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Effect of zinc administration on thyrotropin releasing hormone-stimulated prolactinemia in healthy men

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Abstract

Previous *in vitro* studies have demonstrated zinc (Zn^{++}) inhibition of basal and of potassium (K^{+}) or thyrotropin-releasing hormone (TRH)-stimulated prolactin (PRL) secretion, in a selective, reversible, and dose-dependent manner. Thus, Zn^{++} may regulate physiologically pituitary PRL secretion. Furthermore, studies with patients with uremia, cirrhosis or prolactinoma, have shown the coexistence of hypozincemia and hyperprolactinemia and zinc supplementation did not correct hyperprolactinemia in these patients. In normal individuals Zn^{++} administration produced controversial results on PRL secretion. Here, we investigated whether zinc administration affects TRH-stimulated PRL in healthy men. We found that Zn^{++} administration does not change the TRH-stimulated PRL. Therefore, in normal conditions, Zn^{++} does not inhibit TRH-stimulated prolactinemia. In addition, we found that acute increases of blood PRL and TRH do not alter blood Zn^{++} levels.

Introduction

Zn^{++} is virtually present in all tissues, including the brain, hypothalamus and pituitary (Vallee *et al.* 1993). LaBella *et al.* (1973) have shown that Zn^{++} within bovine hypothalamic extracts inhibited in a dose-dependent manner PRL pituitary secretion, which was later confirmed by Login *et al.* (1983). In addition, Judd *et al.* (1984) and Cooper *et al.* (1987) found that Zn^{++} reversibly inhibited PRL secretion stimulated by TRH and K^{+}, respectively. Based on these findings, Koppelman (1988) proposed the hypothesis that Zn^{++} and PRL were involved in a negative feedback regulatory loop, similar to the one that exists for parathyroid hormone (PTH) and calcium (Ca^{++}).

There are few *in vivo* studies about Zn^{++} and PRL relationship, which resulted in controversial results. In rats, hypozincemia was associated to normal (Root *et al.* 1979), high (Mansour *et al.* 1989) or low plasma PRL levels (Hafiez *et al.* 1989). In humans, hypozincemia and hyperprolactinemia were observed in patients with hepatic cirrhosis (Morley *et al.* 1981), uremia (Caticha *et al.* 1996), prolactinoma (Madureira *et al.* 1993, 1999) and idiopathic hyperprolactinemia (Koppelman *et al.* 1989). Mahajan *et al.* (1985) reported that normalization of zincemia in uremic patients supplemented with Zn^{++} reduced their prolactinemia levels. However, these results were contrasted with other studies (Nishi *et al.* 1988; Koppelman *et al.* 1989; Travaglini *et al.* 1989; Bonomini *et al.* 1993).

The objectives of the present study were to investigate the effect of acute Zn^{++} administration on TRH-stimulated PRL secretion, and, inversely, the effect of acute increment of TRH and PRL on zincemia.

Materials and methods

Subjects

We studied fifteen healthy male volunteers, age 23.53 $\pm$ 2.08 years (mean $\pm$ SD), with body mass index (BMI) of 21.84 $\pm$ 3.68 kg/m^2 (mean $\pm$ SD). They were not taking any medications. The study was approved by Ethical Committee of Faculdade de Medicina de Botucatu. Written informed consent was obtained from all volunteers.

Experimental design

Oral zinc group (Figure 1). Two TRH tests (200 μg, bolus, intravenously) were performed: the first one at 0 min and the second at 210 min, in both control (C) and experimental (E) phases, with a seven-day interval between the phases. At 90 min, saline (20 mL) or elemental Zn^{++} (37.5 mg in 20 mL ultra pure water) were orally administrated in C and E phases, respectively. Dose and route administrations are shown in Figure 1. The 2-h interval between Zn^{++} administration and the second TRH was carried out to achieve the highest serum Zn^{++} levels. Five volunteers were evaluated in this group.

Intravenous zinc group (Figure 2). Two TRH tests were also performed in each phase (control and experimental) with a seven-day-interval between phases: the first at 0 min and the second one at 100 min. At 90 min saline (5 mL; control phase) or Zn^{++} (25 mg in 5 mL; experimental phase) was administered. Dose and route administrations are shown in Figure 2. We chose the 25 mg Zn^{++} dose and the 10 min interval in order to achieve highest serum Zn^{++} levels before the second TRH test. Ten volunteers were evaluated in this group.

Drugs, sample collection and analysis

For these experiments, we utilized $ZnSO_4 \cdot 7H_2O$ (Merck, Germany). The capsules and ampoules were prepared at Analytical Chemistry Laboratory of Pharmaceutics Sciences of São Paulo University, Ribeirão Preto, USP, Brazil. TRH was produced by Biophysics Laboratory of São Paulo Hospital, UNIFESP, São Paulo, Capital, Brazil.

After each TRH administration, blood samples were collected at 15 min interval during 90 min. Blood samples for PRL and Zn^{++} measurements, in control and experimental phases of each group, were determined in the same assay. Serum Zn^{++} was measured by atomic absorption spectrophotometry with sensitivity of 0.01 μg/mL; the intra-assay coefficient of variation of 3.5% and references values of 0.7–1.2 μg/mL. Plasma PRL was determined by radioimmunoassay (Cis-Bio International, France), with sensitivity of 4.9 μIU/mL, intra-assay coefficient of variation of 4.2% and references values of 90–370 μIU/mL. Side effects after acute Zn^{++} administration, by oral or venous routes, were not reported. Transitory miccional urgency, dizziness and nausea were reported after TRH infusion.

Statistical analysis

Statistical analysis was performed using repeated measures tests. Basal, relative peaks and area under the curves (AUC) of PRL, after each TRH injections, were compared. Basal and relative peaks of Zn^{++} levels were compared. It was considered $p < 0.05$ as significant. Results are shown as mean $\pm$ SD.

Results

Zincemia

Oral zinc group. Serum Zn^{++} concentration increased significantly from basal levels of 0.83 $\pm$ 0.10 μg/mL to 1.67 $\pm$ 0.35 μg/mL at 240 min and to 1.54 $\pm$ 0.37 μg/mL at 300 min, $p < 0.05$ (Figure 3A).

Intravenous zinc group. Serum Zn^{++} concentration increased significantly from basal levels of 0.83 $\pm$ 0.15 μg/mL to 6.62 $\pm$ 1.22 μg/mL at 100 min and to 3.30 $\pm$ 0.52 μg/mL at 190 min, $p < 0.05$ (Figure 3B). There were no significant differences of Zn^{++} profiles during the control phase in both groups (Figures 3A and 3B).

Prolactinemia

Oral zinc group. The basal plasma PRL values, before the TRH injection and between phases, did not differ. However, in the control phase, the prolactin levels before the second TRH infusion was significantly lower than the first one, $p < 0.001$ (Figure 4A). After oral zinc administration there was no significant difference between relative peaks, as well as, areas under the curves of PRL, after first and second TRH injections, either in control or in experimental phases, and between these both phases (Figure 4A and Table 1).

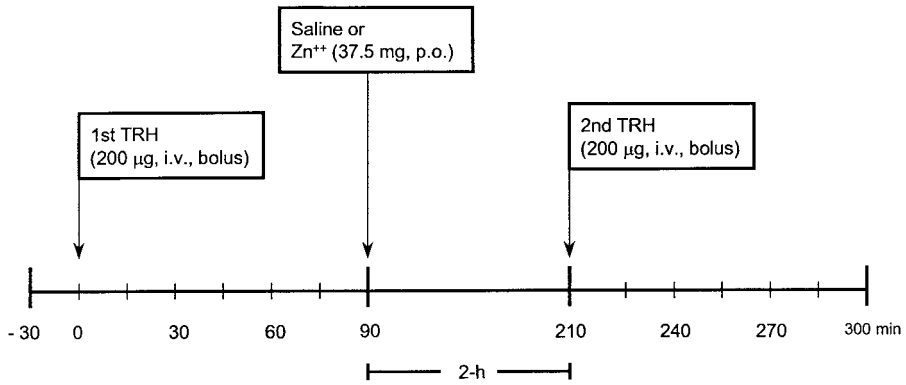

Figure 1. Determination of the effect of oral zinc administration on PRL circulating levels stimulated by consecutive TRH injections.

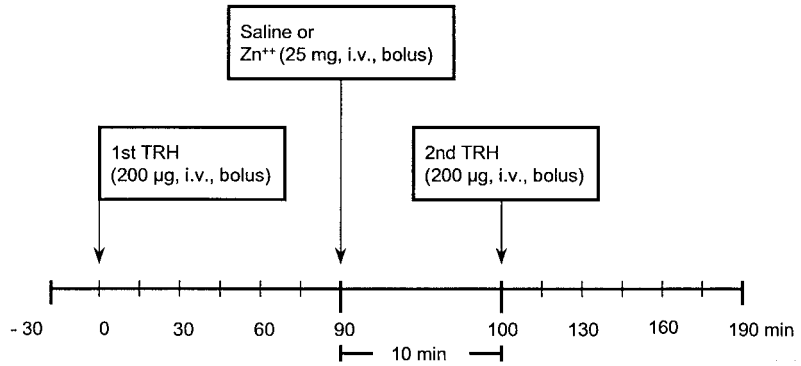

Figure 2. Determination of the effect of intravenous zinc administration on PRL circulating levels stimulated by consecutive TRH injections.

Table 1. Comparison between plasma PRL concentrations at 0 and 120 min, relative peaks and areas under the curves of prolactinemia after acute and consecutive TRH injections in control and experimental phases. At 90 min, placebo or elemental Zn^{++} (37.5 mg) were orally administered

Parameters	Basal			Relative peak			Area under the curve		
Phases	μIU/ml			%			μIU/ml min		
	0 min	210 min	*p*	1st TRH	2nd TRH	*p*	1st TRH	2nd TRH	*p*
Control	188.1 ± 40.9	133.5 ± 43.9	< 0.001*	183.6 ± 101.2	280.9 ± 144.9	0.33	30425 ± 5844	27381 ± 3708	0,12
Experimental	169.5 ± 43.6	128.8 ± 31.4	0.13	230.7 ± 173.8	254.2 ± 92.5	0.80	29999 ± 9057	25427 ± 2397	0,19
p	0.43	0.71	–	0.48	0.26	–	0.63	0.09	–

Values are expressed as mean ± SD.

Table 2. Comparison between plasma PRL concentrations at 0 and 100 min, relative peaks and areas under the curves of prolactinemia after acute and consecutive TRH injections in control and experimental phases. At 90 min, placebo or elemental Zn^{++} (25 mg) were intravenously administered

Parameters	Basal			Relative peak			Area under the curve		
Phases	μIU/ml			%			μIU/ml min		
	0 min	210 min	*p*	1st TRH	2nd TRH	*p*	1st TRH	2nd TRH	*p*
Control	275.0 ± 122.9	297.5 ± 117.4	0.26	265.7 ± 117.7	153.9 ± 96.9	0.007*	50580 ± 20970	37162 ± 15777	0.004*
Experimental	295.6 ± 88.9	300.1± 107.6	0.79	220.7 ± 124.3	119.1 ± 88.3	0.004*	53965 ± 26344	41517 ± 17041	0.02*
p	0.51	0.43	–	0.12	0.09	–	0.32	0.05	–

Values are expressed as mean ± SD.

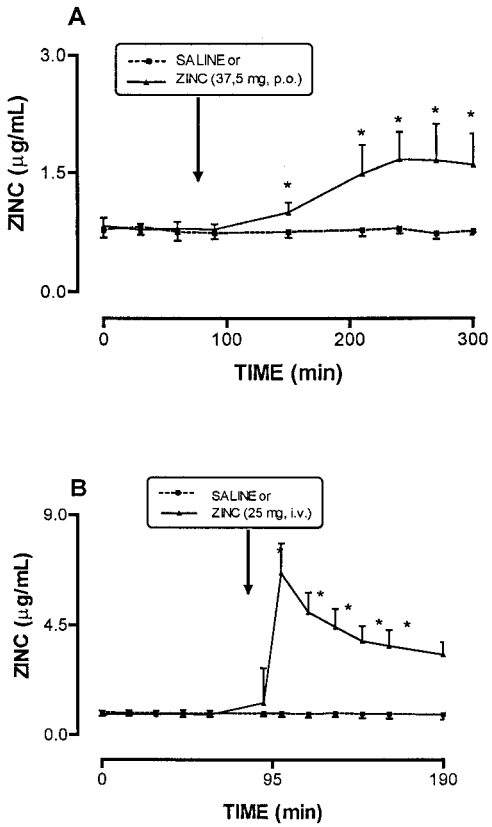

Figure 3. Serum Zn^{++} values during intravenous TRH injections: (A) After oral placebo or elemental Zn^{++} administration or (B) after intravenous placebo or elemental Zn^{++} injection. Values were expressed as mean ± SD.

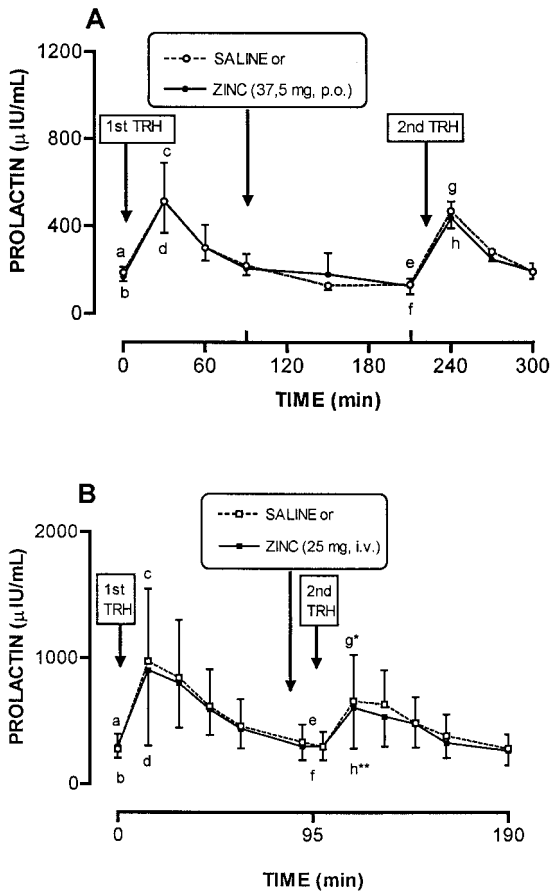

Figure 4. Plasma PRL levels during acute intravenous TRH injections: (A) after oral placebo or elemental Zn^{++} administration. $^{*}p < 0.05 =$ a × e; $p > 0.05 =$ a × b; e × f; c × d; g × h; b × f; c × g; d × h. (B) after intravenous placebo or elemental Zn^{++} administration. *, $^{**}p < 0.05 =$ a × g; d × h; $p > 0.05 =$ a × b; c × d; e × f; g × h; a × e; b × f. Values were expressed as mean ± SD.

Intravenous zinc group. There was no significant difference between PRL levels before the two TRH tests and during control and experimental phases (Figure 4B). The relative peaks and AUC of PRL levels after the second TRH injection were lower than the PRL levels collected after the first TRH test, $p < 0.05$ (Figure 4B and Table 2).

Discussion

The administration of ^{65}Zn was found in the Central Nervous System (CNS), immediately after oral, intravenous or intraperitoneal administration (Vallee & Falchuk 1993; Pérez-Castejón *et al.* 1994; Walsh *et al.* 1994). Moreover, the hypothalamic-pituitary area is not surrounded by the hematoencephalic barrier, which makes it readily permeable to Zn^{++} (Pérez-Castejón *et al.* 1994). Based on these findings, we reasoned that Zn^{++}, as given in the present study, has achieved the hypothalamus and pituitary.

Zn^{++} concentration is maintained within a narrow range due to regulatory mechanisms, which are not completely elucidated. In the intestine, enterocyte metallothyonein (MT) controls Zn^{++} absorption according to the body requirements. On the other hand, Zn^{++} controls MT gene expression (Vallee & Falchuk 1993). This protein has been found in the brain (Walsh *et al.* 1994). We argue that the presence of brain MT may reduce zinc levels in the hypothalamic-pituitary area, similar to what occurs in the intestine (Vallee & Falchuk 1993) and thus explain our findings that high Zn^{++} levels did not change the TRH-stimulated PRL.

Our results are supported by the results of Koppelman *et al.* (1989), who have studied acute Zn^{++} administration on TRH-stimulated prolactinemia in healthy and hyperprolactinemic women. In contrast, we (1989) observed that acute Zn^{++} supplementation can inhibit basal plasma PRL levels. The discrepancies between these studies may be due to methodological differences. In our study, different doses of Zn^{++} were taken into account in the analysis. In addition, both sexes were analyzed as a group, which may complicate the analysis of the results since Zn^{++} and PRL concentrations change during menstrual cycle (Hambidge *et al.* 1986; Aron *et al.* 1997). In contrast to the studies above, we have here studied how Zn^{++} affects plasma PRL levels stimulated by TRH, and no under basal conditions.

The reduction of PRL levels observed after the second TRH injection, in the intravenous zinc group, was not caused by Zn^{++}, because it was also observed in the control phase (Figure 4B). It is likely that the reduction occurred due to the short 10 min interval between the two TRH infusions. This hypothesis is supported by the fact that the same reduction was not observed in the oral zinc group, in which the interval between TRH injections was of 2 h (Figure 4A). The reduction of PRL levels reported here may also be explained by depletion of PRL secretory granules or lactotrope desensitization after the successive TRH injections. Mongioí *et al.* (1983) reported that continuous TRH perfusions did not change PRL levels, but that acute injection of sulpiride, a different secretagogue, raised PRL levels. They attributed these results to lactotrope desensitization and not to depletion of PRL secretory granules.

The paracrine, autocrine and endocrine controls that are involved in PRL secretion may explain differences between *in vitro* and *in vivo* studies. The intercellular communication by chemical mediators was observed in *in vivo* but are difficult to reproduce in *in vitro* experiments (Walsh *et al.* 1994; Habener 1998).

Several studies have shown hormonal effects on Zn^{++} metabolism. It is known that hormones released during infectious or traumatic stresses (cortisol, adrenaline, glucagon, and growth hormone) may lower Zn^{++} levels (Hambidge *et al.*, 1986; Vallee & Falchuk 1993). It is thought that in these situations Zn^{++} may be directed to the intracellular compartment, and thus decrease plasma Zn^{++} levels. However, these authors did not report any PRL effect on Zn^{++} redistribution. Travaglini *et al.* (1991) observed normalization of hypozincemia after normalization of hyperprolactinemia in patients with prolactinoma treated with bromocriptine. They suggested that PRL regulates Zn^{++} turnover. In the present study, an acute increment of blood PRL and TRH levels did not change zincemia during the period of study (Figure 3A and 3B). We argue that the sustained increase of PRL levels in patients with prolactinoma, as observed by Travaglini *et al.* (1991), may have contributed for the discrepancies of results. However, this argument is not supported by the observation of normal zincemia in other studies, which evaluated patients with prolactinoma (Cooper *et al.* 1987; Nishi *et al.* 1988; Koppelman *et al.* 1989).

We conclude that acute Zn^{++} administration does not inhibit TRH-stimulated PRL levels in healthy men, and that acute blood elevation of PRL and TRH levels does not modify serum Zn^{++} concentration during the period of study.

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