

Effect of 1-34 human parathyroid hormone upon first trimester placental human chorionic gonadotrophin secretion *in vitro*: potentiation by epidermal growth factor

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Human chorionic gonadotrophin (HCG) secretion by the early placenta is under multifactorial control. Epidermal growth factor (EGF) has been reported to be involved in regulating the formation and secretion of HCG by first trimester placental explants in culture. The effect of the amino-terminal fragment of parathyroid hormone (1-34 PTH), a calciotropic factor upon HCG secretion, and its possible interaction with EGF were examined in this study, both in static cultures and in superfusion, where it has previously been demonstrated that HCG secretion is spontaneously pulsatile. Gestational age-dependent effects of 1-34 PTH were noted in both models. In static cultures, 1-34 PTH stimulated HCG secretion in 7-9 week placenta, in a biphasic fashion, the maximal effect being noted at 10-25 ng/ml concentrations (250-270%), while at 1 and 100 ng/ml, the effect was mild. In superfusion, the effect of 1-34 PTH added overnight was also stimulatory, as shown by the significantly increased pulse amplitude and area under the curve. Effects of 1-34 PTH at 11-14 weeks were inhibitory. In static cultures at 7-9 weeks, the stimulatory effect of 25 ng 1-34 PTH was increased by 70% when EGF (100 ng/ml) was added. However at 11-14 weeks, this combined effect was inhibitory. We conclude that 1-34 PTH has an endocrine effect on secretion of HCG by the first trimester placental tissue, and this effect is potentiated by the addition of EGF.

Key words: HCG/1-34 PTH/regulation/trophoblast

Introduction

Compared to other placental hormones human chorionic gonadotrophin (HCG) follows an unusual pattern increasing for the first 8 weeks of gestation and maintaining a plateau thereafter. After 11 weeks, HCG decreases and remains low until term. There have been a number of in-vitro studies to investigate the factors involved in the regulation of HCG secretion by the first trimester placenta (Maruo *et al.*, 1987; Siler-Khodr *et al.*, 1987;

Barnea and Kaplan, 1989; Barnea *et al.*, 1990, 1991a,b, 1992). These factors are numerous, suggesting multifactorial control. We have recently reported that epidermal growth factor (EGF), a polypeptide, is involved in regulating the formation and secretion of HCG by the young placenta in culture (Barnea *et al.*, 1990). This growth factor is generally regarded as the promoter of trophoblastic cell differentiation at term (Morrish and Siy, 1985).

Human parathyroid hormone (PTH) is a single chain protein hormone of 84 amino acids which is secreted by the parathyroid gland (Rosenblatt, 1982). The secretion of a lower molecular weight fragment, namely 1-34 PTH, has also been described. This fragment, when compared to the whole molecule, acts as a mitogen on murine mandibular condylar cartilage *in vitro*, in addition to its calciotropic effects which are also ascribed to the whole hormone (Milstone *et al.*, 1989; Shurtz-Swirski *et al.*, 1990). Recently specific binding sites for PTH were identified in the human placenta (Lafond *et al.*, 1988). Also, an increase in HCG secretion and potentiation of EGF action in cells isolated both from early and term placenta have been described (Alsatt *et al.*, 1991).

HCG is a major marker of the placenta, and its secretion *in vitro* is modulated by other mitogens such as platelet-derived growth factor (PDGF) (Taylor and Williams, 1988). Therefore in the present study, we have examined the effect of 1-34 PTH upon HCG secretion and its possible interaction with EGF. This was examined both in static cultures of explants and in superfusion where we have demonstrated that HCG secretion is spontaneously pulsatile and is modulated by endocrine factors like EGF, gonadotrophin-releasing hormone (GnRH) and progesterone (Barnea and Kaplan, 1989; Barnea *et al.*, 1990, 1991a,b). Here we report that 1-34 PTH modulates HCG secretion by the first trimester placental tissue *in vitro*, and that EGF potentiates this effect.

Materials and methods

Chemicals

1-34 PTH, EGF and HEPES were purchased from Sigma (St Louis, MO, USA), HCG kit MAIA clone was purchased from Serono (Rehovot, Israel).

Placental material

Twenty 7- to 14-week-old placentas were studied. After obtaining appropriate consent, elective pregnancy terminations by vacuum curettage were performed. Gestational ages were determined by last menstrual period and ultrasound as previously described

(Fakih *et al.*, 1986). Patients were healthy, did not use any medication, and were non-smokers. Collected tissue was rinsed several times in cold 0.9% NaCl to remove all blood, followed by three additional rinses with culture medium, i.e. Dulbecco's modified Eagle's medium (DMEM; Beit Haemek, Israel), containing 1% antibiotics (penicillin 10 000 IU/ml, streptomycin 10 μ g/ml and amphotericin B 25 μ g/ml).

Explant cultures

Using previously reported procedures (Barnea and Fakih, 1985; Barnea and Kaplan, 1989; Barnea *et al.*, 1990), the placenta was separated from membranes and decidua under a dissecting microscope, and explants of 50–70 mg wet weight were dissected out and rinsed in 2% antibiotic solution. For cultures, explants were placed in DMEM with 1% antibiotic solution either with or without 1-34 PTH (1–100 ng/ml concentration). In some cultures, 100 ng/ml EGF was added alone or with 1-34 PTH. Three to six replicates per test agent or control per placenta were plated at 37°C in an atmosphere of 95% air and 5% CO₂. After 24 h of incubation, the media were collected and stored at –20°C until assay. The tissue was saved for protein analysis. Dishes containing only 10 mM acetic acid served as controls.

In preliminary experiments we found that HCG secretion was linear for the first 48 h. Therefore, in subsequent experiments, the effect of 1-34 PTH was tested at 24 h. Tissue viability was determined by progressive glucose consumption and by staining with haematoxylin–eosin (Barnea *et al.*, 1990).

Superfusion studies

The methodology was recently published (Barnea and Kaplan, 1989; Barnea *et al.*, 1991b). Briefly, a superfusion apparatus (Accusyst, Endotronics, St Paul, MN, USA) with a multichannel peristaltic pump and fraction collector (model 272, ISCO, Durham, NE, USA) were used to study the short-term dynamics of HCG secretion following exposure to 1-34 PTH. Explants (200–300 mg wet weight) preincubated for 24 h with 1-34 PTH or acetic acid only were rinsed and placed into culture chambers, and a HEPES (18 mM)–DMEM solution was washed through in an atmosphere of 95% air and 5% CO₂ at 37°C. Experiments were conducted for a period of 120 min. A 1 ml sample from the effluent was collected every 2.4 min for HCG measurements. In each experiment, one channel served as control and four served as test channels. Collected media were stored at –20°C until assayed.

Assays

HCG was measured by radioimmunoassay as previously reported (Barnea *et al.*, 1989, 1990), with an intra-assay variability of 1.7%. The inter-assay variability was 3.2%. The limit of assay sensitivity was <1 mIU/ml. Cross-reactivity with follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH) was <0.1%.

Placental tissue protein was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistics

Statistical analysis was performed by one-way analysis of variance and Student's *t*-test, with *P* < 0.05 considered statistically

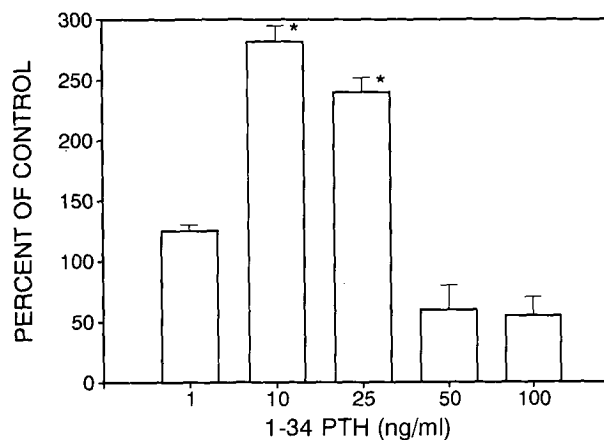


Fig. 1. Biphasic stimulatory effect of various concentrations of 1-34 human parathyroid hormone (PTH) on placental human chorionic gonadotrophin (HCG) secretion by 7–9 week explant cultures (*n* = 10). Data are expressed as percentage of control mean \pm SE. **P* < 0.05 versus control.

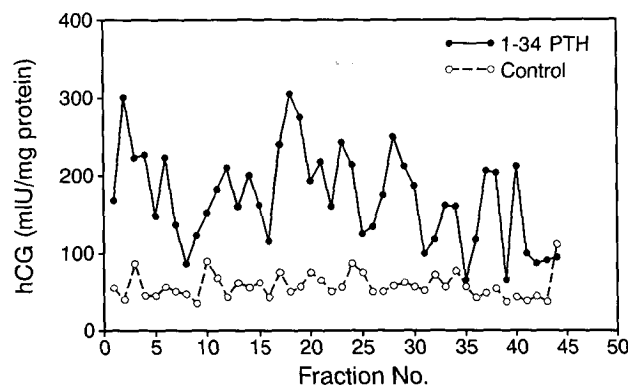


Fig. 2. Human chorionic gonadotrophin (HCG) secretion by superfused 8 week placental explants following pre-exposure to 10 ng/ml 1-34 human parathyroid hormone (PTH) for 24 h (data analysis shown in Table I).

significant. For superfusion experiments, the area under the curve, peak amplitude and peak frequency were calculated using the PULSAR program. Data for HCG concentration were expressed as mIU/mg protein.

Figures from static cultures show data generated in at least three placentas, with three to six explants per placenta per test agent or control. In superfusion experiments the data were representative of at least three placentas run in duplicate with similar results.

Results

Placenta at 7–9 weeks

Figure 1 shows the significant biphasic (bell shape) stimulatory effect of 1-34 PTH upon HCG secretion following 24 h incubation (*P* < 0.05). This was most evident when the explants were treated with 10–25 ng/ml 1-34 PTH. At a lower concentration (1 ng/ml) a mild stimulatory effect (not significant)

Table I. Representative PULSAR analysis of significant peaks, following superfusion of placental explants with or without 1-34 human parathyroid hormone (PTH)

	Pulse frequency	Peak amplitude	Area under curve
A. control	16	29.2	2481
1-34 PTH	13	102.6 ^a	7397 ^a
B. control	16	31.64	1062.5
1-34 PTH	17	14.5 ^a	537.4 ^a

A. = 7–9 weeks gestational age ($n = 10$).

B. = 11–14 weeks gestational age ($n = 10$).

^a $P < 0.05$ versus control.

was noted, while at high concentrations (50–100 ng/ml) no effect was noted.

Figure 2 shows the delayed effect of 1-34 PTH (10 ng/ml) preincubated for 24 h with placental explants upon pulsatile HCG secretion in superfusion. There was a significant increase in amplitude and area under the curve of the significant HCG pulses ($P < 0.05$) when compared to controls. However, no effect on the pulse frequency was noted (Table I).

Placenta at 11–14 weeks

The effect of 10 ng/ml and 25 ng/ml 1-34 PTH added for 24 h upon HCG secretion by placental explants was inhibitory ($P < 0.05$, Figure 3). A lower (1 ng/ml) concentration of the factor was ineffective.

Figure 4 shows that incubation with 10 ng/ml 1-34 PTH, following preincubation with maximal inhibitory dose seen on static culture, decreased HCG secretion in dynamic cultures, as shown by a decrease in mean peak amplitude and area under the curve of significant HCG pulses. However, no change in the pulse frequency was noted (Table I).

Effect of EGF

In placenta at 7–9 weeks addition of 100 ng EGF, which was previously established as being maximally effective (Barnea *et al.*, 1990), together with 25 ng/ml 1-34 PTH in static cultures significantly potentiated the effect of 1-34 PTH alone ($P < 0.01$) (Figure 5). At 10 ng/ml, the potentiating effect of the fragment was mild (data not shown). At 11–14 weeks, however, addition of 100 ng/ml EGF caused a further $30 \pm 7\%$ decrease in HCG secretion when compared to those cultures where 10 ng 1-34 PTH was given alone (data not shown). High (25 ng/ml) or low (1 ng/ml) concentrations of the fragment did not potentiate the EGF effect.

Discussion

The involvement of 1-34 PTH, a calciotropic fragment, in promoting calcified tissue growth has been recently demonstrated (Shurtz-Swirski *et al.*, 1990). Our data provide evidence that this fragment of PTH also has an endocrine effect by stimulating HCG secretion by first trimester placental explants *in vitro*.

The effect of 1-34 PTH on the placental explants was stimulatory or inhibitory depending on the gestational age and it was most effective at concentrations that have recently been shown to have significant mitogenic effects in calcified embryonic

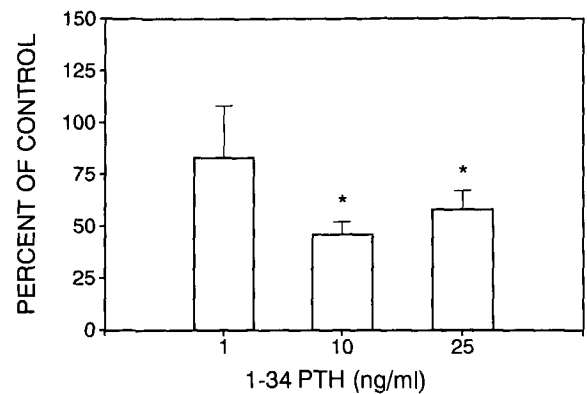


Fig. 3. Inhibitory effect of 10–25 ng/ml 1-34 human parathyroid hormone (PTH) added for 24 h on human chorionic gonadotrophin (HCG) secretion by 11–14 week placental explants ($n = 10$). Data are expressed as percentage of control mean \pm SE. * $P < 0.05$ versus control.

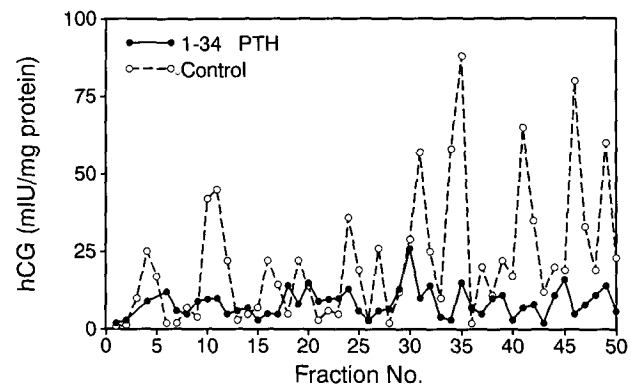


Fig. 4. Delayed inhibitory effect (see text) of 10 ng 1-34 human parathyroid hormone (PTH) added for 24 h on human chorionic gonadotrophin (HCG) secretion by superfused 11 week placental explants (data analysis shown in Table I).

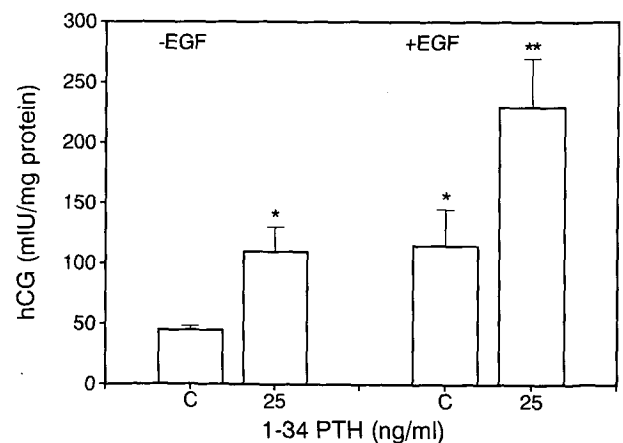


Fig. 5. Additive effect of 100 ng epidermal growth factor on the stimulatory effect on HCG secretion by 7–9 week placental explants induced by 25 ng/ml of 1-34 human parathyroid hormone. Data are expressed as percentage of control mean \pm SE. * $P < 0.05$, ** $P < 0.001$ versus control (C).

tissues (Shurtz-Swirski *et al.*, 1990). The effect was noted both in static cultures (direct contact) and in superfusion (delayed effect) of explants following pre-treatment with the fragment, as shown by increased area under the curve and peak amplitude of HCG pulses. This is of importance since the compound was removed from the media by thorough washing before the superfusion procedure, which suggests that 1-34 PTH action may be mediated by its binding to the placental membrane. Evidence for PTH binding sites was recently provided in the placental brush border membrane and basement membrane (Lafond *et al.*, 1988). The receptor K_d value described was in the 0.1–1 nM range. Indeed, at 7–9 weeks we found a mild (not significant) stimulatory effect on 1 nM concentration of 1-34 PTH which became maximal at 10–25 nM concentration. Lack of effect at 50–100 nM concentration supports the view that the 1-34 PTH action was dependent on binding to the local receptor. The delayed stimulatory action of the fragment may indicate that it has a prolonged effect on HCG secretion locally. We have recently reported that EGF has a similar delayed stimulatory effect (Barnea *et al.*, 1992). The stimulatory effect of 1-34 PTH could have been triggered by the substance acting on the genome, since it has a mitogenic activity through promotion of protein synthesis, as shown in other systems (Burch and Lebovitz, 1983; Lewinson and Silbermann, 1986; Shurtz-Swirski *et al.*, 1990).

Gestational age-dependent effects of 1-34 PTH were noted. Compared to 7–9 weeks, an inhibition of HCG secretion was observed at 11–14 weeks. The effect at 10 weeks was not tested since it is the time of HCG plateau when the effects of β -endorphin are not significant (Barnea *et al.*, 1991). This opposite effect seen at a later gestational stage was not related to changing sensitivity of the placenta, since effectiveness of 1-34 PTH was demonstrated at the same dose range. Other growth factors, e.g. EGF and insulin, also had a gestational age-dependent effect on HCG secretion in both static cultures and superfusion (Barnea *et al.*, 1992; unpublished observation). The gestational age-dependent effect is likely to be caused by changes in expression of the β subunit of the HCG gene, which is turned off after the HCG peak. Also, the ratio of cytosyncytiotrophoblast to syncytiotrophoblast cells is higher in the embryonic stage compared to that in the fetal period, which may also be an influential factor. Roles of growth factors in embryonic and fetal stages may change as, for example, in the later stage emphasis is on growth and not morphogenesis (Barnea *et al.*, 1989).

The finding that, at 7–9 weeks, the 1-34 PTH effect was potentiated by the maximally effective concentration of EGF is of interest. EGF acts on the placenta by binding to specific receptors which have been well characterized (Lai and Guyda, 1984; Maruo *et al.*, 1987). EGF stimulates HCG secretion in the first trimester either when given as short pulses in superfusion, or when added for 24 h in explants, or following long-term exposure to isolated cells (Barnea *et al.*, 1990, 1992). It was recently shown that high (100 ng/ml) 1-34 PTH concentration increased the high-affinity EGF binding sites in isolated cells (Alsatt *et al.*, 1991). At 11–14 weeks the potentiating inhibitory effect of EGF was only mild, which may indicate that at this time HCG production was already maximally blocked by the growth factor.

The action of 1-34 PTH is principally cyclic adenosine

monophosphate (cAMP) dependent (Habener *et al.*, 1984). The effect of EGF is principally tyrosine kinase dependent, which is the intracellular domain of the receptor. Recent studies suggest, however, that these two peptides affect each others' receptors (Halevy *et al.*, 1991). Thus a complex interplay between two peptides is taking place in the placenta; the interaction may take place by reciprocal binding or through events that are mediated at subsequent stages to the receptor. The mechanism involved needs to be further investigated. Furthermore, since both EGF and PTH fragments are present locally (Asa *et al.*, 1990), they may have a trophic regulatory paracrine/autocrine role.

Several immunoreactive fragments of PTH have been described in the circulation, including 1-34 PTH and 53-54 PTH (Rosenblatt, 1982). In the sheep placenta these fragments stimulate calcium transport *in vivo* (Rodda *et al.*, 1988); however, in this study we have described an endocrine role. The changing role of growth factors including 1-34 PTH remains to be further investigated.

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