THE EFFECT OF NITRATE AND THIOCYANATE IONS ON THE RESTING AND ACTION POTENTIALS OF COBALT-TREATED SINGLE NODE OF RANVIER

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The prolongation of the action potential of the single myelinated nerve fibre has been observed for some years (Tasaki, 1957; Brady and Spyropoulos, 1955; Takahashi et al., 1958 and 1960; Spyropoulos, 1961). Among the prolonged action potentials investigated in many other tissues, it has been known in the cardiac and skeletal muscles that anions play an important role for the prolongation (Lubin, 1957; Falk and Landa, 1960a and b; Brady and Woodbury, 1960; Hutter and Noble, 1961). Therefore, it can be supposed that at least, some part of the prolongation of the action potential of myelinated nerve fibre treated with cobalt or nickel ions could be affected by various anions. In the present experiment, the effect of nitrate and thiocyanate on the action potential of the single node of Ranvier under cobalt application was investigated mainly.

On the other hand, though there are some interpretations on the prolongation of the action potential of cobalt- or nickel-treated nerve (for example, Tasaki, 1957; Takahashi et al., 1960), the mechanism is still far from the complete understanding. The authors studied this problem and presented a speculative interpretation on it.

Also, the reports that the depolarization which was brought about by high concentration of potassium could be restored by nickel or TEA application (Tasaki, 1959; Böhm and Straub, 1962) were reinvestigated and the effect of chloride and other anions on this phenomenon was studied.

METHOD

A single nerve fibre was dissected from the sciatic nerve of the frog (Rana nigromaculata). The experimental set-up was the same with that of the previous paper (Hashimura, 1962).

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The normal Ringer solution contained 112 mM NaCl, 2 mM KCl, 2 mM CaCl$_2$ and 2 mM NaHCO$_3$. Chloride-deprived solutions were prepared by replacing chloride with equimolar nitrate or thiocyanate keeping the cations unchanged. Cobalt-added solutions were prepared by adding solid CoCl$_2$ to the normal Ringer and $\text{Co(NO}_3\text{)}_2$ to the chloride-deprived solutions respectively.

The experiments were carried out from 1961 to 1962.

RESULTS

When chloride ions in Ringer were replaced by nitrate, there took place a few millivolts increase of the resting potential (Fig. 1) and a slight decrease of the height of the action potential (Fig. 2). When the solution was thiocyanate Ringer, both the hyperpolarization and the decrease of the spike height became prominent (Fig. 1 and 2). Since the recording electrode (Ag-AgCl) is connected through a Ringer phase, there is a liquid junction which produces a junction potential between the test solution and the electrode when the composition of the irrigating solution is changed. The liquid junction potentials can be calculated from HENDERSON's equation and are 2.3 mV for the nitrate

![Figure 1](image-url)

*Fig. 1.* The upper traces show changes of the resting potential in normal Ringer, nitrate and thiocyanate solutions with or without cobalt. The lower traces in each picture indicate changes of the solution from the normal Ringer to the test solution and then to the former again, all in drops. After about thirty seconds irrigation with the test solution, the normal Ringer was applied again. Upper deflection shows depolarization. All the records are the actually obtained ones, being made no correction for the junction potential.
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FIG. 2. Action potentials in normal Ringer, nitrate and thiocyanate solutions with or without cobalt application. Three different action potentials from the same fibre were superimposed on the same base line without regard to the resting potential. The stray capacity was compensated insufficiently to show the difference of the height of the action potential clearly. R, NO₃ and SCN in the figure represent normal, nitrate and thiocyanate Ringer solutions, respectively. Cobalt concentrations were shown in molar in each record.

and 5.0 mV for the thiocyanate, respectively. These values of the junction potentials are smaller than the potential shifts produced by nitrate or thiocyanate solutions without cobalt or with its low concentrations, as is seen in Fig. 1. Therefore, it is likely that an actual hyperpolarization was brought about by replacing chloride with nitrate or thiocyanate, but no further detailed experiment on this matter was carried out. Although the height of the action potential decreased in the Cl-deprived solutions, the duration of the action potential was hardly influenced, but it seemed to show a slight prolongation. When cobalt ion was added to either normal Ringer or Cl-deprived solutions, there could be seen a marked prolongation of the action potential (Fig. 2 and 3). The optimum concentration for the prolongation was at around the concentration of 10⁻⁴M Co⁺⁺, and above which there was seen a shortening as has been reported by TAKAHASHI et al (TAKAHASHI et al, 1960). In Fig. 2 and 3, one can notice two remarkable points produced by cobalt application; one is the increase of the spike height and the other is the enhanced prolongation of the action potential. In Fig. 2, the action potentials in normal Ringer, nitrate and thiocyanate solutions at certain cobalt concentrations were superimposed.
Fig. 3. Superimposed action potentials in normal Ringer, nitrate and thiocyanate solutions. Each photography is the same with that in Fig. 2. Cobalt concentrations were shown in each record in mole.

Apparently the prolongation is more significant in the chloride-deprived solutions than in normal Ringer in every cobalt concentration. In the figure, one can notice that the height of the shoulder (beginning of the steeper repolarization of the action potential) is higher in order of normal Ringer, nitrate and thiocyanate solutions. When the concentration of cobalt ion was too high, the prolongation became less remarkable; there occurred rather a shortening of the prolongation and in concomitance with it one could note a higher shoulder level. When the shoulder level was compared in the same test solution with various concentrations of cobalt, no marked change could be seen except in very high cobalt ion concentrations (Fig. 3). The repolarization phase after the shoulder was slower in order of normal Ringer, nitrate and thiocyanate solutions. These results may suggest that nitrate and thiocyanate ions are less permeable than chloride ions across the membrane of the node of Ranvier.
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FIG. 4. Effect of membrane potential shift on the prolongation and the height of the shoulder in various concentrations of cobalt in normal Ringer. Normal, cathodal and anodal represent the membrane potential level in their usual sense. Cobalt concentrations were shown in each record in mol.

When the membrane potential level was shifted to either depolarization or hyperpolarization, there could be seen a slight change of the shoulder level; i.e., the shoulder became higher in hyperpolarization and lower in depolarization showing a slight change of duration of the action potential (Fig. 4). However, in this case the relation between the shoulder level and the extent of the prolongation is very slight.

When the sodium concentration of the medium was reduced, the prolongation by cobalt was affected significantly. The lower the sodium concentration was, the shorter the prolongation became (Fig. 5). Without cobalt addition, the duration of the action potential was hardly affected by sodium depletion until the sodium concentration was about 20 mM, but with cobalt ion the duration of the prolonged action potential was shortened already at 50 mM of sodium, though it was longer than that of the normal action potential.

As shortly stated before, the replacement of chloride by nitrate or thiocyanate decreased the height of the action potential, but cobalt application restored it (Fig. 2 and 3). There is a possibility that the sodium carrier system is in inactive state under Cl-depletion, though the mechanism is obscure, so the height of the action potential in the Cl-deprived solutions is lower. If cobalt facilitates activation of the sodium carrier system, recovery of the height of
the action potential will take place. This possibility was tested by measuring the spike height under either anodal or cathodal current application in various solutions, the result being presented in Fig. 6. In the normal Ringer solution, the spike changed its height along the continued line in Fig. 6 and the small effect by hyperpolarization showed that the sodium carrier system was almost in full activation in the normal condition. In the chloride-deprived solutions (broken and dotted lines), on the contrary, the action potential was highly influenced by hyperpolarization and restored its height up to the level of normal action potential, but became inexcitable easily by smaller depolarization. The situation corresponded to the shift of the curve of normal action potential to the left side. When cobalt was applied, every curve shifted to the right in the figure (crosses). This phenomenon seems to show that cobalt activates sodium carrier system like anodal current application. The maximum spike height did not change by cobalt application, so it can be concluded that cobalt makes the inactive sodium carrier system active in some obscure way.

One more point in the chloride-deprived solutions is the decrease of the rheobasic voltage. Observing the critical membrane potential level for firing, it could be seen clearly that the firing level decreased significantly. Since the
The height of the action potential and the membrane potential level. The ordinate shows the spike height, that of the normal action potential in Ringer being taken as one hundred percent. The abscissa is the membrane potential level in millivolts; positive and negative signs show hyperpolarization and depolarization respectively, the zero point indicating the resting potential. The dots and crosses represent without and with cobalt in 10^{-3}M, respective solutions as indicated.

rheobasic voltage can be regarded as indicating a relative value of the critical membrane potential for firing, rheobasic voltages in normal Ringer, nitrate and thiocyanate solutions were measured. An example is shown in Fig. 7. In the Cl-deprived solutions, the membrane behaved as if it were under catelectrotonus, but even after restoration of the spike height by cobalt application there could be seen still a marked decrease of rheobasic voltage compared with that in normal Ringer. Therefore, it may be natural to suppose that in the nitrate and thiocyanate solutions, there is some mechanism which reduces the critical membrane potential level for firing.

Depolarization by isotonic KCl or by KNO₃ showed no marked difference, and when cobalt was applied in various concentrations the depolarization decreased slightly according to the cobalt concentration (Fig. 8). However, the depolarization by isotonic KCl became discontinuous at around 10^{-2}M of cobalt. As is seen in Fig. 8, the depolarization by KCl was small when the concentration of cobalt was high, and at 1.5×10^{-2}M of cobalt the initial small depolarization increased suddenly and shifted to the higher level showing a step. Thus, discontinuity in K-depolarization in Fig. 7 is seen. Contrary, when KNO₃ was used, no marked transition was found at least at around the same concentration as of cobalt (Fig. 7 and 8). These results show that the depolarization
FIG. 7. K-depolarization (right upper), duration of the action potential (left upper) and rheobasic voltage (right lower) were plotted against cobalt concentration in the abscissa. The ordinates were shown in respective blanks. As for the K-depolarization, the straight and the broken lines indicated in the upper most part of the figure.

by excess potassium is influenced by cobalt, and that the influence becomes more significant when chloride is replaced. Therefore, it can be concluded that potassium permeability is influenced by cobalt ion and that chloride takes some marked role for the membrane potential.

The main results in the experiments were summarized and presented in a graph (FIG. 7) in which the duration of the action potential, depolarization by the isotonic potassium and rheobasic voltage were plotted against the cobalt concentration.
DISCUSSION

In several reports (Brady and Woodbury, 1960; Hutter and Noble, 1961; Peterson and Feigen, 1962; Lubin, 1957; Falk and Landa, 1960a and b) the duration of the action potential in other tissues had been investigated by replacing chloride with permeable or impermeable anions, and the results have been discussed mainly with anionic permeabilities, though some of the results in these papers were investigated in some other ways. As described in Results, it has been suggested in the present investigation that nitrate and thiocyanate ions are less permeable than chloride ions across the membrane of the node of Ranvier. Therefore, it can be considered that in the mode of Ranvier, replacement of chloride with less permeable anion like nitrate or thiocyanate would decrease the chloride equilibrium potential retarding partly the repolarization. Thus, part of the prolongation in the chloride-deprived solutions can be understood by considering decreased anionic permeabilities.

However, the prominent prolongation as a whole in cobalt-treated nerve must be explained on one or more other principles, because the anionic contribution for the prolongation is rather small. As the effect of cobalt on the
spike height is very similar to that of hyperpolarization, it could be supposed that cobalt either activates the sodium carrier system or reduces its inactivation. Since the inactivation appears significantly in low sodium concentration, the decreased effect of cobalt in low sodium suggests that the inactivation makes the prolongation smaller. Because the full activation by hyperpolarization does not induce the prolongation in the normal action potential, the prolongation of the action potential in cobalt-treated nerve will be induced probably by the reduction of inactivation of sodium carrier system, though further investigation is necessary for decision and clarification of the mechanism. This consideration is very similar to that in the cardiac muscle in which sustained activation of sodium carrier system was considered (BRADY and WOODBURY, 1960). Another factor for prolongation of the action potential to consider is the retarded and decreased potassium permeability during the action potential. As will be discussed later, the potassium permeability was depressed significantly by cobalt in the resting membrane, so the possibility seems to remain. However, as some reports showed that potassium did not play any role in the repolarization of the action potential of the node of Ranvier (TASAKI and FREYGANG, 1955) or that the muscular action potential did not change even when intracellular potassium concentration was low (KOKETSU and KIMURA, 1960), the discussion on it will be reserved here.

According to the ionic theory, the action potential is initiated when the inward sodium current exceeds outward current. So, the chloride deprivation would make the firing level lower because the countering action of chloride against sodium is taken off. Decrease of the rheobasic voltage, or initiation of spontaneous activity or increase of the rhythm in spontaneously active membrane have been presented either in skeletal or cardiac muscles (CARMELIET, 1961; HUTTER and PADSHA, 1959; PETERSON and FEIGEN, 1962; FALK and LANDA, 1960a and b) and the mechanism has been interpreted in most cases with the depolarization. Though depolarization by chloride depletion in the node of Ranvier could not be observed, the experiment here presented might be still insufficient. The underlying mechanism could be investigated in the same way with these tissues.

The inhibition of potassium depolarization by either nickel or by cobalt (TASAKI, 1959) can be explained if the selective inhibition of potassium permeability by these metal ions is considered. The potassium depolarization results mainly from the change of potassium equilibrium potential and the potassium permeability. In the skeletal muscle, it has been proved that the chloride permeability is appreciable; hence it seems that chloride can play the main role in determining the membrane potential in some conditions (HODGKIN and HOROWICZ, 1959; ADRIAN, 1960). Therefore, if potassium permeability undergoes selective inhibition, making the chloride permeability relatively greater, the chloride equilibrium potential contributes to the membrane potential
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significantly. As the chloride equilibrium potential is near the normal resting potential in the normal chloride concentration, the membrane potential will remain near the resting potential when the potassium permeability is very low, resulting in a tremendous inhibition of potassium depolarization. On the other hand, the potassium permeability increases with the increase of depolarization, so it will develop regeneratively beyond a certain level of membrane potential. The effect of cobalt on the depolarization by KCl can be thus explained. If the chloride is replaced by less permeable anion like nitrate the chloride equilibrium potential will shift. Hence the loss of the inhibition in KNO₃ is expected.

SUMMARY

1. Using the single node of Ranvier, the effect of nitrate and thiocyanate ions with or without cobalt ion was studied.
2. The substitution of chloride ions by these less permeable anions resulted in decrease of the critical membrane potential for firing and decrease of the spike height.
3. The prolongation of the action potential by cobalt was enhanced in the nitrate or thiocyanate Ringer.
4. Chloride depletion is resistant to the inhibition of potassium depolarization by cobalt.
5. These results can be explained by the consideration that chloride permeability plays an important role in the node of Ranvier.
6. The prolongation of the action potential by cobalt application can be explained speculatively by supposing that the inactivation of sodium carrier system is inhibited by cobalt.
7. From the inhibition of potassium depolarization by cobalt, the selective inhibition of potassium permeability was concluded.

REFERENCES


