DEVELOPMENT OF THE CARDIAC BETA ADRENERGIC RECEPTOR IN FETAL RAT HEART

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SUMMARY. Hearts from 13-day-old rat fetuses were shown to specifically bind 7-^{3}H D,L-norepinephrine. In addition, norepinephrine activated adenylate cyclase in homogenates from the same hearts. The activation of the enzyme was abolished by D,L-propranolol. These data demonstrate the existence of a functionally intact cardiac beta adrenergic receptor at a period of time in fetal life prior to the development of inotropic and chronotropic responses to the catecholamines.

Knowledge of the development of fetal cardiac hormone receptors is important in understanding normal fetal cardiovascular physiology and its potential application to normal and pathologic states observed in adult life. Perhaps the most important hormonal modulators of inotropic and chronotropic effects are the catecholamines which act via discrete beta adrenergic receptors to activate the membrane-bound enzyme adenylate cyclase resulting in increased myocardial levels of adenosine 3',5'-monophosphate (cyclic AMP) (1). The cyclic AMP generated initiates other biochemical steps which ultimately result in the physiologic response of the heart (2-4).

The purpose of the present investigation was to determine the development of the beta adrenergic receptor-catecholamine-sensitive adenylate cyclase in fetal rats, a model in which ample data is available in the literature to correlate our results with the known physiologic parameters (5-8). The data in this report show that both specific binding of norepinephrine to cardiac receptors and catecholamine-activation of adenylate cyclase are present by the 13th to 14th day of fetal life, a time when only minimal physiologic responses to catecholamines are noted.

METHODS. Pregnant rats of known gestational age (± 12 hours) were obtained from Holtzman Company, Madison, Wisconsin. The presence of sperm is detected by vaginal smears within 12 hours after mating. The pregnant rats were anesthetized with approximately 0.5 cc of pentobarbital intrathoracically and the fetuses rapidly removed. The heart from each fetus was removed under the visual aid of a dissection microscope (Bausch and Lomb). Only the hearts which were beating at the time of removal were used for these studies. In addition, random hearts were selected for histologic study to confirm the identity of the tissue removed.

Adenylate cyclase assay. Approximately 10-20 hearts were added to 1.0 ml of 0.25 M sucrose and homogenized in a Dounce homogenizer (glass on glass) with 5-7 strokes of the pestle. The homogenate was utilized for the assay of adenylate cyclase in which the conversion of alpha-labeled ^{32}P-ATP to ^{32}P-cyclic AMP is measured (9) under the precise conditions described previously from our laboratory (10, 11).

Norepinephrine binding assay. Norepinephrine binding was determined as previously described (12). Approximately 10-20 fetal hearts were solubilized in a homogenizing solution containing in final concentration sucrose, 0.25M; Tris-HCl, 10mM; pH 7.7; Lubrol-PX, 20 mM, and EDTA-magnesium chloride, 1mM. The homogenate was then applied to a DEAE-cellulose column in order to remove the detergent (11). 7-^{3}H D,L-norepinephrine (13.6 Ci/mmole) was incubated with the fraction containing the solubilized adenylate cyclase (detergent-free, 1M Tris-HCl, pH 7.7 effluent from DEAE-cellulose chromatography) in a final volume of 0.1 ml. After 60 minutes at 37^{o}C, the incubation mixture was added to 5 cm columns of Bio-Gel P2 in a glass column of 0.5 cm diameter equilibrated with 10 mM Tris-HCl, pH 7.7. The column was then washed with 0.6 ml of 10 mM Tris-HCl, pH 7.7. Bound

TABLE 1

7-^{3}H D,L-Norepinephrine (M)	7-^{3}H D,L-Norepinephrine Bound (nanomoles/60 min/mg protein)
5×10^{-5}	125.0 ± 23
5×10^{-6}	112.0 ± 50
5×10^{-7}	18.0 ± 5
5×10^{-8}	2.0 ± 0.2

Binding of 7-^{3}H D,L-Norepinephrine. Each value represents the mean ± SEM of three experiments.

^{3}H-norepinephrine elutes with the fraction containing the solubulized adenylate cyclase (i.e., excluded from the gel), and is collected and counted in a liquid scintillation spectrometer. Control incubations contained 1M Tris-HCl, pH 7.7 in place of the enzyme fraction and indicated that > 95% of the free, unbound ^{3}H-norepinephrine remained in the column gel under these conditions.

MATERIALS. 7-^{3}H D,L-norepinephrine was obtained from New England Nuclear, α-^{32}P-ATP from International Chemical and Nuclear Corporation. Lubrol-PX was a gift from I.C.I. America, Inc.

RESULTS. Binding of 7-^{3}H D,L-norepinephrine. Several recent studies have demonstrated specific binding of norepinephrine to cardiac receptor sites in microsomal preparations of adult dog heart (13,14). Table 1 shows that norepinephrine binding is demonstrable in the hearts from 13-day-old rat fetuses. The binding is concentration related over the range 1×10^{-7}M to 5×10^{-5}M. The specificity of this binding was determined by the ability of unlabeled norepinephrine to decrease the binding of 7-^{3}H D,L-norepinephrine. Table 2 shows that 1×10^{-5}M unlabeled norepinephrine decreased the binding almost completely. Displacement was observed over the broad concentration range of 1×10^{-7}M to 1×10^{-5}M.

TABLE 2

Unlabeled NE (M)	7-^{3}H D,L Norepinephrine Bound (nanomoles/60 min/mg protein)
0	330 ± 80
1×10^{-5}	30 ± 20
1×10^{-6}	200 ± 25
1×10^{-7}	320 ± 100

Displacement of 7-^{3}H D,L-norepinephrine. ^{3}H-norepinephrine was present at 1×10^{-6}M. Each value represents the mean ± SEM of three experiments.

TABLE 3

Norepinephrine (M)	Cyclic AMP Accumulated (picomoles/10 min/mg protein)
0	212 ± 55
5×10^{-5}	506 ± 30
2×10^{-5}	612 ± 30
2×10^{-6}	388 ± 40
0 (+ Propranolol)	259 ± 60
2×10^{-5} (+ Propranolol)	224 ± 45

Norepinephrine Activation of Adenylate Cyclase. Propranolol was present at 5×10^{-6}M. Each value represents the mean ± SE of four samples.

Activation of adenylate cyclase by norepinephrine. Catecholamines activate adenylate cyclase in particulate preparations of adult rat myocardium (1). Table 3 shows that catecholamines activate adenylate cyclase in homogenates of heart from 13-day-old rat fetuses. Maximal concentrations produced approximately a threefold increase above the control. Furthermore, the activation was mediated by a beta adrenergic receptor since the increase in cyclic AMP produced by norepinephrine was abolished by the beta adrenergic blocking drug D,L-propranolol (Table 3). Similar results for both binding and activation of adenylate cyclase are obtained with 18-day-old fetuses.

DISCUSSION. In general, little information is available referrable to the development of cardiac hormone receptors in fetal animals (15). However, a number of related investigations have been reported using fetal rats which provide a good physiologic background in this animal model (5-8). The rat heart begins to beat about the ninth or tenth day after conception. Innervation of the sinus node occurs at sixteen days. In early fetal life there is a slow fixed heart rate, which progressively increases with age. Catecholamines do not produce marked increases in heart rate until about 16-18 days, a time when they have also been shown to have a catecholamine-sensitive adenylate cyclase (16). A recent detailed investigation by Wildenthal in fetal mice (17) demonstrated that there were only minimal chronotropic responses to norepinephrine by 12-14 days, more prominent and significant chronotropic responses occurring after 15-16 days with peak responsiveness at 21-22 days just prior to birth.

The data in this report demonstrate a functionally complete cardiac beta adrenergic receptor is present at 13 days of fetal life prior to the development of chronotropic and inotropic responses to catecholamines. The intact nature of this receptor was demonstrated by the specific binding of norepinephrine to cardiac receptor sites

and the catecholamine-mediated activation of adenylate cyclase. Thus, it would appear that lack of responsiveness to the catecholamines in terms of inotropic and chronotropic effects would reside at a step beyond the generation of cyclic AMP. Since the precise mechanism by which cyclic AMP exerts its effects on the heart is unknown, we are unable to speculate on the specific biochemical site(s) which may not be developed at this time in fetal life (2-4)

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