Histochemical Studies of Monoamine Oxidase during Axonal Reaction

Monoamine oxidase (MAO) is contained in various portions of the central nervous system and is supposed to be concerned with inactivation of amines, such as catecholamines and serotonin. Detailed histochemical mapping of MAO in the normal brain was reported by SHIMIZU et al.[1] and HASHIMOTO et al.[2], indicating 3 characteristic positive systems: autonomic regions, limbic system and paraventricular structures. The present experiment demonstrated specific alteration of MAO activity in the brain and spinal cord undergoing axonal reaction or following deafferentiation.

Experimental animals were adult male white rats. In the main experimental group the unilateral sciatic nerve was sectioned at the level of the trochanter major, while in the supplemental group the unilateral hypoglossal nerve was cut at a crossing point with the digastric muscle. The operated animals were allowed to survive for varying periods of 5, 7, 14, 20, 30, 40 and 90 days. Fresh frozen sections cut from the excized spinal cord and medulla oblongata were stained for MAO activity according to GLENNER et al.[3].

In the normal spinal cord MAO activity is moderately positive in the lateral horn, the substantia gelatinosa of the posterior horn and gray matter around the central canal, while no activity is seen in the anterior horn. 5 days following section of the sciatic nerve, faint MAO activity appears in the dorsolateral cell group of the anterior horn of the lumbal segment on the operated side. After 7 days, moderate to slight MAO activity is demonstrated in both perikarya and surrounding neuropil of the dorsolateral cell group (Figure 1). From 14 to 30 days following neurotomy, MAO activity of the perikarya and adjacent neuropil of the same cell group seems to be more marked than 7 days (Figures 2 and 3). Definite increase of MAO activity in the substantia gelatonisa of the ipsilateral posterior horn of the lumbal segment is demonstrated by 20 and 30 days (Figure 2), being more prominent after 40 and 90 days.

In the medulla oblongata of normal rats MAO activity is very strong in the dorsal nucleus of the vagus nerve, while no activity is in the hypoglossal nucleus. Following section of the hypoglossal nerve, the hypoglossal nucleus of the operated side exhibited changes of MAO similar to that seen in the spinal cord. 7 days following neurotomy, slight activity of MAO appears in the perikarya and neuropil of the hypoglossal nucleus ipsilateral to the operated side. By 14 and 20 days, moderate activity is demonstrated in some perikarya and adjacent neuropil of the hypoglossal nucleus (Figure 4).

In this experiment we could demonstrate characteristic appearance of MAO in some somatic neurons and surrounding neuropil undergoing axonal reaction. Marked increase of MAO activity was also revealed in the substantia gelatinosa of the posterior horn of the corresponding spinal cord following section of the sciatic nerve. MAO in the normal and developing brain is usually accompanied by the concomitant presence of noradrenaline and serotonin (MAEDA[4]), so that it is necessary to study the amines under such experimental conditions. Although many histochemical studies were already performed on the nerve cells undergoing axonal reaction (cf. FRIEDE[5], ADAMS[6]), none have noted the alteration of MAO. It seems interesting to recognize the correlation of the increase of MAO in the perikaryon with that of acid phosphatase and glucose-6-phosphate dehydrogenase, which showed marked enhancement during axonal reaction.

Though MAO activity in the neuropil is difficult to localize in the histochemical sections, its presence within the glial cells was suggested from the study of tissue culture (O'STEEN and CALLAS[7]) and fractionation (HAMBERGER et al.[8]). In this experiment the glial cells adjacent to

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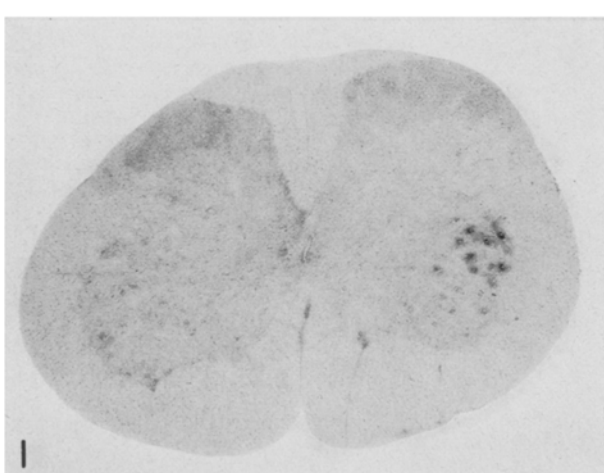

Fig. 1. Cross section of the spinal cord (lumbal segment) stained for MAO. 7 days after section of left sciatic nerve. Nerve cells and surrounding neuropil of the dorsolateral cell group of the ipsilateral anterior horn show moderate MAO activity. ×25.

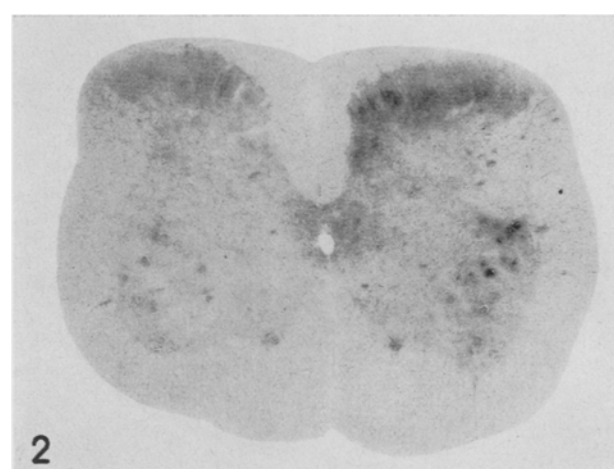

Fig. 2. Cross section of the spinal cord at the same level as Figure 1 stained for MAO. 30 days following neurotomy. Moderate activity is demonstrated not only in the dorsolateral cell group but also in the substantia gelatinosa of the posterior horn on the operated side. ×25.

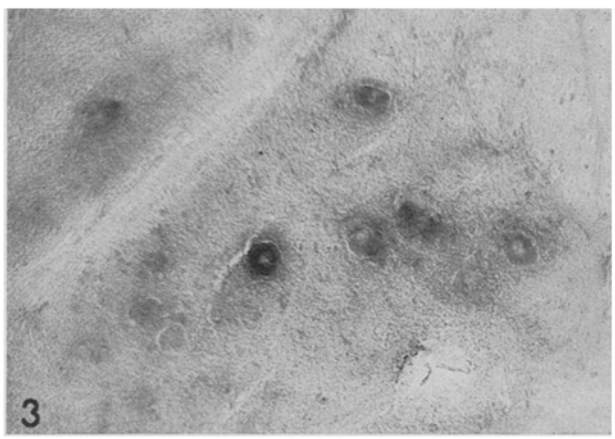

Fig. 3. Enlarged view of Figure 2. Note MAO activity of some perikarya and their surrounding neuropil in the dorsolateral cell group of the anterior horn. ×150.

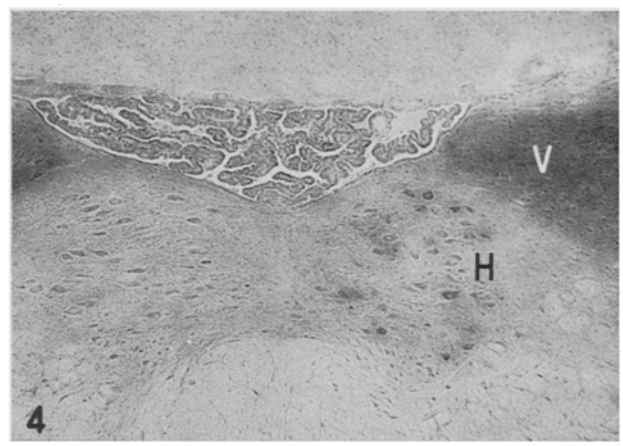

Fig. 4. Cross section of the dorsal part of the medulla oblongata stained for MAO. 20 days following section of left hypoglossal nerve. Most nerve cells and surrounding neuropil of the ipsilateral hypoglossal nucleus (H) showed moderate activity. Strong activity is seen in the dorsal nucleus of the vagus nerve (V). ×60.

the reacting nerve cells and deafferentiated posterior horn might be activated in the metabolism of amines. Similar finding was reported by BARRON and TUNCBAY[9], that glial cells in the anterior and posterior horns showed marked increase of thiamine pyrophosphatase following brachial plexectomy. TOHOYAMA et al.[10] also obtained similar results on thiamine pyrophosphatase activity of the glial cells in the spinal cord and medulla oblongata following neurotomy. Although one of the functional meanings of MAO is the cross-link formation of collagen (BORNSTEIN et al.[11], PAGE and BENDITT[12], etc.), we are unaware whether it is applicable to the perikaryon and neuroglia under regeneration and degeneration. Hence, further study would be necessary for the relation of MAO increase and increased production of neuro- and gliofilament in the central nervous tissues.

Zusammenfassung. Es werden Veränderungen der MAO-Aktivität im Rückenmark oder in der Medulla oblongata von Ratten beschrieben, die durch die Sektion des N. ischiadicus oder des N. hypoglossus verursacht wurden.

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Crystalloid Inclusions in the Connective Tissue of Spider Venom Gland

Crystalline inclusions have been seen in many types of cells and in nearly all compartments of the cell[1,2]. However, we have seen few reports concerning the presence of non-mineralized crystalline inclusions in the matrix of connective tissue. JAKUS[3] has demonstrated the gridlike orientation of collagen units in Descemet's membrane, and HAUST[4] has reported the presence of lattice like profiles in forming elastic tissue. The present report deals with lattice like profiles that are located within the extracellular sheath of venom glands from the brown recluse spider, *Loxosceles reclusa*.

Venom glands were removed from the spiders, sliced into 1 mm thick blocks, fixed in a phosphate-buffered 3% glutaraldehyde solution for 3 h, rinsed in a phosphate-buffered 7% sucrose solution, and postfixed in 2% buffered osmium tetroxide. The blocks were dehydrated in methanol and soaked in propylene oxide prior to being embedded in epoxy resin. Thin sections were stained with combinations of potassium permanganate, uranyl acetate, and lead citrate before being observed in an Hitachi model HU-11B electron microscope.

A brown recluse spider possesses 2 venom glands that are located on either side of the midline in the anterior-superior area of the cephalothorax. Each gland is enveloped with several muscle layers. The extracellular sheath is interposed between the deepest muscle layer and the basal surface of the secretory epithelium. It averages ca.

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