The Occurrence of Rat Spinal Cord Neurons with Strongly Negative-Charged Surface Coats

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Summary. Light microscopy of tissue sections stained with cationic iron colloid (pH 1.0–1.5) showed that the adult rat spinal cord contains some neurons which are provided with strongly negative-charged surface coats. These neurons are distributed preferentially in the posterior and intermediomedial columns of the grey matter. The present study thus supplements our previous study of the rat brain (MURAKAMI et al., 1993b), and proves that the neurons with strongly negative-charged surface coats occur widely in the central nervous system of the adult rat.

MATERIALS AND METHODS

Adult Wistar rats weighing 300–400 g were anesthetized with ethyl ether, and their ascending aorta was cannulated after ligation of the lower end of the abdominal aorta. The animal were then perfused with Ringer’s solution and with 2.5% glutaraldehyde or 4.0% paraformaldehyde solution for 12 h or longer. The blocks thus fixed were embedded in paraffin, cut into sections, incubated in our cationic iron colloid with pH values of 1.0–1.5 (MURAKAMI et al., 1986), immersed in a mixture of K₄Fe(CN)₆ and HCl for Prussian blue reaction, counter-stained with nuclear fast red or carbol-thionin mixture (MURAKAMI et al., 1993b), and observed with a light microscope.

RESULTS

Paraformaldehyde fixation as well as glutaraldehyde fixation allowed favorable staining of tissue anionic sites with our cationic iron colloid (Figs. 1–4).

The neurons with strongly negative-charged surface coats were recognizable in every segment of the spinal cord. More strictly, some of the large-sized neurons in the posterior horn and intermediomedial region of the grey matter of the cervical, thoracic and lumbar segments showed a strong Prussian blue reaction after treatment with our cationic iron colloid at pH values of 1.0–1.5 (MURAKAMI et al., 1993b). This intense Prussian blue reaction was always observed as thin coats or fine meshworks closely associated with the external surface of the cell body and on the roots of cell processes (Figs. 2–4). The meshwork structures of the reaction were clearly noted in the tangentially cut cases (Fig. 4).

The counter-staining with carbol-thionin or nuclear fast red showed that the neurons with the strongly negative-charged surface-coats were well reactive to thionin, revealed images of Nissl bodies (Fig. 3 Inset), and contained a well developed nucleus and nucleolus (Figs. 2–4).
Through the spinal cord, few neurons in the anterior horn and intermediolateral region of the grey matter were reactive to our cationic iron colloid at pH values of 1.0-1.5. Few neurons in the lateral cervical nucleus, substantia gelatinosa or other areas of the white matter or related areas were also reactive to our colloid with such low pH levels.

**DISCUSSION**

This paper demonstrates that some of the large-sized neurons, especially their cell bodies and process roots, in the posterior and intermediomedial columns of the rat spinal cord are coated with certain membrane-associated substances which are strongly reactive to our cationic iron colloid at pH values of 1.0-1.5. Similar neurons with the same surface features have been demonstrated in the visual cortex, hippocampal subiculum, pontine nuclei, cerebellar nuclei and certain other nuclei of the rat brain (MURAKAMI et al., 1993b) as well as in the visual cortex of the human brain (MURAKAMI et al., 1993a).

It is believed that only sulfate-groups can ionize at such low pH levels as 1.0-1.5 (SENO et al., 1985; SENO, 1987). It is therefore reasonable that the membrane-associated substances or strongly negative-charged surface-coats should be sulfated proteoglycans (MURAKAMI et al., 1993b). Recent biochemical or electrophoretic analyses have confirmed that the rat brain...
contains some sulfated proteoglycans such as chondroitin and heparan sulfates (Oohira et al., 1986, 1988). More recent immunohistochemical experiments have demonstrated that the external surfaces of the Purkinje and Golgi cells in the rat cerebellum are stained with a monoclonal antibody against a 600- to 1000-kDa chondroitin sulfate proteoglycan (Maeda et al., 1992). However, our previous study has shown that these surfaces of the Purkinje and Golgi cells are not reactive to our cationic iron colloid with pH values of 1.0–2.0, indicating an advantage with our method for detection of sulfate-groups (Murakami et al., 1993b).

As is well known, neurons of the posterior grey matter, especially its lateral area, mainly form the spinocervical pathway, and project to the lateral cervical nucleus which terminates in the medial lemniscus, thalamus and bulbar brainstem nuclei (Dilly et al., 1968; Webster, 1977). Neurons of the medial area of the posterior grey matter form, together with those of the intermediomedial grey matter, the anterolateral pathways which terminate in the thalamus and bulbar brainstem nuclei (Gwyn and Waldron, 1968; Truex et al., 1970). Judging from their positions, the spinal neurons observed here with their strongly negative-charged surface coats may be included among those neurons forming the spinocervical or anterolateral pathways.

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REFERENCES


