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Actions of lead on transmitter release at mouse motor nerve terminals

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Abstract. The actions of lead (Pb^{2+}) on transmitter release were studied at neuromuscular junctions in mouse diaphragm in vitro. The quantal content of end-plate potentials (EPPs) was reduced by Pb^{2+} in a dose-related manner consistent with inhibition of Ca^{2+} entry into nerve terminals, with a half-maximal effect at 1.4 μM (in 0.5 mM Ca^{2+} and 2 mM Mg^{2+}). Pb^{2+} also inhibited the increased frequency of MEPPs (f_{MEPP} where MEPPs denotes miniature EPPs) produced by Ba^{2+} in the presence of raised K^+, blocking the calculated Ba^{2+} entry half-maximally at 170 μM. However, at concentrations of 50–200 nM, Pb^{2+} often increased f_{MEPP} in 20 mM K^+ in the presence of Ca^{2+} and acted to promote the irreversible effect of lanthanum (La^{3+}) to raise f_{MEPP}. In nominally Ca^{2+}-free solution with 20 mM K^+, brief (1 min) application of Pb^{2+} (20–320 μM) caused rapid dose-dependent reversible rises in f_{MEPP}. With prolonged exposure to Pb^{2+}, f_{MEPP} rose and then slowly declined; after removal of Pb^{2+}, once f_{MEPP} had fallen to low levels, f_{MEPP} responded nearly normally to Ca^{2+} or ethanol, but not to Pb^{2+} itself. In 5 mM K^+, 0 mM Ca^{2+} and varied [Pb^{2+}] (where [ ] denotes concentration), nerve stimulation caused no EPPs, but prolonged tetanic stimulation produced increases in f_{MEPP} graded with [Pb^{2+}] that persisted as a "tail"; results were consistent with growth of f_{MEPP} with the 4th power of intracellular Pb^{2+} and removal of intracellular Pb^{2+} with a time constant of about 30 s. These results suggest that Pb^{2+} acts to block the entry of Ca^{2+} and Ba^{2+} into the terminal via voltage-gated Ca^{2+} channels through which Pb^{2+}, at higher concentrations, also penetrates and then acts as an agonist at intracellular sites that govern transmitter release.

Key words: Lead – Nerve terminal – Transmitter release – Calcium channels

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Introduction

It is now well established that at the neuromuscular junction Ca^{2+} enters the nerve terminal via voltage-gated channels and functions to link transmitter release to nerve terminal depolarization [22]. However, many ions that block depolarization/Ca^{2+} transmitter release, apparently by blocking Ca^{2+} entry, themselves induce or enhance release at depolarized terminals. These include manganese (Mn^{2+}, [3]), cobalt (Co^{2+}, [24]), lanthanum (La^{3+}, [6]), cadmium (Cd^{2+}, [9, 15]) and zinc (Zn^{2+}, [12, 14, 23]). Silbergeld et al. [20] found that Pb^{2+} decreased the force of contraction and increased the latency between nerve stimulation and contraction in mouse and rat in vitro; a blocking activity of Pb^{2+} was subsequently demonstrated at neuromuscular junctions of frog [5, 13] and of rat diaphragm [2]. In addition, Pb^{2+} can also act to increase "spontaneous" transmitter release [1, 2, 5].

The experiments described here were carried out to investigate further the interaction of Pb^{2+} and Ca^{2+} at both extracellular and intracellular sites. The results indicate that Pb^{2+} has several effects on transmitter release, acting, not only as a competitive blocker of Ca^{2+} (and Ba^{2+}) entry at extracellular sites, but also as a substitute for Ca^{2+} in supporting depolarization-induced release. In addition, at a very low concentration range (nanomolar), Pb^{2+} enhances the effectiveness of Ca^{2+} or La^{3+} to raise the frequency of miniature end-plate potentials (f_{MEPP}) in high K^+.

Materials and methods

Experiments were performed upon hemidiaphragms from anaesthetized mice. The techniques used for the mounting and superfusion of the preparation that allowed switching of the bathing solution in a few seconds have been described elsewhere [4]. Intracellular recording of MEPPs and EPPs at end-plates was conventional, using microelectrodes filled with 3 M KCl. Because Pb^{2+} precipitates in bicarbonate/phosphate buffered solutions, experiments were carried out in solutions buffered with 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) and

bubbled with 100% O_2. As in previous work [9, 23] the standard solution for most experiments using raised K^+ had the following composition (mM): $NaNO_3$ 100, KCl 20, $MgCl_2$ 1, glucose 11, HEPES 3, pH 7.3. Sucrose (60 mM) provided isotonicity with solutions containing 150 mM Na^+ and 5 mM K^+. Lowered Na^+ concentration raises the apparent potency of Ca^{2+} for extracellular sites so that half-maximal responses of f_{MEPP} to raised Ca^{2+} are obtained at lower concentrations [8]. Replacement of Cl^- by NO_3^- improves signal/noise ratio for recording MEPPs, which does not significantly affect presynaptic mechanisms [19]. In experiments using nerve stimulation Cl^- was used rather than NO_3^- and $[K^+]$ (where [ ] denotes concentration) was 5 mM. To remove Pb^{2+} the preparation was washed with solution containing Ca-EDTA [usually using 0.15 mM Ca^{2+} and 0.1 mM ethylenediaminetetraacetic acid (EDTA)]. Silen and Martell [21] quote the dissociation constant of the Pb-EDTA complex as $10^{-18.04}$, about 7 orders of magnitude smaller than that for Ca-EDTA ($10^{-10.6}$).

MEPPs were counted by a microcomputer using a program that provided sequential displays of 0.4-s portions of the recorded signal, with lines indicated each MEPP that was counted by the computer; this permitted continuous monitoring of the accuracy of the count. To follow the time course of changes in f_{MEPP}, MEPPs were either recorded continuously at individual junctions or muscle fibres were penetrated randomly and the time of penetration and the f_{MEPP} for each junction recorded. For assessment of quantal content of EPPs we used a computer program that "deconvolutes" the EPP into quantal components, and counts MEPPs at all points in the record. This program will be described elsewhere.

Results

Blockade by Pb^{2+} of Ca^{2+}-dependent release

Pb^{2+} has previously been reported to inhibit Ca^{2+}-induced release of transmitter at neuromuscular junctions of frog [5] and rat [2]. The same phenomenon at a mouse junction is illustrated in Fig. 1a. In the presence of 0.5 mM Ca^{2+} and 2 mM Mg^{2+}, nerve stimulation produced EPPs with a quantal content of 1.5, which was reversibly diminished by 0.5–16 μM Pb^{2+} in a dose-related manner. By interpolation, the half-maximal effect was at 0.24 μM. If transmitter release is proportional to the 4th power of intracellular Ca^{2+} [10] inhibition of Ca^{2+} entry can be calculated from the reduction of the 4th root of the release rate corresponding to the quantal content of the EPP (cf. [23]). On this basis the half-maximal effect of Pb^{2+} to reduce Ca^{2+} entry in this experiment was 1.4 μM Pb^{2+}. The reduction in quantal content by Pb^{2+} was associated with a loss of "facilitation" in short trains, as described by Zengel et al. [25] for Cd^{2+}, the facilitation not being restored with addition of Ca^{2+}. This is illustrated in Fig. 1b which shows, in control, typical facilitation of quantal content in trains of 7 pulses at 100 Hz. Here 100 μM Pb^{2+} reduced the quantal content of EPPs to very low levels. Increasing Ca^{2+} to 2 mM restored the original quantal content but facilitation remained absent. It is notable that the restoration of quantal content by only a quadrupling of $[Ca^{2+}]$, in the presence of 100 μM Pb^{2+}, would appear to contradict the previous value of 1.4 μM for the apparent dissociation constant of Pb^{2+} for block of Ca^{2+} channels. Previously, with Cd^{2+} [10] and with Zn^{2+} [23] it was found that the apparent potency of these ions is diminished by Ca^{2+} to an extent greater than expected simply from competition of Ca^{2+} with the blocking ion at a single site.

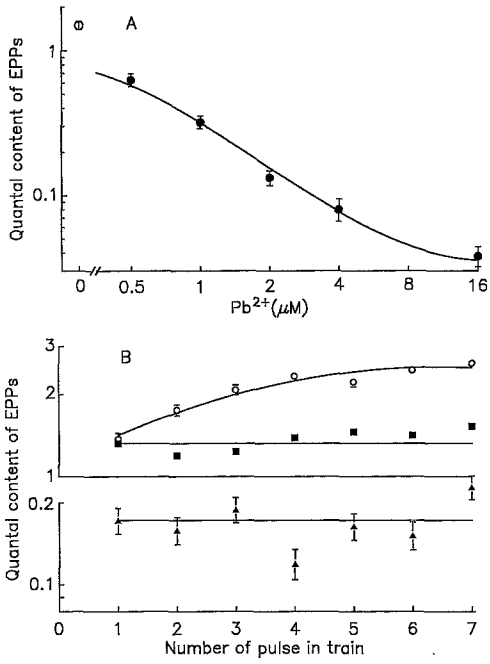

Fig. 1. A Inhibition by Pb^{2+} of end-plate potentials (EPPs) induced by 80-Hz trains in 5 mM K^+, 0.5 mM Ca^{2+} and 2 mM Mg^{2+}. The *line* is a theoretical curve for single-site action of Pb^{2+} to block Ca^{2+} entry, with a half-maximal effect at 1.4 μM, assuming transmitter release is proportional to the 4th power of internal Ca^{2+}. **B** Blockade by Pb^{2+} of facilitation in 100-Hz trains of 7 stimuli, in 5 mM K^+ and 2 mM Mg^{2+}. *Open circles*, (controls) 0.5 mM Ca^{2+}; *solid triangles*, 1 mM Ca^{2+} and 100 μM Pb^{2+}; *solid squares*, 2 mM Ca^{2+} and 100 μM Pb^{2+}

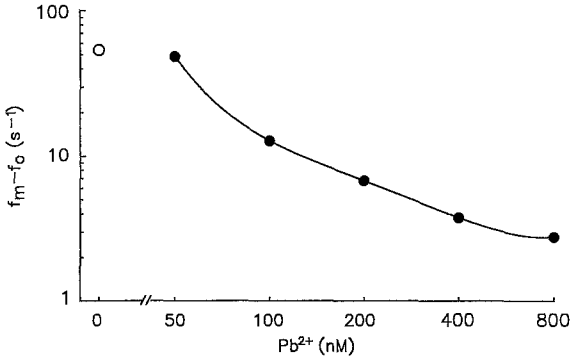

Fig. 2. Inhibition by Pb^{2+} of the increase in the frequency of miniature EPPs (f_{MEPP}) produced by 0.3 mM Ba^{2+} in the presence of 20 mM K^+; data from one junction. The *ordinate* represents the difference between f_{MEPP} in the presence of Ba^{2+} (f_m) and f_{MEPP} in the absence of Ba^{2+} (f_o). *Open circle*, control; *filled circles*, with Pb^{2+}

Effect of Pb^{2+} to increase f_{MEPP} in high K^+

From the above result it would be expected that Pb^{2+} would block the enhanced f_{MEPP}, produced by Ca^{2+} or Ba^{2+} at end-plates depolarized by raised K^+ [10]; with Ba^{2+}, in 20 mM K^+, this was indeed always the case. In the example in Fig. 2 the effect at 50 nM Pb^{2+} is less than

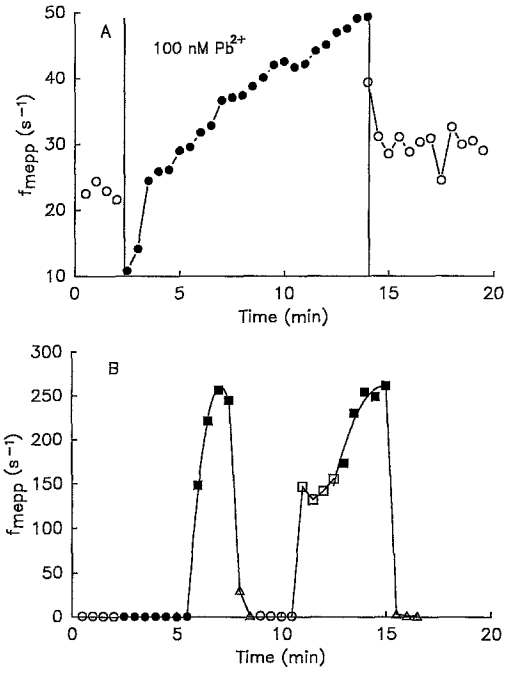

Fig. 3A, B. Effect of Pb^{2+} to increase neurotransmitter release produced by Ca^{2+} in the presence of 20 mM K^+. **A** f_{MEPP} was about 21 s^{-1} in 0.4 mM Ca^{2+} (*open circles*). Addition of 100 nM Pb^{2+} (*filled circles*) caused a transient reduction followed by a rise. f_{MEPP} fell nearly to control values upon withdrawal of Pb^{2+}. **B** f_{MEPP} was about 1 s^{-1} before (*open circles*) and with 100 nM Pb^{2+} (*filled circles*). With 100 nM Pb^{2+} and 1 mM Ca^{2+} (*filled squares*) f_{MEPP} rose to 250 s^{-1}; it returned to control with "wash" with 50 μM Ca^{2+} and 100 μM Ca-EDTA (*open triangles*). 1 mM Ca^{2+} in the absence of Pb^{2+} (*open squares*) raised f_{MEPP} to 150 s^{-1} and subsequent addition of 100 nM Pb^{2+} (*filled squares*) raised f_{MEPP} to 250 s^{-1}

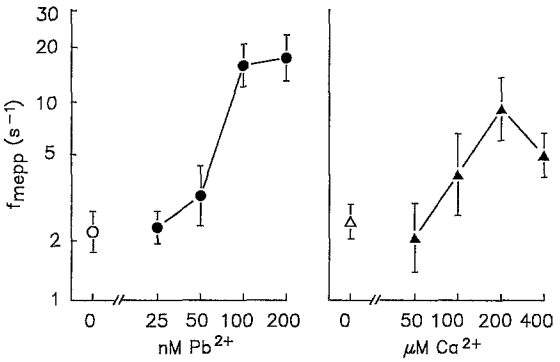

Fig. 4. Pb^{2+} increases the irreversible action of lanthanum (La^{3+}) in 10 mM K^+ to raise f_{MEPP}. Each *point* represents the geometric mean from 22–71 junctions (± S.E.) in a segment of a diaphragm, in 50 μM Ca^{2+} and 100 μM Ca-EDTA, following incubation for 30 min with 0.5 μM La^{3+} alone (control, *open symbol*) or with various concentrations of Pb^{2+} (*filled circles*), or Ca^{2+} (*filled triangles*). The experiment with Ca^{2+} was on a different diaphragm from that with Pb^{2+}. Incubation with Pb^{2+} alone (200 nM, not shown) had no effect to raise subsequent f_{MEPP}

expected from the effects at a higher concentration, but the data otherwise fit half blockade of Ba^{2+} entry at 170 nM (see [23] for method of calculation), if release is proportional to the 4th power of Ba^{2+} entry [17]. The relatively high potency of Pb^{2+} as a blocker when tested versus Ba^{2+} rather than Ca^{2+} was also seen with Zn^{2+} [23] and with Cd^{2+} [10].

The raised f_{MEPP} induced by Ca^{2+} in 20 mM K^+ was also usually inhibited by Pb^{2+}. However, this inhibition was often transient, followed by a rise in f_{MEPP} despite the continued presence of Pb^{2+}. At relatively high concentrations of Pb^{2+} the rise in f_{MEPP} with Pb^{2+} also occurred in the absence of Ca^{2+} (see below) but at low concentrations, e.g. 100 or 200 nM (Fig. 3) the rise did not occur in the absence of Ca^{2+}, but did occur in 0.4 mM Ca^{2+}, 1 mM Ca^{2+}, and sometimes in 2 mM Ca^{2+} (Fig. 3a, b).

To test the possibility that this phenomenon might be related to an effect of Pb^{2+} to enhance entry of other ions into the nerve terminal we examined the effect of Pb^{2+} on the development of the irreversible action of La^{3+} to raise f_{MEPP}; Curtis et al. [6] found that small amounts of Ca^{2+} accelerate the irreversible development of a high f_{MEPP} in the presence of La^{3+} which is attributable to entry of La^{3+} into nerve terminals via Ca^{2+} channels. Preparations were incubated with 0.5 μM La^{3+} (in 10 mM K^+) together with different concentrations of Pb^{2+} for 30 min and f_{MEPP} recorded subsequently in the absence of La^{3+} or Pb^{2+} (with 0.1 mM Ca-EDTA to exclude extracellular Pb^{2+} and La^{3+}). The results (Fig. 4) show that concurrent exposure to Pb^{2+}, in the range of 50–200 nM, increased the effect of exposure to 0.5 μM La^{3+}, with much greater potency, and perhaps more efficacy than Ca^{2+}. In control experiments exposure of preparations for 30 min to Pb^{2+} (200 nM) alone caused no increase in the subsequent f_{MEPP}. Moreover, the effect of Pb^{2+} depended upon its simultaneous presence with La^{3+}; exposure to 200 nM Pb^{2+} for 30 min, followed by "wash" with 0.1 mM Ca-EDTA and subsequent exposure to 0.5 μM La^{3+} gave a maintained f_{MEPP} in the absence of Ca^{2+} [mean $\log_{10} f_{MEPP} = 0.37 \pm 0.09$ (± S.E., $n = 35$)] the same as exposure to only 0.5 μM La^{3+} [mean $\log_{10} f_{MEPP} = 0.40 \pm 0.09$, (± S.E., $n = 43$)]. Thus, Pb^{2+} at about 100 nM, apparently promotes the entry of La^{3+} into nerve terminals, in the same way as Ca^{2+} at about 100 μM, perhaps by promoting opening of channels. A similar action exerted on entry of Ca^{2+} would account for the enhancement by 100 nM Pb^{2+} on f_{MEPP} induced by Ca^{2+} in raised K^+ (Fig. 3), but only if Ca^{2+} itself has less efficacy then Pb^{2+} for this action.

In 20 mM K^+ in the absence of Ca^{2+}, Pb^{2+} at sufficiently high concentrations produced an increase in f_{MEPP} that developed within about 30 s and subsided quickly upon removal of Pb^{2+}. In the example in Fig. 5a, 160 μM Pb^{2+} caused f_{MEPP} to rise from less than 1 s^{-1} to 100 s^{-1} with most of the rise complete in 30 s; upon "washing" with solution containing 0.1 mM Ca-EDTA f_{MEPP} returned to control values in about 1 min. In Fig. 5b average data from 6 junctions are plotted as $f_{MEPP}^{(1/4)} - f_o^{(1/4)}$ (i.e., the increment in the 4th root of f_{MEPP}, which, see below, should be proportional to internal Pb^{2+}) versus external Pb^{2+}. The half maximally effective [Pb^{2+}] in terms of rise of the 4th root of f_{MEPP} was at 85 μM; in terms of f_{MEPP}, the half maximally effective [Pb^{2+}] was at 135 μM. Thus, saturation of this effect of Pb^{2+} occurred at

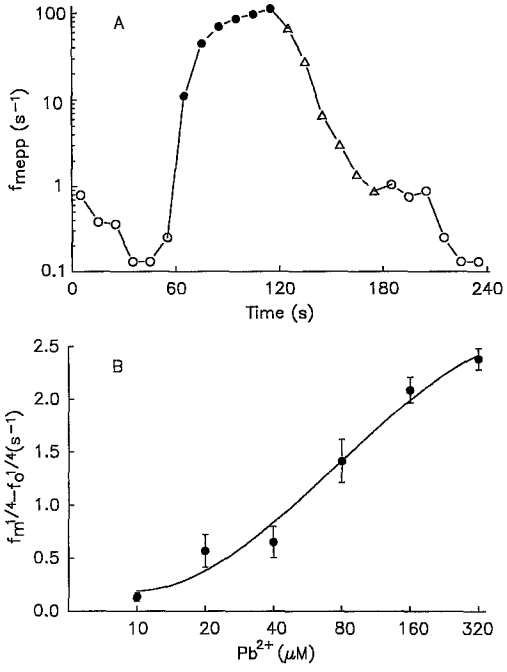

Fig. 5A, B. Rapid and reversible effect of Pb^{2+} to raise f_{MEPP} in the presence of 20 mM K^+ and 0 mM Ca^{2+}. **A** Example of 1-min application of 160 μM Pb^{2+} (*solid circles*) raising f_{MEPP}, with reversal upon removal of Pb^{2+} using 0.1 mM Ca-EDTA (*open triangles*). *Open circles* represent control (no Pb^{2+}). **B** Dose/response curve, where the *points* represent mean $f_{MEPP}^{1/4}$ ($f_m^{1/4}$) at 0–60 s after applying Pb^{2+}, minus 4th root of spontaneous f_{MEPP} in 0 mM Ca^{2+} ($f_o^{1/4}$). Each *point* represents an average from 6 junctions ± S.E.

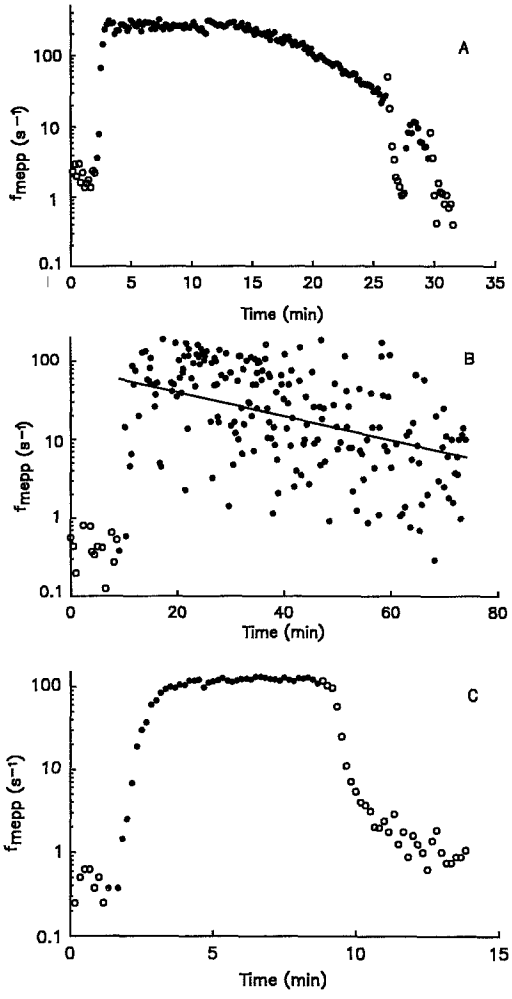

Fig. 6A–C. Response of f_{MEPP} to 100 μM Pb^{2+} in 20 mM K^+ and 0 mM Ca^{2+}. **A** Addition of Pb^{2+} (*solid circles*) caused a rapid increase in f_{MEPP} followed by a decline. f_{MEPP} fell to control or lower upon "wash" with Pb^{2+}, 50 μM Ca^{2+} and 100 μM Ca-EDTA (*open circles*). Repeated applications of Pb^{2+} caused progressively less effect. Data from one junction. **B** Variation between junctions of the effect of 100 μM Pb^{2+} (*filled circles*) to raise f_{MEPP} in 20 mM K^+ and 0 mM Ca^{2+}. **C** Increase of f_{MEPP} by 100 μM Pb^{2+} (*solid circles*) at a junction in a preparation previously incubated for 5 h in 20 mM K^+, 0.1 mM EDTA plus 10 μM Ca^{2+} (*open circles*)

concentrations very much higher than the concentrations that block Ca^{2+} entry.

With prolonged exposure to Pb^{2+}, at 10 μM or greater, f_{MEPP} rose to levels higher than obtained with 1-min exposures, followed by a slow decline despite the continued presence of Pb^{2+}. The effect of Pb^{2+} to raise f_{MEPP} remained reversible; f_{MEPP} rapidly fell to control levels or lower with removal of Pb^{2+} and "wash" with solution containing 0.1 mM Ca-EDTA, even during the declining phase (Fig. 6a). However, once f_{MEPP} had declined in the continued presence of Pb^{2+}, washing with Ca-EDTA and reexposure to Pb^{2+} did not restore the high f_{MEPP} found after the initial exposure to Pb^{2+}. Data obtained by multiple sampling in a diaphragm continuously exposed to 100 μM Pb^{2+} (in 20 mM K^+ and 0 mM Ca^{2+}), is shown in Fig. 6b illustrating the large variations in frequencies attained at different junctions.

Even in nominally Ca^{2+}-free solution, the possibility exists that the effect of Pb^{2+} of increasing f_{MEPP} might be secondary to increase in intracellular Ca^{2+}. Therefore, the effect of Pb^{2+} in 20 mM K^+ was tested in preparations incubated for 5 h in 20 mM K^+ in very low buffered Ca^{2+} (0.1 mM EDTA plus 10 μM Ca^{2+}); responses to 100 μM Pb^{2+} (Fig. 6c) were unaffected.

Although brief (1 min) exposure to 10 μM Pb^{2+} had little effect to raise f_{MEPP} in 20 mM K^+ (Fig. 5b) with more prolonged exposure there was generally a rise that was complete within about 10 min, to about 30 s^{-1}, followed by a fall over the next hour (Fig. 7a, b). In 5 mM K^+, the rise was less and developed more slowly (Fig. 7a), in contrast to the observation of Anwyl et al. [1], suggesting that the effect of Pb^{2+} is secondary to entry into the nerve terminal cytoplasm via voltage-gated channels, presumably those that normally admit Ca^{2+}. In support of this, the response to Pb^{2+} was inhibited by 4 mM Mg^{2+} (Fig. 7b). Following such long exposures to Pb^{2+}, f_{MEPP} fell to control levels after withdrawal of Pb^{2+}.

Effect of Pb^{2+} on f_{MEPP} induced by nerve stimulation

In the presence of Pb^{2+} and absence of Ca^{2+} (5 mM K^+ and 1 mM Mg^{2+}), nerve stimulation caused a rise in f_{MEPP}, much greater than occurred in the absence of Pb^{2+}; this persisted after a train as a "tail" that declined over a

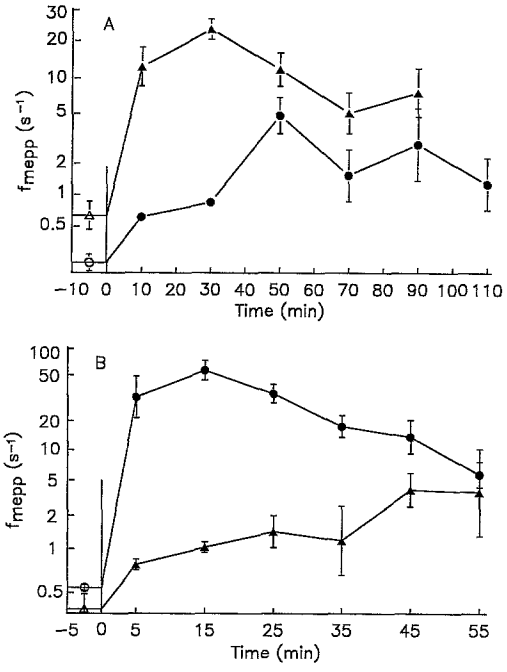

Fig. 7. **A** The rise in f_{MEPP} produced by 10 μM Pb^{2+} in 0 mM Ca^{2+} and 20 mM K^+ (*solid triangles*) or 5 mM K^+ (*solid circles*). **B** Inhibition by 4 mM Mg^{2+} (*solid triangles*) of the rise of f_{MEPP} produced by 10 μM Pb^{2+} (20 mM K^+ and 0 mM Ca^{2+}), compared with 1 mM Mg^{2+} (*solid circles*). In both graphs, each *point* represents the mean from about 20 junctions ± S.E.

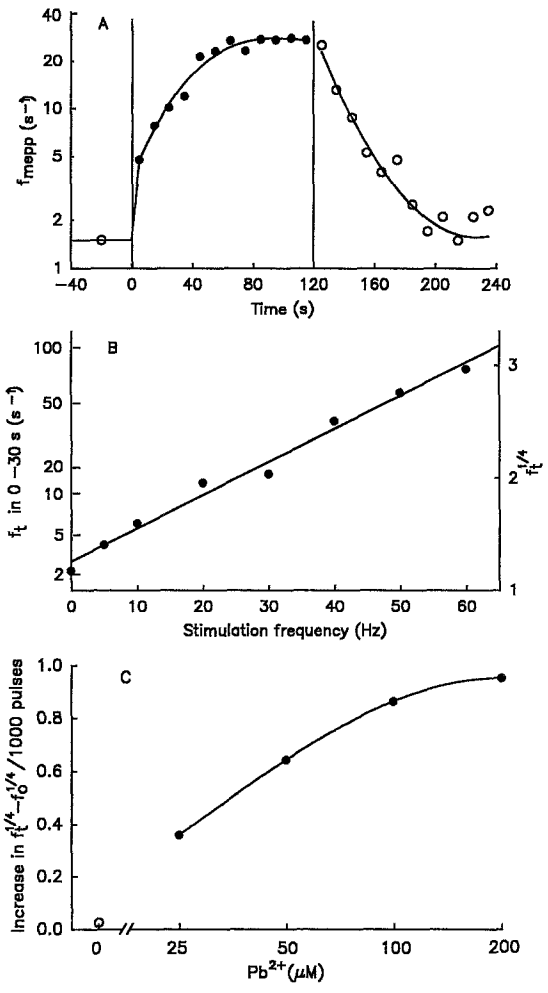

Fig. 8 A–C. The rise of f_{MEPP} produced by nerve stimulation in the presence of 100 μM Pb^{2+}, 1 mM Mg^{2+}, 0 mM Ca^{2+} and 5 mM K^+. **A** Time course of the rise and fall of f_{MEPP} before (*open circle*) during (*filled circles*) and after (*open circle*) nerve stimulation for 2 min at 40 Hz. **B** Linearity with number of stimuli in 30-s trains of the 1/4 power of the "Pb^{2+} tail" intensity, estimated as the average f_{MEPP} in the 1st 30 s after the train. In the absence of Pb^{2+} such trains caused much smaller increases in f_{MEPP}. **C** Gradation of apparent Pb^{2+} entry with external [Pb^{2+}]. Stimuli were given in 30-s trains at a [Pb^{2+}] of 25–200 μM in 0 mM Ca^{2+} and 1 mM Mg^{2+}. "Pb^{2+} entry" per pulse was calculated as the increment of f_{MEPP} raised to the power 1/4 caused by each train of number k as $(f_t^{1/4}-f_o^{1/4})/k$, where f_o is control value of f_{MEPP} and f_t is the f_{MEPP} just after the train by extrapolation (using a 30-s time constant) from the average f_{MEPP} at 0–30 s after the train

period of seconds. The maximum f_{MEPP} attained grew with the number of impulses applied.

An example of time course of development and decline of f_{MEPP} during and after nerve stimulation is shown in Fig. 8a. With nerve stimulation in the presence of Pb^{2+}, the rise and fall of f_{MEPP} were asymmetrical in time course when data were plotted either linearly or semilogarithmically versus time. Here, in 100 μM Pb^{2+}, during a 40-Hz train f_{MEPP} rose from the control value (f_o) with a time constant (τ) of 64 s (by non-linear least squares fitting) but after that the train fell with a τ of 16 s. Plots of $f_m^{1/n}$ versus time (where f_m denotes mean f_{MEPP}) became increasingly symmetrical with increasing n up a value equal to 4, at which τ for growth of $f_m^{1/4}$ was 34 s, while τ for decay of $f_m^{1/4}$ was 32 s; at n equal to 5, τ values were 32 and 33 s, respectively. This corresponds to what would be expected if f_{MEPP} is proportional to the 4th or 5th power of internal Pb^{2+}, which rises with each "injection" of Pb^{2+} by a nerve impulse and is disposed of by a 1st order process with a τ value of 33 s. Graphs (not shown) of the $\log_{10}$ of the absolute value of $(f_m^{1/4}-f_f^{1/4})$ versus time, f_f being maximum f_m value for the rising phase and f_o for the falling phase, showed good fits of both phases to a single exponential with a time constant of 33 s.

An example of the relation of "tail" intensity to number of stimuli in 30-s trains, in 100 μM Pb^{2+}, is shown in Fig. 8b. Designating the f_m value over the period of 30 s after the last pulse in the train as f_t, $f_t^{1/4}$ (or $f_t^{1/5}$, not shown) varied linearly with the stimulation frequency, i.e. with the number of impulses in the train, in the same way as "tails" of raised f_{MEPP} produced by stimulation in the presence of Ba^{2+} and attributable to accumulation of Ba^{2+} in the nerve terminal [17]. The calculated per pulse increment of $f_{MEPP}^{1/4}$ in this example is much the same as that found with 50 μM Ba^{2+} [18] but, in contrast to what was found with Ba^{2+}, this could not be much increased by increase of Pb^{2+} above 100 μM (Fig. 8c).

Corresponding to the low effectiveness (or low entry) of Pb^{2+}, nerve stimulation did not induce any noticeable EPP, even with [Pb^{2+}] raised up to 1 mM; latency histograms of quanta released after nerve stimuli showed no consistent increase in frequency in the period of 0.8–2.5 ms, in which the EPP normally occurs.

Possible intracellular effect on release

After prolonged exposure to Zn^{2+} there occurs a complete blockade of the response of f_{MEPP} to ethanol (cf. [18]) and to Ca^{2+} in raised K^+ [23]. To determine whether a similar effect might be exerted by Pb^{2+}, as suggested by the decline in f_{MEPP} that occurred in the continued presence of Pb^{2+} (Fig. 6), f_{MEPP} was measured in 20 mM K^+ with either added ethanol or Ca^{2+}, before and after a 1-h exposure to 100 μM Pb^{2+}. To ensure the absence of extracellular Pb^{2+}, 0.15 mM Ca^{2+} and 0.1 mM EDTA was added to all solutions except that containing Pb^{2+}. After Pb^{2+} exposure, f_{MEPP} (about 0.7 s^{-1}) was not significantly changed in 0 mM Ca^{2+} but there was a tendency to a lower f_{MEPP} than in the controls, with Ca^{2+} and ethanol. In 2 out of 3 preparations f_{MEPP} in 1 mM Ca^{2+} (normally about 100 s^{-1}) was reduced relative to the controls (to 45% and 54%) and in both preparations tested with 0.8 M ethanol (producing f_{MEPP} of about 50 s^{-1} in controls) f_{MEPP} was reduced after exposure to Pb^{2+}, to 70.5% and 52% of control values. These reductions are small compared to the effects of Ca^{2+} and ethanol to raise f_{MEPP}. This result suggests that there is relatively little (if any) long term accumulation of Pb^{2+} and "toxic" action, in contrast to Zn^{2+}.

Discussion

The present results concur with previous observations that Pb^{2+}, like some other divalent ions, not only inhibits Ca^{2+}-mediated transmitter release, presumably by blocking entry of Ca^{2+} into nerve terminals via voltage-sensitive channels, but also can itself promote release [1, 2, 5]. The effect to inhibit release resembles that shown by Cd^{2+} [10] and Zn^{2+} [23] in that the apparent potency of the blocking ion is much greater when tested versus Ba^{2+} in raised K^+ than when tested versus Ca^{2+} (giving a similar f_{MEPP}) and apparent potency is reduced by Ca^{2+} much more than is compatible with simple competition of the ions at a single site. The action of Pb^{2+} rapidly to promote release in raised K^+, in the absence of Ca^{2+}, resembles that of Zn^{2+} in that it is evident only at concentrations much higher than those that apparently block Ca^{2+} or Ba^{2+} entry by 50%, i.e. it appears that a concentration of Pb^{2+} which blocks entry of other ions does not block its own entry. This phenomenon may be the same as that seen in cardiac Ca^{2+} channels where Ca^{2+} blocks Na^+ flux in the micromolar range but Ca^{2+} flux grades with $[Ca^{2+}]$ in the millimolar range, which is explicable if channels have more than one binding site [11]. Complexity of ion interaction with presynaptic Ca^{2+} channels is also indicated by the sensitivity to Ca^{2+} of the inhibitory effect of Pb^{2+}, Zn^{2+}, and Cd^{2+}, on release mediated by Ca^{2+} or Ba^{2+}.

Pb^{2+} contrasts with Zn^{2+} in its much greater potency and effectiveness in producing a rapid increase in f_{MEPP} in raised K^+, and with Cd^{2+}, for which no such effect is visible, and in the apparently complete reversibility of this effect. Moreover, the long term effects seen with Cd^{2+} and Zn^{2+}, apparently due to irreversible sequelae of ion entry into the nerve terminal, are nearly absent; there is little if any tendency for release to be reduced after prolonged exposure to Pb^{2+}. The effect to raise f_{MEPP} in raised K^+ is consistent with Pb^{2+} acting within the nerve terminal, after entry via voltage-gated channels, in the same way as occurs with tetanic stimulation. The effect of Pb^{2+} to support the temporarily raised f_{MEPP} during and after tetanic stimulation closely resembles the effect seen with Ba^{2+} [16], the only differences being a more prolonged time course and apparent saturation at more than 100 μM or so. Notably, the Hill coefficient for cooperativity of Pb^{2+} to induce release, once inside the terminal, appears to be 4 (or 5) which is the same as for Ba^{2+} [16, 17] and for Ca^{2+} [10]. The lack of an EPP with Pb^{2+} (in contrast to Ba^{2+}) follows from the rather small effect of Pb^{2+} (per pulse) that is attainable in comparison to that with Ba^{2+}. However, we cannot rule out the possibility that the intracellular effect of Pb^{2+} to induce release might be secondary to intracellular release of Ca^{2+}.

The action of Pb^{2+} to block facilitation has been observed previously with Cd^{2+} and Zn^{2+} [25], and presumably reflects the same phenomenon for all three ions. It is not impossible that this action could be secondary to block of Ca^{2+} entry, although restoration of quantal content by added Ca^{2+} in the continued presence of Pb^{2+} does not restore facilitation, since the effect of Pb^{2+} to block Ca^{2+} entry might grow with repetitive stimulation. Alternatively, it is conceivable that Pb^{2+} is much more effective than Ca^{2+} in activating an intracellular mechanism that produces facilitation, and saturates at moderate intracellular Ca^{2+} [7], and therefore obviates the normal facilitation produced by Ca^{2+} entering with each impulse. The action of Pb^{2+} at very low concentrations to increase Ca^{2+}-mediated release, and to promote the development of a high f_{MEPP} in the presence of La^{3+} presumably reflects an increase in the rate of opening of presynaptic Ca^{2+} channels, and supports the idea that the opening of these channels may normally be governed partially by Ca^{2+} [6].

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