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Millimolar concentrations of free magnesium enhance exocytosis from permeabilized rat pheochromocytoma (PC 12) cells

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The role of Mg^{2+} during the final steps of exocytosis was investigated using rat pheochromocytoma cells (PC12) permeabilized with bacterial pore forming toxins. Concentrations of free Mg^{2+} between 0.2 and 2 mM slightly lowered the basal but greatly enhanced the [^{3}H]dopamine release elicited by 8 μM free Ca^{2+}. Maximal effects were obtained at approximately 1 mM free Mg^{2+}. At higher concentrations Mg^{2+} was less potent. Similar effects of Mg^{2+} were obtained in cells permeabilized either for small molecules (by α-toxin) or for large ones (by streptolysin O). It is concluded that millimolar concentrations of cytoplasmic Mg^{2+} play an important role in Ca^{2+} triggered exocytosis.

Presently, the role of Mg^{2+} during intracellular processes is poorly understood. Many intracellular reactions depend on a sufficient supply of this divalent cation but exact data on the free Mg^{2+} concentrations in the cytoplasm, its regulation and role during complex processes such as exocytosis are lacking. It has been reported that fairly high concentrations of Mg^{2+} inhibit Ca^{2+}-induced exocytosis from electrically permeabilized bovine adrenal chromaffin cells [15]. In these cells however, exocytosis requires the presence of ATP and Mg^{2+} [7, 15] which does not allow a clear distinction between effects of free Mg^{2+} alone and Mg^{2+} in concert with ATP. In the rat pheochromocytoma cell line PC12 [14] exocytosis can be stimulated with Ca^{2+} alone, in the absence of Mg^{2+} and ATP [2–5]. Thus, using this cell line the effects of Mg^{2+} can be investigated more precisely.

Stock cultures of pheochromocytoma cells (PC12) were kindly provided by H. Thoenen, Max Planck Institut für Psychiatrie, Martinsried, F.R.G. The cells were cultivated as given earlier [1, 5]. Permeabilization of the cells was achieved by pore-forming bacterial toxins [5, 9]. α-toxin from *Staphylococcus aureus* was prepared as described [16]. Streptolysin O from β-hemolytic streptococci (SLO) was prepared [8]

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and kindly provided by S. Bhakdi (Institut für Mikrobiologie der Universität Gießen). The concentrations of both toxins are given in hemolytic units (HU/ml) obtained with 2.5% rabbit erythrocytes [5, 16]. The free concentrations of Ca^{2+} and Mg^{2+} were calculated using the stability constants given [17] and controlled by ion-selective electrodes. The Mg^{2+}-sensitive carrier (ETH 5214) and the Ca^{2+}-sensitive carrier (ETH 129) were kindly provided by W. Simon, ETH Zurich, Switzerland. They were incorporated into polyvinylchloride membranes as described [6, 18]. Exocytosis was triggered by Ca^{2+} in the presence of different concentrations of free Mg^{2+} in KG (potassium glutamate) buffer containing (mM): KG 150, PIPES 10, EGTA (ethylenglycol-bis(β aminoethylether)-*N,N,N',N'*-tetraacetic acid) 0.5, NTA (nitriloacetic acid) 5, pH 7.2 adjusted with KOH. Further details are given in the Legends of the figures.

One problem encountered in the analysis of effects of low (μM) concentrations of free Ca^{2+} in the presence of high (mM) concentrations of free Mg^{2+} is the precise control of both concentrations of these ions. To overcome this, all the buffers used contained chelators for Mg^{2+} and Ca^{2+} (see above). In addition, their free Ca^{2+} and Mg^{2+} concentrations were measured by ion-specific electrodes [6, 18].

The high selectivity of the electrodes used allowed us to analyze micromolar amounts of free Ca^{2+} in the presence of millimolar amounts of free Mg^{2+}. Thus the effects of Mg^{2+} on Ca^{2+}-stimulated exocytosis observed here are directly correlated to its free concentrations. Representative determinations of Ca^{2+} and Mg^{2+} obtained by the electrodes in the buffers used are listed in Table I.

Mg^{2+} exhibited two effects on exocytosis from α-toxin permeabilized PC12 cells.

TABLE I

CALCULATED VALUES FOR THE FREE Ca^{2+} AND Mg^{2+} CONCENTRATIONS AND THEIR CONTROL BY ION-SPECIFIC ELECTRODES

A: Determination of the free Ca^{2+} concentration in the presence of various amounts of free Mg^{2+}. B: Determination of the free Mg^{2+} concentration in the absence or presence of 8 μM free Ca^{2+}. Mg^{2+} and Ca^{2+} were added to KG buffer to give the calculated free Mg^{2+} or Ca^{2+} concentrations. The pH was adjusted to 7.2 and the concentrations of free Mg^{2+} and Ca^{2+} were checked by the respective electrodes [6, 17].

A.			B.	
Mg^{2+} (mM) calculated	Mg^{2+} (mM) measured		Mg^{2+} (mM) calculated	Ca^{2+} (μM) measured
	No Ca^{2+}	Ca^{2+}		
			–	8
			0.2	7.5
0.2	0.4	0.3	0.5	8.5
0.5	0.5	0.6	1.0	8
1	1.0	1.1	2	8

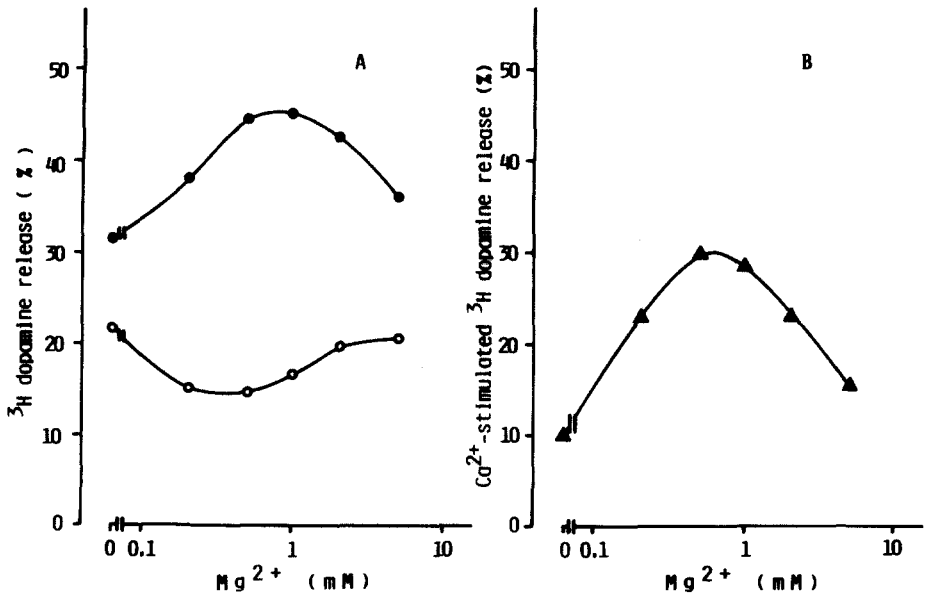

Fig. 1. Effects of various concentrations of free Mg^{2+} on exocytosis from α-toxin-permeabilized PC12 cells. Preloaded PC12 cells were permeabilized with α-toxin (300 HU/ml) in KG buffer (see Table I) in the absence or presence of the indicated concentrations of free Mg^{2+} for 30 min at 30°C. The medium was exchanged for a fresh one containing either no Ca^{2+} or 8 μM free Ca^{2+} plus the amounts of free Mg^{2+} given at the abscissa (A). A gives the percent of dopamine released in the absence (open symbols) or presence (closed symbols) of Ca^{2+}, B the difference of the two curves in A. Values are the mean of two samples. The experiment was repeated 8 times with similar results.

First, at concentrations between 0.2 and 1 mM it slightly lowered the basal release of [^{3}H]dopamine in the absence of Ca^{2+} (Fig. 1). Second, Mg^{2+} drastically enhanced Ca^{2+}-stimulated catecholamine release between 0.2 and 1 mM free Mg^{2+} (Fig. 1A, B). In different experiments 0.2 and 1 mM free Mg^{2+} increased the stimulated dopamine release 1.8 fold ± 0.4 (S.D., $n=5$) and 2.8 fold ± 0.6 (S.D., $n=6$), respectively. Free Mg^{2+} concentrations above 2 mM were less potent, but the effects never dropped to values obtained in the absence of Mg^{2+} (Fig. 1A, B). An inhibitory action of higher Mg^{2+} concentrations was also reported for electrically permeabilized adrenal chromaffin cells [15]. If Mg^{2+} was absent during the 30 min preincubation with α-toxin its effects on basal or Ca^{2+} stimulated release were less pronounced (not shown).

In order to find out whether soluble cytoplasmic proteins are involved in the effects of Mg^{2+} the experiments were also performed with SLO-permeabilized cells. In contrast to α-toxin where only molecules up to 1000 Da can escape during poration, SLO-permeabilized cells loose soluble cytoplasmic proteins as well [3–5, 9]. However, since in SLO-permeabilized cells Mg^{2+} still enhances Ca^{2+}-stimulated [^{3}H]dopamine release (Table II), cytoplasmic proteins are probably not involved in the effects of Mg^{2+}. In this kind of experiment 0.2 and 1 mM free Mg^{2+} increased the stimulated release 2.7 ± 1 and 3.1 ± 1.1 fold, respectively (S.D., $n=4$). It should be noted that

TABLE II

EFFECTS OF VARIOUS CONCENTRATIONS OF FREE Mg^{2+} ON EXOCYTOSIS FROM SLO-PERMEABILIZED PC12 CELLS

Preloaded PC12 cells were incubated with SLO (60 HU/ml) in KG buffer without divalent cations for 5 min at 0°C. The buffer was replaced by a fresh one containing the given amounts of free Mg^{2+} but no Ca^{2+} and the cells were incubated for 20 min at 30°C. Then the buffer was exchanged for a fresh one containing either no Ca^{2+} or 8 μM free Ca^{2+} plus the amounts of Mg^{2+} given. Values are the mean of two samples. Five different experiments gave similar results.

Mg^{2+} (mM)	[^{3}H]Dopamine release (%)		
	Basal	Ca^{2+}-stimulated	Net release
no	35	44.5	9.5
0.2	27.5	46.7	19.2
0.5	28.8	48.4	19.5
1	33.7	53.4	19.7

in SLO-permeabilized cells lower amounts of free Mg^{2+} increased the stimulated release which may be due to the loss of Mg^{2+}-binding material or other factors involved (compare Fig. 1 and Table II).

The improvement of the stimulated release by Mg^{2+} is not due to exchange of Ca^{2+} from intracellular stores or binding sites. In permeabilized PC12 or RIN A2 (a tumour cell line derived from a rat insulinoma) cells concentrations up to 1 mM free Mg^{2+} neither increase nor decrease the free Ca^{2+} concentration as detected by a Ca^{2+}-sensitive electrode [12]. Furthermore the decrease of the basal release due to Mg^{2+} also argues against a liberation of Ca^{2+} from intracellular stores or proteins. Finally, the high capacity of the chelators present in the KG buffer would not allow changes in the free Ca^{2+} concentrations.

In PC12 cells exocytosis was reported to be inhibited by GTPS (guanosin 5'-*O*-(3-thio-triphosphate)) in the presence of free Mg^{2+}. This inhibition is probably due to a pertussis toxin-sensitive GTP-binding protein [2]. Activation of G-proteins depends on the supply of free Mg^{2+} in the millimolar range [2]. Pretreatment of PC12 cells with either pertussis toxin or cholera toxin failed to substantially alter the effects of Mg^{2+} on the basal or Ca^{2+}-stimulated exocytosis. This indicates that the effects of Mg^{2+} reported here are unlikely mediated by G-proteins.

Taking these findings together it can be assumed that the effects of millimolar concentrations of free Mg^{2+} on exocytosis are attributed to binding of Mg^{2+} at the interacting membranes. This may reduce their negative surface charges and subsequently facilitates Ca^{2+}-induced membrane fusion. Another possibility to explain the results would be that similar to the situation seen with charged liposomes [10] Ca^{2+} binding is increased in contacting membranes in the presence of an excess of free Mg^{2+}.

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