CONFIRMATION OF THE HYPOTHETICAL DIFFUSION BARRIER IN THE REGION OF THE RANVIER NODE OF MYELINATED NERVE FIBERS

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Experiments were carried out on single nerve fibers with a "covered" node. The nerve fiber was isolated together with one to three fragments of neighboring fibers adjacent to the node and which were firmly connected to the nodal ends of the myelin sheath by means of numerous connective-tissue membranes, thereby strengthening the node and protecting it against stretching during dissection. In potassium-free solution after-hyperpolarization of the nerve fiber membrane with an amplitude of 2.7 mV develops immediately after the end of the spike. Its origin is connected with maintenance of the increased potassium permeability of the membrane. During repetitive stimulation of the "covered" node the amplitude of after-hyperpolarization falls successively, as a result of a decrease in the outward potassium current caused by a reduction in the potassium electrochemical gradient. These observations are regarded as confirmation of the hypothesis expressed previously on the existence of a diffusion barrier in the region of the Ranvier node due to accumulation of K^+ in the juxtamembranous space.

KEY WORDS: *single Ranvier node; isolated nerve fiber; after-depolarization; after-hyperpolarization; diffusion barrier.*

After-depolarization (AD), lasting only 1-3 msec, is found [7] in single nodes of Ranvier of frog nerve fibers dissected by Tasaki's method [3] (a preparation with "uncovered" Ranvier node). On the basis of these findings and of their comparison with results obtained on the squid giant axon [5, 6] Meves [7] concluded that there is no barrier in the region of the Ranvier node to the free diffusion of ions and that during the spike K^+ flows out into the surrounding solution unhindered.

The writer's experiments on single nerve fibers with "concealed" (not separated from the nerve trunk) and "covered" (strengthened by fragments of two or three neighboring fibers) nodes revealed not only short-term (1.2 msec), but also long-term (48 msec) AD in them [1]. It was postulated on the basis of these findings that the appearance of long-term AD in myelinated nerve fibers with the morphological structure of their Ranvier nodes intact ("concealed" and "covered" nodes) was due to the accumulation of outflowing K^+ in the juxtamembranous space, separated from the external solution by a diffusion barrier formed by processes of Schwann cells and the basement membrane. In single nerve fibers with an "uncovered" node [1, 7], however, this barrier is evidently disturbed during dissection. The problem of whether K^+ can accumulate in the juxtamembranous space of intact nerve fibers assumes great importance in connection with existing views that these ions play a trigger role [8] in metabolic and energetic interactions of various types between the neuronal membrane and Schwann cells.

New experimental evidence of the accumulation of K^+, flowing outward during activity, in the juxtamembranous space of myelinated nerve fibers is presented in this paper.

EXPERIMENTAL METHOD

Experiments were carried out on single nerve fibers of *Rana ridibunda* with "uncovered," "covered," and "concealed" nodes [4], prepared by Tasaki's method [3].

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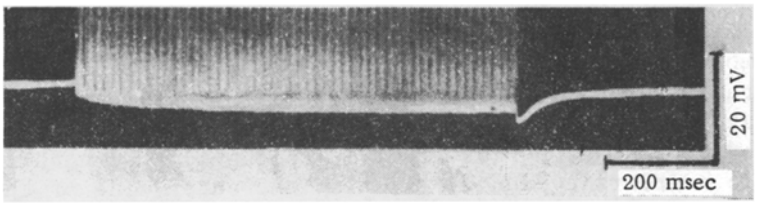

Fig. 1. Successive increase in after-hyperpolarization during repetitive stimulation of single nerve fiber with "uncovered" node and kept in potassium-free Ringer's solution. Frequency of stimulation 100 Hz.

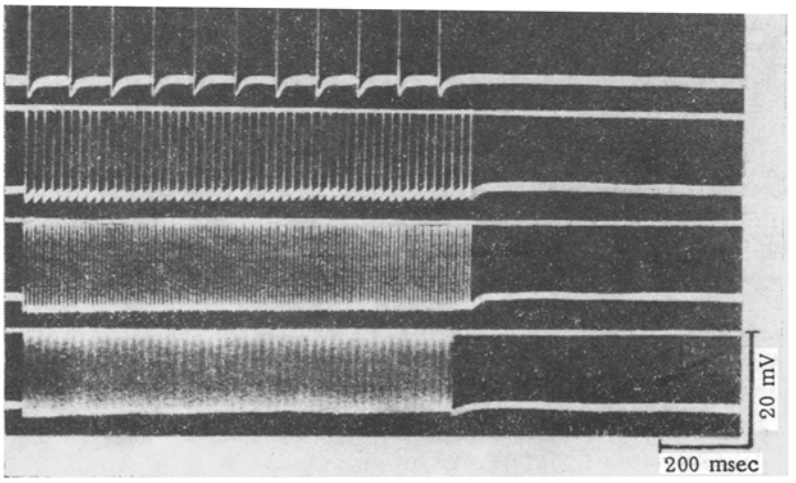

Fig. 2. Decrease in amplitude of after-hyperpolarization of single nerve fiber with "covered" node during each repetitive stimulation. Frequency of stimulation from top to bottom 10, 30, 50, and 100 Hz consecutively. Node bathed with potassium-free Ringer's solution.

EXPERIMENTAL RESULTS

During repetitive stimulation of the isolated frog nerve fiber with an "uncovered" node a shift of membrane potential toward hyperpolarization took place [1, 7]. After the end of repetitive stimulation membrane hyperpolarization increased to 3.2 ± 0.4 mV [1]. On removal of K^+ from the surrounding solution, AH developed after every spike. Its amplitude was 2.7 ± 0.18 mV [2].

During repetitive stimulation of a nerve fiber in potassium-free Ringer's solution a successive increase in after-hyperpolarization also took place (Fig. 1), and when the stimulation ended it increased by a further 1-3 mV. The onset of after-hyperpolarization in all these cases was connected with an over-all increase in the potassium permeability of the membrane. Blocking the potassium channels of the membrane by external application of tetraethylammonium [2] and by intra-axonal injection of Cs^+ ions led to the abolition of after-hyperpolarization.

During stimulation of the "covered" node, on the other hand, the directly opposite picture was observed: After-hyperpolarization diminished successively and the combined resting potential of the membrane shifted toward depolarization (Fig. 2). This was particularly apparent at high frequencies of stimulation of the nerve fiber. The same picture also was found with nerve fibers with a "concealed" node. This fact shows that in fibers with the structure of their Ranvier nodes intact, during repetitive stimulation the outward potassium current falls. This can be explained only on the grounds that K^+ ions leaving the fiber accumulate in the juxtamembranous space of the intact Ranvier node, separated from the external solution by some form of barrier. The same suggestion was put forward previously by Frankenhaeuser and Hodgkin [5] to explain the decrease in after-hyperpolarization of the squid giant axon which they observed during repetitive stimulation. In voltage clamp experiments these workers in fact found a "tail" of inward potassium current, with the same time constant of decline as AD, after the depolarizing stimulus.

In a study of the nature of the "nonspecific current" (I_p) of myelinated frog nerve fibers, Dubous and Bergman [4] obtained evidence that its origin is linked with K^+ accumulation in the juxtamembranous space of the Ranvier node [4]. The direction of I_p is inward and its temporal parameters coincide with those of the short-term AD. These facts are weighty evidence that I_p lies at the basis of the short-term AD of myelinated nerve fibers. The data presented by Dubous and Bergman [4], in conjunction with those examined above, indicate that two ionic barriers evidently exist on the outer side of the membrane in the region of the intact Ranvier node. The first, located nearer to the excitable membrane, has relatively high ($6 \cdot 10^{-3}$ cm/sec) potassium permeability [4]. It is responsible for the generation of I_p and the short-term AD associated with it. Most probably this barrier may be the "unstirred layer" of the outer side of the membrane. The second barrier, located on the outside of the first, has lower ($7.5 \cdot 10^{-4}$ cm/sec) permeability [1]. It is probably formed by processes of Schwann cells and the basement membrane. The second barrier is easily destroyed during dissection of single nerve fibers with an "uncovered" node, whereas under these circumstances the first barrier is evidently completely preserved (the time constant of the short-term AD is the same for "concealed" and "uncovered" nodes).

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