HORMONE-STIMULATED cAMP† PRODUCTION IN HUMAN PLACENTA PERFUSED IN VITRO

J. LEVILLIERS*, E. ALSAT**, Ph. LAUDAT* and L. CEDARD**

** Unité de Recherches sur le Métabolisme des Lipides, U. 35 INSERM*

*** Laboratoire de Chimie Hormonale E.R. 123, CNRS*

and

Faculté de Médecine Cochin-Port-Royal 75014, Paris, France

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1. Introduction

Earlier works have suggested that cAMP would act as a second messenger in the action of gonadotropins on placental metabolism, particularly on oestrogen biosynthesis and glycogenolysis [1].

Adenylate cyclase [2,3] and phosphodiesterase [4] activities have been found in human placenta. The adenylate cyclase is responsive to prostaglandins [5] and catecholamines [3]. The effect of HCG is somewhat controversial; some found no effect [3], others found a significant cAMP accumulation in incubated slices [6,7].

In the present communication we show that LH, HCG, PGE_1 and $PGF_{2\alpha}$ induces a marked increase in the intracellular cAMP concentration of perfused human placenta.

2. Materials and methods

The following chemicals were used: 8-[^{3}H]cyclic AMP (27 Ci/mole) (Radiochemical Centre Amersham, England), cAMP (Calbiochem), beef heart 3′5′-cyclic nucleotide phosphodiesterase (0.45 μunits/mg protein) (Sigma), Indomethacin (Merck, Sharp and Dohme), Nitrocellulose filters (0.45 μm) (Schleicher Schüll, Germany). The protein kinase inhibitor was either prepared according to Gilman [9] or supplied by Sigma. The following products were gifts from: Endopancrine (Paris, Dr Lecoq); HCG, Laboratoires sérobiologiques (Nancy, Dr Pauly); LH (GMM – 40 U/IR P_2, lot 717), Upjohn (Paris); prostaglandins.

2.1. *Perfusion of placenta*

Full term placentas obtained after normal vaginal delivery were perfused within 30 min with 1 litre of a solution containing 9 g NaCl and 2 g glucose, pH 7.4 at 37°C in a closed circuit. Unless otherwise stated the hormone was added at once in the perfusion medium after 20 min equilibration. At the indicated times a piece of approximately 300 mg of tissue was taken off from the same cotyledon, immediately frozen in liquid nitrogen and kept at −80°C until extracted.

2.2. *Purification of protein kinase*

Purification of the protein kinase was performed according to Reimann [8], except that the hydroxylapatite and calcium phosphate steps were omitted. The enzyme was stored frozen at −80°C without loss of cAMP binding activity during more than 18 months.

2.3. *cAMP assay*

The frozen tissue was extracted and assayed according to Gilman [9].

† Abbreviations: cAMP, cyclic 3′5′-adenosine monophosphate; HCG, human chorionic gonadotropin; LH, human luteinizing hormone; PG, prostaglandin.

All determinations were done in duplicate, using different dilutions corresponding to 1–15 mg of placenta. When the amount of tissue was higher than 15 mg per assay an inhibitory effect on the cAMP binding was observed which prevented any estimation. The different substances used in the perfusion have no effect on the cAMP binding assay. In some samples the specificity of the assay was checked by the disappearance, of the substance which competes tes with [^{3}H]cAMP, after hydrolysis by phosphodiesterase.

3. Results and discussion

Fig. 1A shows a two fold increase in cAMP production, obtained 4–6 min after the addition of 40 i.u. LH. The stimulation produced by the introduction of 20 000 i.u. HCG (fig. 1B) is almost equal and occurs at an identical time. The simultaneous addition of 5 mM theophylline potentiated this effect and prevented the rapid decline in the cAMP concentration after the maximum was reached.

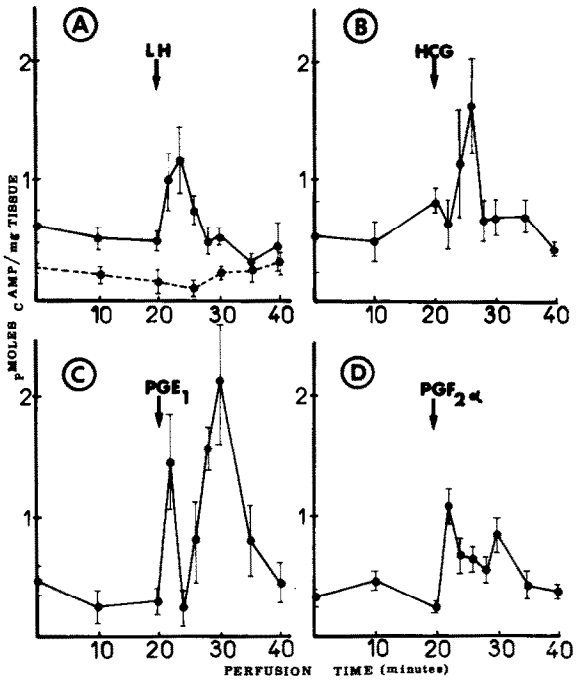

Fig. 1. Time course of cAMP accumulation in human placenta perfused with LH, HCG, PGE_1 and $PGF_{2\alpha}$. After control perfusion (0.9% NaCl, 0.2% glucose) during 20 min, the total amount of hormone was added by flush injection in the recirculating medium at the time indicated by the arrow. cAMP contents expressed as pmoles/mg of wet weight tissue are mean values ±SD of 6–15 determinations on the same sample. A: ——— human LH (40 IU), – – – – – control perfusion; B: HCG (20 000 IU). C: PGE_1 (3×10^{-6} M); D: $PGF_{2\alpha}$ (3×10^{-6} M).

PGE_1 (3×10^{-6} M) elicited a biphasic response (fig. 1C). The first peak occurred 2 min after the introduction of the prostaglandin and the second 8 min later. With the same concentration of $PGF_2\alpha$ we obtained a similar profile although less marked (fig. 1D). At present we have no explanation for the existence of the two peaks.

Although several authors suggested that the action of trophic hormones was mediated through prostaglandins [7,10], no direct evidence could be obtained up to now [11]. In one experiment we also could not suppress the increase in the LH-stimulated cAMP production, by perfusion 20 min before the addition of the hormone, of 50 mg indomethacin (0.15×10^{-3} M), a known inhibitor of prostaglandin biosynthesis. On the contrary the stimulation peak was 3-fold that observed when LH is added alone. The inhibitory effect of indomethacin on the placental cAMP-phosphodiesterase activity cannot account entirely for this stimulation.

Quantitative differences exist in the cAMP concentration in each set of experiments, due to the heterogeneity of the tissue and the physiological state of the placenta, but the shapes of the curves were identical for each perfusion performed in duplicate or triplicate.

The results presented here should be compared to previous findings dealing with the hormone-stimulated production of oestrogen in the perfusion fluid [1]. There is a stricking parallelism between the intensities of the cAMP accumulation in the tissue and the aromatization process elicited by the different substances. In both cases the stimulation by the PGE_1 is markedly higher than that produced by the other hormones. Moreover the duration of the prostaglandin effect on both parameters is longer than that of the gonadotropins, perhaps due to its inhibitory action on the phosphodiesterase (F. Ferré, personal communication).

Simultaneously to the cAMP determinations, in the same perfusion experiments, an activation of the

phosphorylase by these hormones was observed (A. Auguy, L. Cedard, unpublished results). This can explain the increased glucose level in the perfusion fluid, previously measured [1]. It can be assumed that glucose is used, via the pentose pathway, for the production of NADPH necessary to the C_{19}-hydroxylation. These observations can be accounted by the scheme proposed by Haynes et al. [12] and Marsh et al. [13]. This again is in agreement with the similar enhancement in the aromatization process following the addition of a NADPH-regenerating system [1].

All these results support the hypothesis that at least, part of the effects of gonadotropins and prostaglandins on oestrogen biosynthesis are mediated by cAMP.

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