Acta Neuropathol (Berl) (1982) 56:78–80

Acta Neuropathologica

Light- and Electron-microscopic Studies of Intracytoplasmic Acidophilic Granules in the Human Locus Ceruleus and Substantia Nigra

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Summary. Using the brains of 30 patients with mental and neurologic disorders, we studied the intracytoplasmic acidophilic granules in neurons of the substantia nigra and locus ceruleus by light and electron microscopy. The granules were present in all 30 brains, including those with no recognizable pathologic change, there was no correlation between their appearance and the age, sex, disease of, or the medication received by, the patients. In four electron-microscopically examined brains, we noted many small, round electron-dense bodies in the perikarya and neuronal processes of the substantia nigra and locus ceruleus. The bodies were packed tightly within a double membrane; in shape, size, and distribution in the neuronal cytoplasm, they corresponded to acidophilic granules. Some mitochondrial matrices contained one or more similar, but smaller inclusion bodies; larger bodies pushed aside the mitochondrial cristae. We conclude that the acidophilic granules represent highly developed forms of mitochondrial inclusions.

Key words: Intracytoplasmic acidophilic granules – Melanin-bearing neurons – Round electron-dense body – Mitochondrial inclusion

Small acidophilic granules have been described in the cytoplasm of melanin-bearing neurons of the human locus ceruleus and substantia nigra (Marinesco 1902; Foley and Baxter 1958; Lipkin 1959; Lillie and Yamada 1960; Earle 1968; Schochet et al. 1970). They contain protein (Lillie and Yamada 1960); however, their functional significance remains unknown. Schochet et al. (1970) described their ultrastructure as one or more shaves of parallel filaments interconnected by a second set of thinner parallel filaments. It is questionable whether these shaves are comparable to acidophilic granules as they do not have their light-microscopic granular features.

To elucidate the ultrastructure of these granules, we examined the human locus ceruleus and substatia nigra by light and electron microscopy.

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Material and Methods

We used the brains of 30 autopsied patients (age range 7 months to 92 years) with various mental and neurologic disorders. Neuropathologic examinations revealed some of these brains to manifest no pathologic changes. For light microscopy, brains were fixed in 10% formalin, embedded in paraffin, 6 μm coronal sections were cut and stained with HE, PTAH, Azan, or the KB method. If necessary, neuromelanin was bleached with 0.25% potassium permanganate. For electron microscopy, small pieces of the substantia nigra and the locus ceruleus were cut from two fresh and two formalin-fixed brains. Fresh tissue was immersed in 2% glutaraldehyde solution. Specimens from fresh and fixed brains were refixed in 1% OsO_4 solution, and embedded in epoxy resin. Semi-thin sections were HE-stained for light microscopy according to Chang (1972) to confirm the presence of acidophilic granules. Ultrathin sections were contrasted with uranyl acetate and lead citrate.

Results

Acidophilic granules were present in all 30 brains; their appearance did not correlate with the age, sex, disease of, or the medication received by, the patients. Generally, the granules were most numerous in the neuronal perikarya and processes of the locus ceruleus (Fig. 1). Melanin bleaching made them easily recognizable. Granule-bearing neurons were not confined to the substantia nigra or locus ceruleus, but were distributed widely in the brain stem, including the inferior olivary nucleus. PTAH stained the granules; their size varied, the largest were 1.3 μm in diameter.

0001-6322/82/0056/0078/$1.00

Electron microscopy revealed many round bodies of homogeneously high electron density in the neuronal perikarya and dendrites of the substantia nigra and locus ceruleus. Their size also varied in thin sections; it ranged from 0.25 – 1.08 μm in diameter. Usually they were packed tightly within a double membrane (Fig. 2). They were more numerous in the ceruleus neurons. Some mitochondrial matrices contained similar but smaller round bodies, larger bodies occasionally pushed aside the cristae (Fig. 3). In the nigral neurons in one brain we noted filamentous shaves as reported by Schochet et al. (1970); however, they were apparently not associated with round bodies or the mitochondria containing the inclusions.

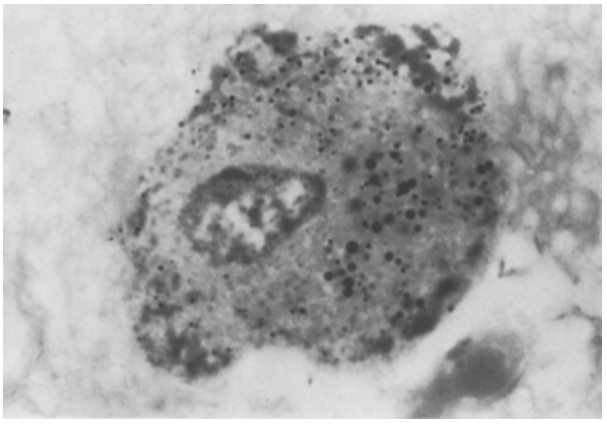

Fig. 1. Acidophilic granules in the perikaryon of a locus ceruleus neuron. Semi-thin epoxy section. HE, ×1,080 (oil immersion)

Discussion

Electron-microscopically, the round bodies appeared to be more numerous in the locus ceruleus than in the substantia nigra, as did the acidophilic granules. Since the round bodies fully corresponded with the acidophilic granules in shape, size, and the distribution pattern in the neuronal perikaryon, they may represent the ultrastructural substratum of the acidophilic granules.

In neurons bearing these round bodies, there also were some mitochondria containing small round inclusions which were as electron-dense as the larger round bodies. Larger inclusions pushed aside the

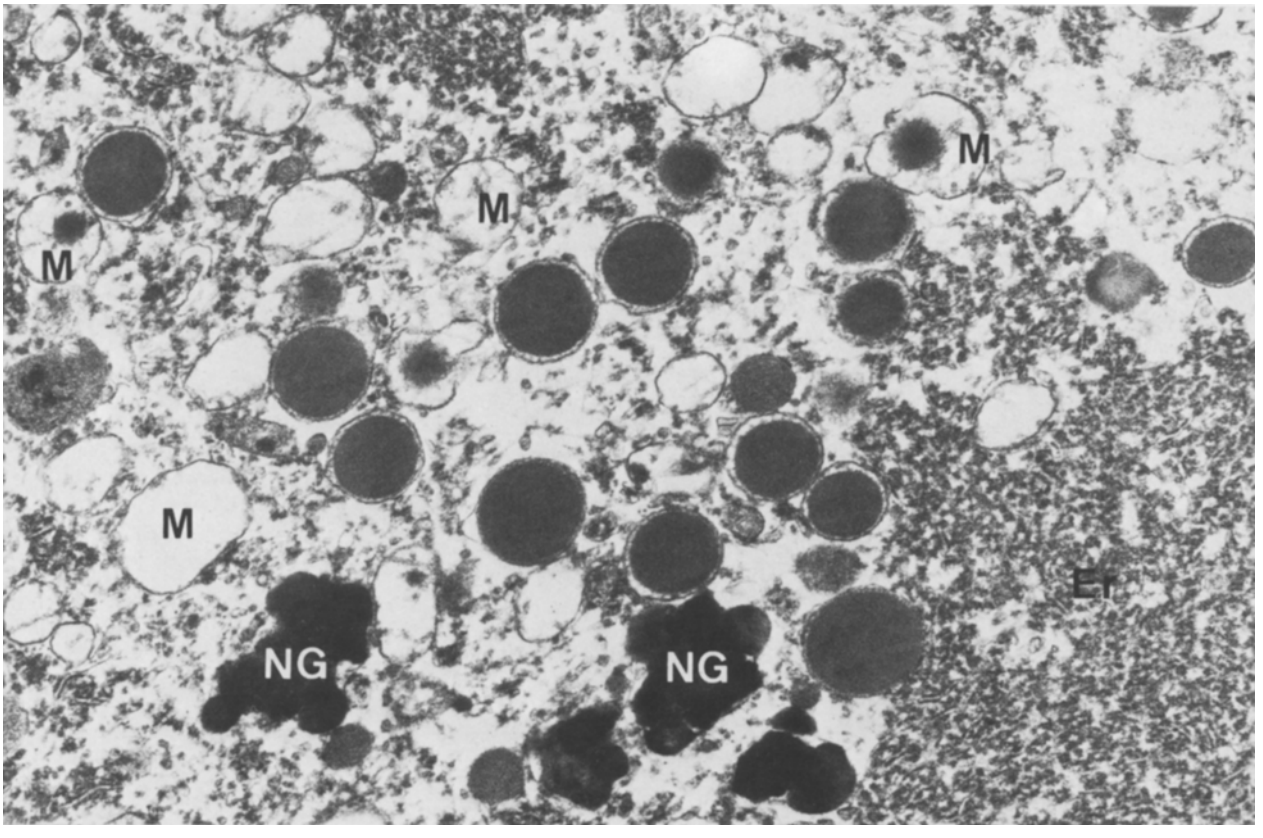

Fig. 2. Round bodies of homogeneously high electron density in the perikaryon of a neuron from the locus coeruleus. They are packed tightly within a double membrane. Some swollen mitochondria contain similar but smaller bodies. ×20,000. *NG* Neuromelanin granules; *M* Mitochondria; *Er* Endoplasmic reticulum

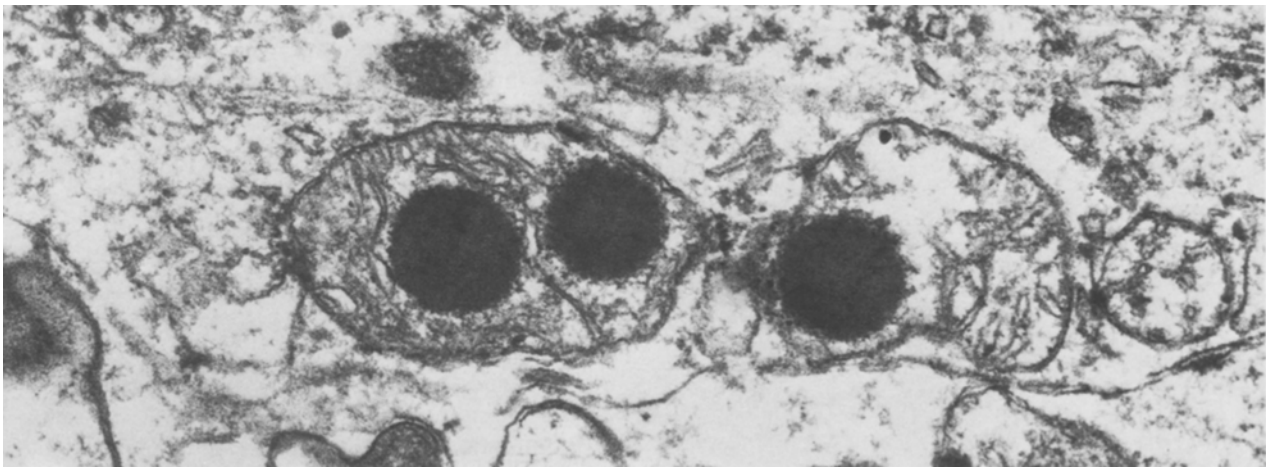

Fig. 3. Round inclusion bodies in mitochondria, pushing aside the mitochondrial cristae. ×65,000

mitochondrial cristae. Therefore, we posit that the larger round bodies are derived by the growth or enlargement of the mitochondrial inclusions.

Similar intramitochondrial inclusions in nerve cells have been reported in the locus ceruleus (Hirosawa 1968; Forno and Norville 1975), and sympathetic ganglia (Pick et al. 1964; Ohsuga et al. 1966; Pick 1967; Forno and Norville 1976), and trigeminal ganglia (Moses et al. 1965; Beaver et al. 1965) of human and several animals.

Forno and Norville (1975) suggested that these intramitochondrial inclusions contained phospholipid. The PTAH-positive staining of the acidophilic granules in our materials indicate they also contain a proteinaceous substance, as had been reported by Lillie and Yamada (1960). Issidorides et al. (1978) documented that protein-rich bodies in the neurons of the substantia nigra and locus ceruleus were noticeably reduced or absent in parkinsonian brains, and that some core of Lewy bodies gave a similar protein reaction.

At present, the functional significance of the acidophilic granules remains unknown.

Acknowledgement. The excellent technical work of Mr. Yoshio Igarashi of Saigata National Sanatorium and Mr. Hisao Imai of Psychiatric Research Institute of Tokyo is greatly appreciated by the authors.

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Received March 25, 1981/Accepted October 20, 1981