DISTRIBUTION OF NUCLEIC ACIDS IN THE HAIR CELLS OF THE ORGAN OF CORTI OF ANIMALS IN RELATIVELY QUIET SURROUNDINGS, AND EXPOSED TO SONIC STIMULATION

Ia.A. Vinnikov and L.K. Titova

From the I.M. Sechenov Institute of Evolutionary Physiology (Director – Academician L.A. Orbeli), Leningrad

(Received September 20, 1957. Presented by Academician L.A. Orbeli)

Research on the distribution of nucleic acids in the neurons of the spiral ganglion has shown that ordinary stimulation leads to increased production of ribonucleic acid (RNA) – the chromidial substance. On the other hand, exposure for three hours to sound of 6000 cps (80 decibels) leads to total disappearance of nucleic acid. Its regeneration within the neurons requires the space of three weeks [16]. We know of no similar studies of the hair cells of the organ of Corti which are directly stimulated by sound, and which transmit the impulses arising from such stimuli to the neurons of the spiral ganglion, through axons situated in the spiral lamina. It is known only that auditory stimulation leads (within 15 minutes) to changes in the dimensions of the nuclei of the hair cells [17, 20], and to changes in their volume [1]. Changes in the distribution of desoxyribonucleic acid (DNA) [16] and of a number of enzymes [2, 3] have also been noted.

The present paper deals with the distribution of DNA and RNA in the hair cells of the organ of Corti under conditions of relative rest and with exposure to sound.

EXPERIMENTAL METHODS

We examined 60 organs of Corti, taken from twenty guinea pigs, five rabbits, and five kittens. Ten animals were taken for each series of experiments. In the first (control) series, the animals were in relatively quiet surroundings. In the second series they were exposed for one hour to sound of high frequency – 1500 cps (95 decibels). In the third series they were exposed to low frequency sound – 300 cps (95 decibels). The animals were killed by decapitation. The specimens were fixed in Carnoy's fluid, and the sections were stained with gallocyanin [9, 15]. As a control, we used crystalline ribonuclease, according to Brachet [12]. Some of the cochleas were treated by Feulgen's method. After fixation and other appropriate treatment, the cochleas were dissected into separate coils, from which we prepared planar total sections of the organ of Corti.

EXPERIMENTAL RESULTS

The organs of Corti taken from the first (control) series all showed the presence of DNA in both preparations treated according to Feulgen, and in those stained with gallocyanin after preliminary treatment with ribonuclease. The nuclei of the inner hair cells contained relatively little DNA (Figure 1). The DNA appeared as fairly large aggregates or granules in the Feulgen-stained preparations; the DNA stained bluish-red and was situated centrally in the round nuclei. The largest one or two aggregates represent the nucleoli. The location of the DNA granules and aggregates was even more clearly evident in gallocyanin-stained preparations, in particular after preliminary treatment with ribonuclease. Such preparations also show the general contours of the cells, of their cytoplasm, and of the nuclear membranes. The DNA particles, and the nucleoli, stain an intense blue color. The sensory

hairs are well defined at the free surfaces of the inner hair cells. Their intensity of staining was not affected by preliminary ribonuclease treatment. A comparison of sections stained with gallocyanin, with and without preliminary ribonuclease treatment, permits the fairly precise determination of the location of RNA in the cells. In the inner hair cells, RNA was found in the apical part of the cell, above the nucleus, in the form of more or less regular rod-shaped formations lying parallel to the long axis of the cells. We did not observe diffuse blue staining of the cells. RNA is diffusely distributed through the nuclei, but after treatment with ribonuclease the background becomes quite clear. This treatment leads to some diminution in the size of the nucleoli.

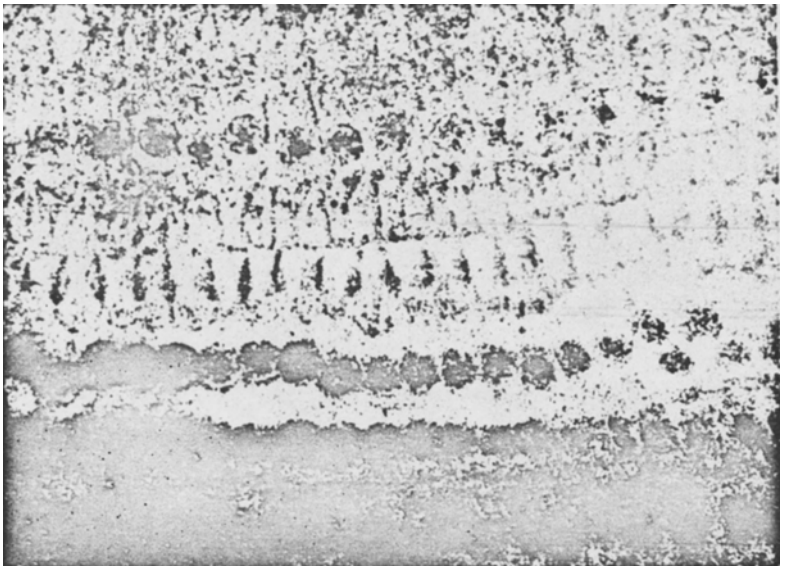

Fig. 1. DNA content of the nuclei of inner and outer hair cells of the organ of Corti of a guinea pig, at the level of the lower coil. Treated with ribonuclease, stained with gallocyanin. Whole surface of the lower coil. Magnification: ocular 7×, objective immersion 90. Photomicrograph.

The nuclei of the outer hair cells are somewhat richer in DNA. The latter appears centrally in the nuclei, in the form of granules and aggregates, together with two to three larger nucleoli. The distribution of DNA in the nuclei of cells treated with ribonuclease and then stained with gallocyanin is the same as in Feulgen-stained cells. In addition, however, the regular, round nuclear membrane, as well as the transparent cytoplasm and the contours of the cell itself, are visible (see Figure 1). In sections stained with gallocyanin after ribonuclease treatment, the sensory hairs of the outer hair cells are clearly to be seen as V-shaped formations situated at the free surface of the cells, with the base of the V directed towards the Hensen cells. The RNA of the nuclei of the outer hair cells is distributed around the nucleoli, and in small amounts throughout the karyoplasm. The RNA of the cytoplasm of the outer hair cells is in the form of slender striated threads, located above the nucleus, and lying more or less parallel to the long axis of the cell. We did not observe diffuse blue staining of the outer hair cells when they were stained with gallocyanin.

DNA is found in large amounts in the nuclei of the pillar cells of the organ of Corti, and the nuclei of the epithelial cells of the vestibular lip are particularly heavily loaded with DNA; this represents a nuclear secretion. The nuclei of the Hensen and Claudius cells, and of the epithelium of the vascular zone are also distinguished by their high DNA content. Less DNA is to be seen in the nuclei of the columnar cells, of Deiter's cells, and of the epithelial cells of Reissner's membrane and of the basilar membrane. Very little RNA could be seen in the supporting cells. It was also absent from the membrane of Corti. There is, nevertheless, a considerable diffuse concentration of RNA, visible as a continuous blue band, along the whole length of the inner cells of the organ of Corti (Figure 2).

In the second series of experiments, involving high-frequency tones, we found marked changes in distribu-

tion of DNA and RNA, chiefly in the hair cells of the outer coil and of the lower part of the central coil of the organ of Corti. The elongated, prismatic shape of the hair cells situated at these levels of the cochlea remained unchanged, and only very seldom was there any alteration in the shape or size of the nucleus of these cells, but the granules and aggregates of DNA were characteristically displaced towards the inner surface of the nuclear membrane. Traces of RNA persisted in the cytoplasm situated above the nucleus, but the regular striation and alignment of the threads were absent. Displacement of the nucleoli was sometimes observed. Judging from the intensity of its staining, the RNA content was greatly diminished. In some of the inner and outer hair cells RNA could not be seen, although the remnants of the colorless striae could be distinguished. The sensory hairs located at the free surfaces of the inner hair cells stained intensively with gallocyanin, as in the control series.

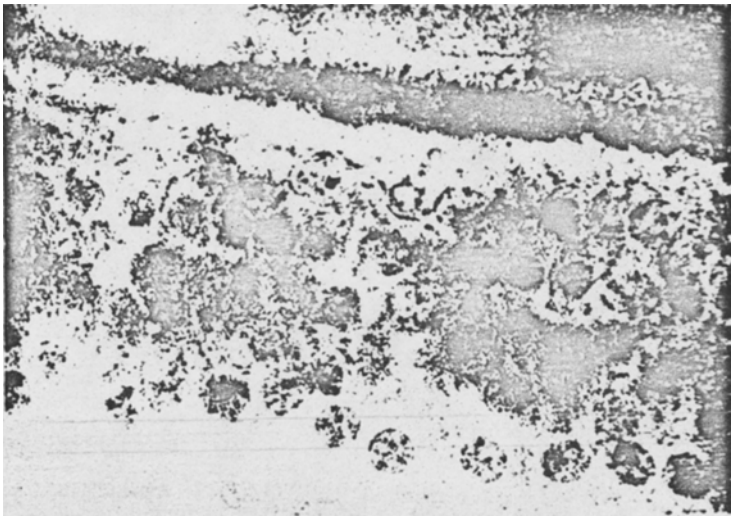

Fig. 2. DNA and RNA present in the cytoplasm and nuclei of inner hair cells and of Hensen cells of the organ of Corti of a guinea pig, at the level of the lower coil. Stained with gallocyanin. Full surface of a section of the lower coil. Magnification: ocular 7x, objective immersion 90. Photomicrograph.

The outer hair cells of the lower coil, and of the lower part of the middle coil of the cochlea, undergo a complex cycle of changes. They respond to sound by contracting, and become more spherical. This was associated in some of the outer hair cells by shrinkage of the nucleus. However, the majority of the outer hair cells seen by us had very swollen nuclei (see Figure 2).

We noticed the following regularities in the location of the nuclei. The inner row of outer hair cells had a marked preponderance of enlarged nuclei (see Figure 2). In this row one to two shrunken nuclei could be seen per field of vision (oil immersion). There were many more such nuclei in the middle row of outer hair cells. Their number was about equal to that of enlarged nuclei (see Figure 2). Finally, the outer row of outer hair cells had a marked preponderance of shrunken nuclei. The proportion of swollen nuclei to be seen in this row was much smaller.

The distribution of DNA in the latter was characteristic. The DNA aggregates and granules were situated marginally at the inner surface of the nuclear membrane, and considerable displacement of the nucleoli was frequently seen. Deformation of the granules and aggregates of DNA was evident; they assumed an unusual shape resembling short, stretched filaments, of irregular shape and of uneven thickness. RNA was distributed diffusely throughout the swollen nuclei, apart from the nucleoli. The RNA content varied from nucleus to nucleus, some staining more or less intensely with gallocyanin. Some nuclei did not contain any RNA, remaining quite transparent after staining. In general, RNA is also absent from the cytoplasm of hair cells having swollen nuclei (see Figure 2).

A distinguishing feature of hair cells with shrunken nuclei is that the granules and aggregates of DNA are scattered more or less uniformly throughout the nucleus; the nucleoli (one to two) can be distinguished by their larger size. The DNA granules are not situated at the inner surface of the nuclear membrane. The karyoplasm of the shrunken nuclei stained diffusely and intensively with gallocyanin. This staining did not appear after preliminary treatment with ribonuclease, which is evidence that it is due to RNA. The cytoplasm of such outer hair cells with shrunken nuclei contained RNA in the form of very slender rod-shaped granules, large numbers of which were distributed randomly around the nucleus. These granules stain intensively with gallocyanin. This staining was not seen after preliminary treatment with ribonuclease. The sensory hairs situated at the apex of the outer hair cells stained deeply with gallocyanin. Owing to the more rounded shape of the shortened hair cells the hairs become crescent-shaped rather than V-shaped. The staining of the sensory hairs is unaffected by treatment with ribonuclease.

The distribution of DNA and RNA in the supporting cells of the organ of Corti differed little from that seen in the control preparations. The DNA aggregates and the nucleoli of the nuclei of Hensen's cells may, however, assume a marginal location (see Figure 2).

The changes seen in the third series of experiments, using tones of low frequency, were of the same kind as in the second series, but they were restricted to the upper coil and to the upper part of the middle coil of the cochlea. The cells of the lower coil and of its lower parts were not affected, as a general rule. Both in the second and in the third series of experiments, we encountered individual outer hair cells in which the distribution of DNA in the nucleus and of RNA in the cytoplasm did not differ from that found in the control series.

It should be noted that the above descriptions are characteristic chiefly of the hair cells of guinea pigs, and to some extent of cats. In rabbits, stimulated hair cells sometimes contained DNA in the form of "lamp brushes" [8]. The alterations in the nuclei of the hair cells seen after exposure to sound of one hour's duration persisted for two to three days, after which the DNA and RNA contents and distribution became the same as for the control animals.

Our findings show that, in distinction from the neurons of the spiral ganglion [16], the hair cells of the organ of Corti react more vigorously to raised functional loading. This excitation reaction is distinguished by changes in the shape of the cells, in the size of their nucleus, and in the distribution of DNA and RNA within them.

The alterations in the size of the nuclei – enlargement (or swelling) and contraction (or shrinkage) – should be regarded as pulsations of the nuclei of the outer hair cells, proceeding fairly rapidly (30-60 minutes), although it cannot be perceived macroscopically. This pulsation may be compared with the recently established phenomenon of rotation of the nucleus, found for different types of cells [21].

The morphological transformations of these nuclei coincide with certain quite important histochemical processes. Displacement of DNA towards the periphery of swollen nuclei, and fall in the RNA content of the cytoplasm of such cells are evidence of intensification of RNA synthesis and of associated protein synthesis, proceeding under the control of the DNA [13]. The synthesized RNA proteins are at once used up by the excited hair cells. Contraction or shrinkage of the nuclei of the hair cells, and enrichment of their nucleus and cytoplasm in RNA may be taken as evidence that such cells catabolize less RNA protein and that they have passed from an excited state to one of relative rest, i.e., such cases represent the terminal stage of the process of nuclear pulsation, a reversion to the initial dimensions following a phase of expansion.

The correspondence between the localization and the changes in DNA and RNA of excited hair cells of the organ of Corti and the changes in their enzyme contents, such as of alkaline [2] and acid [3] phosphatase, makes possible, in conjunction with the findings of other authors [10, 14, 18, 19], the hypothesis that the above-described changes in the distribution of nucleic acids and of their associated proteins may be due basically to transphosphorylation processes, effected by those nonspecific phosphorylases whose location in the organ of Corti is governed by pronounced gradient regularities. This supposition is supported by certain biochemical evidence of the contractile properties of DNA proteins [6], and of the effect of phosphatases on this process [5], which may be responsible for the variations in size ("pulsation") of the nuclei of the hair cells.

Thus, our findings on the distribution of DNA and RNA in the hair cells, taken in conjunction with other histochemical changes recently reported by us [2, 3, 4], are evidence of the participation of nucleic acids in the energetics of the organ of Corti, although this possibility has been rejected by some authors [7].

SUMMARY

Experiments were performed on guinea pigs, rabbits and kittens. It was established that the form and the structure of hair cells of the organ of Corti is changed depending on the condition of the animal (whether it is in condition of rest or stimulated by sound). The content and the distribution of RNA and DNA is changed correspondingly. Depending on the frequency of the sound stimuli the external hair cells in the cochlea become rounded, while their nuclei dilate or, shrink – "pulsate." DNA occupies an external position on the internal surface of the nuclear membrane in the cells with the swollen nuclei. As to RNA – it disappears from the cytoplasm entirely.

Increased content of RNA in the nucleus, as well as in the cytoplasm, is found in the cells with shrunken nuclei.

LITERATURE CITED

[1] Ia.A. Vinnikov and L.K. Titova, Doklady Akad. Nauk SSSR 116, No. 2, 327-330 (1957).*

[2] Ia.A. Vinnikov and L.K. Titova, Biull. Eksptl. Biol. i Med. (1957).*

[3] Ia.A. Vinnikov and L.K. Titova, Biull. Eksptl. Biol. i Med. (1957).*

[4] Ia.A. Vinnikov and L.K. Titova, Doklady Akad. Nauk SSSR 119, No. 3 (1958).*

[5] B.S. Diskina, Biokhimiia 21, 482-490 (1956).*

[6] I.B. Zbarskii and K.A. Perevoshchikova, Biokhimiia 16, 112 (1951).

[7] T.I. Zelikina and V.E. Shungskaia, Reception of Sonic Signals under Various Acoustic Conditions,** (Izd. AN SSSR, 1956), pp. 49-56.

[8] P.V. Makarov, Izvest. AN SSSR, ser. biol. No. 1, 46 (1953).

[9] E. Pearse, Histochemistry (Foreign Literature Press, Moscow, 1956).***

[10] B.J. Axelrod, Biol. Chem. 172, I, 176, 295 (1948).

[11] Chl. Beck, Arch. f. Ohren-Nasen usw. 167, 262-270 (Kunde, 1955).

[12] J. Brachet, Quart. j. of microscop. science 91, 1-10 (1953).

[13] T. Casperson, Chromosoma 5, 562 (1940).

[14] J.F. Danielli, J. exp. biol. 22, 110 (1946).

[15] L. Einerson, Acta ortoped. 28, 82 (Scand. 1949).

[16] C.A. Hamburger, Acta otolaryngol. Suppl. 1945, 61.

[17] U. Merkle, Z. Anat. und Entwicklungsgesch. 117, 504-519 (1954).

[18] O. Meyerhof and H. Green, J. Biol. Chem. 183, 377 (1950).

[19] F. Moog, J. Cell. comp. physiol. 22, 95 (1943).

[20] K. Neubart and E. Wustenfeld, Naturwissenschaften 42, 11, 350 (1955).

[21] C.M. Pomerat, Exp. cell research 5, 191-200 (1953).

*See English translation.

**In Russian.

***Russian translation.