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Regulation of Ornithine Decarboxylase Activity in the Developing Heart of Euthyroid or Hyperthyroid Rats

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SUMMARY

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Ornithine decarboxylase (ODC), the rate-limiting enzyme in the biosynthesis of polyamines, displays high activity early in postnatal development of the heart, followed by a gradual decline to the low levels characteristic of adults. In both 2- and 4-day-old rat pups, kinetic studies revealed the presence of two distinct K_m^{orn} values of 50–60 and about 250 μM. However, by 7 days of age, only one form of the enzyme could be detected, with a K_m^{orn} of 200–250 μM; after this point, the K_m^{orn} remained at the low-affinity value, and further age-dependent reductions in activity occurred due to a progressive decline in the V_{max}. The rate of disappearance of ODC activity, measured after the administration of cycloheximide, did not change with age. Neonatal administration of triiodothyronine (T_3) evoked an initial elevation of ODC followed by depressed activity. As was true of controls, ODC from 2-day-old T_3-treated rats displayed two separate kinetic forms, but the V_{max} values of both forms were elevated above those of controls. At 10 days of age, only low-affinity ODC could be found in T_3-treated rats, and the V_{max} was below that of controls. Cycloheximide treatment revealed only small changes in the half-life of ODC activity after T_3 treatment. The accelerated maturational decline of cardiac ODC in hyperthyroid rats was correlated with depressed RNA synthesis and with subsequent deficits in heart weight. These results indicate that the developmental decline of heart ODC can be explained by the disappearance of the high-affinity kinetic form of the enzyme as well as by a reduction in enzyme synthesis and/or catalytic efficiency; after its initial stimulatory effect, neonatal hyperthyroidism exerts a deleterious effect on ODC activity which may contribute to deficient nucleic acid synthesis and cardiac growth.

INTRODUCTION

Ornithine decarboxylase (ODC; EC 4.1.1.17) catalyzes the formation of putrescine from ornithine, the first and probably rate-limiting step in polyamine biosynthesis (1, 2). The polyamines are distributed ubiquitously in tissues, appear to be associated intimately with cellular growth and differentiation, and play key roles in nucleic acid and protein syntheses (3, 4). Polyamines, nucleic acid, and protein syntheses have been shown to vary in a parallel fashion in a variety of rapidly growing systems, including developing mammalian organs, and generally, ODC activity is stimulated under conditions in which growth is induced and decreases as growth ceases or as the number of actively dividing or growing cells declines. Thus, in the developing heart, ODC activity is high at birth and falls off progressively to the low levels characteristic of adult tissue (5–7). The heart differs from many other tissues and cultured cells in that it goes through two distinct phases of growth. Early in development, DNA replication takes place, but by the 17th day of postnatal age, the heart ceases to synthesize new DNA (8, 9); further growth of the heart therefore represents hypertrophy of preexisting cells. Thus, cardiac tissue offers a unique opportunity to study modes of ODC control in replicating and nonreplicating cells in the same tissue at different stages of development.

In addition to stimulation during general growth, increases in ODC activity have been demonstrated to occur in target organs exposed to specific hormones or neurotransmitters (6, 7, 10–13). In the mature rat heart, triiodothyronine (T_3), an agent which causes cardiac hypertrophy, has been demonstrated to evoke large and persistent increases in ODC (7). In the developing rat heart, however, the elevation of ODC caused by neonatal administration of T_3 is short-lived and is followed by sub-

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sequent declines to subnormal levels (7); after the initial phase of cardiac hypertrophy in the T_3-treated neonate, persistent deficits of heart weight also appear.

Data from a recent investigation indicate that the increases in heart ODC of mature rats responding to cardiac growth stimuli (such as T_3 or isoproterenol administration) or the innately higher ODC of 2-day-old neonates all involve a form of the enzyme with an increased affinity for substrate (14). The present studies suggest that the developmental decline of heart ODC activity is directly related to the disappearance of the high-affinity component of the enzyme as well as to a reduction in the V_{max}. Additionally, the initial elevation and subsequent acceleration of the ontogenetic decline of developing heart ODC evoked by T_3 represent alterations in the V_{max} of the enzyme, which may be involved in deficits in RNA synthesis and cardiac growth.

METHODS

Timed pregnant Sprague–Dawley rats (Zivic–Miller) were housed individually in breeding cages and allowed food and water *ad libitum*. Pups from all litters were randomized at birth and redistributed to the nursing mothers, with the litter size kept at 8 to 10 pups. In each experiment, pups of either sex were selected from several different cages. Neonates were given 3,3′,5-triiodothyronine (T_3) (0.1 mg/kg, s.c., dissolved in 0.01 N NaOH) daily beginning 1 day after birth and continuing for 9 days, while control animals received daily injections of 0.01 N NaOH equal in volume to the T_3 injections (1 ml/kg). The administration of vehicle alone did not affect ODC activity in the rat heart (unpublished data). Pups were weighed and killed by decapitation at intervals of several days; during the course of T_3 administration, animals were sacrificed 24 h after the previous injection. Whole hearts were excised, weighed, and homogenized (Polytron) in 19 vol of ice-cold 10 mM Tris–HCl (pH 7.2) and then centrifuged at 26,000*g* for 20 min. Duplicate aliquots of the supernatant were removed for assay of ODC activity by a modification of the method of Russell and Snyder (2); the incubation mixture contained 0.9 ml of the supernatant preparation and final concentrations of 1.8 mM dithiothreitol, 50 μM pyridoxal-5′-phosphate, and 9.5 μM DL-[^{14}C]ornithine in a final volume of 1 ml. The $^{14}CO_2$ evolved was trapped in hyamine hydroxide and counted by liquid scintillation spectrometry. Under these conditions, activity in the adult and developing tissue samples is linear with time and tissue concentration and formation of putrescine correlates one-to-one with evolution of $^{14}CO_2$ (15). Activities are reported as nanomoles of $^{14}CO_2$ evolved per hour per gram of original wet weight of the tissues. Hearts were pooled from several pups at early developmental stages to obtain sufficient tissue.

The enzyme kinetics of rat heart ODC at various developmental stages in control or T_3-treated pups were determined utilizing a range of ornithine concentrations from 5 to 370 μM. ODC activities were assayed in triplicate for each concentration. Data of the kinetic studies are presented as double-reciprocal plots averaged from three or more experiments; several examples are shown in the figures, with results of all experiments summarized statistically in Table 1. To ensure that the activity measured did, in fact, represent ODC at each age and at each ornithine concentration, the assay blanks contained enzyme prepared from animals given cycloheximide (200 mg/kg, i.p.) 1–2 h before killing. The heart ODC value was then determined as the differences between samples from untreated rats vs cycloheximide-treated rats. Because ODC is virtually unique in having an extremely rapid half-life of 15–20 min (4, 14), this treatment effectively corrects for possible decarboxylation by other, longer-lived enzymes. At very high ornithine concentrations (400 μM or higher), a "nonspecific" component of $^{14}CO_2$ evolution could be detected, which did not display saturation kinetics and did not exhibit a rapid falloff after cycloheximide administration. As this may represent nonenzymatic decarboxylation (16) or activities of other enzymes (17), all assays were conducted with ornithine concentrations below the point (400 μM) where these effects influenced the measured activity significantly and at concentrations where the total activity vanishes 1–2 hr after cycloheximide treatment.

To determine the falloff of ODC activity after cycloheximide or actinomycin D, control and T_3-treated pups either received cycloheximide (200 mg/kg, i.p.) and were killed 5, 10, 15, 20, or 30 min later or were given actinomycin D (10 mg/kg, i.p.) and killed 5 h later; these doses almost totally inhibit cardiac protein synthesis (cycloheximide) or RNA synthesis (actinomycin D) in adult or developing rats (14). In each case, comparisons were made with control or T_3-treated pups given vehicle instead of cycloheximide or actinomycin D.

To determine incorporation of [^{3}H]uridine into the trichloroacetic acid-insoluble fraction which contains RNA, 8- and 12-day-old control or T_3-treated rats were given [^{3}H]uridine (2 mCi/kg, s.c.) 30 min before sacrifice. Hearts were homogenized (Polytron) in 19 vol of Tris buffer and an aliquot was removed for analysis of tissue

TABLE 1

Statistical summary of kinetic data of heart ODC activity in rats at different stages of development

Each value represents the mean ± standard error of three or more experiments, using at least six animals per experiment. For curves in which two kinetic forms were found, the high-affinity form was evaluated from the four or five lowest concentration points and the low-affinity form from the three or four highest.

Source of enzyme	K_m^{orn}	V_{max}
	μM	*nmol/g/h*
2 day old	51 ± 2	20 ± 1
	~250[a]	~60[a]
4 day old	62 ± 5	15 ± 1
	~250[a]	~50[a]
7 day old	201 ± 61	26 ± 8
10 day old	198 ± 32	21 ± 3
20 day old	212 ± 45	21 ± 4
200-g males (~7 week old)	188 ± 51	14 ± 4
340-g males (~10 week old)	195 ± 53	10 ± 3
340-g females (≤14 week old)	255 ± 75	10 ± 0.1
2-day-old T_3 (1 injection)	55 ± 16	48 ± 14
	~240[a]	~180[a]
10-day-old T_3 (9 injections)	259 ± 54	12 ± 3

[a] Values uncorrected for partial contribution of high-affinity form or nonspecific decarboxylation.

uptake of label. Trichloroacetic acid (15% final concentration) was added to 1 ml of homogenate and centrifuged at 15,000*g* for 10 min. The pellet was washed with trichloroacetic acid and recentrifuged twice, digested and with hyamine hydroxide, and counted by liquid scintillation spectrometry.

Statistics. Results are presented as means and standard errors, with levels of significance calculated by two-tailed paired or unpaired *t* tests. In the former test, group means of control and experimental animals were paired by age over the time periods specified; degrees of freedom were calculated as the number of paired means minus one. This mode of pairing enables comparison of the entire time course of development or of trends over an extended time period, as opposed to comparison only of individual age points in the unpaired *t* test. Straight lines are determined by linear regression analysis.

Materials. DL-[1-^{14}C]Ornithine monohydrochloride (52.8 mCi/mmol) and [5,6-^{3}H]uridine (33 Ci/mmol) were obtained from New England Nuclear Corp. and Schwarz-Mann, respectively; 3,3',5-triiodothyronine (sodium salt), pyridoxal-5'-phosphate, cycloheximide, actinomycin D, and DL-ornithine monohydrochloride were purchased from Sigma Chemical Co.; and dithiothreitol was from Bachem Feinchemikalien AG.

RESULTS

Developmental changes. Mature adult rat heart ODC displayed saturation kinetics with a single K_m^{orn} of about 200–250 μM for 340-g male or female rats and a V_{max} of 9–10 nmol/g/h (Table 1). The slightly higher heart ODC activity in 340-g males compared to females did not necessarily represent sex-related differences, but rather could have reflected differences in age; while 340-g male rats are about 10 weeks old, 340-g female rats are generally over 14 weeks old. Indeed, young adult males (200 g, about 6–7 weeks old) also showed a single K_m^{orn} of about 200 μM but an even higher V_{max} of nearly 14 nmol/g/h. Enzyme kinetics of ODC from 2- and 4-day-old rats differed considerably from those of the adults (Fig. 1). Some of the enzyme displayed a high affinity with a K_m^{orn} of 50–60 μM (Table 1), while a component resembling the low-affinity form could be detected at the three highest ornithine concentrations. While the two K_m^{orn} values for ODC appeared to be similar in 2- and 4-day-old rat hearts, the V_{max} of the high-affinity form of the 2

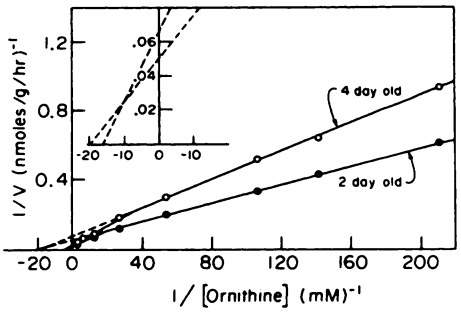

FIG. 1. *Examples of double-reciprocal plots of heart ODC in 2- and 4-day-old neonatal rats*

Each point represents the mean of triplicate determinations of three or more experiments. Kinetic parameters are summarized in Table 1.

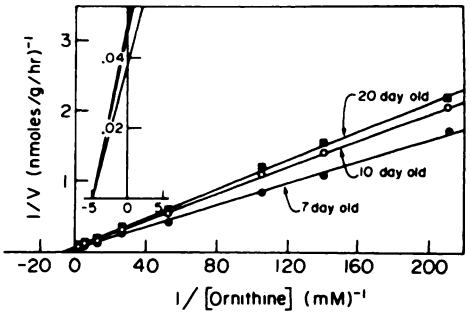

FIG. 2. *Double-reciprocal plot of heart ODC in 7-, 10-, and 20-day-old neonates*

day old was significantly higher than that of the 4 day old ($P < 0.005$ by unpaired *t* test), and the V_{max} of the low-affinity form also appeared greater at the younger age. As shown in Fig. 2, the high-affinity component of ODC observed at 2 and 4 days of age disappeared by 7 days, and at 7, 10, and 20 days of age, rat heart ODC exhibited only a single K_m^{orn} similar to that of the adult (Table 1). However, the V_{max} in the developing rats was still considerably higher than that of the mature adults. It should be noted that, in preparations displaying both kinetic forms of ODC, the parameters for the low-affinity component could not be assessed accurately, as nonspecific decarboxylation of ornithine occurred in assays with substrate concentrations of 400 μM or higher; the values given for approximate K_m^{orn} or V_{max} of the low-affinity form in Table 1 (2 and 4 day olds) represent estimates uncorrected for nonspecific decarboxylation or for partial contribution of the high affinity form.

To determine whether the appearance or disappearance of the two kinetic forms of ODC was associated with shifts in the rates of disappearance of activity after inhibition of protein or RNA synthesis, studies were conducted with cycloheximide or actinomycin D in rats of different ages. As reported previously (14), the falloff of ODC activity after cycloheximide is the same in 2-day-old, 10-day-old, and young adult rats (Fig. 3). After actinomycin D, although there was some variation among the different age groups in the decline of ODC activity, none of the groups was statistically distinguishable from adults (Table 2).

Hormonal effects. The effects of T_3 on the developmental pattern of rat heart ODC (ornithine concentration, 9.5 μM) appear in Fig. 4. T_3 produced an initial 45% elevation of activity followed by a more persistent deficit of 50–90% in the second week of postnatal life. In kinetic studies of ODC, 2-day-old pups treated with a single T_3 injection displayed two forms of the enzyme, with the K_m^{orn} values of the high- and low-affinity forms approximating those observed in the 2-day-old control (Table 1). However, the V_{max} of both components in the treated pups was more than twofold higher than those of the control animals. After nine daily T_3 injections, 10-day-old rats showed a single K_m^{orn} value corresponding to the low-affinity form (260 μM), but the V_{max} of the treated rats was only half that of 10-day-old controls and resembled the value seen in mature adults (Table 1).

In order to assess the rate of disappearance of heart ODC in control and T_3-treated neonates, cycloheximide

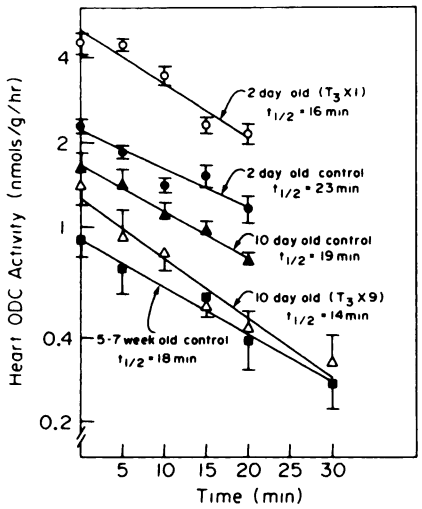

FIG. 3. *Time course of the decline of heart ODC activities in rat heart after administration of 200 mg/kg of cycloheximide i.p.*

Young adult (150 g, 5–7 weeks old), 2-day-old, and 10-day-old controls received saline or T_3 (one or nine daily injections for 2-day- and 10-day-old pups, respectively). All rats received the last saline or T_3 treatment 24 h before cycloheximide. Each point represents the mean ± standard error of six or more animals.

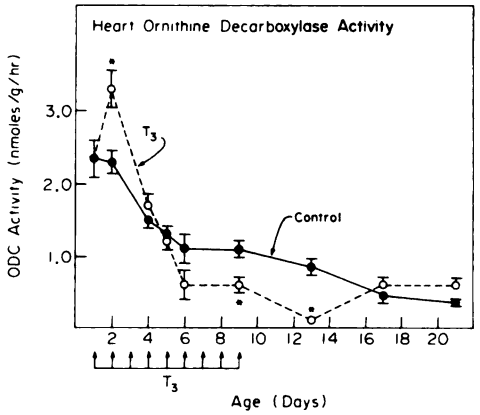

FIG. 4. *Heart ornithine decarboxylase activity at a substrate concentration of 9.5 μM, in developing control rats and rats treated daily with T_3*

Points and bars represent means ± standard errors of 6 to 18 animals. Asterisks denote significant differences ($P < 0.05$ or better) vs controls by unpaired t test. Arrows from day 1 to day 9 denote T_3 administrations. Determinations during the course of T_3 administration were made 24 h after the previous T_3 injection.

was administered and the decline of enzyme activity was examined (Fig. 3). In general, ODC turnover was slightly elevated in T_3-treated rats, as evidenced by small reductions in the half-life of ODC activity in the treated groups. This effect on $t_{1/2}$ could be seen at both a time point at which T_3 elevated ODC activity (2 days) and one where ODC was suppressed (10 days).

Since the altered developmental pattern of rat heart ODC evoked by T_3 (an initial enhancement followed by a subsequent deficit) is one which is usually associated with premature cellular development (18, 19), the effects of T_3 on body weight and on specific organ growth were examined. Control rats increased in body weight from approximately 10 g at 2 days of age to 69 g at 28 days and in heart weight from 50 to 340 mg; heart weight as a percentage of body weight decline slightly with development (Fig. 5). Pups given daily T_3 exhibited an overall pattern of retardation in body weight gains ($P < 0.005$ by paired t test) which continued well after the cessation of drug administration at 10 days of age. Heart weights of T_3-treated pups were enhanced by as much as 45% during drug exposure, but weights were subnormal after cessation of treatment ($P < 0.001$ by paired t test). The heart weight/body weight ratio for T_3-treated pups was elevated significantly ($P < 0.001$ by paired t test) during drug exposure, but was normal from 15 days of age onward.

Cardiac RNA synthesis, monitored by incorporation of [^{3}H]uridine into the trichloroacetic acid-insoluble fraction (containing RNA), was reduced substantially at 8 and 12 days of age in T_3-treated rats compared to controls (Table 3). There was no change in the total uptake of [^{3}H]uridine.

TABLE 2

Falloff of rat heart ODC activity 5 h after actinomycin D (10 mg/kg, i.p.)

Assays were conducted at a substrate concentration of 9.5 μM. Results are expressed in means ± standard errors of the number of animals in parentheses.

Source of enzyme	Control	Actinomycin D	Decline of activity
	nmol/g/h	*nmol/g/h*	%
2 day old	2.91 ± 0.25 (58)	1.30 ± 0.15 (67)	55 ± 5
4 day old	1.73 ± 0.13 (24)	0.512 ± 0.075 (24)	70 ± 4
7 day old	1.24 ± 0.10 (19)	0.616 ± 0.089 (22)	50 ± 7
10 day old	0.891 ± 0.078 (10)	0.347 ± 0.046 (10)	61 ± 5
20 day old	0.645 ± 0.117 (24)	0.390 ± 0.089 (24)	40 ± 14
Adult	0.403 ± 0.033 (8)	0.244 ± 0.052 (8)	40 ± 13

DISCUSSION

The developmental pattern of ODC is thought to provide a sensitive index of the maturation of mammalian organs, and drug-induced perturbations of this pattern have been utilized for teratologic evaluations (5, 13, 18, 19). ODC in the developing rat heart displays initially high activity and a gradual decline with increasing age. The current results provide information about the kinetic mechanisms underlying this pattern, and thus can aid in the interpretation of how drugs or hormones produce shifts in ODC development.

A recent report indicates that elevations of heart ODC in neonatal rats or in adults exposed to growth-inducing stimuli all involve the appearance of a form of the enzyme with a higher affinity for substrate than that found in hearts of untreated mature rats (14); studies with mixtures of enzyme preparations from control and stimulated rats showed that the shift in kinetics did not represent alterations in soluble factors which could influence the activity (14), nor is the stimulation affected by dialysis of the enzyme preparation (unpublished data). The higher affinity therefore may reflect a difference in the properties of the enzyme itself. To determine how shifts in the kinetic properties of ODC contribute to the developmental pattern of overall ODC activity, kinetics of the enzyme

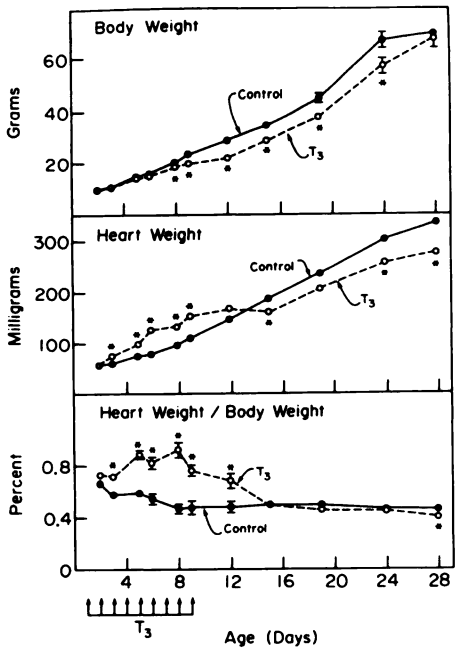

FIG. 5. *Body weight, heart weight, and heart weight as a percentage of body weight in developing control rats and rats treated daily with T_3*

Points and bars represent means ± standard errors of 6 to 18 animals; where standard errors are not indicated, they were less than 5% of the value. Asterisks denote significant differences ($P < 0.05$ or better) vs controls by unpaired t test. Arrows from day 1 to day 9 denote T_3 administration.

were studied at various postnatal ages during the period in which the greatest changes are seen. In the 2- and 4-day-old pups, two forms of ODC were detected; the apparent K_m^{orn} of the low-affinity form resembled that of the mature adult, while the other form exhibited a K_m^{orn} which was four times lower. By 1 week of age, the high-affinity component of ODC diminished to undetectable levels, and only a single K_m^{orn} resembling that of the mature adult was obtained beyond this point. These data indicate that part of the initial elevation and subsequent decline of heart ODC activity during neonatal cellular maturation is associated with the presence and ontogenetic loss of the high-affinity component of the enzyme.

In addition to age-dependent shifts in affinity for substrate, some of the developmental decline in ODC activity resulted from changes in the V_{max}. Thus, the difference between 2- and 4-day-old rats, both of which possess high-affinity ODC, reflected a decline in the V_{max} of both the high- and the low-affinity forms of the enzyme; furthermore, the V_{max} of the one form of ODC present at subsequent ages was twofold higher in 7- to 20-day-old rats than in the mature rat, indicating that further reductions in enzyme activity can occur in the absence of shifts in the K_m^{orn}. Reductions in the V_{max} continue throughout maturation, as the V_{max} declined progressively in 20-day-old, 6- to 7-week-old, 10-week-old, and ≥14-week-old rats; changes in the V_{max} would suggest either that the amount of enzyme declines with age or that catalytic efficiency is age dependent. While no definitive choice of mechanism can be made, a number of possibilities can be ruled out. Since the rate of degradation of enzyme activity after cycloheximide remains virtually unchanged throughout development, the decline of the V_{max} of ODC activity in the developing heart probably does not reflect increased enzyme turnover. Similarly, the alterations of ODC could not be attributed to changes in the rate of degradation of the RNA involved in synthesis of the enzyme, as there was no apparent relationship between age and sensitivity to actinomycin D. Thus, it is most likely that elevations and subsequent declines in the V_{max} of ODC reflect alterations in the rate of enzyme synthesis or in catalytic efficiency of equivalent numbers of ODC molecules.

Kinetic studies also can elucidate the mechanisms by which drugs or hormones elicit perturbations in the ontogeny of ODC and influence cellular growth and functional maturation of developing organs. Neonatal hyperthyroidism is known to accelerate the maturation of various tissues (20) and changes in cellular development are preceded by alterations in ODC (7, 13, 18, 19). Administration of T_3 to neonatal rats elevated heart ODC above control levels during the initial phase of drug treatment but depressed the enzyme activity subsequently. While a slight increase in ODC degradation rate could be detected, the difference was too small to account for the pronounced deficit of ODC activity seen in the second week of postnatal age and certainly could not be responsible for the increase in ODC seen in 2-day-old T_3-treated rats. Kinetic data of the 2-day-old T_3-treated pups revealed the same two forms of ODC as seen in neonatal controls; however, the V_{max} values of both the high- and the low-affinity components of the T_3-treated pups were more than twice normal. These data indicate that T_3 in the neonate increases the synthesis or catalytic efficiency of both forms of the enzyme without altering the affinity characteristics. This can be contrasted with the increase in heart ODC in the mature rat treated with T_3, where a shift in substrate affinity has been clearly demonstrated (14). Effects on V_{max} rather than on K_m^{orn} also account for the decline in ODC activity in hyperthyroid developing rats. In the 10-day-old pups treated with T_3, a single K_m resembling that of the 10-day-old control was obtained, but the V_{max} of the enzyme was 40% lower than that of untreated pups. Hence, the subnormal activity of heart ODC in the 10-day-old T_3-treated pups was primarily related to a reduction in enzyme synthesis or catalytic efficiency. In view of the fact that thyroid hormone accelerates cellular maturation in general, the shift of the V_{max} at 10 days to values similar to those of

TABLE 3

Uptake of [^{3}H]uridine and incorporation into trichloroacetic acid-insoluble material (containing RNA) in hearts of developing control and T_3-treated rats

Rats received daily T_3 or vehicle on days 1-9 postnatally. Data represent means ± standard errors of 6–12 animals.

Age	(dpm/25 mg heart)			
	Total ^{3}H uptake		Trichloroacetic acid insoluble ^{3}H	
	Control	T_3	Control	T_3
8 days	92,000 ± 2,800	82,000 ± 4,000	4,760 ± 560	2,330 ± 300*
12 days	86,100 ± 4,500	75,000 ± 4,000	3,070 ± 150	2,060 ± 230*

* $P < 0.005$ vs control.

the mature rat represents acceleration of the ontogenetic time course of the decline of heart ODC.

In view of the stimulatory and inhibitory phases of T_3 on ODC activity in the developing heart, it would be expected that the treatment should produce a dual effect on organ growth. Initially, heart weights of the neonates were enhanced during exposure to T_3, despite concurrent deficiencies in body weight. A second effect of T_3 was suggested by the subsequent deficits in heart weight; this phase does not appear to be specific to the heart, as the deficits in heart weight were equivalent to those in body weight (no change in the heart/body weight ratio). Although the decline in heart weight was not obvious until after the cessation of T_3 treatment, RNA synthesis (assessed as trichloroacetic acid-insoluble material) was already reduced before the termination of T_3 exposure (at a point where ODC was subnormal). These effects can be contrasted with the continuous elevation of ODC, RNA synthesis, and heart weight in mature rats given thyroid hormones (7, 21). The net effect of T_3 in the developing rat heart appears to be a compression of the time course of maturation, with premature declines in ODC activity and RNA synthesis after the initial stimulatory effect. In view of the postulated roles of polyamines in nucleic acid and protein syntheses (1–4), the alterations in ODC kinetics caused by T_3 administration to neonates may be of functional significance in affecting organ growth.

In summary, the neonatal elevation and subsequent decline of heart ODC activity during development involve shifts in kinetic control of enzyme activity. The high-affinity form present in the neonate disappears soon after birth and subsequent declines in activity of the low-affinity form reflect reductions in enzyme production and/or catalytic efficiency. Neonatal hyperthyroidism accelerates the maturational decline of ODC activity, which may then contribute to perturbations of cardiac development.

REFERENCES

1. Pegg, A. E. and H. G. Williams-Ashman. Biosynthesis of putrescine in the prostate gland of the rat. *Biochem. J.* **100:** 533–539 (1968).
2. Russell, D. H. and S. H. Snyder. Amine synthesis in rapidly growing tissues: Ornithine decarboxylase activity in regenerating rat liver, chick embryo and various tissues. *Proc. Natl. Acad. Sci. USA* **60:** 1420–1427 (1968).
3. Bachrach, U. *Function of Naturally Occurring Polyamines.* Academic Press, New York (1973).
4. Russell, D. H. and B. G. M. Durie. Polyamines as biochemical markers of normal and malignant growth, in *Progress in Cancer Research and Therapy, Vol. 8.* Raven Press, New York (1978).
5. Slotkin, T. A., C. Lau and M. Bartolomé. Effects of neonatal or maternal methadone administration on ornithine decarboxylase activity in brain and heart of developing rats. *J. Pharmacol. Exp. Ther.* **199:** 141–148 (1976).
6. Bartolomé, J., C. Lau and T. A. Slotkin. Ornithine decarboxylase in developing rat heart and brain: Role of sympathetic development for responses to autonomic stimulants and the effects of reserpine on maturation. *J. Pharmacol. Exp. Ther.* **202:** 510–518 (1977).
7. Lau, C. and T. A. Slotkin. Accelerated development of rat sympathetic neurotransmission caused by neonatal triiodothyronine administration. *J. Pharmacol. Exp. Ther.* **208:** 485–490 (1979).
8. Claycomb, W. C. Biochemical aspects of cardiac muscle differentiation: Deoxyribonucleic acid synthesis and cytoplasmic deoxyribonucleic acid polymerase activity. *J. Biol. Chem.* **250:** 3229–3235 (1975).
9. Claycomb, W. C. Biochemical aspects of cardiac muscle differentiation: Possible control of deoxyribonucleic acid synthesis and cell differentiation by adrenergic innervation and cyclic adenosine 3′,5′-monophosphate. *J. Biol. Chem.* **251:** 6082–6089 (1976).
10. Jänne, J., A. Raina and M. Siimes. Mechanism of stimulation of polyamine synthesis by growth hormone in rat liver. *Biochim. Biophys. Acta* **166:** 419–426 (1968).
11. Russell, D. H., S. H. Snyder and V. J. Medina. Growth hormone induction of ornithine decarboxylase in rat liver. *Endocrinology* **86:** 1414–1419 (1970).
12. Maudsley, D. V. Regulation of polyamine biosynthesis. *Biochem. Pharmacol.* **28:** 153–161 (1979).
13. Slotkin, T. A. Ornithine decarboxylase as a tool in developmental neurobiology. *Life Sci.* **24:** 1623–1630 (1979).
14. Lau, C. and T. A. Slotkin. Regulation of rat heart ornithine decarboxylase: Change in affinity for ornithine evoked by neuronal, hormonal, and ontogenetic stimuli. *Mol. Pharmacol.* **16:** 504–512 (1979).
15. Anderson, T. R. and S. M. Schanberg. Ornithine decarboxylase activity in developing rat brain. *J. Neurochem.* **19:** 1471–1481 (1972).
16. Roger, L. J. *The Effects of Protein Hormones on Polyamine Metabolism in the Developing Rat Brain,* Ph.D. dissertation. Duke University, Durham, N.C. (1976).
17. Murphy, B. J. and M. E. Brosnan. Subcellular localization of ornithine decarboxylase in liver of control and growth-hormone-treated rats. *Biochem. J.* **157:** 33–39 (1976).
18. Anderson, T. R. and S. M. Schanberg. Effect of thyroxine and cortisol on brain ornithine decarboxylase activity and swimming behavior in developing rat. *Biochem. Pharmacol.* **24:** 495–501 (1975).
19. Slotkin, T. A. and P. V. Thadani. Neurochemical teratology of drugs of abuse, in *Progress in the Study of Birth Defects: Neural and Behavioral Teratology* (T. V. N. Persaud, ed.). MTP Press, England, 199–234 (1980).
20. Hamburgh, M., L. A. Mendoza, J. F. Burkart and F. Weil. Thyroid-dependent processes in the developing nervous system, in *Conference on Hormones in Development* (M. Hamburgh and E. J. W. Barrington, eds.). Appleton-Century-Crofts, New York, 403–415 (1971).
21. Rabinowitz, M. and R. Zak. Biochemical and cellular changes in cardiac hypertrophy. *Annu. Rev. Med.* **23:** 245–262 (1972).

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