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Regulation of Rat Heart Ornithine Decarboxylase: Change in Affinity for Ornithine Evoked by Neuronal, Hormonal, and Ontogenetic Stimuli

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SUMMARY

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The activity of ornithine decarboxylase (ODC), the rate-limiting enzyme involved in the biosynthesis of polyamines, is markedly elevated in the adult rat heart 4 hr after isoproterenol or 24 hr after triiodothyronine treatment. The rate of degradation of ODC activity, measured after administration of cycloheximide, was not affected by these stimuli: half-lives of the enzyme remained between 18 and 24 min. After isoproterenol or triiodothyronine stimulation of adult rat heart, and in neonatal rats, kinetic data revealed the presence of a different form of the enzyme, with a K_m of 30-50 μM, compared with 260 μM in the adult control. Pretreatment with actinomycin D did not prevent elevation of ODC activity caused by isoproterenol, nor did cyclic AMP appear to be involved in the ODC stimulation. These results suggest that heart ODC regulation involves mechanisms different from those seen in other tissues.

INTRODUCTION

An increasing amount of experimental evidence indicates that the enzyme ornithine decarboxylase (EC 4.1.1.17, ODC)[1] plays a key role in the growth, proliferation and differentiation of all cells (1-3). Ornithine decarboxylase catalyzes the first and probably rate-limiting step in the biosynthesis of the polyamines, the formation of putrescine from ornithine (4, 5). Studies with regenerating liver (5-7), tumor cells (5) and a wide variety of prokaryotic and eukaryotic systems have demonstrated that ODC activity is high during rapid growth and decreases as growth ceases or as the number of actively dividing or growing cells declines.

In some mammalian systems, ODC activity may be regulated by changes in the rate of synthesis of the enzyme, because protein synthesis inhibitors interfere with stimulation of ODC (8). Russell and co-workers have proposed that, in regenerating rat liver, there is transcriptional control of ODC induction that is mediated by type I cyclic AMP-dependent protein kinase (9). However, other studies in liver (10), cultured cells (11), and additional systems have revealed multiple forms of ODC, raising the possibility of posttranslational control of enzyme activity (12). Indeed, putative activators and inhibitors of ODC have been partially characterized in both mammalian and nonmammalian systems (13-16).

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[1] The abbreviations used are: ODC, ornithine decarboxylase; T_3, triiodothyronine; DMSO, dimethylsulfoxide.

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In addition to stimulation of ODC during general growth, recent investigations have shown that ODC can be increased in target organs by exposure to specific hormones or neurotransmitters (3, 7, 17–19). In the adrenal cortex, at least two mechanisms appear to be involved in stimulation of ODC by ACTH: one requires *de novo* RNA synthesis and the other does not (20). In the heart, agents that cause cardiac hypertrophy such as isoproterenol (19) and triiodothyronine (18) have been demonstrated to evoke large increases in ODC. 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 the mature heart is incapable of synthesizing new DNA (21, 22); further growth of the heart represents hypertrophy of pre-existing cells. Thus, this 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. The present studies indicate that the increases in heart ODC in response to different stimuli all involve one common feature: the appearance of a form of the enzyme with an increased affinity for substrate.

METHODS

Treatment of rats. For studies in mature adults, Sprague-Dawley rats (Zivic-Miller) of both sexes weighing 250–380 g were sacrificed by decapitation after various drug treatments as described below. Animals were sex-matched between the control and treated groups within each individual experiment. For studies in developing animals, timed pregnant Sprague-Dawley rats 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 litter sizes kept at 8–10 pups. In each experiment, pups of both sexes were selected from several different cages.

Ornithine decarboxylase (ODC) assay. Hearts or livers were excised, homogenized (Polytron) in 19 volumes of ice-cold 10 mM Tris-HCl (pH 7.2) and then centrifuged at 26,000 × *g* for 15 min. Duplicate or triplicate aliquots of the supernatant were removed for the assay of ODC activity by a modification of the method of Russell and Snyder (5); 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 d*l*-[^{14}C]ornithine in a final volume of 1 ml. In the kinetic studies, [^{14}C]ornithine was isotopically diluted with unlabeled d*l*-ornithine to achieve desired concentrations. The evolved $^{14}CO_2$ was trapped in hyamine hydroxide and counted by liquid scintillation spectrometry. Under these conditions, enzyme activity was linear with time and tissue concentration. Activities are reported as pmols of $^{14}CO_2$ evolved per hour per gram wet weight of tissue.

Protein and RNA synthesis in rat heart. Mature adult rats were divided into groups that received either actinomycin D (10 mg/kg i.p.), cycloheximide (200 mg/kg i.p.) or vehicle. One hr later [^{3}H]uridine (2 mCi/kg s.c.) was given to the actinomycin D-treated and one control (vehicle) group, while [^{14}C]leucine (100 μCi/kg s.c.) was given to the cycloheximide-treated and another control group. In a similar fashion, 2-day-old pups were treated with vehicle or cycloheximide and then with [^{14}C]leucine. Rats were sacrificed 30 min and 1 hr after administration of uridine and leucine, respectively. Hearts were homogenized (Polytron) in 19 volumes of ice-cold 0.3 M sucrose containing 25 mM Tris-HCl (pH 7.4). One-half ml of the homogenate preparation was digested with 1 ml of hyamine hydroxide to determine the isotope uptake into the heart while RNA and proteins were precipitated from another aliquot with 15% trichloroacetic acid, centrifuged, washed twice with trichloroacetic acid and digested with hyamine hydroxide to measure the amount of isotope incorporated into RNA and proteins. The radioactive hyamine hydroxide mixture was then counted by liquid scintillation spectrometry.

Acute stimulation and inhibition of ODC activity. Mature rats were divided into six groups, each consisting of 6–8 animals and treated as follows: (1) saline was given to controls, (2) isoproterenol sulfate (0.2 mg/kg s.c.) was given 4 hr prior to sacrifice, (3)

cycloheximide (200 mg/kg i.p.) was given 5 hr prior to sacrifice, (4) cycloheximide was given 1 hr prior to isoproterenol administration, (5) actinomycin D (10 mg/kg i.p.) was given 5 hr prior to sacrifice, and (6) actinomycin D was given 1 hr prior to isoproterenol treatment. Rat hearts were removed and the ODC activity was assayed as described above.

Determination of half-life of ODC activity after cycloheximide. Because heart ODC activity in fully mature adult rats is low, somewhat younger animals (5–7 weeks old) were used to determine the fall-off of activity after cycloheximide. The young adult rats were treated with isoproterenol (0.2 mg/kg s.c.) or with T_3 (0.1 mg/kg s.c.). For studies in earlier stages of development, 2-day and 1-day old neonates were selected. Several rats in each group were then given vehicle while the rest received cycloheximide (200 mg/kg i.p. given 4 hr after isoproterenol or 24 hr after T_3) and were killed 5, 10, 15, 20 or 30 min later.

Determination of kinetics of ODC. The enzyme kinetics of mature adult and developing rat heart ODC were determined utilizing a range of ornithine concentrations from 6 μM to 400 μM. Additionally, kinetics were determined in adult rats given isoproterenol (4 hr after 0.2 mg/kg s.c.), cycloheximide (15 min after 200 mg/kg i.p.), T_3 (24 hr after 0.1 mg/kg s.c.), actinomycin D (5 hr after 10 mg/kg i.p.) or actinomycin D followed one hr later by isoproterenol. Hearts were pooled from three or four adult rats in each group or from 20 neonates. Ornithine decarboxylase activities were assayed in triplicate for each ornithine concentration. Data of the kinetic studies are presented as double reciprocal plots.

Study with mixed tissue preparations. Hearts from mature adult rats treated with either saline (control), isoproterenol (4 hr after 0.2 mg/kg s.c.), cycloheximide (30 min after 200 mg/kg i.p.) or T_3 (24 hr after 0.1 mg/kg s.c.) or from 2-day-old pups were removed and homogenized as already described. Prior to assay, 0.45 ml aliquots of the control supernatant preparation were mixed with 0.45 ml aliquots of the other groups and the ODC activities then determined.

Cyclic AMP studies. Mature adult rats received s.c. or i.p. injections of saline or DMSO vehicle, dibutyryl cyclic AMP (25 mg/kg in saline), 8-bromo-cyclic AMP (18 mg/kg in saline) or prostaglandin E_1 (50 μg/kg administered in 0.25 ml of DMSO), and were killed 4 hr later. Hearts and livers were assayed for ODC activity. In additional experiments, rats were given a long-acting ganglion blocker (chlorisondamine chloride 5 mg/kg s.c.) to prevent reflex sympathetic stimulation; this was necessary because theophylline produced substantial stimulation of heart ODC by sympathetic nerve activation. These animals were then treated one hr later with theophylline (45 mg/kg i.p.) or vehicle. One hr after theophylline, the rats were given saline or isoproterenol (0.2 mg/kg s.c.), killed one hr after this final injection, and hearts assayed for ODC. The shorter time course was used here to ensure that the stimulation by isoproterenol alone would be submaximal.

Statistics. Results are presented as means and standard errors with levels of significance calculated by the Student's *t*-test (two-tailed, unpaired). Straight lines were determined by linear regression analysis.

Materials. [1-^{14}C]d*l*-ornithine monohydrochloride (52.8 mCi/mmol) was obtained from New England Nuclear Corp., [U-^{14}C]*l*-leucine (312 mCi/mmol) and [5,6-^{3}H]uridine from Schwarz-Mann, chlorisondamine chloride from Ciba Pharmaceuticals, dithiothreitol from Bachem Feinchemikalien AG, and 3,3',5-triiodo-*l*-thyronine (sodium salt), pyridoxal-5'-phosphate, d*l*-ornithine monohydrochloride, d*l*-isoproterenol sulfate, cycloheximide, actinomycin D, dibutyryl cyclic AMP (sodium salt), 8-bromo-cyclic AMP and prostaglandin E_1 from Sigma Chemical Co.

RESULTS

Mature adult rats (250–380 g) had a basal heart ODC activity of 403 ± 33 pmol/g/hr at a substrate concentration of 9.5 μM (Table 1). A sixfold increase in enzyme activity was obtained 4 hr after isoproterenol at this subsaturating ornithine concentration. Pretreatment with actinomycin D failed to prevent this increase; the small reduction

(≅30%) in activity of actinomycin D- + isoproterenol-treated rats compared to isoproterenol alone was similar to the reduction seen in control rats given actinomycin D alone (≃40%). The dose of actinomycin D used in this study reduced incorporation of [^{3}H]uridine into cardiac RNA by 80–95%. Pretreatment with cycloheximide led to total loss of ODC activities in both control and isoproterenol-stimulated animals. Incorporation of [^{14}C]leucine into cardiac proteins was reduced 97% by cycloheximide in adult rat heart and 96% in developing rat heart.

TABLE 1

Effects of actinomycin D (10 mg/kg i.p.) and cycloheximide (200 mg/kg i.p.) on adult rat heart ODC stimulation caused by isoproterenol (0.2 mg/kg s.c.)

Each group represents mean and standard error of 6–8 determinations.

Treatment	Heart ODC activity
	(*pmols/g/hr*)
Control	403 ± 33
Isoproterenol (4 hr)	2653 ± 132[a]
Actinomycin D (5 hr)	244 ± 52[b]
Actinomycin D (5 hr) + Isoproterenol (4 hr)	1834 ± 204[c]
Cycloheximide (5 hr)	9 ± 11[a]
Cycloheximide (5 hr) + Isoproterenol (4 hr)	2 ± 5[d]

[a] $p < 0.001$ vs. control.

[b] $p < 0.05$ vs. control.

[c] $p < 0.001$ vs. control, $p < 0.01$ vs. isoproterenol, $p < 0.001$ vs. actinomycin D.

[d] $p < 0.001$ vs. control, $p < 0.001$ vs. isoproterenol, N.S. vs. cycloheximide.

In order to assess the rate of degradation of ODC activity in young adult (5–7 week old) rat hearts, cycloheximide was administered and the rate of decline of the enzyme activity was examined (Fig. 1). As has been found in rat liver after partial hepatectomy (8), basal heart ODC activity displayed a rapid turnover with a half-life of 18 min. When cycloheximide was given 4 hr after isoproterenol, the half-life of heart ODC was only slightly prolonged (24 min). Similarly, after stimulation of adult heart ODC with T_3 as well as in the untreated developing rat (2 and 10 day old), there was no substantial change in the rate of turnover, with half-lives after cycloheximide administration ranging from 19 min to 23 min. In contrast to drug administration *in vivo*, cy-

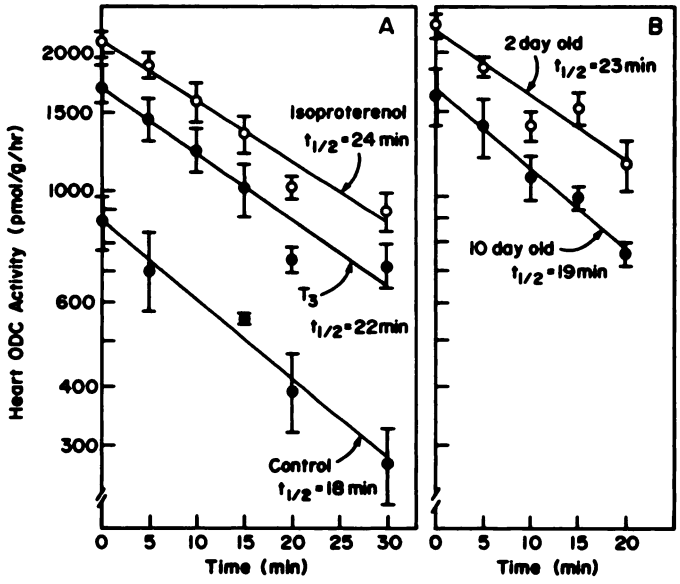

FIG. 1. *Time course of the decline of ODC activities in rat heart after administration of 200 mg/kg of cycloheximide i.p*

A. Young adult (5–7 weeks old) rats given saline (control), 0.2 mg/kg of isoproterenol 4 hr before cycloheximide or 0.1 mg/kg of T_3 24 hr before cycloheximide. B. Two-day and ten-day old neonates. Each point represents the mean and S.E. from a minimum of six animals.

cloheximide added directly to the ODC assay medium in concentrations up to 0.2 mg/L had no effect on enzyme activity (data not shown).

As seen in Figure 2, control mature adult rat heart ODC displayed saturation kinetics with a single K_m of 261 ± 43 μM and a V_{max} of 9.01 ± 1.46 nmols/g/hr. Five hours after treatment with actinomycin D or 15 min after cycloheximide the V_{max} was less than half of control values while K_m remained unchanged. Enzyme kinetics of ODC from rats treated with isoproterenol differed considerably from the controls (Fig. 3). Some of the enzyme appeared to be in a high affinity form, with a K_m of 30 ± 2 μM (range in different batches of adult rats of various sizes and either sex, 30–50 μM), but a component resembling the low-affinity form could be detected at the two highest ornithine concentrations. A similar pattern was obtained 24 hr after treatment with T_3, with some of the ODC displaying an apparent K_m of 46 ± 1 μM and a low-affinity component detectable at high concentrations (Fig. 4). Neonatal heart ODC also appeared to possess a high affinity form, with an apparent K_m of 53 ± 2 μM (Fig. 5). The kinetic parameters of the low-affinity component after isoproterenol or T_3 or in the neonate could not be assessed accurately because of nonspecific decarboxylation of ornithine occurring in assays with substrate concentrations of 400 μM or above.

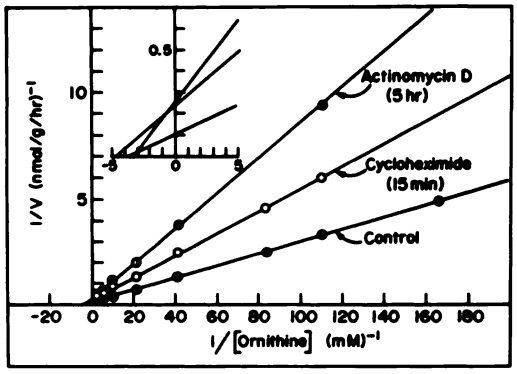

FIG. 2. *Double-reciprocal plot of mature adult heart ODC in control, actinomycin D (5 hr after 10 mg/kg) and cycloheximide (15 min after 200 mg/kg) treated rats*

Each point represents the mean of triplicate determinations. For control, K_m = 261 ± 43 μM, V_{max} = 9.01 ± 1.46 nmols/g/hr. For actinomycin D-treated K_m = 301 ± 39 μM (N.S. vs control), V_{max} = 3.70 ± 0.48 nmols/g/hr ($p < 0.01$ vs control). For cycloheximide-treated, K_m = 213 ± 30 μM (N.S. vs control). V_{max} = 4.07 ± 0.58 nmols/g/hr ($p < 0.01$ vs control).

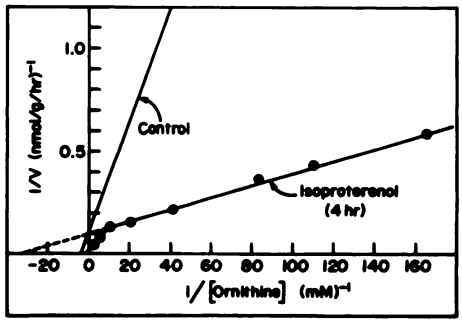

FIG. 3. *Double-reciprocal plot of mature adult heart ODC in control and isoproterenol-treated rats (4 hr after 0.2 mg/kg)*

Lines were fitted by least squares analysis with the three highest concentrations as a separate group. Data points for control appear in Figure 2. For the high-affinity component in isoproterenol treated rats, K_m = 30 ± 2 μM ($p < 0.001$ vs control) and V_{max} = 9.71 ± 0.66 nmols/g/hr.

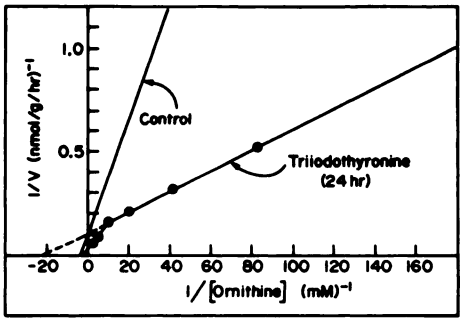

FIG. 4. *Double-reciprocal plot of mature adult heart ODC in control and T_3-treated rats (24 hr after 0.1 mg/kg)*

Data points for control appear in Figure 2. For the high affinity component in T_3-treated rats, K_m = 46 ± 1 μM ($p < 0.001$ vs control) and V_{max} = 9.17 ± 0.25 nmols/g/hr (N.S. vs. control).

To determine if transcription played a role in the alteration of the K_m produced by isoproterenol, kinetics were determined in rats treated with actinomycin D prior to receiving isoproterenol (Fig. 6). Although the apparent K_m of the high-affinity form remained the same as that derived from animals treated with isoproterenol alone, the apparent high-affinity V_{max} of the acti-

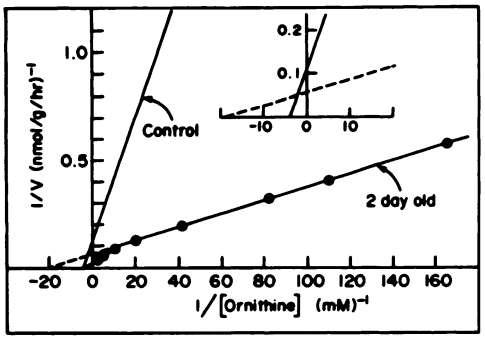

FIG. 5. *Double-reciprocal plot of ODC in mature adult and neonatal (2-day-old) heart*

Data points for adult control appear in Figure 2. For the high affinity component in neonates, K_m = 53 ± 2 μM ($p < 0.001$ vs. adult) and V_{max} = 17.4 ± 0.7 nmols/g/hr.

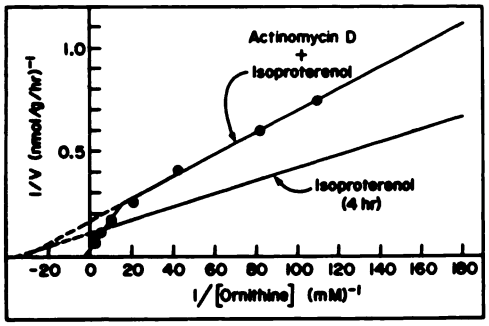

FIG. 6. *Double-reciprocal plot of mature adult heart ODC in isoproterenol-treated rats (4 hr after 0.2 mg/kg) with and without actinomycin D pretreatment (10 mg/kg 1 hr before isoproterenol)*

Data points for rats given isoproterenol alone are presented in Figure 4. For the high-affinity component in rats given actinomycin D and isoproterenol, K_m = 33 ± 3 μM (N.S. vs. isoproterenol alone) and V_{max} = 6.17 ± 0.46 nmols/g/hr ($p < 0.002$ vs. isoproterenol alone).

nomycin D-pretreated rats was reduced by 40% vs. the isoproterenol group, a reduction comparable to that seen in control rats given actinomycin D alone (Table 1, Fig. 2). The curve maintained its biphasic characteristic at high substrate concentrations.

To determine if an excess "activator" or ODC might be present in the stimulated adult heart (isoproterenol or T_3) or in the neonatal heart, or whether an inhibitor might contribute to the decline in activity produced by cycloheximide, some supernatant preparations of the control and of the experimentals were mixed *in vitro* before ODC activities were assayed (Table 2).

TABLE 2

Ornithine decarboxylase assays using mixtures of enzyme samples obtained from rats given various treatments. T_3 (0.1 mg/kg), isoproterenol (0.2 mg/kg), cycloheximide (200 mg/kg) or saline were administered as described in METHODS. Each group represents mean ± standard error of three determinations. Theoretical value is midway between the activities of the two original component samples.

Source of enzyme in assay	ODC activity	Theoretical ODC activity
	(pmols/g/hr)	*(pmols/g/hr)*
100% from control adult rats	382 ± 6	——
100% from isoproterenol-treated adults	3800 ± 60	——
50% from control adults + 50% isoproterenol-treated	2020 ± 50	2090 ± 33
100% from T_3-treated adults	636 ± 18	——
50% control adult + 50% T_3-treated	511 ± 10	509 ± 12
100% from cycloheximide-treated adults	73 ± 6	——
50% from control adult + 50% cycloheximide-treated	241 ± 9	227 ± 6
100% from 2-day-old rats	2360 ± 30	——
50% from control adult + 50% 2 day old	1330 ± 30	1317 ± 18

In each case, the ODC activities of the mixed samples agreed with the theoretical values (midway between the activities of the two original component samples).

Administration of dibutyryl cyclic AMP or 8-bromocyclic AMP failed to cause any significant alteration in heart ODC, but did stimulate liver ODC (Table 3). Similar results were seen with prostaglandin E_1, which increases intracellular cyclic AMP levels. Theophylline, which itself had a small direct stimulatory effect on heart ODC, failed to synergize the effect of isoproterenol, as ODC activity with combined treatment was simply the sum of the two individual drug effects (Table 4).

DISCUSSION

The rapid increases in heart ODC activity in response to stimulation could result from one of two mechanisms: (1) a decrease in the rate of degradation of activity, or (2) activation or induction of the enzyme. The

TABLE 3

Effects of cyclic AMP derivatives and prostaglandin E_1 on adult rat heart and liver ODC activity

Rats were killed 4 hr after treatment. Each group represents mean and standard error of the number of determinations in parentheses.

Treatment	ODC activity	
	Heart	Liver
	(*pmols/g/hr*)	
Saline vehicle	245 ± 49 (11)	616 ± 68 (11)
Dibutyryl cyclic AMP (25 mg/kg s.c.)	249 ± 39 (5)	1179 ± 63[a] (5)
Dibutyryl cyclic AMP (25 mg/kg i.p.)	206 ± 52 (6)	1353 ± 223[b] (6)
8-Bromo cyclic AMP (18 mg/kg s.c.)	190 ± 60 (6)	870 ± 94[c] (6)
DMSO vehicle	185 ± 32 (6)	502 ± 116 (6)
Prostaglandin E_1 (50 μg/kg s.c. in DMSO)	156 ± 41 (6)	1561 ± 343[d] (6)

[a] $p < 0.001$ vs. saline vehicle.
[b] $p < 0.01$ vs. saline vehicle.
[c] $p < 0.05$ vs. saline vehicle.
[d] $p < 0.02$ vs. DMSO vehicle.

TABLE 4

Effect of theophylline on isoproterenol-induced stimulation of adult rat heart ODC

All rats were given chlorisondamine chloride (5 mg/kg s.c.) followed one hour later by theophylline (45 mg/kg i.p.) or vehicle; one hour after theophylline, saline or isoproterenol sulfate (0.2 mg/kg s.c.) were given and rats killed 1 hr after this final injection. Each group represents mean and standard error of 7–8 determinations.

Treatment	Heart ODC activity
	(*pmols/g/hr*)
Chlorisondamine	288 ± 29
Chlorisondamine + theophylline	667 ± 42[a]
Chlorisondamine + isoproterenol	780 ± 58[a]
Chlorisondamine + theophylline + isoproterenol	1230 ± 150[b]

[a] $p < 0.001$ vs. chlorisondamine.
[b] $p < 0.001$ vs. chlorisondamine, N.S. vs. sum of "chlorisondamine + theophylline" and "chlorisondamine + theophylline + isoproterenol" groups.

first hypothesis is unlikely, because the data obtained with cycloheximide show that the half-life of heart ODC does not change substantially during stimulation by a β-adrenergic agonist (isoproterenol), a hormone (T_3), or during postnatal development. In order to determine if the prompt stimulation of enzyme activity involves a new form of ODC, the kinetics of the enzyme were examined in the two adult stimulation models and in the neonate, and were compared with the kinetics of ODC in control adult rats. After treatment with isoproterenol, a new form of the enzyme appeared that had a K_m for ornithine that was lower by a factor of 5 to 8. These data suggest that in the adult the increase in ODC involves, in part, changes in the substrate affinity of the enzyme. The alteration appears to be a general response of the heart to growth stimuli, as nearly identical results were obtained with hormonal stimulation of adult heart ODC by T_3 or in neonatal hearts.

Does the shift in K_m reflect activation of pre-existing ODC? A number of mechanisms have been proposed previously to account for interconversion of different forms of ODC (12) involving various putative activators and inhibitors of the enzyme. In the current study, a preliminary attempt was made to determine if excess activators or inhibitors were present in preparations from control or drug-treated rats. In no case did addition of supernatant from stimulated rats activate ODC from control animals, nor did supernatant from controls affect ODC stimulated rats. Thus, if activation is actually occurring, there appears to be no excess of the activator present in the homogenate.

Another possibility is that the shift in substrate affinity represents differential induction of a second form of the enzyme in the various types of cells present within the heart. In an attempt to determine whether induction of the enzyme participates in stimulation of activity, studies were conducted in which transcription was inhibited by treatment with actinomycin D. Studies in organs and tissues in which ODC can be induced have demonstrated a dependence of the induction upon production of new mRNA (3, 5, 23); thus, increases in ODC activity in regenerating rat liver, in hepatoma cells and in adrenal medulla can be prevented by pretreatment with transcriptional inhibitors (5, 24, 25). In the present

study, treatment with large doses of actinomycin D caused a marked reduction in RNA synthesis in the heart and a partial loss of basal ODC activity, indicating that the drug was effective in reducing transcription-dependent synthesis of the enzyme. However, actinomycin D did not prevent the isoproterenol-induced shift in K_m. Furthermore, the decline in net enzyme activity caused by actinomycin D was approximately equivalent in unstimulated animals and in isoproterenol-stimulated animals, indicating that isoproterenol did not alter the degree of dependence of ODC activity on transcription. Although experiments with transcriptional inhibitors can never be totally definitive (inhibition is never 100%), these results clearly differ from those previously reported in other tissues, in which actinomycin D (in doses 12-fold lower than those used here) totally blocks ODC stimulation (5, 23). Therefore, the production of the high affinity form of ODC in the stimulated adult rat heart is probably not dependent upon transcriptional mechanisms.

Further dissociation of the mechanism of heart ODC stimulation from that of tissues that exhibit actinomycin D-sensitive transcriptional control is provided by the studies with cyclic AMP. In liver, ODC induction appears to be mediated by type 1 cyclic AMP-dependent protein kinase (9), and in the present study, administration of dibutyryl cyclic AMP or 8-bromocyclic AMP produced increases in liver ODC activity. However, no increases occurred in heart ODC activity. Similarly, prostaglandin E_2 stimulated liver, but not heart, ODC. As a final confirmation of the lack of involvement of cyclic AMP in mediating heart ODC stimulation by isoproterenol, inhibition of phosphodiesterase by theophylline did not enhance the effect of isoproterenol. These studies strengthen the view that, unlike the liver, the stimulatory mechanism for heart ODC involves neither transcriptional control of induction nor type 1 cyclic AMP-dependent protein kinase.

The present studies do not necessarily rule out potential participation of translational events in controlling ODC activity, because the high rate of turnover of ODC activity interferes with direct assessment of translational control mechanisms for the enzyme. For example, although cycloheximide undoubtedly prevents the increase in ODC activity caused by isoproterenol (Table 1), this may only reflect the rapid degradation after inhibition of its synthesis, rather than interference with stimulation. Similarly, the kinetic studies do not enable exact conclusions as to whether more enzyme is actually present after stimulation; the higher total V_{max} of the high and low affinity forms, as compared with control V_{max} may represent two conformations of the same enzyme molecule, increased catalytic efficiency, decreased inhibitors, or other factors. Thus, a definitive answer cannot be obtained as to whether enzyme induction plays a role in the appearance of the high affinity ODC.

In summary, rat heart ODC exists in both low affinity and high affinity forms; the latter appears only during neonatal development or in the adult after administration of agents that cause cardiac hypertrophy. The mechanisms involved in regulation of cardiac ODC activity may be different from those demonstrated in other tissues.

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