The difference in shape of spontaneous and uniquantal evoked synaptic potentials in frog muscle

Ronit Cherki-Vakil*, Simona Ginsburg*† and Halina Meiri*‡

*Department of Physiology, Hebrew University-Hadassah Medical School, PO Box 12272, Jerusalem, Israel and † The Open University of Israel, PO Box 39328, Tel-Aviv, Israel

1. Spontaneous and stimulation-induced uniquantal synaptic activity at the frog cutaneous pectoris muscle, treated with neostigmine, was recorded by focal extracellular microelectrodes. A monoexponential curve was fitted to the decay of each synaptic response.

2. A highly significant positive relationship was found between the amplitude and the decay time constant of spontaneous extracellular minature endplate potentials ($MEPPs_o$), whereas the relationship displayed by evoked uniquantal extracellular endplate potentials ($EPPs_o$) was only slightly greater than zero.

3. The difference did not stem from changes in the muscle membrane conductance or from inclusion of outstanding $MEPPs_o$ formed as a result of the block of acetylcholinesterase.

4. The dependence of the rise time on the amplitude was also stronger in $MEPPs_o$ than in $EPPs_o$.

5. In the absence of neostigmine, $MEPPs_o$ exhibited a positive correlation between decay time constant and amplitude, while $EPPs_o$ did not show such a correlation.

6. In view of previously published models of transmitter release, it is suggested that spontaneous secretion of quanta occurs both within and outside the active zones facing postsynaptic areas of variable receptor density.

The quantal hypothesis of transmitter release suggested 40 years ago for the frog motor synapse states that miniature endplate potentials (MEPPs) and stimulation-induced endplate potentials (EPPs) both result from secretion of prepacked quanta of acetylcholine (ACh) from the motor nerve ending. EPPs are induced by several quanta released almost synchronously, and MEPPs by individual spontaneously secreted quanta (del Castillo & Katz, 1954).

To the best of our knowledge, the idea that MEPPs and EPPs originate at the same location on the muscle membrane and result from secretion of similar quanta of transmitter has never been challenged. This view is most probably due to their formal resemblance in shape and their comparable sensitivities to muscle membrane potential and to pharmacological agents (Fatt & Katz, 1952; Magleby & Stevens, 1972 *a*, *b*).

On the other hand, the discovery of 'giant MEPPs', which do not participate in the formation of EPPs, suggests that not all quanta available for spontaneous secretion are suitable for release upon nerve stimulation (Liley, 1956; Pecot-Dechavassine, 1976). Among quanta leading to signals of ordinary shape, it is sometimes possible to distinguish between those released spontaneously and others released due to nerve stimulation. It appears, for instance, that quanta released by stimulation of the motor nerve are preferentially replenished with newly synthesized ACh. With high frequency stimulation, the quantal size of evoked quanta diminishes more slowly than that of spontaneously secreted quanta (Glavinovic, 1987).

Furthermore, the responses of spontaneous and evoked synaptic signals to neostigmine, an acetylcholinesterase (AChE)-blocking agent, are not always alike. Blocking AChE activity causes similar prolongation of the two signals and positive correlations between amplitude and decay are observed in both cases. However, while the positive correlation is evident in the uniquantal MEPPs, in EPPs it is found only when their quantal content is very high (Magleby & Terrar, 1975; Glavinovic, 1984, 1987; Linder, Pennefather & Quastel, 1984).

‡ To whom correspondence should be addressed.

We studied the difference between the unitary components of evoked and spontaneous synaptic signals by recording both through the same focal electrode. It was found that the shapes of the two types of synaptic potential differ, and that the difference is augmented upon block of AChE activity. The results suggest that spontaneous release may occur also at locations outside the active zones involved in evoked release; thus, the quanta released spontaneously and those released by stimulation may face slightly different patches of the postsynaptic membrane.

METHODS

Experiments were performed on the isolated cutaneous pectoris nerve–muscle preparation of the double-pithed frog *Rana ridibunda*. The preparation was dissected in Ringer solution (mM: NaCl, 116; KCl, 2; $CaCl_2$, 1·8; Hepes, 5; pH 7·2). During recording, bath Ca^{2+} concentration was reduced to 0·4 mM, and 2 mM $MgCl_2$ was added to decrease the quantal content of release, so that most stimuli evoked either no response, uniquantal or biquantal synaptic potentials. Neostigmine bromide (10^{-6} mg ml^{-1}) was added to the Ringer solution to prevent hydrolysis of the released acetylcholine.

Suprathreshold stimulus pulses were delivered to the nerve via a suction electrode at frequencies of 0·33–0·5 Hz. Evoked and spontaneous quantal potentials were recorded from superficial endplate regions using focal extracellular microelectrodes (tip diameter 2–3 μm) filled with the extracellular Ringer solution. In some experiments, the extracellular medium contained no added calcium but calcium ions were added to the recording microelectrode for local induction of release. A Zeiss microscope with Nomarski water immersion optics (×400) was employed for positioning the electrode on the muscle cell membrane in

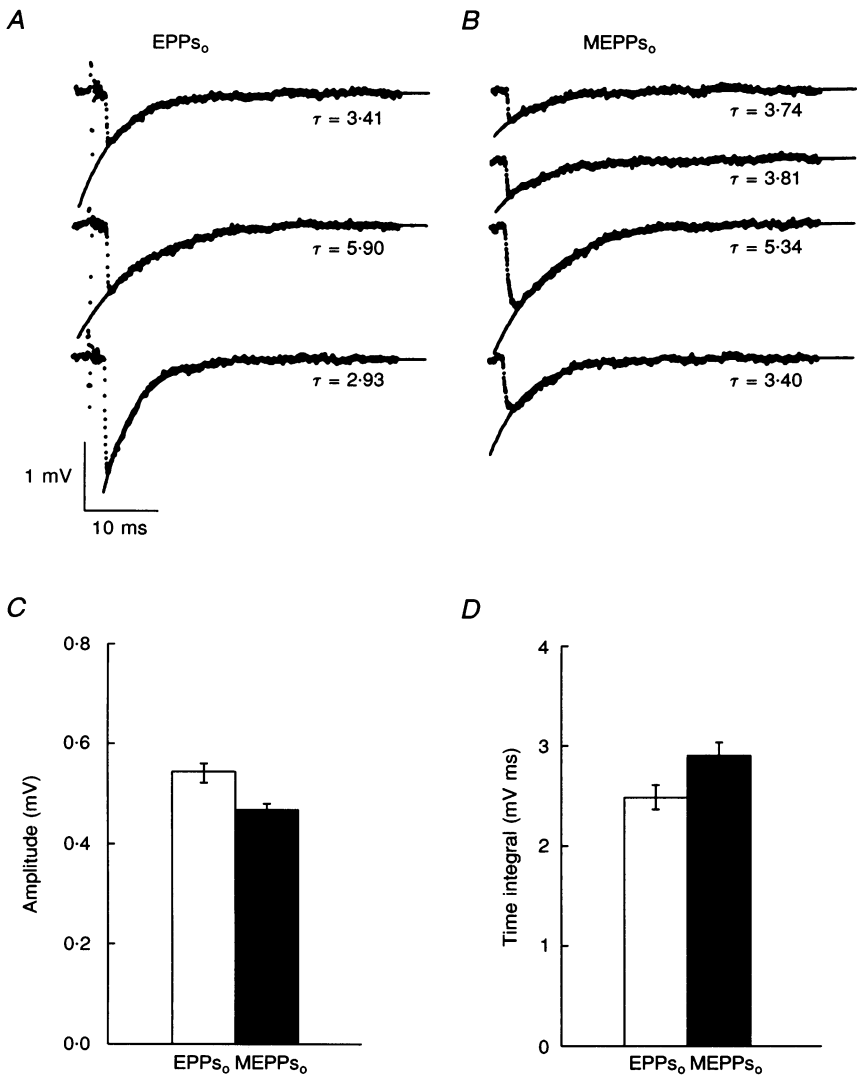

Figure 1. The shapes of uniquantal $EPPs_0$ and $MEPPs_0$ are not entirely similar

Representative $EPPs_0$ (*A*) and $MEPPs_0$ (*B*) and the time constants of their exponential decays. The mean amplitude of successful $EPPs_0$ was higher than that of $MEPPs_0$ (*C*). The mean time integral of 188 $MEPPs_0$ was significantly larger ($P < 0·1\%$) than that of 319 $EPPs_0$ (*D*). Low-Ca^{2+} Ringer solution with neostigmine.

close proximity to the nerve terminal, thus offering optimal recording of the neuronal extracellular nerve terminal potential. The shape of synaptic potentials monitored by this technique reliably represents the time course of local postsynaptic currents (del Castillo & Katz, 1956).

Spontaneous and stimulation-induced synaptic activities were monitored for 20–40 min before the addition of neostigmine to the bath and for 60–90 min in its presence after reaching steady-state conditions. During each recording session 144–1059 extracellular miniature endplate potentials ($MEPPs_o$) and 158–594 successful extracellular evoked endplate potentials ($EPPs_o$) were collected. The electrical signals were fed into a current amplifier (WPI), processed by an A/D converter (Neurodata) and taped on a videotape recorder. The data were later analysed by an IBM-386 computer with TL-1 DMA interface (Axon Instruments) using home-made and commercial programs (Axon-pCLAMP; Sigmaplot). A monoexponential graph was fitted to the falling phase of each individual $MEPP_o$ or EPP_o and the time constant of its decay (τ) was estimated. Subsequently, the amplitude of each synaptic signal was measured and the interrelation between the amplitude and τ was derived from the slope of a linear regression line fitted to the paired observations.

RESULTS

Spontaneous and uniquantal evoked signals are not similar in shape

The major aim of our study was to compare the spontaneous $MEPPs_o$ with stimulation-induced uniquantal $EPPs_o$. Endplates in which uniquantal responses were the most frequent were selected for the comparison. A representative experiment is illustrated in Fig. 1. The quantal content of release at this particular endplate was 0·56. Therefore, in this experiment, the calculated fractions of stimuli that evoked no quantal release, one quantum, double quanta and triple quanta were 57·3, 32, 9 and 1·7%, respectively (del Castillo & Katz, 1954). Upon addition of neostigmine to the solution, the durations of both spontaneous $MEPPs_o$ and evoked $EPPs_o$ were prolonged

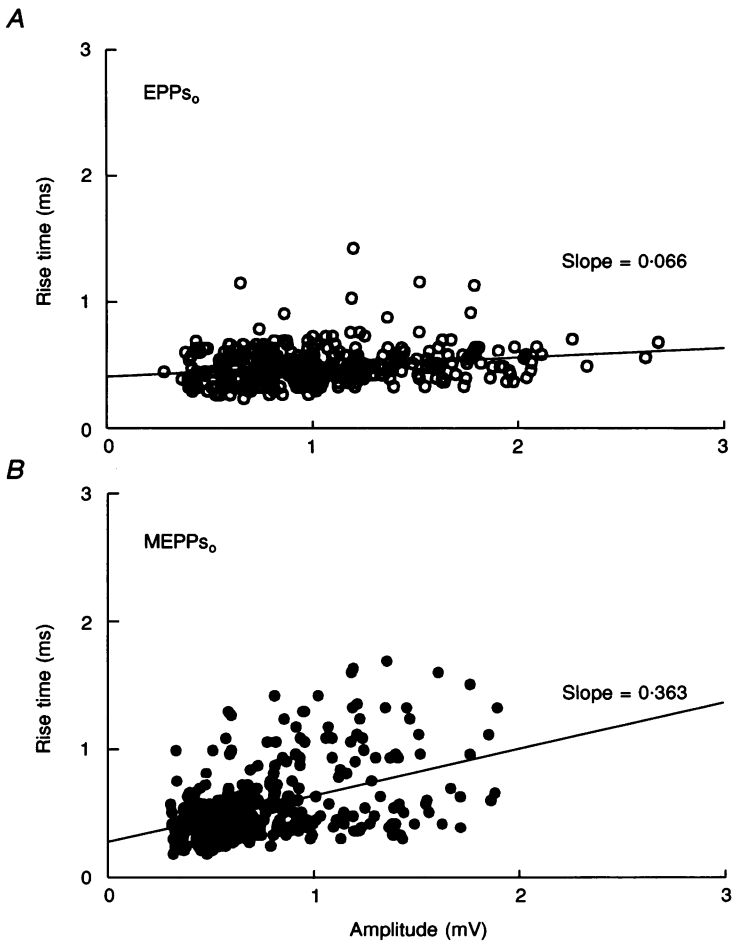

Figure 2. The relationship between rise time and amplitude

$EPPs_o$ (*A*) and $MEPPs_o$ (*B*) recorded at the same location. Each point represents a single synaptic signal (360 for $EPPs_o$ and 320 for $MEPPs_o$). The positive slope of the linear regression line fitted to the experimental points representing $MEPPs_o$ was much larger than the one fitted to $EPPs_o$. Same solution as in Fig. 1.

and the individual values were more variable than in control solution. By casual inspection, the two groups of synaptic responses, $MEPPs_0$ and $EPPs_0$, were similar in shape (Fig. 1*A* and *B*). Nevertheless, a specific difference was unravelled by examining the mean charge transfer (time integral of the signal): the mean charge transfer of $MEPPs_0$ was significantly higher ($P < 0{\cdot}1\%$) than that of $EPPs_0$ (Fig. 1*D*). This finding was particularly surprising in view of the lower mean amplitude of $MEPPs_0$ as compared with the mean amplitude of successful $EPPs_0$ ($P < 0{\cdot}1\%$) evoked mainly by single quanta, but in some cases by two or three quanta (Fig. 1*C*).

The relationship between amplitude and rise time

Larger charge transfer values in $MEPPs_0$ may reflect either longer rise times in $MEPPs_0$ or longer decay times (or both). Since these properties may depend on the amplitude of the signal, we examined their relationships to amplitude. Firstly, we plotted the rise time of each signal against its amplitude and calculated the slope of the linear regression line fitted to this plot. The results illustrated in Fig. 2 show a positive correlation between the rise time and amplitude in $MEPPs_0$ and a much weaker correlation in $EPPs_0$. Therefore, higher charge transfer by $MEPPs_0$ can be attributed to different rise- time properties. The relationship between rise time and amplitude was significantly steeper for $MEPPs_0$ than for $EPPs_0$ in all endplates: the mean ($\pm$ S.D.) slope values of the linear regression lines were $0{\cdot}32 \pm 0{\cdot}17$ ms mV^{-1} for $MEPPs_0$ and $0{\cdot}067 \pm 0{\cdot}07$ ms mV^{-1} for $EPPs_0$.

The relationship between amplitude and decay time

Secondly, we studied the relationship between decay time and amplitude. In all endplates, this relationship was found to be significantly higher in $MEPPs_0$ than in $EPPs_0$. Representative plots are shown in Fig. 3 (*A a* and *B a*). To characterize the relationship between decay time and amplitude further, an exponential curve was fitted to the falling phase of each signal and its decay time constant was noted. Most signals were well described by a single exponential, and an additional fast exponential was necessary only when the $EPPs_0$ were 5- to 7-fold higher

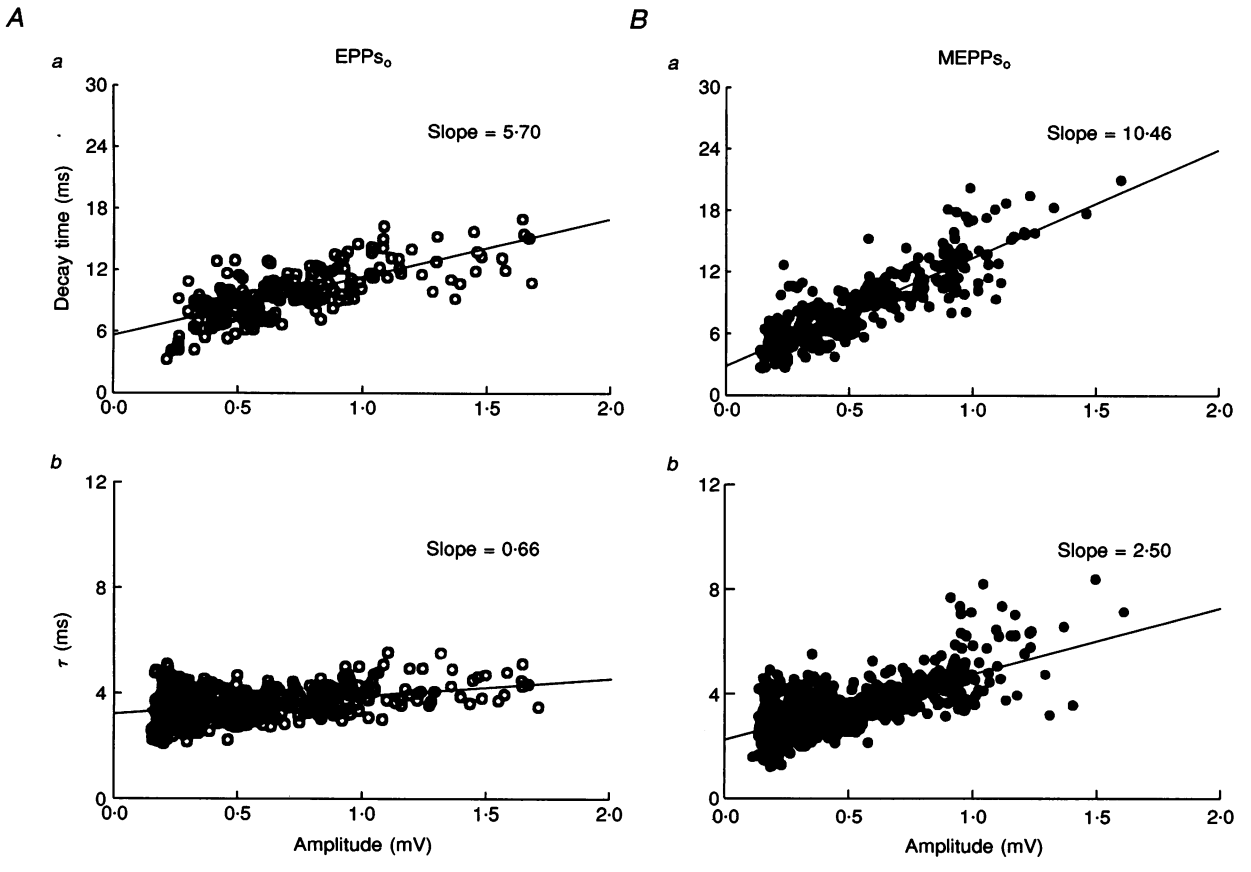

Figure 3. The relationship between decay time and amplitude

An exponential curve was fitted to the falling phase of each signal. The decay time was measured from the peak to the point of 99·5% decay and the exponential decay constant, τ, was noted. Both the decay times and the decay time constants were plotted against amplitude. The positive slope of the decay time *vs.* amplitude for 190 $MEPPs_0$ (*B a*) was higher than for 220 $EPPs_0$ (*A a*). The positive slope of τ *vs.* amplitude was also higher for $MEPPs_0$ (*B b*) as compared to $EPPs_0$ (*A a*). Same solution as in Fig. 1.

than the mean $MEPP_0$. In many experiments, a few giant $MEPPs_0$ appeared which could not be described by any number of exponentials, and these $MEPPs_0$ were omitted from further analysis. The relationship between the exponential time constant of decay (τ) and the amplitude of $EPPs_0$ is presented in Fig. 3*Ab*: the larger the amplitude of the signal, the higher the value of τ. In this particular experiment, the slope of the linear regression line between the two properties was 0·66. The respective value calculated for $MEPPs_0$ was 2·5 (Fig. 3*Bb*), indicating that the dependence of τ on the amplitude is higher in spontaneously occurring synaptic signals than in their stimulation-evoked counterparts.

Results displaying the shape of synaptic signals in twenty-one endplates exposed to neostigmine are summarized in Fig. 4*Aa* and *b*. The mean amplitude of $MEPPs_0$ was significantly lower than that of $EPPs_0$ ($P < 0{\cdot}25\%$), while the decay time constants τ were not significantly different ($5\% < P < 10\%$). The slope between τ and amplitude was much higher for $MEPPs_0$ than for $EPPs_0$ (Fig. 4*Aa*). Clearly, this difference stems from the greater variability of τ in the population of $MEPPs_0$ whose coefficient of variation was significantly higher (Fig. 4*Ab*).

Amplitude and decay time in the absence of neostigmine

To test whether the difference between $MEPPs_0$ and uniquantal $EPPs_0$ is caused by reduced activity of AChE, seventeen endplates were examined both prior to and following the addition of neostigmine. The results illustrated in Fig. 4*Ba* and *b* show that both the τ values and the amplitudes were augmented after the addition of neostigmine (by 2- to 3-fold and 1·1- to 1·3-fold, respectively). The slope of the linear regression line correlating the two properties of $MEPPs_0$ was also lower than that after the addition of neostigmine. Nevertheless, in $MEPPs_0$, decay time and amplitude are positively related, whereas in $EPPs_0$ the relationship is not significantly different from zero, indicating a genuine

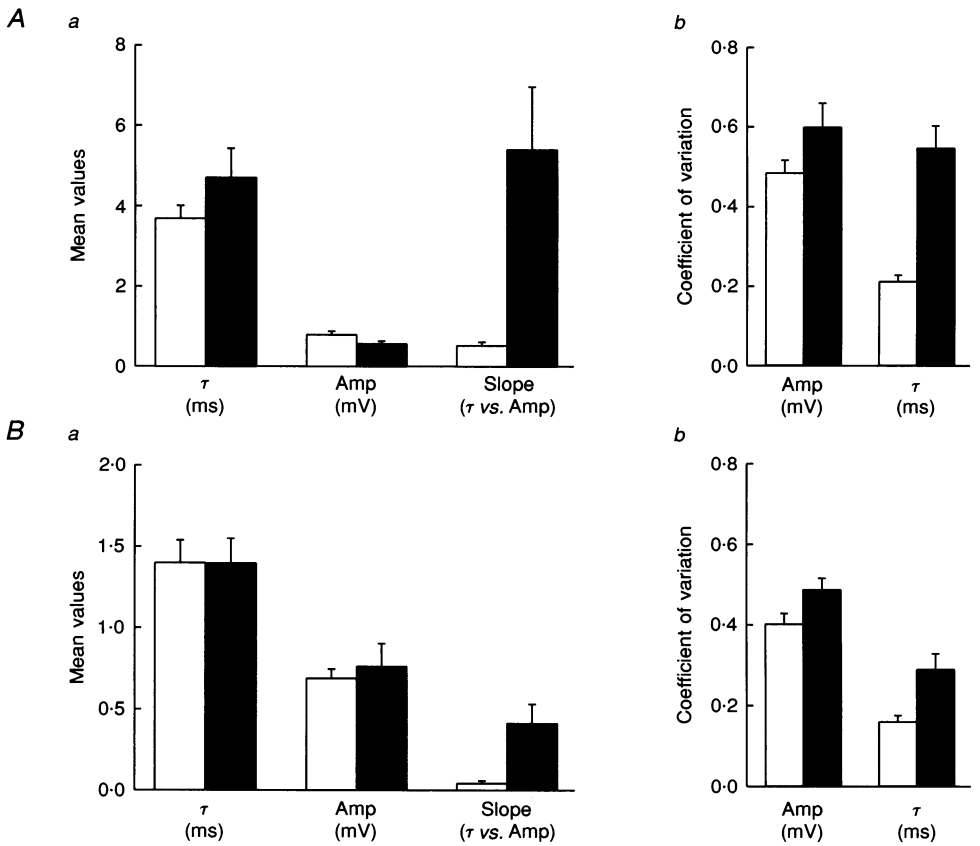

Figure 4. Summary of data in the presence and absence of neostigmine

A a, in the presence of the AChE inhibitor neostigmine, the mean values of the decay constants (τ) were similar for $EPPs_0$ (□) and $MEPPs_0$ (■) and the mean values of the amplitudes (Amp) were not. The mean of the slopes of the τ *vs.* amplitude relationship was profoundly higher for $MEPPs_0$ (21 endplates). *Ba*, in the absence of neostigmine, $MEPPs_0$ and $EPPs_0$ had similar τ values and amplitudes. Nevertheless, the slope of τ *vs.* amplitude was, once more, higher for $MEPPs_0$ (17 endplates). *A b* and *B b*, the variability of the amplitudes of $EPPs_0$ and $MEPPs_0$ was not significantly different. The difference between the variability in τ values of $MEPPs_0$ as compared to that of $EPPs_0$ was high both with (*A b*) and without (*B b*) neostigmine.

difference between the two signals, independent of AChE activity.

Does the difference stem from changes in membrane conductance?

Since the extracellular electrode monitors the magnitude of the electric field rather than the actual ionic current across the endplate membrane, we were concerned by the possibility that, in our experiments, the differences in signal shape may have stemmed from changes in membrane conductance following nerve stimulation. The conductance of the muscle membrane outside the recording area may be higher following nerve stimulation, as a result of the simultaneous release of transmitter at many active spots. If this were the case, $MEPPs_0$ and $EPPs_0$ might assume different shapes even if the currents across the endplate were identical. To test this possibility, we carried out thirteen experiments in which the muscle was immersed in calcium-deficient Ringer solution (with 2 mM $MgCl_2$) and the recording electrode alone contained 18 mM

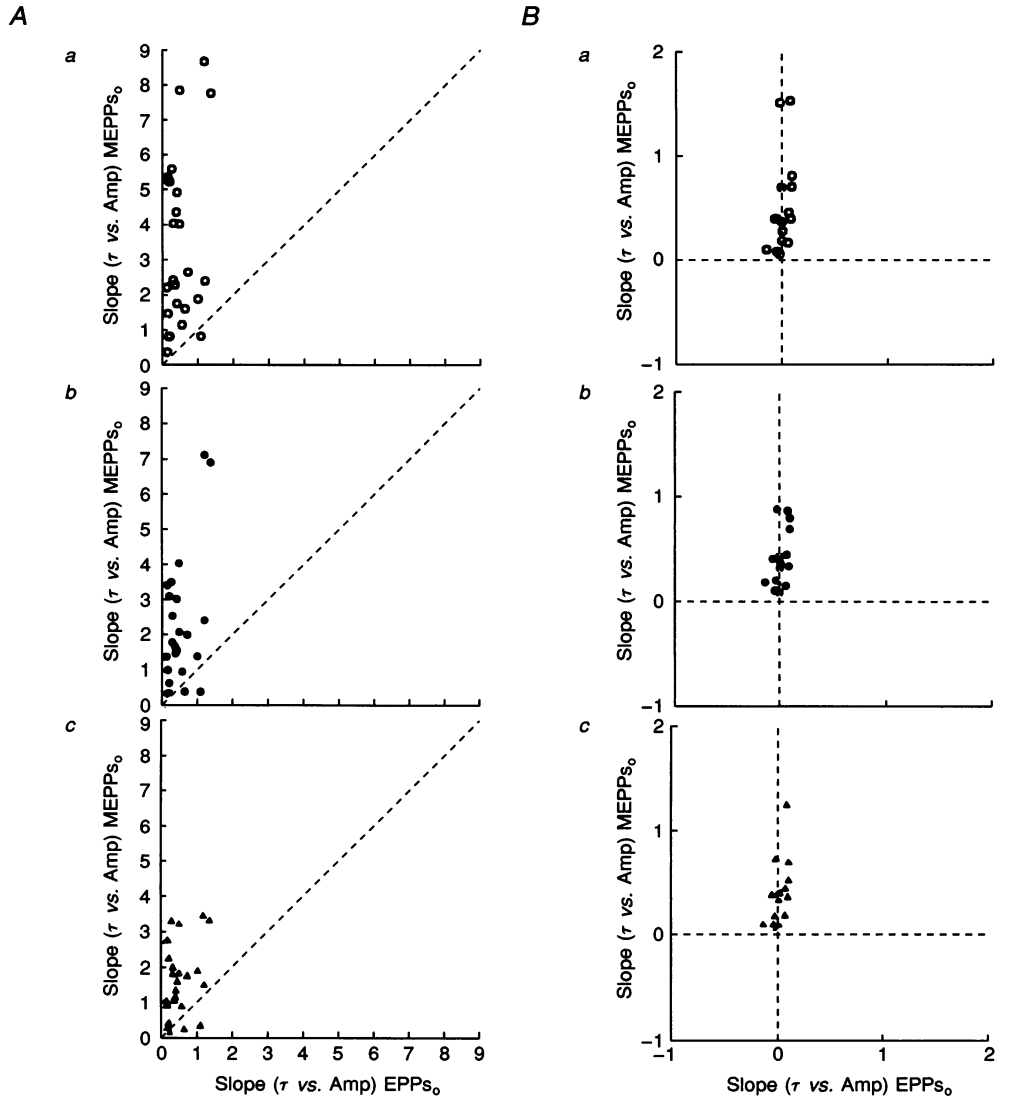

Figure 5. Summary of results subjected to selection procedures

The slope of τ *vs.* amplitude of $MEPPs_0$ was plotted against the corresponding slope of $EPPs_0$ in each experiment. *A*, with neostigmine; *B*, without neostigmine. *A a*, in the presence of AChE inhibitor, all points but one lay to the left of the dashed line, thus indicating that the slopes of $MEPPs_0$ were significantly higher than those of $EPPs_0$. *A b*, the same result was obtained after excluding $MEPPs_0$ from the analysis whose τ values were outside the range of the mean $\pm$ 2 S.D. *A c*, the result persisted when the analysis included only those $MEPPs_0$ whose τ values fell within the range of the τ values of $EPPs_0$. *Ba*, in the absence of neostigmine, the slopes of $EPPs_0$ were not significantly different from 0, whereas those of $MEPPs_0$ were positive in all endplates. *Bb*, this result did not change upon elimination of $MEPPs_0$ whose τ values were outside the range of the mean $\pm$ 2 S.D. *Bc*, the result persisted when only $MEPPs_0$ which had τ values falling within the range of $EPPs_0$ τ values were analysed.

$CaCl_2$. The difference in the shape of $MEPPs_0$ and $EPPs_0$ persisted in these experiments too. The mean slopes of the linear regression lines between τ and amplitude were $5{\cdot}22 \pm 1{\cdot}97$ for $MEPPs_0$ and $0{\cdot}57 \pm 0{\cdot}28$ for $EPPs_0$. These values are similar to those obtained when the extracellular medium contained 0·4 mM Ca^{2+} and 2 mM Mg^{2+}: $5{\cdot}52 \pm 1{\cdot}3$ for $MEPPs_0$ and $0{\cdot}49 \pm 0{\cdot}08$ for $EPPs_0$ (Fig. 4*Aa*). Thus, the time course of the transmembrane synaptic current was not quite the same during the two types of synaptic activity.

Does the difference stem from the presence of outstanding $MEPPs_0$?

We suspected that the difference in τ *vs.* amplitude relationships between $EPPs_0$ and $MEPPs_0$ may have arisen from the inclusion of aberrant spontaneous $MEPPs_0$ in the analysis; thus, the presence of exponentially decaying $MEPPs_0$, with decay times that profoundly deviate from the mean τ, may have in itself changed the relationship between decay time and amplitude. This possibility was tested by exposing the $MEPPs_0$ to two increasingly rigorous selection procedures. Firstly, only $MEPPs_0$ whose τ values were within the range of the mean $\pm$ 2 S.D. were selected and their slope (τ *vs.* amplitude) was compared with that of $EPPs_0$ in each experiment. The results obtained before and after this selection are illustrated in Fig. 5*A a* and *b*. It is clear that the elimination of $MEPPs_0$ with either very high or very low τ values decreased the slope, and in two endplates the slope was even lower than that for $EPPs_0$. Nevertheless, in the majority of experiments (21 out of 23), the dependence of τ on amplitude remained higher for $MEPPs_0$ than for $EPPs_0$.

Secondly, $MEPPs_0$ were selected even more rigorously to include only those signals whose τ values were within the range of those encountered in the $EPPs_0$ themselves (Fig. 5*Ac*). Following this process of selection, both $MEPPs_0$ and $EPPs_0$ had similar amplitudes and similar τ values but the relationship between them was still significantly higher for $MEPPs_0$ in twenty out of twenty-three experiments.

It is interesting to note that the difference between the slopes displayed by $MEPPs_0$ and $EPPs_0$ in the control solution (Fig. 5*Ba*) was also maintained after elimination of $MEPPs_0$ with extreme values of τ (Fig. 5*Bb* and *c*) and the slopes were still positive for $MEPPs_0$ and about 0 for $EPPs_0$ in all experiments. These results strengthen the conclusion that signals induced by spontaneously secreted single quanta of ACh are indeed different from those induced by nerve stimulation.

DISCUSSION

In this work we showed that uniquantal $EPPs_0$ and $MEPPs_0$ recorded at the same location on the muscle endplate are not entirely similar in shape. The most striking finding is the positive correlation between $MEPPs_0$ amplitude and decay time constant and its absence in uniquantal $EPPs_0$.

This difference cannot be attributed to the nerve terminal arborization complexity, since the extracellular focal recording method was employed to monitor exclusively signals originating within several micrometres of the electrode (del Castillo & Katz, 1956). Unspecific external signals which reflect the electric field produced by evoked activity in remote parts of the muscle were very infrequent, due to the low quantal content of release and reproducibility of the results in the virtual absence of calcium ions from the bath solution. These infrequent unspecific responses were discarded from further analysis along with 'giant $MEPPs_0$' produced by blocking AChE activity (del Castillo & Katz, 1956). Since both spontaneous and evoked activities were monitored simultaneously, the difference between the properties of $MEPPs_0$ and uniquantal $EPPs_0$ cannot stem from time-dependent changes in either muscle resting potential or in the 'pipette compression artifact' which obscures diffusion of ACh (Katz & Miledi, 1973).

The positive correlation between the amplitude and decay time constant (τ) of MEPPs upon block of AChE activity was previously related to *en passant* binding of ACh molecules to the double-sited postsynaptic receptors during transmitter diffusion out of the synaptic cleft (Katz & Miledi, 1973; Adams, 1981; Glavinovic, 1984, 1986). A similar relationship between the amplitude and τ in large multiquantal EPPs (in the presence and absence of AChE-blocking agents) was previously ascribed to temporal dispersion of the release of quanta and spatial interaction of several quanta (Katz & Miledi, 1965, 1973; Magleby & Terrar, 1975; Hartzell, Kuffler & Yoshikami, 1975; Magazanik, Nicolosky & Giniatullin, 1984; Giniatullin, Khazipov & Vyskočil, 1993).

The absence of such correlation in endplate currents formed by a smaller number of quanta may be attributed to spatial separation of quanta (Glavinovic, 1985, 1986). Nevertheless, if evoked and spontaneous release of quanta take place at the same location on the nerve terminal, the same relationship between amplitude and τ is expected for $MEPPs_0$ and uniquantal $EPPs_0$.

We propose that the differences of signal shape found in this work may indicate that spontaneous and evoked release occur at different locations of the nerve terminal which face different distributions of the cholinergic receptors.

Quanta of ACh are usually released at the well-differentiated active zones of the presynaptic membrane located facing clusters of cholinergic receptors arranged on the postsynaptic folds (Wernig & Stirner, 1977; Heuser, Reese, Dennis, Jan, Jan & Evans, 1979). However, fusion of synaptic vesicles with the plasma membrane also occurs outside the double-row active zone structure and may evoke calcium-independent release of transmitter (Ceccarelli, Grohovaz & Hurlbut, 1979; Kim, 1986; Ceccarelli, Fesce, Grohovaz & Haimann, 1988; Nystrom & Ko, 1988; Grinnell & Pawson, 1989).

Quanta released at undifferentiated patches of membrane outside the active zone, and those released at the active zones, may face different postsynaptic arrangements. Variations of 14–21 % in receptor density among endplates were suggested in the electrophysiological studies of Matthews-Bellinger & Salpeter (1978). The possibility that cholinergic receptors at the subsynaptic membrane form a non-homogeneous population, namely, that two different populations of receptors are located next to one another, was suggested by Albuquerque & Gage (1978).

Of the many models that have been proposed to simulate synaptic currents (Magleby & Stevens, 1972*a*; Gage, 1976; Steinbach & Stevens, 1976; Rosenberry, 1979; Wathey, Nass & Lester, 1979; Land, Salpeter & Salpeter, 1980, 1981; Land, Harris, Salpeter & Salpeter, 1984; Madsen, Edeson, Lamtt & Milne, 1984; Madsen, Edeson & Milne, 1987), those presented by Land *et al.* (1981, 1984) and Wathey *et al.* (1979) are of particular relevance to the present work, since, in these models, receptor density was one of the parameters explicitly examined. Thus, for example, Land *et al.* (1981) concluded that, when the esterase is blocked, no correlation between miniature endplate current (MEPC) amplitude and rise time is to be expected if receptor density is high, while a positive correlation is anticipated if the density is reduced. This prediction was borne out by their experimental results, using α-bungarotoxin to reduce the effective concentration of ACh receptors (Land *et al.* 1981). In addition, both Land *et al.* (1984) and Wathey *et al.* (1979) showed that, when the esterase is inhibited, a positive correlation between MEPC amplitude and decay time is expected, due to 'buffered diffusion', if receptor density is high, but not when it is low.

At first sight, our results seem to clash with this set of predictions. The absence of any correlation between amplitude and rise time in uniquantal $EPPs_0$ when the esterase is inhibited would suggest that evoked release occurs at locations confronting postsynaptic areas densely packed with receptors. The positive correlations between amplitude and rise time in $MEPPs_0$ would imply that at least some of the spontaneous release takes place at locations facing lower receptor densities. However, if significant numbers of $MEPPs_0$ are indeed produced on such postsynaptic areas of scarcely distributed receptors, the models predict that, contrary to our findings, no positive correlation should be observed between $MEPP_0$ amplitude and decay time.

To reconcile all our results with the theoretical expectations, we must make the following two assumptions. (1) Spontaneous release occurs, at least in part, at locations facing low receptor densities. (2) The ACh molecules in this type of release are highly restricted in their diffusion out of the synaptic cleft and undergo repetitive binding to receptors. Thus, $MEPPs_0$ are produced at postsynaptic patches of sparsely distributed receptors, leading to the positive correlation between amplitude and rise time. In addition, ACh molecules undergo repetitive binding, leading to the positive correlation between amplitude and decay time.

The special morphology of the frog endplate lends some support to the hypothesis of restricted diffusion. Matthews-Bellinger & Salpeter (1978) identified two regions of differential junctional membranes in the frog neuromuscular synapse, which they named 'zone 1' and 'zone 2'. Zone 2 contains the active zones and apposing deep junctional folds, while zone 1 contains portions of interdigitating Schwann 'fingers', but no active zones and no secondary cleft. In zone 1 (see Fig. 1 of Matthews-Bellinger & Salpeter, 1978), the Schwann 'fingers' wrap around the nerve terminal. If spontaneous release occurs in both zones 1 and 2, while evoked release takes place only in zone 2, our findings may be fully reconciled within a single framework. Firstly, spontaneous release would differ from evoked release in facing relatively low receptor densities, since zone 1 lacks the deep folds which are richly packed with receptors. Secondly, the Schwann 'fingers' would restrict the movement of ACh molecules on their way out of the cleft when these molecules are released underneath them, but would not impede the diffusion of transmitter molecules released in zone 2.

In this working hypothesis of receptor density variation and restricted diffusion, we assume that similar vesicles are associated with spontaneous and evoked release. Furthermore, even if different pools of vesicles were recruited in the two types of release, it cannot easily be envisaged how they would contribute to the formation of the different shapes of the synaptic signals.

An alternative explanation of our results may rest on the assumption that receptor density throughout the cleft is homogeneous, but the kinetic nature of the receptors is not. Our findings may be explained if the transmitter molecules released following stimulation encounter a homogeneous population of receptors, while those liberated spontaneously bind to heterogeneous receptors, some of which possess longer open lifetimes, similar to those in extrajunctional channels (Sakmann, 1978; Jackson, Wong, Morris, Lecar & Christian, 1983). The model of Wathey *et al.* (1979, see their Table II) predicts that as the open channel lifetime increases, the amplitude, rise time and decay time of the synaptic signal should all increase. We do not favour this interpretation, since no evidence supports the idea that a kinetically heterogeneous population of receptors is to be found within the primary synaptic cleft. The extrajunctional type of channel has only rarely been detected while monitoring synaptic activity (Colquhoun & Sakmann, 1985).

In summary, we propose that uniquantal evoked synaptic signals and spontaneous synaptic signals differ in their shape as a result both of the different locations at which quanta are released from the presynaptic nerve terminal and of the different receptor densities facing these locations on the postsynaptic muscle. MEPPs are produced, at least in part, at postsynaptic patches of sparsely distributed receptors, hence leading to the positive correlation between amplitude and *rise time*. In addition, ACh molecules of spontaneous release undergo repetitive binding due to their restricted diffusion, leading to the positive correlation between amplitude and *decay time*.

ADAMS, R. R. (1981). Acetylcholine receptor kinetics. *Journal of Membrane Biology* **58**, 161–174.

ALBUQUERQUE, E. X. & GAGE, P. W. (1978). Differential effects of perhydrohistrionicotoxin on neurally and iontophoretically evoked endplate currents. *Proceedings of the National Academy of Sciences of the USA* **75**, 1596–1599.

CECCARELLI, B., FESCE, R., GROHOVAZ, F. & HAIMANN, C. (1988). The effect of potassium on exocytosis of transmitter at the frog neuromuscular junction. *Journal of Physiology* **401**, 163–183.

CECCARELLI, B., GROHOVAZ, F. & HURLBUT, W. P. (1979). Freeze fracture studies of frog neuromuscular junctions during intense release of transmitter. II. Effect of electrical stimulation and high potassium. *Journal of Cell Biology* **81**, 178–192.

COLQUHOUN, D. & SAKMANN, B. (1985). Fast events in single channel currents activated by acetylcholine and its analogues at the frog muscle endplate. *Journal of Physiology* **369**, 510–557.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the endplate potential. *Journal of Physiology* **124**, 560–573.

DEL CASTILLO, J. & KATZ, B. (1956). Localization of active spots within the neuromuscular junction of the frog. *Journal of Physiology* **132**, 630–649.

FATT, P. & KATZ, B. (1952). Spontaneous subthreshold activity at motor nerve endings. *Journal of Physiology* **117**, 109–128.

GAGE, P. W. (1976). Generation of endplate potentials. *Physiological Reviews* **56**, 177–247.

GINIATULLIN, R. A., KHAZIPOV, R. N. & VYSKOČIL, F. (1993). A correlation between quantal content and decay time of endplate currents in frog muscles with intact cholinesterase. *Journal of Physiology* **466**, 95–103.

GLAVINOVIC, M. I. (1984). Prolongation of min. e.p.c.s after cholinesterase blockage is amplitude-dependent. *Journal of Physiology* **354**, 43*P*.

GLAVINOVIC, M. I. (1985). Effect of change in spatial distribution of vesicular release on the variability of miniature end-plate currents in the frog. *Journal of Physiology* **358**, 85*P*.

GLAVINOVIC, M. I. (1986). Variability of quantal events in control solution and after acetylcholinesterase blockade in frog. *Neuroscience* **17**, 519–526.

GLAVINOVIC, M. I. (1987). Synaptic depression in frog neuromuscular junction. *Journal of Neurophysiology* **58**, 230–245.

GRINNELL, A. D. & PAWSON, P. A. (1989). Dependence of spontaneous release at frog junctions on synaptic strength, external calcium and terminal length. *Journal of Physiology* **418**, 397–410.

HARTZELL, H. C., KUFFLER, S. W. & YOSHIKAMI, D. (1975). Postsynaptic potentiation: interaction between quanta of ACh at the skeletal neuromuscular synapse. *Journal of Physiology* **251**, 427–463.

HEUSER, J. E., REESE, T. S., DENNIS, M. J., JAN, Y., JAN, L. & EVANS, L. (1979). Exocytosis of synaptic vesicles captured by quick freezing and correlated with quantal transmitter release. *Journal of Cell Biology* **81**, 275–300.

JACKSON, M. B., WONG, B. S., MORRIS, C. E., LECAR, H. & CHRISTIAN, C. N. (1983). Successive openings of the same acetylcholine receptor channel are correlated in open time. *Biophysical Journal* **43**, 109–114.

KATZ, B. & MILEDI, R. (1965). The effect of temperature on the synaptic delay at the neuromuscular junction. *Journal of Physiology* **181**, 656–670.

KATZ, B. & MILEDI, R. (1973). The binding of acetylcholine to receptors and its removal from the synaptic cleft. *Journal of Physiology* **231**, 549–579.

KIM, Y. I. (1986). Passively transferred Lambert-Eaton syndrome in mice receiving purified IgG. *Muscle and Nerve* **9**, 523–530.

LAND, B. R., HARRIS, W. V., SALPETER, E. E. & SALPETER, M. M. (1984). Diffusion and binding constants for acetylcholine derived from the falling phase of minature endplate currents. *Proceedings of the National Academy of Sciences of the USA* **81**, 1594–1598.

LAND, B. R., SALPETER, E. E. & SALPETER, M. M. (1980). Acetylcholine receptor site density affects the rising phase of miniature endplate currents. *Proceedings of the National Academy of Sciences of the USA* **77**, 3736–3740.

LAND, B. R., SALPETER, E. E. & SALPETER, M. M. (1981). Kinetic parameters for acetylcholine interaction in intact neuromuscular junction. *Proceedings of the National Academy of Sciences of the USA* **78**, 7200–7204.

LILEY, A. W. (1956). An investigation of spontaneous activity at the neuromuscular junction of the rat. *Journal of Physiology* **132**, 650–666.

LINDER T. M., PENNEFATHER, P. & QUASTEL, D. M. (1984). The timecourse of miniature endplate currents and its modification by receptor blockade and ethanol. *Journal of General Physiology* **83**, 435–468.

MADSEN, B. W., EDESON, R. O., LAMTT, S. & MILNE, R. K. (1984). Numerical simulation of miniature endplate currents. *Neuroscience Letters* **48**, 67–74.

MADSEN, B. W., EDESON, R. O. & MILNE, R. K. (1987). Neurotransmission parameters estimated from miniature endplate current growth phase. *Brain Research* **402**, 387–392.

MAGAZANIK, L. G., NIKOLOSKY, E. E. & GINIATULLIN, R. A. (1984). End-plate currents evoked by paired stimuli in frog muscle fibres. *Pflügers Archiv* **401**, 185–192.

MAGLEBY, K. L. & STEVENS, C. F. (1972*a*). A quantitative description of end-plate currents. *Journal of Physiology* **223**, 173–197.

MAGLEBY, K. L. & STEVENS, C. F. (1972*b*). The effect of voltage on the time course of endplate currents. *Journal of Physiology* **223**, 151–171.

MAGLEBY, K. L. & TERRAR, D. A. (1975). Factors affecting the time course of decay of end-plate currents: a possible cooperative action of ACh on receptors at the frog neuromuscular junction. *Journal of Physiology* **244**, 467–495.

MATTHEWS-BELLINGER, J. & SALPETER, M. M. (1978). Distribution of acetylcholine receptors at frog neuromuscular junctions with a discussion of some physiological implications. *Journal of Physiology* **279**, 197–213.

NYSTROM, R. R. & KO, C. P. (1988). Disruption of active zones in frog neuromuscular junctions following treatment with proteolytic enzymes. *Journal of Neurocytology* **17**, 63–71.

PECOT-DECHAVASSINE, M. (1976). Action of vinblastine on the spontaneous release of acetylcholine at the frog neuromuscular junction. *Journal of Physiology* **261**, 31–48.

ROSENBERRY, T. (1979). Quantitative simulation of endplate currents at the neuromuscular junctions based on the reaction of acetylcholine with acetylcholine receptor and acetylcholinesterase. *Biophysical Journal* **26**, 263–290.

SAKMANN, B. (1978). Acetylcholine-induced ionic channels in rat skeletal muscle. *Federation Proceedings* **37**, 2654–2659.

STEINBACH, J. H. & STEVENS, C. F. (1976). Neuromuscular transmission. In *Frog Neurobiology*, ed. LLINAS, R. & PRECHT, W., pp. 33–92. Springer, New York.

WATHEY, J. C., NASS, M. M. & LESTER, H. A. (1979). Numerical reconstitution of the quantal event at nicotinic synapses. *Biophysical Journal* **27**, 145–164.

WERNIG, A. & STIRNER, H. (1977). Quantum amplitude distributions point to functional unity of synaptic 'active zone'. *Nature* **269**, 820–822.

Acknowledgements

We thank Dr Idan Segev for suggesting a crucial control experiment and Professor Rami Rahamimoff for his most helpful suggestions.

Received 16 May 1994; accepted 14 July 1994.