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The Effect of Various Metabolic Inhibitors on the Resting Potential of Frog Muscle*

By Joab Klapp Aronson

Introduction

It is generally known that when the surface of a cell is injured, one is able to measure a potential difference between the site of injury and the intact surface of that cell. The P.D. observed in such instances are of the order of 50-90 mv. The injury is believed to serve as the point of electrical connection between the inside and the outside of the cell, and that the potential exists across the plasma membrance or boundry of the cell.

The difference in the potassium ion concentration on both sides of this cell membrance has been suggested by Davson and Danielli (1943) as the factor which causes the potential to occur. The plasma membrane is permeable to potassium ions but not to the anions associated with them. This ionic dissociation would then give rise to a potential difference across the membrane.

Shanes and Brown (1942) demonstrated that chemical agents which inhibit glycolysis also cause the resting potential to diminish. They used the Sciatic nerve of the frog. They applied monoiodoacetic acid, sodium fluoride and phloridzin to the intact surface of the nerve, and found that there followed a lowering of the resting potential. This decrease is the potential was found to be reversable, providing that the concentrations of the drugs were not large enough to kill the preparation. The method of reversing this inhibition was the addition of lactate and pyruvate, which enabled the cells to bypass the inhibited portion of the glycolytic cycle.

So far the two extreme positions as to the origin of the potential have been mentioned. No doubt each one has some merit and neither tells the whole story. In 1943 and 1944, Shanes performed some more experiments and altered his theories concerning the origin of the potential. The gist of his newer concept was that there are two factors involved in the maintainance of the resting potential. The first one was the movement of potassium ions across the membrane and the second one was involved with glycolysis. His experimental evidence indicated that approximately half of the potential was proportional to the logarithm of the potassium concentration outside

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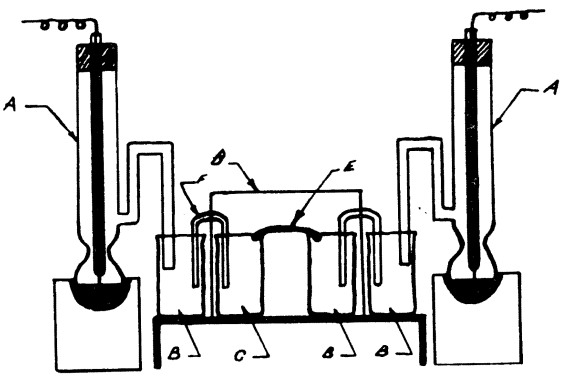
the cell. Other experiments showed that anoxia produced a fall in the potential and that the addition of calcium ion to the media tended to decrease the rate of fall. His later position will be considered at greater lengths at a later point in this report.

This work was undertaken in order to determine whether the information obtained for Frog nerve by Shanes was applicable to Frog muscle.

Methods

In these experiments the sartorius muscle of the frog (Ranapipens) was used. The muscle was dissected with great care and washed frequently to remove the excess ions accumulated because of broken cell, blood vessels, etc. A piece of the pelvic bone was also removed with the muscle as well as its tendenous attachment. This was done in order to avoid any injury to the muscle tissue itself.

After dissections the muscle was placed in Ringer's solution with a ph of 7.2. Oxygen was bubbled thru the solution. The muscle was allowed to equilibrate in this solution for at least one hour. The experimental set-up:



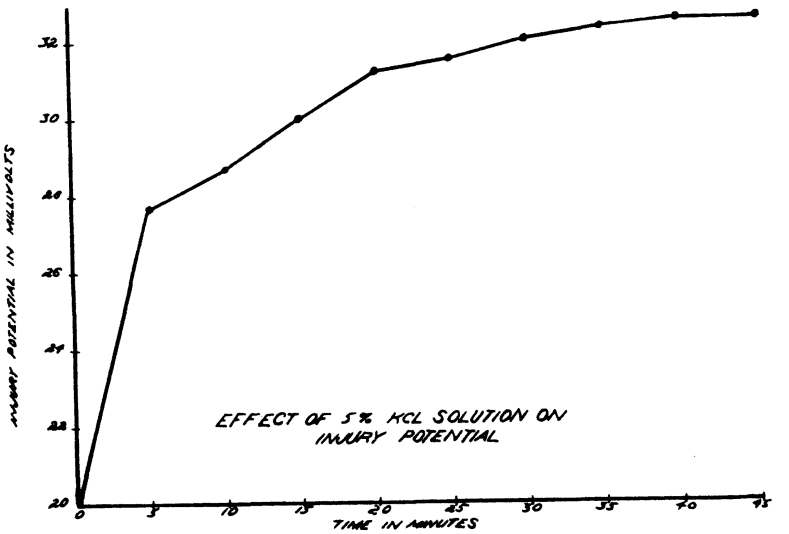
EXPERIMENTAL SET-UP FOR MEASURING INJURY POTENTIAL

Electrical equipment:

L & N Student type potentiometer
Eppley St. Cell
D'Arsenval Galvanometer Mirror suspension box type

Procedure:

In order to produce a constant injury the tendonous end of the muscle was treated with a 5% KCL solution.

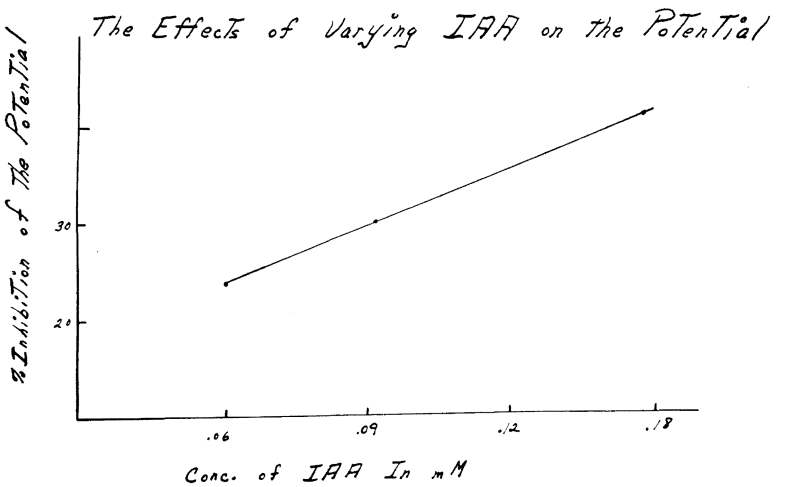


The values observed in this experimental series are not the maximal p. d. known to occur in muscle tissue. This is because the muscle fibers are bound in bundles, and between the fibers, there are the interstitial spaces which would tend to shunt the currents. Using single muscle fibers and micro-electrodes, P.D.'s of around 90 mv are found. In these cases the inserted electrode was placed directly under the one on the surface of the cell, this would tend to do away with all shunting effects.

Another consideration would be the fact that while the muscle is equillibrating in Ringer's solution, the potassium ions of the muscle are slowly leaking out.

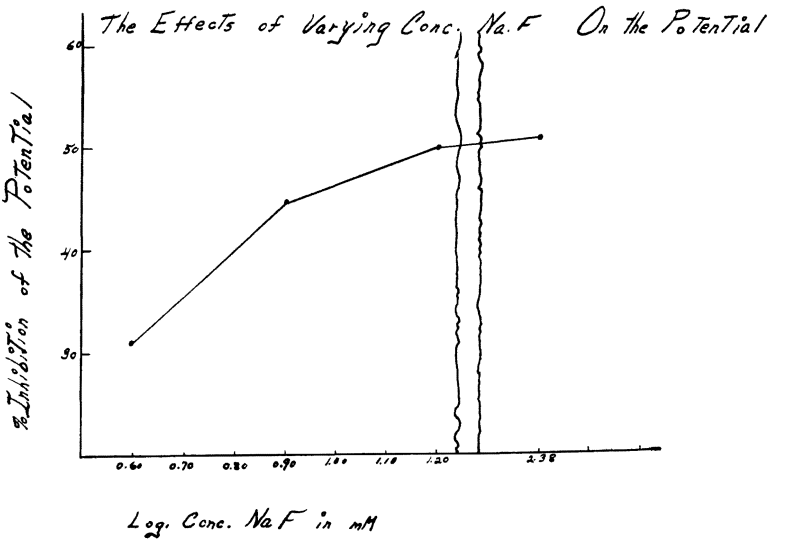
The readings obtained after the constant injury was produced are considered the maximum values in any given experiments, and any percentage change mentioned in this report is related to that value which is considered 100%.

The agent to be tested is added to the vessel containing Oxygentated Ringer's solution in a concentration and volume to produce the desired experimental concentration. Readings are then taken to determine the change in potential with time.



Results

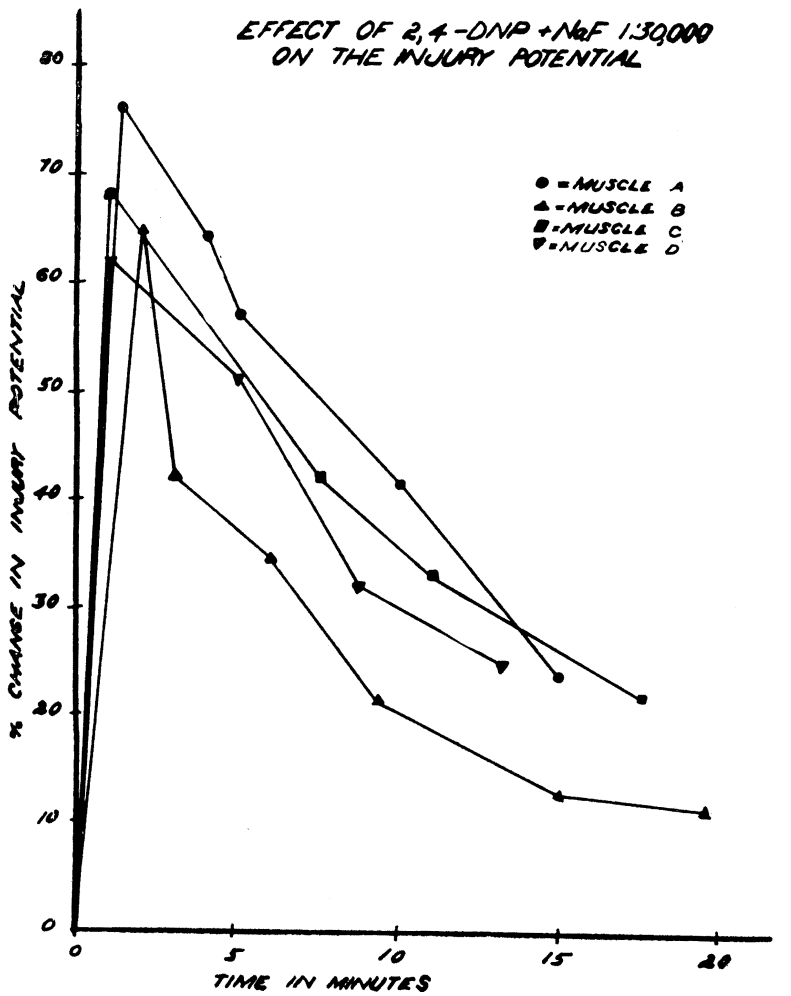
The effects obtained using Monoiodoacetic acid or as termed here IAA are shown on Slide 3. The slide clearly indicated that in the range tested the decrease in the potential is directly proportional to the concentration of the drug. Median Deviation 1.29 mv. The maximum and minimum concentrations were not determined in this case. Lactate and pyruvate were found to return the potential to the normal range.



Slide 4 shows the results obtained when NaF was applied in varying concentrations to the frog muscle. In this case maximal effective concentrations appear to have been reached. M.D. 1.00 77mv. These effects were also found to be reversable in all concentrations except the last one shown on the graph.

When a saturated solution of 2,4 dinitrophenol was applied to the muscle there was no significant change in the potential observed.

When 2,4 Dinitrophenol is applied to the muscle in conjunction with IAA 1:60:000 one finds the resting potential is decreased but not as great a degree as when that concentration of IAA is applied. (A 25.3% #.77 fall as compared to a fall of 28.5 #1.48)



Slide 5 shows the results obtained when 2,4 DNP is applied in conjunction with NaF 1:15000. One first notes an immediate drastic fall in the potential followed by a slow recovery process. The average decrease in the potential was 65.5% in the first 5 minutes and within 20 minutes it returned to 21% of normal.

Incidental to these experiments several other drugs were tried. These results are mere observations and are not to be considered significant since at the most were based on only a few observations.

Peroxide in small concentrations was found to increase the resting potential and in large concentrations to decrease it. Novocaine in concentration of 0.01-1.0% was found to have no effect upon the resting potential.

Discussion

The experimental results just shown indicate that agents such as IAA and NaF inhibit both the resting potential and glycolysis. The relation of the inhibitions is proportional to the concentrations of the drugs used. 2,4DNP appears not to influence the potential to any great extent.

These drugs were applied not to the injured surface of the muscle, but rather to the intact surface of the muscle. This would indicate that their effect was at or near the surface of the cells.

A possible explanation of the relationship between the injury potential and glycolysis was proposed by Shanes. (1942). He felt that the aerobic portion of the potential was due to the formation of pyruvate. The reaction of phosphopyruvate with ADP to form ATP. The ATP thus formed would then react with hypothetical organic radical which would result in a aposhorylation of that radical. Then that phosphorylated radical R would orient assymetrically in the plasma membrane. The potential gradient of this phosphorylated radical would then be its degree of phosphorylation.

It has long been known that IAA interferes with the phosphorylation of glycogen and also in the formation of lactic acid. Green, Needham and Dewan have shown that this substance also prevents phosphorylation of triose phosphate to form 1,3 phosphoglycerate.

Fluoride also causes free phosphates to disappear from the system and also inhibits lactic acid formation.

2,4 DNP is known to interfere with the maintainance of phosphocreatine but also increases the rate of oxygen uptake and carbon dioxide formation and utilization of glucose. (Ronzoni & Ehrenfest, 1941) It in no way affects lactic acid formation, but will in the long run affect the phosphorylation since it is thru the phophocreatine that the cell maintains and stores organic phosphate.

In explaining the effects obtained when 2,4 DNP and NaF were applied simultaneously, one could assume that the first effect was that of the NaF alone, and that the increase in the rate of glycolysis and oxygen uptake enabled the tissue to recover; however the depression of the potential was too great to be caused by that concentration or for that matter any concentration on NaF. One must then assume that there were other unknown factors at work under those conditions.

That sort of an explanation would apply to the results obtained with the use of IAA in conjunction with the 2,4 DNP. It does seem quite apparent that if one added metabolic intermediaries below the point of inhibition that one would obtain a reversal of the inhibition if it were due to the fact that glycolysis was responsible for the potential or for a portion of the potential. And we did find that the addition of lactic acid did cause a reversal of the potential. The action of peroxide would be to increase the rate of oxygen uptake and thus more of the organic radical R, would be formed and thus the potential would be increased. A large concentration of H_2O_2 would denature the proteins and completely destroy the system.

These results would tend to indicate that the system existing in frog muscle is similar to that existing in frog nerve.

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