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Research Article

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Effects of Melanotropic Peptides on Fetal Adrenal Gland

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ABSTRACT Adrenal glands from early, mid, and late fetuses of rabbit, guinea pig, and rat, and from newborn animals of each species, were incubated for 1–4 h with and without 0.1 nM–1 μM ACTH, α- or β-melanocyte-stimulating hormone (αMSH or βMSH). The effects of the peptides were measured on production of glucocorticoids, and on incorporation of labeled thymidine or leucine into DNA or protein, respectively. The findings were similar in all three species. ACTH stimulated synthesis of glucocorticoids throughout fetal life. Potency increased progressively, as reflected by declining minimal effective dose and rising maximal response. In early and mid fetus αMSH and βMSH caused a modest glucocorticoid steroidogenic effect. ACTH and αMSH stimulated DNA and protein synthesis in the early and mid fetal gland. αMSH was more potent than ACTH in these respects, minimal effective dose being generally 10 times less and maximal response 25–200% greater. The effects diminished or disappeared in the late fetal and newborn gland. These data indicate that α- and βMSH possess steroidogenic or growth-promoting properties, or both, for the fetal adrenal gland.

INTRODUCTION

In the adult mammal, ACTH is the specific tropic hormone for the adrenal cortex. α- and β-melanocyte-stimulating hormone (MSH),[1] which are structurally related to ACTH, have only 0.0001% the steroidogenic potency of the latter peptide on the adrenal gland of the adult rat (1, 2).

Challis and Torosis (3) reported that in utero injection of αMSH, but not ACTH, in rabbit fetuses caused a rise in plasma cortisol. As gestation proceeded, plasma cortisol responsiveness to ACTH appeared. Challis and Torosis suggested that αMSH might be the principal hormonal stimulus for adrenal glucocorticoid production in early fetal life. The suggestion is supported by the anatomic prominence of the intermediate lobe in the fetal hypophysis (4).

In the present study, we have investigated the postulated corticotropic function of the MSH in the fetus by measuring the in vitro glucocorticoid steroidogenic potency of ACTH, αMSH, and βMSH on the adrenal gland of fetal rat, rabbit, and guinea pig. In addition, we considered the possibility that the MSH could stimulate other processes besides glucocorticoid production in the fetal glands. In the adult adrenal cortex, ACTH stimulates not only steroidogenesis, but also the synthesis of DNA, RNA, and protein,[2] the breakdown of glycogen, and the oxidation of glucose (9–12). Accordingly, we measured the effect of ACTH, αMSH, and βMSH on the incorporation by fetal adrenals of thymidine and leucine into DNA and protein, respectively. For comparative purposes, other tissues were similarly studied.

METHODS

Hormones

Synthetic preparations of $ACTH_{1-24}$, αMSH and βMSH were obtained from Bachem Fine Chemicals, Torrance, Calif.; they appeared homogeneous by high-pressure liquid chromatography (13). Trypsinized preparations of each peptide, containing <0.1% residual activity by corticotropic or melanotropic assay, were prepared as described previously (14). Synthetic oxytocin and arginine vasopressin were purchased from Sigma Chemical Co., St. Louis, Mo. Purified bovine luteinizing hormone (LH), follice-stimulating hormone (FSH) and growth hormone (GH) were provided by Dr. L. Reichert and Dr. A. E. Wilhelmi (Emory University).

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[1] *Abbreviations used in this paper*: FSH, follice-stimulating hormone; GH, growth hormone; KRBG, Krebs-Ringer bicarbonate buffer plus 200 mg/100 ml glucose; LH, luteinizing hormone; MED, minimal effective dose; RMax, maximal response.

[2] ACTH stimulates adrenal cortical synthesis of these components in vivo. When adrenal cortical tissue is exposed to ACTH in vitro, synthesis of DNA and protein is sometimes inhibited by the local accumulation of glucocorticoids (5–8).

Animals

Female adult albino New Zealand rabbits, Sprague-Dawley rats, and albino guinea pigs were fed ad lib. with Purina rabbit chow, rat checkers, or guinea pig chow (Ralston Purina Co., St. Louis, Mo.), respectively. On specific days they were mated with males of the same strain. The pregnant females were sacrificed by intravenous administration of nembutal (rabbit) or by decapitation (rat and guinea pig) according to the schedule shown in Table I to obtain early, mid, or late fetuses as defined in that table. The fetuses were removed from the uterus and immediately entered into the experiments described below. Newborn animals were studied within 24 h after birth.

Experiments

Preparation of tissues. A litter of four to eight fetuses or newborns were decapitated and the adrenals, liver, kidney, or brain, depending on the experiment, were removed. Except for adrenals, the organ was sliced with a McIlwain chopper (Brinkmann Instruments, Inc., Westbury, N. Y.) set at 0.5 mm; 5–10 mg was placed in 1 ml incubation medium with or without specified dose of hormone. Composition of incubation medium and conditions of incubation depended on the assay (steroidogenesis; DNA or protein synthesis; see below for details). In experiments with adrenals, quartered glands were incubated.

Assay for steroidogenesis. The experiments were done with adrenal glands according to Saffran and Bayliss (15). (Initially we tried the isolated adrenal cell method [2], but could not collect enough free cortical cells from fetal glands to perform the assay.) Adrenals were removed, quartered, and placed in 1 ml of Krebs-Ringer bicarbonate buffer plus 200 mg/100 ml glucose (KRBG) in a polystyrene culture tube. The samples were incubated for 30 min at 37°C. Medium was then aspirated and fresh KRBG (1 ml) containing 0 or 0.1 nM to 1 μM concentration of peptide hormone was added. The tubes were incubated again for 2 h at 37°C. All incubations were carried out in an atmosphere of 95% O_2 and 5% CO_2. After incubation, the medium was removed and extracted by vigorous vortexing with 3 ml methylene chloride for 1 min. Extract was dried under air at 25°C, and analyzed for corticosterone (rabbit and rat) or cortisol (guinea pig) by radioimmunoassay after chromatography according to Vagnucci et al. (16) or Ruder et al. (17), respectively. (These assays were performed at Interscience Institute, Los Angeles, Calif.)

Incorporation of thymidine into DNA. The effect of hormones on incorporation of labeled thymidine into DNA of various organs was measured according to Claycomb (18). Experimental details were as follows. Tissue slices (5–10 mg) or quartered adrenals were suspended in 1 ml of KRBG, and incubated in a metabolic shaker for 2 h at 37°C with 0 or 0.1 nM–1 μM concentration of peptide hormone. After the incubation, the samples were washed twice with 0.15 M NaCl, the supernatant fluid being discarded each time. The samples were resuspended in 1 ml KRBG containing 0.1 μmol 6-[^{3}H]thymidine (5 mCi/μmol sp act) and incubated for 2 more h at 37°C. The samples were then centrifuged at 4,000 *g* for 20 min at 3°C: the supernate was discarded, and residue was resuspended with 2 ml 0.15 M NaCl and homogenized in a Polytron homogenizer (Brinkmann Instruments, Inc.). An equal volume of ice-cold 10% perchloric acid was then added to the homogenate. Samples were mixed well and centrifuged at 4,000 *g* for 20 min. The acid-insoluble residue was washed twice with ice-cold 5% perchloric acid and twice with ethanol-ether mixture (3:1), supernates being discarded after each wash. Finally, the insoluble residue was resuspended in 1 ml 5% perchloric acid, heated to 70°C for 30 min, transferred into counting vials containing 10 ml of Handifluor (Mallinkrodt Inc., St. Louis, Mo.), and the radioactivity, representing [^{3}H]thymidine incorporated into DNA, was measured in a liquid scintillation counter.

Incorporation of leucine into protein. The effect of hormones on incorporation of labeled leucine into protein was measured according to Farese (5). Tissue samples were suspended in 1 ml KRBG for 1 h under 95% O_2 and 5% CO_2 at 37°C. Medium was removed and 1 ml fresh medium was added containing 0.00025 μmol L-4, [5-^{3}H]leucine (5 Ci/μmol sp act) plus zero or 0.1 nM- to 1 μM concentration of peptide hormone. Samples were incubated for 2 h at 37°C under 95% O_2 and 5% CO_2, cooled on dry ice, washed three times in 0.9% NaCl solution, homogenized in 10% trichloroacetic acid (TCA), and centrifuged at 4,000 *g* for 20 min at 4°C. The TCA-insoluble residues were washed twice with 4% TCA, twice with ethanol, and once with diethylether, then resuspended in 1 ml of 1 M NaOH and counted in 15 ml of dioxane scintillation fluid (19).

RESULTS

Rabbit

Glucocorticoid production (Table II). The fetal glands showed the same characteristics under these assay conditions as originally described for the adult gland (15). Preincubation for 1 h before addition of ACTH reduced the basal level of corticosterone production to one-third to one-fifth of base line and increased the corticoid response to 0.1 μM ACTH by a

TABLE I
Duration of Gestation vs. Body Parameters

Species	Early fetus			Mid fetus			Late fetus			Newborn		
	Duration gestation	Body length	Body weight	Duration gestation	Body length	Body weight	Duration gestation	Body length	Body weight	Duration gestation	Body length	Body weight
	d	*cm*	*g*	*d*	*cm*	*g*	*d*	*cm*	*g*	*d*	*cm*	*g*
Rabbit	18–24	3–6	2–14	24–28	6–8	14–35	28–30	8–11	35–50	31	11	50
Guinea pig	35–44	4–7	5–15	44–57	7–9	15–50	57–69	9–14	50–100	70	14	100
Rat	18–19	2–3	1–2	19–20	3–4	2–4	20–21	4–5	4–6	21	5	6

The general relationship between duration of gestation, fetal length, and fetal weight is shown. In the text, fetuses are classified "early," "mid," or "late" on the basis of body weight.

TABLE II

Effect of Hormones on Release of Corticosterone (Rabbit, Rat) or Cortisol (Guinea Pig)

	Concentration of peptide in medium											
		1 nM			10 nM			0.1 μM			1 μM	
	None	ACTH	αMSH	βMSH	ACTH	αMSH	βMSH	ACTH	αMSH	βMSH	ACTH	αMSH	βMSH
Early fetal rabbit	0.2±0.0	0.2±0.0			0.8±0.2*	0.3±0.1	0.2±0.1	2.0±0.2*	0.5±0.2	0.2±0.0	2.0±0.4*	0.4±0.1	0.3±0.1
Mid fetal rabbit	0.4±0.1	0.4±0.1			1.8±0.2*	0.5±0.1	0.3±0.1	2.8±0.4*	1.2±0.2*	1.2±0.1*	3.0±0.3*	1.6±0.2	1.7±0.2*
Late fetal rabbit	1.0±0.2	4.6±0.5*			10.5±1.4*	1.0±0.1	1.8±0.4	9.7±1.1*	0.8±0.1	0.9±0.1	12.3±1.9*	0.8±0.1	1.1±0.1
Newborn rabbit	2.1±0.3	9.0±1.6*			18.5±2.7*	3.0±0.5	3.1±0.4	14.8±3.1*	2.6±0.3	1.5±0.3	17.5±3.8*	2.9±0.3	2.5±0.5
Early fetal guinea pig	0.2±0.1	0.3±0.1			0.4±0.1	0.3±0.1	0.2±0.1	1.6±0.4*	0.3±0.1	0.3±0.1	1.9±0.4*	0.1±0.1	0.5±0.1
Mid fetal guinea pig	0.4±0.0	0.6±0.1	0.6±0.1		1.3±0.2*	1.5±0.2	0.3±0.0	3.2±0.4*	1.2±0.2*	0.9±0.1*	3.0±0.5*	1.3±0.2*	1.4±0.2*
Late fetal guinea pig	3.0±0.4	15.2±2.0*			26.1±4.3*	3.2±0.4	2.9±0.6	28.0±4.0*	2.5±0.3	2.4±0.2	23.5±3.0*	4.0±0.7	3.3±0.2
Newborn guinea pig	5.5±0.3	16.8±2.9*			30.3±4.5*	4.6±0.6	7.3±0.4	32.0±3.9*	6.1±1.0	4.3±0.7		3.9±1.1	6.2±0.4
Early fetal rat	0.5±0.4	0.5±0.1			2.4±0.2*	0.6±0.0	0.7±0.1	4.1±0.6*	1.0±0.2	0.6±0.1	3.8±0.5*	0.8±0.2	0.8±0.2
Mid fetal rat	1.0±0.2	1.5±0.2	1.0±0.4	0.8±0.16	4.0±0.4*	2.0±0.1*	1.6±0.3	7.8±0.2*	2.8±0.1*	3.0±0.4*	8.1±0.9	2.7±0.2*	3.4±0.4*
Late fetal rat	3.1±0.3	12.6±1.0*			15.3±1.0*	2.0±0.3	3.4±0.6	12.1±1.4*	1.8±0.6	1.9±0.5		3.3±0.4	3.5±0.4
Newborn rat	4.1±0.6	10.3±2.1*			26.1±3.4*	5.7±0.7	3.1±0.8	21.9±3.5*	4.2±0.5	3.6±0.5		5.3±0.7	4.1±0.6

Values represent micrograms glucocorticoid/100 mg adrenal gland (wet wt) per 2 h, average±SE; $n = 4$ for all values except for column "none," where $n = 12$. Neither ACTH, αMSH, nor βMSH caused a significant ($P < 0.05$) effect at 0.1 nM.

* $P < 0.05$ for comparison with none.

factor of 4; rate of synthesis of corticosterone in response to 0.1 μM ACTH was linear for at least 3 h. Accordingly, the Saffran-Bayliss assay (15) was used without modification.

The early fetal adrenal gland produced 0.2 μg corticosterone/100 mg adrenal gland per 2 h. This rate was accelerated by ACTH. Potency of ACTH can be expressed in terms of the minimal effective dose (MED) (defined as the lowest concentration that caused an effect significant at $P < 0.05$), which was 10 nM, and in terms of the maximal response (RMax), which was an increment of 1.8 μg corticosterone/100 mg adrenal gland per 2 h. The mid fetal gland responded to all three melanotropic peptides. For ACTH, αMSH, and βMSH, respectively, the MED values were 10 nM, 0.1 μM, and 0.1 μM. Values for RMax were 2.6, 1.2, and 1.3 μg corticosterone/100 mg adrenal per 2 h. In late fetal and newborn glands, ACTH exhibited increasing steroidogenic potency (MED, 1 nM in both instances; RMax 11.3 and 15.4 μg corticosterone/100 mg per 2 h, respectively). In contrast, αMSH and βMSH were inactive.

Trypsinized preparations of ACTH, αMSH, and βMSH were inactive on the mid fetal adrenal gland at 1 μM, as were oxytocin, arginine vasopressin, LH, FSH, and GH.

Incorporation of thymidine into DNA (Table III). With Claycomb's assay (18), basal level of incorporation by the early fetal gland was 19×10^4 dpm/100 mg adrenal per 2 h. Addition of 1 μM ACTH or αMSH increased thymidine incorporation to six to eight times the base line. The experiment was repeated with variations of preincubation and incubation intervals between 1 and 4 h, and the maximal effect was found when these intervals were 1–2 and 2–3 h, respectively. Variations in thymidine concentration between 0.025 and 1 μM did not influence the magnitude of the effect of ACTH or αMSH; higher levels of thymidine reduced the effect. In the rest of this study, accordingly, Claycomb's (18) original assay conditions were used.

To verify that the increase in radioactivity in the TCA supernate of glands exposed to ACTH or αMSH represented accelerated incorporation into DNA, the following experiments were done with mid fetal rabbit glands according to Morley and Kingdon (20). The radioactivity of the TCA supernate as well as of the TCA insoluble fractions was measured; ACTH and αMSH had no effect on the former while increasing the latter by a factor of 5 to 8. When 25 mM hydroxyurea was included in the incubation medium, incorporation of label into the TCA insoluble fraction was totally inhibited both in the absence and presence of ACTH and αMSH.

In the early fetal adrenal gland, αMSH and ACTH stimulated thymidine incorporation with MED of 1 and 10 nM, respectively. RMax expressed as increment in disintegrations per minute thymidine incorporated per 100 mg adrenal/2 h was 165×10^4 and 124×10^4, respectively. As gestation proceeded, both peptides became less potent as reflected by rising MED and falling RMax; the newborn gland was unresponsive to ACTH.

Trypsinized ACTH and αMSH (1 μM) were inactive on the mid fetal gland, as were βMSH, oxytocin, arginine vasopressin, LH, FSH, and GH.

On lung, liver, kidney, and brain from the early fetus,

TABLE III

Effect of ACTH and αMSH on Incorporation of Labeled Thymidine into DNA

	Concentration of peptide in medium								
		1 nM		10 nM		0.1 μM		1 μM	
	None	ACTH	αMSH	ACTH	αMSH	ACTH	αMSH	ACTH	αMSH
Early fetal rabbit	19.1±2.4	15.3±2.7	103.4±18.6*	50.5±8.8‡	192.2±26.5*	150.1±18.6*	170.6±20.4*	143.1±16.5*	183.7±26.0
Mid fetal rabbit	15.6±2.5	19.6±2.4	82.4±9.1*	42.1±8.1§	146.0±18.3*	126.8±11.8*	135.5±1.5*	104.6±15.3*	124.3±15.1
Late fetal rabbit	9.2±1.0	6.4±0.9	12.3±1.0	5.7±0.9	38.9±6.1*	30.4±5.3‡	47.2±5.9*	35.4±3.9*	41.3±5.3*
Newborn rabbit	8.5±0.7		9.8±0.7	6.2±0.8	18.7±1.9*	8.0±1.6	21.6±3.8‡	4.7±0.5	16.7±1.9‡
Early fetal guinea pig	15.1±1.6	18.2±1.6	61.5±8.3*	31.5±6.2‖	90.5±10.5*	52.1±6.9*	87.0±10.0*	56.3±7.0*	81.1±10.1
Mid fetal guinea pig	10.3±2.0	12.3±1.3	41.8±4.9*	21.5±2.0‡	63.4±7.0*	34.2±5.2*	68.5±9.3*	38.5±4.2*	57.2±5.4*
Late fetal guinea pig	6.2±0.7	5.1±0.8	10.1±2.0‖	9.3±1.5	38.8±4.3*	18.0±1.4*	29.7±3.8*	15.0±1.9*	32.1±3.6*
Newborn guinea pig	3.4±0.5		5.4±1.4	5.4±0.8	4.7±0.9	6.2±1.8	4.7±0.9	2.6±0.4	4.2±0.6
Early fetal rat	23.8±3.8	21.7±3.5	52.1±8.2§	42.4±4.0‡	81.6±10.0*	61.0±7.3*	73.7±8.6*	58.0±6.4*	65.3±9.1*
Mid fetal rat	15.2±2.4	16.4±1.8	30.3±3.6‡	31.6±4.0‡	42.9±5.3	42.4±5.8*	38.2±5.1*	35.5±3.6*	49.9±9.5*
Late fetal rat	9.0±0.8	6.9±0.7	8.4±0.5	8.6±1.0	19.2±2.2*	11.1±1.9	26.5±4.2*	6.1±0.8	22.1±2.4*
Newborn rat	5.0±0.6			3.1±0.5	3.5±0.5	3.7±0.7	8.1±1.1‖	2.1±0.3	15.7±1.9*

Values represent dpm × 10^{-4}/100 mg adrenal gland (wet wt) per 2 h. $n = 4$ for all values except column "none," where $n = 12$. Data are given as average±SE. At 0.1 nM, neither peptide caused a significant ($P < 0.05$) effect. βMSH was inactive at 1 μM.

* $P < 0.005$ for comparison with none.

‡ $P < 0.01$ for comparison with none.

§ $P < 0.025$ for comparison with none.

‖ $P < 0.05$ for comparison with none.

ACTH and αMSH at 1 μM had no effect on DNA synthesis under the conditions employed.

Incorporation of leucine into protein (Table IV). In preliminary experiments, early fetal adrenals were tested under the conditions of Farese (5). Basal incorporation of [^{14}C]leucine into protein was 9×10^4 dpm/100 mg adrenals per 2 h. 1 μM ACTH or αMSH doubled the counts incorporated into protein. Radioactivity recovered in the TCA supernatant fraction of the adrenal was not altered. Incubation conditions were then varied as follows: preincubation interval, from 0.5 to 3 h; incubation interval, 0.5 to 3 h; medium leucine, 0.0001 to 0.01 mM. Magnitudes of the ACTH or αMSH effects were not markedly influenced by these alterations of incubation conditions, a 100–200% increase of leucine incorporation being observed in each

TABLE IV

Effect of ACTH and αMSH on Incorporation of Labeled Leucine into Protein (dpm × 10^{-4}/100 mg adrenal per 2 h

	Concentration of peptide in medium								
		1 nM		10 nM		0.1 μM		1 μM	
	None	ACTH	αMSH	ACTH	αMSH	ACTH	αMSH	ACTH	αMSH
Early fetal rabbit	9.0±0.8		6.0±0.4		14.9±1.7*	14.9±1.6‡	18.5±2.0*	16.7±1.8§	20.8±1.6*
Mid fetal rabbit	5.9±0.7		7.2±0.7		9.5±0.8§	6.2±0.4	12.6±1.1*	12.7±1.0*	10.5±1.2‖
Late fetal rabbit	3.5±0.3				2.0±0.8	4.4±0.4	3.2±0.2	2.4±0.3	4.1±5.0
Newborn rabbit	2.6±0.4					1.7±0.3	2.0±0.3	1.8±0.2	2.2±0.3
Early fetal guinea pig	14.2±1.5			12.3±0.9	20.1±1.5	15.8±1.0	30.0±1.8*	19.3±1.7	26.5±2.9§
Mid fetal guinea pig	12.1±0.9			8.1±1.0	5.7±0.7	10.4±1.1	19.9±1.7*	20.5±1.6*	22.6±1.9*
Late fetal guinea pig	9.5±1.1					6.0±0.5	10.3±0.8	11.4±1.3	18.4±1.7*
Newborn guinea pig	6.1±0.5					4.2±0.3	7.8±0.9	3.2±0.2	8.9±1.6
Early fetal rat	18.4±1.6	13.1±1.5	14.8±1.5	17.9±1.3	29.3±3.3	25.6±1.9‖	34.5±3.1*	28.2±2.8‖	28.2±2.9‖
Mid fetal rat	10.4±1.2			8.3±1.1	9.0±0.6	9.1±0.7	20.5±1.8*	9.2±0.7	19.3±0.9*
Late fetal rat	6.4±0.5					5.1±0.7	7.7±0.6	3.5±0.6	7.3±0.5
Newborn rat	3.5±0.4					1.3±0.2	4.2±0.5	1.2±0.1	5.2±1.5

Values represent average±SE; $n = 4$ except for column "none," where $n = 12$. At 0.1 nM, neither peptide caused a significant ($P < 0.05$) effect. βMSH was inactive at 1 μM.

* $P < 0.005$ for comparison with none.

‡ $P < 0.05$ for comparison with none.

§ $P < 0.01$ for comparison with none.

‖ $P < 0.025$ for comparison with none.

instance. Accordingly, the conditions originally described by Farese (5) were used in the rest of this investigation.

In the adrenal gland of the early rabbit fetus, the MED values for the ACTH and αMSH effects were 10 and 10 nM, respectively; maximal responses, expressed as increment in disintegrations per minute per 100 mg adrenal per 2 h, were 7.7×10^4 and 11.8×10^4, respectively. The potency of the peptides declined as gestation proceeded; they were not detectably active in the late fetus and newborn. βMSH was inactive (1 μM) at all stages of fetal life. Trypsinized ACTH and αMSH were inactive at 1 μM on the mid fetal gland, as were oxytocin, arginine vasopressin, LH, FSH, and GH.

ACTH and αMSH at 1 μM did not influence leucine incorporation into protein by slices of mid fetal liver, kidney, or brain.

Guinea Pig and Rat Adrenal Glands (Tables II, III, and IV)

Preliminary experiments, similar to those carried out with rabbit fetal adrenals, established the applicability to guinea pig and rat fetal glands of the Saffran-Bayliss (15), Claycomb (18), and Farese (5) assays for measuring steroidogenesis, DNA synthesis, and protein synthesis, respectively.

The effects of ACTH, αMSH, and βMSH in these assays were generally similar to those in the rabbit. ACTH stimulated glucocorticoid synthesis in all four stages of fetal-newborn life with MED generally 10 nM. Potency increased progressively during gestation as reflected by falling MED and rising RMax. Only in the mid fetal adrenal gland was a glucocorticoid steroidogenic effect of αMSH and βMSH seen. Incorporation of thymidine into DNA was markedly stimulated in the early and mid fetal gland by αMSH (MED 10 nM) and by ACTH (MED 0.1 μM), and to a lesser extent in the late fetal gland. Incorporation of leucine into protein was stimulated in the early or mid fetal gland by αMSH and ACTH; this effect diminished or disappeared in the late fetus or newborn.

DISCUSSION

All three species show a similar picture, as summarized in Table V: (*a*) In the early fetal gland, αMSH and ACTH stimulate the incorporation of thymidine into DNA and of leucine into protein. ACTH stimulates glucocorticoid release. (*b*) In the mid fetal gland, the above effects on thymidine and leucine incorporations persist. A glucocorticoid steroidogenic response to αMSH and βMSH, as well as to ACTH, is present. (*c*) In the late fetal and newborn gland, the effects on DNA and protein synthesis diminish or disappear. Of the three peptides, only ACTH now stimulates glucocorticoid production.

These observations support the suggestion of Challis and Torosis (3) that the MSH are glucocorticoid steroidogenic in fetal life. However, the effect is sharply limited in time to the mid fetal stage. Moreover, the maximal magnitude of the steroidogenic effect of the MSH on the mid fetal gland is only one-tenth to one-third as great as that of ACTH.

The present experiments show that the early fetal gland differs from the late fetal, newborn, and adult glands in two respects: (*a*) specificity of hormonal responsiveness; (*b*) nature of the hormonal responses. (*a*) In the adult gland, αMSH and βMSH possess only 0.0001% of the in vitro glucocorticoid steroidogenic potency of $ACTH_{1-24}$ (1). Present data indicate a similar ratio in the newborn gland. In the mid fetal gland, however, αMSH and βMSH are 10–100% as potent. In this respect the fetal adrenal resembles the frog melanocyte and the rabbit fat cell, which are highly responsive to αMSH and βMSH, as well as to $ACTH_{1-24}$, in terms of melanosome dispersion and lipolysis, respectively (21). (*b*) In vitro glucocorticoid response of the late fetal and newborn gland is not accompanied by accelerated synthesis of DNA and protein. In contrast, when steroidogenesis is stimulated in the isolated early or mid fetal gland by ACTH or αMSH, all three reactions occur together. A possible explanation for this difference in the pattern of in vitro response is the capacity of glucocorticoids to inhibit DNA and protein synthesis (5–8). Thus the magnitude of the steroidogenic response to ACTH increased progressively during fetal life, whereas the DNA and protein responses progressively decreased, then disappeared (Table II). This inverse relation could reflect an inhibitory influence of the intracellular accumulation of glucocorticoid.

Differences in the specificity of the hormonal responses between early fetal and late fetal or newborn glands could have two types of explanation. (*a*) The structural requirement of the corticotropic receptor (22) could change during gestation in all adrenal cells. (*b*) The fetal cortex could contain two or more cell populations with receptors of different specificity, and the relative proportions of these cells could change during gestation. In primates, such cellular heterogeneity is morphologically evident in terms of fetal and definitive zones; the former involutes rapidly after birth (23). Although this anatomic division is not visible in the fetal cortex of rat, rabbit, and guinea pig, hormone-sensitive cell types with different physiological properties could nevertheless be present.

The present data extend earlier reports on the capability of the human fetal adrenal gland to synthesize glucocorticoids (24, 25) and the responsiveness of this

TABLE V

Summary of Responses of Fetal and Newborn Adrenals to ACTH, αMSH, and βMSH

Assay	Species	Stage	ACTH		αMSH		βMSH	
			MED	RMax	MED	RMax	MED	RMax
Steroidogenesis	Rabbit	Early fetal	10 nM	1.8	NR		NR	
		Mid fetal	10 nM	2.6	0.1 μM	1.2	0.1 μM	1.3
		Late fetal	1 nM	11.3	NR		NR	
		Newborn	1 nM	15.4	NR		NR	
	Guinea pig	Early fetal	0.1 μM	1.7	NR		NR	
		Mid fetal	10 nM	2.8	10 nM	0.9	0.1 μM	1.0
		Late fetal	1 nM	25.0	NR		NR	
		Newborn	1 nM	26.5	NR		NR	
	Rat	Early fetal	10 nM	3.5	NR		NR	
		Mid fetal	10 nM	7.1	10 nM	1.8	0.1 μM	2.4
		Late fetal	1 nM	12.2	NR		NR	
		Newborn	1 nM	22.0	NR		NR	
DNA synthesis	Rabbit	Early fetal	10 nM	124.0	1 nM	164.6	NR	
		Mid fetal	10 nM	89.0	1 nM	108.7	NR	
		Late fetal	0.1 μM	26.2	10 nM	32.1	NR	
		Newborn	NR		10 nM	8.2	NR	
	Guinea pig	Early fetal	10 nM	41.2	1 nM	66.0	NR	
		Mid fetal	10 nM	28.2	1 nM	46.9	NR	
		Late fetal	0.1 μM	8.8	1 nM	25.9	NR	
		Newborn	NR		NR		NR	
	Rat	Early fetal	10 nM	34.2	1 nM	41.5	NR	
		Mid fetal	10 nM	20.3	1 nM	34.7	NR	
		Late fetal	NR		10 nM	13.1	NR	
		Newborn	NR		0.1 μM	10.7	NR	
Protein synthesis	Rabbit	Early fetal	10 nM	7.7	10 nM	11.8	NR	
		Mid fetal	10 nM	6.8	10 nM	4.6	NR	
		Late fetal	NR		NR		NR	
		Newborn	NR		NR		NR	
	Guinea pig	Early fetal	NR		0.1 μM	12.3	NR	
		Mid fetal	1 μM	8.4	0.1 μM	10.5	NR	
		Late fetal	NR		1 μM	8.9	NR	
		Newborn	NR		NR		NR	
	Rat	Early fetal	0.1 μM	9.7	10 nM	9.8	NR	
		Mid fetal	NR		0.1 μM	8.9	NR	
		Late fetal	NR		NR		NR	
		Newborn	NR		NR		NR	

NR signifies no response at 1 μM dose. RMax represents maximal increase over base line in these units: steroidogenesis, micrograms corticosterone (rabbit, rat), or cortisol (guinea pig) per 100 mg adrenal/2 h; DNA synthesis, dpm × 10^{-4} of thymidine incorporated into DNA/100 mg adrenal per 2 h; protein synthesis, dpm × 10^{-4} of leucine incorporated into protein/100 mg adrenal per 2 h.

process to ACTH (26, 27). The fetal gland also produces numerous steroids lacking glucocorticoid activity (24, 25). How ACTH, αMSH and βMSH influence formation of steroids other than corticosterone and cortisol in fetal adrenals of various species remains to be studied.

It would be premature to conclude from the present data that αMSH or βMSH function as growth-promoting or steroidogenic corticotropins in fetal life. The MED for the effects of αMSH on synthesis of glucocorticoids, protein, and DNA are 2–200 times higher than the concentrations of these hormones re-

ported in adult rat plasma (28); the rat's circulating level of βMSH is unknown. On the other hand, Silman et al. (29) observed a considerably higher concentration of αMSH in fetal than in adult human pituitary gland. The pituitary intermediate lobe, where the MSH are synthesized and stored, is a prominent structure prenatally in most vertebrates, including man (4). Until the circulating levels of αMSH and βMSH in fetal plasma are measured, their possible corticotropic role in embryonic life will be a matter of speculation.

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