On reversible changes in the steady state ion-conductance of the Ranvier node membrane under low frequency sound fields

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Summary. The influence of sound waves on the stationary membrane conductivity of the axon membrane in myelinated nerve was investigated. Sound fields with frequencies within the auditory limit shift the transition voltage V_{tr} and correspondingly the threshold voltage of the action potential in polarisation direction. The results indicate that these changes of the electrical membrane behavior are due to a direct mechanical interaction between low frequency sound fields and membrane structure.

Steady state ion conductance of nerve membranes are voltage-dependent, showing 2 stable states, a 'high ohmic' state for the hyperpolarized and a 'low ohmic' state for the depolarized membrane. Within a certain voltage range both states continuously change from one to the other. The position of this transition range with respect to the resting potential (V = 0) can be defined by the transition voltage V_{tr}[2]. Formally this conductivity transition is consistent with the electrical behavior of liquid crystals and is also a condition for bistability and differential-negative resistance[2,3].

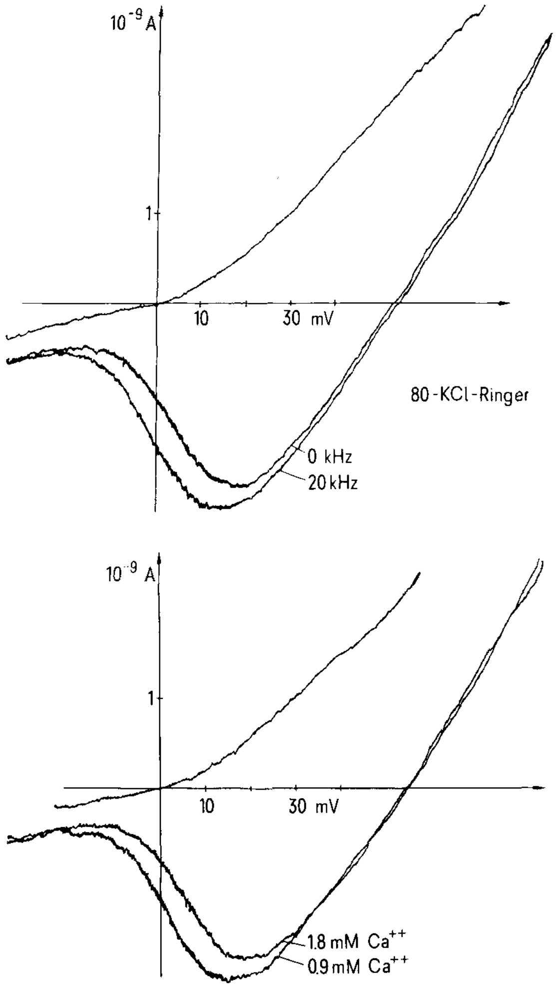

Fig. 1. Stationary I-V characteristics of a nerve membrane at normal and 80-KCl Ringer's solution. The V_{tr}-shift caused by decreasing the $|Ca^{++}|_a$ (below) is compared with the V_{tr}-shift due to a sound field (above).

In light of the visco-elastic properties of liquid-crystals[4,5], the study of the effect of sound waves on the transition voltage V_{tr} seems to be a promising approach to a better insight into the mechanism of stationary membrane conductivity. In this communication we report preliminary results of such experiments.

Methods. A single myelinated nerve fibre (N. ischiadicus of male *Rana esculenta*) was mounted in a 3-electrode device with air gap isolation[6]. The test node, N2, in the central tube could be alternatively rinsed by different solutions. The subsequent nodes on both sides are in throughs with Ringer's solution. In measuring the stationary current voltage (I–V) curves, the impressed voltage V across the axon membrane of N2 was varied continuously by a ramp generator with a velocity $dV/dt <$ 3 mV/sec and recorded simultaneously with the membrane current I by a X-Y recorder[7]. Normal Ringer's solution is used to determine the resting potential (V = 0). The actual V_{tr} was then determined as the inflection point of the I-V-curve within the transition region. To get an accurate measure of this inflection point, the I-V-curve has to show a pronounced region of differential negative resistance. Therefore the node was depolarized by Ringer's solution in which 80 mM Na^+ are replaced equimolarly by K^+; isosmolarity is not changed.

Sound fields with frequencies between 200 Hz and 2 MHz are produced by a quartz-ceramic and coupled to the Ringer's solution of one side trough. This trough was mechanically isolated from the rest of the apparatus; therefore the sound was conducted to N2 only by the nerve fibre bridging the air gap. The quartz-ceramic could be excited to mechanical oscillations of different amplitudes and frequencies by means of a function generator (datapulse).

Most of the experiments are carried out using sound frequencies in the range between 200 Hz and 50 kHz (see results). Within this range, the relative sound intensity was almost independent of frequency. An increase of intensity with frequency is important only for higher frequencies (resonance frequency of the quartz ceramic $\simeq$ 2 MHz). Variations in the sound intensity, which might be possible even at low frequencies due to the special geometry of the quartz oscillator, could be ruled out experimentally by using several oscillators of

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different size and form. Because of the theoretical and experimental difficulties in measuring the sound energy actually entering the tissue[8], no attempt has been made to measure the sound intensity on an absolute scale.

Results. Looking at the I-V characteristic of the depolarized membrane (in 80 KCl-Ringer's solution, see method), sound waves in the audiofrequency range alter the transition region provided that the sound-intensity exceeds a certain minimum value. These alterations resemble, even in detail, those produced by decreasing the external Ca^{++} concentration, $|Ca^{++}|_a$[9]. This is seen in figure 1, which shows I–V characteristics from the same nerve preparation, demonstrating the similarity of sound- and Ca^{++}-effect. In normal Ringer's solution, the same sound fields cause a reversible depolarisation of the membrane of about the same order as observed by decreasing the $|Ca^{++}|_a$[10]. In both cases, sound field and $|Ca^{++}|_a$-decrease, the membrane depolarisation is accompanied by similar alterations of the I–V characteristic. Both, the specific alterations of the I–V characteristic and the depolarisation of the membrane in normal Ringer's solution, can be explained by a shift of only 1 parameter, V_{tr}, in polarisation direction[9]. In agreement with these V_{tr}-shifts, the threshold voltage of the action potential was observed to be shifted in the same direction. Due to this shift, the minimum amplitude of a stimulating pulse is decreased. This also could be verified experimentally. All observed sound-dependent changes of the membrane behavior are reversible provided that the sound-intensity does not exceed an upper limit. In addition it could be shown experimentally that these changes are not due to secondary electrical effects of the sound field.

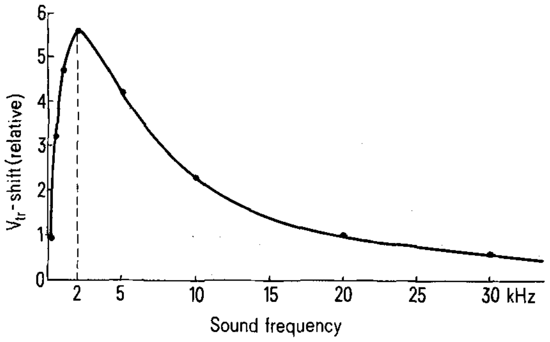

Fig. 2a. Relative V_{tr}-shift as function of sound frequency.

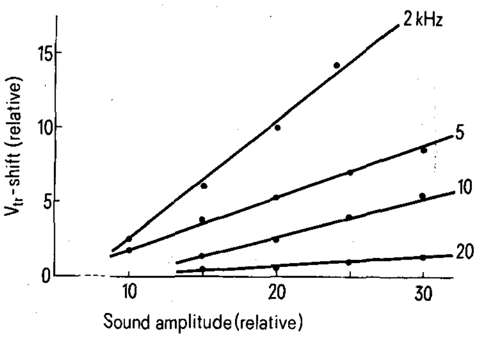

Fig. 2b. Relative V_{tr}-shift as function of sound amplitude at different frequencies.

Since the sound-intensity at the axon membrane of N2 could not be measured on an absolute scale, the analogy between sound- and Ca-dependent alterations of the I-V-curves was used to normalize the sound-dependent V_{tr}-shifts to the V_{tr}-shift caused by a change of $|Ca^{++}|_a$ from 1.8 mM to 0.9 mM as measured at the same node. Figure 2a shows the dependency of V_{tr}-shifts on sound frequency. The points denote V_{tr}-shifts (relative to the dependence of V_{tr} on $|Ca^{++}|_a$) which are determined from I-V-curves measured at the same nerve preparation. Due to unavoidable differences in the acoustical coupling of the sound to N2, the relative V_{tr}-shifts are slightly different for different preparations; the form of the curve in figure 2a, however, remains the same. The V_{tr}-shift shows a maximum at a frequency $\nu = 2$ kHz ($\pm$ 0.5 kHz); at $\nu > 50$ kHz no changes of the I-V-curves could be observed, even with sound-intensities increased by a factor of 10. The dependency of the V_{tr}-shifts on the relative sound amplitude is seen in figure 2b for different frequencies. All measured points are obtained from experiments on one node. The linearity of this dependency is independent of the actual preparation.

Similar V_{tr}-shifts are also observed in the presence of 10^{-6} g/ml TTX in the external solution. Replacing the 80 mM KCl-Ringer by normal Ringer's solution, and depolarizing the membrane by adding 10^{-4} g/ml veratridine, equivalent sound-dependent V_{tr}-shifts are found.

Discussion. Shifts of the transition region caused by variations of the external ion-concentration (divalent cations shift about 20fold stronger than monovalent cations[11]) are normally referred to the interaction between negative fixed charges at the membrane surface and the external ions[12]. We therefore had to test the reasonable assumption that the sound field acts on the axon membrane by changing the surface charge density. This was done using a micro-electrophoretic method to measure the effect of similar sound waves on the ζ-potential of myelin membrane-vesicles from the same nerve material[13]. Since this ζ-potential has the same dependency on external ion concentration as V_{tr}, we assume a similar behavior of the surface charges for both the axon and the myelin membrane. However, no influence of the sound-fields on the ζ-potential of the myelin membranes could be observed. This result corresponds with morphological observations; the morphological alterations of the Ranvier node by a change of the external Ca-concentration[14] are not observed during sound field application. We therefore assume that the sound field acts directly on those membrane structures which control the steady state conductivity. In addition, the form of the curve in figure 2a (resonance curve) and the linear dependency of the V_{tr}-shift on sound amplitude (figure 2b) point to a direct mechanical interaction between sound field and membrane structure.

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