# GENERATION OF ACTION POTENTIALS IN SINGLE NODES OF RANVIER OF ISOLATED FROG NERVE FIBERS UNDER THE INFLUENCE OF NICKEL AND CADMIUM IONS*

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In recent years considerable progress has been made in the study of the ionic permeability of the nerve membrane during excitation. However, the mechanism of these changes and the nature of the underlying processes remain unexplained [3, 5]. Here we present results which seem to us to be important in connection with the ionic mechanism of the generation of action potentials (AP) in single nodes of Ranvier of isolated frog nerve fibers.

## EXPERIMENTAL METHOD

The work was carried out on isolated nerve fibers from the sciatic nerve of Rana temporaria. A fiber was placed on two air insulator bridges [16]. A diagram of the arrangement for polarization, stimulation, and for leading off potentials from a single node of Ranvier N are shown in Fig. 1. A dc amplifier with a cathode follower input was employed. For stimulation and polarization we used direct current pulses of variable strength and duration. All solutions were made up with normal Ringer. The experiments were continued from May 1962 to April 1963.

## EXPERIMENTAL RESULTS

The influence of Ni and Cd ions on the duration of the phase of repolarization of the AP. It has been shown that ions of Ni, Co, and Cd prolong the repolarization of the AP forming a marked plateau, which in some cases is of considerable duration [4, 9, 11-15, 17, 18]. Our experiments confirmed these findings. We also found that under the influence of Ni the duration of the AP plateau increases with increase in the strength and duration of the stimulus (Fig. 1, A and C). Cooling potentiates this effect in the case of Ni a constant cathodic potential or by an excess of potassium ions reduces the duration of the plateau (Fig. 1, B). A similar effect is observed when the condition of the node deteriorates during a prolonged experiment. On the other hand when the membrane is hyperpolarized by an anode the duration of the plateau is as a rule considerably increased (Fig. 1, D). In this respect the results of our experiments differ from those of Takahashi and his-co-workers [15], who found no changes in the duration of the plateau during an electronus.

Change in the critical level of depolarization. Nickel and particularly cadmium ions raise the threshold of excitation (rheobase) of a node of Ranvier. As our experiments have shown the cause of this increased excitability is an increase in the critical level of depolarization of the membrane. Evidence of this increase is the fact that under conditions in which Ni and Cd are present there is no change of the resting potential, but there is a considerable increase in the threshold change of membrane potential (depolarization threshold) necessary for the initiation of an action potential.

Changes in the rate of rise and in the amplitude of an AP. Nickel and cadmium ions ($10^{-3}$-$5 \cdot 10^{-3}$ M $NiCl_2$ and $CdCl_2$) cause some reduction in the rate of rise of the AP, and as a rule cause an increase in amplitude,†

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† We measured the amplitude of the AP from the critical level of depolarization of the membrane to the top of the spike, and the level of the top of the spike was calculated from the initial level of the action potential.

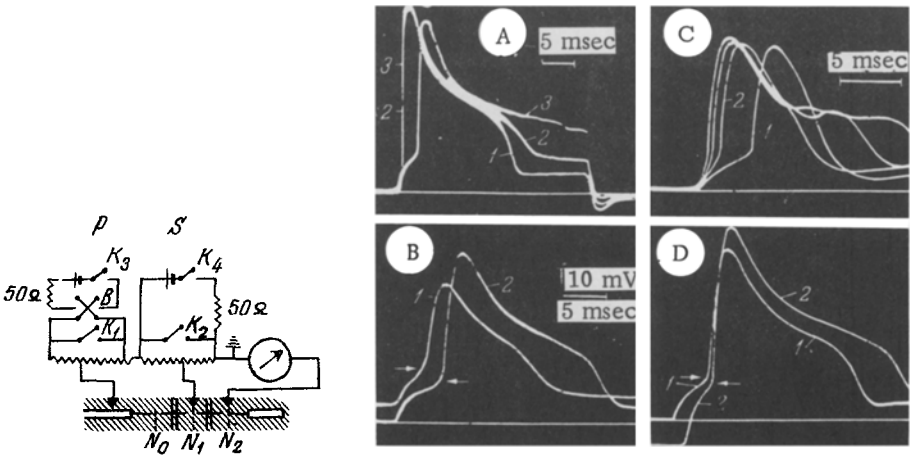


Fig. 1. Diagram of the experimental set-up. Nodes $N_0$ and $N_2$ in 0.2% novocaine solution. A direct current for polarization (circuit P) and for stimulation (circuit S) are adjusted in strength by means of potentiometers, and in duration by a Helmholtz pendulum (keys $K_1$, $K_2$, $K_3$, and $K_4$). The nonpolarizable electrodes consist of $Cu-CuSO_4-$ kaolin. The internodes of the nerve fiber lie on air bridges as insulators. The width of the middle section of the chamber in which the node $N_1$ lies is 0.8 mm. A) Effect of a constant stimulating current on the duration of the AP plateau in $10^{-3}$ M $NiCl_2$. The records are superimposed on a single frame. Stimulus strength 1, 1.5, and 2 times the rheobase (curves 1, 2, 3). Experiment made on December 13, 1962, temperature 10°; B) ditto, experiment on October 26, 1962, temperature 20°. As the current strength increases during the repolarization phase slow waves of depolarization appear; B) influence of subthreshold cathodic stimulation on the critical level of depolarization (indicated by an arrow), and on the amplitude and duration of the AP of the node in a $10^{-3}$ M $NiCl_2$ solution; D) ditto under anodic influence. 1) Before switching on the polarizing current; 2) one second after the onset of polarization. Displacement of the baseline of curve 2 indicates the shift of the resting potential under the influence of the applied current.

particularly in the height of the peak. The increased amplitude of the AP is greater the smaller its initial value. This observation led us to undertake experiments on nodes of Ranvier whose AP had been either reduced or completely suppressed by various agents.

The influence of Ni and Cd ions on nodes of Ranvier treated with novocaine, with an excess K, with solutions poor in Na ions, or damaged mechanically. Nodes of Ranvier treated with 0.01% novocaine are unable to generate an AP. Stimulation of such a node evokes only local responses which increase gradually with increase of stimulus strength (Fig. 2B). The addition to the novocaine solution of up to $10^{-3}$ M $NiCl_2$ restores the ability of the node to generate an AP. Then the resting potential remains unchanged (Fig. 2C). The threshold of depolarization and the duration of the recovery of the AP are considerably increased in comparison with their original value (Fig. 2A and C), but similar changes occur also under the influence of Ni and Cd ions on intact nodes. When the sequence of actions is reversed, i.e., when to a solution of $10^{-3}$ M $NiCl_2$ or $CdCl_2$ novocaine of strength up to 0.01% is added there is some reduction in the amplitude and the duration of the AP; however the threshold of depolarization usually remains unchanged.

Ni and Cd ions are able to restore the power of nodes of Ranvier which have been damaged mechanically by careless preparation to generate action potentials. Of course the effect achieved depends upon the extent of the damage (Fig. 2D, E, and F).

An increase of K ions in the solution leads to depolarization of the membrane and to a reduction in amplitude of the AP (Fig. 3B). The addition to a solution containing an excess of K of up to $10^{-3}$ M $NiCl_2$ causes a considerable increase in the amplitude and duration of the AP despite the fact that depolarization of the membrane brought about by an excess of K ions is not thereby eliminated (Fig. 3D).

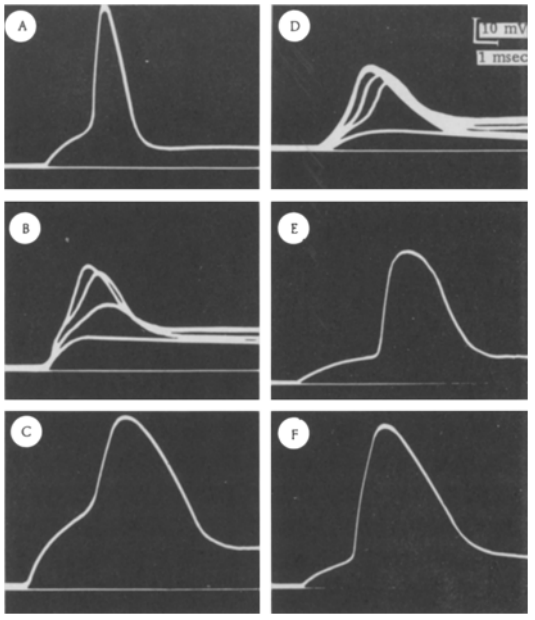


Fig. 2. Recovery of the power to generate an action potential in a damaged node of Ranvier; A) AP of a node in Ringer, stimulus of rheobase strength; B) local responses of the same node in a 0.01% novocaine solution, the responses increase with increase of stimulus strength; C) effect of replacing the novocaine solution by a solution of novocaine and $10^{-3}$ M $NiCl_2$; D) responses of a node damaged mechanically by insufficient care in the preparation; E) one minute after addition of $5 \cdot 10^{-3}$ M $NiCl_2$ to this node; F) ditto, after 5 minutes.

These established facts run counter to the findings of Takahashi and his co-workers [15] who found that in the presence of cobalt and nickel urethane, cocaine, and potassium chloride bring about considerably less change in amplitude of the AP than is caused under normal conditions (experiments on nerve fibers of the Japanese toad).

Of the treatments which we used it was only a reduced concentration of Na ions in the medium (down to 5-10% of the original concentration) which caused a reduction in the amplitude of the AP, which could be restored neither by the additions of Ni or Cd ions nor by the application of a strong direct current from an anode.

Influence of Ni and Cd ions on changes of the critical level of depolarization of the membrane and on the AP during electrotonus. Cathodic depolarization of the critical level and to a reduction in the height reached by the peak of the AP, whereas anodic hyperpolarization causes a reduction of the first index and an increase of the second [12].

Under the influence of Ni and Cd ions these changes are greatly weakened (Fig. 3E). A reduction in the changes in the critical level and AP under the influence of the cathode are due to an increased stability of the membrane to the depressive action of prolonged depolarization. By contrast the reduction of the anodic changes of the AP are apparently due to the fact that Ni and Cd ions themselves greatly increase the height of the peak of the AP, raising it in many cases to a value close to the maximum potential obtained for the given node under the influence of a strong anodic current. Therefore application of an anode after the action on the node of Ni or Cd cannot evoke any further considerable increase in the AP. Evidence for the correctness of this explanation is afforded by the fact that reduction of the influence of the anode in the presence of Ni or Cd is better shown the greater the amplitude of the AP.

All the effects of Ni and Cd considered are eliminated by $10^{-2}$-$10^{-3}$ cysteine.

According to a widespread view [5, 8] the phase of depolarization of an AP in a nerve fiber is due to an increased permeability to sodium ($P_{Na}$), whereas the phase of repolarization is related to a cessation of $P_{Na}$ and to a limited increase of potassium permeability ($P_K$). Experiments carried out by clamping the membrane potential, and theoretical calculations enabled Dodge [5] to explain the changes in the shape and duration of the AP under the influence of nickel ions to a reduction (approximately by half) of the velocity constants of the changes of $P_{Na}$ and $P_K$ during depolarization. In particular the marked extension of the phase of repolarization and the appearance of a long plateau were associated with a delay in the development of inactivation and of increase of $P_K$ [9]. From this point of view it is evidently understandable that the increased stability of the node of Ranvier to the suppressive influence of the cathode which we have discovered can be understood because the cathodic depression (elevation of the critical level of depolarization of the membrane and reduction of the AP) is usually interpreted as a consequence of inactivation and increase of $P_K$ [2, 5, 8].

However the facts described concerning the restorative and enhancing influence of Ni and Cd ions on modified nodes seems to us to indicate that in the action of these ions not only is there a reduction in the rates of development of inactivation and of increase of $P_K$ but that inactivation, which is already under way, is greatly reduced. It is known that the strength of the rising sodium current ($I_{Na}$) is greatly reduced during excitation under the influence of novocaine [10], mechanical damage to the fiber [6, 8], or by depolarization of the membrane by an excess of K ions [7]. In the terminology of Hodgkin and his followers, these actions reduce that fraction (h) of maximum sodium

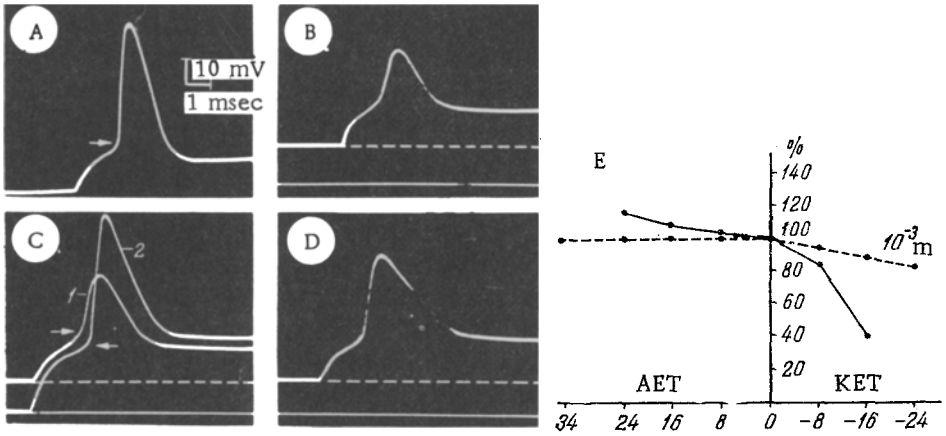


Fig. 3. Effect of $10^{-3}$ M $NiCl_2$ on an AP at a node of Ranvier treated with an excess KCl in Ringer. A) Initial value of the AP; B) response of the node in 30 mM KCl. Displacement of the baseline (dotted line) shows the degree of depolarization of the membrane by an excess of potassium ions; C) recovery of the AP of the node during repolarization of the membrane by direct current from an anode. Lower continuous line – initial level of the resting potential. 1) Before application of the anode; 2) during anodic repolarization of the membrane; D) increase in amplitude of the AP during replacement of the KCl solution by a mixture of KCl and $10^{-3}$ M $NiCl_2$. The AP of the node increases despite the fact that the depolarization of the membrane brought about by the KCl is not eliminated by the Ni ions; E) effect of $10^{-3}$ M $NiCl_2$ on the electrotonic changes in amplitude of the AP. Abscissa – strength of polarizing current. KET – catelectrotonus, AET – anelectrotonus. Ordinate – amplitude of AP.

permeability of the membrane which may be activated by stimulation (sudden depolarization). The ions of Ni and Cd apparently enhance h, and reduce the degree of inactivation of the I – h membrane, which also has the effect of increasing the amplitude, or of restoring the AP in a modified node.

This effect may also be brought about by the reduced rate of increase of $P_K$ due to ions of Ni and Cd during excitation. However the value of this factor could not be ascertained without further investigations.

The fact that ions of Ni do not restore the AP of nodes in a medium deficient in sodium ions agrees well with the hypothesis advanced here, because the cause of such a reduction of the AP is a reduction in the concentration of the gradient ($Na_{external}/Na_{internal}$); the cause is not an increase in the initial inactivation of the (I–h) membrane, because this takes place under the influence of novocaine and other agents [1].

Hodgkin and his co-workers have not proceeded beyond a formal mathematical description of the processes of inactivation and change of $P_K$. However the question of the nature of these processes remains unexplained. The fact that Ni, Co, and Cd ions bind SH-groups of proteins (formation of weakly dissociated complexes of the type S–Ni–S) while cysteine reduces the physiological effects of these ions [1] constitutes a reason for supposing that some physicochemical processes involving sulfhydril groups is responsible for changes of ionic permeability of the membrane during excitation.

The classical agent for restoring the AP of a modified nerve fiber has always been anodic current. According to Hodgkin and Huxley [8] its action depends on its ability to eliminate inactivation (reduction of I–h) and to restrict the outflowing current of potassium. However in anodic action these changes are secondary, and result from hyperpolarization of the membrane. In contrast to this ion, Ni and Cd do not change the resting potential, but apparently exert a direct inhibitory action on the chemistry of these processes.

## SUMMARY

By experiments on single nodes of Ranvier of isolated nerve fibers of the frog we showed that in addition to a marked increase in the duration of repolarization after an action potential Ni and Cd ions also caused a rise in the critical level of membrane depolarization, an increased action potential level, an increase of amplitude of action

potential, and a somewhat reduced steepness of its rising phase. Ni and Cd ions restored the ability to generate action potentials in nodes of Ranvier which had been modified by a 0.01% procaine solution, by an excess of potassium ions (30 mM/l KCl) or slightly injured mechanically during dissection. It was only when the Na concentration in the medium was reduced that the action potential could be restored. Ni and Cd ions greatly slowed and reduced the cathodic rise in the critical level and reduced the amplitude of action potentials. Cysteine ($10^{-2}$-$10^{-3}$ M) eliminated all the effects of the ions we have mentioned. We suggest that by binding the SH-groups of the proteins of the nerve fiber, nickel and cadmium reduce the rate of inactivation and the increase of potassium permeability during polarization, and, in addition, weaken the initial inactivation of the membrane (I—h), whenever this was brought about by modifying influences.

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