Histochemical Demonstration of Some Dehydrogenases and NAD-H Diaphorase in Cat Pacinian Corpuscles

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Received November 12, 1968

Summary. Using histochemical methods for G-6-P-DH, NAD-HD, M-DH, S-DH, L-DH and G-DH the author has examined the participation and distribution of these enzymes in Pacinian Corpuscles of male cats. The reactions for G-6-P-DH, L-DH, M-DH and NAD-HD show similarity regarding the intensity and the character of intrareceptoral distribution. The nerve fibre and its ending show the highest activity. Three other zones might be differentiated round them. The first zone situated in close proximity to the axon coincides with the inner core of the Corpuscles and shows higher activity than the others (second and third zones). The activity of the latter ones diminishes towards the periphery of the receptor.

The reaction for S-DH is strongly expressed at the nerve fibre and its ending and weaker at the inner core of the Corpuscles.

The reaction for G-DH is moderately expressed in the inner core whereas the nerve fibre and the outer core show the feeble activity.

The author discusses the obtained results in relation to the necessity of energy and the ways it is obtained for the excitation of the axonal membrane.

The nature of the oxidative processes in the sensory encapsulated nerve endings has been scarcely studied. The concept of HALLOWELL (1961) of the necessity of a large amount of energy for the excitation of the axonal membrane and its terminals, during the appearance of the generation potential, needs histochemical confirmation. The limited number of papers, dealing with the histochemical localization of single oxidative enzymes such as S-DH (succinic dehydrogenase) (MICHAEL, 1965; MONTAGNA and YUN, 1962; PORTUGALOV and JAKOYLEV, 1955; STEIGLEDER and SCHULTIS, 1958; SHANTHAVEERAPPA and BOURNE, 1966; ULUMBEKOV, 1964), cytochrome oxydase (MICHAEL, MONTAGNA and YUN, 1961; SHANTHAVEERAPPA and BOURNE, 1966; STEIGLEDER and SCHULTIS, 1958; ULUMBEKOV, 1964), NAD-HD (nicotilamideadenindinucleotide diaphorase) (MICHAEL and ULUMBEKOV, 1964) reveal only partially the aerobic mechanism of carbohydrate degradation as an energetic source in the sensory encapsulated nerve endings.

Whereas some of the authors (MICHAEL, MONTAGNA and YUN, 1961, 1962; STEIGLEDER and SCHULTIS, 1958) consider only the presence or the absence of a definite enzyme in the encapsulated nerve endings, others (PORTUGALOV and JAKOYLEV, 1955; SHANTHAVEERAPPA and BOURNE, 1966; ULUMBEKOV, 1964) examine more extensively the intrareceptoral distribution of the enzymes. The findings of the latter are at variance as to the precise localization of the above mentioned enzymes in different structural elements of the Vater-Pacini Corpuscles.

PORTUGALOV and JAKOYLEV have found that the inner core of Corpuscles is a main site of S-DH activity, and ULUMBEKOV, SHANTHAVEERAPPA and BOURNE prove the same for the axon and its endings. ULUMBEKOV asserts that the inner core is almost devoid of S-DH activity, whereas SHANTHAVEERAPPA and BOURNE
are of the opinion that the latter as well as the lamellae of the outer core possesses moderate activity.

The histochemical demonstration of L-DH (lactic dehydrogenase) has been only incompletely mentioned in the papers of Shanthaveerappa and Bourne, and Michael. The latter one reports on G-6-P-DH (glucose-6-phosphate dehydrogenase) activity in the Vater-Pacini Corpuscles in brief. The intrareceptoral distribution of L-DH has been discussed by Shanthaveerappa and Bourne.

Glutamic dehydrogenase (G-DH) occupies a prominent position in the amino acid synthesis: it is responsible for the transamination of $\alpha$-ketoglutaric acid and its conversion into glutamic acid (Gerebtzoff and Brochti, 1966). It is known that glutamic acid plays an important role in the catabolism and degradation of a great number of amino acids via the mechanism of transamination. Its decarboxylation, on the other hand, leads to the formation of $\gamma$-aminobutyric acid, which is considered to act as an inhibitor of the nerve impulse transmission.

The role played by glutamic acid and G-DH respectively, in carbohydrate and protein metabolism determines their significance and requires exact histochemical visualization in animal tissues. The number of publications, dealing with the histochemical demonstration of G-DH in the peripheral nervous system, are mainly concerned with the distribution and the functional role of the enzyme in vegetative nervous system (Rodriguez, 1966) or in the spinal cord nerves and ganglia (Gerebtzoff and Brochti, 1966). We found no data in the available literature about G-DH histochemical demonstration in the sensory receptors.

The limited number of publications on the participation of the enzymes in the carbohydrate and protein metabolism of the encapsulated nerve endings and the lack of clear cytological characteristics of the intrareceptoral distribution of these enzymes made us undertake the present investigation.

**Material and Methods**

The mesentery from adult male cats was used, because of the dense population of Vater-Pacini Corpuscles round the vessels. Following chloral hydrate (10%) narcosis of the animals the abdominal cavity was opened and small pieces of the mesentery were isolated. They were immediately placed on dry ice at $-20^\circ$C and put in a cryostat. Cryostat sections of 10—15 microns were cut. They were incubated at $+37^\circ$C in incubation baths as follows: G-6-P-DH (according to Weismann and Gerezt, 1961) for 30 minutes at pH = 7.8, L-DH (by the method of Seligman et al., 1958, as modified by Barka, 1963) for 30 minutes at pH = 7.0, S-DH, M-DH (malic dehydrogenase) and G-DH (by the method of Nachlas et al., 1958, as modified by Barka, 1963) for 60, 30 and 60 minutes at pH = 7.0, 7.0, 7.0 respectively, and NAD-HD (by the method of Farber and Louvriere, 1956) for 30 minutes at pH = 7.4. After incubation the sections were washed in saline, then fixed in 10% neutral formol and mounted in Canada balsam.

**Results**

Glucose-6-phosphate Dehydrogenase, Nicotinamide-adenindinucleotide Diaphorase, Malic Dehydrogenase. Since the reactions for G-6-P-DH, NAD-HD and M-DH show similarity regarding the intensity and the character of intrareceptoral distribution they will be described simultaneously. The Corpuscles give a strongly positive reaction for G-6-P-DH (Figs. 2 and 10), NAD-HD (Fig. 1) and M-DH (Figs. 4 and 5). The final product represents formasan granules equal in form and size which depending on the density of localization give a strong, moderate or