An Analysis of Factors which Determine the "Voltage Sensitivity" of the End-Plate Current

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Abstract. The half-time ($t_{1/2}$) of the end-plate current (e. p. c.) is an exponential function of the membrane potential (V). This "voltage sensitivity" of the e. p. c. was reported to follow the equation $t_{1/2} = Ae^{BV}$, A and B being constants. While in some laboratories it was shown that $V/\log t_{1/2}$ relation was a linear function, it was reported from other laboratories that the $V/\log t_{1/2}$ relation was linear only at negative levels of the membrane potential. At positive levels of membrane potential the $V/t_{1/2}$ relation was no more exponential and $t_{1/2}$ approached a constant value. It appeared that another equation, where $t_{1/2} = Ae^{BV} + C$, the latter being another constant, could provide a better description of the voltage sensitivity of e. p. c. The e. p. c. was recorded in frog muscle at membrane potentials from about +50 mV to about −150 mV in Tris-HCl buffered Ringer solution. In all muscle fibres examined, the equation $t_{1/2} = Ae^{BV} + C$ could be much better fitted to experimental data than the equation $t_{1/2} = Ae^{BV}$. It is suggested that the transmitter-receptor interaction(s) or the conformational transitions of the receptor are speeded up at strongly positive levels of the membrane potential and could eventually reach their maximum rate. Thus, the constant C seems to reflect the maximum rate of these processes.

Introduction

The introduction of microelectrode technique into electrophysiology made it possible to record the end-plate potential (e. p. p., Fatt and Katz 1951) and the underlying end-plate current (e. p. c., Takeuchi and Takeuchi 1959). Quantitative data on neuromuscular transmission have been recently summarized by Steinbach and Stevens (1976). It seems that the transmitter, after being released into synaptic cleft, undergoes at least three different processes: i) reversible binding to junctional receptors, ii) enzymatic destruction and iii) removal from the synaptic cleft by diffusion. The time course of the e. p. c. apparently depends on these three processes and on the rate of release of the transmitter into the synaptic cleft.

The e. p. c. is a relatively complex function consisting of a rising and a falling phase. The former seems to depend mainly on the spatial and temporal spread of the released transmitter (Takeuchi and Takeuchi 1959; Kordaš 1972b; Gabrovec

et al. 1975; c. f. also reviews by Hubbard et al. 1969; Gage 1976). The falling phase is an exponential function (Kordaš 1969; Magleby and Stevens 1972a, b; Kordaš 1972a, b; Anderson and Stevens 1973; Gage and McBurney 1975; Reviewed by Gage 1976) and seems to depend, at least under normal conditions, mainly on the rate of degradation of the receptor-transmitter complex. A mathematical analysis of the kinetic scheme, comprising the release of the mediator, its removal from the synaptic cleft by hydrolysis and diffusion, and the receptor-transmitter interaction(s)supports this view (Kordaš 1977a).

It has been repeatedly shown that the decay of the e. p. c. (expressed as the rate constant α, the time constant τ or half-time $t_{1/2}$ of decay) is slowed down if the mambrane potential is made more negative (Kordaš 1969, Magleby and Stevens 1972a, b; Kordaš 1972a, b; Anderson and Stevens 1973; Gage and McBurney 1975; Kordaš 1977a, b; Humar et al. 1980). It is thought that the rate of degradation of the receptor-transmitter complex or its conformational transition(s) become(s) slower when the membrane potential is made more negative and vice versa. Re-arranging the equation suggested by Magleby and Stevens (1972a), $t_{1/2}$ of the e. p. c. is an exponential function of the membrane potential (V):

$$t_{1/2} = Ae^{BV} \tag{1}$$

As A and B are constants, log $t_{1/2}$ should be a linear function of V.

In several laboratories the validity of this equation was tested over a range of membrane potentials from about $+50$ mV to about -150 mV. Magleby and Stevens (1972a), Anderson and Stevens (1973) and Gage and Mc Burney (1975) reported the V/log $t_{1/2}$ relation to be linear. On the other hand, Scuka (1975), Kordaš (1977a, b) and Humar et al. (1980) reported that the V/log $t_{1/2}$ relation was linear only at negative levels of the membrane potential. At its positive levels, however, the "voltage sensitivity" of the e. p. c. was decreased or lost. Thus, at strongly positive levels of the membrane potential the $t_{1/2}$ seems to approach a finite, constant value. If this is true, it seems that the effect of the membrane potential on $t_{1/2}$ of the e. p. c. could be described by a similar, but more complex equation:

$$t_{1/2} = Ae^{BV} + C, \tag{2}$$

where C is another constant.

Careful examination of data from other laboratories (Albuquerque and Oliveira 1979, Fig. 10; Cully-Candy et al. 1979, Fig. 6) showed that a non-linear relation of V/log $t_{1/2}$ could be observed in some of the muscle fibres studied. The authors, hower, did not discuss this aspect of their results.

In the present study it was attempted i) to find out which of the two equations described above could be a better description of the $V/t_{1/2}$ relation in frog skeletal

muscle and, if equation (2) applies, ii) to define the factor(s) which determine the constant C.

Material and Methods

Experiments were performed on m. extensor long. dig. IV of the frog (*Rana esculenta*). To abolish muscle contractions evoked by indirect stimulation of the muscles, they were "glycerol-pretreated" (Howell and Jenden 1967) by using 800 mmol/l glycerol in Ringer solution as described (Kordaš et al. 1975). Different experiments were deliberately performed at different temperatures in the range of 20—25°C. However, the temperature of Ringer solution in the muscle chamber was kept constant throughout each single experiment. In some muscle fibres the $V/\log t_{1/2}$ relation was recorded at a certain temperature in the above range and than the temperature was decreased to about 12—14°C. Following this the $V/\log t_{1/2}$ relation was recorded again. Details about temperature in single experiments are given in Table 1.

Table 1. Values of constants A, B and C, calculated by fitting the equation (2) to experimental data (11 end-plates) by using the method of least squares. To demonstrate which of the two equations discussed in the paper showed a better fit, the sum of least squares was calculated for each experiment not only if equation (2) was applied ($\Sigma x^2_{(2)}$), but also if equation (1) was applied ($\Sigma x^2_{(1)}$) to experimental data.

Exp. N°.	Temp. (°C)	A (ms)	B (mV^{-1})	C (ms)	$\Sigma x^2_{(2)}$	$\Sigma x^2_{(1)}$
1	23.9	0.14	−0.0137	0.37	0.004	0.046
2	23.3	0.42	−0.0096	0.27	0.009	0.027
3	24.4	0.19	−0.0156	0.20	0.003	0.009
4	22.5	0.19	−0.0163	0.44	0.041	0.651
5	20.6	0.26	−0.0150	0.31	0.021	0.387
6	23.3	0.17	−0.0164	0.58	0.089	0.517
7	21.7	0.21	−0.0171	0.38	0.030	0.501
8	25.0	0.27	−0.0158	0.37	0.184	0.434
9	23.8	0.27	−0.0133	0.25	0.004	0.025
	13.9	0.81	−0.0169	0.96	0.118	1.842
10	22.2	0.27	−0.0138	0.15	0.006	0.060
	12.8	0.74	−0.0183	1.19	0.485	4.986
11	21.1	0.25	−0.0162	0.26	0.059	0.296
	12.5	0.86	−0.0184	0.43	0.663	3.751

In all experiments the muscle in the muscle chamber was superfused with a Tris-HCl buffered Ringer solution (NaCl 116 mmol/l; KCl 2 mmol/l; $CaCl_2$ 1.8 mmol/l; Tris 4 mmol/l; HCl 3.3 mmol/l; pH 7.4).

The voltage-clamp set-up used in the present experiments (Fig. 1) was the same as described (Kordaš 1977a). To be able both to check the efficiency of the clamp and to see the actual displacement of the resting membrane potential, two oscilloscopes connected in parallel were used. One of them (Tektronix R 5031) served as a conventional recording oscilloscope (e. p. p. and membrane potential changes on the lower beam, e. p. c. on the upper beam). The other oscilloscope (Tektronix 502 A) was used as the feed-back amplifier (open loop gain 10^4 at the input selector setting of 0.2 mV/cm). To test the frequency response of the system a square pulse could be applied across the resistor connecting the

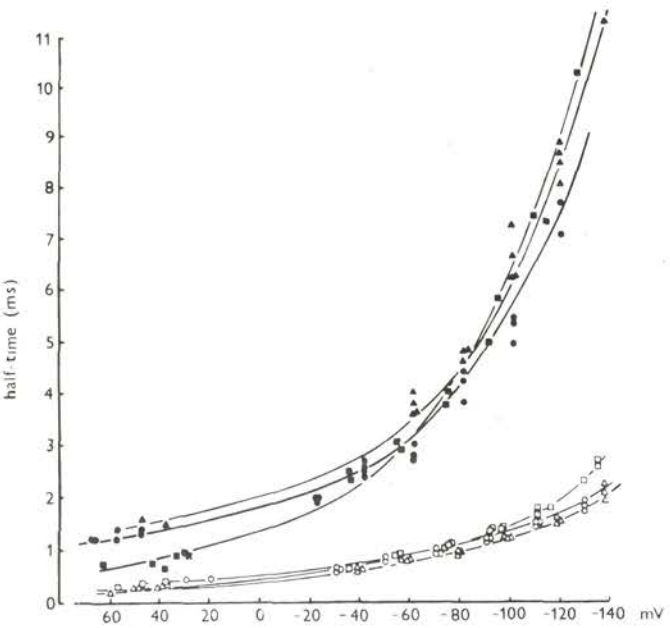

Fig. 3. The effect of the membrane potential on the half-time of the e. p. c. recorded at two different temperatures in three end plates (Exp. No. 9, ○●; No. 10, △▲; No. 11, □■; Table 1). Details about the temperature in particular experiments can be found in Table 1. Open symbols: Temperature about 22°C; full symbols: Temperature about 12°C. Solid lines connecting individual points are drawn according to the equation $t_{1/2} = Ae^{BV} + C$, the value of constants A, B and C being as shown for these three experiments in Table 1.

operative, but the decay of the e. p. c. seems to be determined by factor i). This is because the value of its rate constant is much less than the value of the over-all rate constant of processes ii) and iii). By making the membrane potential strongly positive, the value of the rate constant of process i) is increased. But this increase is certainly not infinite. At some strongly positive level of the membrane potential the maximum value of this rate constant must be reached. This maximum value can be, first either higher than the value of the over-all rate constant determining processes ii) and iii), or second, lower than the value of the over-all rate constant determining processes ii) and iii).

In the case of the first possibility, process i) would be fast in comparison with process ii) and iii), when the membrane potential is made strongly positive. Therefore, the latter processes would determine the decay of the e. p. c. and the constant C. As these processes are relatively temperature insensitive, the constant C should be relatively temperature-insensitive.

In the case of the second possibility, process i) would be speeded up when the membrane potential is made strongly positive and would, eventually, reach

maximum vaule of its rate constant, but it would be still slow in comparison with processes ii) and iii). Therefore the process i), being the rate-limiting step, would determine the decay of the e. p. c. and the constant C. As this process is highly temperature-sensitive (Takeuchi and Takeuchi 1959; Kordaš 1972b; Anderson and Stevens 1973; Kordaš 1977a), constant C should be highly temperature-sensitive. The "voltage sensitivity" of the e. p. c. would be lost as soon as the maximum rate of process i), effected by making membrane potential strongly positive, is reached.

Present preliminary, but also some earlier data (Kordaš 1977a) obtained by recording the $V/t_{1/2}$ relation at at two different temperatures, support the second possibility. Thus, the constant C seems to reflect the maximum value of the rate constant of process i).

It is also clear that in the present investigation the constants A and B have the same meaning as originally suggested by Magleby and Stevens (1972a). In their work the value of A, when expresseed in the same units as in the present paper, ranged from 0.25 ms to 1.31 ms, and that of B from 0.0054 mV^{-1} to 0.0119 mV^{-1} at about 25°C. Thus it seems that the individual variation of values of A and B was slightly more pronounced in the experiments of Magleby and Stevens (1972a) than in the present work.

Anderson and Stevens (1973) have shown that the values of the constants A and B are highly temperature-sensitive. They reported one experiment in which a decrease of the temperature from 18°C to 8°C made A increase from 1.65 ms to 2.67 ms, and B from 0,0057 mV^{-1} to 0.0150 mV^{-1}. Qualitatively, a similar increase in the values of A and B was observed also in the present preliminary experiments, performed at two different temperatures. Quantitatively, however, the absolute value of A was slightly smaller in the present experiments, but its increase, effected by a decrease in temperature was much larger than in the experiment of Anderson and Stevens (1973). Further, the value of B was only slightly increased in the present experiments, while it was almost doubled in the experiments of Anderson and Stevens. It remains to be determined whether these quantitative differences are due to differences in experimental procedure or to differences in muscle preparation.

At present it is also not clear why in some experiments (Magleby and Stevens 1972a, b; Anderson and Stevens 1973; Gage and McBurney 1975) the $V/t_{1/2}$ relation followed the equation (1), while in others (Scuka 1975; Kordaš 1977a, b; Humar et al. 1980) it followed the equation (2). It is clear that any factor decreasing the value of C would make the $V/t_{1/2}$ relation approach the relation by equation (1). It remains to be established whether the value of C depends on the species of the frog or on the experimental procedure.

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