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The giant miniature endplate potentials frequency is increased in aged rats

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HIGHLIGHTS

- Neuromuscular transmission efficiency is compromised only in elderly.
- GMEPPs frequency is markedly increased in aged rats.
- Both amplitude and quantal content of EPPs are decreased in aged rats.
- EPPs failures in aged rats are ~3 times lower than expected by mathematical prediction.
- High occurrence of GMEPPs might act as a compensatory mechanism.

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ABSTRACT

At the neuromuscular junction, spontaneous giant events (GMEPPs) are enhanced in different conditions when degenerative and/or remodeling processes take place, but no one investigated their incidence upon aging. In the present work, we evaluated evoked and spontaneous neuromuscular transmission events recorded from single muscle fibers. Phrenic-diaphragm preparations of 3–4, 12–16, 36–40 and 70–80 weeks old rat males were used. We found that the occurrence of GMEPPs significantly increases in aged rats. Moreover, in old rats the neuromuscular transmission was significantly impaired due to a significant decrease in the amplitude and quantal content of evoked endplate potentials. Interestingly, the number of observed EPPs failures were ~3 times lower than the predicted value based on the quantal content. This discrepancy was not observed in infant or adult rats. The coincidence of a high GMEPPs frequency with a lower than expected EPPs failure rate suggests that GMEPPs events are needed to preserve effective neuromuscular transmission in aged animals.

1. Introduction

Giant miniature endplate potentials (GMEPPs) scarcely occur at endplates of mammalian and amphibian muscle fibers. In 1957, Liley [1] studied GMEPPs at endplates of rat muscle fibers and concluded that they consist of summated normal MEPPs. Later, Jansen and Van Essen [2] reported that the rate of rise of GMEPP in the rat diaphragm is consistently lower than that of modal MEPPs or evoked EPPs [3]. Consequently, the authors concluded that GMEPPs should not result from spontaneous action potential firing in the motor neuron, since EPPs of similar size invariably take less time to peak. Unlike the frequency of MEPPs, the frequency of GMEPPs is neither affected by nerve terminal depolarization, nor by changes in extracellular calcium concentration [4,5]. Rather it seems to reflect changes in the presynaptic terminal namely an intracellular Ca^{2+} dysregulation [1,6,7]. In 1996, Sellin and colleagues [8] suggested a possible origin of these giant events. In their opinion, GMEPPs are not caused by the "regulated transmitter release" which is controlled by nerve stimuli triggering calcium entry through presynaptic N- and P-type channels. Instead,

Abbreviations: ALS, amyotrophic lateral sclerosis; EPPs, endplate potentials; MEPPs, miniature endplate potentials; GMEPPs, giant miniature endplate potentials.

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GMEPPs would be examples of "constitutive neurotransmitter secretion", resulting from early endosomes, which are normally present in motor nerve terminals, probably outside the "active zones". Almost 20 years after this report, the cause and physiological role of GMEPPs remain controversial. Increased GMEPPs have been reported in different pathological conditions such as paralysis [7], nerve terminal sprouting and synapse remodeling [9] and nerve terminals degeneration [10] due to motor neuron diseases [11], including amyotrophic lateral sclerosis (ALS) [12]. Whether the aging process modifies the occurrence of these potentials is, however, not yet known. Aging is generally associated with muscle weakness and in many cases with neuromuscular transmission alterations [13]. Thus, in the present work we evaluated both MEPPs and GMEPPs through the aging process in rats.

2. Materials and methods

2.1. Animals

The experiments were performed on isolated preparations of the phrenic nerve-diaphragm from male Wistar rats obtained from Harlan Interfauna Iberia, SL (Barcelona). Four groups of rats were used: infant (3–4 weeks old), adult (12–16 weeks old), older adult (36–40 weeks old) and aged (70–80 weeks old). The animals were handled according to European Community guidelines and Portuguese Law on animal care and killed by decapitation under halothane anesthesia. A strip of the left hemidiaphragm was isolated together with the phrenic nerve and mounted in a 3 ml perspex chamber. A physiological perfusion solution flowed continuously at a rate of 3 ml/min via a roller pump and it was maintained at room temperature (22–23 °C). The bath volume was kept constant by suction.

2.2. Electrophysiology recordings

Evoked end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) were recorded in the conventional way [14] with intracellular electrodes, filled with KCl (3 M) and 10–20 MΩ resistance, inserted in the motor end-plate. The reference electrode was an Ag–AgCl pellet. The nerve was stimulated supramaximally (rectangular pulses of 20 μs duration applied once every 2 s) through a suction electrode. Muscle fibers with a resting membrane potential less negative than −60 mV were rejected. EPPs and the resting membrane potential were continuously monitored throughout the experiment and digitally stored on a personal computer with the Clampex® program (pCLAMP 10 Axon Instruments, Foster City, CA). The neuromuscular junctions from which data was selected for further analysis met the following criteria: (1) stability of the resting membrane potential, which could not spontaneously vary by more than 5% during the recording process, (2) initial value of the membrane potential, which ranged between −60 mV and −80 mV, (3) initial amplitude of EPPs, which ranged between 2 mV and 4 mV. The results were analyzed off-line and each 60 consecutive EPPs were averaged. The proportion of EPPs "failures" and "successes" was calculated by counting the number of total failures of EPP in response to 30 consecutive stimuli. MEPPs were detected through an event detection protocol. The threshold for detection of MEPPs was set within the range of 0.25–1 mV of amplitude whereas for detection of GMEPPs it was set at 1 mV. To prevent the contamination of the signal with electric noise, only events with more than 2 ms of duration were considered. GMEPPs were not considered for the calculation of MEPPs mean amplitude, since they do not contribute to the components of evoked release [3]. Mean MEPP and GMEPP frequency were measured by counting the number of MEPPs and GMEPPs acquired in gap free mode for 100 s periods. The ratio between the frequency of GMEPPs and the frequency of MEPPs was calculated to estimate the probability of giant events occurrence throughout age. The mean amplitude of MEPPs was calculated considering the average of the mean amplitude of 100 consecutive MEPPs. A change in MEPP frequency that was not accompanied by a change in MEPP amplitude was interpreted as a change in spontaneous transmitter release. Quantal contents of EPPs were estimated as the ratio of the average evoked response amplitude to the average amplitude of the MEPPs recorded during the same period.

2.2.1. Mathematical prediction of EPPs failures

The electrophysiological recordings were done in the presence of high Mg^{2+} (19 mM) concentration, whereby the quantal content of EPPs is low. We therefore calculated EPPs "failures" by the indirect method proposed by del Castillo and Katz [15] using the following equation: [f_{EPP} = no. of nerve impulses/e^m], where f_{EPP} indicates the number of failures and m the quantal content of EPPs. Since some discrepancy may arise between the direct observation and the mathematical EPPs failures prediction, comparisons among age groups were done based on a rectification value. The rectification value was calculated using the following equation: [Rectification $f_{EPP} = f_{EPP(ageX)}/f_{EPP(adult\ rats)}$], which takes the adult age group as a reference group.

2.3. Physiological solutions

The bathing solution contained (mM): NaCl 117, KCl 5, $NaHCO_3$ 25, NaH_2PO_4 1.2, glucose 11, $CaCl_2$ 2.5, $MgCl_2$ 1.2. It was continuously gassed with 95% O_2 and 5% CO_2 (pH 7.4) and kept at room temperature (22–25 °C). Muscle twitches were prevented by increasing the bath concentration of Mg^{2+} to 19 mM.

2.4. Statistical analysis

The n value in statistical analyses indicates the number of experiments, which corresponds to a single neuromuscular junction per preparation/animal. Data are expressed as mean ± SEM from n experiments. The significance of the differences between means was evaluated by one-way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons when differences were significant. Values of $P < 0.05$ were considered to represent statistically significant differences.

3. Results

3.1. Age-dependent changes in spontaneous release of ACh

The mean resting membrane potential (RMP) remained unchanged through aging (Table 1, $P > 0.05$; one way ANOVA).

As shown in Fig. 1A and B, there was a monotonous decrease in the frequency of MEPPs upon aging, which represents a significant reduction (by about 66%) in aged rats in relation to infant rats (Table 1, $P < 0.05$; one way ANOVA followed by Tuckey's test). The ratio between the frequency of GMEPPs and the frequency of MEPPs was calculated to investigate whether the probability of GMEPPs varied in the different groups. As shown in Fig. 1C, the occurrence of GMEPPs relative to MEPPs was proportionally higher in aged animals than in other age groups (Table 1, $P < 0.05$; one way ANOVA followed by Tuckey's test), indicating an abnormal spontaneous synchronized release of ACh in aged rats. The MEPP amplitude distribution fitted a Gaussian distribution (Fig. 1D) in all groups. There was no significant difference in the mean amplitude of MEPPs ($P > 0.05$; one way ANOVA) among groups (Table 1), which excludes age-related post-synaptic changes.