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Modelling endplate currents: dependence on quantum secretion probability and decay of miniature current

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Abstract. Quantification of the time course and amplitude of endplate currents (EPC) was made with respect to dispersion of quanta secretion and to changes in the exponential decay of miniature endplate currents (τ_{mepc}). The relationship between RPC amplitude and τ_{mepc} follows a double-exponential curve with $\tau_1 = 0.3$ ms and $\tau_2 = 6$ ms. If the amplitude of fully synchronised EPC is taken as 100%, then the loss of EPC amplitude is already 42% with "physiological" parameters of dispersion (the half-rise and decay constant of distribution of secretion probability = 0.5 ms, $\tau_{mepc} = 1$ ms). This loss is even more substantial if secretion is more dispersed or miniature endplate currents decay faster.

Key words: Model endplate current – Transmitter secretion

Introduction

At the adult vertebrate neuromuscular junction with intact cholinesterase (Giniatullin et al. 1993), the duration and amplitude of the multiquantal postsynaptic response is limited by a relatively short mean open time and conductivity of ACh receptor channels (Katz and Miledi 1972; Sakmann and Witzemann, 1989) and by a sufficiently synchronized release of tens (mammals) or even hundreds (amphibia) of quanta from active zones at the nerve terminal (Katz and Miledi 1967; Katz 1969). Any distortion of the optimal pattern of pre- and postsynaptic kinetics might alter the amplitude and time course of synaptic currents (Del Castillo and Katz 1954; Cohen et al. 1981 a, b).

We therefore followed the kinetics of pre- and postsynaptic processes using a computer model of the vertebrate EPC at room temperature (see references in the section "Materials and methods"). The aim was to determine how changes in the time distribution of quantal release and the time course of a single quantum response (which reflects the mean open time of the ACh receptor channel (Anderson and Stevens 1993; Sakmann and Witzemann 1989)) may affect the resulting EPC. These two factors were chosen and examined together because both of them are variable during natural patterns of synaptic activity (Van der Kloot 1988a; Giniatullin et al. 1993) and both of them might be changed by a number of pharmacological agents (e.g. Fu 1994; Van der Kloot 1990).

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Materials and methods

The computer model was created to simulate the EPC by a convolution of two curves: the distribution of secretion probability (DSP) and the miniature endplate current (MEPC). The parameters of DSP and MEPC were made variable to study their effect on EPC. The EPC was described in terms of three parameters: amplitude of the EPC, rise time (measured from the time at which the EPC reaches 10% of its amplitude to the time at which it reaches 90%), and time constant of the EPC exponential decay (τ_{epc}). In accordance with physiological data (Del Castillo and Katz 1954; Giniatullin and Khazipov 1991; Giniatullin and al. 1993) the EPC quantal content was chosen to be at least 100 independent identical quanta (MEPC). The shape of EPC did not change with the number of quanta, whereas the smoothness of the EPC curve increased when the number of quanta was raised.

DSP describes the changes of the quanta release probability with time. It is a complex curve having an S-shaped rising phase (Barret and Stevens 1972; Dudel 1986) and an exponential decay (Katz and Miledi 1965; Datyner and Gage 1980; Dudel 1986; Van der Kloot 1988a). The rising phase was calculated as an integral of the density of a normal Gaussian distribution, according to the following equation:

$$E(t) = \left(\frac{1}{\sigma\sqrt{2\pi}}\right) e^{-\frac{(t-m)^2}{2\sigma^2}}$$

where t is time, π is equal to 3.1418288129, σ is the standard deviation (or dispersion) and m is the mean or mathematical expectation.

The function $F_{(t)}$ was digitally integrated over time from $t=0$ to $t=2\ m$ (equal to the double mean), while the standard deviation was chosen to be half of the mean ($\tau=m/s$). Then, the integrated area was 95% of the total area under the $F_{(t)}$ function curve. With the parameters indicated in the first paragraph of the Results and discussion section, the resulting S-shaped cumulative curve fits well with the experimentally observed shape of the rising phase of DSP (Barret and Stevens 1972; our unpublished data). In accordance with others (e.g. Dudel 1986), the DSP rising phase was linear in probability-versus-time double-logarithmic plotting.

The variations in DSP were done by changing the slope of either its rise or decay time, or both. MEPC was described as a single postsynaptic event in response to the release of one transmitter quantum. Since the the decay phase usually conforms to about 90% of the MEPC duration (Gage and Armstrong 1968), we assumed MEPC rise to be instantaneous. The exponential decay time constant τ_{mepc} varied from 0.01 to 6.00 ms.

Results and discussion

With parameters close to the natural conditions at the frog phasic neuromuscular junction at 20 °C (the half-rise and decay constant of the DSP curve = 0.5 ms, $\tau_{mepc}=1$ ms (Barret and Stevens 1972; Van der Kloot 1988a, b; Gage and Armstrong 1968)), the delay from the start of the release up to 10% of EPC maximum amplitude was 0.85 ms, rise time 0.46 ms and τ_{epc} 1.24 ms. An asynchronous release of quanta given as the DSP curve resulted in a substantial loss of EPC amplitude. If the fully synchronized EPC was taken as 100%, then asynchronous release resulted in an EPC amplitude decrease of 42% (Fig. 1 EPC).

MEPC decay variations (τ_{mepc} between 0.01 and 6 ms) were tested whilst keeping the half-rise and decay of DSP at 0.5 ms. Resulting EPC parameters are given in Fig. 2A, B, C. The loss of amplitude (synchronous release versus asynchronous) was substantial even at $\tau_{mepc}=6$ ms. Reducing τ_{mepc} below the physiological value of 1 ms, e.g. during depolarization (Magazanik and Vyskočil 1969; Magleby and Stevens 1972); or during pharmacologically induced shortening of open channel time (Adams and Feltz 1980; Beránek and Vyskočil 1968)) led to a sharp decline of EPC amplitude. The EPC rise was also increased at longer τ_{mepc} (Fig. 2B), but the slope of the curve was less steep. The τ_{epc} was positively correlated with τ_{mepc} within 1 and 6 ms ("physiological" range) and the correlation coefficient was 0.98. A slight deviation was found at τ_{mepc} less than 1 ms (Fig. 2C).

The pattern of ACh release is constant at a given temperature (Datyner and Gage 1980; Van der Kloot and Cohen 1984), but it is likely to be altered during repetitive activity (Giniatullin and Khazipov 1991; Ruzzier and Scuka, 1979; Ruzzier et al. 1982; Van der Kloot 1988). Three types of DSP changes with the assumption of independence of the rise and decay phase of DSP (Van der Kloot 1988a, b) were tested: variations in DSP rise, DSP rise and decay or DSP decay alone (Fig. 1A, B, C, respectively). The smallest changes of EPC amplitude were found with variation due to DSP rise (Fig. 2D, open symbols) while the highest changes were observed with variations due to DSP decay (Fig. 2D, triangles). By contrast, EPC rise time (Fig. 2E) was affected mostly by DSP rise variations and EPC decay (Fig. 2F) was changed mostly by variation of DSP decay.

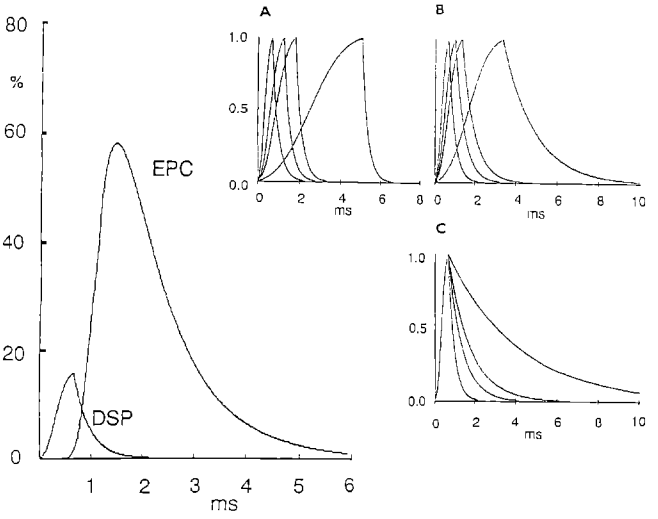

Fig. 1A–C. Distribution of secretion probability (DSP) of individual quanta and the resulting multiquantal endplate current (EPC) computed from input parameters: DSP rise time = 0.5 ms, DSP exponential decay constant = 0.5 ms, MEPC exponential time constant = 1.0 ms. Abscissa – time in ms, ordinate – number of quanta released (for DSP curve) and relative amplitude of EPC (for EPC curve) as percentage of "ideal", fully synchronous release. For DSP, the amplitude indicates the non-dimensional, relative number of quanta released, as normalised according to the maximal value, which is 1.0, as in the inset.
Inset – three types of release probability variations. **A** changes in rise time of DSP, **B** identical changes in rise time and exponential decay constant of DSP, **C** changes of exponential decay constant of DSP only. Abscissa – time of release distribution in ms, 0 = time of release of the first quanta after "stimulation". Ordinate – non-dimensional, relative number of quanta released, as normalised according to the maximal value

The physiologically important observation concerns the loss of EPC amplitude due to asynchronous release, which is 42% of optimal size when parameters are close to those of adult muscles at room temperature ($\tau_{mepc}=1$ ms, DSP rise and decay = 0.5 ms). Using real experimental data for DSP distribution (Katz and Miledi 1967), the observed loss of amplitude was similar, by 46% (details not given). This loss might be even more substantial when τ_{mepc} is shorter (e.g. during depolarization (Cohen et al. 1981; Magazanik and Vyskočil 1969)) and might contribute to the drop of EPC size by open channel blockers (e.g. Adams and Feltz, 1980; Magazanik and Vyskočil 1969) when the initial part of the MEPC decay is shortened.

The model relationship between EPC amplitude and τ_{mepc} (Fig. 2A) may be used as a "calibration" for how much the EPC amplitude would change in real experiments when shortening or prolongation of MEPC occurs. The

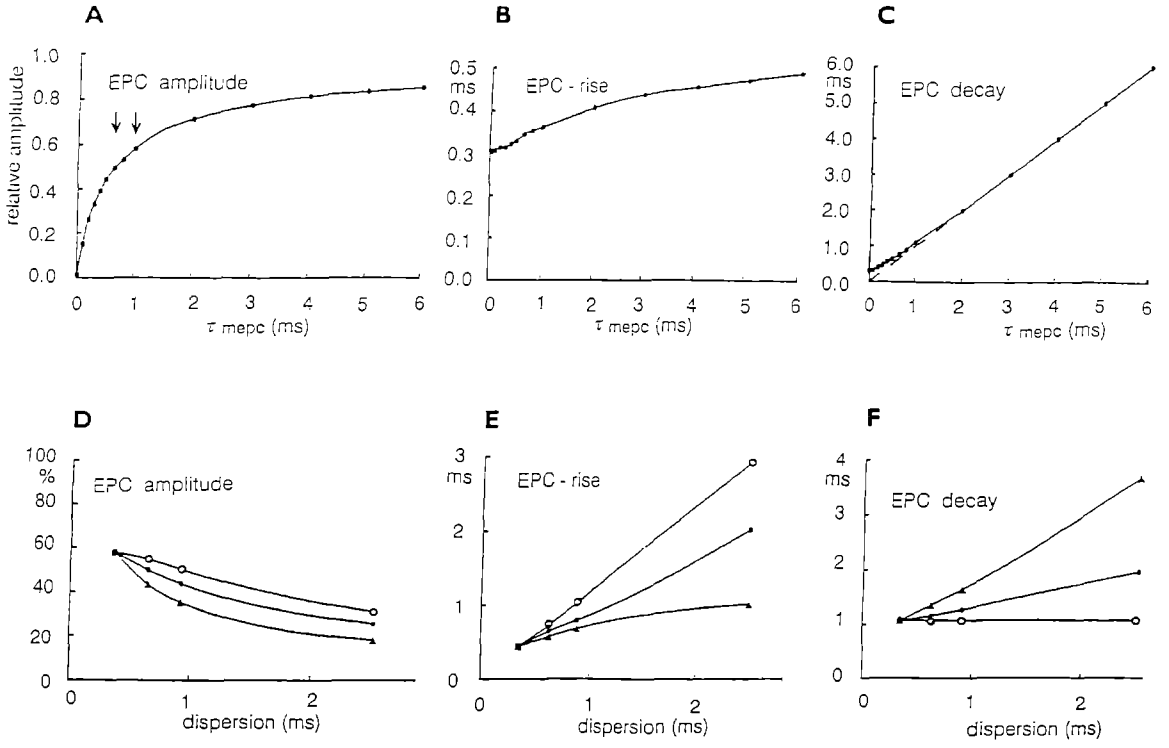

Fig. 2. A–C. The dependence of model multiquantal EPC amplitude (**A** ordinate in relative amplitude, 1.0 = amplitude of EPC at fully synchronous release), EPC 10–90% rise time (**B** ordinate in ms) and EPC decay time constant τ_{epc} (**C** ordinate in ms) on the decay constant of single-quantum miniature endplate current (τ_{mepc}, abscissa in ms) at constant secretion probability (both DSP rise time and decay = 0.5 ms). Arrows at A indicate the flattenned part of the curve, corresponding to the intersection of slow and fast exponentials. **D, E, F** amplitude (**D** ordinate – relative amplitude to synchronous release), 10–90% rise time **E** and decay time constant (**F** τ_{EPC}) of EPC during three types of release pattern changes: *open circles* – release dispersion changed by rise time of DSP (cf. Fig. 1A), *filled circles* – dispersion changed by rise time and decay of DSP (cf. Fig. 1B), *triangles* – dispersion changed by DSP decay (cf. Fig. 1C). *Abscissa* – dispersion defined as the duration (in ms) of the release measured from the time at which DSP reaches half amplitude to the time at which DSP decays to half amplitude

curve has a fast (τ_1=0.3 ms) and a slow (τ_2=6 ms) exponential component, separated by a brief flatter segment at τ_{mepc} 0.6–1 ms (arrows). This intersection corresponds to both open channel time and τ_{mepc} at resting membrane potential (e.g. Sakmann and Witzemann 1989; Fig. 1). In the fast exponential component of the curve (left), even a slight shortening of τ_{mepc} may dramatically decrease the EPC amplitude. The right part of the curve is a slow exponential and corresponds to the prolongation of τ_{mepc} by anticholinesterases or by longer channel openings (embryonic receptors with γ subunit (Mishina et al. 1968; Sakmann and Witzemann 1989)). The EPC amplitude in this region is affected to a much smaller extent (Giniatullin et al. 1989).

When the time dispersion of release is increased, then the EPC amplitude loss is even more pronounced. This is in accordance with the speculation that amplitude depression during short trains is partly due to higher asynchrony of release (Giniatullin and Khazipov 1991). It has also been found that during short trains, the EPC rise time subsequently rose without change in the EPC decay (Giniatullin and Khazipov 1991; Van der Kloot 1988). This fits well with what was found in the model when only the ascending phase of DSP was lengthened (Fig. 2E, F, open symbols). Interestingly, this type of release variation affects the EPC amplitude less than the other two variations (Fig. 2D, open symbols vs. filled symbols). Thus, the prolongation of EPC rise time up to 1.5 fold in train experiments (Giniatullin and Khazipov 1991; Giniatullin et al. 1993) may contribute to only a 6% decrease of the EPC amplitude.

The reported model might complement deconvoluting methods (Van der Kloot 1988a, b) to estimate the timing of quantal release and describe EPC changes seen at the living neuromuscular synapse under specific physiological and pharmacological conditions.

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