(*13*), or *Azotobacter agilis* (*14*), which also have internal membrane systems. Phosphatidyl choline has also been found in several species of *Agrobacterium* (*1*), but no details of the membrane structure of these organisms are available. Since phosphatidyl choline is the major phospholipid of the endoplasmic reticulum of higher organisms, its presence in a number of bacteria with extensive intracytoplasmic membranes is suggestive of an important role for this phospholipid in the elaboration of cytoplasmic membranes (*15*).

P-O. HAGEN
HOWARD GOLDFINE
Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts
P. J. LE B. WILLIAMS
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

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Tetraethylammonium and Tetrodotoxin: Effects on Cochlear Potentials

Abstract. *Tetraethylammonium chloride, which is believed to decrease potassium conductance, and tetrodotoxin, which apparently decreases sodium conductance in nerve fibers, were introduced iontophoretically into the organ of Corti or the scala media of guinea pig cochlea. The former depressed the direct-current endocochlear potential and also the alternating-current cochlear microphonics (the receptor potential of the ear), but tetrodotoxin was ineffective except on the nerve impulses.*

It is well known that endolymph of the cochlear duct has a high concentration of potassium, nearly 30 times that of the perilymph (*1*). The physiological significance of this pattern of electrolytes is not obvious, yet the richness in potassium seems indispensable for the high sensitivity of the cochlear microphonics (CM) (*2*). Furthermore, some experimental results show that the CM responses may be modified by increasing or decreasing the endocochlear potential by external current or by KCl injected into the scala tympani (*3*).

Katsuki *et al.* (*4*) recently developed the method of iontophoretic introduction of various pharmacological agents in the vicinity of the cochlear hair cells. Using the same technique, we applied tetraethylammonium chloride (TEA) and tetrodotoxin (in the citrate buffer solution, in which tetrodotoxin acts as a cation) to the hair-cell region in order to clarify the ionic mechanism of cochlear responses. We had a frog nerve preparation as a control on the effect of the iontophoretic introduction of tetrodotoxin.

It has been reported that TEA prolongs the spike potential of myelinated nerve fibers of vertebrates (*5*), of muscle fibers of crustaceans and vertebrates (*6*), and of giant axons of squid (*7*); and experiments with *Onchidium* neurons suggest that these effects of TEA probably result from suppression of the increase in K-conductance of the treated cell membrane (*8*).

Tetrodotoxin is known to block impulse activity in nerve and muscle fibers (*9*) and is considered to be a blocking agent for the sodium channel in the membrane (*10*). It has also been shown that in two receptors, the crustacean stretch receptor and the pacinian corpuscle, tetrodotoxin acts selectively on the all-or-none component —that is, it blocks the action potential but leaves the generator potential unaffected. It follows that the spike and the receptor-generator potential in these two receptors are independent events subserved by different mechanisms (*11*).

The CM responses and the neural action potential (N_1) were recorded

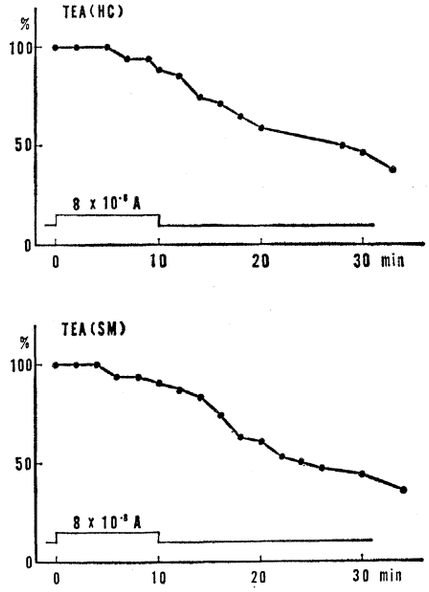

Fig. 1. Effect of TEA on the cochlear microphonics; it was introduced iontophoretically into the hair-cell region (*TEA-HC*) and the scala media (*TEA-SM*) with a current of 8×10^{-6} amp for 10 minutes. Ordinate, amplitude of microphonics as a percentage of that of the control.

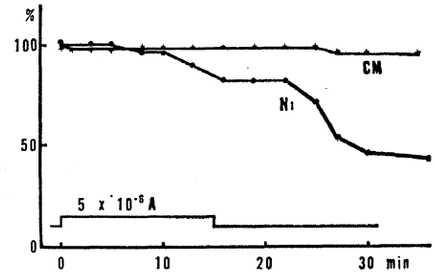

Fig. 2. Effect of tetrodotoxin on the cochlear microphonics (*CM*) and the action potentials (N_1). Tetrodotoxin was introduced iontophoretically into the hair-cell region. *A*, ampere. Ordinate, amplitude as a percentage of that of the control.

from the basal turn of guinea pig cochlea by means of vestibulo-tympanic leads, in the manner of Tasaki (*12*). When TEA was introduced into either the hair-cell region of the organ of Corti or the scala media, with a current of 8×10^{-6} amp for 10 minutes, there was a gradual irreversible decrease of the CM responses, which lost about 50 percent of their initial value within 30 minutes (Fig. 1). The decrease in CM responses, caused by TEA, was almost the same for these two regions, although remarkable differences are evident when acetylcholine is administered to the same two regions (*4*). The effects of TEA on the N_1 responses, not marked, resembled those following application of acetylcholine (*4*). The endocochlear d-c potential was decreased to 50 percent of its original value after the introduction of TEA into the scala media, but no change followed introduction into the hair-cell region.

Following iontophoretic introduction of tetrodotoxin into the hair-cell region, the CM showed almost no change, while the action potentials diminished gradually to less than 50 percent of their original amplitudes 30 minutes after the application commenced (Fig. 2). This depression of N_1 was irreversible.

From these results we can draw certain inferences concerning the receptor mechanism of the hair cells in the cochlea. Tetrodotoxin caused no change in the CM responses, which we regard as receptor potentials; this finding suggests that CM involves an ionic mechanism different from that of nerve action potential; it also agrees with results obtained with other receptor organs (*11*) in which the receptor-generator potentials were not affected by tetrodotoxin.

If TEA suppresses the increase in K-conductance, depression of the CM responses by TEA suggests that the CM represent changes in the membrane potential of hair cells, caused by changes in the rate of movement of potassium ions through this membrane, controlled by movements of the hairs on the cells.

When applied to the hair-cell region TEA did not decrease the endocochlear potential; in this instance the reduction in CM response was presumably caused only by the change in K-conductance of the hair cells. When TEA was introduced into scala media, however, the reduction in endocochlear potential probably contributed to the decrease in CM response. Presumably K-conductance is implicated in maintenance of the endocochlear potential by stria vascularis.

Y. KATSUKI
K. YANAGISAWA
Department of Physiology, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo
J. KANZAKI
Department of Otolaryngology, Keio University Medical School, Shinanomachi, Shinjuku-ku, Tokyo

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Electroretinogram of the Frog during Embryonic Development

Abstract. *Eyes removed from frog embryos at various stages of development gave a definite pattern of change in the electroretinogram. From the 7th to 9th days the electroretinogram consisted of slow, purely cornea-negative potentials. From the 9th to 10th days the responses were negative but included a prominent fast, negative component superimposed on the slow potentials. During the 11th to 17th days positive potentials appeared and developed. From the 20th day on, the typical electroretinogram of the adult obtained.*

During development of the vertebrate retina the electroretinogram (ERG) undergoes striking changes in form. In the house mouse, for example, the first appearance of the ERG at the 13th to 14th postnatal days of development revealed a response comprised of a prominent, initial, cornea-negative *a*-wave which was elicited even at low light-intensities (*1*). At similar intensities for the eye of the 21-day-old mouse the ERG consisted of large positive *b*- and *c*-waves without preliminary negative activity. The ERG of the 10-day-old albino rat was also significantly different from that of the adult, consisting of a small, duplex, negative response containing an initial fast wave followed by a slower wave (*2*). Predominantly negative activity in the early ERG was observed in the dog, rabbit, and chick (*3*). Contrary to these results are reports that the *b*-wave, rather than the *a*-wave, is the first to appear in the kitten and the albino rat (*4*).

We are here concerned especially with the results reported for the developing frog eye. The early ERG from *Rana temporaria* showed no negativity but only a small, monophasic, positive wave (*5*). For the same species of frog, Muntz (*6*) made the definite statement that "as soon as the ERG could be recorded at all, the components of the adult ERG were immediately apparent. . . ." Is the frog, then, an exception to the developmental sequence observed in the mouse, rat, dog, rabbit, and chicken? Our work was initiated to clarify this point. We have found that the earliest ERG of *Rana pipiens* does, in fact, consist only of negative components, that there is both a slow and fast wave in this ERG, and that a very orderly sequence of change occurs in the ERG of the developing eye.

We studied 141 isolated eyes obtained from embryos of accurately known age. Four different batches of eggs were employed; fertilization times were known to be on 23 April 1964, 13 February 1965, 10 March 1965, and 29 March 1965. Starting with the