Suppression of Serum Immunoreactive Human Epidermal Growth Factor by Acute Increase in Prolactin in Women

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Abstracts

Epidermal growth factor (EGF) is known to stimulate proliferation of various mammalian cells and secretion of prolactin (PRL) from rat anterior pituitary tumor cells. The effect of an acute increase in serum PRL induced by thyrotropin releasing hormone (TRH) or metoclopramide (MCP) on the serum immunoreactive EGF concentration was examined in nine hyperprolactinemic patients and eight normoprolactinemic women. The basal level of serum EGF in normoprolactinemic subjects was 472.8 ± 51.1 pg/ml (Mean $\pm$ SEM), which was not significantly different from that in hyperprolactinemic patients (487.8 ± 22.5 pg/ml). The serum EGF concentration was decreased to 40-50% of the basal level after the abrupt increase in serum PRL induced by the injection of TRH or MCP in normoprolactinemic subjects, but no significant change in serum EGF occurred in hyperprolactinemic patients after MCP injection, in spite of a significant increase in PRL. These results suggest that an acute increase of serum PRL in normoprolactinemic women, but not in hyperprolactinemic patients, suppresses serum EGF.

Epidermal growth factor (EGF), a single chain polypeptide with a molecular weight of 6045, first isolated from the submandibular gland of male mice, is a potent stimulator of the growth of various types of cells (Cohen *et al.*, 1974; Carpenter, 1978). EGF has also been shown to stimulate the secretion of prolactin (PRL) and inhibit the production of growth hormone *in vitro* in cultures of rat anterior pituitary tumor cells (Agnes *et al.*, 1980). In addition, EGF has been demonstrated to

have an inhibitory effect on estrogen production by ovarian granulosa cells and on testosterone production by testicular Leydig cells (Hsueh *et al.*, 1981). Hormones, such as androgen, progestin and α -adrenergic agents, were shown to increase the production of EGF in submandibular glands with a concomitant increase in the plasma concentration in male mice (Byyny *et al.*, 1974). A similar polypeptide hEGF, was subsequently purified from human urine (Starkey *et al.*, 1975). The immunoreactive hEGF concentrations in extracellular fluids, saliva, milk and urine have been reported (Starkey *et*

al., 1977; Dailey *et al.*, 1978; Gregory *et al.*, 1979; Carpenter, 1980; Hirata *et al.*, 1979). Tissues which contain a higher concentration of EGF are the submandibular gland, thyroid gland, duodenum, pancreas and kidney. The localization of hEGF in the anterior pituitary gland was studied immunocytochemically (Kasselberg *et al.*, 1985). However, the origin and regulation of serum hEGF production are still unclear. The present study was designed to examine the effect of rapid prolactin secretion on the serum immunoreactive EGF concentration in normal women and hyperprolactinemic patients by employing thyrotropin releasing hormone (TRH) and dopamine antagonist metoclopramide (MCP).

Materials and Methods

Subjects and samples

Eight normoprolactinemic (PRL < 30 ng/ml) women 22 to 37 years old (mean, 29.0 ± 4.5 years) and nine with pituitary adenomas and hyperprolactinemia (16 to 36 years old; mean, 27.6 ± 5.8 years) were studied with the consent of subjects. All studies were started at 10:00 AM after overnight starvation. The normoprolactinemic subjects were given a bolus of 100 μ g of gonadotropin-releasing hormone (GnRH) intravenously during the early follicular phase and then 500 μ g of thyrotropin releasing hormone (TRH) 2 days later. The hyperprolactinemic patients were subjected to the GnRH-TRH test on their first visit to the hospital. Blood samples were taken before, and 15, 30, 60 and 120 min after the test. Normoprolactinemic and

Fig. 1. Standard curve for hEGF radioimmunoassay (solid line). Binding of ^{125}I -hEGF to antibody was not affected by other hormones [i.e., PRL, FSH, LH, GH, TSH, ACTH, LH-RH, TRH, hCG, insulin and insulin-like growth factor (IGF)] and no cross reaction was observed with mEGF even at a concentration over 1000 times higher (dotted line). Dilutions of serum gave a curve parallel to that of the standard. B: Fraction of total ^{125}I -hEGF bound to antiserum in the presence of hEGF. B_0 : Fraction of total ^{125}I -hEGF specifically bound to antiserum in the absence of unlabelled hEGF. N: Fraction of total ^{125}I -hEGF bound to normal rabbit serum.

hyperprolactinemic subject were also given the dopamine antagonist metoclopramide (MCP, 10 mg) during their next early follicular phase of the menstrual cycle, and blood samples were taken before, and 15, 30, 45 and 60 min after MCP administration. Three normoprolactinemic subjects and two of hyperprolactinemic patients were studied for diurnal changes in the PRL and EGF concentration. An indwelling cannula was inserted into the antecubital vein and kept patent throughout the sampling period by slow infusion of isotonic saline. Blood samples were drawn every 15 min for 2 hours. The sera were separated and stored at -20°C until assays of PRL and EGF.

RIA for PRL

The serum PRL concentration was determined with a homologous radioimmunoassay kit generously supplied by NIAMDD (Sinha *et al.*, 1973).

Our assay system could detect as little as 2.0 ng/ml of hPRL, V-L-S No. 1, and the coefficient of variation was 4.3% (intra-assay) and 9.4% (inter-assay), respectively (Aono *et al.*, 1977).

RIA for hEGF

Synthetic-gene recombinant hEGF, generously provided by Wakunaga Co. (Hiroshima, Japan), was used as a standard and iodinated tracer in the RIA. Rabbit anti-hEGF serum was also supplied by Wakunaga Co., hEGF was radioiodinated by the chloramine-T method (Hunter *et al.*, 1962), and ^{125}I -hEGF was purified on a Sephadex G-25 fine gel column. The concentration of hEGF in the serum was assayed by double antibody radioimmunoassay. Briefly, a sample of 200 μl or unlabelled hEGF standards ranging from 1.65 fmole (0.01 ng) to 1.65 pmole (10 ng), was mixed with 100 μl of rabbit anti-hEGF serum diluted to 1:

20,000 with PBS-BSA buffer containing 2% normal rabbit serum. 100 μl PBS containing ^{125}I -labelled hEGF (approximately 20,000 cpm) was then added. The reaction mixtures were incubated for 2 days at 4°C . Antibody-bound and free ^{125}I -EGF were separated by adding 200 μl of 2% goat antirabbit serum and incubating the mixtures at 4°C for an additional 24 hr. The resulting insoluble material was collected by centrifugation at 3,000 rpm for 30 min and the supernatant was aspirated. Radioactivity in the precipitate was measured in a gamma counter. A standard curve was made using various amounts of hEGF standard ranging from 1.65 fmole to 1.65 pmole. No cross reaction with other peptides or mouse EGF was noted (Fig. 1). The assay system had a sensitivity of 1.65 fmole (10 pg/ml) EGF, and the mean ($\pm$ SEM) recovery rate was $90.6 \pm 2.2\%$. The intra-assay

Fig. 2. Percent changes (Mean $\pm$ SEM) in serum PRL and EGF in normoprolactinemic subjects after GnRH injection ($\bullet$ — $\bullet$) and in normoprolactinemic subjects and hyperprolactinemic patients after saline infusion ($\circ$ — $\circ$).

and inter-assay coefficients of variation were 9.7% and 11.3%, respectively. Serum PRL and hEGF were measured in duplicates and all samples were measured in the same assay run. Statistical significance was examined by Student's *t*-test.

Results

Mean ($\pm$ SEM) serum PRL in hyperprolactinemic patients was 183.4 ± 41.4

ng/ml, which was significantly ($p < 0.01$) higher than that in normoprolactinemic subjects (13.2 ± 1.6 ng/ml). The mean ($\pm$ SEM) serum immunoreactive EGF concentration in hyperprolactinemic patients (487.8 ± 22.5 pg/ml) was not significantly different from that in normoprolactinemic subjects (472.8 ± 51.5 pg/ml). GnRH administration in eight normoprolactinemic subjects and saline infusion in three normoprolactinemic subjects and two hyperpro-

lactinemic patients resulted in no significant change in serum PRL or EGF (Fig. 2). The injection of TRH into the normoprolactinemic subjects induced a marked increase in PRL from 13.2 ± 1.6 ng/ml to 104.2 ± 23.2 ng/ml at 15 min (Fig. 3, top), with a concomitant decrease in immunoreactive EGF to $50.7 \pm 6.3\%$ of the basal level at 120 min (Fig. 3, lower). In the nine hyperprolactinemic patients, the administration of GnRH and TRH did not cause any significant change in serum PRL or EGF (Fig. 3). In normoprolactinemic subjects, MCP injection produced a 31.2-fold increase in serum PRL after 30 min, and a significant decrease in serum immunoreactive EGF to $42.1 \pm 7.0\%$ of the pre-injection level after 60 min (Fig. 4.) In hyperprolactinemic patients, MCP injection did not induce any significant change in immunoreactive EGF even though PRL increased significantly (Fig. 4).

Fig. 3. Percent changes (Mean $\pm$ SEM) in serum PRL and EGF after TRH injection in normoprolactinemic subjects and after injections of GnRH and TRH in hyperprolactinemic subjects. The asterisks indicate significant differences from the basal level ($p < 0.01$).

Normo : normoprolactinemia, Hyper : hyperprolactinemia.

Discussion

In the present report, we observed that the administration of TRH or MCP decreased serum immunoreactive EGF after

an abrupt increase in serum PRL in normoprolactinemic subjects. However, in hyperprolactinemic patients, the serum immunoreactive EGF level did not change at all even when there was a significant increase in PRL following the injection of MCP.

There was no significant difference between the basal immunoreactive EGF concentrations in these two groups. These findings suggest that an abrupt increase in PRL may inhibit either the production or release of EGF from its site(s) of origin in the normal control. The only tissue proven to be related to the production of EGF is the submaxillary gland in mouse. Following excision of the gland, the basal EGF concentration in mouse body fluid was reduced somewhat but remained within the detectable range. This suggests the presence of alternative sites for EGF synthesis in mouse (Perheentupa *et al.*, 1985). The main origin of hEGF in serum is not clear, although hEGF is reported to be present in the anterior pituitary gland, submaxillary gland, thyroid gland, duodenum, pancreas and kidney. In the anterior pituitary gland, most cells shown to contain hEGF immunocytochemically also stained for hFSH, hLH and hTSH, but not for hPRL (Kasselberg *et al.*, 1985).

PRL was increased after injection of either

Fig. 4. Percent changes (Mean $\pm$ SEM) in serum PRL and EGF in normoprolactinemic and hyperprolactinemic subjects after MCP injection.

* $p < 0.05$, ** $p < 0.01$ compared with the basal level.

Normo : normoprolactinemia, Hyper : hyperprolactinemia.

TRH or MCP, but their effect is induced by different pathways. TRH has a direct stimulatory effect on PRL secretion at the pituitary level, whereas antidopaminergic agents such as MCP stimulate PRL release by blocking dopamine receptors in hypothalamus (MacLeod, 1976; Labrie *et al.*, 1978). In normal individuals, two types of pharmacological stimulation of PRL release induce the same effect on serum EGF, suggesting that serum EGF concentration may be regulated by the pituitary and/or extrapituitary concentration.

Another possibility is that the acute increase in serum PRL may increase EGF clearance from the kidneys. A previous study showed that thyroxine injection decreased EGF by enhancing excretion of EGF from the kidneys in female mice (Perheentupa *et al.*, 1984). In our subjects, urinary EGF did not change at all within 24 hours after the injection of TRH or MCP (data not shown).

The PRL concentration in normoprolactinemic woman gradually increased to a peak during sleep, and was about 4 times the basal concentration (Sassin *et al.*, 1973), but EGF showed no diurnal change (Hirata *et al.*, 1980). Hyperprolactinemic patients are known to be less sensitive to TRH and MCP than normal cycling women. In the present study, we demonstrated that serum immunoreactive EGF did not decrease after the administration of TRH or MCP in hyperprolactinemic patients.

Taken together, these results suggest that the secretion of PRL may have regulatory effects on maintaining the EGF concentration, but the precise mechanism of the effect of PRL on EGF secretion is not clear. The origin and biological function of EGF in man are unknown at present time. Further studies are required to characterize the role of EGF in relationship to PRL and other hormones in normal and pathological conditions.

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References

- Agnes, S., K. Margo, M. W. Joanne and H. Y. Jr. Armen (1980). Epidermal growth factor and thyrotropin-releasing hormone act similarly on a clonal pituitary cell strain. *J. Cell. Biology.* 85, 786-797.
- Aono, T., T. Shioji, T. Shoda and K. Kurachi (1977). The initiation of human lactation and prolactin response to suckling. *J. Clin. Endocrinol. Metab.* 44, 1101-1106.
- Byyny, R. L., D. N. Orth, S. Cohen and E. S. Doyne (1974). Epidermal growth factor: Effect of androgens and adrenergic agents. *Endocrinology* 95, 776-782.
- Carpenter, G. (1978). The regulation of cell proliferation: Advances in the biology and mechanism of action of epidermal growth factor. *J. Invest. Dermatol.* 71, 283-287.
- Carpenter, G. (1980). Epidermal growth factor is a major growth-promoting agent in human milk. *Science* 210, 198-200.
- Cohen, S. and C. R. Jr. Savage (1974). Part II Recent studies on the chemistry and biology of epidermal growth factor. *Recent Progress in Hormone Research* 30, 551-575.
- Dailey, G. E., J. W. Kraus and D. N. Orth (1978). Homologous radioimmunoassay for human epidermal growth factor (urogastrone). *J. Clin. Endocrinol. Metab.* 46, 929-936.
- Gregory, H., S. Walsh and C. R. Hopkins (1979). The identification of urogastrone in serum, saliva and gastric juice. *Gastroenterology* 77, 313-318.
- Hirata, Y. and D. N. Orth (1979). Epidermal growth factor (urogastrone) in human tissues. *J. Clin. Endocrinol. Metab.* 48, 667-672.
- Hirata, Y., G. W. Moore, C. Bertagna and D. N. Orth (1980). Plasma concentrations of immunoreactive human epidermal growth factor (urogastrone) in man. *J. Clin. Endocrinol. Metab.* 50, 440-444.
- Hsueh, A. J. W., T. H. Welsh and P. B. C.

- Jones (1981). Inhibition of ovarian and testicular steroidogenesis by epidermal growth factor. *Endocrinology* 108, 2002-2004.
- Hunter, W. M. and F. C. Green (1962). Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* 194, 495-496.
- Kasselberg, A. G., D. N. Orth, M. E. Gray and M. T. Stahlman (1985). Immunocytochemical localization of human epidermal growth factor/urogastrone in several human tissues. *J. Histochem. Cytochem.* 33, 315-332.
- Labrie, F., L. Lagace, L. Ferland, M. Beaulien, J. Massicotte and V. Raymond (1978). New aspects of the control of pituitary hormone secretion. *Ann. Clin. Res.* 10, 109-119.
- MacLeod, R. M. (1976). Regulation of prolactin secretion. In: Ganong, W. F. (ed.). *Frontiers in Neuroendocrinology* vol 4, 169-194. Raven Press, New York.
- Perheentupa, J., J. Lakshmanan, S. B. Hoath and D. A. Fisher (1984). Hormonal modulation of mouse plasma concentration of epidermal growth factor. *Acta endocr., Copenh.* 107, 571-576.
- Perheentupa, J., J. Lakshmanan and D. A. Fisher (1985). Epidermal growth factor in mouse urine: Non-blood origin and increase by sialadenectomy and T₄ therapy. *Acta endocr., Copenh.* 108, 428-432.
- Sassin, J. F., A. G. Frantz, S. Kapen and E. D. Weitzman (1973). The nocturnal rise in human prolactin is dependent on sleep. *J. Clin. Endocrinol. Metab.* 37, 436-440.
- Sinha, Y. N., F. W. Selby, U. J. Lewis and W. P. Vanderlaan (1973). A homologous radioimmunoassay for human prolactin. *J. Clin. Endocrinol. Metab.* 36, 509-516.
- Starkey, R., S. Cohen and D. N. Orth (1975). Epidermal growth factor: Identification of a new hormone in human urine. *Science* 189, 800-802.
- Starkey, R. and D. N. Orth (1977). Radioimmunoassay of human epidermal growth factor (urogastrone). *J. Clin. Endocrinol. Metab.* 45, 1144-1153.