RAPID COMMUNICATION

Effect of Hypertonicity on Augmentation and Potentiation and on Corresponding Quantal Parameters of Transmitter Release

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Cheng, Hong and Michael D. Miyamoto. Effect of hypertonicity on augmentation and potentiation and on corresponding quantal parameters of transmitter release. J. Neurophysiol. 81: 1428–1431, 1999. Augmentation and (posttetanic) potentiation are two of the four components comprising the enhanced release of transmitter following repetitive nerve stimulation. To examine the quantal basis of these components under isotonic and hypertonic conditions, we recorded miniature endplate potentials (MEPPs) from isolated frog (Rana pipiens) cutaneous pectoris muscles, before and after repetitive nerve stimulation (40 s at 80 Hz). Continuous recordings were made in low Ca2+ high Mg2+ isotonic Ringer solution, in Ringer that was made hypertonic with 100 mM sucrose, and in wash solution. Estimates were obtained of m (no. of quanta released), n (no. of functional release sites), p (mean probability of release), and var_p (spatial variance in p), using a method that employed MEPP counts. Hypertonicity abolished augmentation without affecting potentiation. There were prolonged poststimulation increases in m, n, and p and a marked but transient increase in var_p in the hypertonic solution. All effects were completely reversed with wash. The time constants of decay for potentiation and for var_p were virtually identical. The results are consistent with the notion that augmentation is caused by Ca2+ influx through voltage-gated calcium channels and that potentiation is due to Na+−induced Ca2+ release from mitochondria. The results also demonstrate the utility of this approach for analyzing the dynamics of quantal transmitter release.

INTRODUCTION

Repetitive stimulation of motor nerves causes an increase in the amount of transmitter released in the poststimulation period. This can be measured as an increase in the endplate potential (EPP) amplitude or in the frequency of miniature endplate potentials (MEPPs) (Hubbard 1963). The increase can be separated into four components: fast facilitation, slow facilitation, augmentation, and potentiation (Magleby 1987). Even though there have been many studies of these components, the specific events responsible for these phenomena remain unestablished.

Another well-investigated but enigmatic phenomenon is the effect of hypertonic solutions on transmitter release. Moderate hypertonicity causes an increase in MEPP frequency and nerve-evoked EPPs, but very strong hypertonic solutions produce a decrease in nerve-evoked quantal output (Hubbard et al. 1968). The effects are transient in the mammal but more sustained in the frog (Kita and van der Kloot 1977). The study of transmitter release under hypertonic conditions is important, because it may elucidate some of the processes involved in exocytosis.

We have recently developed a method of estimating quantal release parameters during the brief periods associated with augmentation and potentiation. The aim of this study was to use this method to examine the effect of hypertonicity on these components and on the corresponding quantal release parameters.

METHODS

We employed isolated cutaneous pectoris muscles from Rana pipiens in these experiments. Animals were killed by decapitation followed by rapid double pithing. Muscles with attached nerves were dissected free, mounted in a 1-ml Sylgard-lined chamber, and perfused with Ringer solution using a roller pump (1.5 ml/min). The normal Ringer solution was modified by decreasing Ca2+ and adding Mg2+ to prevent muscle contraction. The resulting solution contained (in mM) 110 NaCl, 2.5 KCl, 0.3 CaCl2, 6.0 MgCl2, 5.6 glucose, and 2.0 tris(hydroxymethyl)aminomethane (Tris), pH 7.2 (226 mOsm). Hypertonic Ringer solution was made by adding 100 mM sucrose to the above solution (320 mOsm).

Intracellular 3M KCl-filled microelectrodes were used to record MEPPs before and after tetanic stimulation. Nerves were stimulated (40 s at 80 Hz with supramaximal 0.10-ms pulses) using a suction electrode and Grass S-88 stimulator. Each trial consisted of continuous single-cell recording in which data were recorded after 20 min each in control Ringer, hypertonic Ringer, and wash. All experiments were carried out at room temperature (22–24°C). Data were recorded with a modified video recorder and results were analyzed off-line using an R.C. Electronics Data Acquisition System.

Augmentation and potentiation were defined as the fractional increase in poststimulation over prestimulation transmitter release, when the other component equaled zero. This was calculated by \( V_m = (V_t/V_c) - 1 \), where \( V_m \) was the fractional increase in MEPP frequency at time \( t \), \( V_t \) was MEPP frequency at time \( t \), and \( V_c \) was control or prestimulation MEPP frequency. Potentiation was determined empirically, from semilogarithmic plots of \( V_m \) versus time, as the component with the slowest time constant. Augmentation was determined as the component with the next slowest time constant (Magleby 1987).

To determine the quantal release parameters MEPPs were recorded for 70 s after stimulation. The numbers of MEPPs in 10-ms intervals were counted and 300 sequential counts were used for each estimate. The “moving bin” technique of Lev-tov and Rahamimoff (1980) (bin size = 3 s, \( \Delta\bin = 1 \) s) was incorporated into the computer analysis (Provan and Miyamoto 1993) to smooth out point fluctuations. Because the midpoint of the first bin occurred 1.5 s after stimulation, examination of the two...
components of facilitation (decay constants \(< 1 \text{s}\)) was not feasible with this approach.

According to Brown et al. (1976), quantal analysis must take into account spatial variance in \(p\) and temporal variance in \(n\) and \(p\). This problem was addressed by using very short time bins (10 ms) to essentially eliminate temporal variance and by using third moment equations to derive independent estimates of spatial variance in \(p\) \((\text{vars}_p)\) (Provan and Miyamoto 1993).

R E S U L T S

The effect of hypertonicity on augmentation and potentiation is shown in Fig. 1, A and B. In both panels MEPP frequencies are expressed as \(V_m\), the fractional increase over control (see METHODS), and plotted as a function of time after stimulation. In Fig. 1A, the open circles show the expected two-phase decay in MEPP frequency after stimulation. Exposure to hypertonic solution (\(\bullet\)) caused a shift to a one-phase decay, i.e., the first phase was abolished, whereas the second phase was unaffected. Finally, washing with control solution (\(\triangle\)) restored the two-phase decay.

In Fig. 1B, the results are replotted on semilogarithmic axes, so that the exponential components can be expressed as straight lines. For the time between 20 and 70 s, the data obtained in control, hypertonic, and wash solutions were clustered around a line representing a single exponential component. This slower component (shown as \(P\)) presumably represented potentiation, and the deviation from this line presumably reflected augmentation (Magleby 1987). The time constant of decay for \(P\) was 23.7 s, whereas that for augmentation was 4.3 s (after correction for \(P\)). As indicated by the points between 0 and 20 s, exposure to

FIG. 1. Effect of moderate hypertonicity on augmentation and potentiation. Miniature endplate potentials (MEPPs) were continuously recorded for 70 s following stimulation (80 Hz for 40 s). Points represent means for data from 6 single junction experiments obtained in control (○), hypertonic (●), and wash (▲) situations (error bars omitted for clarity). Ordinates are expressed in \(V_m\), the fractional increase in poststimulation over prestimulation MEPP frequency. Control and wash solutions consisted of low Ca\(^{2+}\), high Mg\(^{2+}\) Ringer, and hypertonic solution made by adding 100 mM sucrose. A: results show shift from two-phase decay in \(V_m\) to one-phase decay in hypertonic solution and return to two-phase decay after wash. B: results in A are replotted on semilogarithmic coordinates to show that decay is two-component exponential. Solid line shows regression fit to points representing potentiation (\(P\)).

FIG. 2. Effect of hypertonicity on quantal release parameters. Results are obtained from same data used to generate Fig. 1. Effect on A: number of quanta released (\(m\)); B: number of functional release sites (\(n\)); C: mean probability of release (\(p\)); and D: spatial variance in \(p\) \((\text{vars}_p)\). Note that \(n\) and \(p\) remain relatively elevated (compared with control) for 70 s, whereas \(\text{vars}_p\) is significantly decreased after 30 s. Overlap of points in control (○) and wash (▲) solutions indicates reversible nature of short-term (30 min) exposure to moderate hypertonicity (●). Results represent means ± SE from 6 preparations.
hypertonic solution eliminated augmentation without affecting potentiation.

The effects of hypertonicity on the quantal release parameters are shown in Fig. 2. The data are from the same experiments used to produce Fig. 1, A and B. Because \( m \) is defined as the number of MEPPs in a prescribed interval (Provan and Miyamoto 1993), the results in Fig. 2A are identical to those in Fig. 1A except they have not been normalized to fractional release over control.

Figure 2 shows that hypertonicity caused a significant increase in each of the quantal release parameters. The initial poststimulation increase in \( m \) was fivefold greater in hypertonic than in control solution (Fig. 2A). This was associated with a 2-fold increase in \( n \) (Fig. 2B) and a 2.5-fold increase in \( p \) (Fig. 2C). The increases in \( m, n, \) and \( p \) in hypertonic solution were well sustained for the duration of the recording period; i.e., in terms of percent of initial value, \( m \) was still at 37% after 70 s (vs. 7% for isotonic control), \( n \) was at 58% (vs. 7% for control), and \( p \) was at 63% (vs. 24% for control). For both \( n \) and \( p \), the time course of decay in hypertonic solution could be fitted by a one-component exponential.

There was also a marked increase in \( \text{var}_m, \text{var}_p \) (Fig. 2D), but the time course of decay was more rapid than with \( m, n, \) or \( p \). After 30 s, \( \text{var}_p \) had decreased to 23% of the initial value and was at baseline within 60 s. Regression fit of the points in hypertonic solution (Fig. 2D, ○) to a one-component exponential yielded a decay time constant of 24.0 s. The effects of hypertonicity were again completely reversible, as the values obtained after 20 min of wash were indistinguishable from their respective controls (Fig. 2, A–D).

**Discussion**

Because of the large number of trials needed with single junction recording, a handful of investigators have used surface-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987). Our results show three advantages with the face-recorded EPPs to delineate the time course of decay of facilitation, augmentation, and potentiation (reviewed by Magleby 1987).

The finding (Fig. 1B) that moderate hypertonicity abolished augmentation but not potentiation supports the idea that these phenomena are associated with different processes (Magleby 1987). The results shown in Fig. 2 show that hypertonicity caused a marked and sustained increase in \( m \) due to the sustained increases in \( n \) and \( p \). Although hypertonicity also caused a large increase in \( \text{var}_n, \text{var}_p \), this increase was not linked to the increase in \( n \) or \( p \), because the decay of \( \text{var}_n, \text{var}_p \) was much more rapid.

The following scenario is suggested to explain these results. Moderate hypertonicity produces cell shrinkage which increases cytoplasmic \( \text{Ca}^{2+} \) ([\( \text{Ca}^{2+} \)]. In the frog, this is reflected by a sustained increase in MEPP frequency and nerve-evoked EPPs (Kita and van der Kloot 1977). A sustained increase in \( \text{[Ca}^{2+}] \), would explain the prolonged increase in \( p \) (Fig. 2C), if \( p \) is related to \( \text{[Ca}^{2+}] \), at the active zones (Provan and Miyamoto 1993). Similarly, a sustained increase in \( \text{[Ca}^{2+}] \), could explain the increase in \( n \) (Fig. 2B), if \( n \) is related to the number of functional (having docked vesicles) release sites (Brown et al. 1976). In this case, increased \( \text{[Ca}^{2+}] \), would lead to phosphorylation of synapsin I and migration of vesicles to release sites (Llinás et al. 1991). The sustained increase in \( \text{[Ca}^{2+}] \), implies that the processes that normally restore \( \text{[Ca}^{2+}] \), to resting levels may be overloaded during hypertonic conditions. If so, differences in the ability to regulate cell volume or \( \text{[Ca}^{2+}] \), may explain the contrasting effects seen in mammals and frogs. Finally, the gradual decrease in \( m \) and \( p \) in hypertonic solution (Fig. 2, A and C) may be because of a slow reduction in \( \text{[Ca}^{2+}] \), resulting from the overloaded but operational \( \text{Ca}^{2+} \) regulatory processes. Such a reduction in \( \text{[Ca}^{2+}] \), might also explain the gradual decrease in \( n \) if, as suggested, \( n \) reflects the number of docked vesicles that result from \( \text{Ca}^{2+} \)-induced migration to the active zones.

Rosenmund and Stevens (1996) have suggested that hypertonicity may promote quantal release in the absence of \( \text{Ca}^{2+} \), conceivably by producing a conformational change in a protein involved in exocytosis. This possibility does not obviate the present suggestion that the changes in \( n \) and \( p \) observed in the presence of \( \text{Ca}^{2+} \) (Fig. 2, B and C) may be because of an increase in \( \text{[Ca}^{2+}] \). In fact, Brosius et al. (1992) have demonstrated sustained increases in \( \text{[Ca}^{2+}] \), in neurons exposed to hypertonic solution in the presence or absence of extracellular \( \text{Ca}^{2+} \).

Augmentation is believed to depend on \( \text{Ca}^{2+} \) influx through voltage-gated calcium channels (Magleby 1987). Accordingly, the disappearance of augmentation with hypertonicity (Fig. 1, A and B) indicates that \( \text{Ca}^{2+} \) influx may be reduced or eliminated under these conditions. In support of this, Brosius et al. (1992) and Rosenmund and Stevens (1996) have shown that voltage-gated \( \text{Ca}^{2+} \) currents are reduced by hypertonicity because of mechanical effects on the channel and/or reduction in the \( \text{Ca}^{2+} \) gradient (as a result of the increase in \( \text{[Ca}^{2+}] \)).

Agents that inhibit mitochondria under nonstimulated conditions produce an increase in \( \text{var}_p \), presumably by inhibiting the uptake or promoting the release of mitochondrial \( \text{Ca}^{2+} \) (Provan and Miyamoto 1995). Under normal conditions, the uptake of cytoplasmic \( \text{Ca}^{2+} \) by these organelles is intensified during tetanic stimulation (David et al. 1998), conceivably because the increase in \( \text{Ca}^{2+} \) influx leads to more available \( \text{Ca}^{2+} \) (increased \( \text{[Ca}^{2+}] \)). Hypertonicity may enhance this process by further increasing \( \text{[Ca}^{2+}] \), by means of cell shrinkage. Accordingly, the dramatic increase in \( \text{var}_p \) (Fig. 2D) may be because of efflux of \( \text{Ca}^{2+} \) from mitochondria that are fully loaded during tetanic stimulation under hypertonic conditions. In this regard, Tang and Zucker (1997) have suggested that potentiation is due to the slow release of \( \text{Ca}^{2+} \) from mitochondria. Such an explanation is consistent with the results in Fig. 2D which show a remarkable similarity between the time constant of decay for \( \text{var}_p \) (24.0 s) and the time constant of decay for potentiation (23.7 s).

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