Table 2. Sperm-releasing activity in hypophysectomized frogs treated with vehicle (PDX); hypophysis homogenate (PD), Buserelin (GnRH*) or GnRH* plus an antagonist (GnRHA)

PDX		PDX + GnRH*		PDX + PD		PDX + GnRH* + GnRHA	
n		n		n		n	
+	−	+	−	+	−	+	−
0	10	15	4	10	1	1	9
a		b		b		a	

a vs b, $p < 0.001$; a vs a, N.S.; n = sample size; + and − represent spermiating and non-spermiating animals.

Table 3. Sperm-releasing activity in intact (I) and hypophysectomized (PDX) frogs treated with GnRH* twice (at 0 h and 24 h later)

PDX		I + GnRH*		PDX + GnRH*	
n		n		n	
+	−	+	−	+	−
0	4	4	0	0	4
a		b		a	

a vs b, $p < 0.05$; a vs a, N.S.; n = sample size; + and − represent spermiating and non-spermiating animals.

Table 4. Sperm-releasing activity in hypophysectomized frogs treated with vehicle (PDX), hypophysis homogenate (PD), hCG, GnRH* plus hCG or GnRH* plus PD

PDX		PDX + hCG		PDX + hCG + GnRHA		PDX + PD		PDX + PD + GnRHA	
n		n		n		n		n	
+	−	+	−	+	−	+	−	+	−
0	25	18	9	2	23	27	2	25	2
a		b		a		b		b	

a vs b, $p < 0.001$; a vs a, N.S.; n = sample size; + and − represent spermiating and non-spermiating animals.

frogs locally[5–11]. The finding of multiple sites of action is in agreement with results in male and female rats[4,14]. In particular, it is interesting to note that in the female rat GnRH* induces ovulation via plasminogen activator induction[14]. A similar activity may be responsible for the sperm release from Sertoli cells in frogs.

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Effects of estradiol on parathyroid cell activity

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Summary. Beta-estradiol-3-benzoate provoked an initial centrifugal membrane shift in rat parathyroid cells and, later, enlargement of the compartments concerned with parathyroid hormone secretion, which suggests that estradiol modulates not only transport and release of parathyroid hormone but also the capacity for its synthesis, packaging and storage.
Key words. Estradiol; parathyroid; exocytosis; membrane synthesis; rat.

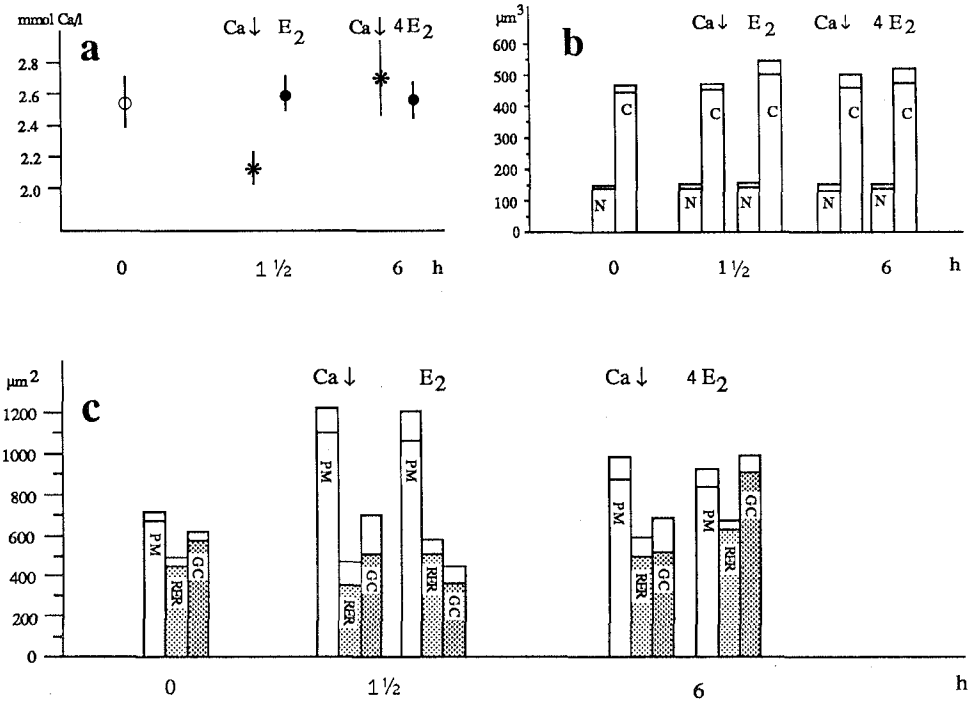
Parathyroid (PT) glands are responsible for maintaining calcium homeostasis by secretion of parathyroid hormone (PTH) which acts on kidneys, intestine and bones[1]. The secretory activity is largely controlled by the serum calcium concentration via a unique feedback mechanism[2]. There are many other secretagogues such as mono-, di- and trivalent cations, catecholamines and hormones, e.g. glucagon, prostaglandins and serotonin,

modifying PTH secretion[3]. Recent studies in vitro suggest that estrogens may modulate, per se, the release of PTH[4,5].

PTH is synthesized on ribosomes of the rough endoplasmic reticulum (RER), packaged in the Golgi complex into secretory granules, transported to the cell periphery, and released by exocytosis[6]. The membrane-bound transport of PTH, and its subsequent release, are associated with a centrifugal membrane shift which was demonstrated morphometrically by a significant increase in the surface area of plasma membrane in rat PT cells 1–1.5 h after stimulation by decreasing the serum calcium concentration[8,9]. In this study, a similar centrifugal membrane shift was estimated by electron microscopic morphometry in PT cells of male rats 1.5 h after application of a single dose of β-estradiol-3-benzoate (2.5 µg/100 g of b. wt) (fig., c). No changes in serum calcium concentrations (fig., a) were recognized by spectro-photometric determination using o-cresolphthalein complexon as indicator[10]. In the next stage of acute stimulation, the centrifugal membrane shift is compensated by enhanced internalization of plasma membrane by endocytosis[11], reaching an equilibrium in PT cells within 2 h after decrement of serum calcium[9] or after estradiol application (data not shown).

The centrifugal membrane shift is considered to be an indicator of enhanced PTH release as a response of PT cells to decreased serum calcium, or to a single dose of estradiol. It has been suggested that estrogens mediate inhibition of bone resorption[12], leading to hypocalcaemia which, in turn, stimulates parathyroid secretory activity. In our experiments, we did not find any changes in serum calcium and, more important, the time course would probably be too short for the development of hypocalcaemia via inhibited bone resorption. Modulation of PTH release by estrogens, therefore, is unlikely to be mediated by serum calcium. This idea was initially put forward because estradiol stimulated PTH release from isolated human[4] and bovine[5] PT tissue as determined by radioimmunoassays.

PTH release may be modulated within minutes[6,13], but modulation of PTH synthesis requires hours[6]. Since PTH is synthesized on ribosomes of the RER, packaged in the Golgi complex, stored and transported in secretory granules, an enlargement of these three compartments is considered to be an indicator of activated PTH synthesis[14]. In a previous study, we examined the effect of a transient serum calcium decrease on PT cell morphology and showed that more than 6 h but less than 12 h are required to induce an apparent increase of the surface



a Serum calcium concentration is decreased 1.5 h after starting EGTA infusion[8] or after administration of sodium phosphate[15] and returns to normal level 6 h after administration of sodium phosphate, Ca↓ Serum calcium concentration remains unchanged 1.5 h after a single i.m. application of estradiol, E_2, and after 4 applications of the same dose (2.5 µg/100 g b. wt) at intervals of 1.5 h, 4 E_2.

b Mean nuclear volume, N, and mean cell volume, C, were estimated by point counting analysis[21] and calculated on the basis of the numerical density obtained by the method of Weibel and Gomez[22] (for equations see ref. 23). Neither parameter is changed in PT cells of rats 1.5 h (n = 5) or 6 h (n = 5) after decrement of serum calcium concentration or after a single (n = 5) or 4 consecutive doses (n = 5) of estradiol compared to untreated controls (n = 8).

c The cell surface area, PM, is increased ($p < 0.001$) 1.5 h after initial decrement of serum calcium concentration and after estradiol application. The cell surface area is also increased ($p < 0.01$) 6 h after initial decrement of serum calcium concentration and after 4-fold treatment with estradiol. PT cells respond with a significant increase in surface area of RER ($p < 0.001$) and Golgi complex ($p < 0.001$) in rats treated with estradiol, 4 E_2, whereas these compartments are not increased at 6 h after initial decrement of serum calcium concentration.

area in membranes of the RER, Golgi complex and secretory granules[15]. In this study, we did not find any quantitative changes in membranes of these compartments in PT cells examined 3, 6 and 12 h after a single application of estradiol (data not shown). However, four consecutive applications of the same dose of estradiol at intervals of 1.5 h provoked an increase of the surface area in plasma membranes by 25%, in RER membranes by 40%, in Golgi membranes by 59% (fig., c), and in membranes of secretory granules by 72% within 6 h after the initial application. It must be borne in mind that the number of secretory granules is low in PT cells of rats and is, therefore, difficult to assess for use as a reliable parameter. Their surface area was estimated to be $58 \pm 8\ \mu m^2$ in controls, $66 \pm 36\ \mu m^2$ 6 h after decrement of serum calcium induced by intraperitoneal application of phosphate, and $100 \pm 19\ \mu m^2$ 6 h after quadruplicate estradiol treatment.

The increase of surface area in plasma membrane, RER, Golgi complex and secretory granules is considered to result from de novo membrane synthesis and implies enhanced capacity for PTH synthesis, packaging, storage and transport. It is thus concluded that both estradiol and decrement of serum calcium concentration may induce de novo membrane synthesis. The time delay in manifestation, however, suggests a different mechanism. Whether estradiol modulates parathyroid activity directly, or whether it acts via a mediator other than calcium, is unknown. One might speculate that there is a mechanism similar to that in other cells[16], namely, that estrogens bind to receptors[17,18] leading to a genomic response which finally promotes synthesis of membrane constituents. This might possibly explain the late event in the response of PT cells to estradiol, the enlargement of membrane compartments, but not the early event, the centrifugal membrane shift.

Estradiol also appears to have rapid non-genomic effects similar to those in MCF-7 breast cancer cells[19] or in neurons in the rat arcuate nucleus where the number of exo-endocytic pits increased within 1 min of perfusion with estradiol[20]. β-Estradiol-3-benzoate prepared as a crystalline suspension in water is rapidly absorbed after intramuscular administration and rapidly metabolized, the half-life time being 50 min. One pulse of estradiol was sufficient to induce an early membrane shift considered to be associated with exocytotic PTH release, whereas at least 4 pulses were required to induce enlargement of the compartments concerned with PTH synthesis, packaging, storage and transport, indicating that duration of signalling is one important factor in the response of PT cells to estradiol. This idea is supported by primary results showing that higher doses of estradiol did not lead to enhanced enlargement of the membraneous compartments whereas prolongation (4 pulses at intervals of 2 h) resulted in markedly increased surface area of RER and Golgi complex. PTH release in tissue exposed to estradiol for 3 h in vitro[4,5], which coincides with the early centrifugal membrane shift, and the increment of membranes concerned with PTH secretion, imply that estrogens play an important role in the modulation of PT activity and, consequently, in the maintenance of calcium homeostasis.

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