Prolactin release in man: influence of cimetidine, thyrotrophin-releasing hormone and acute hypercalcaemia

Sven Röjdmark

Department of Internal Medicine II, Södersjukhuset, 100 64 Stockholm

Abstract. The responsiveness of anterior pituitary lactotrophs and thyrotrophs to cimetidine (Cim) was investigated in healthy volunteers. Four-hundred mg Cim, injected iv, raised the serum prolactin level (Prl) from $14 \pm 2$ to $58 \pm 9$ ng/ml ($P < 0.001$), but left the serum thyrotrophin level (TSH) unaffected. Acute hypercalcaemia, induced by iv infusion of calcium, blunted this Cim-elicited Prl response by $35 \pm 4\%$ ($P < 0.01$). Iv injection of 25 $\mu$g thyrotrophin-releasing hormone (TRH) had similar Prl-releasing potency as 400 mg Cim, and raised the Prl level from $14 \pm 1$ to $51 \pm 6$ ng/ml ($P < 0.001$). In contrast to Cim, TRH also increased the TSH level significantly. Although oral pre-treatment with Cim for 3 days (1000 mg/day) failed to affect the Prl response to TRH in this study, iv injection of the drug more than doubled the above mentioned Prl response to TRH. The TSH response to TRH remained unaffected both by oral and by iv administration of Cim. These results imply that acute changes in serum calcium affect the release pattern of Prl, and that iv administration of Cim may add Prl-releasing power to TRH in healthy individuals.

Although previous studies have shown that calcium ions are necessary for the in vitro secretion of both Prl (Ostlund et al. 1978; Parsons 1969; Wakabayashi et al. 1972) and TSH (Schrey et al. 1978; Vale et al. 1967), a high extracellular level of calcium appears to stabilize bovine anterior pituitary secretory granules containing growth hormone and Prl, and decrease the hormone release from these granules (Lemay et al. 1974). Studies performed in vivo have shown that endogenous hypercalcaemia, caused by Leydig cell tumour transplantation in Fischer rats (Sowers et al. 1980a), and exogenous hypercalcaemia, caused by iv infusion of calcium chloride in anaesthetized Sprague-Dawley rats (Sowers et al. 1980b), inhibit TRH-elicited Prl and TSH responses. Also in man exogenous hypercalcaemia blunts TRH-induced Prl (Röjdmark et al. 1981) and TSH (Röjdmark & Andersson 1982a) responses by a mechanism which appears to involve potentiation of the dopaminergic signal (Röjdmark & Andersson 1982a). Since recent findings in man imply that cimetidine (Cim) induces Prl release indirectly by decreasing the dopaminergic tone (Röjdmark & Andersson 1982b), it is reasonable to assume that exogenous hypercalcaemia inhibits such Cim-induced Prl release. It is also possible that Cim adds Prl-releasing power to TRH, inasmuch as Cim appears to use the dopaminergic route to affect the lactotrophs, whereas TRH influences these cells directly (Frohman 1981).

The present investigation was undertaken in order to find out whether acute hypercalcaemia actually inhibits Cim-induced Prl release in healthy subjects. Another intention was to elucidate whether oral and/or iv administration of Cim potentiates the Prl-releasing effect of TRH in normal individuals.

Material and Methods

Subjects studied

Thirteen healthy subjects (8 women and 5 men) volunteered for the study. They were divided into two groups. Group 1 comprised 6 individuals, aged 22–36 years. Group 2 comprised 7 subjects, aged 19–32 years. No
participant was obese, and all were free of medication. They were informed of the purpose of the study and gave their free consent.

**Study protocol**

The experiments started at 08.00 h after an overnight fast with the participants resting in a supine position. Short plastic catheters were inserted in both antecubital veins. These catheters were kept patent by slow drips of saline. One of the catheters was used for blood sampling; the other for various infusions and injections. All participants were allowed to equilibrate for approximately 30 min before the experiments commenced.

**Group 1.** Two experiments (A and B) were performed on separate days and in random order on each subject in this group. In exp. A, 400 mg Cim (Tagamet®, Smith, Kline & French Ltd., Welwyn Garden City, England) was injected at 90 min, on an iv background infusion of saline, infused between 0 and 150 min. In exp. B, an identical dose of Cim was injected at 90 min, on a background infusion of calcium (Calcium Sandoz®, Sandoz AG, Basel, Switzerland; 3.75 mg/kg·h, infused between 0 and 150 min). Blood samples for serum Prl, TSH, calcium, and blood glucose were obtained before (–10, –1, and 89 min), and after the Cim injection at time intervals outlined in Fig. 1.

**Group 2.** Three experiments (C, D and E) were carried out on separate days and in random order on each subject in this group. In exp. C, 25 μg TRH (Thyrefact®, Hoechst AG, Frankfurt am Main, West Germany) was injected at 0 min. In exp. D, an identical dose of TRH was given after 3 days of oral medication with Cim (1000 mg/day, plus 200 mg on the morning of the 4th day, 1 h before the test). In exp. E, 25 μg TRH and 400 mg Cim were injected together in one of the antecubital veins. Blood samples for Prl, TSH, and glucose were collected before (–10 and –1 min), and after the injections at time points shown in Fig. 2.

**Assay procedures**

Prl was measured by RIA (Prolactin Kit, Biodata Serono Diagnostics, Milano, Italy) as previously reported (Röjdmark et al. 1981). RIA was also used for measuring TSH (TSH Kit, Diagnostic Products Corp., Los Angeles, USA). The sensitivity of the assay was 1.0 μU/ml as reported in a previous paper (Röjdmark & Andersson 1982a). Blood glucose was determined enzymatically with a commercial glucose oxidase preparation (Kabi Reagents, Stockholm, Sweden). A flame photometric method was used for measuring calcium.

**Statistics**

Incremental values above basal were first calculated in each participant. Inter-experimental differences were then estimated and statistically evaluated by use of Student’s t-test for paired data.

**Results**

In exp. A, iv infusion of saline had no significant effect on the basal levels of Prl (14 ± 2 ng/ml), TSH (1.0 ± 0.0 μU/ml), glucose (4.2 ± 0.1 mmol/l), or calcium (2.16 ± 0.03 mmol/l). IV injection of Cim, however, increased Prl to a maximum of 58 ± 9 ng/ml (P < 0.001; Fig. 1), but left the other parameters unaffected.

In exp. B, iv infusion of calcium raised serum calcium progressively from 2.16 ± 0.01 to 2.76 ± 0.04 mmol/l at 150 min (P < 0.001). The hypercalcaemia per se had no significant influence on the basal TSH (1.0 ± 0.0 μU/ml) or glucose concentrations (4.3 ± 0.2 mmol/l), but caused Prl to fall from

---

**Fig. 1.**

Serum Prl and calcium increments (mean ± SEM) in response to 400 mg Cim, injected iv at 90 min, on an iv background infusion of saline (— — —), and calcium (○—一○), in healthy subjects. *P < 0.05; **P < 0.01; ***P < 0.001.
In exp. D, where the participants were pre-treated with oral Cim, iv TRH induced a Prl increase from 12 ± 1 to 58 ± 5 ng/ml (P < 0.001), and a TSH increase from 1.4 ± 0.2 to 6.6 ± 0.8 μU/ml (P < 0.001). Neither of these responses differed significantly from the corresponding ones obtained in exp. C (Fig. 2). The blood glucose level (4.1 ± 0.1 mmol/l) did not change significantly in this experiment either.

In exp. E, the combined iv administration of TRH + Cim provoked a Prl increase from 15 ± 2 to 110 ± 13 ng/ml (P < 0.001), and a TSH increase from 1.2 ± 0.1 to 7.0 ± 0.9 μU/ml (P < 0.001). The Prl response exceeded the one obtained in exp. C significantly (P < 0.01; Fig. 2). It also exceeded the one obtained in another study, where 400 mg Cim was injected alone in normal subjects, by a factor of 1.9 (Röjdmark & Andersson 1982b). As in exp. C and D, TRH + Cim did not affect the blood glucose concentration (4.1 ± 0.1 mmol/l) in this experiment either.

Discussion

The present investigation confirms previous studies showing that Cim, when administered iv in doses varying from 200 to 400 mg, provokes a significant rise in serum Prl (Burland et al. 1979; Carlson & Ippoliti 1977; Ferrari et al. 1979; Masala et al. 1980; Pontiroli et al. 1981; Rolandi et al. 1979), but leaves serum TSH unaffected (Carlson & Ippoliti 1977). It also demonstrates that acute hypercalcaemia blunts the Prl response, which follows upon a rapid iv injection of 400 mg Cim, by approximately 35%. Although the exact mechanism behind this calcium-induced Prl inhibition is unknown, a few feasible explanations will be considered.

1. It is possible that acute hypercalcaemia, by stabilizing secretory granules (Lemay et al. 1974), and by tightening cytoplasmatic membranes (Manery 1966), blocks hormone release from pituitary cells. In favour of this hypothesis recent in vivo studies show that acute hypercalcaemia not only inhibits hormone secretion from Prl-producing pituitary cells in anaesthetized rats (Sowers et al. 1980b), but also inhibits basal human Prl secretion (Ajlouni & El Khateeb 1981), and blunts TRH-induced Prl (Röjdmark et al. 1981), and TSH release (Röjdmark & Andersson 1982a) in awake
normal subjects. If hypercalcaemia inhibits hormone release from pituitary cells in vivo, it is reasonable to assume that calcium-antagonistic agents, such as verapamil (Singh et al. 1978), may augment hormone release from these cells in response to various secretagogues. Contrary to this assumption, verapamil appears to inhibit TRH-induced TSH release (Barbarino & DeMarinis 1980), and to leave TRH-stimulated Prl release unaffected in normal individuals (Röjdmark et al. 1981). Although this does not necessarily exclude a direct effect of calcium on human lactotrophs, alternative explanations for the inhibitory effect of acute hypercalcaemia on the lactotrophs must be considered.

II. The mechanism underlying Cim-elicited Prl release is presently controversial. A direct stimulatory action on the pituitary itself has been suggested (Bateson et al. 1977; Burland et al. 1978). Another possibility is that Cim influences the lactotrophs indirectly by blocking the dopaminergic signal from the hypothalamus to the anterior pituitary (Bateson et al. 1977). Although in vitro studies, carried out by use of pituitary glands from Wistar rats, have failed to demonstrate an effect of Cim on dopamine receptors (Delitala et al. 1979a), recent studies in man have shown that Cim, under normal conditions, stimulates the release of Prl via a reduced dopaminergic inhibition of the lactotrophs (Röjdmark & Andersson 1982b). Since acute hypercalcaemia appears to influence the dopaminergic signal in the opposite direction (Röjdmark & Andersson 1982a), this may explain why exogenous hypercalcaemia blunts Cim-elicited Prl release in man.

In this study iv administration of 25 μg TRH raised the Prl level from 14 ± 1 to 51 ± 6 ng/ml in 15 min. Oral pre-treatment with Cim for three days did not affect this Prl response significantly. By contrast, 400 mg Cim, administered iv together with TRH, more than doubled the above mentioned TRH-induced Prl response. It is a well known fact that TRH acts on specific TRH receptors on the lactotrophs to release Prl from these cells (Frohman 1981). The Prl response is clearly dose-dependent, showing a progressive increase for TRH doses ranging between 6.25 and 100 μg. A plateau in Prl response occurs above this point (Jacobs et al. 1973). As mentioned above, Cim appears to reduce the dopaminergic tone, and consequently influences the lactotrophs indirectly. If thus TRH and Cim stimulate the lactotrophs along different routes, it is reasonable to assume that Cim, if given together with TRH, adds Prl-releasing power to TRH, provided that a sub maximal Prl-releasing dose of TRH (< 100 μg) is used. This was the case in the present investigation as stated above. However, when other investigators stimulated the Prl release by use of a much higher TRH dose (200 μg), simultaneous iv infusion of Cim, either failed to influence the TRH-induced Prl response (Delitala et al. 1979b), or augmented this response only slightly (Scarpignato et al. 1979). Based on these results it may be concluded that Cim, administered in the form of an iv bolus dose, appears to add Prl-releasing power to TRH only when given together with a small TRH dose, which elicits a submaximal Prl response when given alone.

Acknowledgments

The author gratefully acknowledges the skilful technical assistance of C. Eriksson, K. Lamminpää, A. C. Larsson and C. Nyström.

References


Received on August 23rd, 1982.