AN APPLICATION OF A NEW METHOD FOR RECORDING THE ACTION POTENTIAL OF THE SINGLE MYELINATED NERVE FIBER TO DETECTING THE COBALT-ION EFFECT

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The action potential of the single myelinated nerve fiber has been heretofore recorded as a current flow along the longitudinal axis of the fiber, using the so-called Tasaki's bridge insulator method. In this method a great magnification of the amplifier is required as the current thus led is extremely small.

Stämpfli (1), Tasaki and Frank (2) devised a new recording method capable of obtaining a height of the spike as great as that obtained by the intracellular recording technique. Their method is the same in principle as the intracellular microelectrode technique.

The use of the intracellular microelectrode was proved not always suitable by several authors (3, 4, 5), since the impairment by the insertion of the electrode into such a small fiber is great. The authors adopted a new method of recording the action potential extracellularly using the pre-amplifier made for the intracellular microelectrode. The new method enables us to record the potential as great as the source of the action potential itself. It was found that some heavy-metal ions have specific action on the node of Ranvier. In this paper the abnormal prolongation of action potential of the single myelinated nerve fibers of toad and heavy-metal ion effects on them is mainly dealt with.

EXPERIMENTAL METHOD

Isolation technique of the single nerve fiber: Tasaki's orthodox method, simplified by Ichioka, was used. The same nerve trunk could repeatedly be used for the preparation of single fibers. When the technique failed at a portion of nerve trunk, another preparation could be successfully made at another portion.

Recording method of the action potential: The new type of the pre-amplifier devised by Dr. Matsuo, Tohoku University, was used. This was originally designed for the intracellular electrode. The wave-form distortion by the big electrode-resistance of more than 300 MΩ was perfectly compensated by negative capacity type head-amplifier, as shown in fig. 1. Bridge insulator and the pre-amplifier above-mentioned enable us to pick up the source potential of the single fiber, as has been beautifully explained by Tasaki (2). Fig. 2 shows the circuit diagram of the pre-amplifier A and other equipments B and C.

Received for publication April 8, 1958.

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319
K. UCHIZONO AND Y. MATSUMOTO

It has been reported that the optimum value of the intracellular electrode is 20-50 MΩ. The resistance of the single nerve fiber mounted on the bridge insulator varies proportionally to the length of the air-gap. According to Tasaki the resistance of the single nerve fiber is 290 MΩ/mm.

FIG. 1. Compensation of the square-pulse deformation.
A: No compensation, input resistance 100 MΩ (pure resistor).
B: Compensated square pulse by adjusting the capacitor—C in fig. 2 (arrow).
Time-mark 1 msec.

FIG. 2. Set-out of the pre-amplifier and its accessories.
A: Three-stage pre-amplifier. Arrow shows the compensator.
B: Adjustor of the resting potential.
C: Eliminator source of the amplifier.

This extracellular recording method enables us, theoretically at least, to pick up the potential of the source of the action potential through the single nerve fiber of which the resistance is enormously high.

Generally it is very difficult to use the high resistance electrode owing to floating capacity in the input circuits. Tasaki (2) eliminated this trouble by his ingenious "driven shield." The authors avoided the difficulty by employing a new "negative capacity type" pre-amplifier of Dr. Matsuo.
RESULTS

The simple dissection method adopted here might seem to be too rough to get a good single fiber preparation, but it was proved that the sample thus prepared had good activity for a long time. For example, single fibers dissected by this method showed the action potential of more than 100 mV for more than 24 hours.

**Normal action potential of the single nerve fiber:** The action current led off from a single nerve fiber was in the order of $10^{-6}$ A (6). It was predicted and later actually demonstrated by Tasaki (5, 6, 7) and others (1) that the action potential of the single myelinated nerve fiber was in the order of 100 mV. The resistance of the single fiber mounted on the air-gap of the bridge insulator was in the order of $10^8$ Ω. Then it is naturally deduced that the magnitude of the potential $E$ is

$$E = 10^{-6} \times 10^8 = 0.1 \text{ V}$$

The height of the action potential recorded by the method shown in the diagram in fig. 2 was more than 100 mV.

Fig. 3 shows the action potential of the single nerve fiber. The amplitude increased gradually by gentle fanning and attained the final value of about 100 mV or more (from A to E in fig. 3). But the height was again reduced gradually after stopping the fanning. This can be explained by the fact that the external resistance of the single fiber mounted on the air-gap varies according to the grade of its dessication.

**Fig. 3.** Increase of the spike-height by dessication.

The spike height of the single fiber was gradually increases as it is dessicated by gentle fanning of the internodal portion mounted on the air-gap, from A to D. These figures show the conducted diphasic action potentials. E is monophasic action potential, induced by increased dessication. Conduction of the impulse is blocked by decrease of the external current along the axis cylinder owing to the increase of the external resistance. Time-mark 1 msec. Calibration of the spike height 50 mV.

Fig. 4 shows the normal action potentials. A is the mononodal action potential, whereas in B the conducted impulses are shown along with the non-conducted monophasic spikes.

This seems to be direct evidence of the saltatory conduction of the nerve
impulse. The conduction can be easily blocked by gentle fanning, due to reduction of the outward current in the distal nodal membrane. In the critical condition the conduction and its block occurred alternately as shown in fig. 4, B.

The form of the mononodal action potential: As shown above it was easy to get a mononodal spike without the application of depolarising agents. This is preferable to protect the node of Ranvier from damage by drug action. In fig. 4 showing the typical action potentials of single fiber, it may be noticed that in the middle of the falling phase of the spike there exist slight humps (shown by arrows). Beside there is another smaller hump at the foot of the rising phase (B in fig. 4), which seems to be due to the action potential of the preceding node of Ranvier.

Negative after-potential: The normal action potential seems to have no appreciable after-potential as shown in fig. 3, 4. But sometimes the authors recorded action-potentials with clear-cut negative after-potential as shown in fig. 5, 6. The magnitude of this after-potential was in the range from several mV to 20 mV. The duration of the after-potential paralleled the magnitude of the negative after-potential (of fig. 5 A and B). These after potentials were summated by repetitive stimulation. This was shown in fig. 6. There was no positive after-potential as revealed by Gasser (8).

Cobalt-ion effect on the single nerve fiber: As was already reported by one (9) of the authors it often occurred that the duration of the single fiber response was enormously prolonged to reach the final value as great as 500 msec. The exact nature of this peculiar phenomenon remained to be seen. Spyropoulos (10) reported on the change in the duration of the action potential of the single nerve fiber. Del Castillo (11), Lorente de No (12) studied on the effect of various metallic ions on the nerve fiber. Takahashi et al. (13) revealed the plateau formation of the spike by the application of sulfhydryl reagent on nerve fibers.

The present authors had much interest to see whether the prolongation of the spike duration which was incidentally discovered by them had any relationship with drug actions. Fig. 7 shows the variation of the spike form by the
In normal spikes the remarkable negative after-potentials were often recognized. Its amplitude and duration were not constant and the exact measurement of the latter was impossible because of the asymptotic decrease of the potentials. Time-mark 1 msec.

Repetitive stimuli were given at the frequency of about 100 c/s. No positivity as reported by Gasser was recognized at the end of stimuli (arrow).

Cobalt chloride solution of relatively high concentration was added into the Ringer-pool in which a single fiber was maintained. Note the immediate appearance of dramatic configurations of the spike.

This wave-form deformation (A to D) was nearly completely recovered by the application of the cysteine solution (E). 50 mV calibration was shown after the spike (A and B).
Fig. 8. The effect of cobalt chloride solution on the node of Ranvier.

Two-step falling phase of the spike was clearly seen after the application of 1 drop of 1% cobalt chloride solution in Ringer pool in which the node of Ranvier was maintained. Voltage-calibration 100 mV.

Time-mark msec.

Fig. 9. I: After the application of cobalt chloride solution the normal spike as shown in fig. 4 was deformed in its characteristic form. Two successive stimuli were given at various intervals. Very small spike in height and duration was shown in C. II: No such small spike was found in this case. Time-mark 20 msec.
application of CoCl₂ solution. The change occurred almost instantaneously (within several seconds). The main deformation of the spike was the prolongation of the falling phase of the spike. No appreciable change was recognized on the rising phase. These changes were almost completely reversible. The wave-form deformation was recovered by rinsing the fiber with Ringer solution. The most interesting fact was that the recovery was especially prompt and complete when the cysteine solution (about 0.05-0.1%) was added in the Ringer pool. Fig. 8 shows the dramatic change in the spike form by the application of 1 drop of 1% CoCl₂ solution in the Ringer pool (about 1 ml.). On the falling phase of the spike there appeared two humps, the first hump at the middle of the falling phase and the second one at a height of one-half of the first hump. It is worth noting that cysteine, but not cystine, solution is effective for the recovery of the spike.

Recovery process of the spike: In general the duration of the spike was lengthened by application of cobalt chloride solution. Fig. 9, I, II shows the recovery process of these abnormal responses. It often occurred that the spike duration elicited by test shocks were relatively short compared with the first response evoked by conditioning shocks. When the shock intervals were lengthened to some limit, there appeared large spikes with all or none character. It was later revealed clearly by the double air-gap method (9) that the small spikes seen at the foot of the rising phase was due to the preceding node of

![Fig. 10. The separation of two spikes.](image)

Soon after the application of cobalt chloride solution about 0.1% the notch appeared at the rising phase of the spike (at about one-third of the main spike). With the lapse of time these notches became more remarkable and ended separated completely. Then the main impulse disappeared.

Voltage calibration 100 mV.
Time-mark 1 msec.
Ranvier. This is also clear in fig. 10. The rising phase with a step of the spike shows the summation of the spikes of the two nodes. Generally speaking, in normal spikes it is difficult to see clearly these humps. The authors could see these phenomena distinctly by application of cobalt chloride solution. It seemed to be due to the rise of the threshold of the spike and the lengthening of the latency of the action potential of node. The height of the step which seemed to be due to the preceding node of Ranvier was about one-third of the spike of the observed node (fig. 10). The direct effect of drug on the node of Ranvier was shown in fig. 11. When the drug was applied to the distal node in the diphasic action potential the prolongation of the spike-duration occurred separately on the positive phase of the spike as shown in B and D in fig. 11. A and C show the prolongation of the spike when the drug was applied to the proximal node.

**Fig. 11. Localization of the drug action.**

Upper: Cobalt chloride solution was given at a node or Ranvier. Two distinct notches appeared on the falling phase of the spike.

Lower: The drug was given at a distal node of Ranvier. The positive phase of the spikes which show the activity of the distal node was enormously prolonged without influencing the proximal node.

Time-mark 1 msec.

**DISCUSSION**

Stämpfli (1) and Henatsch (14) reported independently that the duration of the spike of the single nerve fiber was increased by the application of sodium solution of higher concentration. It is clear that the duration of the spike varies according to the temperature of the surrounding medium. For example, Schoepfle (15) recorded the spike of the longer duration of 10 msec. at 3° C.
It was also reported by Tasaki (7) that the duration of the spike was prolonged by application of such drugs as sinomenine, brucine etc. Konishi (16) reported recently that the duration was lengthened by anelectrotonus.

The lengthening of duration of the spike reported by these authors was at the highest less than 10 msec. or so. The prolongation of the spike-duration reported here by the present authors was extraordinarily great. It was very much interesting that the prolongation of the spike-duration by CoCl₂ solution was reduced to its original value by the application of cysteine, but not by cystine. On this point, Takahashi et al. (17) reported an attractive hypothesis.

Throughout the experiment it was almost in the falling phase of the spike that the extraordinary prolongation of the spike-duration occurred. In the rising phase the authors could not recognize any appreciable change. It was impossible by drug action to see enormous prolongation such as 100 msec. or more, as it was reported by one of the present authors (9). In any case the prolongation and accordingly the plateau-formation of the spike occurred on the falling phase at the height of one-half or one-third of the spike. The extraordinary prolongation of the spike as shown in fig. 5 reminded us of the plateau of the intracellularly recorded action potential of the heart muscle. Del Castillo et al. (11) explained that the block of nerve impulse by heavy-metal ions was due to their action on the SH-group. It is interesting that their explanation suggests the enzymic action of SH-group on the initiation of the nerve impulse. It is further interesting that the deterioration of the spike by SH-group reagents was recovered by application of sulfhydryl group such as cysteine.

CONCLUSION

1. The new extracellular recording method of the single myelinated nerve fibers was introduced to analyse the drug action on them.
2. The duration of the spike of the single myelinated nerve fiber was prolonged enormously especially in the falling phase of the spike by the application of CoCl₂ solution.
3. The prolongation of the spike-duration by SH-blocking agents such as CoCl₂ was recovered almost completely by application of SH-group such as cysteine.
4. These facts seem to suggest the enzymic mechanisms of the nerve impulse.

ACKNOWLEDGEMENT

The authors would express their sincere thanks to Dr. Matsuo by whose technical help their studies were much improved.

Part of expenses was defrayed from the Education Ministry.

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