EXPERIMENTAL BIOLOGY

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

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The present communication presents the results of histochemical examination of the organ of Corti for alkaline phosphatase by the Gomori method [4].

Thirty organs of Corti from 30 animals including 18 cats (10 kittens and 8 adult), 6 rabbits and 6 guinea pigs, were examined in whole-area preparations.

In the first, control, series of experiments the 10 animals concerned were maintained in a state of relative rest prior to decapitation. In the second series of experiments the same number of animals was subjected to auditory stimulation by sounds of high frequency – 1500 cps (95 decibel) – for one hour prior to decapitation. In the third series a similar number of animals was subjected to auditory stimulation by low frequency sounds – 300 cps (95 decibel) – for one hour prior to decapitation.

Whole-area preparations of the organ of Corti in the case of control animals show a clear picture which indicates, at first glance, diffuse activity of the enzyme which becomes gradually attenuated in the middle and particularly the inferior convolutions. A concentration gradient is thus observed which is spirally arranged with respect to intensity being most marked in the superior convolution and declining towards the inferior one. There is no doubt that the presence of such a gradient is connected with functional differences between the elements of the organ of Corti dependent on their situation on one or other level of the cochlea.

The Gomori stain gives rise to the greyish-brown coloration in all the structural elements of the organ of Corti which resembles closely the iron hemotoxylin stain. The intensity of color depends on the length of incubation: prolonged incubation (18 hours) is associated with deep coloration of the preparation, shorter incubation (6 hours) with a lighter stain. At high magnification it becomes apparent that despite the generalized staining of the preparation individual structural elements of the organ of Corti are markedly distinct, in other words the concentration of alkaline phosphatase in various structural elements of this organ is far from identical, indicating differences in the enzyme's activity in various cellular structures of the organ.

Alkaline phosphatase is well demonstrated in the epithelium of Reissner's membrane facing the endolymph cavity of the organ of Corti. Alkaline phosphatase activity is clearly seen at intercellular borders which have the appearance of fine, regular dark brown lines delineating polygonal epithelial cells. These borders are considerably more definite than those seen in preparations stained by silver nitrate impregnation. The cytoplasm of the epithelial cells is vacuolated, clear and contains practically no enzyme although isolated granules with high content of the enzyme are at times encountered. The nucleus is distinguished by its large size with clearly defined nuclear membrane. The karyoplasm consists of more or less regular granules with considerable concentration of the enzyme. Against their background one or more nucleoli stand out with high enzyme concentration. The enzyme is absent from the connective tissue part of the membrane.

It is also absent from the endothelium facing the perilymph aspect of the scala vestibuli. The endothelial nuclei contain the enzyme.

The basilar membrane is distinguished by alkaline phosophatase activity at the intercellular borders of the endothelium facing the perilymph of the scala tympani. The enzyme is also present in the cytoplasm of the endothelial cells and especially in the nuclei. There is undoubted enzyme activity in the connective tissue radial fibers of the basilar membrane. These are uniformly stained throughout their length to a brown color. Alkaline phosphatase is absent from the basal substance of the basilar membrane.

Fig. 1. Alkaline phosphatase in the outer hair cells of the organ of Corti at the level of the middle (2nd) convolution of a young rabbit in a state of relative rest. Whole-area preparation. Average magnification (oc. 7x, obj. 40). Microphotograph.

No alkaline phosphatase activity can be demonstrated in the tectorial membrane of the organ of Corti. It remains absolutely clear irrespective of the length of incubation. The enzyme is also completely absent from the Kolliker membrane. Low concentrations of the enzyme are seen in the inner hair cell cytoplasm; higher concentrations are present in the nucleus, the nuclear membrane and particularly in the nucleolus. The enzyme concentration is high in the sensory hairs of the inner hair cells. It must be pointed out that there is slight diffuse staining of the phalanges and phalangeal cells which indicates the presence of alkaline phosphatase.

The outer hair cells stand out prominently owing to enormous concentration of the enzyme. They are stained deep brown and together form 3 dark strips, divided by light interlayers of the phalanges, which extend along the whole organ of Corti on all the convolutions of the cochlea (Fig. 1). However, the concentration of alkaline phosphatase in the hair cells follows a definite downward trend along the spiral from the upper to the lower convolutions (spiral downward gradient). At high magnification each hair cell can be studied individually. The hair cell is distinguished by dark, faintly granular cytoplasm with uniformly distributed concentration of the enzyme. The nucleus is sharply defined by the nuclear membrane. The karyoplasm is granular and contains one or several nucleoli with marked enzyme activity. The apical surface of the hair cells shows the sensory hairs very clearly owing to high concentration of the enzyme. The hairs of each cell form together a characteristic pattern in the form of the letter V with its apex pointing towards the Hensen cells (Fig. 2). In the case of the outer hair cells, however, the concentration of the enzyme increases on approaching the Hensen cells; the enzyme concentration is higher in the second layer than in the first and is still higher in the third one (see Fig. 1). There is thus a radial gradient of phosphatase content in the outer hair cells. The content and distribution of glycogen coincide remarkably with the content and distribution of alkaline phosphatase in these cells.

Low concentrations of the enzyme are found in the pillar cells where the inner hair cells are separated from the outer hair cells by a light, regular strip corresponding to the tunnel. The alkaline phosphatase activity is also slight in the cells of Deiters. It is considerably higher in the cells of Hensen and Claudius. Enzyme activity is demonstrable in the region of the intercellular borders and in the granular cytoplasm of the Hensen cells and also the cells of Claudius. The cell nuclei are sharply defined by the nuclear membrane and show granular karyoplasm and intensely stained nucleoli.

The vascular strip shows extraordinarily high alkaline phosphatase activity, particularly in the vascular endothelium. The borders between the epithelial cells, however, are not discernible, their cytoplasm and nuclei being deeply stained even on short incubation.

Fig. 2. Alkaline phosphatase in the hairs of the outer hair cells of the organ of Corti at the level of the inferior (3rd) convolution in a young cat. Whole-area preparation. Immersion magnification. Microphotograph.

The enzyme is found, in low concentration, in many ganglion cells of the spiral ganglion. Its activity is slight both in the cytoplasm and in the nucleus, although it is somewhat higher in the cytoplasm of the nerve cells than in the nucleus. The latter shows greater activity in the nucleolus. No neurofibrils are seen. Considerably higher enzyme activity is noted in the cytoplasm and nuclei of the satellites, the connective tissue and the endothelium of the surrounding blood vessels.

No alkaline phosphatase is, as a rule, found in the nerve fibers situated in the vicinity of the ganglion cells. It is, however, often found in the dendrites which pass to the basilar membrane and end on the hair cells. The individual nerve fibers here are seen as fine, winding threads forming T-shaped or knee-shaped ramifications; such fibers are seen both in the region of the tunnel and under the hair cells at the base of which they may end in pinhead-like enlargements. Such is the picture of alkaline phosphatase distribution in the organ of Corti of control animals.

The differences between our data concerning the alkaline phosphatase picture in the organ of Corti in cats, rabbits and guinea pigs and data obtained for bats [5] should, evidently, be explained by the difficulties encountered in the preparation of paraffin sections from the inner ear of bats. The advantage of the whole-area preparation method is quite obvious.

In the second series of experiments – following auditory stimulation by sounds of high frequency – the character and distribution of alkaline phosphatase in the organ of Corti underwent a change. The concentration of the enzyme diminished to some extent in Reissner's membrane, at the intercellular borders and in the cytoplasm as well as the nuclei of the epithelium. The connective tissue radial fibers of the basilar membrane in the region of the third and part of the second convolution show marked decrease of enzyme concentration with complete disappearance of the enzyme at the end of the third convolution. This was associated

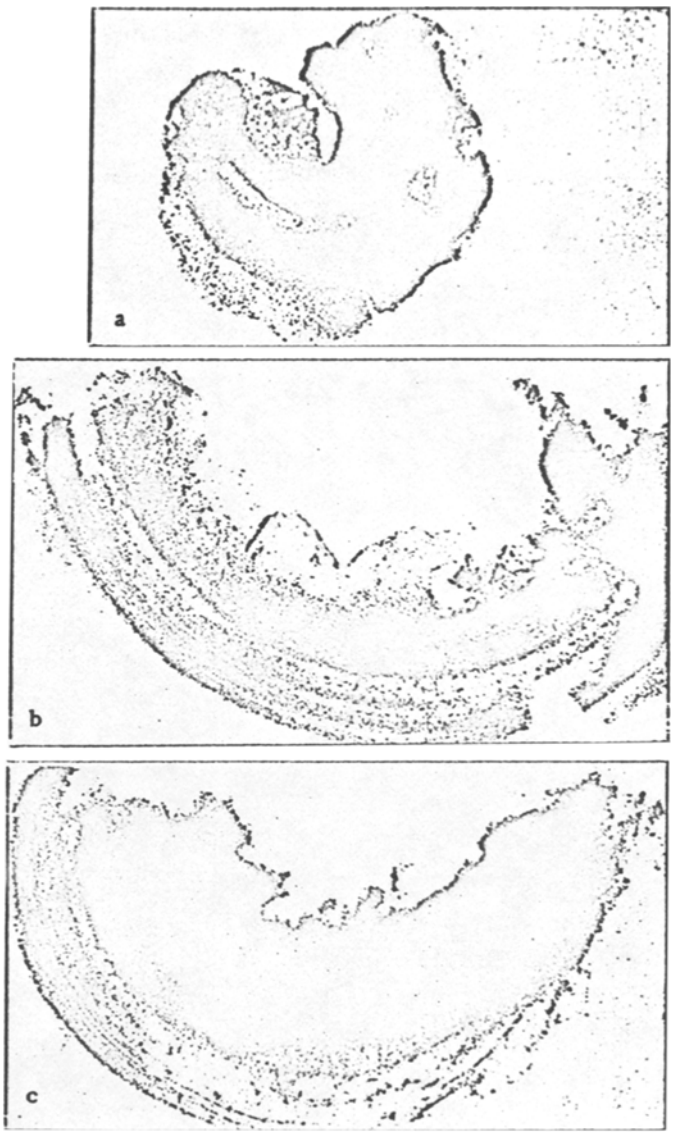

Fig. 3. Gradient of diminution of alkaline phosphatase concentration in the organ of Corti of young cat: a) superior (1st) convolution; b) middle (2nd); c) inferior (3rd).
Whole-area preparations. Magnifying lens, low magnification.

with a general drop in the concentration of alkaline phosphatase in the structural elements of Corti's organ situated on the basilar membrane (Fig. 3, a, b, c). In other words, the concentration of the enzyme did not differ from the control in the region of the superior convolution (Fig. 3 a) but to some extent in the middle convolution (Fig. 3, b) and particularly in the inferior convolution (Fig. 3, c) all the structural elements of the organ of Corti, including the hair cells, showed general diffuse lowering of alkaline phosphatase concentration which, in the case of the most inferior region amounted to practical disappearance.

Such a general fall in alkaline phosphatase activity coincides with a number of features characteristic of the excited state of the hair cells in the inferior and to some extent the middle convolutions. The inner hair cells usually show little change apart from some general decrease of coloration. The outer hair cells not

only show progressive decrease of coloration in ascending the spiral of the cohclea but also acquisition of a rounded shape as the result of contraction; this rounded shape is characteristic of the state of excitation. The cell cytoplasm is vacuolated. Most of the nuclei are, as a rule, enlarged considerably while some tend to shrink. The karyoplasm of the enlarged nuclei is lighter than in the control and this is also true of the nuclear membrane. The lighter nucleoli are displaced towards the periphery of the nucleus to lie adjacent to its membrane. The shrunken nuclei are more deeply staining. Their structure cannot be discerned. The hairs situated at the apical surface of the cells also show a gradual fall in enzyme concentration. The angle formed by them becomes obtuse instead of acute. It must be mentioned that the concentration of enzyme is always somewhat higher in the hair cells than in the surrounding phalanges (Fig. 3,b,c). In those cases where the enzyme is present it is found in larger quantities in the outer layer of the hair cells (radial gradient).

Consistent decline in enzyme concentration is also observed in the pillar cells and the cells of Deiters, Hensen and Claudius. A similar fall in concentration is noted in the elements of the vascular strip especially in the region of the third convolution. The concentration of the enzyme is markedly lower in the ganglion cells of the spiral ganglion, in the satellites and in the nerve fibers passing to the basilar membrane.

In the third series of experiments, in which auditory stimuli of low frequency were used, analogous changes were seen to occur. But, unlike the experiments in the second series, these changes were confined to the superior and part of the middle convolutions of the cochlea. The concentration of alkaline phosphatase was thus lower than control only in the lower convolutions of the cochlea in the second series of experiments and in the superior convolution in the third series. The middle convolution occupies an intermediate position. Consequently changes in enzyme concentration in the organ of Corti are related to the effect of sound stimuli of different frequencies but the general gradient relationships established in control experiments are maintained.

It seems to us that this cannot be unrelated to the similar gradient for glycogen which has been found by us in the organ of Corti [1]. The concentration of glycogen in the hair cells is also connected with the functional changes in the organ. This coincidence no doubt indicates that alkaline phosphatase participates in the processes of breakdown and synthesis of phosphate esters, i.e., in carbohydrate metabolism [4, 6]. There is suggestive evidence that changes in the alkaline phosphatase concentration in the cytoplasm and particularly in the nucleus presumably indicate a connection between this enzyme and nuclein metabolism [3, 5]. The fact that the fall of enzyme concentration does not constitute a fall of its enzyme activity in the true sense of the word, as shown by the experiments described, is of great interest. Quite to the contrary, enzyme concentration declines at the very areas of the organ which are functioning at the given moment with the greatest intensity and utilize the maximal amount of alkaline phosphatase. The lowest concentration of the enzyme is thus found to be connected with its greatest activity i.e., with its maximal participation in catalytic reactions. Such a histochemical interpretation of the "activity" of the enzyme (alkaline phosphatase) would seem to find support in similar data obtained on Vater-Paccini corpuscles [1].

SUMMARY

The distribution gradient of alkaline phosphatase was established as a result of histochemical investigation of the organ of Corti.

The character and the distribution of alkaline phosphatase in the organ of Corti changed after stimulation by high frequency sound waves. The concentration of the enzyme in the superior convolution did not differ from that in the control group. There was a decrease of the enzyme in the inferior convolution and its complete absence in the lowest portion. The medial convolution occupied an intermediate position.

Analogous changes were observed when the stimulation was accomplished by sound of low frequency.

The concentration of acid phosphatase in the organ of Corti underwent a change depending on the difference in frequency of the sound used for stimulation. However, the gradient ratio which was established in control animals remained unaltered.

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