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Isoproterenol Stimulation of Ornithine Decarboxylase Blocked by Propranolol during Ontogeny of the Murine Heart

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SUMMARY

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Fluctuations in the activity of fetal mouse heart ornithine decarboxylase (ODC) are positively correlated to growth rate. ODC activity was maximal (27 pmoles/min/mg of protein) at 13 days of embryogenesis, the earliest time that hearts could be dissected. Stimulation of fetal cardiac ODC activity was used as an index of cardiac responsiveness to adrenergic stimulation. The ability of either maternally injected or fetally injected isoproterenol to stimulate an increase in fetal heart ODC activity in embryos close to term was concentration-dependent. The *beta*-adrenergic stimulant, 10 mg/kg maternally or 2 mg/kg fetally, promoted a maximal response (300–400%). Maternal injections were used routinely, therefore, for ease of experimentation. The development of the fetal heart trophic response to *beta*-receptor agonists was examined after maternal injections of 2 or 10 mg/kg of isoproterenol from 13 to 21 days of gestation. Isoproterenol promoted an increase in myocardial ODC activity at all times monitored during fetal development. The highest drug-stimulated activity, 57 pmoles/min/mg of protein, occurred at 16–17 days of gestation. After this, the absolute specific enzyme activity achieved after isoproterenol declined, although the magnitude of the response remained at 2.5- to 3-fold above control until birth. The stimulation was blocked by propranolol (10 mg/kg s.c.). The maximal *in vivo* coupling of the *beta*-receptor to murine ODC activity, apparent from Day 13 of gestation, precedes the development of its *in vitro* coupling to the chronotropic response, first apparent after 17 days.

INTRODUCTION

The appearance of *beta*-receptors in the fetal mouse heart, as determined by radioactive ligand binding methods (1, 2), precedes any demonstrable effect of catecholamines on the cardiac chronotropic or inotropic response (1–3). Adrenergic stimulation of developing heart tissue has profound effects on cellular nucleic acid and protein synthesis patterns (4, 5), and the catecholamines have been postulated to play a key regulatory role in cardiac cell proliferation and differentiation (4, 5). However, the time during embryogenesis at which adrenergic stimulation can actually influence cardiac development is unknown.

In the adult animal, catecholamines have a trophic effect on the heart, promoting increases in tissue protein, RNA, and polyamine biosynthesis (6–8). One of the earliest biochemical changes detectable in the cardiac trophic response after *beta*-receptor stimulation by administration of isoproterenol or norepinephrine is an increase in the activity of ODC[4] (EC 4.1.1.17), the rate-limiting enzyme in the polyamine biosynthetic pathway (9–12). There is a strict parallelism, shown to exist in a large variety of species and tissues, between the rates of tissue RNA and protein synthesis and the measurable cellular polyamine content (13–15). Because of this stringent relationship and the rapid and large fluctuations that occur in the enzyme after tissue stimulation as a result of its short turnover time (i.e., 10–20 min) (16), the activity of ODC has been suggested as a sensitive marker in assessing the presence or absence of a tissue response to a pharmacological stimulus (17, 18).

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[4] The abbreviation used is: ODC, ornithine decarboxylase.

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in assessing the presence or absence of a tissue response to a pharmacological or physiological stimulus (17, 18).

The present study was undertaken to determine the time of *beta*-receptor coupling to cardiac biosynthesis during embryonic development of the mouse heart. The ODC activity in the fetal heart was used as an indicator of the acquisition of cellular responsiveness to adrenergic input.

METHODS

CD-1 mice obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.) were housed on a 12-hr photoperiod. Gestational age of the fetuses was timed from a 24-hr period of exposure of the female to 12 males. Females with vaginal plugs at the end of the 24-hr period were separately caged and the day of plug observation was counted as Day 1. Drugs were administered by s.c. injection of the mother except where placental transfer was evaluated. In these placental transport studies, the mother was anesthetized with ether, the fetuses were exposed by laparotomy, and each fetus was injected in the area of the hip. The abdominal incision was closed with a continuous suture and the mother was allowed to recover from the anesthetic. Controls treated with 0.9% NaCl solution were subjected to sham laparotomy. At the appropriate time following drug administration, fetuses of both sexes were delivered by caesarean section and killed by cervical dislocation. The hearts were rapidly excised, blotted and weighed, frozen by immersion in liquid nitrogen, and stored at −80°. The frozen tissue (5–10 mg) was homogenized with a Tissumizer (Tekmar Company, Cincinnati, Ohio) at 4° in 1 ml of 50 mM $NaHPO_4$-$KHPO_4$, pH 7.2, containing 5 mM NaF, 0.1 mM EDTA, 2 mM dithiothreitol, and 0.06 mM pyridoxal phosphate. Homogenates were centrifuged at 10,000 × *g* for 5 min and two 200-μl aliquots of the resulting supernatant were assayed for ODC activity. Incubations were conducted at 37° for 60 min. The reaction was initiated by the addition of 0.2 μCi of L-[^{14}C]ornithine (50 mCi/mmole) (Amersham/Searle Corporation, Des Plaines, Ill.) and cold ornithine to a final concentration of 0.25 mM. Pyridoxal phosphate-independent release of $^{14}CO_2$ was determined by incubating an equivalent amount of tissue supernatant in the presence of 4-bromo-3-hydroxyl-benzyloxyamine dihydrogen phosphate, and the amount of release was subtracted from the sample value. The reaction was terminated by the addition of 0.6 ml of 1 M citric acid. The reaction was continued for another 15 min and the $^{14}CO_2$ evolved was trapped by 20 μl of 2 N NaOH on a 3 MM filter paper (Whatman Inc., Clifton, N. J.) suspended above the reaction mixture in a plastic well (Kontes Company, Vineland, N. J.). The filter paper was then placed in toluene-Omnifluor (New England Nuclear Corporation, Boston, Mass.) and radioactivity was determined in a liquid scintillation spectrometer. Specific activity of the enzyme is expressed as picomoles of $^{14}CO_2$ liberated per minute per milligram of protein.

Protein content of tissue supernatants was determined by the dye-binding method of Bradford (19). The rate of heart growth is very rapid (e.g., 2- to 3-fold increase per 24 hr) during the time of embryogenesis under study, and the gestational age was estimated over a 24-hr period. Additionally, interindividual differences in cardiac development have been noted to be fairly large, even in the same litter (20). Variations from litter to litter in postnatal studies of ODC sensitivity to drug stimulation have been noted (21). Therefore, to normalize the data so that ODC activity could be compared with the exact growth status of the organs assayed, all of the data of protein content per heart obtained during the study were plotted versus the estimated age (Fig. 1). The gestational age for each embryonic heart assayed was then determined from the averaged curve based on the individual heart protein content.

RESULTS

Figure 2 shows the results of an experiment in which the developmental pattern of ODC in the basal (unstimulated) heart was determined. At all stages of embryogenesis examined, there was a significant amount of enzyme activity present in the fetal mouse heart. At 13 days of gestation, the earliest time that hearts could be dissected, ODC activity was maximal (27 pmoles/min/mg of protein) and then declined progressively throughout the remainder of gestation. The change in the basal activity of the enzyme paralleled the changing growth rate of the heart, as determined by the increment in organ protein content (Fig. 2).

The concentration of isoproterenol required to stimulate an increase in fetal heart ODC was determined in a dose-response experiment conducted with embryos close

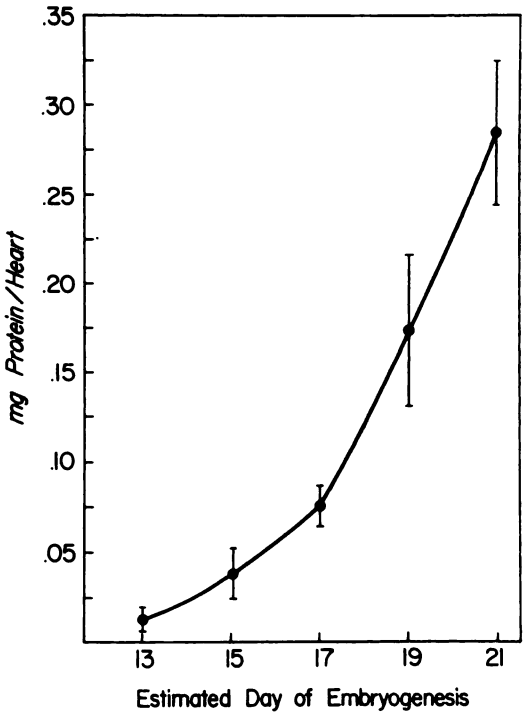

FIG. 1. *Protein content of the fetal mouse heart during embryogenesis*

The protein content of the 10,000 × *g* supernatant from the homogenized fetal hearts was determined by the dye-binding method of Bradford (19), normalized per heart, and plotted versus the estimated day of embryogenesis. Each *data point* represents the mean ± standard deviation, *n* = (Day 13) 10, (Day 15) 7, (Day 17) 10, (Day 19) 16, (Day 21) 46.

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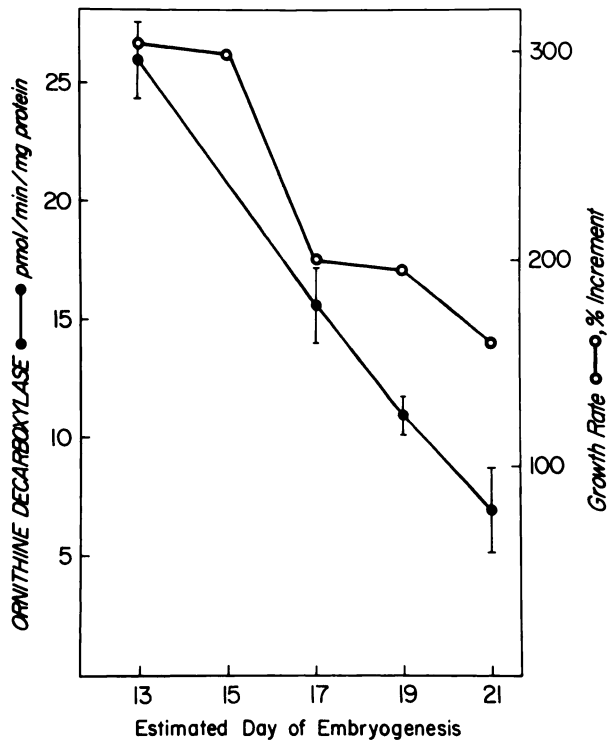

FIG. 2. *Changes in ODC activity in the fetal mouse heart during embryogenesis*

The activity of ODC (●) in mouse hearts obtained at the indicated day of gestation was assayed as described under Methods. Each *data point* represents the mean ± standard deviation of measurements of three or four heart pools. The rate of growth of the heart (○) was calculated as the percentage increment in heart protein during each 2-day period as given in Fig. 1.

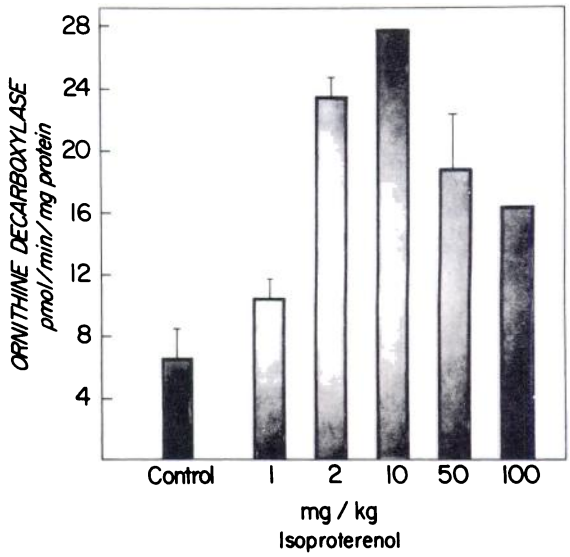

FIG. 3. *Isoproterenol stimulates a dose-dependent increase in ODC activity of the fetal mouse heart*

Mothers were injected s.c. with the indicated dose of isoproterenol or 0.9% NaCl solution (control) 4 hr prior to assay of the fetal heart ODC activity. *Bars* represent the mean ± standard deviation of the measurements of four to six separate heart pools, except for the highest dose, at which there was significant fetal death.

to term (average 0.23 mg of protein per heart, estimated 20 days of gestation). The mothers were injected with varying amounts of the *beta*-adrenergic agonist (s.c. in 0.5 ml of 0.9% NaCl solution) and, 4 hr later (the time of maximal response in adult animals) (9–12), ODC activity of the fetal heart was determined. As shown in Fig. 3, 1 mg/kg, the lowest dose producing a consistent response, promoted a 60% elevation in enzyme activity, whereas maximal ODC levels (420% of control) were achieved with 10 mg/kg of isoproterenol. The response to the drug was biphasic, since higher concentrations were less effective. This decrease in efficacy could be the result of drug-induced cytotoxicity, since, at concentrations of 100 mg/kg or greater, fetal death frequently occurred.

To ensure that maternal injection was comparable to fetal administration, i.m. injections of 2 mg/kg and 10 mg/kg were given to fetuses at 17 days of gestation. All of those receiving 10 mg/kg died, but ODC activity was stimulated in hearts of fetuses given 2 mg/kg (Table 1). Stimulation after direct fetal injection did not differ from that of injected mothers. These experiments were added in proof of stimulation of fetal *beta*-receptors after maternal injection. Propranolol blocked the increase in ODC activity in response to isoproterenol after either fetal or maternal injection.

In order to study the maturation of the heart trophic response to *beta*-receptor stimulation, the ability of maternal injections of isoproterenol to elevate fetal heart ODC was examined throughout embryogenesis. As shown in Fig. 4, the *beta*-agonist promoted an increase in cardiac enzyme activity at all times assayed during fetal development. Although the basal ODC activity declined from 13 to 17 days of embryogenesis, administration of 10 mg/kg of isoproterenol elevated the enzyme activity to levels higher than the peak basal levels detectable at 13 days. The highest drug-stimulated activity (57 pmoles/min/mg of protein) occurred at 16–17 days of gestation. After this, the elevation of ODC activity detectable in response to isoproterenol administration declined, although the magnitude of the stimulation remained at a level approximately 2.5- to 3-fold above control until birth. Companion litters at all stages of embryogenesis also were tested with 2 mg/kg of isoproterenol to evaluate for possible changing sensitivity to adrenergic stimulation during maturation. A response pattern similar to that at 10 mg/kg was obtained with

TABLE 1

Fetal heart ornithine decarboxylase activity after fetal or maternal injection

Fetuses were injected i.m. in the hip ar (2 mg/kg). The average weight of fetuses at 17 days was 500 mg, so they received 5 μg/25 μl. Mothers received 10 mg/kg of isoproterenol s.c. Propranolol (10 mg/kg s.c. maternal or i.m. fetal) was given 10 min prior to isoproterenol in the mothers, but simultaneously in fetuses.

	Ornithine decarboxylase activity
	pmoles $^{14}CO_2$/min/mg protein
Isoproterenol	49.5 ± 6.3 (n = 8)
0.9% NaCl solution	12.1 ± 2.5 (n = 6)
Isoproterenol + propranolol	6.2 ± 0.9 (n = 6)
Mother	
Isoproterenol	54.0 ± 4.7 (n = 5)
0.9% NaCl solution	8.2 ± 0.9 (n = 5)
Isoproterenol + propranolol	5.8 ± 0.06 (n = 5)

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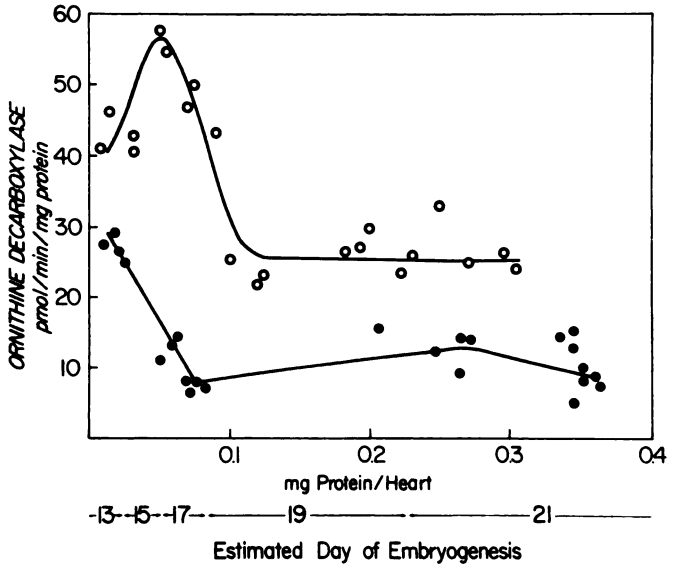

FIG. 4. *Isoproterenol stimulation of ODC induction in the fetal mouse heart at different times of gestation*

Isoproterenol (10 mg/kg; ○) or 0.9% NaCl solution (control; ●) was administered i.p. to mothers at the indicated days of gestation. The activity of ODC in the fetal heart was measured 4 hr after administration of the drug.

the lower dose, although the extent of the ODC increase was never as great as that promoted by the higher dose.

The trophic response of the fetal mouse heart to *beta*-adrenergic stimulation is compared with the chronotropic response in Fig. 5. Studies (1, 2) of the chronotropic response were conducted with fetal hearts obtained from mice of the same colony. Therefore, for the *in vitro* chronotropic studies, the timing of developmental events was under conditions identical with those for the *in vivo* trophic response studies. The heart rate response was not statistically elevated over the basal rate at 13 days after isoproterenol administration (10^{-3} M), even though a 107% increase was observed. At 17 days, the chronotropic response was 131% above basal at a time of ODC stimulation to 300% of control. These data compare favorably with those of Wildenthal (3), who demonstrated a 110% increase in heart rate at 13 days with 10^{-6} M isoproterenol and a 108% increase at 13 days with 10^{-5} M norepinephrine. The *beta*-receptor is coupled to the trophic response as assessed by the marked increase in cardiac ODC activity which was measurable from 13 days of gestation through birth, with peak effects demonstrable at 16–17 days of development. In contrast, there is a minimal effect of *beta*-receptor stimulation on mouse heart rate at 13 days of gestation (1–3). The chronotropic response to isoproterenol of 130% of basal rate (3), apparent after 17 days, increases progressively in magnitude until birth.

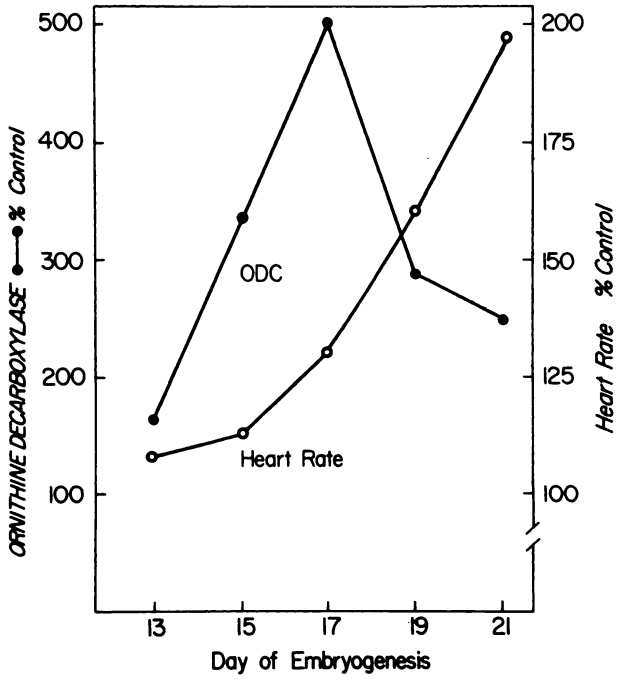

FIG. 5. *Changes in the trophic and chronotropic response of fetal mouse hearts to isoproterenol during embryogenesis*

The percentage increase stimulated by isoproterenol above basal ODC activity in the fetal heart at the indicated days of embryogenesis was calculated from the data in Fig. 4. The data showing the change in heart rate of the fetal mouse heart in response to a maximally stimulating dose (10^{-3} M) of isoproterenol are taken from Roeske *et al.* (22).

DISCUSSION

Catecholamines produce marked increases in cardiac macromolecular biosynthesis in both the adult and developing animal (4–12). However, the time at which the heart becomes responsive to trophic stimulation by adrenergic agents is unknown. As discussed by Bartolome *et al.* (11) and Slotkin (17), the developmental factors governing heart responsiveness to adrenergic stimulation include (a) establishment of sympathetic innervation, (b) development of cardiac *beta*-receptors, and (c) acquisition of cellular competence to respond to receptor stimulation. In the developing mouse heart, atrial innervation is thought to be complete after 15–16 days of gestation (3, 20) and the appearance of cardiac *beta*-receptors precedes this, with 14% of the adult density level apparent at 13 days (1). These early receptors are functionally coupled to adenylate cyclase activation (2), but do not mediate a marked heart rate response (1, 3). The present study examined the ontogeny of *beta*-receptor coupling to the heart biosynthetic response.

The induction of ODC, the rate-limiting enzyme in polyamine biosynthesis, is a ubiquitous component of the growth response (reviewed in refs. 17 and 18). Changes in cellular polyamine content have been shown to parallel rates of RNA and protein synthesis in both embryonic systems and in hypertrophy or hyperplasia of adult tissues (reviewed ref. in 13 and 14), including cardiac hypertrophy (23–26). Therefore, we used the sensitivity of cardiac ODC to adrenergic input as an index of whether the *beta*-receptors present in the fetal tissue were coupled to the cardiac trophic response. The trophic responsiveness of the fetal heart was found to be functionally coupled to the *beta*-receptor from 13 days of gestation through birth. The peak responsiveness, in terms of the magnitude of the increase in enzyme activity promoted, occurred at 17 days. This could reflect either a more efficient coupling of the adrenergic receptor to the trophic process or changes in the intracellular components regulating RNA and protein synthesis.

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The dose of maternally injected isoproterenol required to promote a maximal increase in the fetal heart ODC (10 mg/kg) is 50- to 100-fold greater than that which promotes an increase in the adult heart (9–12). The fetus has been reported to be less sensitive to sympathomimetic amines than the adult (27); however, the affinity of the murine fetal cardiac *beta*-receptors for adrenergic agonists has been shown to be identical with that of the adult (1, 2). Therefore, this large difference in efficacy more likely represents the inefficiency of placental transport of the drug. Direct fetal injections of 2 mg/kg were comparable to 10 mg/kg s.c. to the mother. The question of whether there is any transport at all from the maternal circulation to the fetus has been a subject of controversy (28–30). However, studies conducted using radioactively labeled catecholamines (29, 30), as opposed to those depending on a fetal physiological response (28) to measure possible transport, have shown that the drug crosses the placenta and accumulates in the fetal heart. The ability of direct fetal injection to produce stimulation of fetal heart ODC activity similar to that produced by maternal injection, as well as the ability of propranolol to block this increase after either procedure, substantiates the occurrence of stimulation of fetal heart *beta*-receptors after maternal injection.

The development of the cardiac trophic response to *beta*-adrenergic stimulation appears to precede expression of the chronotropic response. This is in harmony with the ability to detect *beta*-receptors during murine heart development prior to the development of significant responsiveness to *beta*-agonists as measured by a detectable heart rate response (1). Although the comparison of *in vitro* findings concerning the chronotropic response with *in vivo* trophic stimulation is subject to question, measurement of heart rate changes on freshly isolated hearts under stable, controlled conditions avoids the confusing influences of secondary changes in heart rate produced by neural and humoral factors. This comparison is further substantiated by almost identical results on the chronotropic responses which were obtained by this (1, 2) and another laboratory (3), even though the timing of developmental studies might be different in separate laboratories. Further studies are in progress to assess whether the receptors coupled to the cardiac trophic response have pharmacological properties different from those coupled to the chronotrophic response.

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