendrite-like process (d) is devoid of precipitates (arrowhead). x 44,000

the dopamine release on target cells in the striatum.

References

Beninato M. and Spencer R.F. (1987). A cholinergic projection to the rat substantia nigra from the pedunculopontine tegmental nucleus. Brain Res. 412, 169-171.

Beninato M. and Spencer R.F. (1988). The cholinergic innervation of the rat substantia nigra: a light and electron microscopic immunohistochemical study. Exp. Brain Res. 72, 178-184.

Bolam J.P., Francis C.M. and Henderson Z. (1991). Cholinergic input to dopaminergic neurons in the substantia nigra: a double immunocytochemical study. Neuroscience 41, 483-494.

Bolam J.P. and Smith Y. (1990). The GABA and substance P input to dopaminergic neurones in the substantia nigra of the rat. Brain Res. 529, 57-78.

Clarke P.B.S., Hommer D.W., Pert A. and Skirboll L.R. (1985). Electrophysiological actions of nicotine on substantia nigra single units. Br. J. Pharmacol. 85, 827-835.

Clarke P.B.S., Hommer D.W., Pert A. and Skirboll L.R. (1987). Innervation of substantia nigra neurons by cholinergic afferents from pedunculopontine nucleus in the rat: neuroanatomical and electrophysiological evidence. Neuroscience 23, 1011-1019.

Ehringer H. and Hornykiewicz O. (1960). Verteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des Extrapyramidalen. System. Wiener. Klin. Wochenschr. 38, 1236-1239.

Gould E., Woolf N.J. and Butcher L.L. (1989). Cholinergic projections to the substantia nigra from the pedunculopontine and laterodorsal tegmental nuclei. Neuroscience 28, 611-623.

Graybiel A.M. (1990). Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci. 13, 244-254.

Henderson Z. and Greenfield S.A. (1987). Does the substantia nigra have a cholinergic innervation? Neurosci. Lett. 73, 109-113.

Javoy F., Agid Y., Bouvet D. and Glowinski J. (1974). Changes in neostriatal DA metabolism after carbachol or atropine microinjections in the substantia nigra. Brain Res. 68, 253-260.

Lichtensteiger W., Felix D., Lienhart R. and Hefti F. (1976). A quantitative correlation between single unit activity and fluorescence intensity of dopamine neurones in zona compacta of substantia nigra, as demonstrated under the influence of nicotine and physostigmine. Brain Res. 117, 963-968.

Lichtensteiger W., Hefti F., Felix D., Huwyler T., Melamed E. and Schlumpf M. (1982). Stimulation of nigrostriatal neurones by nicotine. Neuropharmacology 21, 963-968.

Martínez-Murillo R., Villalba R.M. and Rodrigo J. (1989). Electron microscopic localization of cholinergic terminals in the rat substantia nigra: an immunocytochemical study. Neurosci. Lett. 96, 121-126.

Ohoka T. and Tsuji S. (1988). Specificity of ionic fixation with silicotungstic acid for cytochemical localization and identification of acetylcholine in synaptic vesicles. Biomed. Res. 9, 335-341.

Smith Y. and Bolam J.P. (1991). Convergence of synaptic inputs from the striatum and the globus pallidus onto identified nigrocellular cells in the rat: a double anterograde study. Neuroscience 44, 45-73.

Tsuji S., Alameddine H.S., Nakanishi S. and Ohoka T. (1983). Molybdic and tungstic heteropolyanion for «ionic fixation» of acetylcholine in cholinergic motor nerve terminals. Histochemistry 77, 51-56.

Tsuji S. and Anglade P. (1989). Acetylcholine in neurons and paraneurons: A histochemical study. Arch. Histol. Cytol. 52 (Suppl.), 75-83.

Woolf N.J. and Butcher L.L. (1986). Cholinergic systems in the rat brain: III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia and basal forebrain. Brain Res. Bull. 16, 603-637.

Accepted February 25, 1993