three major steps: a) the recognition of glucose as a stimulus and glucose metabolism to generate cofactors such as ATP and NADPH; b) alterations of ionic fluxes across the plasma membrane and membranes of intracellular organelle, in particular resulting in cytosolic accumulation of Ca^{++}; and c) contractility of microtubular-microfilamentous system to translocate insulin granules and their eventual release by exocytosis. The present study suggests that apparently adrenergic receptors and cyclic AMP are not involved in the mechanism by which ethanol inhibits insulin secretion. However, based upon well documented studies on the metabolic effects of ethanol [24], it is conceivable that ethanol may interfere with insulin release by its action at other sites of the sequence of events involved in insulin release.

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Comparison of the time courses of luteinizing hormone (LH) secretion rates during continuous stimulation by LH-releasing hormone (LH–RH) in vivo and in vitro

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Summary. The patterns of LH secretion during constant stimulation of the pituitary glands of estradiol-treated ovariectomized rats with a maximally stimulating amount of LH–RH in vivo and in vitro correspond with each other qualitatively and quantitatively. In vitro the changes with time of the LH secretion rate are somewhat retarded, especially the occurrence of desensitization.
Key words. Pituitary gland; luteinizing hormone (LH); luteinizing hormone-releasing hormone (LH–RH); LH–RH releasing activity in vivo vs in vitro.

Biochemical studies of the processes which are activated by stimulation of the pituitary gland with LH–RH are mostly performed in vitro[1,2]. The ultimate result of the activation of those processes, the secretion of LH, has a very characteristic pattern which, however, varies according to the experimental design (hemi-pituitary glands, dissociated pituitary cells) used[2–6], and depends on the endocrine state (e.g. intact, gonadectomized, hormone-treated) of the animal from which the pituitary glands have been collected[7–10]. From the cited literature it also appeared that the patterns of LH secretion in vivo are often different from those observed in vitro. Consequently, the close relationship between the biochemical processes studied in vitro and the pattern of LH secretion in vivo remains unclear. In the present study, a first step was made towards clarifying that relationship by investigating the kinetics of LH secretion rates during continuous stimulation with LH–RH both in vitro, by incubating hemi-pituitary glands with constant amounts of LH–RH, and in vivo, by infusing LH–RH at a constant rate.

Materials and methods. Animals. Adult female rats from the Wistar-derived colony kept in the Department of Pharmacology in Leiden were used. They were allowed free access to food and water in an animal room illuminated from 05.00 to 19.00 h, at a constant temperature of 22 °C. The animals were ovariectomized (OVX) irrespective of the stage of the ovarian cycle and used 14 days afterwards; at that time they weighed about 200 g. For methodological reasons (increased responsiveness of the pituitary glands to LH–RH) the animals were s.c. injected with estradiol-17β-benzoate (OB; Organon, Oss, The Netherlands; 7 µg in 0.2 ml of arachis oil) 24 h before the start of the experiment.

Infusions. A maximally active amount of LH–RH[11] (Beckman, Geneva, Switzerland) was infused at a constant rate of 1000 ng/h via a cannula inserted into the right jugular vein. Blood samples were taken through a cannula inserted into the right carotid artery. One hour before the start of the infusion and, if relevant 4 h later, the animals received (an) i.p. injection(s) of sodium phenobarbitone (80 mg/kg b.wt) in order to suppress endogenous LH–RH release.

Incubations. The animals were killed by decapitation. Each anterior pituitary gland was halved and placed in an incubation flask containing medium TC 199 (Boehringer, Mannheim, W. Germany). They were preincubated in the same medium until 20 min following decapitation. Then the medium was replaced by 1 ml medium with or without a maximally active concentration of LH–RH[7,8] (1000 ng/ml) and a 4-h incubation followed. The incubations were carried out at 37 °C under continuous shaking in an atmosphere of O_2:CO_2 (95%:5%).

Assay of LH. The assay used was essentially the same as that described by Welschen et al.[12]. Specific anti-ovine LH was a generous gift from Drs J. Dullaart and J.Th.J. Uilenbroek

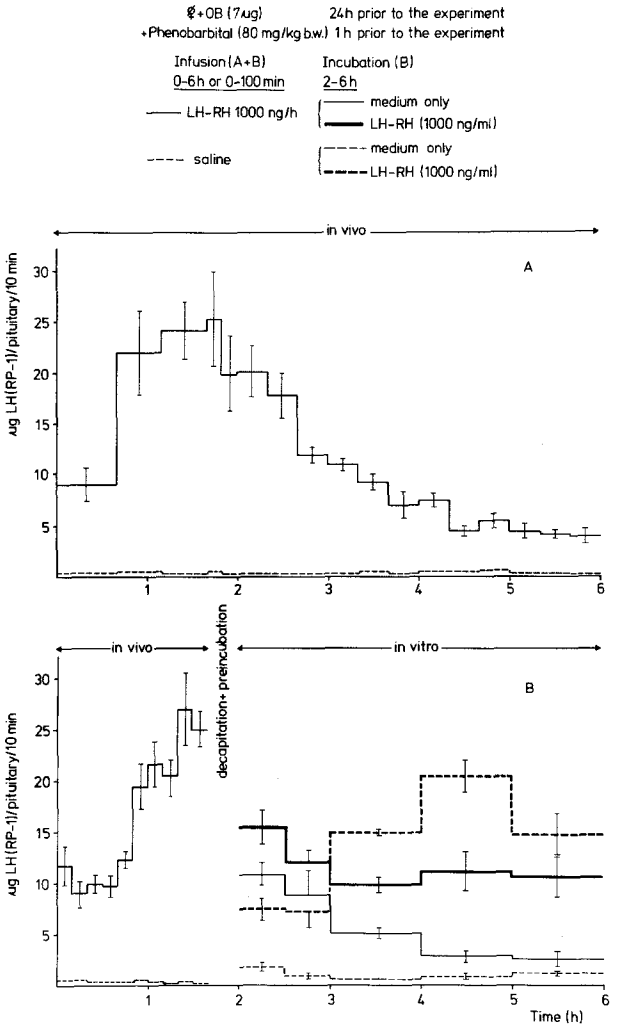

Figure 1. Patterns of LH secretion rates in vivo and in vitro. The results are expressed as µg LH–RP-1/pituitary gland/10 min (± SEM; N = 4). The length of the horizontal lines indicates the interval between two subsequent samples.

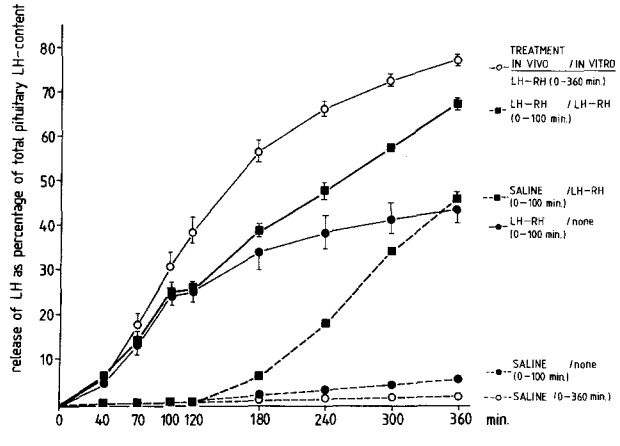

Figure 2. A summary of LH release expressed as the percentage (±SEM) of total LH content (i.e. residual pituitary LH content at the end of the experiment plus the total amount of LH released). Data obtained from the same experiment as described in figure 1.

(Erasmus University, Rotterdam, The Netherlands). Rat LH–I-6 and rat LH–RP-1, both kindly provided by Dr A. F. Parlow and the NIAMDD, were used for iodination and as a standard preparation respectively. The results of the estimations are expressed in terms of this standard ± SEM (N = 4). Samples of plasma, of medium and of saline extracted pituitary tissue were stored frozen until required for assay of LH. The concentrations of LH measured in the media were corrected for changes in volume due to sampling. The LH secretion rates in vivo were calculated according to the procedure described by Koiter et al.[13] using an elimination constant of 0.0462 (half-life of LH: 15 min).

Statistical analyses. Statistical comparisons were made by analysis of variance and then by Duncan's multiple comparison test[14]. A difference was considered to be significant when analysis of variance showed significant heterogeneity for the whole group, and the multiple comparison test gave a value of $p < 0.05$ for the two groups concerned.

Experimental design. The experiment was carried out on 4 separate days with 1-week intervals. On each day, 6 different treatments were performed in duplicate and each treatment was repeated on one of the other days in order to allow direct comparison between all groups. The following experimental series were carried out: 0–6 h infusions with either saline (group 1) or LH–RH (group 2), samples being taken at 0, 40, 70, 100, 110, 120 min and subsequently every 20 min; 0–100 min infusions with either saline, after 20 min followed by incubation in medium only or with LH–RH (groups 3 and 4 respectively), or LH–RH, after 20 min followed by incubation in medium only or with LH–RH (groups 5 and 6 respectively). During the infusion period samples were taken every 10 min and during the subsequent preincubation and incubation periods at 2, 2½, 3, 4, 5 and 6 h, relative to the start of the experiment.

Simultaneously additional control series were carried out to measure the pituitary LH content after each treatment in vivo and in vitro, and that of untreated animals. Stimulation by LH–RH in vivo and/or in vitro caused a severe and significant LH depletion; however, during the experiment the total amount of LH (i.e. total amount released added to that remaining in the glands) did not change significantly. So it was also demonstrated in vitro that basal and LH–RH-stimulated LH release in untreated and saline-infused animals were similar (results not shown).

Results. 1) *LH secretion in vivo.* Figures 1A and 1B show that during infusion with LH–RH (group 2) the initial LH secretion rate is high for about 10 min (phase I), then becomes lower and constant during the next 30 min (phase II), then increases for 50 min (phase III), after which it declines rapidly (phase IV). During saline infusion the LH secretion rate remains low and constant (group 1).

2) *LH secretion in vitro after saline infusion in vivo.* LH–RH-induced LH release in vitro (group 4) is relatively low and constant for 1 h (phases I and II), then increases for the next 2 h (phase III), after which it declines (phase IV) (fig. 1B). Basal release of LH in vitro (group 3) is more or less constant and about twice as high as in vivo (group 1).

3) *LH secretion in vitro after LH–RH infusion in vivo.* Figure 1B shows a high initial LH secretion rate from glands incubated in medium alone (group 5) which decreases until stabilized after about 2 h, at a level twice that of basal LH release in vitro. The LH secretion rate of the pituitary glands in medium containing LH–RH (group 6) is already stabilized after 1 h but at a three times higher level than that of the animals which were continuously exposed to LH–RH in vivo (group 2).

4) *Cumulative LH release in percentage of total content.* Figure 2 presents the amounts of cumulative LH releases expressed as percentages of total LH contents (i.e. total amount of LH released added to that present in the glands at termination of the experiment). The figure demonstrates that after 2 h infusion with LH–RH (group 2) when about 39% of LH is released the rate of LH release declines (see also fig. 1A). In group 6 (LH–RH in

vivo and in vitro) this percentage is reached after 3 h; however, the rate of LH release in vitro does not decline.

Discussion. During continuous stimulation with LH–RH the LH secretion rate in vivo displays the characteristic multiphasic pattern as described before[13]. Similar patterns have been recognized in rats and other species including man[15]. However, these patterns were generally considered to be biphasic: a first phase of low response for 1–2 h (here phases I and II) and a second phase of increased response followed by desensitization (here phases III and IV). The significance of the very early short phase of relatively increased LH release (representing only a very small amount of LH) observed in vivo (phase I) and also in vitro during more frequent sampling[3–5] is not clear. It is unlikely, however, that it represents the first phase of the biphasic response of the human studies described above.

The present study shows that the overall patterns of LH secretion rates in vivo and in vitro fit reasonably well, although there are some differences in the timing of events: the occurrence of phases III and IV is retarded. These differences are probably not the result of poor penetration of LH–RH into the inner area of the glands, since the heights of the phase II responses in vivo and in vitro are similar, indicating an equally efficient induction of LH release. Moreover, pretreatment of the pituitary glands with LH–RH in vivo failed to cause a clear desensitization during subsequent incubation with LH–RH, even if the same percentage of LH had been released as in vivo at the time desensitization had become apparent. Therefore, the delay in occurrence of phases III and IV is rather the result of less efficient processing of LH release under in vitro conditions. A similar phenomenon has been observed in both static incubation[7,8,16] and perifusion[17] experiments with hemi-pituitary glands from OVX rats. Taking these results together, they may point to environmental differences between in vivo and in vitro conditions influencing the efficacy of processing the increment (phase III) and subsequent decrease (phase IV) in the pituitary LH response to LH–RH. Moreover, comparing previous data from Schuiling et al.[18], who also used hemi-pituitary glands from OB-pretreated rats, with the present data shows that perifusion experiments may procure an even better resemblance to LH secretion patterns obtained in vivo, indicating that agents released by the pituitary gland itself interfere in the efficacy of the action of LH–RH.

In conclusion the present results show that the overall patterns of LH secretion rates in vivo and in vitro (using hemi-pituitary glands) fit reasonably well. Allowing for the timing differences, the results indicate that in vitro studies of biochemical aspects of the LH secretion mechanism using hemi-pituitary glands provide results which are pertinent to the in vivo situation.

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Increase in cellular cyclic GMP level by potassium stimulation and its relation to ciliary orientation in *Paramecium*

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Summary. Physiological roles of cyclic GMP in the control of ciliary movement in *Paramecium caudatum* were investigated. We found that 1) an increase in cellular cyclic GMP level was observed in association with recovery from the ciliary reversal produced by K stimulation, and 2) the presence of cyclic GMP inhibited the Ca-induced ciliary reversal in triton-extracted models. These results suggest that cyclic GMP plays a key role in the control of the Ca-mediated ciliary reversal mechanism.
Key words. Cyclic GMP; calcium; *Paramecium;* triton-extracted model; ciliary reversal; excitable membrane.

Recent biochemical studies revealed that cilia of ciliate protozoa contain various proteins and enzymes which interact with Ca^{2+} and cyclic nucleotides, such as Ca/calmodulin-regulated guanylate cyclase[2], adenylate cyclase[3], cGMP and cAMP-dependent protein kinases[4]. Many investigators demonstrated that Ca^{2+} is a mediator of ciliary reversal in ciliate protozoa. Triton-extracted models of *Paramecium* exhibit ciliary reversal when the Ca^{2+} concentration of the reactivation medium is raised above a certain threshold level (10^{-6}–10^{-5} M)[5]. In live specimens of *Paramecium* ciliary reversal always occurs in association with an entry of Ca^{2+} into cilia through activated voltage-dependent Ca channels in the ciliary membrane[6,7]. In order to understand the role of cyclic nucleotides in the Ca-mediated control of ciliary movement in *Paramecium*, we measured the amount of cellular cGMP in relation to ciliary reversal produced by K stimulation.

Materials and methods. Wild-type *Paramecium caudatum* (stock G3, syngen 3, mating type V, trichocyst nondischarge) was cultured at 25 °C in bacterized lettuce infusion. Cells were harvested in the early stationary phase of growth by filtration, and incubated for at least 2 h in an equilibration medium containing 0.5 mM KCl, 1.0 mM $CaCl_2$ and 1.0 mM Tris-HCl (pH 7.2). A 24-well tissue culture cluster (Costar, No. 3524) was used for the stimulation experiments. 0.25 ml of cell suspension containing about 600 cells was introduced into each well of the culture