The Action of Acetylcholine on the End-Plate Potential

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=ABSTRACT=

The failure of the action potential at the end-plate membrane to reach the sodium equilibrium potential is due to the stimulating action of acetylcholine on Na^+-K^+ pump. This action of acetylcholine causes an enormous increase in the K^+ transport rate. The quantitative amount of potassium ions in the inside of the end-plate membrane prevented the permeability of sodium ions during the depolarization phase of the action potential. It would favor the changes in the shape of action potential by acetylcholine which are always toward a fixed potential slightly below the zero line. The increased Na^+-K^+ pump activity by acetylcholine is responsible for the hypopolarization of membrane. This reduces the membrane resistance of the end-plate during transmitter activity.

Fatt and Katz (1951) has been shown that in an uncurarized muscle the end-plate potential is usually greater than threshold strength, and an action potential arises out of the end-plate potential as it crosses threshold. The threshold potential at the end-plate is the same whether determined by indirect or direct stimulation. However, the shape of the action potential recorded at the end-plate in response to indirect stimulation differs from the shape of the potential recorded following direct stimulation of the muscle. The changes in the shape of the indirect action potential are always toward a fixed potential slightly below the zero line.

This altered shape is confined to the end-plate region; an action potential recorded a few millimeters away, has a normal shape, no matter what the mode of stimulation and also indicated that the membrane resistance of the end-plate is greatly reduced during transmitter activity. It has been postulated that the acetylcholine causes a large nonselective increase in ion permeability to explain the end-plate depolarization in skeletal muscle. However, later evidence indicates that the permeability changes are limited to increases in P_{Na} and P_K to quite larger values (Fatt and Katz, 1952).

The nature of final steady level of the end-plate potential is slightly less than zero and reduction in membrane resistence of the end-plate during transmitter activity are less well understood.

On the other hand, Skou (1957) fund an adenosine triphosphatase (ATPase) in the microsomal fraction of crab nerve which was stimulated by Na^+, and this Na^+ stimulation was much greater if K^+ was also present. Skou suggested that this ATPase may be related to the Na^+-K^+ pump. Later studies on erythrocyte membranes by several investigators(Hoffman, 1960; Post et al, 1960; Dunham and Glynn, 1961) established that the pump and ATPase share many features in common. These studies on the erythrocyte membrane

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and work by many other investigatiors on a variety of preparation from many tissues have left litter doubt that this ($Na^+ + K^+$)-activated adenosine triphosphatase (NaK ATPase) is somehow involved in the $Na^+ - K^+$ pump and may in fact be the $Na^+ - K^+$ transport machine itself (Hokin and Hokin, 1963; Skou, 1965; Albers, 1967; Glynn, 1968).

The purpose of the present work is the attempt to understand better the nature of the end-plate potential. For this purpose, the relationship between the effects of acetylcholine on membrane potential and on NaK ATPase activity in skeletal muscle has been evaluated.

METHODS

Animal preparation; The experiments were carried out with the sartorius muscle of Rena temporaria for measurement of membrane potential, with the rabbit skeletal muscle for preparation of microsomes and with the rabbit blood for preparation of ghosts.

(a) Electrodes

These experiments were conducted at the physiological institute, Tübingen University, Tübingen (1958). The microelectrodes were drawn from 0.8 mm melting point pyrex glass tubing with hands. The microelectrodes regularly used were filled with 3 M KCl and had comperatively low resistance, usually between 4 and 100 megohms.

Potentials were measured between one Ag-AgCl electrode with a 3 M KCl bridge to the solution inside the microneedle and another in the external Ringer solution. Experimental solutions containing 2.5 mM KCl; 115 mM NaCl; 1.8 mM $CaCl_2$; 0.85 mM NaH_2PO_4, and pH 7.4. The membrane potentials were recorded by photographing the pattern on the cathode ray oscilloscope, as shown in Fig. 1.

(b) Preparation of microsomes

Microsomes from rabbit skeletal muscle were prepared as described by Inesi et al (1966).

(c) Preparation of ghosts

Rabbit blood was obtained by cardiac puncture. Clotting was prevented with heparin. The blood was centrifuged and the plasma and buffy coat removed by suction; the cells

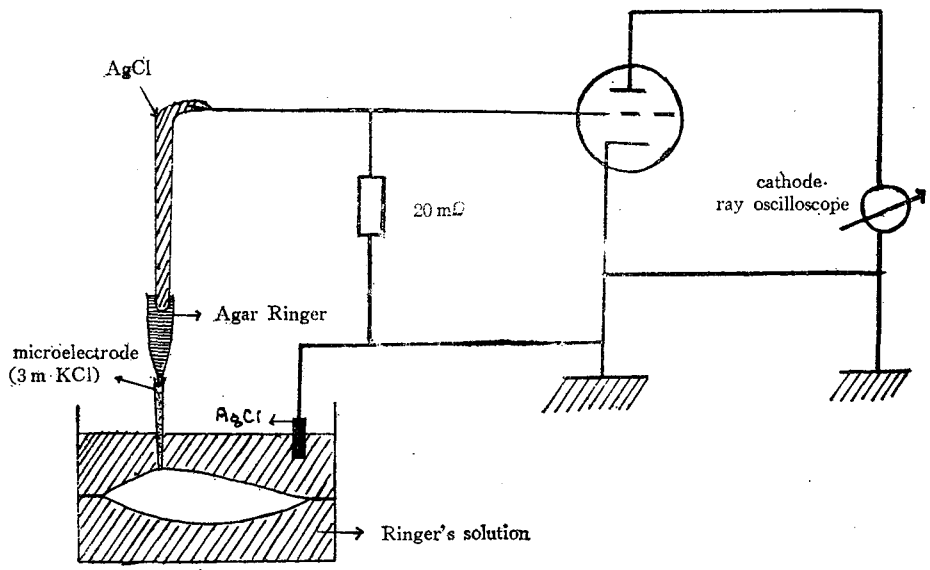

Fig. 1. Schematic diagram of resting potential recording system.

were washed two times with isotonic saline and then washed two times with isotonic $MgCl_2$ solution containing 1 mM EDTA. The cells were lysed by adding them quickly to 30 volumes of 15 mOsM tris-HCl buffer solution. The haemolysate was allow to stand for 60 min and the cells were then spun down (10 min at 10,000 g) and washed two times with 15 mOsM tris-HCl buffer solution containing 1 mM EDTA and then washed one time with 15 mOsM tris-HCl buffer solution (pH 7.5) at 4°C. The fluffy white precipitate was obtained in this way and used in these experiments.

(d) Measurement of ATPase activity

Incubation was carried out in 5 ml glass tubes with gum stopers at 44°C for to addition of ATP in a volume of fluid equal to one tenth of total volume, so that the cooling effect was small, at the end of the incubation period the tubes were cooled in iced water for 1 min., and then ice cold 50% of trichloroacetic acid was added to make the final concentration in 5%. The tubes were kept in iced water untill it was convenient to spin down the precipitated protein and estimate the inorganic phosphate in a portion of the supernatant by the method of Fiske & Subbarow (1925). The cooling before the addition of trichloroacetic acid was found to be necessary to get complete deproteinization. A small blank correction was also made, to allow for the trace of color developed in supernantans from control tubes to which no ATP had been added.

In experiments involving large numbers of tubes, the ATP was added serially at 15 sec. intervals at the begining of the experiments and the tubes were removed from the water bath to ice water at 15 sec. intervals at the end. Trichloroacetic and was added to the tubes in the same order exactly 1 min. later.

RESULTS

Effects of acetylcholine on the membrane potential

In figure 2 resting membrane potential of single frog skeletal muscle fiber recorded with intracellular electrode is presended. In the

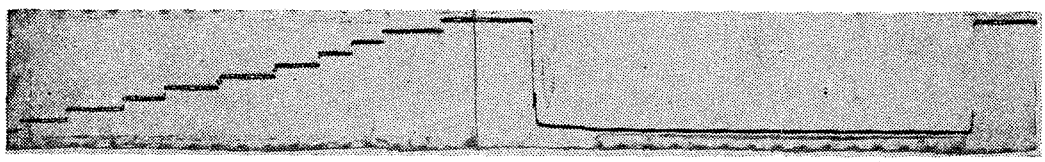

Fig. 2. Resting membrane potential of single skeletal muscle fiber recorded with intracellular microelectrode. Temp. 23°C; Ordinate scale, 10 mV steps from voltage calibrator.

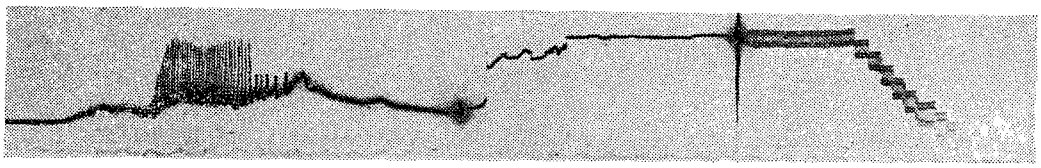

Fig. 3. Action membrane potential of single skeletal muscle fiber recorded with intracellular microelectrode in the presence of ACh (10^{-4}g/ml). Temp. 22°C; Ordinate scale, 10 mV steps from voltage calibrator.

control study shown in figure 2 the resting potential amounted to 90 mV.

The action potential of single frog muscle fiber in the presence of acetylcholine is showned in figure 3.

In this experiment, it has been showned that the action potential of skeletal muscle fiber induced by stimulating in the presence of acetylcholine (10^{-4}g/ml). It should be note that the shape of action potential induced by acetylcholine dffers from the shape of the action potential recorded following direct stimulation of muscle. The change in the shape of the action potential induced by acetylcholine is toward a slightly below the zero line. This altered shape of the action potential by acetylcholine in the medium is not differs from the action potential at end-plate region.

Effect of concentration of acetylcholine on NaK ATPase in skeletal muscle microsomal fraction.

Figure 4 shows the effect of acetylcholine concentration on NaK ATPase activity in skeletal muscle microsomal fraction. The activity of NaK ATPase increases with increasing acetylcholine concentration till 5 mM-acetylcholine and then levels off. Figure 4 shows the effect of acetylcholine concentration on the maximal stimulation of the NaK ATPase activity at a concentration of 5 mM. The results reported in this study show that the acetylcholine stimulated the activity of NaK ATPase, which is involved in sodium and potassium ions transport across cell membrane.

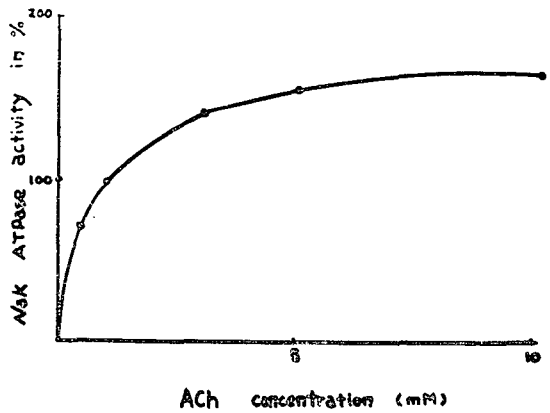

Fig. 4. The effect of concentration of ACh on NaK ATPase in the skeletal muscle microsomal fraction. Temp. 44°C; pH 6.5; ATP 3 mM; Mg 6 mM; Na 100 mM; K 10 mM; EDTA 0.1 mM. Duration 1 hr.

Effect of lysine in the presence of acetylcholine on the NaK ATPase activity of skeletal microsome fraction.

The effect of lysine in the presence of acetylcholine on the NaK ATPase activity is showned in figure 5.

Figure 5 represents an experiment designed to test the effect of acetylcholine on the reac-

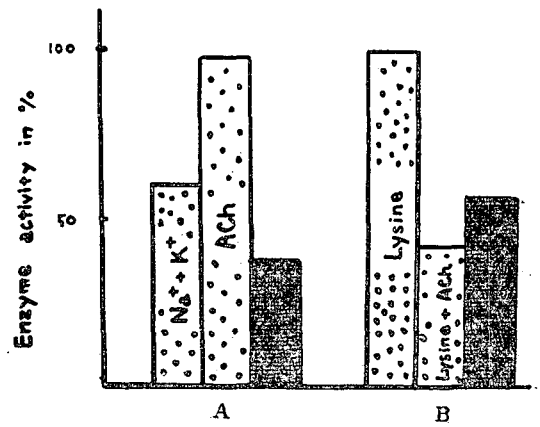

Fig. 5. The effect of lysine on NaK ATPase in the presence of ACh in skeletal muscle microsomes. Temp. 44°C; pH 6.5; ATP 3 mM; Mg 6 mM; Na 100 mM; K 10 mM; EDTA 0.1 mM; Lysine 15 mM; ACh 5 mM. Duration 1 hr. Each column is an average of 4 experiments.
▩ : Percentage of difference.

tive side of NaK ATPase. The activity of NaK ATPase was stimulated by acetylcholine (Fig 5, A) and the activity of NaK ATPase was also stimulated by lysine alone, which may be concerned with compensatory effect on this enzyme, but the activity of this enzyme was markedly inhibited by acetylcholine in the presence of lysine (Fig 5, B). The percentage of differences between two groups was not similar. It has been shown that the action of acetylcholine on NaK ATPase may be due to reaction with the NH_2 groups of this enzyme..

Effect of lysine on the NaK ATPase of red cell ghosts in the presence of acetylcholine

The effect of lysine in the presence of acetylcholine on the NaK ATPase activity of red cell ghosts is shown in figure 6. Figure 6 shows that the activity of NaK ATPase is inhibited by actylcholine (Fig 6, A) and the activity of this enzyme by lysine alone has no effect but the activity of NaK ATPase was inhibited by lysine in the presence of acetylcholine (Fig 6, B). The percentage of differences between two groups was not similar. These observations indicate that the inhibitory effect of acetylcholine on the NaK ATPase activity in red cell ghosts is also due to reaction with the NH_2 group of this enzyme.

The effect of aspartic acid in the presence of acetylcholine on NaK ATPase in red cell ghosts

In figure 7 shows that the effect of aspartic acid in the presence of acetylcholine on NaK ATPase in red cell ghosts.

Figure 7 indicates that the activity of NaK ATPase is inhibited by acetylcholine (Fig 7, A) and the activity of this enzyme is slightly stimulated by aspartic acid alone, which may

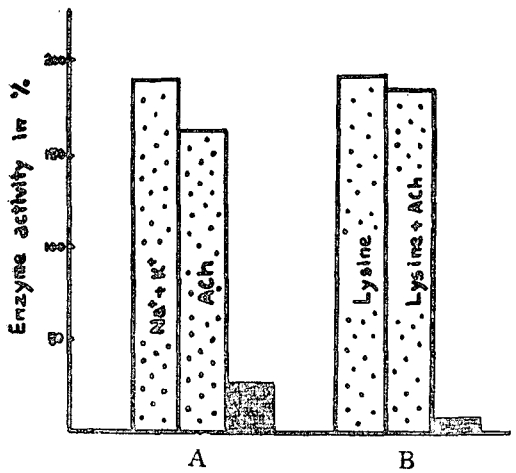

Fig. 6. The effect of lysine in the presence of acetylcholine on the NaK ATPase activity of the red cell ghosts. Temp. 44°C; pH 7.6; ATP 1.5 mM; Mg 2 mM; Na 80 mM; K 17 mM; ACh 5 mM. Duration 1 hr. Each column is an average of 4 experiments.
■ : Percentage of difference.

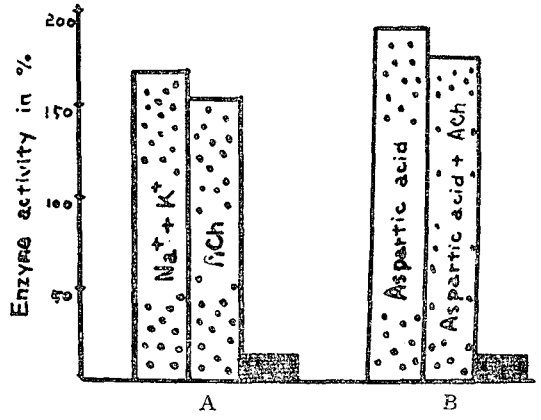

Fig. 7. The effect of aspartic acid in the presence of acetylcholine on the NaK ATPase activity in the red cell ghosts. Temp. 44°C; pH 7.6; ATP 1.5 mM; Mg 2 mM; Na 80 mM; K 17 mM; ACh 5 mM. Duration 1 hr. Each column is an average of 4 experiments.
■ : Percentage of difference.

be concerned with the compensatory effect on this enzyme, but the activity of this enzyme was inhibited by acetylcholine in the presence of aspartic acid (Fig 7, B). The percentage of differences between two groups was similar. It has been shown that the inhibitory action of acetylcholine on NaK ATPase in red cell ghosts was not related to the COOH groups of this enzyme.

Effect of cysteine in the presence of acetylcholine on the activity of NaK ATPase in the red cell ghosts

Figure 8 shows that the effect of cysteine in the presence of acetylcholine on the NaK ATPase in red cell ghosts. These results indicate that the activity of NaK ATPase is inhibited by acetylcholine (Fig 8, A) and the activity of NaK ATPase is stimulated by cysteine alone, which may be concerned with the compensatory effect on this enzyme, but the activity of enzyme is inhibited by acetylcholine in the presence of cysteine(Fig 8, B). The percentage of differences between two groups was not different. It has been shown that the inhibitory action of acetylcholine on NaK ATPase in red cell ghosts was not related to the SH groups of this enzyme.

In experiments decribed above (Fig 7, 8), the action of acetylcholine on the NaK ATPase activity was not related to the COOH groups or SH groups of this enzyme.

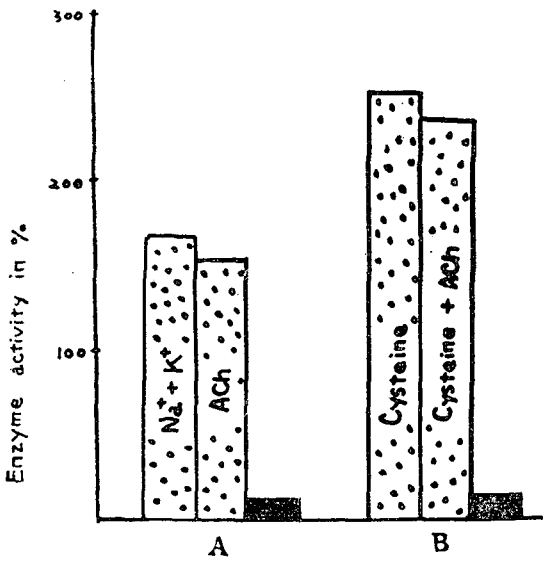

Fig. 8. Effect of cysteine in the presence of acetylcholine on the NaK ATPase activity in the red cell ghosts. Temp. 44°C; pH 7.5; ATP 1.5 mM; Mg 2 mM; Na 80 mM; K 17 mM; ACh 5 mM. Duration 1 hr. Each column is an average of 4 experiments.
▩ : Percentage of difference.

DISCUSSION

The present results using intracellular microelectrode has been shown that the changes in the shape of the action potential by acetylcholine are always toward a fixed potential slightly below the zero line. This altered shape of action potential by acetylcholine in the medium is not differs from the action potential at the end-plate region.

Fatt and Katz (1951) have been observed that the acetylcholine causes a large nonselective increase in ion permeability, such as has been postulated to explain the end-plate depolarization in the skeletal muscle. However, later evidence indicates that the permeability changes are limited to increases in P_{Na} and P_K to quite larger values (Fatt and Katz, 1952).

Takeuchi and Takeuchi (1960), using the voltage clamp technic, have also indicated that at the motor end-plate of skeletal muscle, the end-plate potential produced by acetylcholine is due to an increase in permeability to Na^+ and K^+ but not to Cl^-. In 1969, Kerkut, Brown and Walker have been suggested that at some invertebrate postsynaptic sites, a

metabolically dependent, electrogenic sodium pump may contribute to the potential change induced by the transmitter.

Present study indicated that the acetylcholine stimulated the activity of NaK ATPase in the skeletal muscle microsomal fraction and suggesting that also increases the rate of the Na^+—K^+ pump in plasma membranes of skeletal muscle. The stimulating action of acetylcholine on NaK ATPase activity is due to reaction with NH_2 groups of this enzyme.

On the other hand, Hodgkin and Katz(1949) propcsed that the depolarization phase of the action potential is brough about by a brief and highly specific increase in the membrane permeability to Na^+. This increase would permit Na^+ to enter the cell at a greatly increased rate, driven both by the concentration and voltage gradient and thus charge the membrane toward the sodium equilibrium potential.

It has been suggested that, at the first stage, the acetylcholine diffuses across the small gap between nerve ending and the end-plate, and then reacted with end-plate membrane. This would permit Na^+ to enter the cell at a greatly increased rate and thus charge the membrane toward the sodium equilibrium potential. Simultaneously, the acetylcholine also reacted with NH_2 groups of NaK ATPase and then activating the rate of Na^+—K^+ pump in the end-plate membrane. This would permit K^+ to enter the cell. The quantitave amount of potassium ions prevented the action potential to reach the sodium equilibrium potential. It would favor the changes in the shape of action potential by acetylcholine which are always toward a fixed potential slightly below the zero line.

At the second stage, the acetylcholine stimulated the activity of NaK ATPase in plasma membrane. The stimulating action of acetylcholine on the activity of NaK ATPase resulting in an increases rate of Na^+—K^+ pump. This produces an increase in intracellular potassium ions, causes a decrease the negative charges on the inside of membrane and simultaneously an decrease the positive charges on the outside of the membrae. The lesser the intracellular-to-extracellular potassium ion ratio, the lesser the magnitude of the membrane potential.

It would favor hypopolarization of the membrane and lesser the normal tendency of the membrane to depolarize, thus excitabilizing the membrane. This induced reduction in membrane resistance of the end-plate during transmitter activity.

On the other hand, there is a little evidence suggesting that an increase in the rate of active Na^+ transport may vary with membrane potential (Woodbury, 1963).

The relation between the action potential by acetylcholine and the action of acetylcholine on the NaK ATPase activity in the skeletal muscle has been determined. It has been indicated that the acetylcholine increased the Na^+—K^+ pump by enhancing activity of NaK ATPase and may contribute to the changes in the shape of the end-plate potential.

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