Differences in luteinizing hormone release stimulated in rat anterior pituitary cells by leukotriene C_4 and by gonadotropin-releasing hormone in vitro

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Summary. Continuous administration of leukotriene C_4 (LTC_4, 10^{-10} M) to superfused rat anterior pituitary cells increased LH release for 40 min only, whereas in a parallel experiment gonadotropin-releasing hormone (GnRH, 10^{-9} M) evoked a continuous increase in hormone secretion. In contrast to GnRH, LTC_4 did not desensitize rat anterior pituitary cells. The secretory action resulting from the administration of LTC_4 (10^{-10} M) was abolished for 40 min after previous stimulation. The results documented the dual action of LTC_4 on LH exocytosis.
Key words. Leukotriene C_4; luteinizing hormone; gonadotropin releasing hormone; desensitization; rat anterior pituitary cells.

Luteinizing hormone (LH) is secreted in the pituitary in a pulsatile manner. This is the physiological mode of LH release[1-3]. Unphysiologically, permanent administration of gonadotropin releasing hormone (GnRH) to rat anterior pituitary cells sustained LH release on an elevated level for a few hours[4]. Rat anterior pituitary cells mainly metabolize free arachidonic acid by oxidative pathways[5], and the release of LH can be attenuated by inhibition of 5-lipoxygenase[6]. These findings initiated further studies which showed that leukotrienes, 5-lipoxygenase metabolites of arachidonic acid, stimulate LH[7] and prolactin[8] release. Arachidonic acid (AA) and its other metabolites also induce LH secretion[9]. Recent reports proposed both leukotrienes and protein kinase C activation as parallel intracellular GnRH signal triggers in rat anterior pituitary cells[4,7,11]. Therefore, the aim of the work was to compare the action of LTC_4 and GnRH on LH secretion.

Materials and methods

A single cell suspension was prepared from rat anterior pituitary glands by trypsinization[10]. The cells were cul-

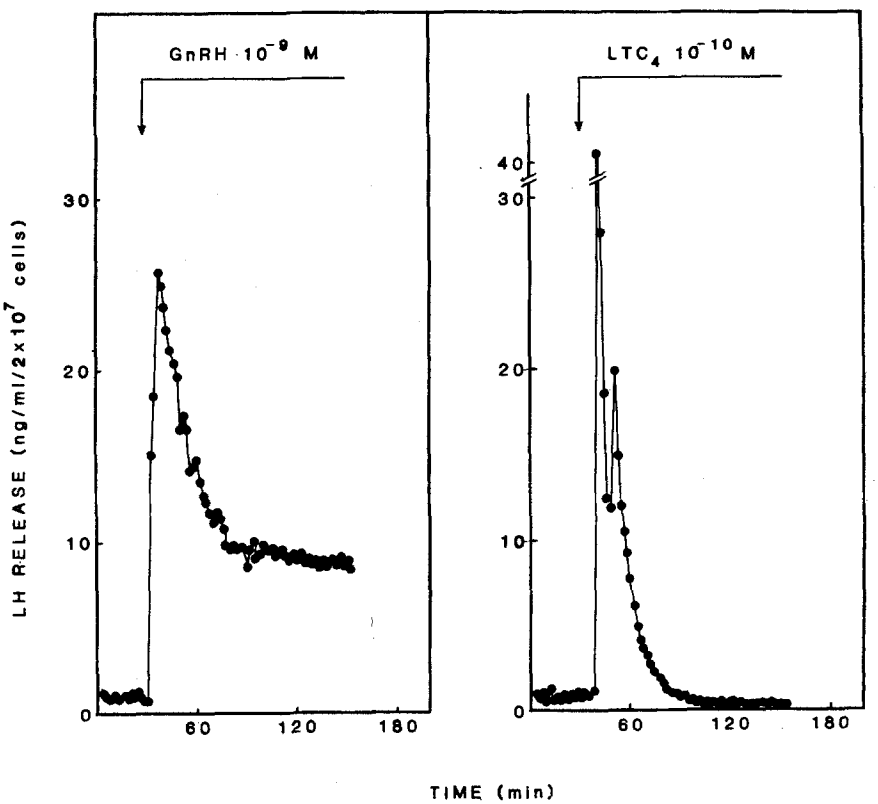

Figure 1. Continuous stimulation of superfused pituicytes by GnRH (10^{-9} M) (left panel) and by leukotriene C_4 (10^{-10} M) (right panel).

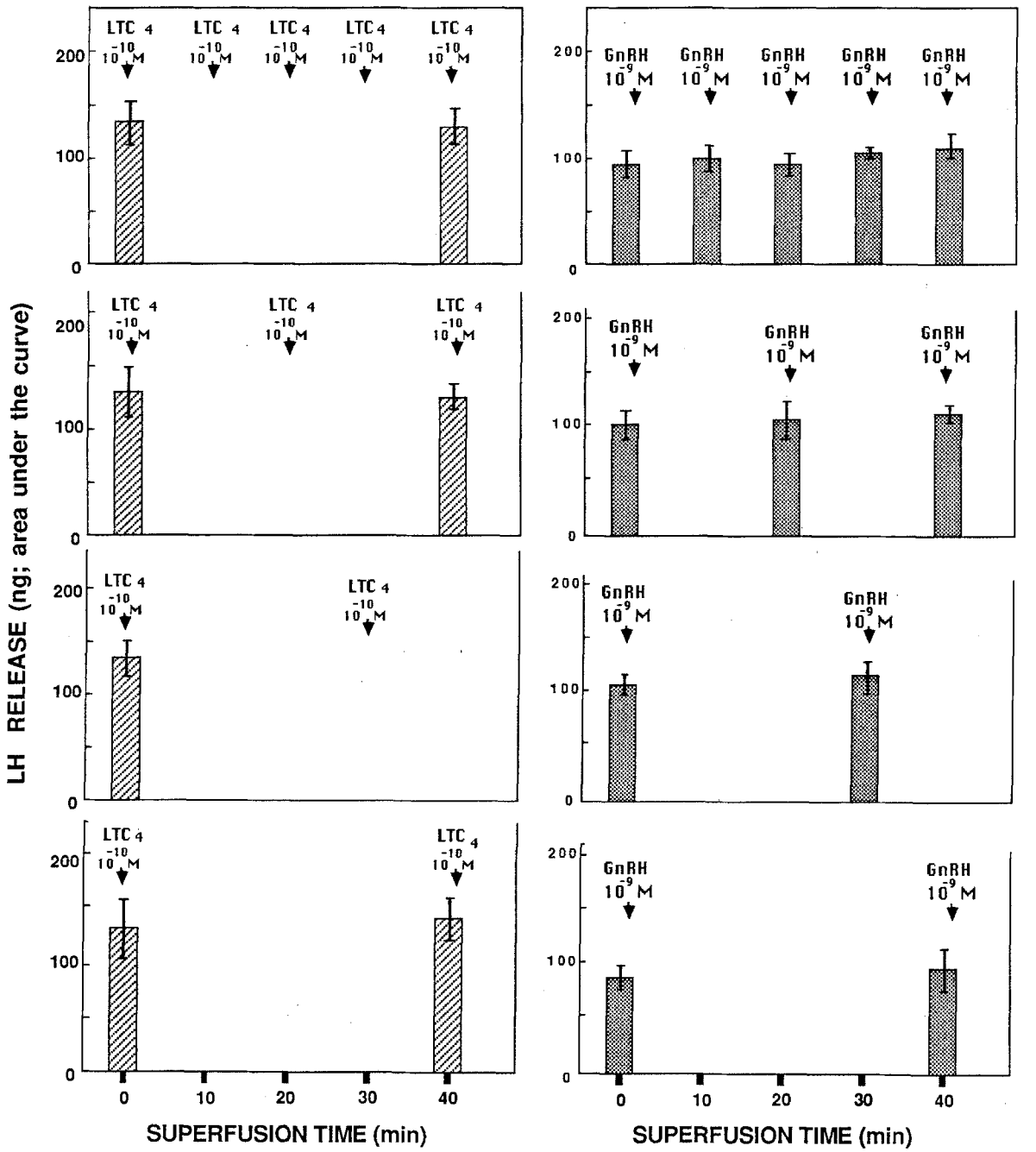

Figure 2. Release of luteinizing hormone from rat pituitary cells in superfusion experiment, stimulated by LTC_4 (10^{-10} M) or by GnRH (10^{-9} M) in different time intervals: 10, 20, 30 and 40 min.

tured on cytodex beads. After 72 h cells were transferred to columns and allowed to recover for 60 min while being superfused at a speed of 0.5 ml/min with medium 199 Earle supplemented by 20 mM Hepes. GnRH (Peninsula Laboratories, San Carlos, CA, USA) was dissolved in distilled water; leukotriene C_4 (LTC_4) was dissolved in ethanol; both agents were further diluted in medium prior to use. Stimulants were added for 4 min to the superfusion medium in ethanol. The final concentration of ethanol was 0.01% approximately. No effect of ethanol 0.01% alone on superfused rat pituitary cells was detected. Fractions were collected every 2 min and assayed for LH by RIA. Desensitization was achieved by addition of GnRH (10^{-9} M) for 24 h on the day prior to the superfusion. In the same manner cells were pretreated by LTC_4 (10^{-10} M). All experiments were repeated five times.

Results

Superfusion of rat anterior pituitary cells (2×10^7 cells/column) in the continuous presence of GnRH (10^{-9} M) resulted in an increase of LH release with peak values at 1–2 min (fig. 1, left panel). LH secretion decreased after

Release of luteinizing hormone in superfusion experiment from rat pituitary cells pretreated for 24 h by stimulants: LTC_4 (10^{-10} M) or GnRH (10^{-9} M).

	Pretreated 24 h by GnRH 10^{-9} M Stimulated by GnRH 10^{-9} M	Pretreated 24 h by LTC_4 10^{-10} M Stimulated by LTC_4 10^{-10} M
Pretreated	15.6 ± 2.8*	138.2 ± 15.6*
Control (non-pretreated)	96.5 ± 12.4*	132.1 + 17.3*

*ng LH, calculated in superfusion experiments as area under the curve.

the peak and remained at an elevated level thereafter. Using the same conditions, the continuous administration of LTC_4 (10^{-10} M) induced the rapid rise of LH in the perifusates with a second smaller peak (fig. 1, right panel). After 40 min gonadotropin levels decreased below basal levels.

Pituitary cells pretreated for 24 h by GnRH (10^{-9} M) were no longer sensitive to stimulation by GnRH in this concentration. In contrast, pretreatment of pituitary cells by LTC_4 (10^{-10} M) did not evoke desensitization to LTC_4 and release of LH by LTC_4 (10^{-10} M) was unchanged (table).

The stimulation of secretory action by LTC_4 (10^{-10} M) was abolished for a short time after previous stimulation. A refractory period of at least 40 min was necessary before LH secretion could again be evoked by LTC_4 (10^{-10} M) (fig. 2, left panel). Stimulation of LH secretion by GnRH was possible as often as necessary (fig. 2, right panel).

Discussion

We used LTC_4 as a stimulant because leukotrienes were proposed as an intracellular GnRH signal trigger in rat anterior pituitary cells [7, 11]. The results obtained confirm that LTC_4 stimulates LH release. However, despite its continuous presence in the medium, this agent is not able to sustain LH secretion on an elevated level. These findings support the proposal of Chang et al., that AA metabolism is responsible for mediating the immediate rapid LH response to GnRH, whereas activation of protein kinase C is necessary for the prolongation of GnRH-induced LH secretion. Our findings furthermore characterize the response curve of rat pituitary cells to LTC_4, demonstrating a double-peak effect, observed previously for GnRH [12] and reflecting an action on two different intracellular pools of gonadotropins. Our previous unpublished results have shown that leukotrienes are produced in rat pituitary cells and involved in the intracellular effector system of GnRH action. Moreover, the observed mode of action of LTC_4 seems to be in conformity with the physiological pulsatile LH release in the organism [1–3].

The experiment performed reveals that it is best to apply the superfusion method in order to test the effect of leukotrienes on LH secretion.

As shown, pretreatment of pituitary cells by LTC_4 does not cause desensitization to this agent. This suggests that LTC_4 is not involved in the mechanism of desensitization.

A second stimulation of secretion by LTC_4 was possible after a 40-min refractory period. No similar results have previously been reported. The possible mechanism of the observed phenomenon is that LTC_4, after a short period of stimulation, inhibits membrane fusion and exocytosis of LH. Possibly two different subtypes of LTC_4 receptors are present, which are coupled with the two different functions of stimulation and inhibition of membrane fusion. The refractory period is necessary to reverse the inhibitory changes in membranes. We have shown earlier that LTC_4 receptors are present in rat anterior pituitary cells (unpublished data). Recently Tsai [13] similarly proposed the existence of two separate subtypes of LTB_4 receptors in human neutrophils mediating the two different functions.

On the other hand, it cannot be fully ruled out that the observed phenomenon of refraction is a kind of desensitization. We must consider the possibility that LTC_4 desensitizes its receptors and attenuates calcium fluxes, as was shown for LTB_4 in human leukemic cells [14], so it cannot stimulate LH secretion. However, the short refraction time and the abolition of the stimulation of hormone secretion by LTC_4 during this time, together with the complete secretory reactivity of the cells after that, are arguments against the hypothesis of desensitization as a possible mechanism for the refraction phenomenon. Moreover, we did not show any intermediate diminished action of LTC_4 which would indicate desensitization.

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1 Veldhuis, J. D., Evans, V. S., Johnson, M. L., Wills, M. R., and Rogol, A. D., J. clin. Endocr. Metab. *62* (1986) 881.
2 Clark, I. J., and Cummins, J. T., Endocrinology *111* (1982) 1737.
3 Gambacciani, M., Liu, J. H., Swartz, W. H., Tueros, V. S., Yen, S. S. C., and Rasmussen, D. D., Neuroendocrinology *45* (1987) 402.
4 Chang, J. P., Graeter, J., and Catt, K. J., Endocrinology *120* (1987) 1837.
5 Vanderhoek, J. K., Kiesel, L., Naor, Z., Bailey, J. M., and Catt, K. J., Prostaglandins Leukotrienes Med. *15* (1984) 375.
6 Naor, Z., Kiesel, L., Vanderhoek, J. K., and Catt, K. J., J. Steroid Biochem. *23* (1985) 711.
7 Kiesel, L., Przylipiak, A., Rabe, T., and Runnebaum, B., Gynec. Endocr. *1* (1987) 25.
8 Przylipiak, A., Kiesel, L., Przylipiak, M., Rabe, T., and Runnebaum, B., Acta endocr. *114*, suppl. 283 (1987) 71.
9 Kiesel, L., Przylipiak, A., Emig, E., Rabe, T., and Runnebaum, B., Life Sci. *40* (1987) 847.
10 Denef, C., Hautekeete, E., DeWolf, A., and Vanderschuerren, B., Endocrinology *103* (1978) 724.
11 Hulting, L., Lindgren, J. A., Hökfelt, T., Eneroth, P., Werner, S., Patrono, C., and Samuelsson, B., Proc. natl Acad. Sci. USA *82* (1985) 3834.
12 Naor, Z., Katikineni, M., Loumaye, E., Garcia Vela, A., Dufau, M. L., and Catt, K. J., Molec. Cell Endocr. *27* (1982) 213.
13 Tsai, B. S., Keith, R. H., Villani-Price, D., Heack, R. A., Bauer, R. F., Leonard, R., Abe, Y., and Nicolaou, K. C., Prostaglandins *37* (1989) 287.
14 Winkler, J. D., Sarau, H. M., Foley, J. J., Mong, S., and Crooke, S. T., J. Pharm. exp. Ther. *246* (1988) 204.

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