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EFFECTS OF ZINC CHLORIDE ON THE HYDROLYSIS OF CYCLIC GMP AND CYCLIC AMP BY THE ACTIVATOR-DEPENDENT CYCLIC NUCLEOTIDE PHOSPHODIESTERASE FROM BOVINE HEART

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Summary

In the presence of 10 μM Ca^{2+} and 5 mM Mg^{2+} (or 0.25 mM Mg^{2+}), the addition of 100 μM Zn^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Cu^{2+} or 1 mM Mn^{2+} resulted in varying degrees of stimulation or inhibition of 10^{-6} M cyclic GMP and cyclic AMP hydrolysis by the activator-dependent cyclic nucleotide phosphodiesterase from bovine heart in the absence or presence of phosphodiesterase activator. The substrate specificity of the enzyme was altered under several conditions. The addition of Zn^{2+} in the presence of 5 mM Mg^{2+} and the absence of activator resulted in the stimulation of cyclic GMP hydrolysis over a narrow substrate range while reducing the V 65% due to a shift in the kinetics from non-linear with Mg^{2+} alone to linear in the presence of Zn^{2+} and Mg^{2+}. Zn^{2+} inhibited the hydrolysis of cyclic GMP and cyclic AMP in the presence of activator with K_i values of 70 and 100 μM, respectively. Zn^{2+} inhibition was non-competitive with substrate, activator and Ca^{2+} but was competitive with Mg^{2+}. In the presence of 10 μM Ca^{2+} and activator, a K_i of 15 μM for Zn^{2+} vs. Mg^{2+} was noted in the hydrolysis of 10^{-6} M cyclic GMP. Several effects of Zn^{2+} are discussed which have been noted in other studies and might be due in part to changes in cyclic nucleotide levels following phosphodiesterase inhibition.

Introduction

We have previously reported [1,2] that the substrate specificity of the activator-dependent cyclic nucleotide phosphodiesterase from bovine heart depends on the divalent metal used to support enzyme activity in the absence

Abbreviation: EGTA, ethyleneglycol-bis(β-aminoethylether)-N,N'-tetraacetic acid.

or presence of phosphodiesterase activator. The enzyme preferentially hydrolyzed cyclic GMP in the presence of Mg^{2+} while approximately equal amounts of cyclic GMP and cyclic AMP were hydrolyzed in the presence of Mn^{2+}, Zn^{2+}, Co^{2+} or Ni^{2+}. Fe^{2+}, Cu^{2+} and Ca^{2+} did not support enzyme activity alone. The magnitude of the increase in enzyme activity due to activator and Ca^{2+} also depended on the divalent metal used to support enzyme activity without activator. Human heart has been reported by Tipton and Cooke [3] to contain, in total, 7.8 mM Mg^{2+}, 1.2 mM Ca^{2+}, 1 mM Fe^{2+}, 0.5 mM Zn^{2+}, 60 μM Cu^{2+}, 4.6 μM Mn^{2+}, 1 μM Ni^{2+} and 1 μM Co^{2+}. The high levels of Mg^{2+} and Zn^{2+} in vivo suggest that these divalent metals may be able to influence enzyme activity provided that a significant amount of the metal is free for interaction with the enzyme. The low levels of Ni^{2+}, Co^{2+} and Mn^{2+} in vivo suggest that these metals probably have little physiological role in this system unless they are compartmentalized in association with the enzyme. The use of a single divalent metal to monitor the properties of this enzyme is not physiological and therefore this report describes the effects of various divalent metals on the hydrolysis of cyclic GMP and cyclic AMP in the presence of optimal and suboptimal levels of Mg^{2+}. The high levels of Mg^{2+} in vivo dictated its use as the point of comparison in these studies. The significant stimulatory and inhibitory effects of Zn^{2+} observed in the presence of Mg^{2+} were examined in some detail to assess their possible physiological importance.

Materials and Methods

Cyclic AMP, cyclic GMP and *Crotalus atrox* venom were purchased from Sigma. Cyclic [^{3}H]AMP and cyclic [^{3}H]GMP were from Amersham/Searle. $ZnCl_2$ was from Fisher Scientific.

Phosphodiesterase activity was assayed by a procedure adapted from Russell et al. [4]. An appropriate dilution of enzyme was incubated in 40 mM Tris/Cl, pH 7.4, and 5 mM $MgCl_2$ containing $1 \cdot 10^{-8}$—$3 \cdot 10^{-8}$ M cyclic [^{3}H]AMP or cylic [^{3}H]GMP (50 000 to 150 000 counts/min) in a total volume of 1 ml. When higher concentrations of cyclic nucleotides (usually 10^{-6} M cyclic AMP or cyclic GMP) were required, the indicated amounts of unlabelled cyclic nucleotides were included. Other additions to, or alterations of, this assay procedure are as indicated in the legends to the figures and tables. After 10 min at 30° C, the reaction was terminated by boiling for 3 min. After cooling in ice for 15 min, 0.1 ml of *C. atrox* venom (1 mg/ml in H_2O) was added to each sample and incubated for 30 min at 30° C. The reaction was terminated by boiling for 3 min. Following cooling in ice, undegraded cyclic nucleotides were removed by centrifugation at $1200 \times g$ for 5 min following the addition of 1.0 ml of Dowex 1 × 8 ion exchange resin (1 : 2 slurry in H_2O). [^{3}H]Adenosine or [^{3}H]guanosine in the supernatant were detected by liquid scintillation spectrometry. Corrections were made for the binding of approx. 20—30% of the [^{3}H]adenosine or [^{3}H]guanosine to the ion exchange resin. The amount of phosphodiesterase used was adjusted so that no more than 15% of the cyclic nucleotide was hydrolyzed during the incubation. None of the divalent metals, at the levels studied, interfered with the 5′-nucleotidase activity of snake venom.

Activator-dependent cyclic nucleotide phosphodiesterase from bovine heart was prepared through the step of rechromatography on DEAE-cellulose as described previously [2]. The active peak was pooled and concentrated using a PM-10 membrane in an Amicon Ultrafiltration chamber. This enzyme preparation was found to be free of phosphodiesterase activator as described previously [2]. The concentrated enzyme preparation (20 ml) was then dialyzed against 8 l of 1 mM EGTA, pH 7.4, and 20 mM potassium phosphate buffer, pH 7.4, for 8 h at 4°C and then dialyzed against 8 l of 20 mM potassium phosphate buffer.

Phosphodiesterase activator was prepared from bovine liver as described previously [5]. This preparation was dialyzed against 1 mM EGTA, pH 7.4, and 20 mM potassium phosphate buffer, pH 7.4, and then dialyzed against 20 mM potassium phosphate buffer, pH 7.4.

Results

The combined effects of Mg^{2+} and various divalent metals on the hydrolysis of 10^{-6} M cyclic GMP and cyclic AMP by the activator-dependent cyclic nucleotide phosphodiesterase from bovine heart in the absence and presence of a saturating amount of phosphodiesterase activator are presented in Tables I and II. The concentrations of Mg^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+} and Co^{2+} used were those previously found to maximally support enzyme activity when used alone in the absence of activator [2]. Fe^{2+} and Cu^{2+}, which did not support enzyme activity alone [2], were arbitrarily studied at 100 μM. In the presence of 5 mM Mg^{2+} and 10 μM Ca^{2+} (Table I) the enzyme hydrolyzed 3.0-fold more cyclic GMP than cyclic AMP in the absence of activator and 4.7-fold more in the presence of activator. In the absence of activator, the addition of 100 μM Cu^{2+} decreased the hydrolysis of cyclic GMP by 75%. The addition of 1 mM Mn^{2+} or 100 μM Ni^{2+}, Co^{2+} or Fe^{2+} did not significantly affect cyclic GMP hydrolysis while 100 μM Zn^{2+} stimulated cyclic GMP hydrolysis 15%. The stimulation by Zn^{2+} reached 35% in some enzyme preparations and was found to be a labile property of the enzyme as the stimulation was lost after 4 days of storage at −70°C. Cu^{2+}, which decreased cyclic AMP hydrolysis by 70%, was the only metal to significantly alter the hydrolysis of cyclic AMP. In the presence of activator, the addition of Zn^{2+}, Mn^{2+}, Fe^{2+} or Cu^{2+} significantly decreased the hydrolysis of cyclic GMP (51, 25, 45 and 83%, respectively). The hydrolysis of cyclic AMP was decreased 45, 38 and 72% by Zn^{2+}, Fe^{2+} and Cu^{2+} respectively. The enzyme became less specific for cyclic GMP upon the addition of Cu^{2+} in the presence of activator.

As the ratio of the Mg^{2+} level present in association with the phosphodiesterase in vivo to the levels of other divalent metals is not known, we also examined the effects of the same levels of Mn^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+} and Cu^{2+} in the presence of 20-fold less Mg^{2+} (250 μM, Table II). 5 mM Mg^{2+} is optimal for the hydrolysis of both cyclic AMP and cyclic GMP in the absence of phosphodiesterase activator; however, 0.25 mM Mg^{2+} is optimal for only cyclic GMP hydrolysis as the hydrolysis of cyclic AMP is reduced 50%, and therefore the enzyme is more cyclic GMP-specific at the lower Mg^{2+} concentration. In the presence of activator there were only small differences between

TABLE I

Combined effects of Mg^{2+} (5 mM) and various divalent metals on the hydrolysis of 10^{-6} M cyclic GMP and cyclic AMP in the absence and presence of a saturating amount of phosphodiesterase activator (4 μg). All samples contained 10 μM $CaCl_2$. The results are expressed as pmol/mg per min and are the mean ± S.E. of 12 determinations from three experiments.

Metals	Minus activator			Plus activator		
	Cyclic GMP	Cyclic AMP	cGMP/cAMP	Cyclic GMP	Cyclic AMP	cGMP/cAMP
Mg^{2+} 5 mM	518 ± 22.8	173 ± 8.5	2.99	2468 ± 131.2	525 ± 31.8	4.70
Mg^{2+}, Mn^{2+} 1 mM	385 ± 19.2	190 ± 10.2	2.02	1843 ± 95.6 *	475 ± 27.8	3.88
Mg^{2+}, Zn^{2+} 100 μM	609 ± 21.6	191 ± 12.9	3.19	1202 ± 87.5 *	289 ± 21.2 *	4.15
Mg^{2+}, Ni^{2+} 100 μM	462 ± 20.3	180 ± 9.8	2.56	2546 ± 162.1	588 ± 29.5	4.33
Mg^{2+}, Co^{2+} 100 μM	502 ± 25.4	172 ± 5.3	2.92	2452 ± 103.6	562 ± 31.5	4.36
Mg^{2+}, Fe^{2+} 100 μM	440 ± 21.6	170 ± 7.9	2.58	1358 ± 115.6 *	325 ± 21.7 *	4.18
Mg^{2+}, Cu^{2+} 100 μM	125 ± 8.3 *	50 ± 4.8	2.50	421 ± 31.2 *	150 ± 9.6 *	2.80

* Significantly different from the control with Mg^{2+} alone ($p < 0.05$).

TABLE II

Combined effects of Mg^{2+} (250 μM) and various divalent metals on the hydrolysis of 10^{-6} M cyclic GMP and cyclic AMP in the absence and presence of a saturating amount of phosphodiesterase activator. All samples contained 10 μM $CaCl_2$. The results are expressed in pmol/mg per min and are the mean ± S.E. of 12 determinations from three experiments.

Metals	Minus activator			Plus activator		
	Cyclic GMP	Cyclic AMP	cGMP/cAMP	Cyclic GMP	Cyclic AMP	cGMP/cAMP
Mg^{2+} 250 μM	500 ± 24.8	91 ± 4.9	5.49	2312 ± 153.7	612 ± 28.5	3.78
Mg^{2+}, Mn^{2+} 1 mM	325 ± 18.3 *	163 ± 10.4 *	1.99	1735 ± 85.7 *	501 ± 32.9	3.46
Mg^{2+}, Zn^{2+} 100 μM	212 ± 12.5 *	100 ± 6.1	2.12	735 ± 51.4 *	288 ± 19.5 *	2.55
Mg^{2+}, Ni^{2+} 100 μM	500 ± 31.2	220 ± 14.5 *	2.27	2305 ± 131.6	925 ± 58.7 *	2.49
Mg^{2+}, Co^{2+} 100 μM	525 ± 19.8	172 ± 12.1 *	3.05	2125 ± 87.2	662 ± 45.3	3.21
Mg^{2+}, Fe^{2+} 100 μM	388 ± 18.6	106 ± 6.5	3.66	1406 ± 55.6 *	305 ± 23.6 *	4.62
Mg^{2+}, Cu^{2+} 100 μM	75 ± 5.4 *	21 ± 2.2 *	3.57	390 ± 31.6 *	150 ± 8.5 *	2.60

* Significantly different from the control with Mg^{2+} alone ($p < 0.05$).

the hydrolysis of cyclic GMP and cyclic AMP in the presence of 5 mM or 0.25 mM Mg^{2+}. In the absence of activator, the addition of 1 mM Mn^{2+} in the presence of 0.25 mM Mg^{2+} resulted in the hydrolysis of 35% less cyclic GMP but 80% more cyclic AMP, and therefore the enzyme was less specific for cyclic GMP. The addition of Zn^{2+} and Fe^{2+} decreased the hydrolysis of cyclic GMP by 60 and 23%, respectively while Ni^{2+} and Co^{2+} increased the hydrolysis of cyclic AMP by 140 and 65%, respectively, all ions resulting in the enzyme being less specific for cyclic GMP. Cu^{2+} inhibited the hydrolysis of both cyclic GMP and cyclic AMP by 85 and 77%, respectively. In the presence of activator the addition of Mn^{2+}, Zn^{2+}, Fe^{2+} or Cu^{2+} was inhibitory to the hydrolysis of both cyclic GMP and cyclic AMP, with Zn^{2+} and Cu^{2+} being the most inhibitory. In contrast, the addition of Ni^{2+} resulted in a 50% increase in cyclic AMP hydrolysis. The addition of Zn^{2+}, Ni^{2+} or Cu^{2+} resulted in the enzyme being less specific for cyclic GMP hydrolysis.

The effects of Zn^{2+} on the kinetic parameters of the enzyme were examined in the absence and presence of 5 mM Mg^{2+}. In the absence of activator, a Hofstee plot [6] (Fig. 1) of enzyme activity with 50 μM Zn^{2+} against a wide range of cyclic GMP concentrations (10^{-7}—10^{-4} M) gave linear kinetics indicating an apparent K_m of 5 μM for cyclic GMP. As reported prevously [2], non-linear kinetics were observed with 5 mM Mg^{2+}, indicating two apparent K_m values (1 and 15 μM) for cyclic GMP. The V value obtained with Zn^{2+} was 76% below that obtained with Mg^{2+}. The combination of Mg^{2+} and Zn^{2+} gave linear kinetics (apparent K_m = 3 μM) and was stimulatory over a narrow substrate range (10^{-7}—10^{-6} M) compared with Mg^{2+} alone but was inhibitory at higher

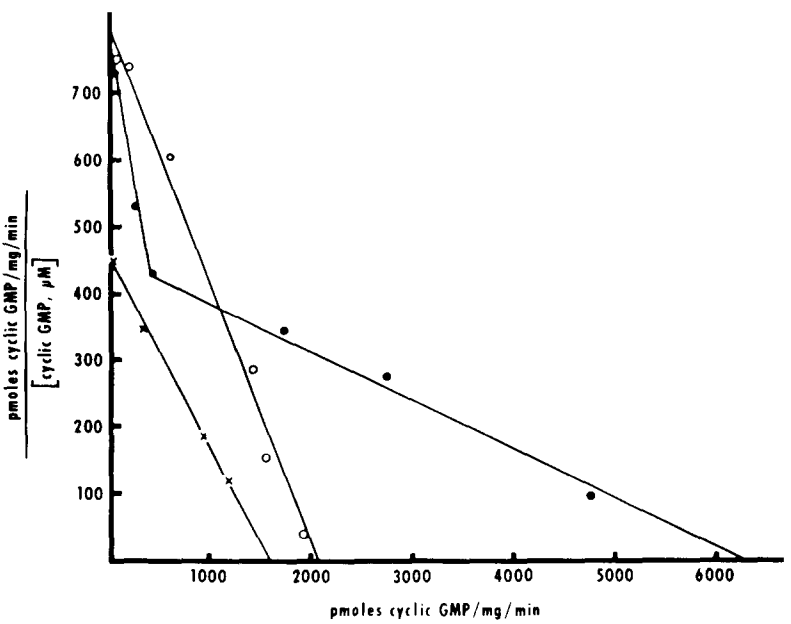

Fig. 1. Hofstee plot [6] of cyclic GMP (10^{-7}—10^{-4} M) hydrolysis in the absence of phophodiesterase activator in the presence of 5 mM Mg^{2+} (●———●), 50 μM Zn^{2+} (X———X) and 5 mM Mg^{2+} and 50 μM Zn^{2+} (○———○). All samples contained 10 μM $CaCl_2$.

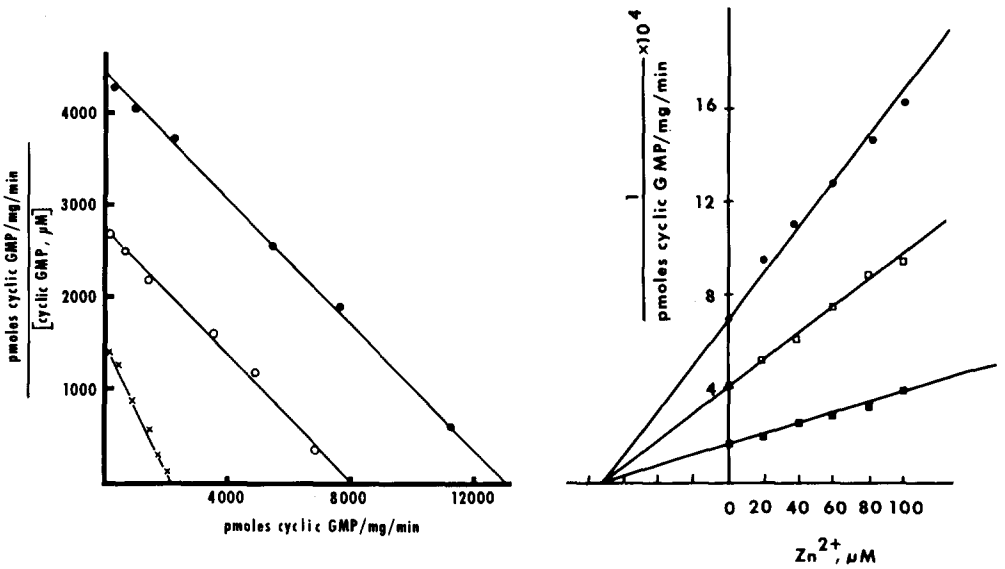

Fig. 2. Hofstee plot [6] of cyclic GMP (10^{-7}—10^{-4} M) hydrolysis in the presence of phosphodiesterase activator (4 μg) in the presence of 5 mM Mg^{2+} (●———●), 100 μM Zn^{2+} (X———X) and 5 mM Mg^{2+} plus 100 μM Zn^{2+} (○———○). All samples contained 10 μM $CaCl_2$.

Fig. 3. Dixon plot [7] of the effect of increasing concentrations of Zn^{2+} on cyclic GMP hydrolysis in the presence of phosphodiesterase activator (4 μg). The concentrations of cyclic GMP were 0.5 μM (●———●), 1 μM (□———□) and 5 μM (■———■). All samples contained 10 μM $CaCl_2$.

substrate levels as V was reduced by 65% from that with Mg^{2+} alone. In the presence of activator, linear kinetics of cyclic GMP hydrolysis were observed, as the combination of Mg^{2+} and Zn^{2+} was inhibitory over the entire substrate range (Fig. 2). No change in K_m for cyclic GMP (3 μM) was observed with the combination of Mg^{2+} and Zn^{2+} although the V value was decreased by 38% from that with Mg^{2+} alone.

The nature of the inhibition of cyclic nucleotide hydrolysis by Zn^{2+} was examined. The Hofstee plot (Fig. 2) demonstrated that Zn^{2+} inhibition was non-competitive with cyclic GMP as substrate in the presence of phoshpodiesterase activator and 10 μM Ca^{2+}. Zn^{2+} inhibition was also found to be non-competitive with cyclic AMP as substrate. A Dixon plot [7] of cyclic GMP hydrolysis in the presence of 5 mM Mg^{2+}, 10 μM Ca^{2+} and phosphodiesterase activator indicated a K_i of 70 μM for $ZnCl_2$ (Fig. 3). The K_i for $ZnCl_2$ was 100 μM with cyclic AMP as substrate. The Dixon plots also indicated the non-competitive nature of inhibition by Zn^{2+} with cyclic AMP and cyclic GMP as substrates. A double reciprocal plot of cyclic GMP hydrolysis versus increasing levels of activator in the presence of 5 mM Mg^{2+} and 10 μM Ca^{2+} demonstrated that Zn^{2+} inhibition was non-competitive with activator (Fig. 4). Similarly, a double reciprocal plot of the effects of 30, 50 and 70 μM $ZnCl_2$ on the hydrolysis of 10^{-6}M cyclic GMP versus increasing concentrations of Ca^{2+} (0.5—20 μM) in the presence of 5 mM Mg^{2+} and phosphodiesterase activator demonstrated that Zn^{2+} inhibition was non-competitive with Ca^{2+}.

A double reciprocal plot of the effects of Zn^{2+} on the hydrolysis of 10^{-6} M

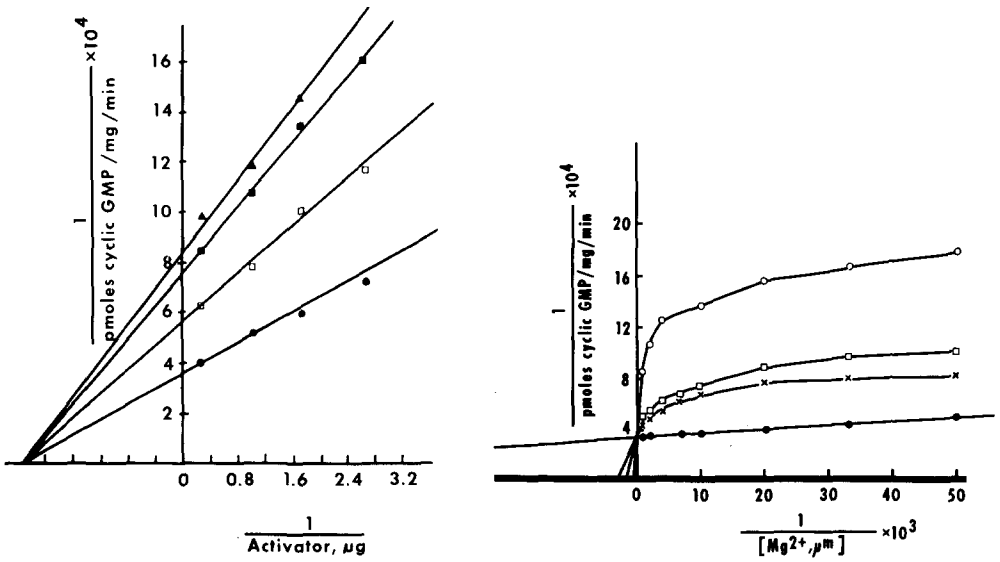

Fig. 4. Double reciprocal plot of the effect of 0 (●———●), 30 μM (□———□), 50 μM (■———■) and 70 μM (▲———▲) Zn^{2+} on the hydrolysis of 10^{-6} M cyclic GMP in the presence of increasing levels of phosphodiesterase activator. All samples contained 10 μM $CaCl_2$.

Fig. 5. Double reciprocal plot of the effect of 0 (●———●), 20 μM (X———X), 30 μM (□———□) and 60 μM (○———○) Zn^{2+} on the hydrolysis of 10^{-6} M cyclic GMP in the presence of increasing levels of Mg^{2+}. All samples contained phosphodiesterase activator (4 μg) and 10 μM $CaCl_2$.

cyclic GMP versus increasing concentrations of Mg^{2+} in the presence of phosphodiesterase activator and 10 μM Ca^{2+} is presented in Fig. 5. A linear plot was obtained with Mg^{2+} alone, indicating an apparent K_m of 10 μM for Mg^{2+}. The plots were non-linear in the presence of Zn^{2+}; however, V was unchanged, sug-

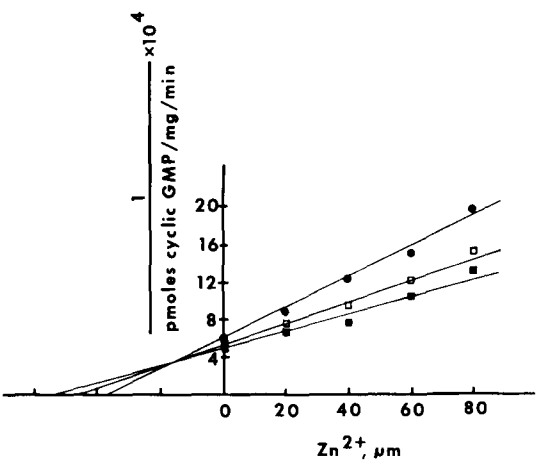

Fig. 6. Single reciprocal plot of the effect of increasing concentrations of Zn^{2+} on the hydrolysis of 10^{-6} M cyclic GMP in the presence of phosphodiesterase activator (4 μg) and 50 μM (●———●), 250 μM (□———□) and 500 μM (■———■) Mg^{2+}.

gesting that Zn^{2+} competes with Mg^{2+} in the inhibition of enzyme activity. A plot (Fig. 6) of the reciprocal of 10^{-6} M cyclic GMP hydrolysis in the presence of 10 μM Ca^{2+} and activator versus increasing concentrations of Zn^{2+} in the presence of varying Mg^{2+} levels indicated a K_i of 15 μM for Zn^{2+} vs. Mg^{2+}.

Discussion

The intracellular levels of cyclic AMP and cyclic GMP are, in part, regulated by the activity of the various cyclic nucleotide phosphodiesterases [2]. The activity of these enzymes is dependent on the presence of a divalent metal. For example, we have noted [2] that Mg^{2+}, Zn^{2+}, Mn^{2+}, Ni^{2+} and Co^{2+} support the activity of the activator-dependent cyclic nucleotide phosphodiesterase from bovine heart. Mg^{2+} probably has the dominant role in vivo in the support of the activity of this enzyme due to its high level in vivo compared to the other metals. However, this report indicates that Zn^{2+}, Mn^{2+}, Fe^{2+} and Cu^{2+} can significantly alter the activity of this enzyme observed in the presence of varying levels of Mg^{2+}. Therefore these metals, in addition to Mg^{2+}, may regulate the activity of this enzyme in vivo provided that a significant amount of the metals is available for interacton with this enzyme.

The results suggest that Zn^{2+} may predominantly function as an inhibitor of phosphodiesterase activity. Although Zn^{2+} can satisfy the divalent metal requirement of the enzyme in the hydrolysis of cyclic AMP or cyclic GMP, it does so with a V value lower than that observed with Mg^{2+} alone. In the presence of 5 mM Mg^{2+} and phosphodiesterase activator, Zn^{2+} inhibits the hydrolysis of cyclic GMP and cyclic AMP, with K_i values of 70 and 100 μM, respectively. Figs. 5 and 6 indicate that Zn^{2+} inhibition is competitive with Mg^{2+}, although in Fig. 5 the data were not linear in the presence of Zn^{2+}. This non-linearity is to be expected, as at low Mg^{2+} levels Zn^{2+} partially satisfies the divalent metal requirement of the enzyme and supports enzyme activity, but with higher Mg^{2+} levels, the lower V observed with Zn^{2+} results in inhibition of enzyme activity. Due to the competitive nature of this interaction, V was not changed by Zn^{2+} with increasing Mg^{2+} concentrations (Fig. 5).

The inhibitory potency of Zn^{2+} is therefore dependent on the Mg^{2+} concentration. If the Mg^{2+} level interacting with the enzyme is below 5 mM, then the K_i of Zn^{2+} observed in the hydrolysis of cyclic GMP and cyclic AMP would be below 70 and 100 μM, respectively. The amount of Mg^{2+} available for interaction with this cyclic nucleotide phosphodiesterase in vivo may be considerably below the total Mg^{2+} concentration (7.8 mM) reported in human heart [3], as many other enzymes require Mg^{2+} for optimal enzyme activity either through binding of the Mg^{2+} to the enzyme or binding to the substrate. As the total level of Zn^{2+} in human heart is 0.5 mM [3] and the K_i of Zn^{2+} is probably less than 100 μM in the hydrolysis of cyclic GMP and cyclic AMP, Zn^{2+} may function in vivo to maintain a degree of inhibition of the activity of the enzyme. As the inhibition by Zn^{2+} is reversible, changes in the intracellular level of Zn^{2+} might also result in changes in the activity of this enzyme.

Several effects of Zn^{2+} have been noted which might be due in part to changes in cyclic nucleotide levels. In humans, Zn^{2+} deficiency is associated with hypogonadism and decreased release of pituitary gonadotropins [8].

LaBella et al. [9] have shown that the addition of Zn^{2+} to bovine pituitary extracts increased the release of growth hormone, thyrotropin, gonadotropins and ACTH. The secretion of gonadotropins [10,11], thyrotropin [12], growth hormone [13—16] and ACTH [17] from rat anterior pituitary glands have been shown to be enhanced by cyclic AMP, and therefore Zn^{2+} may function in the secretion of these hormones, in part, by increasing intracellular cyclic AMP following inhibition of phosphodiesterase activity.

Zn^{2+} apparently stabilizes the lysosomal membrane of hepatocytes [18] preventing the release of lysosomal enzymes. Ignarro and co-workers [19—21] have studied the effects of cyclic nucleotides and agents which alter cyclic nucleotide levels on lysosomal enzyme release in isolated rat liver lysosomes [20] and lysosomes from guinea pig polymorphonuclear leukocytes and have suggested that a reciprocal relationship exists between cyclic AMP and cyclic GMP with cyclic AMP inhibiting and cyclic GMP enhancing release. Therefore if the effect of Zn^{2+} to stabilize the lysosomal membrane is through a cyclic nucleotide-dependent mechanism then it may be the net effect of the opposing influences of increased levels of both cyclic AMP and cyclic GMP following inhibition of phosphodiesterase activity.

Zn^{2+} acts as a mitogen in lymphocytes [23—25] while cyclic AMP and cylic GMP have been found to have regulatory roles in cellular proliferation. Cyclic GMP apparently is an intracellular mediator of the proliferative process in lymphocytes as the potent mitogens phytohemagglutinin (PHA) and concanavalin A have been observed to elevate levels of cyclic GMP more than 10-fold within several minutes after addition to human lymphocyte cultures [26]. Only small changes in cyclic AMP levels occur at this time [27]. Further study has implicated cyclic GMP in the stimulation of both nuclear acidic protein phosphorylation [28] and RNA polymerase activity [29,30] which occur early upon induction of proliferation by mitogens. Several studies suggest that cyclic AMP may have an inhibitory effect in cellular proliferation. For example, the addition of cyclic AMP analogues or agents that raise cyclic AMP levels slows the logarithmic growth rate of lymphocytes and fibroblasts [31]. Conversely, rapidly growing cells have low levels of cyclic AMP [32]. Berger and Skinner [25] noted that the addition of Zn^{2+} in a narrow range of concentrations ($1.5—4.5 \cdot 10^{-4}$ M) resulted in a greatly increased blastogenic transformation and mitosis of lymphocytes which was comparable to the effects of PHA. Therefore the effects of Zn^{2+} on cellular transformation might be due, in part, to changes in cyclic nucleotide levels following inhibition of phosphodiesterase activity.

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