The voltage and temperature dependence of the end-plate current in frog skeletal muscle

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Abstract. The effect of membrane potential (V) on the half-time (t1/2) of the falling phase of the end-plate current (e.p.c.) was found to obey the equation

\[ t_{1/2} = A \cdot e^{BE/V} + C \]

where A, B and C are constants.

The temperature dependence of t1/2 was found to follow the Arrhenius equation. The activation energy (E_a) varied from about 50 kJ/mol to about 120 kJ/mol.

At membrane potentials between about -40 mV and -140 mV, the Ea/V relation was similar in all end-plates investigated: Ea increased if membrane potential was made more negative. At membrane potentials between about +60 mV and -40 mV, however, the Ea/V relation was different in different end-plates: If membrane potential was made more negative, Ea was either increased, or not affected, or decreased.

It is concluded that at negative levels of membrane potential the decay of the e.p.c. depends on average life-time of ionic channels, opened up by the action of acetylcholine on junctional receptors. At strongly positive levels of membrane potential, however, the decay of the e.p.c. can be determined by the average life-time of ionic channels or by the clearance of transmitter from the synaptic cleft, or both. Either of these processes can be reflected in the value of constant C in the above equation.

Key words: Neuromuscular junction - End-plate current - Acetylcholine receptor

Introduction

During neuromuscular transmission the conductance of postsynaptic membrane is temporarily increased. The time course of this conductance change can be followed by recording the end-plate current (e.p.c.; Takeuchi and Takeuchi, 1959). In an attempt to elucidate the molecular events during neuromuscular transmission, in the recent past the e.p.c., particularly its falling phase, has been intensively studied in a variety of experimental conditions. So it seems established that the released acetylcholine (Ach) is reversibly attached to junctional receptors (R) to form the acetylcholine-receptor complex (AchR). After being formed the conformation of this complex fluctuates between its "closed" (AchR) and "open" (AchR') state (for details see Steinbach and Stevens 1976; Sakmann and Adams 1979; Sakmann et al. 1980; Colquhoun 1979, 1981). During the falling phase of the e.p.c. the following reactions seem to take place:

\[ \text{AchR}' \rightleftharpoons \text{AchR} \rightleftharpoons \text{Ach} + R. \]

It has been generally accepted that in normal conditions the reaction AchR'\rightleftharpoons AchR is relatively slow, while clearance of Ach from the synaptic cleft is relatively fast. Thus in these experimental conditions the exponential decay of the e.p.c. reflects both the rate constant of the conformational step in the above scheme as well as the average life-time of ionic channels (Magleby and Stevens 1972a, b; Kordaš 1972a, b; Anderson and Stevens 1973; Gage and McBurney 1975; reviewed by Gage 1976; Peper et al. 1982). It has also been shown that the decay of the e.p.c. is highly dependent on temperature, membrane potential, and, at least in some species, on hydrostatic pressure (Takeuchi and Takeuchi 1959; Kordaš 1969; Magleby and Stevens 1972a, b; Anderson and Stevens 1973; Ashford et al. 1982). The decay of the e.p.c. is slower at lower temperatures, at more negative membrane potentials, and at higher hydrostatic pressures. It seems that in these experimental conditions the average channel life-time is lengthened and consequently the rate constant of the reaction AchR'\rightleftharpoons AchR is decreased. However, if junctional cholinesterase is inhibited, the average channel life time is not affected, while the rate of the reaction AchR'\rightleftharpoons AchR is strongly decreased because of the "repetitive" action of Ach in the synaptic cleft (Katz and Miledi 1973).

To elucidate the mechanism whereby membrane potential affected the decay of the e.p.c., Magleby and Stevens (1972a, b) provided a quantitative description of this effect. If expressed as half-time (t1/2) of decay, the effect of membrane potential (V) can be described as

\[ t_{1/2} = A \cdot e^{BE/V}, \]

where A and B are constants. It follows, then, that log t1/2 should be a linear function of V.

While this has been shown to be the case in some laboratories (Magleby and Stevens 1972a, b; Anderson and Stevens 1973; Gage and McBurney 1975), in other laboratories (Scuka 1975; Kordaš 1977a, b; Smetkov 1977; Humar et al. 1980) it was shown that the log t1/2/V relation was linear only at negative levels of membrane potential. At positive levels, however, the "voltage sensitivity" of the e.p.c. did not obey Eq. (1). Examination of data from other laboratories (e.g. Albuquerque and Oliveira 1979; Cull-Candy et al. 1979) seemed also to show a non-linear relation of the log t1/2/V plot. Thus it seems, at least in some species of...
experimental animals, that at strongly positive levels of membrane potential \( t_{1/2} \) approached a finite, constant value.

It has recently been suggested (Kordaš, 1982) that the effect of \( V \) on \( t_{1/2} \) could be described by a modified Eq.

\[
t_{1/2} = A \cdot e^{BV} + C,
\]

where \( C \) is another constant. It follows, then, that log \( t_{1/2} \) should not be a simple linear function of \( V \).

It should be borne in mind that the experiments mentioned above were performed in different species of experimental animals, mostly on isolated skeletal muscle of toad (Bufo marinus) or frog (Rana pipiens, Rana temporaria, Rana esculenta). Stevens (1978) reported that in Rana pipiens the log \( t_{1/2}/V \) relation was linear, while in Rana temporaria this relation was not linear.

It would be of interest to know which of the two equations, mentioned above, applies for a given species of experimental animals. Further, it would be also of interest to identify reactions that determine the decay of the e.p.c. In connection with this it is useful to remember that the falling phase of the e.p.c. shows a relatively high \( Q_{10} \) (about \( 2 - 4 \)) and activation energy \( (E_a; \) about 80 kJ/mol; Takeuchi and Takeuchi 1959; Gage and McBurney 1975; Gage 1976; Peper et al. 1982). Some data seem to indicate that \( E_a \) increases if membrane potential is made more negative (cf. Anderson and Stevens 1973). Thinking in terms of the Arrhenius equation for the rate constant it can be concluded that if there is only one reaction which determines the decay of the e.p.c., and if this reaction is voltage sensitive, the \( E_a/V \) relation should be approximately linear. If, however, this relation were not linear, probably at least two reactions determine the decay of the e.p.c.

The present investigation had three aims: First, to study the \( t_{1/2}/V \) relation in a large series of skeletal muscle of frogs, identified taxonomically, and to find out which one of the two equations mentioned above gives the better description of the \( t_{1/2}/V \) relation. Second, if in these animals Eq. (2), but not Eq. (1) applied, the temperature dependence of \( t_{1/2} \) might be different from that described earlier (for review see Gage 1976; Peper et al. 1982). Thus it seemed of interest to see whether in our experimental animals \( t_{1/2} \) fitted an Arrhenius plot and, if so, to determine \( E_a \). Third, it seemed appropriate to study the \( E_a/V \) relation to see whether \( E_a \) increases on hyperpolarization of the muscle fibre.

Methods

All experiments were performed on m. extensor longus digiti IV of frogs, obtained from the Kočani region, Macedonia, Yugoslavia. Before the experiment, the animals were kept for at least a few days in a refrigerated room at 4°C, and after being sacrificed, each animal was identified taxonomically according to Sket (1967). The majority of frogs were identified as Rana ridibunda (Pallas), and a few of them as Rana esculenta (L).

To abolish muscle contraction evoked by indirect stimulation, the muscles were “glycerol-pretreated” (Howell and Jenden 1967) using 800 mM glycerol in Ringer solution (NaCl 116 mM; KCl 2 mM; CaCl₂ 1.8 mM; Tris 4 mM; HCl 3.3 mM; pH 7.4) as described (Kordaš et al. 1975).

The voltage-clamp set-up was the same as before (Kordaš 1982). For the negative feed-back an amplifier having maximum gain of \( 10^4 \) and maximum output voltage \( \pm 100 \) V was used throughout this investigation.

The “voltage sensitivity” of the falling phase of e.p.c., as recorded at one end-plate, is shown in Fig. 1. It is clear that at strongly positive levels of membrane potential the log \( t_{1/2}/V \) relation is no longer linear, suggesting that Eq. (2) applies. The values of constants \( A, B \) and \( C \), obtained in 16 end-plates are summarized in Table 1. Comparing the sum of least squares for the setting \( C = 0 \) and \( C \neq 0 \) in single experiments it can be concluded that in 14 out of 16 measurements Eq. (2) is a better description of the \( t_{1/2}/V \) relation.