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Endocrine Interaction Between Zinc and Prolactin

An Interpretative Review

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ABSTRACT

Zinc plays a very important role in animal and human metabolism. Nowadays, it is one of the most extensively studied trace element, since its sphere of action has been demonstrated to be very broad. From the biochemical standpoint, it controls more than 300 different enzymes, many of them involved with intermediary metabolism, DNA and RNA synthesis, gene expression, and immunocompetence. It also plays a significant role in hormonal homeostasis, since it can interact with almost all hormones. Zn^{2+} is closely related to the thyroid and steroid hormones, insulin, parathormone, and pituitary hormones, particularly prolactin (PRL). Zn^{2+} can inhibit PRL secretion within a range of physiologically and pharmacologically relevant concentrations. This property has raised the possibility of clinical applications of zinc. In this article, we review the literature on the subject in an attempt to provide a comprehensible general view.

Index Entries: Zinc; prolactin synthesis, storage, and secretion; prolactinoma; normal individuals.

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RELATIONSHIP BETWEEN ZINC AND PROLACTIN

There is an inverse physiological relationship between zinc and prolactin. Therefore, it has been suggested that this trace element is part of the negative feedback regulatory loop *(1)*. The PRL release from the anterior hypophysis depends on the tonic inhibitory control of dopamine and on the coordinated activity of the calcium–calmodulin complex, enzymes, and calcium channels and pump, among others *(2)*. The hypothalamus and hypophysis are Zn^{2+}-rich areas *(3,4)*. The physiological plasma Zn^{2+} concentration ranges from 10 to 15 μM *(5)*, and it has been observed that values between 1 and 60 μM have the ability to inhibit the secretion of newly synthesized PRL in vitro. The first researchers to observe these facts were LaBella et al. *(4)* in 1973, when they incubated bovine pituitary extracts with 60 μM Zn^{2+}. These findings were corroborated by Login et al. *(6)*, who succeeded in inhibiting the PRL synthesis secretion from normal female rat anterior pituitary glands by using 1–100 μM Zn^{2+} in an acute selective dose-dependent manner. The Zn^{2+} capacity to inhibit PRL at physiological and pharmacological concentrations brought about the idea that this ion plays an important role in PRL regulation in vivo.

PROPOSED MODES OF ACTION ON LACTOTROPH CELLS—IN VITRO STUDIES

There are several possible ways Zn^{2+} can act on PRL synthesis and secretion. Each step is separately approached below and illustrated in Fig. 1.

Calcium Channels

Ca^{2+} channels, responsible for cell membrane excitability, have an important role in PRL release. Perifusion of pituitary cells with a Ca^{2+}-free medium or the addition of Ca^{2+} channel blockers inhibits the release of PRL *(7)*. There is a relationship among Zn^{2+}, Ca^{2+}, and Ca^{2+} channels. Regarding Ca^{2+}, the Zn^{2+} ions are 20 times less permeable to the Ca^{2+} channels, but their affinity is 10 times stronger, turning Zn^{2+} into a Ca^{2+} antagonist *(8)*. By acting in this manner, Zn^{2+} alters the permeability and contractility of these channels, and thus, inhibits PRL secretion.

Exchangers

There are innumerable exchangers in the plasma membrane. Most of them use the energy stored in the Na^{+} electrochemical gradient to move an ion against a concentration gradient. Zn^{2+} can also inhibit the functionality of the Na^{+}/Ca^{2+} exchangers by altering the permeability of lac-

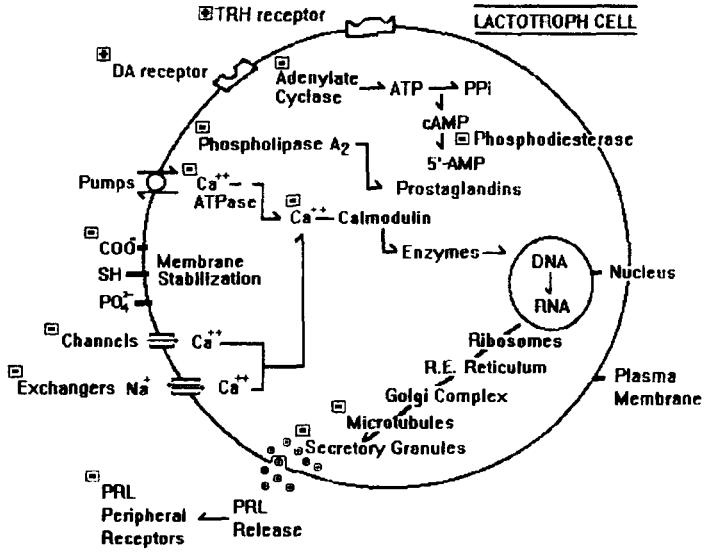

Fig. 1. Possible modes of Zn^{2+} action on lactotroph cells. Zinc acts by inhibiting (⊖) the synthesis, secretion, and peripheral action of PRL. At first, this ion inhibits the function of membrane enzymes (adenylate cyclase, phospholipase A_2, Ca^{2+} ATPase), Ca^{2+} channels, Na^+/Ca^{2+} exchanger, Ca^{2+}-calmodulin complex, microtubules, and secretory granules, and increases stabilization of lactotroph membranes (interacting with COO^-, SH, and PO_4^{3-} groups). Finally, zinc also stimulates (⊕) DA and TRH receptors (even though it inhibits intracellular Ca^{2+}), and inhibits peripheral PRL receptors. These conclusions were obtained from in vitro studies. For more details, *see text.*

totroph membranes *(9)*. This event, similarly to the antagonism presented by the Ca^{2+} channels, explains the inhibition of PRL secretion.

Calcium–Calmodulin Complex and Enzymes Regulated by It

Many of the Ca^{2+} intracellular effects are possible only when Ca^{2+} is bound to calmodulin, forming the Ca^{2+}–calmodulin complex, whose activity is regulated by cellular Ca^{2+} flow *(10)*. Calmodulin itself has no biological effect, and it needs Ca^{2+} to be activated. It is in the helicoidal conformation that camodulin is able to activate a large number of enzymes and stimulate many intracellular events *(10)*. The relationship between zinc and the Ca^{2+}–calmodulin complex plays an important role, because zinc can act at three levels:

1. It competes with Ca^{2+} in binding to calmodulin *(11)*;
2. It binds to the complex after enzyme complexing *(12)*; and
3. It binds to the target enzymes *(12)*

In this process, Zn^{2+} decreases the activity of the enzymes activated by the complex *(13,14)*. It is known that the Ca^{2+}–calmodulin complex is closely related to PRL synthesis *(15)* and the main enzymes involved in the process are Ca^{2+}-ATPase, adenylate cyclase, and phospholipase A2. These enzymes are attached to plasma membranes and the enzyme activities are also inhibited by Zn^{2+} *(13,16)*. Ca^{2+}-ATPase is generally believed to be the Ca^{2+} pump, and there are two distinct types of Ca^{2+}-ATPase: one present in the plasma membrane, which ejects Ca^{2+} outside the cell, and the other present in the sarcoplasmic reticulum, which drives Ca^{2+} ions from the cytoplasm into the sarcoplasmic reticulum *(10)*. Adenylate cyclase is the enzyme that catalyzes the synthesis of 3′,5′-adenosine monophosphate (cAMP) from adenosine triphosphate (ATP), and cAMP is an important nucleotide that stimulates PRL release *(17)*. On the other hand, cAMP is degraded by phosphodiesterase, an enzyme that is also inhibited by Zn^{2+} *(13)*. Phospholipase A_2 participates in prostaglandin synthesis, and catalyzes the deacylation of phosphoglycerides to produce a lysophosphatide and a free fatty acid, such as arachidonic acid, which stimulates PRL synthesis *(18)*. This inhibitory effect of Zn^{2+} on the enzymes explains the immobilization of energy-dependent activity of the lactotroph membrane and increases the integrity of the membrane structure. Thus, Zn^{2+} acting on all these intracellular mechanisms negatively interferes with PRL synthesis and secretion.

Gene Expression

Many cellular processes, such as transcription, RNA processing, and replication, are regulated by protein–DNA and protein–protein interactions. Many such proteins have embedded in their structure a domain (or motif) that serves to bind to the DNA molecule *(19)*. This motif for the DNA recognition sequence is now known as the "zinc-finger." A common feature of these types of structural motifs is the use of cysteines and/or histidines for zinc binding to nucleic acids *(20)*. For instance, Cys2-Cys2 motif may bind exclusively to DNA, and Cys2-His2 motif could have the ability to bind to both DNA and RNA *(21)*. The Cys2-His2 zinc-finger is the most widely occurring DNA binding motif, and two or three zinc-fingers may be sufficient for specific DNA binding *(22)*. The zinc atom is in the center of each unit, and each of these domains is usually characterized by the conservation of its tertiary structure *(23)*. Independent studies have resulted in the identification, characterization, and structure determination of nuclear receptor DNA binding domains. The nuclear receptor supergene family includes receptors for hormones, such as steroid hormones, thyroid hormone, and receptors for several other ligands, and they have similar mechanisms of action *(24)*.

Microtubules

Microtubules are cylindrical cytoskeletal elements whose main function is to favor cell motility and division, as well as intracellular transportation. Despite the existence of microtubule-associated proteins (MAPs), the major protein of the microtubules is tubulin, which is composed of α and β subunits *(25)*. Zn^{2+} has been found in high concentrations in tubulin-rich areas, mainly in the brain *(26)*, and the ion Zn^{2+} *per se* can bind to microtubule proteins and induce microtubule assembly *(27)*. However, the contractile elements of this system depend on the action of Ca^{2+}. Since Zn^{2+} antagonizes many Ca^{2+}-mediated processes, it eventually hampers the transport of the secretory granules, as well as the excitability of the lactotroph plasma membrane itself.

Secretory Granules

The secretory granules of the lactotroph cells work as a PRL pool. The presence of Zn^{2+} has also been detected in these granules *(28)*. In addition, it has also been demonstrated that this ion has a great inhibitory effect on the PRL release from bovine and rat secretory granules *(29,30)*. The effect of Zn^{2+} may occur both on the proteins of the granule membrane and on the PRL oligomers within these granules, inhibiting thioldisulfide interchange mechanisms *(31)*. Although the matter has not yet been fully settled, some investigators believe that this is the main sphere of action of Zn^{2+} *(32)*.

Membrane Stabilization

Zn^{2+} interacts with some intrinsic components of the cellular plasma membrane. It binds predominantly to ligands containing sulfur, nitrogen, and, to a lesser extent, oxygen. Because of these properties, Zn^{2+} prefers proteins, lipoproteins, and phospholipids. This means that it may bind to SH, COO–, and PO_4^{3-} groups, stabilizing biomembranes and biostructures *(9,33)*. In this respect, the finding that zinc protects the peroxidation of unsatured fatty acid of the phospholipid moiety *(34)* was a fundamental advance. Another good example of its capacity to stabilize membranes is its inhibitory effect on drug-induced histamine release from mastocytes *(33,35)*. This same phenomenon may be proposed to occur in lactotroph cells. Depolarization of pituitary cells with K^+ increased Ca^{2+} uptake *(36)* and, consequently, induced Ca^{2+}-dependent PRL release *(37)*. However, when 50 μ*M* Zn^{2+} was added to the perifused medium containing dispersed female rat pituitary cells, Zn^{2+} inhibited prolactin secretion stimulated by 50 μ*M* K^+ *(38)*.

PRL Peripheral Receptors

The first step toward hormonal action is binding to target structures through receptors. The hormone–receptor interaction may be influenced

by many factors, such as ion strength, pH, and temperature *(39)*. However, Zn^{2+} has been shown to be a powerful peripheral PRL inhibitor through a purely competitive mechanism. This has been demonstrated in prostate *(40)*, ovarian, and adrenal tissue *(41)*.

Lactotroph Cell-Surface Receptors

Several receptors at the plasma membrane level have been postulated to function as a gate to transmit the information to the intracellular space, and the structural and functional integrity of many of them is maintained by Zn^{2+} *(9)*. Thyrotropin-releasing hormone (TRH) plays the role of a positive stimulus, and dopamine (DA) acts as a negative stimulus in the neural control of PRL. The action of TRH on lactotroph cells involves only intracellular Ca^{2+} mobilization *(42)*. When Zn^{2+} is related to this hormonal mechanism, two events are observed. The first shows that Zn^{2+} has the property to increase TRH affinity for its synaptic membrane receptors *(43)*. The second shows that this same ion is able to inhibit the effects of TRH on PRL synthesis when perfused in dispersed female rat pituitary cells *(38)*. These same authors demonstrated that 50 μM Zn^{2+} is enough to block the stimulus provided by 10 n*M* TRH, when added to the medium. This phenomenon could be plausibly explained by the competitive role played by Zn^{2+} in intracellular Ca^{2+}.

As for DA, it is known that there are receptors on the plasma membrane and in the cytosol, as well as in the secretory granules of lactotroph cells *(44)*. Zn^{2+} was found in these granules *(28)*. However, the effects of this ion on DA and its receptors remain unknown. Studies undertaken on adenohypophyses of lactating rats have suggested that Zn^{2+} and DA have common mechanisms inhibiting both phases of PRL secretion *(32)*. It is plausible to admit that Zn^{2+} also increases the affinity of this neurotransmissor for its lactotroph receptors. This simultaneous effect of DA would inhibit PRL in the secretory granules *(32)*, as well as affect intracellular cAMP *(45)*.

CLINICAL IMPLICATIONS OF ZINC FOR PRL IN ANIMALS AND HUMANS—IN VITRO STUDIES

There are few reports relating in vivo Zn^{2+} to PRL. In some species, e.g., the rat, it was evidenced that chronic hypozincemia did not increase PRL levels in blood, keeping them within the limits of normality *(46,47)*. However, in humans, this relationship was found to be different. Zinc-deficient alcoholic cirrhotic patients had significantly increased PRL levels *(48)*. Mahajan et al. *(49)* observed in 1985 that patients with chronic renal failure (CRF) presented low blood Zn^{2+} levels and high PRL. When the patients were supplemented with 50 mg Zn^{2+}/d for 18–24 mo, Zn^{2+} and PRL levels returned to normal in all the zinc-treated patients. Hypozincemia and hyperprolactinemia were found in another popula-

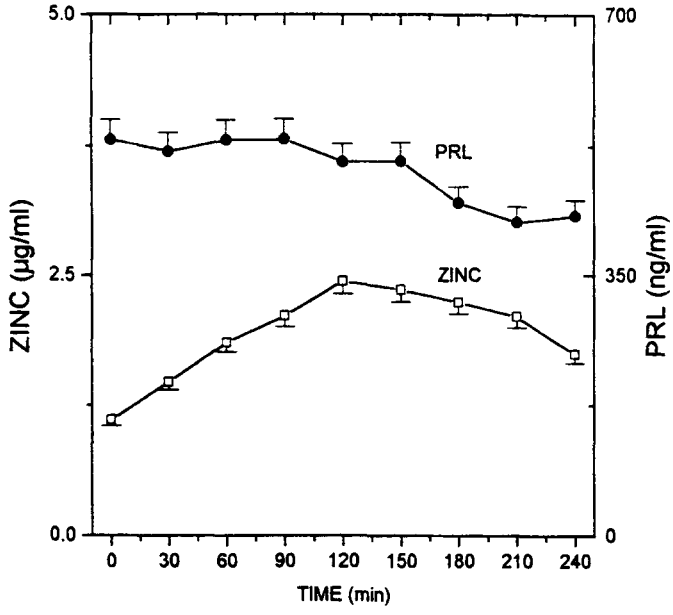

Fig. 2. Plasma zinc and PRL values during the oral zinc tolerance test in eight patients with hyperprolactinemia. Values are expressed as means. A zinc increment was unable to decrease PRL levels to the normal range.

tion of patients with CRF also under basal conditions *(50)*. However, these authors did not achieve the same results obtained by Mahajan et al. *(49)* with Zn^{2+} supplementation, i.e., oral Zn^{2+} administration did not modify basal or TRH-stimulated serum PRL levels. This discrepancy might be owing to the different periods of Zn^{2+} administrations, amount of stress, sleep, and/or the different numbers of individuals. In normal subjects and lambs, chronic or acute Zn^{2+} administration provoked a significant fall in basal plasma PRL *(51–54)*, but this same procedure did not have similar effects on patients with prolactinomas. Zn^{2+} was unable to decrease basal PRL values *(55–57)* and those in TRH-stimulated patients *(55,56)*.

Some comments should be made at this point. The hypozincemia and hyperprolactenemia relationship under basal conditions was also observed by Travaglini et al. *(56)* in 1991 and Madureira *(57)* in 1993 (Figs. 2 and 3). In contrast, the patients reported by Koppelman et al. *(55)* in 1989 presented normal plasma Zn^{2+} levels despite PRL elevation in blood. Also important was the fact observed by Travaglini et al. *(56)* in 1991, while administering bromoergocriptine for 9 mo to 20 patients with microprolactinomas. There was a fall in blood PRL and an elevation in Zn^{2+}, with the inverse relationship between them persisting. In these studies, it was observed that the number of patients with prolactinoma

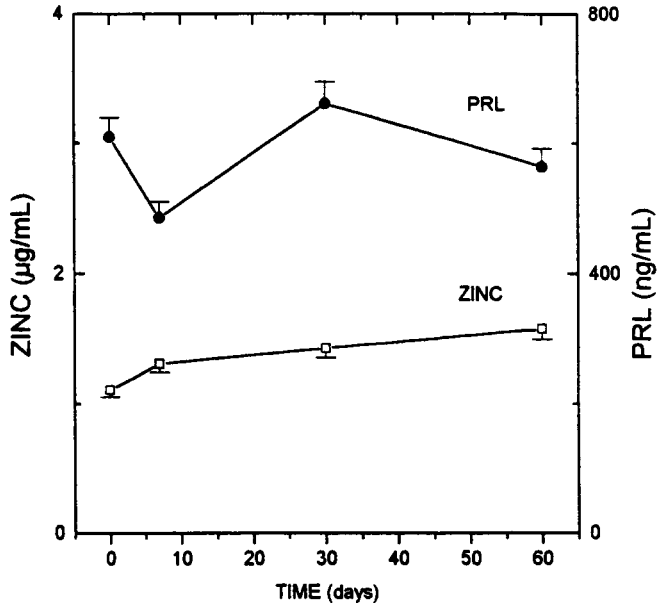

Fig. 3. Plasma zinc and PRL levels on days 0, 7, 30, and 60 after oral administration of 165 mg heptahydrated zinc sulfate. Values are expressed as means. Chronic zinc administration was not effective in decreasing plasma PRL values to the normal range.

and supplemented with Zn^{2+} was small, varying from six to eight. Perhaps this was a limiting methodological factor because Travaglini et al. *(56)* in 1991 detected a serum PRL fall ranging from 95 ± 8 to 75 ± 9 µg/L, a high though not statistically significant decrease.

CONCLUSIONS

On the basis of the above reports, two distinct situations appear, i.e., differences between the results obtained in vitro and in vivo. All the in vitro studies demonstrated the existence of an inverse relationship between Zn^{2+} and PRL. The mechanisms involved in this process have clearly shown that Zn^{2+} interferes physiologically and pharmacologically with practically all the steps involved in the synthesis, storage, release, and peripheral action of PRL. That means that Zn^{2+} interacted with the functions of Ca^{2+} channels, Na^{+}/Ca^{2+} exchangers, Ca^{2+}-calmodulin complex, Ca^{2+}-ATPase, adenylate cyclase, phosphodiesterase, and phospholipase A_2 activities, secretory granules, microtubule excitability, membrane stabilization, and membrane receptors. The only unknown mechanism in this vast process is that involving the action of DA. Regarding the in vivo studies, the inhibitory effects on PRL synthesis and secretion were not

conclusive. Patients with hyperprolactenemia (cirrhosis, CRF, and prolactinoma) had hypozincemia. A subsequent pharmacological elevation of Zn^{2+} caused PRL values to fall only in patients with CRF and in the controls. There was no fall in patients with prolactinoma. In rats, contrary to expectation, Zn^{2+} deficiency in blood did not increase plasma PRL levels. The clinical studies have been conducted on a wide spectrum of patients under nonstandardized conditions, all of which can affect prolactin levels. For this reason, the conclusion is that further studies are necessary to determine the precise role of Zn^{2+} in normal and abnormal regulation of prolactin release in vivo.

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