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INTERLEUKIN 1 BETA INHIBITION OF TRH-STIMULATED PROLACTIN SECRETION AND PHOSPHOINOSITIDES METABOLISM

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Summary: The effect of interleukin 1 beta on prolactin secretion and on phosphoinositide turnover in anterior pituitary cells was evaluated. Interleukin 1 beta significantly inhibited TRH-stimulated prolactin secretion assessed by the reverse hemolytic plaque assay. In particular, the cytokine reduced the percentage of plaque forming cells, the plaque mean area, the large plaques percentage. TRH-stimulated inositol phosphate production was also significantly inhibited by interleukin 1 beta. This study shows that interleukin 1 beta reduces TRH-induced prolactin secretion through a direct action on pituitary cell, and attenuates the TRH-stimulated phosphoinositide breakdown. This latter effect may suggest that the reduced lactotropes sensitivity to TRH action may be partially due to interleukin 1 beta inhibition of phosphatidylinositol breakdown.

Interleukin 1 beta (IL1), a monokine produced by macrophages and monocytes during the acute phase of the inflammatory response, is involved in a wide range of non immunological activities (1). Recently, the existence of a regulatory loop between immune and neuroendocrine systems has been suggested (2,3). Indeed, cells of the immune system produce neuroendocrine hormones mRNAs, such as that for ACTH, GH, TSH, prolactin (PRL) and also express receptors for these hormones (3). IL1, whose receptors have been found in the brain (4,5) and pituitary (6), seems to be involved in the control of the hypothalamic-hypophyseal-adrenal axis (7). IL1 immunoreactive fibers have been shown in the hypothalamus (8), including the median eminence, suggesting

ABBREVIATIONS: IL1 = Interleukin 1 beta; PRL = prolactin.

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that IL1 may be released directly into the hypophyseal portal vessels affecting anterior pituitary hormones secretion, likely through an interaction with pituitary IL1 receptors (6). However, the data about the influence of IL1 on pituitary hormone secretion are still conflicting. Bernton et al reported that IL1 stimulated GH, LH, TSH, ACTH release from primary cultures of dispersed rat normal anterior pituitary cells, while inhibited PRL secretion (9). In perifusion studies they were unable to reproduce such results on prolactin secretion (10). Other authors failed to observe any change of hormonal secretion in primary culture of anterior pituitary cells (11,12,13). However, we previously showed that IL1 inhibited basal and VIP-stimulated PRL secretion from single lactotropes (14,15,16). The molecular mechanisms whereby IL1 could modulate the secretory processes have not yet been completely clarified. The coupling of IL1 receptors with adenylate cyclase activity (17) and calcium mobilization have been suggested in a PRL-secreting pituitary cell line (235/1)(18). Other authors reported that IL1 affects phosphoinositides turnover in isolated rat pancreatic islets (19,20), while it induces diacylglycerol production in a transformed human T cell leukemia line and in mesangial cells via a novel mechanism, involving the hydrolysis of phosphatidylcholine and phosphatidylethanolamine, respectively (21,22).

In this report we studied IL1 modulation of PRL release, from single lactotropes, in TRH-stimulated conditions, by means of reverse hemolytic plaque assay (RHPA). Since TRH enhances phosphatidylinositol hydrolysis in pituitary (23) and GH3 (24) cells, we evaluated whether IL1 could affect TRH-stimulated PRL release through a modulation of inositol phosphates compounds production.

MATERIALS AND METHODS

- Primary cultures of anterior pituitary cells
Male Wistar rats (200-250 gr.) were decapitated and the anterior pituitary glands quickly removed in sterile conditions and the neurointermediate lobe was discarded (25). The tissue was cut into cubic millimeter fragments and enzymatically dispersed by serial incubations with trypsin (Worthington, Freehold NJ, USA) (2.5mg/ml, 15 min. at 37°C) and pancreatin (2mg/ml, 12 min. at 37°C); the fragments of tissue were further dispersed by mechanical trituration through plastic pipette tips in few mls of SMEM (Gibco). Cell viability was greater than 95% as measured by the trypan blue exclusion test.

- Reverse hemolytic plaque assay (RHPA)
RHPA was performed according to the method described by Leong et al (26). Briefly, after trypsin dispersion, 1 ml pituitary cells (about 500000 cells) was mixed with 1 ml suspension of 12% protein A coated ovine RBC (SCLAVO) and the mixture was introduced in Cunningham chambers, pretreated with poly-L-lysine. One hour later each chamber was incubated with medium (RPMI 1640 + 0.1% BSA + trypsin inhibitor + antibiotics) with antiserum (PRL-Ab diluted 1:100) and test substances for 2 h, followed by incubation with medium plus guinea pig complement (diluted 1:15) for 40-50 minutes. Plated cells were fixed in ice-cold RPMI 1640 with 1% glutaraldehyde . After staining pituitary cells were viewed and counted with the aid of a Leitz Diavert microscope. The number of plaque-forming cells was expressed as a percentage of all cells (randomly counting always 200 cells/slide). Plaque area was measured by a microscope (Zeiss) connected with a computer (Contron) by a videoplan IBAS I system, enumerating 100 randomly chosen plaque areas per slide. The frequency distributions of PRL plaque areas were also drawed in order to show eventual changes in small and large plaque areas percentages.

- Inositol phosphates assay
The anterior pituitary cells previously dispersed were plated in 24 multiwells in HAM F-10 supplemented with 15% of foetal calf serum for 24 hours. The medium was then changed with HAM F-10 containing 0.1% of foetal calf serum and 5uCi/ml of 3H-D-myo-inositol (Amersham) and the cells were incubated for further 36 hours (27). The day of the experiment the cells were washed with Krebs Ringer bicarbonate plus HEPES (NaCl 120mM; KCl 5mM; NaHCO3 2.5mM; MgSO4 1.2mM; KH2PO4 1.2mM; CaCl2 2mM) and incubated in 1 ml of Krebs Ringer bicarbonate containing 10 mM LiCl. The reaction was stopped by addition of 1 ml of ice-cold methanol, the wells were then scraped and the inositol phosphates were extracted by using chloroform, methanol and water (1:1:0.65). Inositol phosphates were separated by using Dowex anion exchange resin (AGX 108 formate form). Inositol phosphates were eluted using 1.0 M ammonium formate and 0.1 M formic acid (28). The experiments were carried out in quadruplicate and repeated at least three times.

- Statistics
The mean of at least three independent experiments for each group of treatment was calculated. The results were expressed as the mean +/- SEM. Statistical analysis was performed by means of ANOVA followed by the Newman and Keuls test. A P value less than 0.05 was considered statistically significant. As the frequency curves of the plaque mean areas were not normally distributed, all of the plaque area data were transformed to log-normal before statistical analysis.

- Chemicals
Aliquots of human recombinant IL1 (1,000,000 UI/ml) were stored at -80° C till use. For inositol phosphate assay IL1 was diluted in Krebs Ringer bicarbonate, while in RHPA experiments it was diluted in RPMI medium.
All the substances, unless otherwise specified, were purchased by Sigma.

RESULTS AND DISCUSSION

In this study we evaluated the effects of IL1 on PRL secretion from single pituitary cell by using the RHPA technique. In particular, we evaluated three parameters: 1) the number of plaque-forming cells, which represent the percentage of PRL-secreting cells; 2) the mean area of the plaques, which is proportional to the amount of the secreted hormone; 3) the frequency distribution of plaque mean area, expressed by the percentages of plaques with individual areas smaller than the mean area (small plaques), and by the percentages of plaques with an individual area larger than the plaque mean area (large plaques).

IL1 did not affect the percentage of PRL secreting cells (plaque forming cells) in basal conditions (control 29+/-1.5 %; IL1 1pM 27+/-2 %). As reported in Fig.1, TRH induced a significant increase in the percentage of PRL secreting cells, due to the TRH acceleration in the rate of plaques formation (29). In these stimulated conditions, IL1 showed an inhibition only at the concentration of 0.01pM ($P<0.05$).

TRH (100nM) also significantly increased the plaque mean area (Fig.2). This effect was clearly reverted by the lowest concentrations of IL1 (0.01pM, 1pM, 100pM), while the highest concentration of IL1 (10nM) failed to significantly inhibit PRL secretion (Fig.2).

Recently, lactotropes have been reported to differ in the capacity to secrete PRL and to be heterogeneous with respect to TRH responsiveness; indeed, TRH increases the fraction of

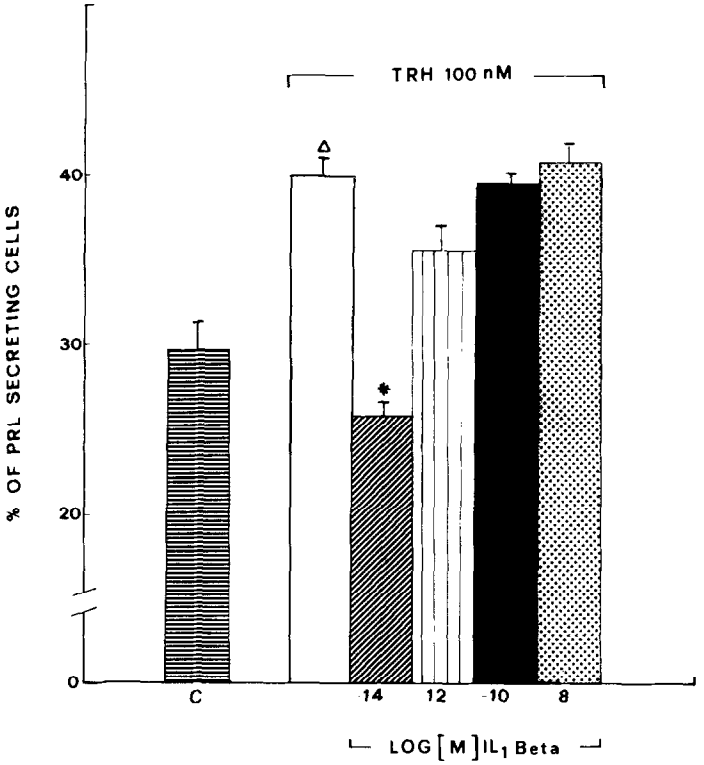

Figure 1: Effect of different IL1 concentrations on TRH increase in the percentage of PRL secreting cells.
* P<0.05 versus TRH; Δ P<0.05 versus control.

lactotropes forming large plaques (30). In Fig.3 it is shown that the percentage of large plaques, enhanced by TRH (from 38% to 86%), is clearly reduced by IL1, suggesting that the cytokine reduces the sensitivity of the large plaque forming lactotropes to TRH stimulation.

These findings confirm our previous data (14,15,16) about a direct action of IL1 on anterior pituitary gland, reinforcing the concept that IL1 reduces PRL secretion acting at level of single lactotrope cell. However, the use of RHPA, which avoids any paracrine interactions among pituitary cells, does not exclude the existence of such interaction in vivo.

TRH is able to stimulate the production of inositol phosphate in primary cultures of anterior pituitary cells (23), in GH3

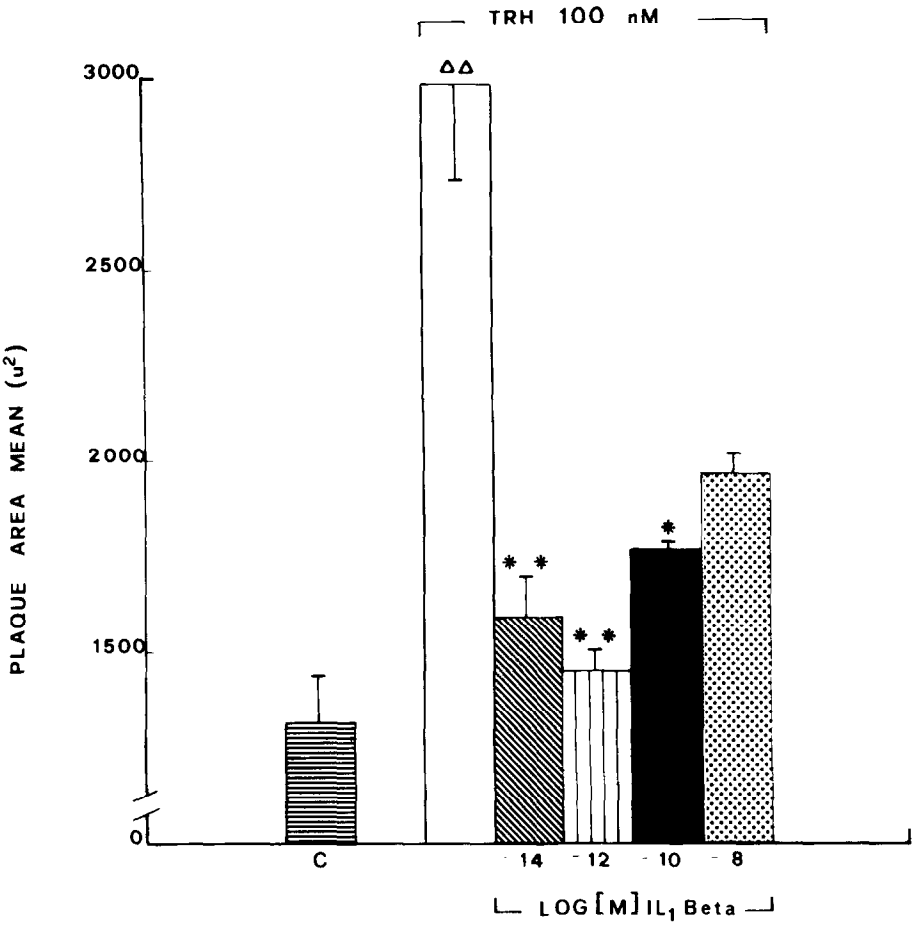

Figure 2: Inhibitory effect of different IL1 concentrations on the increase of the plaque mean area induced by TRH.
* P<0.05 and ** P<0.01 versus TRH; ΔΔ P<0.01 versus control.

cells (24), and in lactotropes enriched population (31). Thus, to assess the possible involvement of phosphoinositide turnover in IL1-induced reduction of TRH-stimulated PRL secretion, we studied the effect of the monokine on phosphoinositide metabolism, both in basal and in TRH-stimulated conditions.

In basal conditions, IL1 did not significantly modify inositol phosphate production, showing only a slight inhibitory trend (control 2193+/-148 cpm/well; IL1 1pM 2001+/-137 cpm/well). TRH (100nM) significantly stimulated inositol phosphate production in normal anterior pituitary cells in culture, and this stimulation

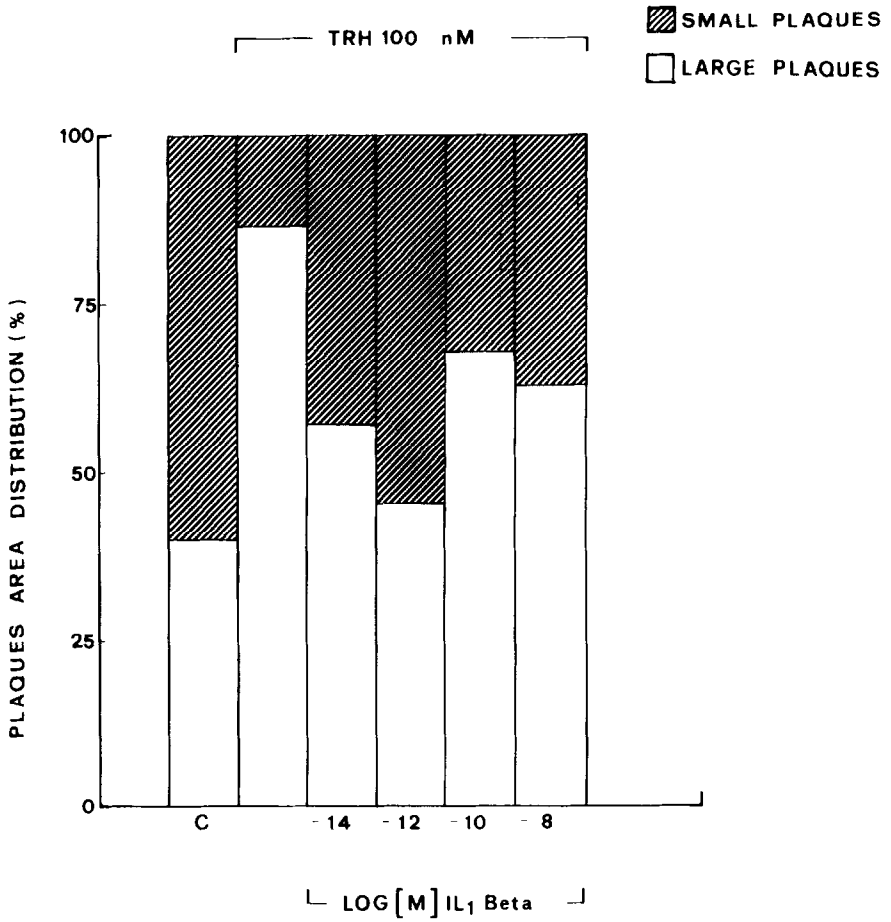

Figure 3: Effect of different IL1 concentrations on the percentage of large (open bars) and small (full bars) plaque areas formed by PRL secreting cells stimulated by TRH. The percentages of plaques with individual areas smaller than the control mean area (small plaques) and the percentages of plaques with an individual area larger than the control plaque mean area (large plaques) express the frequency distributions of plaque areas. The control mean area was 1528 umq. (Cfr. text).

was clearly inhibited by IL1 at the concentration of 0.01pM and 1pM; conversely, higher (100pM and 10nM) concentrations of the monokine were ineffective in this respect (Fig.4). IL1 (1pM) inhibition of TRH-induced inositol phosphate production was more pronounced with an higher TRH concentration (1uM)(Fig.5). The lack of efficacy of the highest concentrations of IL1 on phosphoinositide hydrolysis could be ascribed to the existence of two receptors for IL1, with low and high affinity (1). Indeed, it

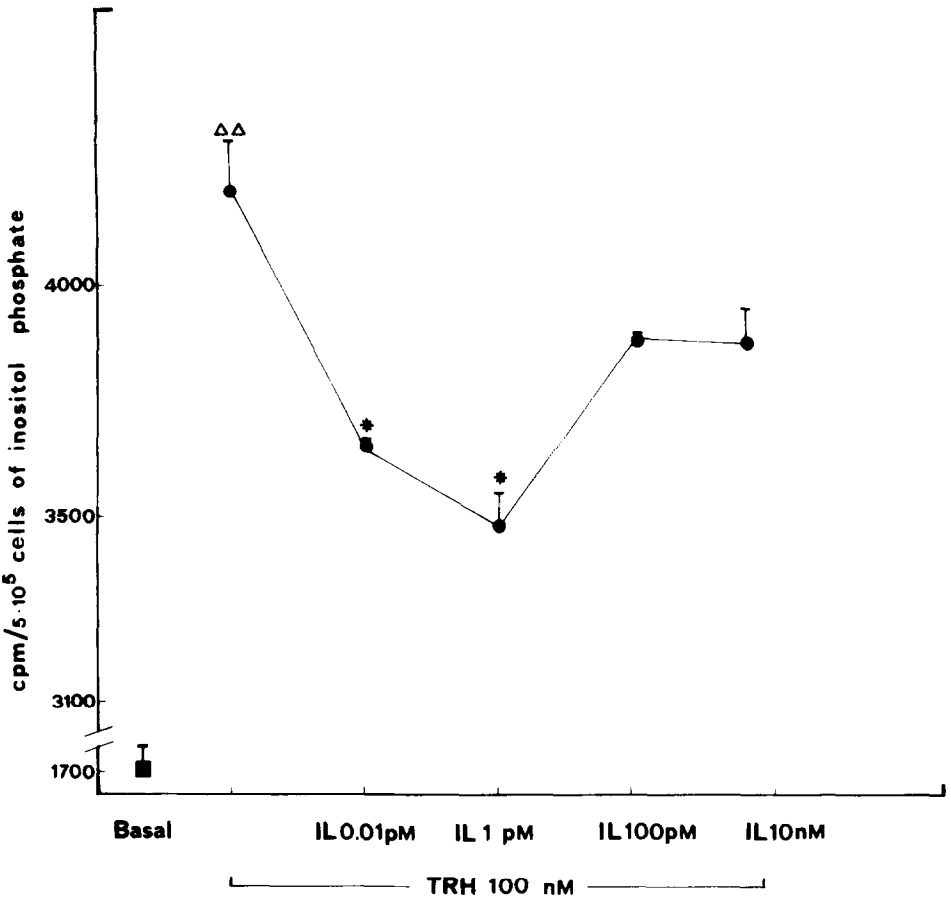

Figure 4: Effect of different IL1 concentrations on TRH (100nM) stimulated inositol phosphate accumulation. IL1 was added 4 minutes before TRH, whose exposure lasted 12 minutes.
* $P<0.05$ versus TRH; $^{\Delta\Delta}$ $P<0.01$ versus basal.

is possible that, when IL1 is given at high concentrations, the stimulation of a low affinity binding site for IL1 could balance the inhibitory effect of the activation of an high affinity receptor for the cytokine.

These findings, together with our previuos reports, suggest a pleomorphic action of IL1 on second messenger systems at pituitary level, since different transducing mechanisms, such as adenylate cyclase system and calcium fluxes (14,15,16), besides phosphoinositide turnover seem to be involved.

In conclusion, our data show that IL1 inhibits TRH-stimulated PRL secretion and phosphoinositide hydrolysis. The correspondence

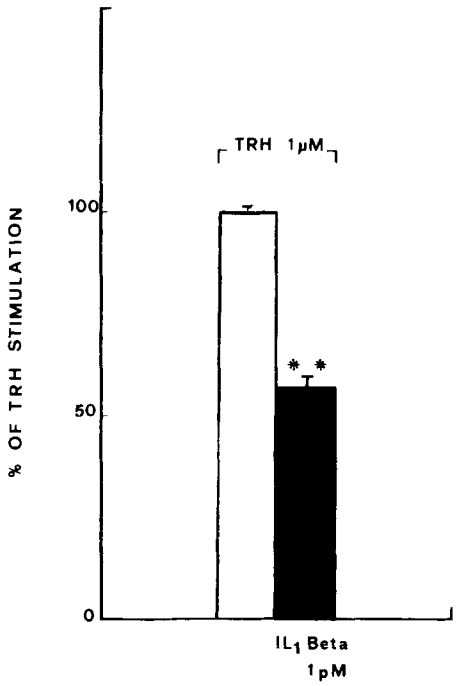

Figure 5: Effect of IL1 on TRH (1uM) stimulated inositol phosphate accumulation.
** P<0.01 versus TRH.

between IL1 concentrations effective on PRL release and IL1 concentrations able to attenuate phosphoinositide hydrolysis suggests that the reduced lactotropes sensitivity to TRH action may be partially due to the IL1 attenuation of TRH-stimulated phosphoinositide breakdown.

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