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Distribution of Zinc Enriched (ZEN) Neuron Somata in the Medulla Oblongata of Rat

Hyun Wook Cho and Gorm Danscher[1]

Department of Biology, Suncheon National University, Suncheon, Cheonnam 540-742, Korea; [1]The Steno Institute, University of Aarhus, Aarhus, Denmark

Autometallography method with intraperitoneal injection of sodium selenite was employed to investigate the localization of somata of zinc enriched (ZEN) neurons in the medulla oblongata. The distribution patterns of the labeled neurons were variable from rostral to caudal regions. The labeled cells by the method were found in C1 adrenaline cells, gigantocellular reticular nucleus, inferior olive, paragigantocellular nucleus, prepositus hypoglossal nucleus, raphe obscurus nucleus, and reticular nucleus regions.

KEY WORDS: Autometallography, ZEN Neurons, Medulla Oblongata

Identification of endogenous zinc pool in the central nervous system (CNS) has been demonstrated with histochemical technique, autometallography (AMG) (Haug, 1973; Danscher, 1984; Christensen *et al.*, 1992; Mandava *et al.*, 1993; Danscher and Montagnese, 1994) or atomic absorption spectrophotometry (Hu and Fride, 1968; Danscher *et al.*, 1976). The AMG is a process by which nanometer sized gold particles and crystal lattices of sulfide or selenide of silver, mercury, or zinc encyst themselves in silver (Danscher and Montagnese, 1994). The zinc is located in the boutons of the zinc enriched (ZEN) neurons and more precisely, within the vesicles of the boutons (Slomianka *et al.*, 1990; Danscher and Montagnese, 1994). The zinc localization in the CNS can be identified histochemically by the Timm-Danscher (Danscher, 1981), selenium (Danscher, 1982), dithizone (Frederickson and Howell, 1984) and quinoline (Frederickson *et al.*, 1987) methods. The Timm-Danscher and selenium methods are based on autometallographic silver enhancement of zinc bound in situ, either as zinc sulfide or zinc selenide, whereas the dithizone and quinoline methods are based on chelation of zinc (Danscher and Montagnese, 1994).

In rats that are allowed to survive for 24 hr after a intraperitoneal selenite injection, zinc-selenium reaction products in the vesicles of ZEN neurons are retrogradely transported, and the loaded somata of the ZEN neurons can be rendered visible by silver amplification (Danscher, 1984; Slomianka *et al.*, 1990; Christensen *et al.*, 1992).

The functions of the zinc in the CNS are not well understood. But relationship the zinc with spatial behavior (Valdes *et al.*, 1982), NMDA receptor (Christine and Choi, 1990), Alzheimer's disease (Constantinidis, 1990), and neurotransmission (Sloviter, 1985; Khulusi *et al.*, 1986; Frederickson and Danscher, 1990; Vener and Loeb, 1992) were reported.

Previous works to describe the ZEN neurons in the telencephalon have relied on conventional lesion-degeneration methods (Howell *et al.*, 1991; Mandava *et al.*, 1993). Slomianka *et al.* (1990) reported the labeling and localization of the neurons in the telencephalon by intraperitoneal injection of sodium selenite. The primary aim of

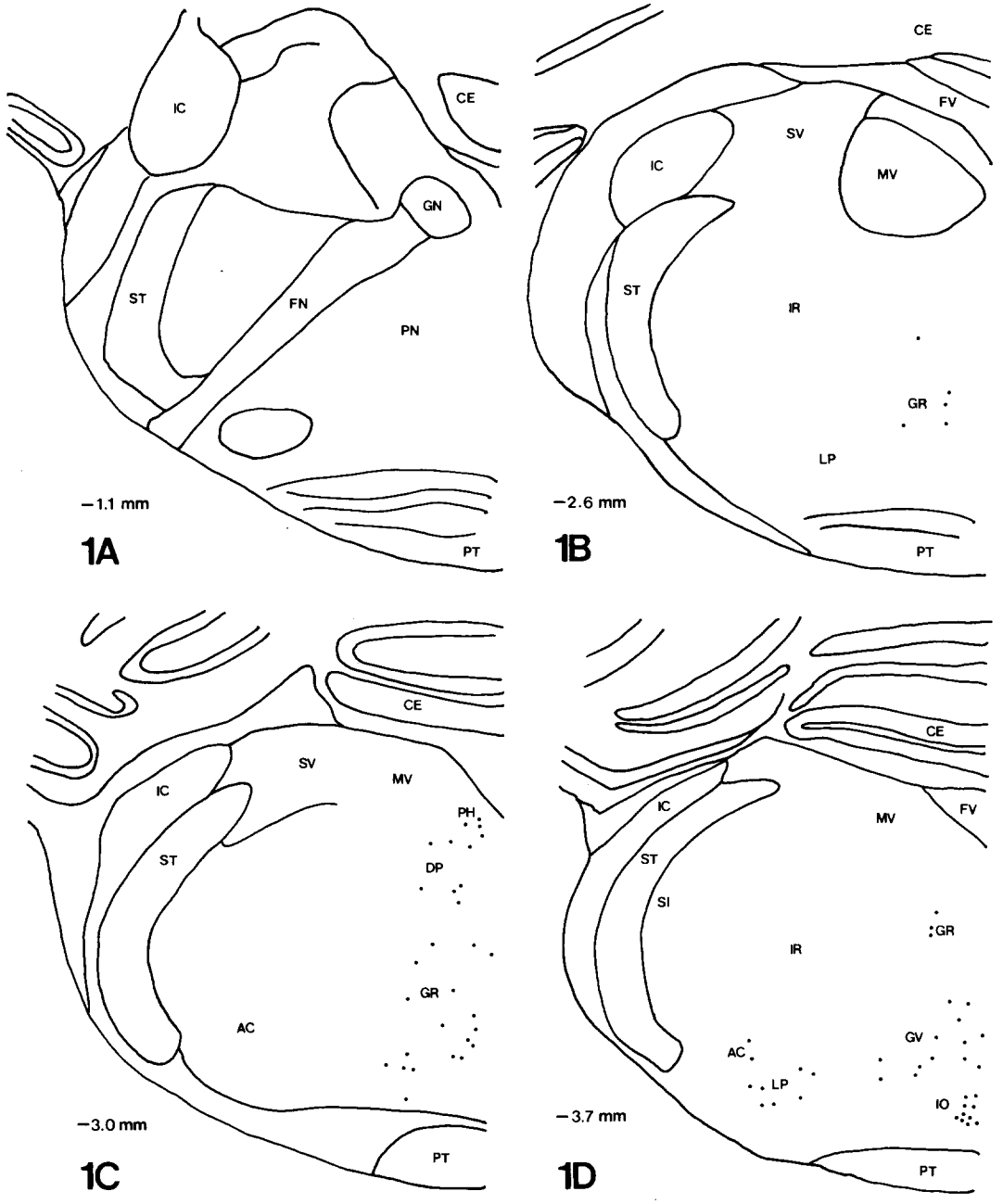
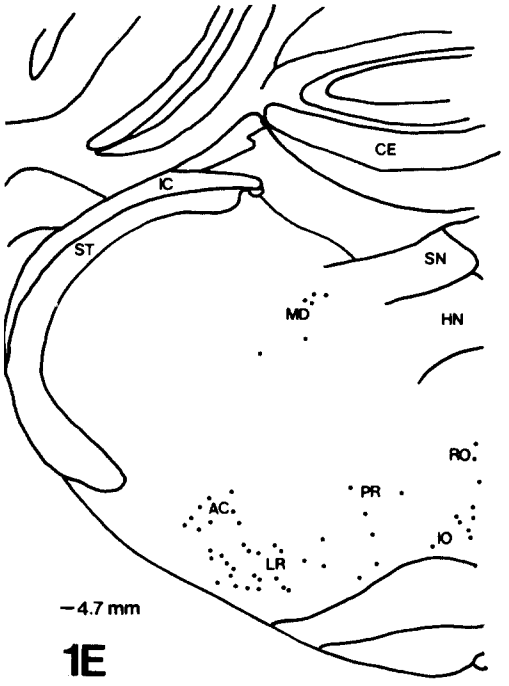
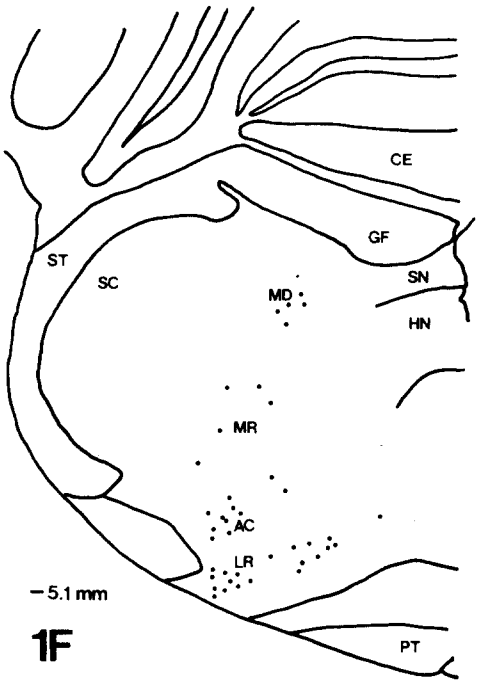
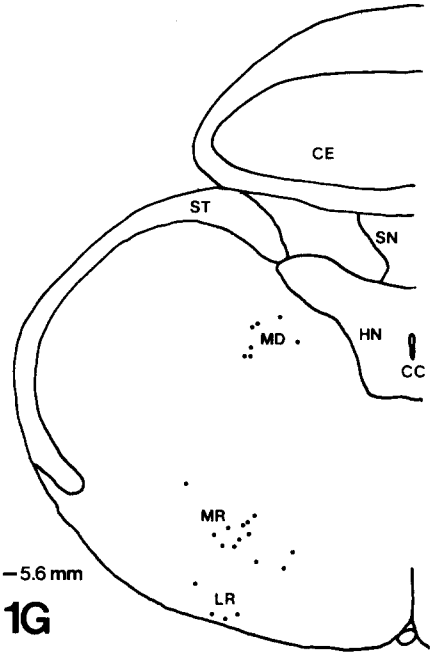
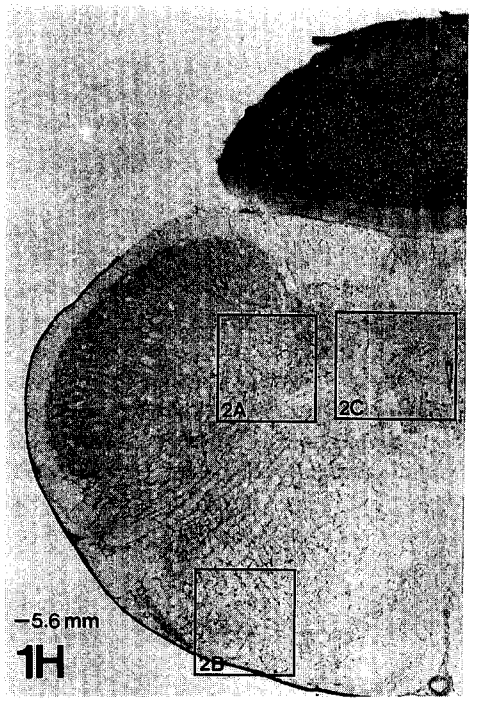


Fig. 1. Schematic drawings and a photomicrograph of coronal sections of the medulla oblongata. A-G, Numbers indicate the estimated distance in mm from interaural line of rat. The minus (–) means posterior to the line. Dots on the drawings of the sections indicate the distribution of labeled ZEN neuron somata. H, Photomicrograph corresponds to a drawing of section G. The framed areas are enlarged in Fig. 2. Toluidine blue counterstain. × 20.
AC, C1 adrenaline cells; CC, Central canal; CE, Cerebellum; DP, Dorsal paragigantocellular nucleus; FN, Facial nerve; FV, Fourth ventricle; GF, Gracil fasciculus; GN, Genu facial nerve; GR, Gigantocellular reticular nucleus; GV, Gigantocellular reticular nucleus, ventral; HN, Hypoglossal nucleus; IC, Inferior cerebellar peduncle; IO, Inferior olive;









IR, Intermediate reticular nucleus; LP, Lateral paragigantocellular nucleus; LR, Lateral reticular nucleus; MD, Medullary reticular field, dorsal; MR, Medullary reticular nucleus, ventral; MV, Medial vestibular nucleus; PH, Prepositus hypoglossal nucleus; PN, Pontine reticular nucleus; PR, Paramedian reticular nucleus; PT, Pyramidal tract; RO, Raphe obscurus nucleus; SC, Spinal trigerminal nucleus, caudal; SI, Spinal trigerminal nucleus, interpolar; SN, Solitary tract nucleus; ST, Spinal trigerminal tract; SV, Spinal vestibular nucleus.

this study is to clarify the distribution of ZEN neuron somata in the medulla oblongata of rat using intraperitoneal injection of sodium selenite followed by silver amplification (Danscher, 1982).

Materials and Methods

Ten adult Kyoto rats with average 300 g were used in this experiment. Ten mg sodium selenite (Na_2SeO_3) were dissolved in 1 ml deionized water, and the solution was used for the intraperitoneal injection. Rats were intraperitoneally injected with 8 mg/kg sodium selenite and allowed to survive for 24 hours. After the survival times, the rats were deeply anesthetized with sodium pentobarbital and then sacrificed by decapitation.

The brains were quickly removed from the skull and frozen with carbon dioxide (Slomianka *et al.*, 1990). Transverse cryostat sections of 30 μm thickness were cut, thawed onto Farmer-cleaned glass slides and air-dried for 15 min to 2 hours. The sections were fixed in 70% ethanol for 30 min, rehydrated and coated with 0.5% gelatin before the autometallographic development. For the autometallographic development, the sections were immersed in a physical developer composed of 60 ml protecting colloid solution, 10 ml citrate buffer and 15 ml reducing agent. Immediately before development, 15 ml silver ion supply was added to the the developer (Danscher, 1982; Danscher and Montagnese, 1994).

Development proceeded in a dark box for 1 hour at 26°C. After development, the sections were rinsed in running tap water, in deionized water, exposed to a 2% Farmer solution for 10 sec, rinsed in deionized water, counterstained with 1% toluidine blue, rinsed in deionized water, dehydrated and mounted in Dammar resin.

Results

The regions of medulla oblongata were identified according to Paxinos and Watson's guide (1986). The loaded perikarya of the neurons were shown as black dots on the left halves of the sections (Fig. 1).

All of the labeled ZEN neurons were composed of Group I neurons classified by Slomianka *et al.* (1990). The neurons were loaded with zinc-selenium granules that were distributed through the cytoplasm and processes of the cells (Fig. 2A and B). Detection of the labeling in the processes was little compared to the heavy labeling in the perikarya of the neurons. The density of zinc-selenium granules was decreased abruptly between perikarya and proximal processes.

Loaded somata of the ZEN neurons were not located in posterior 1.1 mm section (Fig. 1A) from interaural line (Paxinos and Watson, 1986) or in some regions (Figs. 2C, D). The loaded somata began to appear in Fig. 1B. The labeled somata of the neurons were observed in C1 adrenaline cells, dorsal paragigantocellular nucleus, gigantocellular reticular nucleus, gigantocellular reticular nucleus (ventral), inferior olive, lateral paragigantocellular nucleus, lateral reticular nucleus, medullary reticular field (dorsal), medullary reticular nucleus (ventral), prepositus hypoglossal nucleus, paramedian reticular nucleus, and raphe obscurus nucleus (Fig. 1B-G). The loaded somata were located in the gigantocellular reticular nucleus of rostral section (Fig. 1B), and the lateral reticular nucleus, medullary reticular field (dorsal), and medullary reticular nucleus (ventral) of caudal section (Fig. 1G).

Discussion

For the histochemical localization of zinc in ZEN neurons of CNS, four methods, Timm-Danscher (Danscher, 1981), selenium (Danscher, 1982), dithizone (Frederickson and Howell, 1984) and quinoline (Frederickson *et al.*, 1987) were used. In rats subjected to intraperitoneal injection of sodium selenite and with a survival time of 24-48 hr, somata of ZEN neurons in CNS can be labeled by the autometallographic development (Danscher, 1984; Slomianka *et al.*, 1990). In neurons that do not have zinc in their boutons, zinc-selenium reaction products do not form. Therefore only ZEN neurons are retrogradely labaled by the present method.

In the present results, the ZEN neurons in the

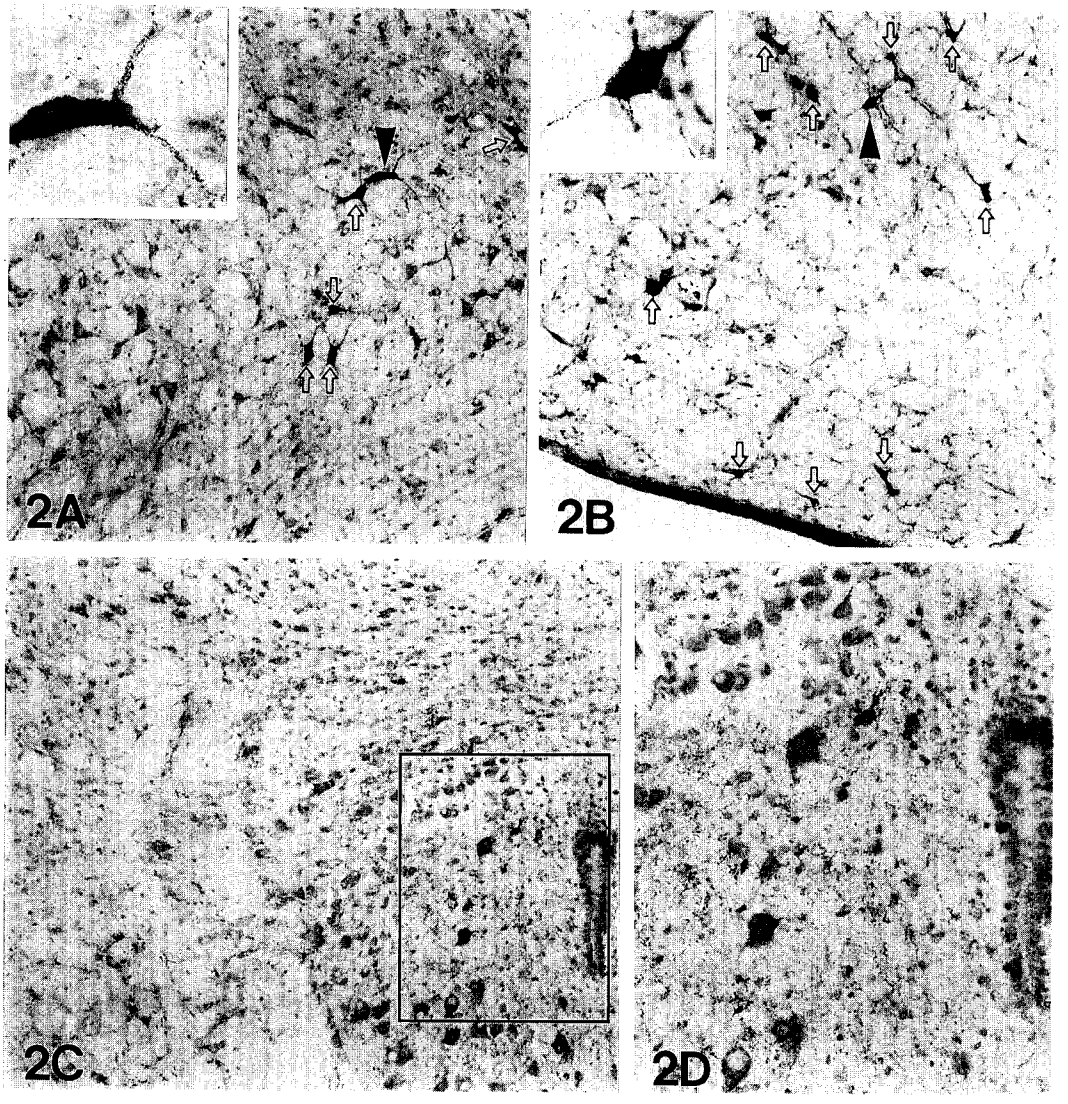


Fig. 2. A, Labeled ZEN neurons somata (arrows) in the MD region. The somata (arrowhead) is shown at higher magnification in inset. The zinc-selenium accumulations by retrograde transport are densely appeared in the cytoplasm. The density of the granules are decreased abruptly between somata and proximal processes. × 100 and × 400. B, Distribution of the somata (arrows) in the LR and MR regions. The labeled somata (arrowhead) in the MR region is shown in inset. × 100 and × 400. C, The loaded neurons were not appeared in the HN and around the CC regions. The zinc-selenium reaction products were not precipitated in cells of this regions. × 100. D, Enlarged part of figure C. × 200.

medulla oblongata appeared to be Group I neurons classified by Slomianka *et al.* (1990). According to previous work, Group II neurons contain some coarse precipitates like those found in Group I neurons, but they are marked by the presence of numerous fine precipitate particles which give rise to a dusty appearance of the stain.

To determine the pathway of ZEN neurons, intracerebral injection and iontophoresis was conducted (Howell *et al.*, 1991; Christensen *et al.*, 1992). The use of conventional lesion-degeneration methods, combined with zinc

histochemistry, has allowed localization of the origins and trajectories of the ZEN neuron pathways (Frederickson and Danscher, 1988; Howell and Frederickson, 1989; Christensen *et al.*, 1992). The lesion-degeneration methods coupled with intracerebral injection of selenium compounds have advantages for identification of the ZEN neurons afferent pathways to a specific region. However the lesion methods are less suitable for demonstrating the localization of all ZEN neurons in the CNS.

The aim of this work was to map the distribution of the somata of the ZEN neurons in the medulla oblongata by selenium method. The distribution patterns of the labeled perikarya were markedly variable from region to region within the medulla oblongata. In the present selenium method with intraperitoneal injection, origin of the neurons was distributed in the dorsal paragigantocellular nucleus, gigantocellular reticular nucleus and the prepositus hypoglossal nucleus of the rostral, and lateral reticular nucleus, medullary reticular field (dorsal) and medullary reticular nucleus (ventral) of caudal regions by coronal sections.

The ZEN neurons originated in the medulla oblongata were labeled by the selenium method. The terminal or pathway of the neurons in this area may be identified by the lesion-degeneration method combined with zinc histochemistry. The present results, showing the distribution of ZEN neurons somata, may facilitate the histochemical approach to an understanding of zinc functions.

Acknowledgements

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쥐의 연수에서 아연이 풍부한 뉴런(ZEN neuron) 세포체의 분포

조현욱 · Gorm Danscher[1](순천대학교 자연과학대학 생물학과, [1]Steno Institute University of Aarhus, Denmark)

쥐에 sodium selenite 를 피하주사하여 연수에 분포하는 아연이 풍부한 뉴런(ZEN neuron) 의 세포체 위치를 autometallography 방법으로 조사하였다. 표지된 세포체의 분포양상은 앞쪽 부위에서 뒤쪽 부위까지 다양하였다. 표지된 세포체는 C1 adrenaline cells, gigantocellular reticular nucleus, inferior olive, paragigantocellular nucleus, prepositus hypoglossal nucleus, raphe obscurus nucleus, 및 reticular nucleus 부위에 나타났다.