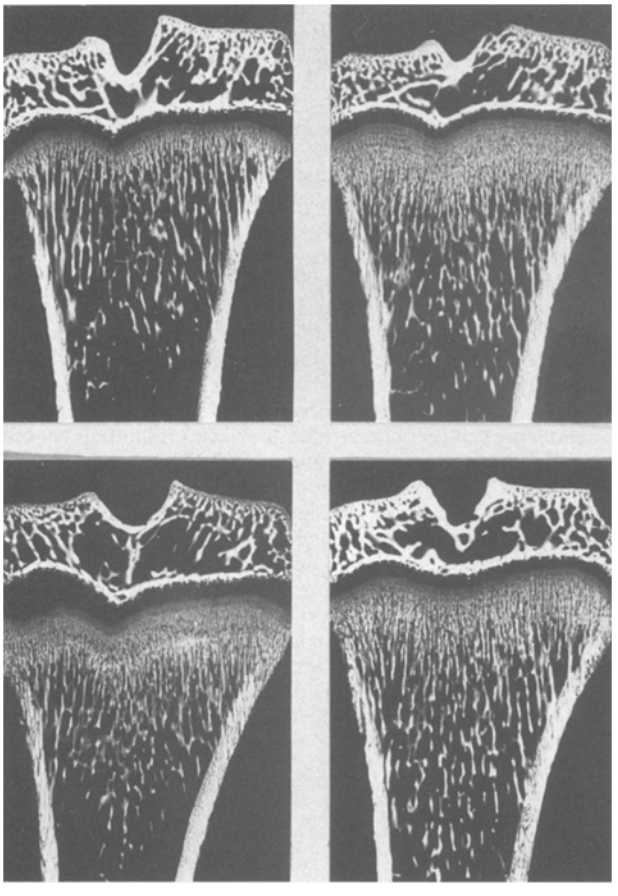
In conclusion, the dihalogenmethylenbisphosphonates Cl_2MBP, and to some extent Br_2MBP and F_2MBP differ from the monohalogen ClMBP and other analogous bisphosphonates without halogens by their stronger potency for increasing the alkaline phosphatase activity and the fatty acid oxidation of calvaria cells in culture. The three dihalogenbisphosphonates show similar activity in inhibiting bone resorption. However, ClMBP and other compounds such as MBP or HMBP, although they are also inhibitors of bone resorption[5,27], show no such cellular effect. There is therefore no general relation between the two processes as regards the effects of bisphosphonates. Thus, the parameters measured may play no role in the effect of bisphosphonates on resorption, or these compounds may act through more than one mechanism, making such a comparison only relatively meaningful.



Contact microradiographs of the proximal tibia in rats. Top left: Control, treated with NaCl; top right: Cl_2MBP; left bottom: Br_2MBP; right bottom: F_2MBP. All bisphosphonates were given for 7 days at a s.c. dose of 161 μmol/kg b. wt/day.

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Studies on the modulation of the desensitization of the pituitary gland by luteinizing hormone-releasing hormone in the ovariectomized rat

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Summary. In ovariectomized rats the desensitization of the LH cells in vivo, which develops during constant rate infusion of LHRH, 1) does not depend on a concomitant depletion of the pituitary LH stores, 2) proceeds normally when the hypothalamo-pituitary connection has been severed and 3) is a process in which LH itself is not involved.
Key words. Luteinizing hormone release; luteinizing hormone-releasing hormone; ovariectomized rat; desensitization; pituitary stalk section; pituitary autotransplantation; human chorionic gonadotropin.

Luteinizing hormone-releasing hormone (LHRH) can both in vivo and in vitro lower the responsiveness of its target cells in the pituitary gland[1,2]. It has been suggested that downregulation by LHRH of the number of its receptors is the biochemical basis of this phenomenon of 'desensitization'[3]. However, other factors can also modulate or even induce the process of desensitization. It has for instance been shown that administration of estradiol during infusion of LHRH induces desensitization[4] almost at once and that prolactin has a negative effect on the sensitivity of the pituitary gland for LHRH[5]. The present study with ovariectomized rats aims at an investigation of three other factors which might influence the induction of desensitization by LHRH.

It was first investigated whether the time of onset of desensitization during constant rate infusion of LHRH is related to the extent of depletion of the pituitary LH stores. In another experiment we looked for a modulating influence of the hypothalamus, in particular for a putative LH-release-inhibiting factor (cf. prolactin, growth hormone), although so far no such hypothalamic factor has been isolated. We looked for evidence of such a factor by studying the time course of luteinizing hormone (LH) release during constant rate infusion of LHRH, in rats in which the hypothalamo-pituitary connection had been severed, either by autotransplantation of the gland or by cutting the pituitary stalk. Finally we investigated the possibility of an autofeedback effect of LH on the pituitary gland (such an autofeedback has been shown to exist in rabbits[6]) by measuring the effect of a large dose of the LH-related hormone human chorionic gonadotropin (hCG) on the LH response to a subsequent constant rate infusion of LHRH.

Materials and methods. Locally-bred Wistar rats were ovariectomized bilaterally at the age of 3 months and used for experiments 4–5 weeks later when they weighed 200–250 g. The ovariectomized rat was chosen as the experimental model to preclude possible interference of ovarian hormones with the phenomena under investigation.

LHRH (Beckman Instruments Ltd, Switzerland) was infused at a constant rate into the jugular vein. Blood samples (300 µl) were taken from the carotid artery. Cannulation was performed under light ether anesthesia 2–3 h before the start of the experiments. At the end of the experiments 1 and 2 the pituitary glands were removed and homogenized and LH was extracted in saline. Pituitary extracts and plasma samples were stored at −20 °C until assay.

Plasma LH concentrations and LH contents of pituitary extracts were measured by double antibody radioimmunoassay[7].

The LH-RP-1 standard and LH-I-4 for iodination were donated by the NIADDK and the antibody against LH by Drs Dullaart and Uilenbroek of the Erasmus University at Rotterdam.

Prolactin was also measured by double antibody radioimmunoassay using the method of Kwa and coworkers[8,9]. As reference preparation rPRL-RP-3 was used. The antibody rPRL-S-8 and prolactin for iodination rPRL-I-5 were also gifts of the NIADDK.

LH release during LHRH infusion was judged according to the 'area-under-the-curve' (AUC or integrated LH release) of the LH curves. The AUC (expressed in arbitrary area units; AU) was calculated by dividing the area under the LH curves into right angled trapezes, the areas of which were added. Under the present experimental conditions the AUC is proportional to the quantity of LH released during the infusion period[10].

In experiment 1 the time at which the maximal plasma LH concentration was reached was used as a parameter for the onset of desensitization. Differences in timing between the groups was tested by the Mann-Whitney U-test (with $p < 0.05$). Differences between means were analyzed by the unpaired, two-tailed Student's t-test at a level of significance of 0.05.

Experimental design. Experiment 1. 5 weeks after ovariectomy six groups of rats were infused with LHRH at the rate of either 10.4 (n = 3), 52 (n = 5), 104 (n = 5), 416 (n = 5), 728 (n = 7) or 1040 (n = 6) ng/h for 20 h. To damp hypothalamic LHRH secretion the animals were anesthetized with sodium-phenobarbitone during the LHRH infusion. A first dose of 80 mg/kg was injected i.p. 1 h before the start of the infusion; a second dose of 30 mg/kg was administered 5 h later.

Experiment 2A. Six rats were hypophysectomized 3 weeks after ovariectomy (surgery was performed by a pharyngeal approach and the pituitary gland was gently aspirated). The pituitary glands were subsequently implanted under the kidney capsule.

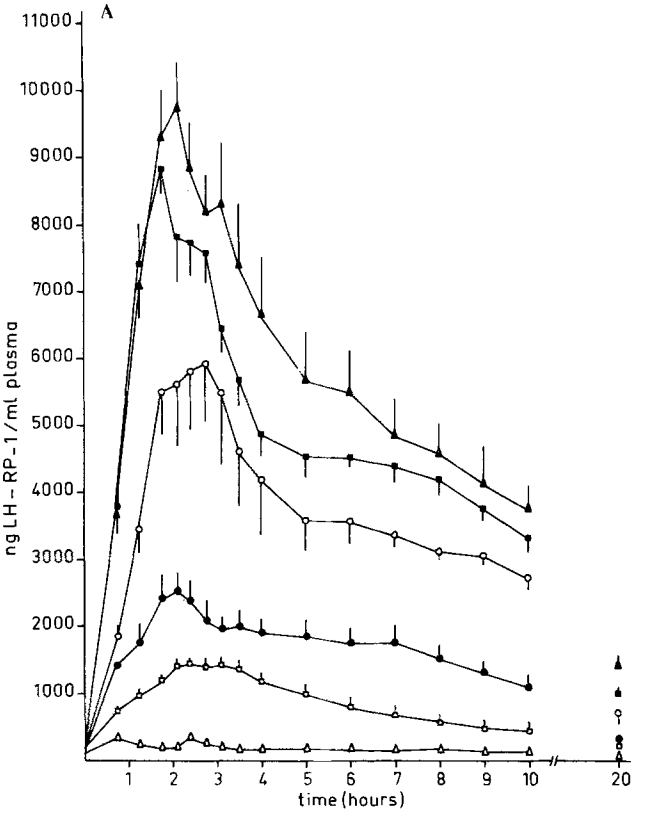

Figure 1. Time courses of the mean plasma LH concentrations during infusion of LHRH at the constant rates of 10.4 (△), 52 (□), 104 (●), 416 (○), 728 (■) or 1040 ng/h (▲) in ovariectomized rats. Means ± SEM.

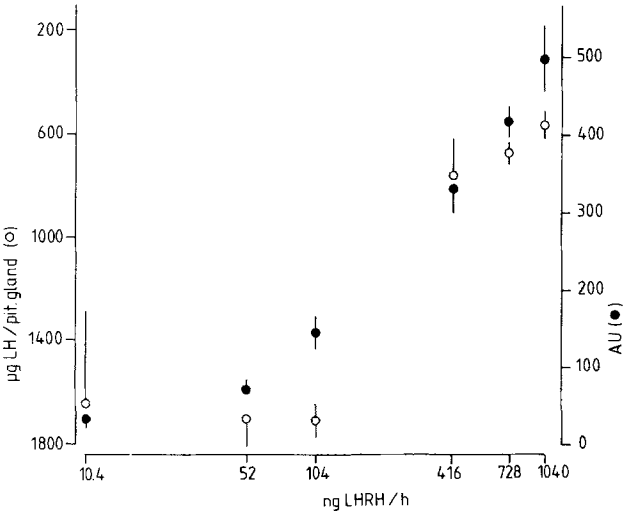

Figure 2. The relationship between LHRH infusion rates and (a) the area under the curve (AUC; expressed in area units, AU) of the LH responses depicted in figure 1 (●) and (b) pituitary LH content at the end of the infusions (○) in ovariectomized rats. Means ± SEM.

At the same time an Alzet minipump (model 2001; Alza Corp., Palo Alto, California, USA) was implanted s.c. The minipump was filled with an LHRH-saline solution, releasing 1 µl or 10 ng LHRH per h, so that the pituitary gland remained exposed to (low) levels of LHRH, which might promote the integrity of the LH cells. Although pulsatile infusion might be a more 'physiological' method[11], we preferred, for practical reasons, the present method of low constant rate infusion of LHRH, as, in ovariectomized rats, it is equivalent to pulsatile infusion: it neither affects the pituitary LHRH-responsiveness (own observations) nor induces LHRH-receptor-downregulation[12]. Five control rats were also operated on, but the pituitary gland was left in situ. These rats also received an Alzet minipump. After surgery all rats had free access to a 3%-glucose-saline solution. 6 days after surgery LHRH was infused for 22 h at the rate of 104 ng/h. At the end of the experiment the sellae of the experimental rats were checked for remnants of the pituitary gland (none were found).

Experiment 2B. 3 weeks after ovariectomy the pituitary gland was exposed in eight rats by a pharyngeal approach, the pituitary stalk cut and a piece of aluminium paper applied between the pituitary gland and the hypothalamus to prevent regeneration of the hypothalamo-pituitary connection. At the same time an Alzet minipump, releasing LHRH at the rate of 10 ng/h, was implanted s.c. Three control rats were also implanted with a minipump, filled with an LHRH solution. After surgery the rats had free access to a 3%-glucose-saline solution. 6 days after surgery LHRH was infused at the rate of 104 ng/h for 22 h. Only those rats which exhibited both a low LH (< 100 ng/ml) and a high prolactin (> 10 ng/ml) plasma concentration just before the LHRH infusion (both parameters are indicative of a severed hypothalamo-pituitary connection), were retained in the experimental group for evaluation. Only four out of the original eight rats thus remained.

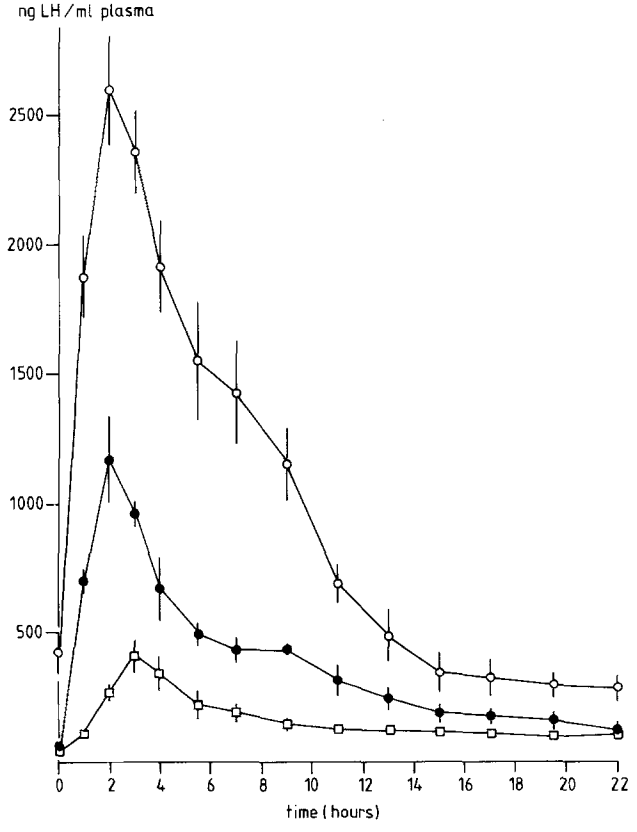

Figure 3. Time courses of the plasma LH concentrations during constant rate infusion of LHRH (104 ng/h) of ovariectomized rats with an autotransplanted pituitary gland (□), ovariectomized rats, of which the pituitary stalk has been sectioned (●) and control rats (○). Means ± SEM.

The two control groups of experiment 2 were statistically not different and they were combined in the table and figure 3.

Experiment 3. 5 weeks after ovariectomy four rats were infused with 700 IU of hCG over a period of 6 h. Four control rats received a saline infusion. 12 h later LHRH was infused at the rate of 104 ng/h for 24 h.

Results and discussion. Infusion of LHRH at a constant rate caused a dose-dependent release of LH, which was associated with a similar dose-dependent depletion of the pituitary LH stores (fig. 2). However, the rise and fall of the LH release with time and hence the onset of desensitization was similar at all doses (fig. 1; cf. Schuiling and Gnodde[13]). Apparently, desensitization occurs irrespective of the amount of LH released or of the extent of depletion of the pituitary LH content. In incubation experiments with (hemi)pituitary glands it was shown that desensitization occurs earlier when the LHRH stimulus is higher[14]. In that in vitro model, however, diffusion of LHRH might influence stimulation: at lower doses LHRH may not readily reach the inner cells of the preparation. This would surely affect the kinetics of LH release.

Isolation of the pituitary gland from the hypothalamus (by autotransplantation or by cutting the pituitary stalk) did not affect the change with time of the release of LH during constant rate infusion of LHRH (fig. 3). This suggests that the hypothalamus does not interfere with the course of the process of desensitization. In both experimental groups, however, the total amounts of released LH were much smaller than in sham-operated rats, but this reduction cannot be considered as desensitization, as it is associated with a similarly reduced pituitary LH content (see table; cf. Koiter et al.[15]). Taken together, these data do not suggest the existence of an LH-release-inhibiting factor. The decrease of the LH stores of such isolated pituitary glands may be caused by the stress of the experimental procedures, leading to a severe and prolonged depletion of the pituitary LH content and loss of tissue. Repletion of the LH content of pituitary glands in situ after such a severe depletion (due to stimulation with LHRH) can occur within days[15]. However, autotransplantation of the pituitary gland leads to a sustained loss of gonadotropic functions (synthesis and release) unless the transplant has been placed in the medial basal hypothalamus[16,17]. Furthermore, retransplantation of the gland, which had initially been implanted under the kidney capsule, to its original site near the eminentia mediana induces a restitution of those gonad-

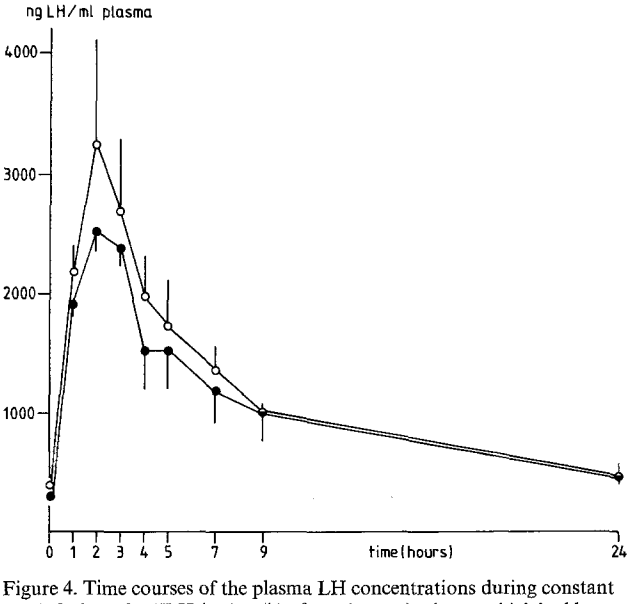

Figure 4. Time courses of the plasma LH concentrations during constant rate infusion of LHRH (104 ng/h) of ovariectomized rats, which had been infused with hCG (700 IU over 6 h; ●——●) or with saline (○——○) 15 h before the start of the LHRH infusion. Means ± SEM.

otropic functions[18]. These observations suggest that some hypothalamic factor(s) are (is) needed for the integrity of the pituitary gonadotropin system[19].

LHRH is a likely candidate for such an hypothalamic factor and indeed it has been found that LHRH can stimulate the synthesis of LH (e.g. Khar and Jutisz[20], Moes et al.[21]). In the present experiments LHRH was supplied to the isolated glands at a low and constant level, that is at a level which induces no desensitization nor downregulation of the number of LHRH receptors in ovariectomized rats[12]. Still, this procedure did not normalize the pituitary LH stores in the isolated pituitary glands during the present relatively short experimental period. As it is also not known whether better results might have been obtained if LHRH had been given in a different fashion (e.g. at a higher concentration or as pulses), it still remains uncertain whether LHRH is involved in maintaining the integrity of the pituitary gland.

Infusion of a large amount of hCG did not affect the LH response to an LHRH infusion 15 h later (fig. 4). However, when a similar large amount of endogenous gonadotropins is released by the pituitary gland, due to stimulation with LHRH, the gland remains desensitized for days[15]. Apparently, a high gonadotropin concentration in the plasma for a prolonged period of time is not sufficient to induce desensitization. Also, exposure of the pituitary gland to rLH just prior to the proestrous LH surge in the female rat did not affect the gonadotropin response to a subsequent LHRH infusion[22]. Thus, it appears that neither hCG nor LH exhibit a negative feedback effect on the hypothalamo-pituitary axis of the female rat; this is in contrast with such observations in the rabbit and in man[6, 23].

Maximal plasma LH concentrations (height; ng/ml) and area under the curve (integrated LH release or AUC; in arbitrary area units, AU) during 22 h of infusion of LHRH at the constant rate of 104 ng/h and pituitary content of LH (Pit, content; µg LH) at the end of the infusion of ovariectomized rats with an isolated pituitary gland (by stalk section: St.; by autotransplantation: Transpl.) and of control rats (C). Means ± SEM

Kind of rats	Number	Height	AUC	Pit. content
C	8	2631 ± 210	208 ± 21	594 ± 136
St.	4	1172 ± 166	86 ± 2	216 ± 25
Transpl.	6	419 ± 65	36 ± 4	99 ± 14

For all columns holds: C > St. > Transpl. ($p < 0.05$).

In conclusion one might infer that the desensitization of the pituitary gland, as caused by LHRH, is not affected by the extent of the concomitant depletion of the LH stores; furthermore, there is no evidence for a hypothalamic factor influencing the kinetics of the process of desensitization, or for any autofeedback effect of secreted LH.

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Changes in patterns of ecdysteroid secretion by the ring gland of *Drosophila* in relation to the sterol composition of the diet

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Summary. Brain-ring glands from *Drosophila* larvae reared on a defined diet containing campesterol (24-methyl-cholesterol) as the major sterol, secreted – in addition to ecdysone – a compound identified previously as a 24-methyl analogue, 20-deoxy-makisterone A. Using ergosterol or cholesterol as the sterol component of the diet, only ecdysone was detectable in cultures of brain-ring glands.
Key words. Ecdysteroid; sterols; *Drosophila melanogaster;* ring gland.

20-hydroxyecdysone is widely regarded as the moulting hormone of insects; ecdysone, synthesized from cholesterol[1] and secreted by the prothoracic glands or, in Diptera, the ring gland, is hydroxylated at C20 by the fat body and other tissues to produce the active moulting hormone[2]. Recent studies on the phytophagous hemipterans *Oncopeltus fasciatus, Dysdercus cingulatus* and *Dysdercus fasciatus* indicate that the moulting hormone in these insects is a C24-methylated analogue of 20-hydroxyecdysone, makisterone A (20-hydroxy-24-methyl-ecdysone)[3–5]. Evidently, campesterol (24-methyl-cholesterol) in the diet is utilized for ecdysteroid production without removal of the methyl group at C24 of the side chain[5].

Of the Diptera, it has been shown that *Sarcophaga* ring glands secrete ecdysone in vitro[6] and in *Drosophila melanogaster*, 20-hydroxyecdysone is apparently the major ecdysteroid at around the time of puparium formation[7]. However, brain-ring glands from *D. melanogaster* larvae reared on a corn meal-yeast diet secrete, in addition to ecdysone, two less polar ecdysteroids (LP1 and LP2), one of which (LP2) is likely to be 20-deoxy-makisterone A (24-methyl-ecdysone)[8]. Here, I present evidence that