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HORMONE- AND FLUORIDE-SENSITIVE ADENYLATE CYCLASES IN HUMAN FETAL TISSUES

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SUMMARY

Adenylate cyclase activities have been assayed in the human fetal adrenal, heart ventricle, brain, liver, testis, kidney, skeletal muscle and lung during the first trimester of pregnancy. The requirements for adenylate cyclases are similar to those reported in all adult tissues. Of all tissues studied, heart ventricle had the highest level of enzymatic activity, and this tissue was most responsive to hormonal stimulation. Although adenylate cyclases from all of these tissues were stimulated by F^- *in vitro*, hormonal stimulation was observed only in the liver, adrenal and heart ventricle. The presence of hormone-responsive adenylate cyclase in human fetal tissues suggests that cyclic AMP may be involved in gene expression.

INTRODUCTION

Development of an organism from fertilization to maturity is characterized by an orderly progression of enzyme protein formation, which eventually leads to acquisition of the complete metabolic machinery characteristic of differentiated tissue. In fetal rat liver, the enzymes responsible for glucose oxidation and amino acid metabolism (*e.g.* tryptophan oxygenase, tyrosine aminotransferase, and glucose-6-phosphatase) are absent but rise postnatally to adult levels[1–4]. It has been shown that the formation of tyrosine aminotransferase and glucose-6-phosphatase can be induced prematurely by the administration of glucagon to fetal rats. Injection of dibutyryl cyclic AMP directly into fetal rats also causes premature formation of the above enzymes[5]. From these experiments, it was inferred that cyclic AMP is a mediator of glucagon action in the fetal rat liver, which in turn causes premature appearance of amino acid metabolizing enzymes.

Since cyclic AMP has been shown to play a role in the differentiation of hepatic enzymes in the fetus, it was of interest to investigate the presence of hormone-sensitive adenylate cyclases in developing human fetal tissues. The present investigation of

Abbreviations: HCG, human chorionic gonadotropin; ACTH, adrenocorticotropic hormone; LH, luteinizing hormone.

adenylate cyclases was undertaken to determine (a) if human fetal tissues at early stages of differentiation possess these enzymes, (b) *in vitro* requirements for their activity and (c) their responsiveness to appropriate hormones and to F^-.

MATERIALS AND METHODS

Human tissues

Three human fetuses of 12, 13, and 12 weeks estimated gestational age were obtained at the time of hysterotomy performed for psychosocial reasons. The tissues were removed and immediately frozen in an alcohol–solid CO_2 mixture and subsequently stored at -80 °C until assayed.

Chemicals

[U-^{14}C]ATP was purchased from New England Nuclear Corp. Pyruvate kinase and cyclic AMP were purchased from Sigma Chemical Co. and phosphoenol pyruvate was obtained from Cal Biochem. Luteinizing hormone (LH) (N.I.H.-LH-B7) was generously donated by the Endocrine Study Section, National Institutes of Health. Human chorionic gonadotropin (HGG) (Antuitrin "S"), adrenocorticotropic hormone (ACTH) and epinephrine were purchased from commercial sources. All other chemicals were conventional commercial products.

Preparation of enzyme

Fetal tissues were homogenized in 5 vol. of 0.01 M Tris–HCl, pH 7.5 for 1 min at 0 °C in a glass homogenizer with a teflon pestle using a TRI-R motor. The homogenates were strained through four layers of cheesecloth. These preparations were assayed within 10 min.

Assay of adenylate cyclase

The enzyme assays were performed by the procedure described by Drummond and Duncan[6]. The assay mixture contained the following substances in a final volume of 150 μl: 1 μCi of [U-^{14}C]ATP (16 Ci/mole), 14 mM $MgCl_2$, 35 mM Tris–HCl, pH 7.5, 8.0 mM theophylline, 7 mM KCl, 7 mM KF, 18 mM phosphoenolpyruvate, 3.0 units of pyruvate kinase and 2 mM cyclic AMP. In those experiments in which hormonal responses were tested, the incubation mixture did not contain F^-. After the incubation (usually 15 min), the tubes were heated in a boiling water bath for 3 min and then centrifuged for 5 min at 4000 rev./min in a Sorvall centrifuge. The supernatant solution (100 μl) was then applied to Whatman No. 40 filter paper and chromatographed in 1 M ammonium acetate (pH 7.5)–ethanol (3 : 7, by vol.) for 16 h at room temperature. Authentic samples of ATP, ADP, AMP, cyclic AMP, adenosine and inosine were chromatographed parallel to the incubation mixture. After drying the chromatograms, the areas corresponding to cyclic AMP were marked on the chromatograms, cut out and placed in scintillation vials. The radioactivity on the chromatograms was determined by liquid scintillation spectrometry after the addition of 15 ml of scintillation fluid (4 g of PPO and 50 mg POPOP in 1 l toluene).

Characterization of cyclic [^{14}C]AMP

For characterization purposes, the areas on chromatograms corresponding to cyclic AMP were eluted with water[7] and then lyophilized. The residues were then

dissolved in water and incubated with cyclic nucleotide phosphodiesterase as described by Butcher and Sutherland[8]. The incubation mixtures were then chromatographed in the same system as described for the adenylate cyclase and the areas corresponding to AMP were then eluted and the radioactivity determined.

RESULTS

Requirements for the enzyme

Like the adenylate cyclases from other sources[6,9-12], those derived from fetal tissues required Mg^{2+} for activity (Table I). Mn^{2+} also had a similar effect. The Mg^{2+} concentration curve for adenylate cyclase in fetal heart is shown in Fig. 1A. The adenylate cyclases from human fetal tissues were also stimulated by F^-. The concentration curve for F^- stimulation in human fetal heart is shown in Fig. 1B.

TABLE I

REQUIREMENTS FOR Mg^{2+} FOR *IN VITRO* ADENYLATE CYCLASE ACTIVITY IN HUMAN FETAL TISSUES

Experimental conditions are described in text. Data presented are from tissues of one fetus. The data from the other two fetuses were similar.

Tissue	*Mg^{2+} addition**	*Enzymic activity***
Heart ventricle	−	6
	+	800
Liver	−	3
	+	100
Adrenal	−	4
	+	138
Brain	−	6
	+	209
Testis	−	4
	+	156
Skeletal muscle	−	4
	+	93
Lung	−	6
	+	132
Kidney	−	4
	+	132

* All incubations were performed in the presence of F^-. When Mg^{2+} was present, the concentration was 17 mM.

** Enzymic activity expressed as pmoles of cyclic AMP formed per min per mg protein. The identity of cyclic AMP was established by the procedure described in the text.

Effect of hormones

Since adenylate cyclases from adult mammalian tissues are responsive to a wide variety of protein, polypeptide and neurohormones, the influences of some of these stimulants were tested in appropriate human fetal tissues. Since the amount of available tissue from each fetus was too small to permit replicate determinations

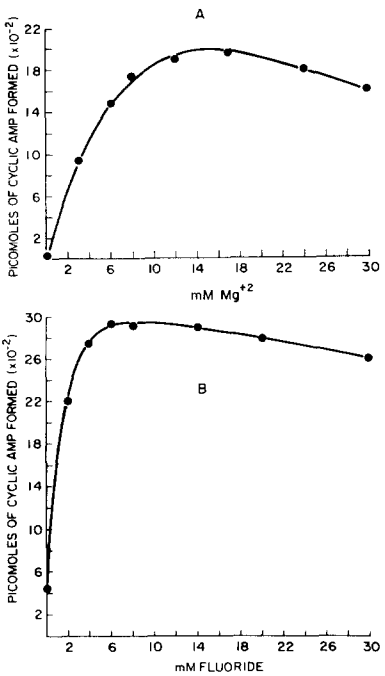

Fig. 1. (A) Effect of Mg^{2+} concentration on adenylate cyclase activity in human fetal heart ventricle at 12 weeks of gestation. (B) Effect of F^- on adenylate cyclase activity in human fetal heart ventricle at 12 weeks gestation.

statistical analysis was precluded. The findings from three individual fetuses are described.

Human fetal heart adenylate cyclase was responsive *in vitro* to both epinephrine and glucagon in all three fetuses studied as shown in Fig. 2. The extent of

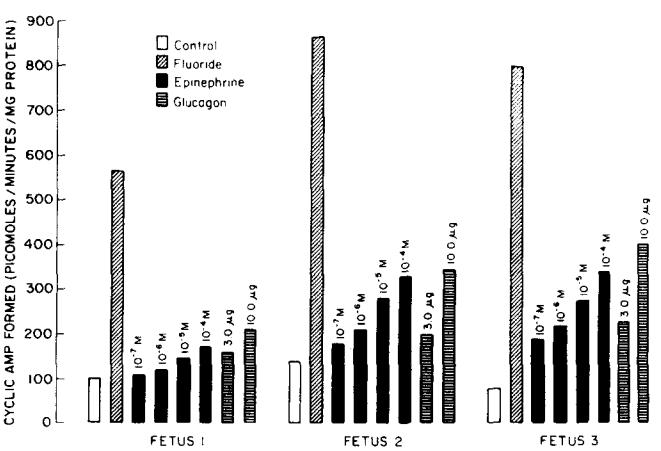

Fig. 2. Effects of F^-, epinephrine and glucagon on adenylate cyclase activities in heart ventricles of three human fetuses.

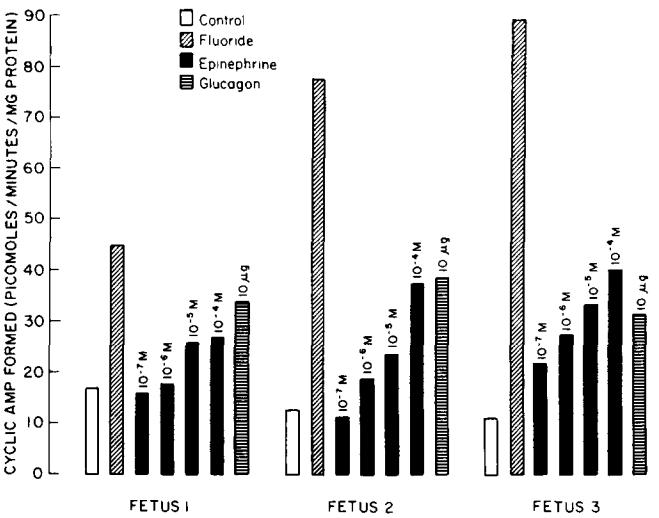

Fig. 3. Effects of F^-, epinephrine and glucagon on adenylate cyclase activities in liver from three human fetuses.

stimulation by epinephrine was a function of the added hormone. Maximal stimulation of adenylate cyclase was observed at 10^{-4} M. The extent of stimulation caused by F^- was far greater than that caused by maximal concentrations of hormones.

Fetal liver adenylate cyclases from the three fetuses were responsive to glucagon and epinephrine as shown in Fig. 3. Again, the effect of F^- was greater than that produced by the hormones. Fetal adrenals were responsive to ACTH, and this effect

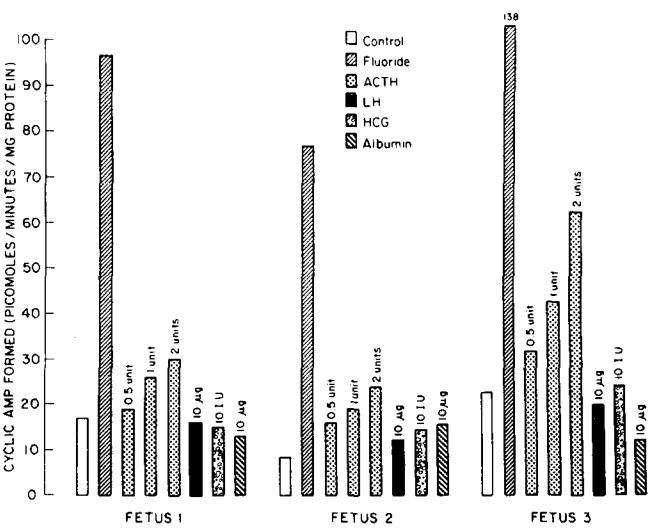

Fig. 4. Effects of ACTH and other substances on the adenylate cyclase activities in adrenals from three human fetuses.

was concentration dependent and specific as LH, HCG and albumin were without any effect (Fig. 4).

The adenylate cyclases from testis, lungs, skeletal muscle, kidney and brain were not stimulated by the *in vitro* addition of hormone. The hormones tested were LH and HCG in the testis, epinephrine in lungs, vasopressin in kidney, catecholamines and glucagon in skeletal muscle, and neurohormones in the brain. The areas of brain studied were cerebrum, cerebellum and medulla oblongata. There were no significant differences in the adenylate cyclase activities in these different areas of brain. All these tissues were responsive to F^-.

DISCUSSION

This study has been aimed at understanding developmental aspects of adenylate cyclase in human fetal tissues. Attention was focused on the earliest periods in human fetal growth at which tissues were morphologically differentiated and sufficient amounts of tissue for performance of the assays could be identified and obtained.

One interesting aspect of the study is the close similarity in the requirements for adenylate cyclase with respect to the concentrations of F^- and Mg^{2+} in the human fetus and tissues from other species. The data presented here are in agreement with the findings of Drummond and Duncan[6] for guinea pig ventricle and Menon and Smith[12] for salmon testis.

Of all the fetal tissues studied, the heart ventricle had the highest levels of adenylate cyclase activity. Additionally, the heart ventricle was the most responsive tissue to catecholamine stimulation. Fetal liver was responsive to both glucagon and epinephrine. The adenylate cyclases from fetal adrenal were stimulated by ACTH and this effect appeared specific, as HCG, LH and albumin were without any effect. By this stage of gestation, the human fetal adrenal plays a key role in feto-placental steroidogenesis. The observation that ACTH stimulated adenylate cyclase suggests establishment of the pituitary–adrenal axis by this stage of development. The adenylate cyclase from skeletal muscle was only slightly responsive to catecholamines (8 %). This is in contrast to the adult in which several hormones are reported to stimulate cyclic AMP production in this tissue. Catecholamines did not stimulate adenylate cyclase in the various areas of fetal brain studied. It has been reported that a homogenate from brain tissue was not stimulated by catecholamines[13], but a slice preparation readily responded to these hormones[14–16]. We have not studied possible hormonal influence in a slice preparation of brain. Adenylate cyclase from fetal testis did not respond to LH. As testosterone can be synthesized *de novo* in the human fetal testis by this gestational age[17], and as testicular androgen is important in sexual differentiation, failure to observe activation of adenylate cyclase in the testes is of interest. Whether this is because the fetal testis is functioning autonomously at this stage of gestation, or whether it is due to an artifact of the incubation system used, awaits further elucidation. Furthermore, oxytocin did not stimulate adenylate cyclase in the fetal kidney and epinephrine was ineffective in lungs. These findings stand in contrast to those in adult tissues of other species.

Adenylate cyclases from all the fetal tissues responded to F^- and in those experiments in which hormones did activate adenylate cyclases, the extent of stimulation caused by F^- was always greater than the hormonal responses. Schmidt and

Robison[18] reported that the adenylate cyclase from rat brain was stimulated by F^- only 10 days after birth but not in the fetus, nor in earlier postnatal life. This situation is in contrast to the F^- responsiveness of adenylate cyclase observed in the human fetal brain.

The presence of adenylate cyclase in human fetal tissues, especially their activation by hormones, may have important biological ramifications. The presence of adenylate cyclase suggests that cyclic AMP may control developmental processes such as gene expression[5]. The hormonal responsiveness in the heart, adrenal and liver can be interpreted as an indication that the hormone recognition site (discriminator) may be present in these tissues from early stages in fetal development.

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