# Prostaglandins and Their Effects on Human Placental Adenyl Cyclase

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ABSTRACT Prostaglandins increased adenyl cyclase activity in human term placental homogenates in a dose-dependent manner during 10-min incubation periods. The potency of the prostaglandins examined was demonstrated to be in the ascending order, prostaglandin $F_{1\alpha} < A_2$, $F_{2\alpha}$, $B_2 < A_1 < E_2 < E_1$. Although no specific trophic or regulating factors for placental function have been described as yet, it is possible that prostaglandins which are synthesized in decidual tissue could play such a physiological role.

## INTRODUCTION

We have observed that the human term placenta has an adenyl cyclase system which can be stimulated by catecholamines and fluoride, but not by human chorionic gonadotropin (1). Much interest has recently been focused on the relationship between prostaglandins and the ubiquitous adenyl cyclase system which is responsible for the formation of adenosine 3',5'-cyclic monophosphate (cyclic AMP) (2). The purpose of the present study was to test the effects of prostaglandins on this newly recognized placental adenyl cyclase.

## METHODS

The enzyme system was prepared as described elsewhere (1). Briefly, human term placental tissue (2.5 g) was homogenized in 10 vol of 0.25 M sucrose in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 3000 *g* for 10 min at 0°C. The sediment was washed once with 10 vol of 0.25 M sucrose and centrifuged at 6000 *g* for 10 min at 0°C. The pellet was suspended in 4 vol of 0.25 M sucrose. This suspension was used as the enzyme preparation. The incubation mixture for measuring adenyl cyclase activity consisted of 42 mM Tris HCl (pH 7.4), 1 mM [8-$^{14}$C]-adenosine triphosphate (ATP) (1 $\mu$Ci, approximately 1.6 $\mu$Ci/$\mu$mole), 3.3 mM $MgSO_4$, 5 mM aminophylline (equivalent to 10 mM theophylline), 10 mM phosphocreatine, 0.17 mg/ml creatine phosphokinase, 0.1% w/v bovine serum albumin, 100 $\mu$l of the enzyme preparation (derived from 25 mg of placental tissue), and one of the prostaglandins in a total volume of 600 $\mu$l. Incubations were continued for 10 min at 30°C in air in a metabolic shaker and terminated by immersion in a boiling water bath for 3 min. Determination of the labeled cyclic AMP formed was carried out according to the method of Krishna, Weiss, Brodie, and Birnhaumer (3, 4).

The identity of the labeled enzymatic reaction product as 3',5'-cyclic monophosphate was confirmed by chromatography on Dowex 50-$H^+$ column, isolation from the supernatant fluid after treatment with $BaSO_4$-$Zn(OH)_2$, conversion by an adenosine 3',5'-cyclic nucleotide diesterase to $^{14}$C-5'-AMP with retention of constant specific activity throughout.

Placental adenyl cyclase activity has been shown to be maximally stimulated by NaF with linearity of response for 15 min (1). Utilization of an ATP-generating system provided optimal conditions for testing prostaglandin effects in this study over the 10 min incubation time. Statistical evaluation was by the Student's *t* test. A variety of prostaglandins were utilized ($PGE_1$, $PGE_2$, $PGA_1$, $PGA_2$, $PGF_{1\alpha}$, $PGF_{2\alpha}$, and $PGB_2$), dissolved in 95% ethanol and 10 $\mu$l each of the solutions was added to the incubation medium. The incubation mixture with prostaglandins was equivalent to 1.6% v/v ethanol solution and the range of each prostaglandin tested was from $2.7 \times 10^{-8}$ M up to $2.8 \times 10^{-4}$ M at an interval either of 10- or 5-fold increase in its concentration.

## RESULTS

Fig. 1 shows the stimulatory effects of different concentrations of prostaglandins on the human placental adenyl cyclase system. A dose response of adenyl cyclase activity to the prostaglandins was clearly demonstrated. In $PGE_1$-treated samples there was no significant difference between the control activity and that after treatment with $2.8 \times 10^{-8}$ M $PGE_1$. (The control refers to the adenyl cyclase activity measured with 1.6% v/v ethanol, i.e., 331 ±14.6 pmoles/25 mg of tissue per 10 min. The value represents the mean ±standard error of four replicate determinations.) As the dose of $PGE_1$, however, was increased from $2.8 \times 10^{-7}$ M to $1.1 \times 10^{-4}$ M, there were significant increases in adenyl cyclase activity over the control ($P < 0.001$). The other prostaglandins were active only at higher concentrations. Increasing amounts of $PGE_2$ from $2.7 \times 10^{-6}$ M to $1.2 \times 10^{-4}$ M were shown to be significantly effective over the

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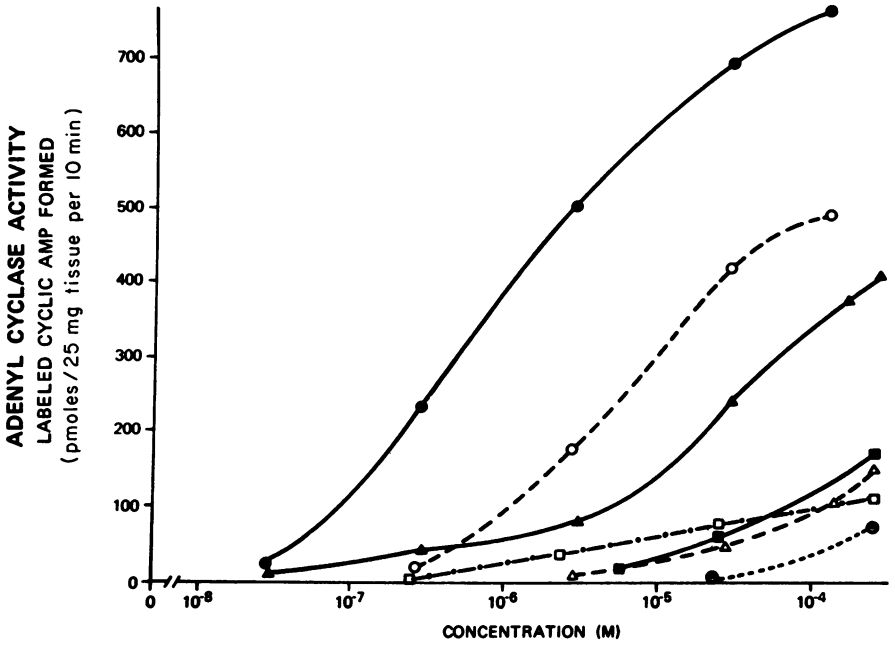


FIGURE 1 Dose response of adenyl cyclase activity to $PGE_1$ (●), $PGE_2$ (○), $PGA_1$ (▲), $PGF_{2\alpha}$ (△), $PGA_2$ (□), $PGB_2$ (■), and $PGF_{1\alpha}$ (⊙) in human term placental homogenate. Each point is the mean either of two or three duplicate determinations.

control ($P < 0.001$). With $PGA_1$-treated samples responses were detected at concentrations from $2.9 \times 10^{-6}$ M to $2.8 \times 10^{-4}$ M ($P < 0.01$). $PGA_2$ and $PGB_2$ were demonstrated to be significantly effective on adenyl cyclase activity at concentrations from $2.5 \times 10^{-5}$ M to $2.5 \times 10^{-4}$ M ($P < 0.01$) and $PGF_{2\alpha}$ at concentrations from $1.4 \times 10^{-4}$ M to $2.5 \times 10^{-4}$ M activated the enzyme system significantly over the control ($P < 0.01$). In comparison to the other prostaglandins tested here, $PGF_{1\alpha}$ seemed to be least active in stimulating the enzyme system. This compound produced a slight but significant stimulation at $2.3 \times 10^{-4}$ M ($P < 0.05$).

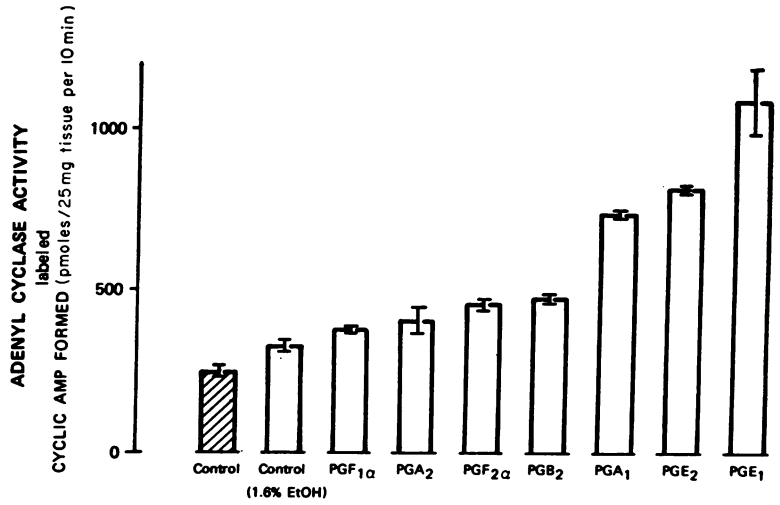


FIGURE 2 The stimulation of adenyl cyclase in human term placental homogenate either at the highest concentration of each prostaglandin examined or by 1.6% ethanol. Open and hatched columns represent adenyl cyclase activity in the presence and absence of 1.6% ethanol, respectively. The results are the mean ±SE of two or four duplicate observations.

The relative potency of the prostaglandins tested was calculated in the same manner as used by Murad, Chi, Rall, and Sutherland (5). The relative potencies of the seven prostaglandins as shown in Fig. 1 were: $PGE_1$, 1000; $PGE_2$, 60; $PGA_1$, 10; $PGB_2$, $PGF_{2\alpha}$, $PGA_2$, 1.0; and $PGF_{1\alpha}$, 0.2. The adenyl cyclase activity observed either at the highest concentration of each prostaglandin examined or by 1.6% ethanol is shown in Fig. 2. We have demonstrated that ethanol (3.2%) activated placental adenyl cyclase (1) after 30 min incubation, as previously reported for liver adenyl cyclase (6). As shown in Fig. 2, ethanol at a concentration of 1.6% increased adenyl cyclase activity during 10 min by a statistically significant amount ($P < 0.001$).

## DISCUSSION

There have been several reports describing the effects of prostaglandins on adenyl cyclase and cyclic AMP content in various endocrine tissues. In the rat ovary $PGE_1$ and $PGE_2$ had the same potency to maximally stimulate cyclic AMP formation, although the accumulation of the nucleotide at low concentrations was greater for $PGE_2$ (7). Prostaglandins at a concentration of 20 $\mu$g/ml appeared to increase rat anterior pituitary cyclic AMP concentration in the order of ascending potency, $PGF_{1\alpha} < PGA_1$, $PGB_1 < PGE_1$ (8). In thyroid tissue, $PGE_1$ and $PGE_2$ were shown to have the ability to elevate cyclic AMP levels, whereas $PGB_1$ and $PGF_{1\alpha}$ seemed to be inactive at 10 $\mu$g/ml (9, 10). As far as the action of prostaglandins on steroid synthesis is concerned, the order of ascending potency, $PGA_1 < PGF_{2\alpha} < PGE_1 < PGE_2$ was observed in bovine corpus luteum (11) and $PGE_2$ was also reported to be more potent than $PGE_1$ and $PGF_{2\alpha}$ in rat adrenal steroidogenesis (12). Prostaglandins appear to have different actions in vivo and in vitro, as was demonstrated with regard to a luteolytic activity in vivo (13) and a steroidogenic effect in vitro by $PGF_{2\alpha}$ on rat ovaries (14). With respect to pregnancy $PGF_{2\alpha}$, $PGE_1$, and $PGE_2$ have been used successfully for induction of labor and therapeutic abortions (15–18), although the mechanism for this is unknown.

The blood vessels of the human umbilical cord and term placenta in addition to amniotic fluid and decidua were found to contain $PGF_{1\alpha}$, $PGF_{2\alpha}$, $PGE_1$, and $PGE_2$ (19). Although no data are thus far available on a physiological role of prostaglandins in the placenta, it is suggested from the present study that prostaglandins may act as regulators of adenyl cyclase and could, accordingly, be involved in a control mechanism of placental function.

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