Identification of β -Adrenergic Receptors Using [3 H]Dihydroalprenolol in Fetal Sheep Heart: Direct Evidence of Qualitative Similarity to the Receptors in Adult Sheep Heart

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Summary

The fetal sheep heart responds to β -adrenergic stimuli; however, *in vivo* studies show the response of the fetal heart is less than that of the adult heart. We used [3 H]dihydroalprenolol (DHA) to study directly β -adrenergic receptors in heart particulates of fetal sheep at term and adult sheep. [3 H]DHA binding to fetal heart particulates was rapid, reversible ($t_{1/2} = 2.9 \pm 0.3$ min), stereoselective, saturable (101.2 ± 7.4 fmoles/mg protein), and of high affinity (4.8 ± 0.4 nM). The rank order of agonists competing for [3 H]DHA binding was isoproterenol (0.32 ± 0.10 μ M) > epinephrine (1.19 ± 0.23 μ M) = norepinephrine (2.67 ± 0.69 μ M), which is compatible with β_1 -adrenergic potencies. [3 H]DHA also bound to the adult sheep heart in a manner expected for β_1 -receptors. No difference in the binding affinity of [3 H]DHA or agonists' competition was demonstrated between the fetal and adult sheep heart. Comparison of the concentration of β -adrenergic receptors in fetal and adult hearts was confounded by the choice of the denominator for unit expression. The concentration was higher in the adult when expressed as a function of protein content or 5'-nucleotidase activity (0.52 ± 0.07 versus 1.12 ± 0.06). However, there was no difference when tissue weight, $\text{Na}^+ - \text{K}^+$ -ATPase, or NaF-stimulated adenylate cyclase was used. Furthermore, isoproterenol-stimulated adenylate cyclase and cardiac contractile response to a threshold dose of isoproterenol were identical in the fetal and adult sheep heart. We conclude that β -receptors can be studied with [3 H]DHA in the fetal sheep heart, this receptor is qualitatively similar to the β -receptor in the adult sheep heart, and it is unlikely that there is a difference in the concentration of β -adrenergic receptors in fetal and adult sheep heart.

Speculation

The interactions of adrenergic agents with cardiac β -adrenergic receptors in the term fetus are identical to those in the adult, and the receptor-adenylate cyclase coupling system reaches maturity before birth.

The fetal sheep heart differs from the adult heart in its response to physiologic and pharmacologic adrenergic stimuli. Isoproterenol, a β -adrenergic agonist, increases the heart rate of chronically instrumented fetal sheep as early as 60 days of gestation (18). However, the heart of the fetus is less responsive to isoproterenol than is that of the neonatal or adult sheep (26). Furthermore, the ability of propranolol, a β -adrenergic antagonist, to decrease heart rate is less in early gestation (<120 days of gestation) than in the term fetus or neonate (23). *In vitro* cardiac contractile response to isoproterenol is similar in both fetus and adult; however, norepinephrine is more potent in the fetal heart (9). Moreover, the *in vitro* effect of propranolol on isoproterenol-induced cardiac con-

tractile response in fetus is similar to its effect in adult, but the fetal heart is more sensitive to the negative inotropic property of propranolol (9). It is difficult to evaluate the role of β -adrenergic receptors in these differences by conventional pharmacologic techniques because the response may be modified by factors such as reflex activity, muscle function, endogenous humoral substances, and differences in circulation between the fetus, neonate, and adult.

We have utilized radioligand binding techniques with the β -adrenergic antagonist, [3 H]dihydroalprenolol (DHA) to examine directly β -adrenergic receptors in hearts of the term fetus and adult sheep to determine if qualitative or quantitative changes in the receptor could account for differences observed in adrenergic responses. We found that [3 H]DHA binds to fetal and adult myocardial preparations with characteristics consistent with β -adrenergic interactions. The β -adrenergic receptor as identified by [3 H]DHA binding is qualitatively similar in the fetus and adult. The concentration of the receptors expressed as a function of NaF-stimulated adenylate cyclase activity also appears to be similar. Sensitivity of adenylate cyclase to isoproterenol and cardiac contractile response to a threshold dose of isoproterenol are similar in both preparations.

MATERIALS AND METHODS

Pregnant ewes (one 132 days and three 140 days of pregnancy) were anesthetized with sodium pentobarbital (60 to 120 mg/kg IV). Hearts were removed from the fetus and mother (an adult with a similar hormonal environment) and were placed immediately in ice cold 0.9% NaCl solution for heart particulate preparation or in an oxygenated Krebs-Ringer medium (30°C) for an isometric contraction experiment. Atria, fat, and large coronary vessels were trimmed from the ventricle.

VENTRICULAR MUSCLE PARTICULATE PREPARATION

Ventricular muscle particulate was prepared by a modification of the method described by Sharma and Banerjee (20). Ventricles were minced and homogenized by Polytron (Brinkmann, PCU-2) set at full speed for 10 sec in 20 volumes of ice-cold 50 mM Tris-HCl (pH 7.4). The homogenate was passed through four and then eight layers of cheesecloth, and the filtrate was centrifuged twice at $48,200 \times g$ for 15 min with washing of the intermediate pellet. The final pellet was resuspended at a protein concentration of 5 to 10 mg/ml and stored at -70°C . [3 H]DHA binding to the preparations was unchanged up to 6 months. The protein concentration was determined by the method of Bradford (5) using bovine serum albumin as the standard.

Using this procedure, the protein recovery was 40.7 ± 9.1 mg/g for fetus and 25.7 ± 6.3 mg/g for adult ($n = 3$ to 4).

BINDING ASSAY

Binding assays were performed in a 250 μ l volume containing heart particulate (protein concentration at 1.5 to 2 mg/ml), 50 mM Tris-HCl (pH 7.4), 1 mM ascorbic acid, and [3 H]DHA (0.9 to 16 nM for equilibrium experiments; 1.2 to 2.4 nM for competition experiments) with 2% ethanol at 30°C for 15 to 20 min. At the end of incubation, 5 ml of 50 mM Tris-HCl (4°C) were added to the assay tube, and samples were immediately filtered through Whatman GF/C glass fiber filters to separate free and bound [3 H]DHA. Filters were rapidly washed with 15 ml of 50 mM Tris-HCl (4°C), dried, and placed in scintillation vials containing Econofluor (New England Nuclear Corp.), and counted in a Packard Tris-Carb Liquid Scintillation Counter (model 3320) at 54% machine efficiency.

Specific binding, defined as binding in the absence of (-)-alprenolol minus binding in the presence of 10 μ M (-)-alprenolol, was about 80% of total binding in fetal and adult sheep hearts.

ENZYME ASSAY

Adenylate cyclase activity in sheep heart particulate was determined at a protein concentration of 30 to 60 μ g/ml in a 10-min incubation as described by Insel *et al.* (13). The incubation medium for generating cyclic adenosine 3',5'-monophosphate (cAMP) contains 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 8), 10 mM MgSO₄, 0.1 mg/ml bovine serum albumin, 1 mM RO-20 (a phosphodiesterase inhibitor), 0.7 mM EGTA, 1 mM ATP, 1 mM dithiothreitol, and 100 μ M GTP. cAMP generated was measured by a competitive protein binding method (10).

5'-Nucleotidase activity was assayed at pH of 9.0 (21) at a protein concentration of 0.16 to 0.2 mg/ml for 20 min. Free inorganic phosphorus (Pi) was determined by the method of Chen *et al.* (6).

Na⁺, K⁺-ATPase activity was determined by the method described by Besch *et al.* (4). The specific activity of Na⁺-K⁺-ATPase, defined as activity in the absence of 10 μ M ouabain minus activity in the presence of ouabain, was about 30% of total activity. Protein concentration was 0.1 to 0.3 mg/ml, and incubation time was 30 min. The method used to determine Pi content was similar to the procedure of Harris and Popat (12).

Product formation (cAMP or Pi) was a linear function of protein concentration and time under the conditions described for all assays.

ISOMETRIC CONTRACTION

Ventricular muscle strips were suspended in oxygenated Krebs-Ringer medium (30°C) at 1 g tension and electrically driven at 10 mV with a pulse duration of 5 msec and a frequency of 1.6/sec by a Grass stimulator. Isometric contractions were recorded with strain-gauge transducers (Grass FT03) connected to a four-channel Grass polygraph. After 60 min equilibration, the responses to cumulative doses of isoproterenol (10^{-11} to 10^{-5} M) and norepinephrine (10^{-8} to 10^{-5} M) were determined.

STATISTICS

The null hypothesis was that [3 H]DHA binding sites, adrenergic agonist competition for binding, and isoproterenol-stimulated adenylate cyclase were similar in the fetal and adult sheep hearts and was tested by an unpaired Student's *t* test. The potencies of selected adrenergic agonists competing for [3 H]DHA binding was evaluated by an analysis of variance by ranks and a nonparametric multiple comparison (Table 2) (28). All values are presented as mean $\pm$ S.E.

CHEMICALS USED

(-)-Norepinephrine HCl, (-)-epinephrine bitartrate, (-)-isoproterenol HCl, and (-)-alprenolol-(+)-tartrate were purchased from Sigma Chemical Co. (-)-Propranolol HCl and (+)-propran-

olol HCl were gifts from Ayerst Pharmaceutical Co. (-)-[propyl-1,2,3- 3 H]Dihydroalprenolol HCl (51.1 Ci/mmol) was purchased from New England Nuclear Corp. Stock solutions and serial dilutions of adrenergic agonists and antagonists were made in 1.0 mM HCl.

RESULTS

CHARACTERISTICS OF [3 H]DHA BINDING TO PARTICULATE PREPARATIONS OF FETAL SHEEP HEART

Binding Kinetics. [3 H]DHA bound rapidly to heart particulates. Equilibrium was reached within 5 to 20 min at 30°C (Fig. 1A). Inasmuch as binding slowly declined after 20 min, 15 to 20-minute incubations were used in subsequent experiments.

The rate constant for association (k_1) calculated from a pseudo-

Fig. 1. Kinetic analysis of [3 H]DHA binding to fetal sheep heart particulates.

A, [3 H]DHA binding as a function of time. Heart particulates (protein concentration at 2 mg/ml) were incubated with 4.26 nM [3 H]DHA for the time indicated. Data are expressed as a percentage of [3 H]DHA specific binding at equilibrium (44.9 fmoles/mg protein). Inset, pseudo-first-order association rate plot. B, bound [3 H]DHA; Beq, bound [3 H]DHA at equilibrium. The slope of the line (k_{ob}), determined by linear regression, was used to calculate the rate constant for association (k_1) (25).

B, dissociation of [3 H]DHA binding as a function of time. Heart particulates (2 mg/ml) were incubated with 4.26 nM [3 H]DHA until equilibrium was reached (10 min). Dissociation was started ($t = 0$ min) by adding (-)-alprenolol (final concentration, 10 μ M). Inset, first-order dissociation rate plot. The slope of the line determined by linear regression was the rate constant for dissociation (k_2).

Points mean of duplicate determinations from a representative experiment ($n = 3$ to 4).

first order plot was $0.10 \pm 0.01 \text{ nM}^{-1} \text{ min}^{-1}$ ($n = 3$). [^3H]DHA dissociated from the binding sites with a half-time ($t_{1/2}$) of $2.9 \pm 0.6 \text{ min}$ ($n = 3$) in the presence of excess (–)-alprenolol (Fig. 1b) giving a rate constant for dissociation (k_2) of $0.25 \pm 0.04 \text{ min}^{-1}$ ($n = 4$) (25). The dissociation constant (K_d) calculated from the ratio of k_2 to k_1 was 2.5 nM , which is in good agreement with the value obtained in subsequent equilibrium experiments.

Saturation of Binding Sites. As shown in Figure 2, [^3H]DHA binding to fetal sheep heart preparations was saturable and of high affinity. Scatchard analysis of the data resulted in a single line indicating the presence of a single population of binding sites. A Hill plot of the data was also a straight line ($n = 0.998$) with a slope of 1.0, further suggesting that cooperative interactions were not present in [^3H]DHA binding to sites in these preparations (3).

In the fetal sheep heart particulates, the binding affinity of [^3H]DHA in four separate experiments was $4.8 \pm 0.4 \text{ nM}$, and the concentration of binding sites was $101.2 \pm 7.4 \text{ fmoles/mg protein}$.

Stereoselectivity and Drug Specificity of the [^3H]Binding Site. The results of competition experiments with selected adrenergic agonists and antagonists in these preparations are shown in Figure 3. The (–) stereoisomer of propranolol competed 200 times more effectively than the (+) stereoisomer when 50% of the sites were occupied. Phentolamine, an α -adrenergic antagonist, had little effect on [^3H]DHA binding.

The order of potency for agonist competition for [^3H]DHA binding was isoproterenol > epinephrine $\approx$ norepinephrine, which is similar to the order of potency of these agonists in stimulating cardiac muscle contraction and is characteristic of β_1 -adrenergic receptor interactions (16).

COMPARISON OF β -ADRENERGIC RECEPTORS IN FETAL AND ADULT SHEEP HEARTS

Cardiac Contractile Responses to Isoproterenol and Norepinephrine. The effects of isoproterenol and norepinephrine on isolated ventricular strips are shown in Figure 4. The threshold dose of isoproterenol required to evoke a contractile response was similar in the fetus ($5 \times 10^{-10} \text{ M}$, 10^{-8} M , and $5 \times 10^{-8} \text{ M}$) and adult (10^{-9} M , $5 \times 10^{-9} \text{ M}$, and 10^{-8} M) ($n = 3$). However, the dose of norepinephrine required to evoke a contractile response was 50 times higher for adult ventricular strips compared to fetal ventricular strips ($n = 3$ each).

Comparison of [^3H]DHA Binding. [^3H]DHA binding to adult

Fig. 2. Scatchard analysis of [^3H]DHA binding to fetal sheep heart particulates. Scatchard analysis of data from an experiment in which fetal sheep heart particulates (2 mg/ml) were incubated with increasing concentrations of [^3H]DHA (0.9 to 15.5 nM) in the presence or absence of $10 \mu\text{M}$ (–)-alprenolol. Specific binding was determined as described in "Materials and Methods." Inset, direct plot of same data. Points mean of duplicate determinations from a representative experiment ($n = 4$).

Fig. 3. Inhibition of [^3H]DHA binding by adrenergic agonists and antagonists in fetal sheep heart particulates. Heart particulates (1.5 to 2 mg/ml) were incubated with 1.24 to 2.43 nM [^3H]DHA in the presence or absence of $10 \mu\text{M}$ (–)-alprenolol and the listed agents. Percent specific binding is binding in the absence of competitor divided by binding at the indicated concentration of agents. Points, mean of duplicate determinations from a representative experiment ($n = 3$).

and fetal heart preparations was similar. Scatchard analysis indicated that binding was to a single class of sites without evidence of cooperativity. The K_d for adult myocardium was $4.9 \pm 1.6 \text{ nM}$ ($n = 3$) which was not different from the K_d in the fetus ($P > 0.5$). However, a significantly higher concentration of binding sites was found in the adult when sites were expressed per mg protein (Table 1).

To further compare the concentration of [^3H]DNA binding sites in the particulates of fetal and adult sheep hearts, we measured activities of the plasma membrane marker $5'$ -nucleotidase, Na^+/K^+ -ATPase, and NaF -stimulated adenylate cyclase. The concentration of binding sites were expressed as a function of these markers and gram of tissue wet weight (Table 1). A higher concentration of binding sites was found in the adult when sites were expressed as a function of $5'$ -nucleotidase activity, but no statistical difference was demonstrated between the fetus and adult when the data were expressed as a function of Na^+/K^+ -ATPase activity, NaF -stimulated adenylate cyclase activity, or gram of tissue wet weight. The ratio of receptor concentration to isoproterenol-stimulated adenylate cyclase activation in the two was not significantly different (Table 1). In addition, we did not demonstrate a difference in concentration between the two when the data were expressed as a function of basal adenylate cyclase activity ($0.80 \pm 0.27 \text{ fmoles/pmol cAMP/min}$ versus $0.58 \pm 0.21 \text{ fmoles/pmol cAMP/min}$).

The concentration-related effect of adrenergic agonists in competing for [^3H]DHA binding in the adult was tested, and their potency also fulfilled the pharmacologic criteria for β_1 -adrenergic receptors (Table 2). There was no demonstrable difference in the ability of these agents to compete for binding in the fetus and adult heart preparations ($P > 0.3$ for each comparison).

Comparison of Adenylate Cyclase Activity. There was no demonstrable difference in the activity of basal (16.0 ± 4.3 versus $29.8 \pm 8.9 \text{ pmoles cAMP per mg per min}$) or specific NaF -stimulated adenylate cyclase (68.9 ± 11.5 versus $75.8 \pm 8.0 \text{ pmoles cAMP per mg per min}$) between the fetal and adult sheep heart preparation.

As shown in Figure 5, isoproterenol elicited a concentration-dependent increased activity of adenylate cyclase in both particulates. The concentration of isoproterenol required for half-maximal stimulation (dose required to inhibit stimulation by 50%) was similar in fetus ($0.42 \pm 0.20 \mu\text{M}$) and adult ($0.17 \pm 0.01 \mu\text{M}$) ($n = 3$ for each value; $P > 0.3$). The specific maximally stimulated adenylate cyclase expressed per mg of protein was also similar ($P > 0.1$) in fetus ($22.5 \pm 5.1 \text{ pmoles cAMP per mg per min}$) and adult ($29.2 \pm 6.6 \text{ pmoles cAMP per mg per min}$).

Fig. 4. Comparison of contractile responses to isoproterenol and norepinephrine in fetal and adult sheep ventricular muscle preparations. Isoproterenol and norepinephrine at indicated final concentrations were added to tissue baths and contractile response determined in continuously stimulated fetal or adult myocardial strips as described in "Materials and Methods" (higher concentrations are not shown). The first increased contraction to agents was defined as threshold response.

DISCUSSION

[³H]DHA has been used successfully to examine directly β -adrenergic receptors in crude membrane preparations from hearts of adult mammals including rat (22), guinea pig (15), and dog (2). We found that [³H]DHA bound to fetal sheep heart particulates in a manner which fulfilled pharmacologic criteria expected for binding to cardiac β -receptors. First, the binding was rapid, reversible, saturable, stereoselective, and exhibited high affinity. Second, adrenergic antagonists competed with [³H]DHA for binding at these sites with specificity, i.e., propranolol $\gg$ phentolamine. Third, the rank order of adrenergic agonists competition was isoproterenol $>$ epinephrine $\approx$ norepinephrine, which is similar to their β_1 -adrenergic pharmacological potencies (16). Therefore, [³H]DHA appears to be a suitable tool to study directly the properties of β -receptors in the fetal sheep heart.

Most evidence indicates that cardiac β -receptors are localized within the plasma membrane and stimulation of the receptor may result in increased contractility through activation of adenylate cyclase (8). In this study, we found similar values in fetal and adult sheep hearts for receptor affinity (K_d), inhibitory constants for adrenergic agents (K_i), and the concentration of isoproterenol required to half-maximally stimulate adenylate cyclase. No qualitative difference in the receptor and enzyme activation in the fetal and adult sheep hearts could be demonstrated. Ahumada *et al.* (1) has found that in the ventricular muscle particulate of fetal sheep epinephrine (10 μ M) and NaF (10 mM) but not glucagon (0.1 μ M) stimulate the activity of adenylate cyclase and the maximal response to NaF was similar in fetal and adult sheep.

Investigation of the concentration of receptors in an ontogenic study is complicated by the possibility of a differential loss of plasma membranes which might occur during homogenization and centrifugation of tissues at different stages of development. In this study, we used a relatively crude particulate to examine β -receptors in an effort to minimize this possible loss in the fetal and adult sheep hearts and the recovery of plasma membrane enzyme markers NaF-stimulated adenylate cyclase and Na^+ - K^+ -ATPase was similar in the two preparations (Table 1, footnote).

In addition, the choice of a "denominator" to normalize the concentration of binding sites presents potential difficulties because the "constant" chosen may also vary with development. We approached this problem by expressing the concentration of sites per mg protein or gram tissue wet weight, both of which are used most commonly, and also as a function of the activity of the membrane marker enzymes 5'-nucleotidase, Na^+ - K^+ -ATPase, or NaF-stimulated adenylate cyclase. There were no significant differences in the concentration of binding sites as a function of wet

Table 1. Comparison of the concentration of binding sites in fetal and adult heart particulates when the data are expressed as a function of gram wet weight, mg protein, and activities of membrane enzyme markers

Denominator ¹	Concentration of binding sites		
	Fetus	Adult	P
fmole/mg protein	101.2 $\pm$ 7.4 ²	138 $\pm$ 7.7	<0.02
pmole/ μ g of P _i /min (5'-nucleotidase)	0.52 $\pm$ 0.07	1.12 $\pm$ 0.06	<0.01
pmole/ μ g of P _i /min (Na^+ , K^+ -ATPase)	0.18 $\pm$ 0.04	0.20 $\pm$ 0.04	N.S. ³
fmole/pmole cAMP/min (by NaF)	1.63 $\pm$ 0.32	1.85 $\pm$ 0.11	N.S.
pmole/g of wet weight	4.0 $\pm$ 0.4	3.2 $\pm$ 0.3	N.S.
fmole/pmole cAMP/min (by isoproterenol)	5.52 $\pm$ 1.17	5.26 $\pm$ 1.23	N.S.

¹ Each preparation was assayed for enzymes and normalized with the corresponding concentration of [³H]DHA binding sites determined by Scatchard analysis. Protein contents per gram of tissue wet weight were 40.7 $\pm$ 9.1 and 25.7 $\pm$ 6.3 mg/g for fetus and adult, respectively. Specific activity of 5'-nucleotidase was 0.20 $\pm$ 0.02 μ g of P_i per mg per min for fetus and 0.12 $\pm$ 0.00 μ g of P_i per mg per min for adult. Specific activity of Na^+ , K^+ -ATPase was 0.63 $\pm$ 0.09 μ g of P_i per mg per min for fetus and 0.76 $\pm$ 0.21 μ g of P_i per mg per min for adult. Specific activity of NaF-stimulate adenylate cyclase was 68.9 $\pm$ 11.5 pmoles and 75.8 $\pm$ 8.0 pmoles cAMP per mg per min for fetus and adult, respectively.

² Mean $\pm$ S.E. of three to four experiments.

³ Not significant, $P > 0.1$.

Table 2. Comparison of K_i values for adrenergic agonists in fetal and adult sheep heart particulates

Agonist	K_i (μ M) ¹	
	Fetus	Adult
(-)-Isoproterenol	0.32 $\pm$ 0.10 ²	0.20 $\pm$ 0.05
(-)-Epinephrine	1.19 $\pm$ 0.23 ³	1.47 $\pm$ 0.27 ³
(-)-Norepinephrine	2.67 $\pm$ 0.69 ³	2.42 $\pm$ 0.78 ³

¹ K_i values were calculated from formula $K_i = \frac{I_{50}}{1 + (L)/K_d}$ (25).

² Mean $\pm$ S.E. of 3 experiments.

³ Significant at $P < 0.05$ compared with the corresponding value of (-)-isoproterenol.

Fig. 5. Comparison of isoproterenol-stimulated adenylate cyclase in fetal and adult sheep heart preparations.

Values are expressed as percentage maximum of isoproterenol-stimulated adenylate cyclase at indicated concentrations. Data are from three experiments.

weight, $\text{Na}^+\text{-K}^+\text{-ATPase}$ or NaF -stimulated adenylate cyclase, yet a higher concentration of binding sites was found in the fetal heart when the sites were expressed as a function of 5'-nucleotidase or mg protein. Since both sensitivity (dose required to inhibit stimulation by 50%) and maximal response of isoproterenol-stimulated adenylate cyclase were similar in the fetal and adult sheep heart particulate, two possibilities could be suggested: (1) the β -receptor concentration in the two preparations is similar; (2) the β -receptor concentration is increased in the myocardium of adult sheep, but the efficiency of receptor-mediated cyclase activation is reduced proportionally, resulting in similar cyclase activation in the adult and fetus. We believe the first explanation is more likely because: (1) in the systems reported previously alterations in β -receptor concentration are usually accompanied by an altered sensitivity and/or maximal response of isoproterenol-stimulated adenylate cyclase (14, 26); and (2) the coupling between the β -receptor and cAMP formation has recently been demonstrated to be highly efficient in the myocardium of adult mammals (24). It is possible that increased 5'-nucleotidase activity in the fetal heart particulate is the result of ontogenic changes in this enzyme unrelated to the β -receptor. The small (37%) increase in binding sites per mg protein in the adult sheep heart particulate may be due to the fact that the protein measured does not proportionally reflect the membrane protein. Our results indicate that when receptor concentrations are examined for an ontogenic study, the choice of denominator may determine the conclusion. NaF -stimulated adenylate cyclase has previously been proposed as the appropriate membrane marker enzyme (21), and we have interpreted our data using this marker because of the similarity of β -adrenergic activation of adenylate cyclase in the fetal and adult myocardium. However, the conclusion must be tentative because balanced changes in coupling and receptor concentration could also yield the same result.

Because the affinity for adrenergic agonists (K_i), the concentration of binding sites (when expressed as a function of stimulated adenylate cyclase), and activation of adenylate cyclase are similar in the fetal and adult heart particulates, the decreased response to isoproterenol *in vivo* (27) and the increased response to norepinephrine *in vitro* in the fetus (9) would seem to be due to differences beyond the receptor. The decreased fetal cardiac response to isoproterenol may be related to a dampening effect of umbilical-placental circulation and vascular shunts (27). In the fetal sheep ventricular strip, the increased inotropic response to norepinephrine is probably associated with a decreased norepinephrine neuronal uptake (9) and possibly the presence of a relatively high concentration of cardiac α -adrenergic receptors (7).

It appears from our study that functional β -receptors in the sheep fetus reach maturity in terms of concentration, affinity for adrenergic agonists and antagonists, and the subsequent receptor mediated cyclase maturation near term. The maturation of β -receptors and receptor-mediated cyclase activation adenylate cyclase activation in fetal heart may occur earlier than in other tissues such as the brain (11). Fetal sheep heart is similar to fetal human heart in many respects including ontogeny of nerve development (17, 19) and therefore may be a good model for understanding the development of functional adrenergic receptors in the human heart. In addition, the relatively large body of information available on pharmacologic responses of fetal sheep myocardium *in vivo* is useful to correlate developmental changes in the receptor and adenylate cyclase activation with adrenergic contractile response.

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