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Inhibitory effect of calcium on serum prolactin

Klaus Kruse and Ute Kracht

Departments of Paediatrics, University of Kiel, Schwanenweg 20, D-2300 Kiel, Federal Republic of Germany

Abstract. The effect of calcium (Ca) infusions on serum prolactin (Prl) was studied in normal controls and children with disorders of Ca metabolism: Three patients with secondary hyperparathyroidism (vitamin D deficiency rickets), 2 children with idiopathic hypoparathyroidism, 13 epileptic patients with anticonvulsant drug induced inhibition of calcitonin (CT) secretion and 1 patient with vitamin D resistant rickets with normal CP and low PTH secretion.

Ca induced a significant decline of serum Prl in most subjects which could not be explained by the associated increase of CT or decrease of iPTH. The important role of Ca for the in vitro secretion of dopamine has been established for a long time. It is speculated that the inhibitory effect of Ca infusion on serum Prl may be due to dopamine release from nerve tracts in the hypothalamus.

During the last ten years many physiological and pharmacological factors have been detected to affect human prolactin (Prl) secretion (reviewed by Frantz 1978). Among the most important physiological conditions, all known to be stimulatory to Prl release, are stress, sleep, pregnancy and nursing. Many pharmacological substances were found to be inhibitory or stimulatory to Prl secretion.

Recently a clear-cut increase of plasma Prl during parathyroid hormone infusion (Isaac et al. 1978) and a significant decrease of Prl during calcitonin administration (Isaac et al. 1980) have been demonstrated in man, suggesting that Prl may be involved in calcium metabolism. Since a requirement for calcium (Ca) has been shown for Prl secretion in vitro (Gautvik & Tashjian 1973; Parsons 1970), we studied the influence of Ca on Prl secretion in vivo. This investigation reports an inhibitory effect of Ca infusions on serum Prl in children. This effect is probably not due to the associated changes in circulating calcitonin and parathyroid hormone serum levels.

Reprint requests:
Dr. K. Kruse, Universitäts-Kinderklinik,
Schwanenweg 20, D-2300 Kiel,
Federal Republic of Germany.

Subjects and Methods

Informed consent to the investigations was given by the parents of all patients and controls.

Subjects

Twenty three children were studied (age range 1–16 years), 14 boys and 9 girls. The clinical and laboratory data are presented in Table 1.

The *epileptic children* received long-term treatment with primidone or phenobarbital in combination with other anticonvulsant drugs like valpoic acid, carbamazepine and ethosuximide. Three patients were also treated with phenytoin. All these anticonvulsant drugs have no influence on Prl secretion, since fasting baseline Prl levels and 24-h Prl patterns were found to be normal in epileptic children on treatment with these drugs (Kruse et al. 1981). Serum calcium (Ca) levels were normal or low-normal, the concentrations of immunoreactive parathyroid hormone (iPTH) and phosphate (P) in serum were normal, the alkaline phosphatase activities (AP) were normal or slightly increased. Serum calcitonin (CT) was undetectable in 4 patients (< 40 pg/ml), and measurable in the others.

Table 1.
Clinical and laboratory data of epileptic children, patients with disorders of caclium metabolism and controls.

subjects	age (year)	sex	serum levels				
			Ca (mmol/l) (2.10–2.60)*	*P* (mmol/l) (1.3–1.9)	AP (U/l) (150–525)	iPTH (pmol/l) (<0.8–5.0)	CT (pg/ml) (<40–430)
epileptics							
1	15	m	2.32	1.69	333	1.6	<40
2	14	m	2.49	1.44	286	4.3	<40
3	14	f	2.58	1.29	240	2.3	50
4	11	m	2.51	1.56	813	2.8	290
5	16	m	2.35	1.27	408	3.4	390
6	16	m	2.29	1.26	185	3.4	70
7	13	m	2.29	1.60	437	2.5	120
8	13	f	2.19	1.52	355	3.4	120
9	11	m	2.22	1.63	602	3.0	60
10	12	m	1.98	1.63	468	2.8	100
11	9	f	2.34	1.83	719	2.7	<40
12	13	f	2.27	1.36	521	5.5	140
13	14	f	2.31	1.45	222	1.5	<40
HP							
1	10	m	1.72	2.15	328	<0.8	230
2	8	m	1.80	2.00	338	<0.8	160
VDRR							
1	7	f	2.21	0.89	783	<0.8	210
VDR							
1	1 2/12	m	2.06	0.97	826	13.2	40
2	1 3/12	f	2.20	0.93	3220	8.4	140
3	1 5/12	f	2.03	0.90	917	7.2	40
controls							
1	9	f	2.41	1.47	313	2.0	140
2	5	m	2.25	1.50	359	2.6	120
3	10	m	2.40	1.34	294	1.3	40
4	14	m	2.51	1.36	332	1.9	40

HP = hypoparathyroidism, VDRR = vitamin D resistant rickets, VDR = vitamin deficiency rickets,
* = normal range, Ca = calcium, P = inorganic phosphate, AP = alkaline phosphatase,
iPTH = immunoreactive parathyroid hormone, CT = calcitonin.

Patients with disorders of calcium metabolism. Two brothers with idiopathic hypoparathyroidism (HP) demonstrated hypocalcaemia, hyperphosphataemia and undectable iPTH serum levels. A girl with vitamin D resistant rickets (VDRR) presented with small stature, manifest osteomalacic changes of bone, marked hypophosphataemia, normocalcaemia and low iPTH serum levels at the time of study. Three children with severe clinical and radiologic signs of untreated rickets were diagnosed as having vitamin D deficiency rickets (VDR) because of low 25-hydroxyvitamin D serum levels, secondary hyperparathyroidism, hypophosphataemia and elevated serum AP. The serum creatinine concentrations were normal in all patients and controls.

Study protocol

After an overnight fast an indwelling neddle was placed into the forearm vein of patients and controls and was kept patent by the slow administration of 0.9% saline. Ca was infused at a dose of 0.45 mmol/kg body weight from 0900–1200 h and blood samples were collected 15 and 0 min before and hourly until 1400 h during and after the infusion for the determination of Prl, Ca, iPTH and CT. In the three patients with VDR only two blood samples were taken before and at the end of the infusion (1200 h).

Methods

Ca was determined by atomic absorption spectrophotometry, P (test kit from Harleco, Merz and Dade, Munich, FRG) and AP (Hausamen et al. 1967) photometrically. Serum iPTH was measured by RIA according to Hehrmann et al. (1976), using the antibody S_{478}, which is reactive against carboxy regional fragments and the intact PTH molecule. The intraassay coefficient of variation calculated from 48 determination was 12.4% and the interassay variance was 18.2% (n = 17). Serum CT was assayed by RIA (Raue et al. 1978), employing the test kit of Byk-Mallingrodt (Dietzenbach, FRG). The method is sensitive to about 40 pg/ml, the intra- and interassay-variance being below 10%.

Serum Prl was assayed by a specific double antibody RIA unsing the Serono test kit. One nanogram of Serono antigen corresponds to 32.5 IU/ml of WHO 75/504. Intra- and interassay coefficients of variation were 2.8% and 12.5%, respectively, the sensitivity of the assay being 1 ng/ml.

All samples from each child were investigated in duplicate in the same assay.

Results

As shown in Table 2, the basal Prl serum levels were within the normal range in patients and

Table 2.
Influence of a calcium infusion (0.45 mmol/kg/3h) on serum prolactin in epileptic children, patients with disorders of calcium metabolism and controls.

	serum prolactin (ng/ml)						maximal increase of serum calcium (Δ mmol/l)
	hour after starting the infusion						
	0*	1	2	3	4	5	
Epileptics							
1	5.3	4.2	3.3	2.2	3.3	2.2	0.41
2	7.1	6.4	4.9	3.2	3.6	4.2	0.89
3	6.0	5.1	4.3	2.3	2.4	< 1.0	1.15
4	5.7	3.4	2.7	2.1	2.4	4.4	0.80
5	6.9	6.7	5.7	4.7	5.7	6.0	1.00
6	9.1	9.6	7.8	5.9	5.6	9.5	1.12
7	5.7	6.4	4.2	5.4	3.7	4.5	1.23
8	3.9	3.3	2.8	3.3	2.5	2.3	1.20
9	6.8	3.9	3.2	2.7	2.6	7.0	0.94
10	5.6	5.8	3.8	4.7	3.0	3.8	0.62
11	12.7	6.4	7.8	3.9	5.5	12.6	1.05
12	9.7	5.7	5.7	4.4	7.4	8.9	2.03
13	7.5	7.5	4.6	3.7	4.7	5.0	1.12
HP							
1	9.2	5.9	4.4	3.8	3.9	3.9	0.87
2	8.8	4.2	3.0	2.2	3.4	3.9	1.02
VDRR							
1	5.7	3.1	1.6	1.6	3.2	6.4	0.71
VDR							
1	7.2			2.3			0.73
2	14.2			7.6			0.45
3	16.0			3.0			1.58
controls							
1	4.5	3.4	2.9	3.3	2.2	3.7	0.43
2	13.0	11.3	8.3	11.2	11.2	9.1	0.71
3	12.6	11.0	6.8	5.2	6.9	12.3	0.48
4	7.3	4.5	3.7	2.6	2.5	5.4	0.70

* The mean basal concentration of Prl was determined from two basal samples – except patients with VDR; the normal range for children aged 6–16 years is 2.4–13.5 ng/ml and for children aged 1–2 years 7.0–22.5 ng/ml.

controls. Infusions of Ca induced a decline of Prl in all subjects, with a nadir 2–3 h after starting the infusion (Table 2). Serum Prl thereafter increased again but remained slightly lower than the baseline value in most subjects. In terms of the maximal percentage decrease from baseline (mean ± SD) the Ca induced hormone suppression was 53.5 ± 14.7% in the epileptics, 58.7 and 75% in the patients with HP, 71.9% in the girl with VDRR, 68.1, 46.5 and 81.3% in the children with VDR, 53.0 ± 12.7% in the controls and 56.9 ± 14.6% in all 23 subjects.

The maximal net decrement of all children is shown in Fig. 1.

There was a highly significant positive correlation ($r = 0.84$, $P < 0.001$, $n = 23$) between the baseline Prl concentration and the maximal absolute Prl decrease, expressed in Δ ng/ml (Δ Prl), as shown in Fig. 2.

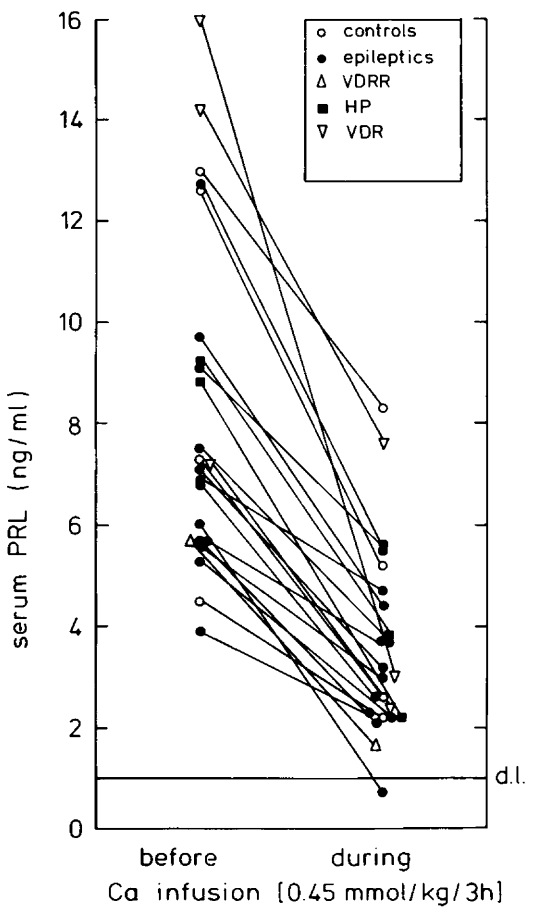

Fig. 1.
Maximal net decrement of serum Prl during Ca infusion in 23 children. d.l. = detection limit.

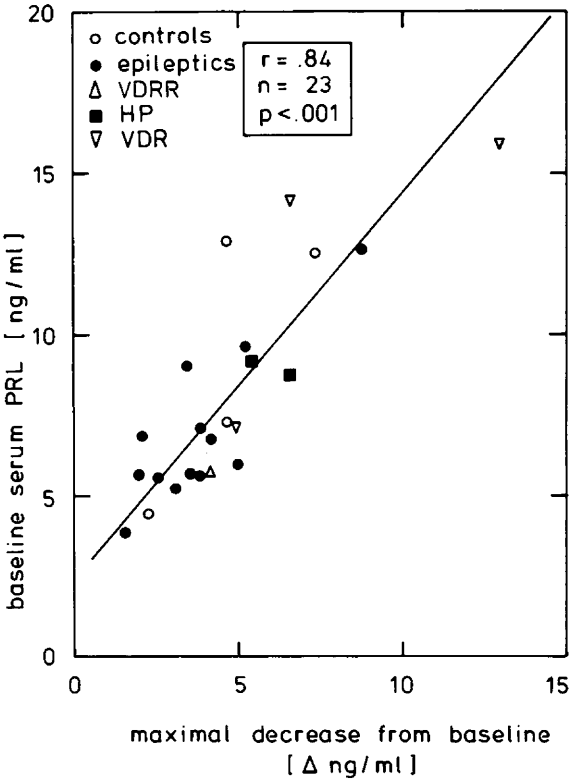

Fig. 2.
Relationship between baseline serum Prl and Ca-induced maximal absolute Prl decrease, expressed in Δ ng/ml, in 23 children.

The maximal serum Ca increase of the 23 children is summarized in Table 2.

The serum iPTH concentration, expressed as the maximal percentage decrease from baseline, declined significantly in the epileptics (56 ± 18%, mean ± SD), the children with VDR (77, 39 and 64%) and the controls (46 ± 16%, mean ± SD) and remained undetactable in the patients with HP and VDR.

Despite an exaggerated increase of serum Ca the CT serum levels did not rise in 6 and increased only slightly (Δ 10–50 pg/ml) in the other 7 epileptic children. A normal rise of CT in relation to the Ca increase was found in the remaining children, the maximal increase being 730 and 290 pg/ml in HP, 130 pg/ml in VDRR, 30, 120 and 140 pg/ml in VDR and 20–140 pg/ml in the controls.

In the 23 children Prl was neither correlated to the maximal increase of Ca ($r = 0.25$) or CT ($r = 0.14$), nor to the maximal decrease og iPTH ($r = 0.22$).

Discussion

The present study demonstrates that Ca is effective in lowering serum Prl levels in children. Our findings are in contrast to in vitro experiments showing stimulatory effects of Ca on Prl release from the pituitary gland (Parsons 1970) or pituitary cells in culture (Gautvik & Tashjian 1973). Therefore the inhibitory effect of Ca in vivo has to be explained by other factors associated with the acute increase of serum Ca. Ca infusions stimulate the secretion of calcitonin (CT) which in consequence may suppress serum Prl (Isaac et al. 1980). Serum CT concentrations increased normally in relation to the serum Ca increment in our controls and patients with disorders of Ca metabolism but not in the epileptic children, supporting our earlier findings that anticonvulsant drugs may inhibit CT secretion (Kruse et al. 1980a,b). However, the Prl suppression by Ca, expressed as a percentage of baseline value, was similar in the epileptic children as in the controls as well as most of the patients with disorders of Ca metabolism. Furthermore there was a lacking correlation between the maximal CT-increase and Prl-decrease in the whole study population, suggesting that CT was of no major influence on serum Prl.

The suppression of serum PTH, a stimulating factor of Prl secretion (Isaac et al. 1978) cannot explain the effect of Ca on serum Prl in our subjects, because (a) the patients with HP and VDRR demonstrated the same Prl decrease as the children with normal or elevated serum iPTH levels and (b) no correlation was found between Prl- and iPTH-suppression in all children.

Prl is the only anterior pituitary hormone which is under predominantly inhibitory control by the hypothalamus. The physiological Prl-inhibition factor (PIF), which is liberated by the hypothalamus as a result of afferent dopaminergic impulses, seems to be dopamine (DA) itself (Clemens & Shaar 1980). DA is secreted into the portal vessels by the tuberoinfundibular neurons and acts directly at the adenohypophysis to inhibit Prl secretion (MacLeod 1976). Many pharmacological agents have been found to inhibit Prl secretion from the pituitary by stimulating DA release or by inhibiting DA re-uptake (for example nomifensine) or by acting on the DA receptors (for example apomorphine and bromocriptine) (reviewed by Clemens & Shaar 1980). The important role of Ca for the in vitro secretion of neurohormones (Douglas 1966; Douglas & Poisner 1964) and neurotransmitters (Burn & Gibbons 1965; Dodge & Rahanimoff 1967; Rubin 1970) has been well established. As shown by Philippu & Heyd (1970) Ca enhances the release of DA from in vitro suspensions of striatal DA-storing vesicles, and additional experiments have demonstrated that Ca seems to be necessary for the release of DA from nigrostriatal nerve terminals (Baldessarini & Kopin 1967; Bustos & Roth 1972; Farnebo & Hamberger 1971).

In connection with these findings we speculate that the inhibitory effect of Ca-infusions on serum Prl may be due to a Ca-induced DA-release from DA-containing nerve tracts in the hypothalamus. This may explain the discrepancy between the stimulatory effect of Ca on Prl secretion in vitro to our study, because DA-releasing agents are known to inhibit Prl release only in vivo (Clemens & Shaar 1980). Ajlouni & Hagen (1975) demonstrated a stimulatory effect of intravenous calcium on growth hormone levels in normal adults, supporting our speculation of Ca-induced DA-release, since growth hormone is stimulated by an increase of the dopaminergic activity.

The degree of the inhibitory effect of Ca on Prl secretion seems to be determined by the basal Prl level, as a significant positive correlation ($r = 0.84$, $P < 0.001$) was found between the baseline Prl levels and the maximal Prl decrease during Ca infusion in our subjects. The same relationship was demonstrated by Judd et al. (1978) and Quigley et al. (1980), investigating the Prl response to DA infusions in normal and hyperprolactinaemic women, where the inhibition of Prl by DA was correlated with basal Prl levels. Furthermore the mean maximal values for Prl suppression by DA infusion were similar in the normal women (56%) and the Prl decrease in our subjects during Ca infusion (56.9%).

The lack of correlation between Prl suppression and maximal serum Ca-increase in our subjects may be due to the demonstrated dependency of the Prl decrease on the basal Prl levels, which possibly reflect the endogenous DA inhibition of Prl secretion (Quigley et al. 1980). Infusions of Ca at different doses in subjects with comparable baseline Prl serum levels are needed to demonstrate a possible concentration-related inhibitory effect of Ca on Prl secretion.

Such studies may also decide wether the Prl inhibition is only an effect of superphysiological serum Ca levels (as in our patients and controls) or

can be already produced by serum Ca levels within the upper normal range. The letter would support the possibility that changes in Ca homeostasis might affect Prl under physiological conditions.

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