

5. G. Glick and E. Braunwald, *Circ. Res.*, 16, 363 (1965).
6. D. F. Leon, J. A. Shaver, and J. J. Leonard, *Am. Heart J.*, 80, 729 (1970).
7. G. Mancina, J. Ludbrook, A. Ferrari, et al., *Circ. Res.*, 43, 170 (1978).
8. T. G. Pickering, B. Gribbin, and P. Sleight, *Cardiovasc. Res.*, 6, 277 (1972).
9. A. M. Scher, W. W. Ohm, K. Bumgarner, et al., *Fed. Proc.*, 31, 1219 (1972).
10. S. F. Vatner, C. B. Higgins, and E. Braunwald, *Cardiovasc. Res.*, 8, 153 (1974).
11. H. Vidrio and I. Tena, *Clin. Pharm. Ther.*, 28, 587 (1980).
12. A. J. Zweifler and L. Hansson, *Clin. Pharm. Ther.*, 19, 403 (1976).

FREQUENCY FACILITATION IN NEUROMUSCULAR SYNAPSES OF THE FROG SARTORIUS MUSCLE

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Frequency facilitation, or an increase in mediator liberation in response to a stepwise change in stimulation frequency from 0.5 to 10 Hz, is one form of facilitation in the neuromuscular junction. It has been suggested that this form of facilitation is based on mobilization of the mediator, i.e., "migration" of quanta of mediator toward the points of liberation, or their preparation for liberation [4, 5, 8, 9]. In this case, in accordance with the statistical hypothesis of mediator liberation [6], the increase in quantum composition of end-plate potentials (m) during frequency facilitation ought to be accompanied by an increase in the reserves of accessible mediator (n) without any change in the probability of liberation of a mediator quantum (P), and this has been demonstrated experimentally [5].

However, determination of the binomial parameters of mediator liberation at relatively low frequencies of stimulation (5-10 Hz) revealed an appreciable increase in the parameter P also [3]. This suggests that frequency facilitation may reflect not only mobilization of the mediator, but also a change in the probability of liberation of a mediator quantum. The investigation described below was carried out to test this hypothesis.

EXPERIMENTAL METHOD

Neuromuscular preparations of the sartorius muscle of *Rana ridibunda* were perfused with Ringer's solution of the following composition (in mM): NaCl 115; KCl 2; CaCl₂ 0.9-1.8; MgCl₂ 4-6; pH 7.2-7.4. End-plate potentials (EPPs) and miniature EPPs (MEPPs) were recorded intracellularly by a standard microelectrode technique from the synaptic regions of superficial muscle fibers. To investigate frequency facilitation, the motor nerve was stimulated by series of pulses (600 stimuli) at frequencies of 0.5, 2, 4, 6, and 8 Hz. The last 100-200 EPPs were recorded in each series [9]. Assuming an exponential relationship between the evoked liberation of mediator (m) and the frequency of stimulation (f)

$$m = m_0 e^{kf},$$

the coefficient of frequency facilitation (k) and the value of m at zero frequency (m₀) were determined by the method of least squares. The method of determining binomial parameters of mediator liberation was fully described previously [1-3, 5]. Values of facilitation after single and paired (interval 200 msec) stimulation also were determined. The testing pulse was applied 16-600 msec after the conditioning stimulus. The intervals between stimulations were 12-30 sec. The dynamics of facilitation in this case was described by the equation $F_t = F_0 e^{t/\tau}$ [10], where F_t denotes facilitation during time t after conditioning stimulation, F_0 facilitation at t = 0, and τ is the time constant of k of facilitation. Changes in the parameters m, n, and P during facilitation (F_m , F_n , F_p) were calculated by the equations:

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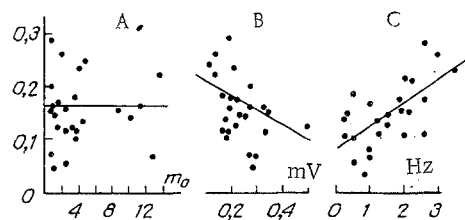


Fig. 1. Coefficient of frequency facilitation as a function of quantum composition of EPP at zero frequency (A), of amplitude of MEPP (B), and frequency of MEPP (C). Abscissa: A) m_0 , B) amplitude of MEPP (in mV), C) frequency of MEPP (in Hz); ordinate, coefficient of frequency facilitation (k).

TABLE 1. Dynamics of Binomial Parameters of Mediator Liberation during Frequency Stimulation (values at frequency of 0.5 Hz taken as 100%; $m \pm M$; $n = 9$)

| Binomial parameter | Frequency of stimulation of motor nerve, Hz | | | | |
|--------------------|---|-----------|------------|------------|------------|
| | 0,5 | 2 | 4 | 6 | 8 |
| m | 100 | 126,3±6,4 | 171,6±21,1 | 248,5±34,8 | 335,5±49,9 |
| P | 100 | 137,0±9,4 | 141,9±11,1 | 161,5±18,3 | 144,9±18,2 |
| n | 100 | 95,1±13,5 | 123,3±13,5 | 159,0±20,0 | 238,0±29,4 |

$F_m = (m_t - m_1)/m_1$; $F_n = (n_t - n_1)/n_1$; $F_p = (P_t - P_1)/P_1$, where m_1 , n_1 , and P_1 are the binomial parameters of the first EPP and m_t , n_t , and P_t are the same for the testing EPP with an interval of t (in milliseconds) between stimulations. All the results were subjected to statistical analysis by the Nairi computer.

EXPERIMENTAL RESULTS

When mediator liberation was sharply reduced ($Mg^{++} = 4-6$ mM; $Ca^{++} = 0.9$ mM) the value of m was found to be an exponential function of the frequency of stimulation of the motor nerve. Values of the coefficient of frequency facilitation (k) varies from 0.04 to 0.29, and the mean quantum composition at zero frequency (m_0) varied from 0.7 to 14.0. Analysis revealed no significant relationship between k and m_0 (coefficient of correlation +0.02; $P > 0.1$; Fig. 1A). Negative correlation was found between k and the initial amplitude of MEPP and positive correlation with the frequency of MEPP (coefficients of correlation -0.42 and +0.59, respectively; $P < 0.05$; Fig. 1B, C). The frequency and amplitude of MEPP are known to depend on the extent (area) of synaptic contact [7]. The unequal values of k may therefore be due to the different sizes of the synapses studied.

Determination of the binomial parameters of mediator liberation showed that during frequency facilitation the increase in m was accompanied by changes in P and n (Table 1; Fig. 2A). At relatively low frequencies of stimulation (0.5-2 Hz) the parameter P increased without any appreciable changes in the parameter n . A further increase in the frequency of stimulation led to an even more marked increase in the parameter P , followed by a small decrease at frequencies of over 6 Hz. The maximal increase in the parameter P was $161.5 \pm 18.3\%$. At frequencies of over 2 Hz there was a steady increase in the parameter n , which reached $238.0 \pm 29.4\%$ of the initial value at a frequency of 8 Hz (Table 1).

The results of investigation of two neuromuscular synapses with different values of frequency facilitation are given in Fig. 2. In synapses with relatively low values of k the increase in the parameters P and n was small and was observed at relatively high values of P (close to unity) and low values of n (Fig. 2B). It can be postulated on the basis of the calcium hypothesis of facilitation of mediator liberation [10, 11] that in endings with low values of k and high initial values of P secretion reaches saturation level on account of Ca^{++} ions entering the nerve ending even at relatively low frequencies of stimulation, and a further increase in the frequency of stimulation is not accompanied by any increase in P [1, 3]. The small rise in n in these synapses can be explained by the small number of liberation zones, which correlates with the smaller area of synaptic contact [1]. The presence of

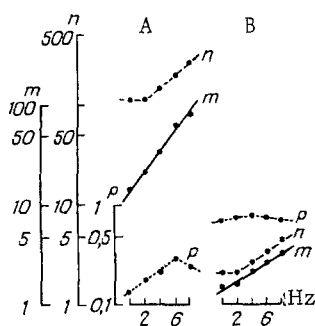


Fig. 2

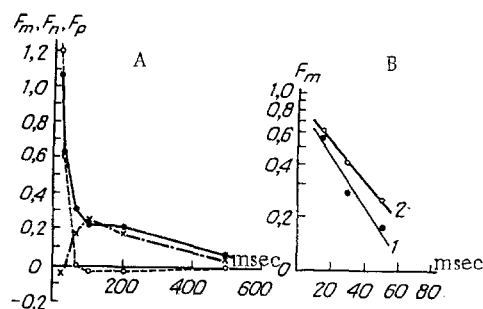


Fig. 3

Fig. 2. Changes in binomial parameters of mediator liberation in two neuromuscular synapses with different degrees of frequency facilitation. A) $k = 0.26$, amplitude of MEPP 0.18 mV, frequency of MEPP 4 Hz; B) 0.1, 0.35 mV, and 0.3 Hz respectively. Abscissa, frequency of stimulation of motor nerve (in Hz); ordinate, parameters m , n , and P .

Fig. 3. Facilitation of mediator liberation in neuromuscular synapses of the frog sartorius muscle. A) Changes in binomial parameters m (filled circles), n (empty circles), and P (crosses); B) dynamics of decay of first component of facilitation after single (1) and paired (with interval of 200 msec; 2) stimulation. Results of six experiments. Abscissa: A) F_m, F_n, F_p ; B) F_m . Ordinate, interval between testing and conditioning stimulation (in msec).

synapses with low values of k explains results obtained in some investigations of frequency facilitation, in which no change was found in the parameter P [5].

The increase in mediator secretion during repetitive stimulation is due to summation of facilitation after each single pulse [10], and this is also accompanied by an increase in the statistical parameters n and P [2]. Facilitation after a single stimulation is made up of two components: The first arises immediately after the conditioning pulse and decays exponentially to 100 msec, while the second begins much later, reaches a maximum after 120-200 msec, and ends after 1.0-1.5 sec [10]. We showed that the increase in n coincides with the development of the first component of facilitation and the increase in P with the course of the second component [2]. The results of a separate experiment to study changes in the binomial parameters during facilitation after a single pulse are illustrated in Fig. 3A.

Comparison of changes in the statistical parameters during frequency facilitation and after a single pulse showed that the increase in P during a stepwise increase in the frequency of stimulation was due to summation of P during the second component of facilitation after each separate pulse. In fact, at frequencies of stimulation of 2-6 Hz (interval between pulses 500-166.7 msec) P increased. The increase in n observed with stimulation at a frequency of 4-8 Hz (interval between stimulations 250-125 msec) still remains unexplained. Since the first component of facilitation with paired stimulation, due to the increase in n , ends after 100 msec, at the frequencies of stimulation used there should be no increase in the parameter n . It might be supposed that during repetitive stimulation the time course of the component of facilitation would be protracted.

An investigation of the dynamics of decay of the first component of facilitation after paired conditioning stimulation with an interval of 200 msec confirmed the validity of these hypotheses. It was found that the use of two pulses as conditioning stimulation led to an increase in the time constant of decay of the first component of facilitation (τ). For instance, whereas after a single pulse the values of τ were 26.1 ± 5.1 msec, after double stimulation they were 36.0 ± 7.9 msec (Fig. 3B). The differences were statistically significant ($P < 0.05$). Lengthening of the time course of the first component of facilitation during repetitive stimulation was associated, in all probability, with the accumulation and delayed utilization of Ca^{++} in the nerve ending. This is shown, in particular, by the increase in τ of the first component of facilitation with an increase in the Ca^{++} concentration in the surrounding solution [11].

The results thus suggest that frequency facilitation is accompanied by two processes: an increase in the reserves of accessible mediator and an increase in the probability of liberation of the acetylcholine quantum. The increase in P is due to summation of the second component of facilitation whereas the increase in n is due to superposition of the first component, which follows a more protracted course during repetitive stimulation. The increase in n is evidently a process of mobilization of the mediator and is linked with preparation of acetylcholine quanta for liberation, whereas the increase in P is associated with activation of the secretion mechanism on account of an increased rate of Ca^{++} inflow into the nerve ending.

LITERATURE CITED

1. A. L. Zefirov, *Fiziol. Zh. SSSR*, 66, 531 (1980).
2. A. L. Zefirov, *Neirofiziologiya*, 12, 324 (1980).
3. A. L. Zefirov and S. N. Zemskova, *Fiziol. Zh. SSSR*, 65, 1220 (1979).
4. K. Alkadni and R. L. Volle, *Arch. Int. Pharmacodyn.*, 229, 261 (1977).
5. D. D. Branisteanu, M. D. Miyamoto, and R. L. Volle, *J. Physiol. (London)*, 254, 19 (1976).
6. J. Del Castillo and B. Katz, *J. Physiol. (London)*, 124, 560 (1954).
7. T. Maeno, *J. Neurophysiol.*, 32, 793 (1969).
8. T. Maeno and C. Edwards, *J. Neurophysiol.*, 32, 785 (1969).
9. A. Mallart and A. R. Martin, *J. Physiol. (London)*, 193, 679 (1967).
10. R. Rahamimoff, *J. Physiol. (London)*, 195, 471 (1968).

BIORHYTHM OF THE PARTIAL PRESSURE OF OXYGEN IN UTERINE AND FETAL TISSUES

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It has long been known that the body exhibits great resistance to hypoxia and anoxia during antenatal and early postnatal periods of development [6, 9, 11, 12, 16]. The cause of this phenomenon has not yet been explained. Some workers attribute the increased tolerance to differences in the energy metabolism of newborn infants, to which an important relative contribution is made by anaerobic glycolysis [15, 18]. Other workers consider that neonatal tissues have a low level of oxidative metabolism [17]. The glycogen content in the heart and liver of newborn infants also is known to be 10 times higher than in the adult [5, 14]. However, the mechanism of stimulation of reactions which readjust the metabolism of the fetus in order to maintain its viability under conditions of oxygen lack has never been explained. Now, after much research, it has been shown that preliminary training under conditions of moderate hypoxia increases the resistance of the body to more severe hypoxia and to various other pathogenic factors [3, 4, 7, 8, 10]. The resistance of the body has been shown to be increased irrespective of the conditions of creation of hypoxia, i.e., it is independent of whether the hypoxic state is continuous for a certain period of time or whether it is induced by repeated short exposures to oxygen insufficiency [1].

The uterus in sexually mature animals is known to exhibit continuous contractile activity. During pregnancy considerable contractile activity of the uterus, both isotonic and isometric, also is observed [2]. There are data in the literature on the partial pressure of oxygen (pO_2) in the tissues of the uterus and fetus, which show that pO_2 falls during each contraction, even if only for short duration [13]. The biological significance of the

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