The role of parathyroid hormone and calcitonin in magnesium absorption in the rat small intestine

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Summary. We studied duodenal and ileal magnesium (Mg) absorption in intact, parathyroidectomized (PTX), thyroid- (TX) and thyroparathyroidectomized (TPTX) rats with iodine hormones replaced, and, additionally, in PTX rats receiving bovine parathyroid hormone 1-34 and 1,25-dihydroxyvitamin D_3, respectively. Mg absorption was reduced after PTX and TPTX in the duodenum, but not in the ileum, whereas TX had no influence on duodenal or ileal Mg absorption. Both bovine parathyroid hormone 1-34 and 1,25-dihydroxyvitamin D_3 increased Mg absorption in the duodenum and the ileum in PTX rats.

The hormonal regulation of intestinal magnesium (Mg) absorption has not yet been clarified completely. The possible stimulatory influence of vitamin D metabolites is judged to be controversial, while with respect to parathyroid hormone (PTH) and calcitonin (CT) there are only few and contradictory results[2-5]. In this study we measured the Mg absorption in the duodenum and the ileum of intact, thyroidectomized (TX), parathyroidectomized (PTX), and thyroparathyroidectomized (TPTX) rats, and, additionally, in PTX rats receiving bovine PTH 1-34 (b-PTH 1-34) and 1,25-dihydroxyvitamin D_3 (1,25-D_3) respectively. The results indicate that endogenous PTH is involved in the regulation of intestinal Mg absorption.

Material and methods. Male Wistar rats (*Mus rattus*, Brunnthal, FRG), weighing 260-300 g, were fed a commercial chow (No. 1324; Altromin, Lage, FRG) and tap water, both ad libitum.

Three different experimental trials were carried out: a) Evaluation of the effect of TX, PTX and TPTX on intestinal Mg absorption; b) evaluation of the effect of exogenous b-PTH 1-34 and 1,25-D_3 on intestinal Mg absorption in PTX rats; c) influence of the in vivo isolation of an intestinal loop per se on the viability of the intestinal mucosa

a) Four different rat models were used: thyroparathyroid-intact (intact) controls, TX, PTX and TPTX rats[6]. In TX and TPTX rats iodine hormones were replaced by l-thyroxine (600 µg/l drinking water). This dose was shown internally (development of body weight; free fractions of iodine hormones) to achieve a euthyroid state. The effect of surgery upon serum Ca was not monitored prior to the measurement of the Mg absorption, but for the calculation of the results only animals with serum Ca levels in PTX and TPTX lower than 1.5 mM/l, and in TX not different from intact rats were included. 17 days after removal of the glands, intestinal Mg absorption was measured by an in vivo loop technique[7] using the disappearance of radio-Mg (^{28}Mg) out of an in situ isolated duodenal and ileal loop, respectively, within 120 min (see below). In addition, in intact, TX and PTX rats kinetics of the absorption were determined from values measured at 30, 60 and 120 min respectively.

b) In this trial Mg absorption was determined on the 17th day after PTX. On the 15th and 16th days, the rats were injected i.p. with b-PTH 1-34 and 1,25-D_3 or vehicle (=controls), respectively. Doses: b-PTH 1-34: 15 IU/100 g b.wt, vehicle 0.2 ml 0.6% NaCl, containing 0.66% glucose, 0.17% glutathione, 0.4% BSA[8], at 08.00 h, 16.00 h and 22.00 h on each day, on the 17th day at 08.00 h; 1,25-D: 7.5 pM/100 g b.wt, vehicle 25 µl 96% ethanol, at 08.00 h and 20.00 h on each day, on the 17th day at 08.00 h.

Measurement of intestinal Mg absorption: In brief; rats, fasted overnight (water allowed) were anesthetized (sodium-pentobarbital, 50 mg/kg b.wt) and subjected to laparotomy. The pancreatic duct was then ligated and from both the duodenum and the ileum an isolated loop (length

Table 1. Kinetics of disappearance of magnesium-28 (^{28}Mg) out of the in vivo isolated duodenal loop in thyroparathyroid-intact (intact), parathyroidectomized (PTX) and thyroidectomized (TX) rats

	Disappearance of ^{28}Mg[a] cpm × 10^{-3}/g wet wt of loop			cpm × 10^{-3}/g mucosal dry wt		
Surgery	30 min	60 min	120 min	30 min	60 min	120 min
Intact	5.8 ± 0.6 (5)	8.3 ± 0.8 (6)	15.1 ± 1.4 (7)	61 ± 7 (5)	103 ± 12 (6)	163 ± 15 (7)
PTX	1.9 ± 0.1[c] (4)	6.7 ± 1.1 (5)	10.6 ± 0.8[b] (5)	23 ± 1[c] (4)	73 ± 15 (5)	121 ± 4 (4)
TX	5.5 ± 0.8 (5)	7.8 ± 1.0 (5)	13.9 ± 1.3 (5)	63 ± 10 (5)	96 ± 12 (5)	166 ± 12 (5)

[a]See Methods section; ( ), number of observations; values are means ± SEM; [b]$p \leq 0.05$; [c]$p \leq 0.01$ vs intact.

Table 2. Effect of bovine parathyroid hormone 1-34 (b-PTH 1-34) and 1,25-dihydroxyvitamin D_3 (1,25-D_3) on the disappearance of magnesium-28 (^{28}Mg) out of the vivo isolated duodenal and ileal loop in parathyroidectomized (PTX) rats

Disappearance of ^{28}Mg[a]	Vehicle (6)	b-PTH 1-34 (6)	Vehicle (9)	1,25-D_3 (8)
Duodenum				
cpm × 10^{-3}/g wet wt of loop	10.2 ± 1.0	12.3 ± 0.8	9.8 ± 0.8	14.2 ± 1.2[d]
cpm × 10^{-3}/g mucosal dry wt	128 ± 12	159 ± 12[b]	130 ± 12	186 ± 17[d]
Ileum				
cpm × 10^{-3}/g wet wt of loop	13.2 ± 0.7	17.7 ± 1.0[e]	10.9 ± 0.8	16.4 ± 2.3[c]
cpm × 10^{-3}/g mucosal dry wt	153 ± 10	184 ± 12[b]	132 ± 10	231 ± 35[d]

[a]See Methods section; ( ), number of observations; values are means ± SEM; [b]$p \leq 0.05$; [c]$p \leq 0.02$; [d]$p \leq 0.01$; [e]$p \leq 0.001$ vs vehicle resp.

about 6 cm) was prepared, into which 0.5 ml of a solution ($MgCl_2$, 2 mM/l; NaCl, 150 mM/l; KCl, 5 mM/l) with ^{28}Mg (40,000 cpm; Kernforschungsanlage Jülich) was injected. Thereafter the abdomen was closed and the animal kept at 24–26 °C. After 2 h the abdomen was reopened, blood was drawn from the abdominal aorta, the loops were removed in toto and their ^{28}Mg radioactivity was measured. The wet weight of the loops was then determined, and after scraping off the mucosa and drying it for 24 h at 100 °C, mucosal dry weights too.

As a measure for the Mg absorption the disappearance of ^{28}Mg (cpm; corrected for radioactive decay) was calculated. In order to adjust for differences in the size of the loops the disappearance was additionally related to the wet weight of the loops and mucosal dry weights, respectively.

c) Intraintestinally instilled glucose is rapidly absorbed by an active transport mechanism[9]. This process requires the expenditure of energy and can be blocked by metabolic inhibitors such as ouabain, potassium cyanide and 2.4-dinitrophenol[10,11]. In addition, it has been shown that intestinal glucose absorption is impaired when the intestinal mucosa is structurally damaged[12]. Therefore, the disappearance of glucose out of an in situ isolated intestinal loop can be considered as a measure for the viability of the mucosa and the integrity of the transport processes. In this experimental trial we measured it immediately after the isolation of a duodenal loop, and again after 60 and 120 min.

The experimental procedure was that employed for the measurement of the Mg absorption (see above), with modifications: 1. the instilled solution additionally contained glucose (25 mM/l); 2. the duodenal loop was resected already 5 min after the instillation, its content gently expressed, and after spinning down cellular debris, volumes and glucose concentrations were determined; 3. from the volume and glucose concentration the amount of glucose recovered was calculated, and from this the disappearance of glucose (μmoles). This latter was related to the wet weight of the loop and to mucosal dry weight.

Analyses. Serum Ca was measured by atomic absorption photometry (FL 6; Zeiss, Oberkochen, FRG), ^{28}Mg radioactivity with a gamma counter (BF 5300; Berthold, Wildbad, FRG), glucose using the oxidase method (Glucoanalyzer; Beckman, Palo Alto, USA).

Statistics. Results represent means ± SEM. The significance of differences was obtained from application of Student's 2-tailed test.

Results. a) Influence of TX, PTX and TPTX on intestinal Mg absorption. The disappearance of ^{28}MC out of the duodenal loop was significantly reduced after PTX and TPTX, both when related to the wet weight of the loop and the mucosal dry weight (fig.). In contrast there was no effect of PTX and TPTX on ileal Mg absorption. TX had no influence on Mg absorption, either in the duodenum or in the ileum.

The kinetic data of Mg absorption show that in PTX rats the mean duodenal absorption is also reduced after 30 and 60 min. Owing to the small number of observations, however, the decrease is not statistically significant in either case (table 1). In contrast, Mg disappearance in TX rats is identical with that in intact rats after 30 and 60 min also.

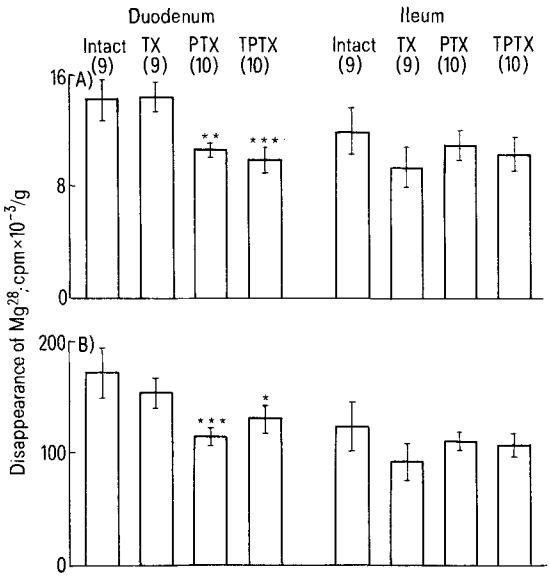
b) Influence of exogenous b-PTH 1–34 and 1,25-D_3 on intestinal Mg absorption in PTX rats. In PTX rats both duodenal and ileal Mg absorption were increased by exogenous b-PTH 1–34 and 1,25-D_3 (table 2).

c) Influence of the in vivo isolation of an intestinal loop per se on the viability of the intestinal mucosa. The disappearance (μmoles/g wet wt of loop) of glucose determined immediately after the in situ isolation of a duodenal loop (at 0 min), and 60 and 120 min later, was of about the same magnitude: 0 min – 16.3 ± SEM 1.0, 60 min – 15.5 ± 2.2, 120 min – 16.5 ± 2.9. Values in μmoles/g mucosal dry weight are (same order): 226 ± 26, 215 ± 31, 241 ± 48.

Discussion. At present the role of PTH and CT in the regulation of intestinal Mg absorption is not clear. Concerning PTH there are contradictory reports. It has been suggested that after TPTX, besides Ca absorption, Mg absorption is also reduced[13]. In agreement with this would be that Mg absorption out of the in vivo isolated duodenal, jejunal and ileal loop in PTX rats was higher after PTH administration[14,15]. But, in contrast to these reports, there are balance studies in rats showing unchanged[16] or even elevated[17,18] Mg absorption after PTX. Concerning CT, so far only very preliminary results are available, showing a decrease in Mg absorption following the administration of CT, but only in one parathyroid-intact and in one PTX pig[13].

In the present investigation we studied the influence of TX, PTX and TPTX as well as of exogenous b-PTH 1–34 and 1,25-D_3 after prior PTX on the intestinal Mg absorption in rats. Mg absorption was determined as the disappearance of ^{28}Mg out of in situ isolated duodenal and ileal loops respectively during 120 min. The long period of time for absorption has the advantage of relatively high and, in consequence, easily observable disappearance values. Moreover, the glucose disappearance measured immediately after isolation of a duodenal loop and 60 and 120 min later shows that mucosal viability is not impaired by the technical manipulations per se. On this basis our results demonstrate that in rats, CT most likely has no effect on small intestinal Mg absorption: when related to the wet weight of the loop or mucosal dry weight the disappearance of radio-Mg out of the duodenal and ileal loops was not changed following TX, a situation hitherto considered as reflecting CT-deficiency. CT immunoreactivity from extrathyroidal sources has been reported[19], but its biological significance is still unknown.

In contrast to endogenous CT endogenous PTH is apparently involved in the regulation of intestinal Mg absorption: after the removal of the parathyroid glands, i.e. in



Disappearance of magnesium-28 (^{28}Mg) out of the in vivo isolated duodenal and ileal loop in thyroparathyroid-intact (intact), thyroidectomized (TX), parathyroidectomized (PTX) and thyroparathyroidectomized (TPTX) rats. *A* Disappearance related to the wet weight of the loop. *B* Disappearance related to mucosal dry weight. (): number of observations. Values are means ± SEM. *$p \leq 0.05$; **$p \leq 0.02$; ***$p \leq 0.001$.

PTX and TPTX rats, duodenal Mg disappearance was significantly reduced - which is in accordance with the present observation and earlier reports[14,15] that in PTX rats Mg absorption is increased by the administration of PTH. However, the effect of PTH seems not to be a direct one, but to be mediated by the intestinally highly active 1,25-D_3, as it has been shown that PTH stimulates the renal synthesis of 1,25-D_3 in vitro[20] and in vivo[21], and as the effect of PTH on intestinal Mg absorption of PTX rats can be mimicked by the administration of 1,25-D_3 (this study). Administration of b-PTH 1-34 and 1,25-D_3 in this study and others[14,15] increases Mg absorption not only in the duodenum but also in the ileum, a bowel segment whose Mg absorption seems not to be controlled by endogenous PTH (fig.). However, this effect may be the expression of supraphysiologic doses of b-PTH 1-34 and 1,25-D_3, and a final interpretation would necessitate the determination of the blood values achieved by the exogenous administration of each substance. Nevertheless, our results support the view of a differential influence of endogenous PTH on small intestinal Mg absorption depending on the anatomical region under study.

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Interrelationship between plasma and ovarian cholesterol in a teleost fish

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Summary. The profiles of plasma and ovarian cholesterol altered similarly as a result of seasonal influence. Fish pituitary extract and LH significantly depleted plasma and ovarian free cholesterol only, esterified cholesterol remaining unaltered. Findings indicated that plasma cholesterol was the primary source of sterol for ovarian steroidogenesis.

Circulatory cholesterol is the potential source for ovarian steroidogenesis and sterol ester storage by the mammalian ovary[2-4]. Considerable insight into the relationship between circulatory and ovarian cholesterol in steroidogenic tissues has also been gained from studies with mammals[3-7]. Again, investigation with mammals have clearly demonstrated the regulatory role of gonadotropin hormone (GtH) in the utilization of cholesterol as a substrate during steroidogenesis[8-10]. For fish, no such information is yet available. Recently we have reported that the dynamics of ovarian free (f) and esterified (e) cholesterol in a teleostean fish differ markedly from that of the mammal[11,12]. Information regarding the relationship between circulatory and ovarian cholesterol is essential in order to comprehend the pattern of cholesterol utilization in ovarian steroidogenesis. Therefore, the present work was designed to follow the profiles of circulatory and ovarian cholesterol at different stages of the reproductive cycle of a seasonally breeding teleost and after treatment with GtH and GnRH.

Materials and methods. To determine the plasma and ovarian cholesterol in different stages of the reproductive cycle, specimens of *Channa punctatus*, a commonly available murrel, were collected from ponds near this University. Prior to sacrifice within 24 h after collection, blood from each fish was drawn from the caudal vein with a heparinized syringe. After sacrifice, the ovary of each fish was dissected out, homogenized and subjected to low speed centrifugation (1000 × g). The supernatant was saved for cholesterol assay.

For the hormonal treatments pituitaries were collected from *C. punctatus* (at the preparatory stage; weight of the freshly collected pituitary being between 350 and 400 µg) and stored in cold acetone. Each pituitary (p) was separately homogenized with 1 ml of 0.6% saline and centrifuged at 1000 × g. The supernatant was injected i.m. Each fish received 1 p/day and the treatment was continued for 7 days. Other treatments included a) ovine LH (NIAMDD-oLH-22) at a dose of 1 µg/100 g/day for 7 days; and b) LH/FSHRH (GnRH, 21-103-DH-NIAMDD) at a dose of 1 µg/100 g, for 10 days. Injections were always given in the morning to the MS 222 (Sandoz Ltd) anesthetized fishes in order to minimize handling and injection shock. The sample size in the experiments varied from 7 to 10.

At the end of each treatment, collection of plasma and ovarian homogenization was done as described above. Determination of the cholesterol content and separation of (f) and (e) cholesterol by TLC was carried out as described by us recently[11,12]. The data were analyzed by Student's t-test[13].