THE PRESENCE AND DISTRIBUTION OF ACID PHOSPHATASE IN THE ORGAN OF CORTI IN ANIMALS IN A STATE OF RELATIVE REST AND UPON AUDITORY STIMULATION

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It was established in previous work that under the influence of auditory stimulation a series of cyto-physiologic changes was undergone by the organ of Corti hair cells; these changes characterized the transition of the cells into a state of excitation. Then glycogen which must be regarded as the endogenous energy substrate of the organ of Corti was found in these elements [1]. Finally, the distribution of alkaline phosphatase in the organ was investigated, since it would most probably participate in the maintenance of balance both of glycogen and nucleoproteins [2, 7].

It has been suggested that unlike the tissue distribution of alkaline phosphatase (A.P.), the distribution of acid phosphatase (Ac. P.) is localized mainly in the body of the cell, i.e., that acid phosphatase is directly associated with cell metabolism only. Lately, since the discovery of considerable Ac. P. activity in vegetative neurones, histochemical methods revealing its presence have been recommended as replacement of silver impregnation technics [3, 6]. It is thought that Ac. P. characterizes different functional states of neurones and their peripheral endings [3, 4, 6].

Results of histochemical examination of the organ of Corti for Ac. P. are reported in the present communication.

EXPERIMENTAL METHODS

Acid phosphatase was demonstrated by the Gomori method [5] using different incubation periods. Thirty organs of Corti were studied in serial cross section preparations from 18 cats (10 kittens and 8 adult cats), 6 rabbits and 6 guinea pigs. In the first, control series of experiments 10 animals were maintained in a state of relative rest before being sacrificed by decapitation. In the second series of experiments 10 animals were subjected to one hour of auditory stimulation by high frequency stimuli (1500 cps, 95 decibels) prior to decapitation. In the third series of experiments 10 animals were subjected to low frequency (300 cps, 95 decibels) auditory stimulation for one hour prior to decapitation.

EXPERIMENTAL RESULTS

In the control series of experiments the serial cross sections of the organ of Corti showed reddish-brown passing into black, precipitate (of orthophosphoric acid plumbate) corresponding to the distribution of Ac. P. The enzyme was present in low concentration – light brown precipitate – on the superior convolution of the

organ. Its concentration increased gradually on the middle convolution and reached a maximum on the inferior convolution where the precipitate was black in color. Acid phosphatase was thus distributed along a spiral gradient, its concentration increasing from the superior convolution toward the inferior one. In this respect the distribution gradient for Ac. P. in the organ of Corti proved to be a mirror image of the gradient found by us for alkaline phosphatase. The gradient included all the structural elements of the convolution. There is no doubt that the presence of a distribution gradient reflects functional differences between the elements of the organ of Corti depending on the position of these elements at the level of one or other of the cochlear convolutions. The part of the Reissner membrane facing the endolymph was lined with squamous epithelium and acid phosphatase was only found in the nuclei of the latter. The enzyme was concentrated on the nuclear membrane in karyoplasm aggregates and particularly in the nucleoli (Fig. 1). No acid phosphatase was detected in other parts of the Reissner membrane. The enzyme was also totally lacking from the structural elements of the basilar membrane, the tectorial membrane and the Kelliker membrane.

Fig. 1. Distribution of acid phosphatase in the external hair cells of Corti's organ at the level of the inferior (third) convolution of a young rabbit subjected to sound stimulation (1500 cps, 95 decibels) for 1 hour. Oil immersion objective. Cross section preparation. Microphoto.

Acid phosphatase was traced to the inner sensory hair cells where it was found in the cytoplasm, the nucleus and in the hairs. It was found in low concentration in the phalanges and phalangeal cells. The enzyme can be demonstrated most clearly in the outer hair cells in which it is concentrated in the vacuolated cytoplasm and in the nucleus. The nuclear membrane is sharply delineated. The karyoplasm consisted of nonuniformly colored aggregates and 1-2 outlined nucloli. The sensory hairs in the outer hair cells showed considerable concentration of acid phosphatase.

It should be pointed out that concentration of acid phosphatase increased spirally not only from the superior convolution toward the inferior one, but sometimes also in the radial direction – from the inner hair cells toward the outer hair cells. This radial gradient, however, was not constant. At times other areas were found within the limits of the same convolution in which acid phosphatase concentration increased in the region of the inner hair cells. No acid phosphatase was found in the pillar cells, Deiters, Hensen and Claudius cells; nor was it detected in the vascular strip. Likewise no acid phosphatase was found in the structures enumerated above either in the second or third series of experiments.

Sometimes the presence of acid phosphatase could be detected in some neurones of the spiral ganglion adjacent to one or other convolution and also in nerve fibers passing toward the organ of Corti. It was concentrated in the cytoplasm and the nucleus showing marked preponderance to the area of the nucleolus and at the site of origin of the peripheral process. Acid phosphatase was absent from satellites, blood vessels and connective tissue of the ganglion.

In the second series of experiments, using sounds of high frequency (1500 cps), the distribution gradient for acid phosphatase was in general similar to that established in the control series. Concentration of the enzyme remained practically unaltered in the superior convolution while in the inferior and to some extent in the middle convolutions it was somewhat diminished; the spiral gradient was nonetheless maintained. The cytoplasm of the hair cells of Corti's organ, both inner and outer, became more transparent in the inferior and to some extent in the middle convolutions. The size of the nuclei was changed: some of the nuclei were swollen and markedly enlarged whereas others diminished and shrunk. The outline of the nuclear membrane became pale. The karyoplasm became homogeneous and the nucleoli became paler. The sensory hairs continued to preserve a fairly high concentration of acid phosphatase. The angle formed by the hairs acquired an elliptical shape owing to contraction of the body of the outer hair cells which assumed a more spherical shape, these changes being characteristic for a state of excitation. Changes of acid phosphatase concentration in the phalangeal cells must be pointed out; they often showed alternation of pale areas with darker ones.

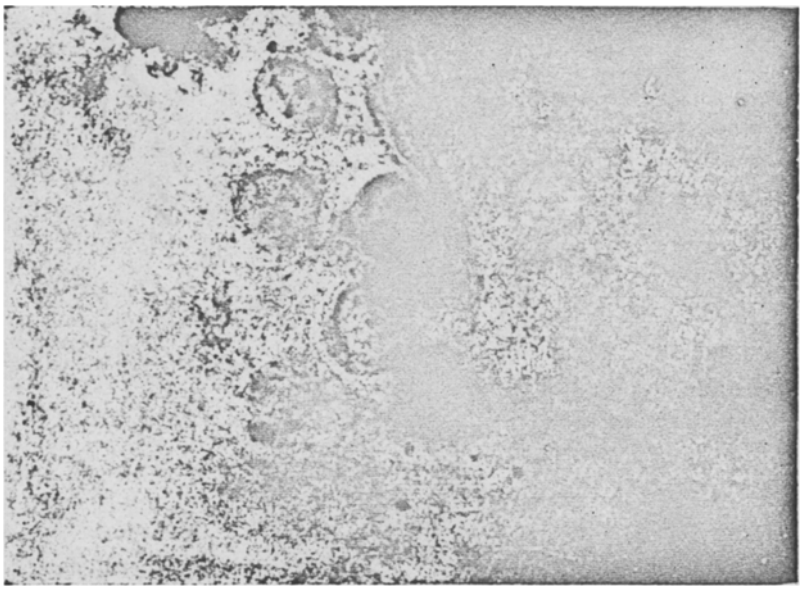

Fig. 2. Acid phosphatase distribution in the nuerones of the spiral (eighth) ganglion at the level of the superior (first) convolution in a young rabbit subjected to sound stimulation (300 cps, 95 decibels) for 1 hour. Immersion magnification. Total, cross section preparation. Microphoto.

In the neurones of the spiral ganglion of the inferior and partly of the middle convolutions concentration of acid phosphatase in the cytoplasm, the nucleus nucleoli and dendrites of the nerve cells could often be observed. In the more translucent zones of Corti's organ it was sometimes possible to trace separate fine fibers intertwining with one another in the area at the base of the outer and inner hair cells. These fibers with definite concentration of acid phosphatase represent parts of the outer and inner spiral plexus of Corti's organ. They apparently emerge in connection with functional loading of the corresponding parts of the cochlea.

In the third series of experiments in which sounds of low frequency (300 cps) were employed the spiral gradient was also, on the whole, preserved, as was also the radial gradient (in parts). But in this case there was

a sharp fall in the concentration of acid phosphatase in the region of the hair cells of the superior convolution, while it remained practically unchanged in the middle and superior convolutions. The fall in acid phosphatase concentration in the helicotrema region was so marked that it was almost entirely absent from the hair cell cytoplasm and was barely discernible in their nuclei and particularly the nucleoli. The nuclei were in some cases enlarged, in others diminished. The acid phosphatase concentration also decreased in the hairs, phalanges and phalangeal cells with consequent transparency of the tunnel. It should, however, be mentioned that within the limits of the first convolution, on its descending part, there was already noticeable increase in acid phosphatase concentration.

Changes in the spiral ganglion at the level of the superior convolution proved to be of particular interest. In association with the general fall in concentration it was possible to trace successfully numerous bipolar neurones containing acid phosphatase. Concentration of the enzyme in individual neurones was seen to be unequal. In some its content was extremely high, particularly in that part of the cytoplasm from which the dendrite comes off; in this case the neuronal nucleus was well outlined by its membrane and the nucleolus was well traced (Fig. 2). In other neurones the concentration of acid phosphatase was lower, although it was present both in the cell body and in the nucleus and nucleolus. In the neurones with high acid phosphatase concentration a peripheral process was clearly visible which sprouted from that pole of the cell which was distinguished by high concentration of the enzyme. The axon was at the opposite pole and was more transparent. Bundles of fibers with increased acid phosphatase content were found between the neurones and the processes coming off from their cell bodies; these fibers evidently originated from other parts of the cochlea or, perhaps, represented the end branching of the vegetative bundle of Rasmusen (Fig. 3).

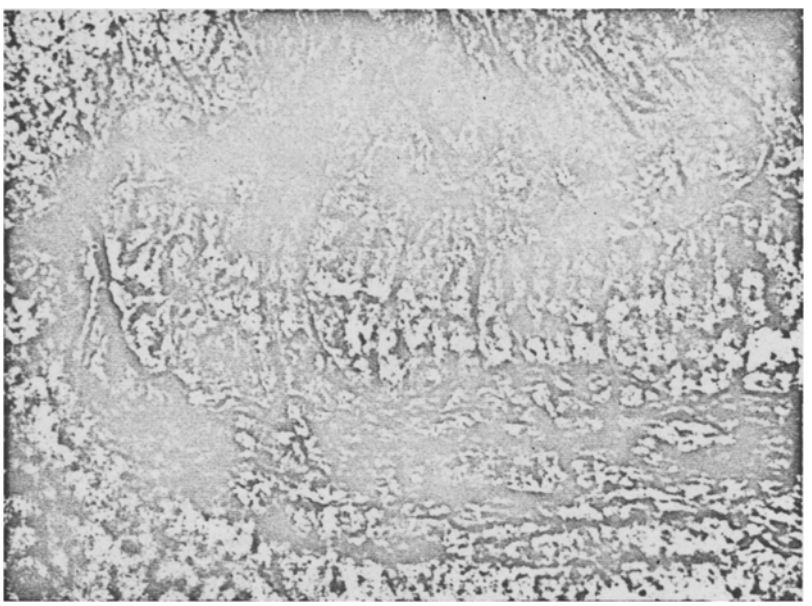

Fig. 3. Acid phosphatase distribution in the nerve fibers of the inner and outer spiral plexus of Corti's organ at the level of the superior (first) convolution in the helicotrema area of a young rabbit subjected to sound stimulation (300 cps, 95 decibels) for 1 hour. Immersion magnification. Cross section preparation. Microphoto.

It proved possible to trace successfully the further course and endings of the spiral neurone dendrites in the region of the superior convolution. Leaving the ganglion they formed a characteristic, widening radial bundle in the region of the spiral lamina; the fibers of this bundle pierced the habenula perforata and passed toward the inner and outer cells of Corti's organ. In the helicotrema region it was possible to note a fairly

large bundle which skirted the edge of the inner hair cells and passing directly under the outer hair cells at their base followed the spiral downward along the convolution. The radial fibers mentioned above, which approached the inner hair cells, formed under their base a very definitely translucent, fairly dense inner spiral plexus. It proved possible to trace the way in which separate fibers of this plexus ended in flask-shaped swellings at the base of the outer hair cells. Part of the fibers from the inner spiral plexus bridged the tunnel and passed under the base of the outer hair cells; here the fibers bent downward at an angle or, forming T-shaped branches, joined those fibers, mentioned above, which had entered in the helicotrema region and formed together the outer spiral plexus. In the latter, three bundles can be clearly discerned which correspond to three rows of outer hair cells as well as individual terminal enlargements which can embrace several receptor elements simultaneously.

The differences in acid phosphatase concentration in the region of the superior convolution evoked by functional "loading" thus permit exceptionally clear demonstration of a number of distinctive structural features of Corti's organ, inaccessible to the usual histologic methods of investigation. Differences in acid phosphatase content of the hair cells in Corti's organ on the one hand, and in the nerve fibers and cell bodies of the spiral ganglion neurones on the other can, in our view, be explained as follows. In the receptor elements of Corti's organ the acid phosphatase is completely used up in catalytic enzymatic processes in connection with increased work. Therefore, its absence may be linked with local prolonged excitation of receptor elements. In the neurones and fibers of the spiral ganglion on the other hand excitation spreads by way of alternating impulses. During the "intervals" between these some part of the enzyme can, apparently, undergo restoration.

Consequently, the results obtained from studies on cochlear convolutions, receptor cells and neurones of Corti's organ permit consideration of differences in acid phosphatase concentration in the structural elements studied as an indicator of different functional significance.

SUMMARY

A spiral gradient of the distribution of acid phosphatase was discovered in the structural elements of the organ of Corti in animals which were in condition of relative rest.

Low concentration of the enzyme was found in the superior (first) convolution of the cochlea.

Its quantity gradually increases in the medial (second) convolution while the highest concentration is revealed in the inferior (third) convolution of the cochlea. Stimulation of animals by sounds of high frequency (1500 cps, 95 decibels) for one hour causes a decrease of the concentration of acid phosphatase in the hair cells of the inferior convolution of the cochlea. Stimulation by sounds of low frequency (300 cps , 95 decibels) on the contrary, causes a decrease in concentration of the acid phosphatase in the cells of the superior convolution. However, stimulation by sound makes the concentration of acid phosphatase more elective in certain neurones of the eighth ganglion, as well as in the nerve fibers of the internal and external spiral network of the organ of Corti.

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