HUMAN PLACENTAL cAMP PHOSPHODIESTERASE ACTIVITY KINETIC PROPERTIES AND SENSITIVITY TO SOME DRUGS AND HORMONES

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1. Introduction

In human full-term placentas, cyclic AMP was demonstrated to be involved in the regulation mechanisms which control the rate of estrogens biosynthesis [1]. The cAMP intracellular concentration which regulates the extent of several physiological phenomena is the result of both enzymatic activities: synthesis and degradation of the cyclic nucleotide.

Previous studies have shown the presence in the human placenta of an adenylate cyclase activity [2] stimulated by different hormones such as catecholamines [3,4] and prostaglandins [5]. An increase of cAMP formation was also demonstrated by [^{14}C]adenine incorporation after addition of HCG [6].

A cyclic AMP phosphodiesterase activity was found in placental homogenates [7], and the present work was undertaken to determine its kinetic properties and its sensitivity to different drugs and hormones.

2. Materials and methods

2.1. *Chemicals*

(8-[^{3}H]) cyclic 3′5′-adenosine monophosphate (spec. act. 27 Ci/mM) and [^{14}C]adenosine (spec. act. 385 mCi/mM) were supplied by CEA Saclay France. Anion exchange resin (AG1-X2 200–400 mesh) was obtained from Bio-Rad. Adenosine 3′5′, cyclic monophosphoric acid (cAMP), adenosine 5′ monophosphoric acid (5′AMP), imidazole, snake venom (*Crotalus atrox*), aluminum oxide (neutral activity grade I), insulin (bovine pancreas) were purchased from Sigma Chemical Co. and other chemicals and drugs from Calbiochem.

Indomethacin, prostaglandins and flufenamic acid were respectively given by Merck Sharp Dohme, Upjohn and Parke-Davis Laboratories.

2.2. *Enzymatic preparation*

The preparation of the placental homogenates was carried out as previously described [2].

Freezing at −20°C is not followed by any detectable loss of enzymatic activity during two months.

2.3. *Determination of cAMP phosphodiesterase activity*

In the two-stage isotopic procedures employed according to Thompson and Appleman [8]: assay I or to Filburn and Karn [9]: assay II, the 5′AMP formed from cAMP is converted into adenosine by the 5′nucleotidase present in the crotalus atrox venom. In both methods, reaction mixture contained 5×10^{-3} M Mg-acetate, 8×10^{-2} M Tris–HCl pH 8.0, 1.5 μCi/ml [^{3}H]cAMP, unlabeled cAMP at various concentrations and enzymatic preparation. At the end of the reaction [^{14}C]adenosine (0.01 μCi/tube) was added as a radioactive tracer. Enzymatic activity was calculated from the conversion of [^{3}H]cAMP into [^{3}H]adenosine corrected by the recovery of [^{14}C]adenosine (about 68% in assay I, 92% in assay II) and substraction of the blank values. All the assays were carried out in linearity conditions with respect to time and protein concentration, allowing measurement of the initial rates of reaction. Identical results were obtained with the two procedures. Proteins were determined by the method of Lowry et al. [10] using bovine serum albumin as standard.

3. Results

Kinetic analysis of cAMP phosphodiesterase activity vs cAMP concentration by double reciprocal plots according to Lineweaver and Burk shows two different slopes (fig.1). In the range of cAMP concentration from 1×10^{-7} M to 2×10^{-5} M and apparent K_m I of $0.97 \pm 0.12\ 10^{-5}$ M and a V_{max} of 629 ± 81 pM/min/mg of protein is obtained corresponding to the high affinity form. In the range of 2×10^{-5} M to 5×10^{-4} M cAMP a second series of constants is determined revealing a second form of enzymatic activity having a lower affinity: K_m II = $1.36 \pm 0.3 \times 10^{-4}$ M, V_{max} = 1752 ± 360 pM/min/mg of proteins.

In different tissues, cAMP phosphodiesterase activity is stimulated by Mg^{2+}. In placental homogenates the concentration of this ion determined by atomic absorption method is 0.044 ± 0.013 mg/g of wet tissue. Therefore in our experimental conditions of incubation the endogenous Mg^{2+} concentration is in the 10^{-4} M range. This fact can explain that without any addition of Mg^{2+} half of the full activity is expressed. Fig.2A shows that the two forms of phosphodiesterase activity requires additions of 5×10^{-3} M Mg^{2+} in the incubation medium to reach maximum activation.

1×10^{-2} M imidazole stimulates the low affinity form about 35% but has no significant effect upon the high affinity form (fig.2B). Theophylline is found to be a non-competitive inhibitor of the high affinity form: $K_m = 7.1 \times 10^{-4}$ M, while it appears to be a competitive one for the low affinity form: $K_i = 3.6 \times 10^{-3}$ M (fig.3). Caffeine is somewhat less effective and inhibits non-competitively the two enzymatic activities. K_i values determined for both low range and high range concentrations of substrate are respectively 2.7×10^{-3} M and 5.2×10^{-3} M (fig.4).

Prostaglandins are weak inhibitors of cAMP phosphodiesterase activity (table 1). PGE_1 is more potent than PGE_2 and the effect of $PGE_{2\alpha}$ is not significant. The percentage of PGE_1 inhibition begins to increase from 1×10^{-9} M to 1×10^{-8} M, does not change between 1×10^{-8} M and 1×10^{-6} M and increases again till 1×10^{-3} M. Flufenamic acid, indomethacin and progesterone have a strong inhibitory action; on the contrary estradiol 17β is less effective. The sensitivity of the enzyme, especially to PGE,

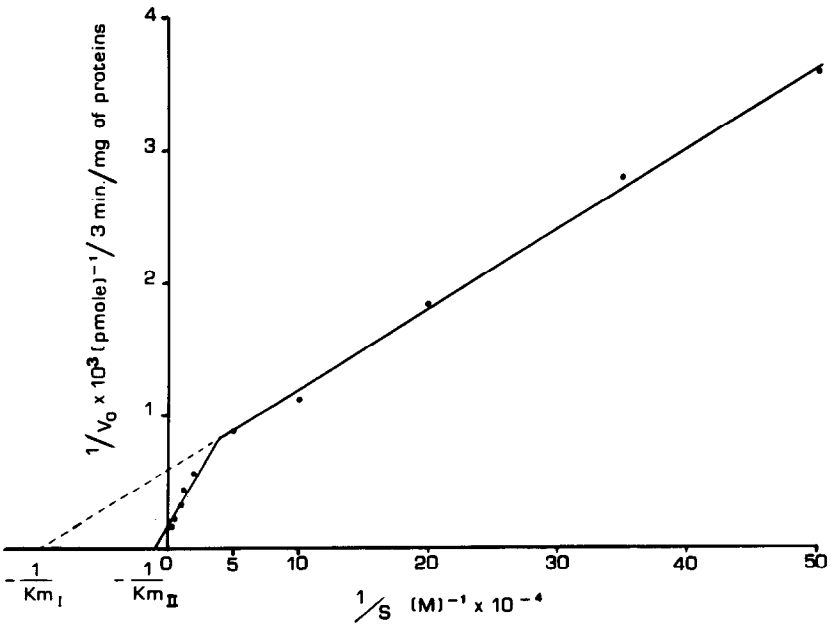

Fig.1. Kinetic analysis by double reciprocal plot of cAMP phosphodiesterase activity in human placental homogenates.

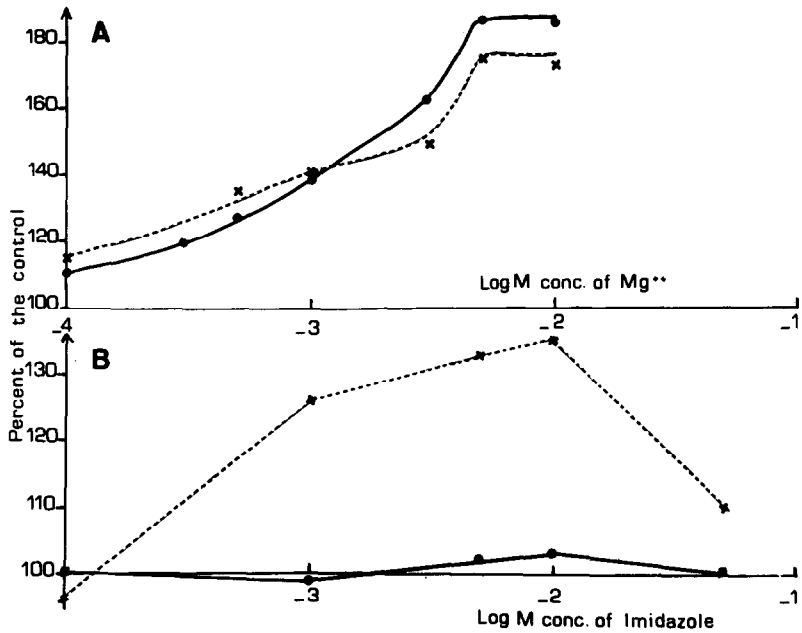

Fig. 2. Effect of Mg^{2+} (A) and imidazole (B) on human placental cAMP phosphodiesterase activity; on high affinity conditions: 8×10^{-6} M cAMP (●——●) and low affinity conditions: 4×10^{-4} M cAMP (X - - - X). Results are expressed in 100% of activity measured in the absence of added compound.

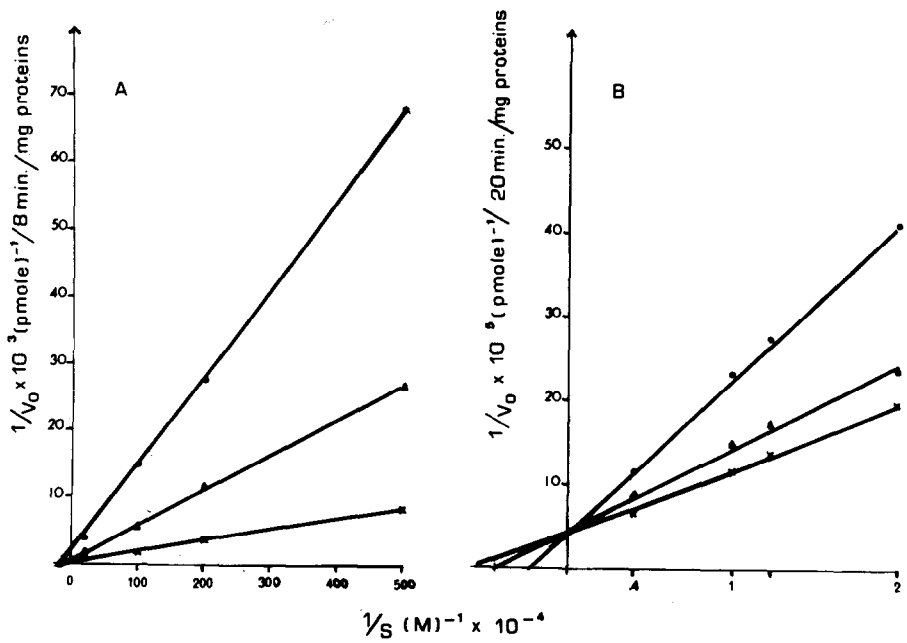

Fig. 3. Inhibition of cAMP phosphodiesterase activity by theophylline in high (A) and low (B) affinity conditions. The concentrations of theophylline used are (X——X) none, (▲——▲) 1×10^{-3} M theophylline, (●——●) 5×10^{-3} M theophylline.

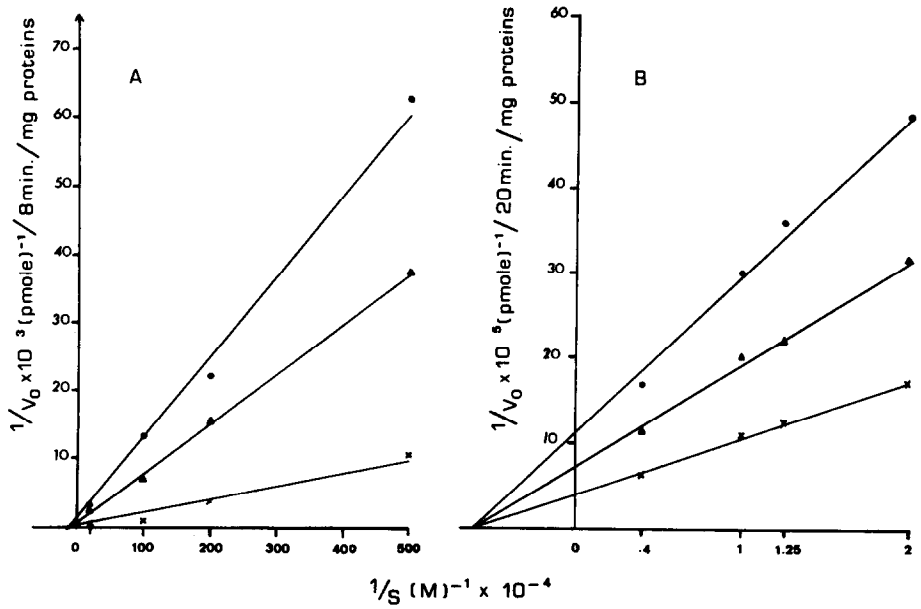

Fig.4. Inhibition of cAMP phosphodiesterase activity by caffeine in high (A) and low (B) affinity conditions. The concentrations of caffeine used are (X——X) none, (▲——▲) 4 X 10^{-3} M caffeine, (●——●) 1 X 10^{-2} M caffeine.

flufenamic acid, indomethacin and progesterone, is more important at low substrate concentration corresponding to the high affinity activity.

L-epinephrine (1 X 10^{-4} M – 1 X 10^{-3} M), norepinephrine (1 X 10^{-4} M – 1 X 10^{-3} M), FNa (1 X 10^{-8} M to 1 X 10^{-3} M) and insulin (180 μU/ml to 1800 μU/ml) were tested and do not exhibit any inhibitory effect of both enzymatic activities.

Table 1
Effect of some inhibitors of placental cAMP phosphodiesterase activity at low and high substrate concentration

Additions	Concentrations	Enzyme activity (% of the control)	
		Low K_m conditions 8 X 10^{-6} M cAMP	High K_m conditions 5 X 10^{-4} M cAMP
PGE_1	10^{-5} M	78 ± 5	89 ± 4
	10^{-3} M	68 ± 4	80 ± 5
PGE_2	10^{-3} M	86 ± 5	90 ± 6
$PGF_{2\alpha}$	10^{-3} M	95 ± 7	96 ± 9
Flufenamic acid	10^{-3} M	39 ± 4	76 ± 5
Indomethacin	10^{-3} M	34 ± 4	64 ± 7
Progesterone	10^{-4} M	66 ± 3	72 ± 4
	10^{-3} M	48 ± 5	65 ± 5
Estradiol 17β	10^{-4} M	84 ± 3	85 ± 2
	10^{-3} M	74 ± 5	82 ± 3

Each value represents the mean ± SD of duplicate experiments in four different placentas.

4. Discussion

Kinetic analysis of the cAMP hydrolysis has shown in placental homogenates, as in many other organs, the presence of two cAMP phosphodiesterase activities. This suggests a regulation depending of the substrate concentration. But, it is also possible that there are several types of enzymes different by kinetic parameters. The placental homogenates contain several types of cells which possess perhaps different cAMP phosphodiesterase. An other explanation would be a different regulation of the part of activity which has been demonstrated to be associated with particulate fractions [7].

Both low and high affinity phosphodiesterase activities require Mg^{2+} but imidazole stimulates only the low affinity form.

A complex inhibition by theophylline similar to that we observed was found by Schonhofer in isolated fat cells [11]. Our results show that caffeine acts non-competitively at low and high substrate concentration. The high affinity activity is more sensitive to inhibitory effect of methylxanthines, prostaglandins, flufenamic acid, indomethacin and progesterone and is probably more responsible of physiological and pharmacological events during pregnancy.

The weak inhibitory effect of prostaglandins of the E series [12] is of great interest with regard to the results of Satoh and Ryan [5] who found a stimulatory action of those compound on placental adenylate cyclase activity. The intra-tissular increase of cAMP level obtained during placental perfusion experiments, after addition of PGE_1 [13] is therefore the consequence of at least two different effects on the enzymes responsible of formation and breakdown of the cyclic nucleotide. The inhibition of placental cAMP phosphodiesterase activity by indomethacin and flufenamic acid which play a role in the synthesis or/and the action of prostaglandins is similar to that observed in bovine heart preparation [14] and could explain several paradoxal effects obtained with those compounds [15].

Acknowledgements

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