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MYOCARDIAL ORNITHINE DECARBOXYLASE OF HYPERTENSIVE
AND DIABETIC RATS: EFFECTS OF INSULIN AND
TRIIODOTHYRONINE TREATMENT

BY

MICHAEL E. CHRISTE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTERS OF SCIENCE
IN
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1989

MASTER OF SCIENCE THESIS

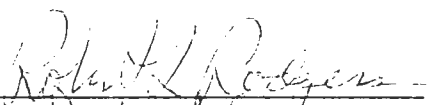
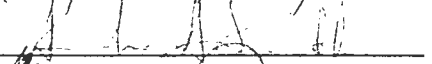

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UNIVERSITY OF RHODE ISLAND
1989

ABSTRACT

Ornithine decarboxylase (ODC) is the first and rate-limiting enzyme in the synthesis of polyamines, which play a regulatory role in nucleic acid and protein synthesis. The enzyme may be involved in normal and abnormal growth of the myocardium, in the relationship between mechanical stress and protein synthesis, and in the development of left ventricular hypertrophy (LVH). Results from this and other laboratories suggest that experimental diabetes has a more profound effect on mechanical function and cellular growth of hypertrophic heart muscle than it does on nonhypertrophic tissue. Diabetes is associated with reduced serum levels of both insulin and thyroid hormone. Both of these hormones are demonstrated regulators of ODC activity. Despite this, relatively little is known about the effects of diabetes on cardiac ODC activity and polyamine synthesis in the presence or absence of LVH. Therefore, we have hypothesized that: 1) ODC activity of hypertrophic left ventricle in spontaneously hypertensive rat (SHR) is higher than that of nonhypertrophic left ventricle in the normotensive rat strains, Wistar Kyoto (WKY) and Sprague Dawley (SD); 2) Untreated diabetes will reduce left ventricular ODC activity of SHR and of nonhypertensive rats, but its effects on the former will be greater in magnitude; 3) The effects of diabetes on left ventricular ODC activity, of either SHR or normotensive rats, will be preventable with insulin treatment, and at least partially preventable by thyroid hormone treatment, *in vivo*. Diabetes was induced in SHR and WKY by Streptozotocin (STZ) at 15 weeks of age. Subgroups were treated with insulin or

triiodothyronine (T_3). After 8 weeks of treatment, left and right ventricles were assayed for ODC activity, by measuring the rate of evolution of $^{14}CO_2$ from ($1-^{14}C$) L-ornithine. The results show that left ventricular ODC activity of nondiabetic SHR was not significantly different from that of either the WKY or SD normotensive rats. Streptozotocin-induced diabetes reduced left ventricular ODC activity to about the same extent in the hypertrophic SHR and in the nonhypertrophic WKY, depressing V_{max} without affecting the apparent K_m of the enzyme in both strains. However, experimental diabetes had no effect on right ventricular ODC activity in either the SHR or WKY strains. Both insulin and T_3 treatment were partially effective in preventing the reductions in ODC activity caused by diabetes. The results show that the depression in ODC activity caused by untreated diabetes : 1) Is selective for the left ventricle perhaps because of its relatively greater workload; 2) Is not selective for the hypertrophic ventricle in the SHR strain; and 3) May be related, in part, to the effects of the hypothyroidism which attends diabetes. These results do not support a causal relationship between left ventricular ODC activity and the maintenance of hypertrophy, nor do they support a predisposition of left ventricular hypertrophy to influences of diabetes on ODC activity (Hypotheses 1 and 2). The results do support a participation of attendant hypothyroidism in the effects of diabetes on left ventricular ODC activity (Hypothesis 3).

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The author would like to thank Dr. Robert Rodgers for his utmost patience, moral support, and guidance throughout the course of this study.

I would like to dedicate this thesis to my wife, Barbara. Without her love, support, and understanding, this work could not have been completed. Finally, I would like to thank special friends Amy Davidoff, Mark Mariani, and Francine Pinault for all of their help.

PREFACE

This thesis was prepared according to the manuscript format and is being submitted to the American Journal of Physiology.

TABLE OF CONTENTS

	PAGE
ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
PREFACE.....	v
TABLE OF CONTENTS.....	vi
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
ABSTRACT.....	2
INTRODUCTION.....	4
MATERIALS AND METHODS.....	8
RESULTS.....	13
DISCUSSION.....	23
REFERENCES.....	34
APPENDIX-RESULTS ANCILLARY TO MANUSCRIPT.....	41
BIBLIOGRAPHY.....	54

LIST OF TABLES

	PAGE
MANUSCRIPT	
TABLE 1: Serum glucose, insulin, T_3 and T_4	16
TABLE 2: Systolic arterial pressure and heart rates.....	17
TABLE 3: Body and heart weight ratios.....	18
TABLE 4: Left ventricular ODC activities.....	19
TABLE 5: ODC kinetics.....	22
APPENDIX	
TABLE 6: ODC activity at saturating ornithine concentration.....	50
TABLE 7: Right ventricular ODC activities.....	51
TABLE 8: Subcellular localization of ODC.....	52
TABLE 9: Myocardial arginase activity.....	53

LIST OF FIGURES

	PAGE
MANUSCRIPT	
FIGURE 1: Ventricular ODC kinetics.....	21
APPENDIX	
FIGURE 2: Characterization of ODC.....	43
FIGURE 3: Effect of increasing concentrations of enzyme supernatant on ODC activity.....	45
FIGURE 4: Verification of linearity of enzyme assay with time.....	47
FIGURE 5: Verification of $^{14}\text{CO}_2$ recovery by base- trap technique.....	49

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ABSTRACT

Ornithine decarboxylase (ODC) is the first and rate-limiting enzyme in the synthesis of polyamines, which play a regulatory role in nucleic acid and protein synthesis. The enzyme may be involved in normal and abnormal growth of the myocardium, in the relationship between mechanical stress and protein synthesis, and in the development of left ventricular hypertrophy (LVH). Results from this and other laboratories suggest that experimental diabetes has a more profound effect on mechanical function and cellular growth of hypertrophic heart muscle than it does on nonhypertrophic tissue. Diabetes is associated with reduced serum levels of both insulin and thyroid hormone. Both of these hormones are demonstrated regulators of ODC activity. Despite this, relatively little is known about the effects of diabetes on cardiac ODC activity and polyamine synthesis in the presence or absence of LVH. Therefore, we have investigated the possible role of ODC in LVH in the spontaneously hypertensive rat (SHR) and in the non-hypertensive Wistar Kyoto (WKY) and Sprague Dawley (SD) strains. We also examined the effects of diabetes on cardiac ODC activity in the presence and absence of LVH. The results show that left ventricular ODC activity of nondiabetic SHR was not significantly different from that of either the WKY or SD normotensive rats. Streptozotocin-induced diabetes reduced left ventricular ODC activity to about the same extent in the hypertrophic SHR and in the nonhypertrophic WKY, depressing V_{max} without affecting the apparent K_m of the enzyme in both strains. However, experimental diabetes had no effect on right ventricular ODC activity in either

the SHR or WKY strains. Both insulin and triiodothyronine (T_3) treatment were partially effective in preventing the reductions in ODC activity caused by diabetes. The results show that the depression in ODC activity caused by untreated diabetes : 1) Is selective for the left ventricle perhaps because of its relatively greater workload; 2) Is not selective for the hypertrophic ventricle in the SHR strain; and 3) May be related, in part, to the effects of the hypothyroidism which attends diabetes.

INTRODUCTION

The polyamines - putrescine, spermidine, and spermine - play an important regulatory role in nucleic acid and protein synthesis and cellular growth (23, 47, 72, 73). The initial step in their formation is catalyzed by ornithine decarboxylase (ODC, EC 4.1.1.17), which converts ornithine to putrescine, and appears to be the rate-limiting enzyme for polyamine synthesis (44, 61). ODC is unique in that its half-life is approximately 10-20 minutes, the shortest of any known mammalian enzyme (40, 64). The activities of ODC and putrescine fluctuate rapidly and specifically in response to a variety of hormonal and metabolic factors that alter cell growth (73). Thus ODC is markedly increased in tissues in which growth is accelerated, and its activity is usually low when the rate of growth is slow (11).

Cardiac hypertrophy is an early adaptive response to increased functional demand on the heart, such as pressure or volume overload (52, 80). The message by which increased mechanical stress is translated into increased protein synthesis and hypertrophy is not clear (60, 84). As a growth-regulating enzyme, ODC is a plausible point of control in the hypertrophy process. However, the extent to which ODC may contribute to the development and maintenance of myocardial hypertrophy is not well defined. The activity of ODC is normally higher in the left ventricle than in the right, which seems to be a reflection of the relatively greater work required of this chamber (6). A rise in ODC activity is a well documented biochemical

event in the development of hypertrophy in a variety of tissues (3, 21). Previous studies have shown an increase in polyamine concentration and cardiac ODC activity in the hypertrophic response to aortic constriction (17, 40), catecholamines (3, 4, 9, 22, 30), thyroid hormone (12, 13, 45, 46, 54) and hypoxia (32, 55). The fact that stimulation of myocardial ODC activity precedes the development of hypertrophy suggests that increased polyamine synthesis may be an important regulatory component of cardiac hypertrophy (39, 40, 60, 62). In fact, a temporal relationship has been described by Calderera et al. (10) between an increase in polyamine concentration which precedes an increase in RNA synthesis during the early stages of exercise-induced cardiac hypertrophy. In addition to these acute studies, the results of Ruskoaho et al. (60) show that the onset of left ventricular hypertrophy over time in the spontaneously hypertensive rat (SHR) model of hypertension is associated with elevated left ventricular ODC activity. Boucek et al. (7) have shown that changes in tension development alone in isolated rabbit papillary muscles can stimulate ODC activity.

It is well known that diabetes diminishes protein and RNA synthesis (16, 43) and accelerates protein degradation (81) in a variety of tissues, including cardiac muscle. Previous studies in this and other laboratories have also shown that diabetes reduces heart size, and in the SHR model of hypertension may also reverse left ventricular hypertrophy (15, 58, 70). Despite this, relatively little is known about the effects of diabetes on cardiac ODC activity and polyamine synthesis in the presence or absence of hypertension. Indeed, the influence of diabetes on ODC activity in any tissue is

not well characterized. Levine et al. (36) and Brosnan et al. (8) and others (25, 59) have reported an increase in ODC activity in liver and kidney of diabetic normotensive rats. However, Sochor et al. (69) and Conover et al. (14) both reported profound decreases in ODC activity in liver and heart of diabetic rats which could be prevented with insulin treatment. The effect of diabetes on myocardial ODC activity has not been investigated in any model of cardiac hypertrophy.

An attendant hypothyroidism, or "low thyroid state" (53), is often associated with human and experimental diabetes. Hypothyroidism alone decreases myocardial ODC activity (13), and thyroid hormone is an established regulator of myocardial ODC activity (12, 13, 54). Thus, studies of the effects of experimental diabetes on ODC activity should be designed to take into account the possible influence of attendant hypothyroidism.

The central hypotheses of the proposed study can be stated as follows: 1) ODC activity of hypertrophic left ventricle in SHR, at a given age, is higher than that of nonhypertrophic left ventricle in normotensive rat strains; 2) Untreated diabetes of moderate severity and eight weeks duration will reduce left ventricular ODC activity of SHR and of nonhypertensive rats, but its effect on the former will be greater in magnitude; 3) Effects of diabetes on left ventricular ODC activity, of either SHR or normotensive rats, will be preventable with insulin therapy, and at least partially preventable by thyroid hormone treatment, in vivo. Secondary aims of the study include comparisons of left and right ventricular ODC activities of

nondiabetic SHR and normotensive rats, and analysis of the kinetics of ODC obtained from nondiabetic and diabetic rat left ventricle.

MATERIALS AND METHODS

Experimental Groups and Treatments. Male Spontaneously Hypertensive rats (SHR) and age-matched normotensive control Wistar Kyoto (WKY) and Sprague Dawley (SD) rats were obtained from Charles River Breeding Laboratories (Wilmington, MA.). The animals were housed in temperature-controlled animal rooms (22°C) with a 12-hr light-dark cycle (8:00 a.m. to 8:00 p.m.). They were kept in group cages by strain and maintained on standard rat chow (Agway Inc., Syracuse, N.Y.) and tap water ad libitum. At 15 weeks of age, animals were matched according to body weight (BW) and blood pressure (SAP) within strains and divided into either diabetic or nondiabetic groups. Diabetes was induced in the SHR and WKY rat strains via a single tail vein injection, under light ether anesthesia, of Streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO.). The dose of STZ for each strain was adjusted in order to induce similar degrees of diabetes as assessed by serum glucose concentrations. Based on preliminary results, the doses used in this study for SHR and WKY strains were 45 mg STZ/kg BW and 50 mg STZ/kg BW respectively. The STZ was dissolved in citrate buffer (0.1 M at pH 4.5) just prior to use. The nondiabetic animals were also anesthetized and were injected with a similar volume of citrate buffer. The duration of either untreated or treated diabetes was eight weeks and the extent of diabetes was monitored weekly by urinary glucose measurements (Diastix^(R)) and verified by serum glucose and insulin levels at time of sacrifice.

A series of three studies, consisting of separate treatment groups, were carried out as follows (see Table 4): 1) A preliminary study of the effects of diabetes on left ventricular ODC activities in the SHR and WKY rats; 2) The effects of insulin and triiodothyronine (T_3) treatment of diabetic SHR; 3) The effects of insulin and T_3 treatment of diabetic WKY. In addition, kinetic analyses were performed in Study 3 as described below. The animals were divided into experimental and control groups as follows. The nondiabetic animals from both SHR and WKY consisted of untreated controls and those treated with daily administration of 3,5,3'-triiodothyronine sodium (T_3). Those animals treated with STZ were subdivided into diabetic and insulin- and T_3 -subgroups. Daily injections of T_3 (10ug/kg BW, Sigma Chemical Co., St. Louis, MO.) (74) and Protamine Zinc Insulin (I; 10 U/kg BW, Eli Lilly, Indianapolis, IN.) (75) were administered subcutaneously. This dose of T_3 was selected in previous studies in this laboratory according to its ability to prevent diabetes- induced bradycardia and to restore low serum thyroid hormone levels of diabetic rats to normal (15).

In vivo measurements. Systolic arterial pressure (SAP), heart rate (HR) and body weight (BW) were taken just prior to tail vein injections, and then four and eight weeks after STZ injection. A standard tail-cuff sphygmomanometer within a temperature controlled chamber (34°C) was used to measure SAP and HR, after the animals were prewarmed for 20 minutes (51). The SAP reported for each rat is the mean of at least six consecutive measurements. All measurements were recorded prior to the daily injections of T_3 or insulin.

Serum assays. After eight weeks of diabetes (at approximately 23 weeks of age), the animals were sacrificed by decapitation and their serum collected and stored at -20°C . Treated animals were always sacrificed 15-20 hours after their last daily injection. Serum titers of glucose were determined by the glucose oxidase assay (Sigma) and used to evaluate the degree of diabetes. Animals were classified retrospectively as diabetic if serum glucose values exceeded 300 mg/dL. Radioimmunoassays were used to determine serum insulin (Micromedic Systems Inc., Horsham, PA.) and total T_3 and total thyroxine (T_4) concentrations (Cambridge Medical Diagnostics, Billerica, MA.). Rat insulin was generously supplied by Dr. R. Chance (Eli Lilly) and was used as the standard for the insulin assays.

Enzyme assays. The heart was quickly excised, rinsed in ice cold 25 mM HEPES buffer, pH 7.2

(N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid, Research Organics, Cleveland, OH.), and blotted dry. The left ventricle with septum was carefully isolated from the right ventricle and each weighed separately. The left ventricle was diced and a 5% homogenate prepared in hypotonic 25mM HEPES at pH 7.2, using a Bio-Homogenizer (Biospec Products, Bartlesville, OK.) and centrifuged at 36,000g for 20 minutes. Triplicate aliquots of the supernatant fluid were used as the source of the enzyme. Right ventricles were quickly frozen in liquid nitrogen, stored at -80°C and assayed later.

The activity of ODC was determined by a modification of the procedure of Russell and Synder (61). The assay is based on the rate of evolution of $^{14}\text{CO}_2$ from ($1\text{-}^{14}\text{C}$) L-ornithine (Amersham Corp.,

Arlington Heights, IL.). The supernatant fluid was added to the reaction mixture in a 7 mL glass vial sealed with a rubber stopper through which was suspended a plastic center well (Kontes Glassware, Vineland, NJ.) containing 0.35 mL of 1M KOH and a small piece of filter paper. The assay mixture contained the following at the designated reaction concentrations in a final volume of 0.5 mL (all reagents from Sigma): dithiothreitol, 0.5 mM; pyridoxal phosphate, 0.05 mM; sodium bicarbonate, 3.0 mM; (1-¹⁴C) L-ornithine, 25 uM, 200 uM, or 400 uM (0.1 uCi; approximately 16,000, 2000, or 1000 cpm/nmol); and enzyme preparation, 0.3 mL, equally approximately one mg of protein. (Values for left ventricular ODC activity at 200 uM and 400 uM substrate concentration are in Appendix). In the kinetic studies (1-¹⁴C) L-ornithine was isotopically diluted with unlabelled L-ornithine to achieve the desired concentrations (6.25 to 300 uM; approximately 64,000 to 1,350 cpm/nmol). After diluting the (1-¹⁴C) L-ornithine, the open vial was gently stirred at room temperature in a fume hood for one to two hours in order to release free ¹⁴CO₂ and minimize background (78). Assay blanks contained 0.3 mL of 25 mM HEPES in place of cardiac enzyme supernatant and normally averaged 50 cpm/tube/hr. The lowest signal-to-noise ratio was usually in the range of 300 to 500 cpm above background for 25 uM ornithine and 150 to 200 cpm for 200 uM ornithine.

The reaction was allowed to proceed for one hour at 37°C and was terminated by the injection of 0.3 mL of 3.0 M citric acid through the rubber stopper. The ¹⁴CO₂ generated from the decarboxylation of (1-¹⁴C) L-ornithine was distilled into the center well during a second incubation of one hour. Approximately 96% of the generated

$^{14}\text{CO}_2$ was recovered in one hour (see Appendix). The center wells were transferred to scintillation vials for quantification of radioactivity (counting efficiency - 85%). Preliminary experiments verified that the rate of $^{14}\text{CO}_2$ generation was linear with time and enzyme concentration (see Appendix). Activity of ODC is expressed as pmol of $^{14}\text{CO}_2$ generated/hr/mg protein in the cell-free supernatant fluid after determination of protein content by the Lowry method (37).

Statistical analysis. The effects of diabetes and the hormone treatments were evaluated by one- or two-factor analysis of variance (ANOVA) with repeated measures when appropriate, and the Student Newman-Keuls test (Statistical Analysis Systems, Cary, NC.) for multiple comparisons. Straight lines for kinetic analysis were determined by linear regression. A level of significance of $p < 0.05$ was considered sufficient. Lower probabilities were not reported.

RESULTS

Serum assays. The effects of STZ-induced diabetes of 8 weeks duration on measurements of serum glucose, insulin, T_3 and T_4 of SHR and WKY rats are summarized in Table 1. Injection of STZ increased serum glucose, and reduced serum insulin of SHR and WKY rats to the same extent. By these criteria, the degree of diabetes was equivalent in both strains. The diabetic animals of both strains were also characterized by depressed serum T_3 and T_4 levels. The magnitude of attendant hypothyroidism was about the same in the two diabetic groups, but the T_3 levels of nondiabetic SHR were significantly higher than those of the nondiabetic WKY.

Treatment with T_3 had no effect on serum glucose or insulin levels of any of the experimental groups (Table 1). Treatment of diabetic SHR with T_3 restored the depressed serum T_3 levels to normal, and also aggravated the decline in serum T_4 , probably by inhibiting TSH production by the pituitary and T_4 secretion from the thyroid gland. T_3 treatment of nondiabetic SHR had the same depressing effect on T_4 levels, but had no effect on the other serum measurements. However, the same dose of T_3 , when administered to either nondiabetic or diabetic WKY, significantly increased serum T_3 levels as well as exacerbating the decline in serum T_4 levels.

Insulin treatment of diabetic SHR did not correct the hyperglycemia although it caused a pronounced increase in serum immunoreactive insulin activity (Table 1). Insulin treatment did not restore serum T_3 and T_4 levels to normal. In contrast, treatment of

diabetic WKY with the same dose of insulin effectively reversed the hyperglycemia and depressed serum insulin levels, and corrected the reductions in serum T_3 and T_4 levels.

Arterial Pressure and Heart Rate In Vivo. Nondiabetic SHR were hyper-tensive and tachycardic compared to nondiabetic WKY and SD rats at both Week 0 and Week 8 (Table 2). Diabetic SHR were characterized by depressed SAP and HR after 8 weeks, confirming previous results (58). Diabetes had no significant effect on either SAP or HR in the WKY strain. Treatment of diabetic SHR with either insulin or T_3 was sufficient to prevent the decrease in both SAP and HR. Treatment of nondiabetic SHR with T_3 had no effect on either SAP or HR, but administration of the same dose of T_3 to diabetic and nondiabetic WKY rats did cause significant elevations in both SAP and HR.

Body and Heart Weights. Left ventricles of SHR were hypertrophic, both relatively (LVW/BW) and absolutely (LVW/RVW) compared to left ventricles of the nonhypertensive WKY and SD rats (Table 3). Diabetes reduced BW, left ventricular weight (LVW), and right ventricular weight (RVW) of SHR, but did not have a significant effect on indices of left ventricular hypertrophy. Diabetes reduced BW in WKY rats but had no effect on left or right ventricular weights. The effects of diabetes on heart weights (LV and RV) were more pronounced in the SHR than they were in the WKY strain.

Insulin treatment of diabetic SHR either partially or fully restored BW, LVW, and RVW, but had no effect on LVW/BW or LVW/RVW (Table 3). Insulin treatment effectively restored BW of diabetic WKY rats. Treatment of diabetic SHR with T_3 caused a slightly greater

decline in BW and an increase in LVW, leading to a restoration of LVW/BW. Treatment of nondiabetic SHR with T_3 increased RVW significantly, and increased LVW/BW. Treatment of nondiabetic and diabetic WKY with T_3 induced a relative left ventricular hypertrophy (LVW/BW), due apparently to a reduction in body weight.

Ornithine Decarboxylase Activity. Left ventricular Ornithine Decarboxylase (ODC) activity in 23 week old nondiabetic SHR was not significantly different from that of either the WKY or the more outbred SD rat strain (Table 4, Study 3), confirming previous results (60). Diabetes reduced left ventricular (LV) ODC activity in both SHR and WKY to about the same extent (Table 4, Studies 1, 2, and 3). Insulin treatment prevented the decline in LV ODC activity in the WKY (Study 3), but not in the SHR group (Study 2). Treatment with T_3 was partially effective in preventing the decline in LV ODC activity in both the SHR and WKY strains (Studies 2 and 3). The results of Table 4, obtained at a substrate concentration of 25 μ M, were confirmed at a saturating concentration of 200 or 400 μ M ornithine (see Table 6, Appendix). Right ventricular ODC activity was not significantly affected either by diabetes or by any treatment (see Table 7, Appendix). The activity of the crude enzyme preparation seemed to display Michaelis-Menten kinetics in both the nondiabetic and diabetic state (Figure 1). The inset in Figure 1 depicts the Lineweaver-Burk plots for nondiabetic and diabetic SHR. It demonstrates that diabetes caused a decrease in V_{max} , but had no effect on the apparent K_m for ornithine, in the SHR strain. A similar pattern was evident in the WKY strain (Table 5).

TABLE 1. Serum glucose, insulin, triiodothyronine and thyroxine levels of nondiabetic, diabetic and treated SHR, WKY and SD.

Strain Group	n	Glucose (mg/dl)	Insulin (μ U/ml)	T ₃ (ng/dl)	T ₄ (μ g/dl)
<u>SHR</u>					
Nondiabetic	12	131 \pm 16 ϕ	83 \pm 22	112 \pm 22 ϕ	3.95 \pm 0.49
Diabetic	8	633 \pm 55*	20 \pm 10*	46 \pm 12*	1.46 \pm 0.45*
Diabetic + T ₃	6	666 \pm 77*	31 \pm 14*	125 \pm 45	0.17 \pm 0.11**
Diabetic + I	9	676 \pm 42*	193 \pm 74**	61 \pm 18*	3.07 \pm 0.31**
Nondiabetic + T ₃	6	145 \pm 33	77 \pm 14	101 \pm 21	0.21 \pm 0.11**
<u>WKY</u>					
Nondiabetic	12	170 \pm 23	117 \pm 78	86 \pm 15 ϕ	3.89 \pm 0.49
Diabetic	11	636 \pm 60*	23 \pm 6*	46 \pm 9*	3.05 \pm 0.40*
Diabetic + T ₃	7	645 \pm 45*	41 \pm 29*	133 \pm 42**	0.03 \pm 0.01**
Diabetic + I	7	176 \pm 48	> 300 **	86 \pm 12	3.70 \pm 0.61
Nondiabetic + T ₃	7	187 \pm 23	164 \pm 57	147 \pm 26**	0.11 \pm 0.08**
<u>SD</u>					
Nondiabetic	9	160 \pm 14	141 \pm 37	42 \pm 12 ϕ	3.35 \pm 0.57 ϕ

Values are means \pm SD, obtained 8 weeks after initiation of diabetes or treatment from animals used in Studies 2 and 3. T₃, triiodothyronine; T₄, thyroxine; I, protamine zinc insulin.

* Significantly different from the nondiabetic group (within strain; p < 0.05).

**Significantly different from the nondiabetic and diabetic groups (within strain; p < 0.05).

ϕ Significantly different from nondiabetic groups (between strains; p < 0.05).

TABLE 2. Systolic arterial pressure and heart rates of nondiabetic, diabetic and treated SHR, WKY and SD.

Strain Group	n	SAP (mmHg)		HR (beats/min)	
		Week 0	Week 8	Week 0	Week 8
<u>SHR</u>					
Nondiabetic	12	175 ± 16Φ	198 ± 13Φ	399 ± 32Φ	449 ± 37Φ
Diabetic	8	179 ± 24	144 ± 15*	389 ± 26	299 ± 38*
Diabetic + T ₃	6	185 ± 16	184 ± 26	374 ± 27	468 ± 37
Diabetic + I	9	175 ± 16	191 ± 13	379 ± 25	407 ± 31
Nondiabetic + T ₃	6	174 ± 18	203 ± 14	402 ± 20	472 ± 45
<u>WKY</u>					
Nondiabetic	12	127 ± 11	143 ± 11	294 ± 19Φ	300 ± 22Φ
Diabetic	11	124 ± 11	129 ± 9	310 ± 12	270 ± 18
Diabetic + T ₃	7	127 ± 9	180 ± 15**	307 ± 17	370 ± 41**
Diabetic + I	7	128 ± 18	143 ± 10	301 ± 12	309 ± 18
Nondiabetic + T ₃	7	124 ± 9	174 ± 12**	321 ± 20	421 ± 51**
<u>SD</u>					
Nondiabetic	9	131 ± 7	149 ± 14	363 ± 25Φ	351 ± 26Φ

Values are means ± SD, obtained before (Week 0) and 8 weeks after initiation of diabetes or treatment from animals used in Studies 2 and 3. SAP, systolic arterial pressure; HR, heart rate; T₃, triiodothyronine; I, protamine zinc insulin.

* Significantly different from the nondiabetic group (within strain; p < 0.05).

**Significantly different from the nondiabetic and diabetic groups

(within strain; p < 0.05).

Φ Significantly different from nondiabetic groups (between strains; p < 0.05).

TABLE 3. Body and heart weights of nondiabetic, diabetic and treated SHR, WKY and SD.

Strain Group	n	BW (g)	LV (mg)	RV (mg)	LV/RV (mg/mg)	LV/BW (mg/g)
<u>SHR</u>						
Nondiabetic	12	342 ± 31 Φ	980 ± 92 Φ	202 ± 27	4.94 ± 0.77 Φ	2.87 ± 0.14 Φ
Diabetic	8	191 ± 23*	510 ± 69*	133 ± 26*	3.95 ± 0.91	2.68 ± 0.17
Diabetic + T ₃	6	179 ± 24*	571 ± 91*	143 ± 33*	4.11 ± 0.76	3.18 ± 0.18**
Diabetic + I	9	299 ± 23	756 ± 65**	191 ± 27	4.01 ± 0.56	2.53 ± 0.13
Nondiabetic + T ₃	6	331 ± 30	1018 ± 118	243 ± 44**	4.31 ± 0.91	3.08 ± 0.25**
<u>WKY</u>						
Nondiabetic	12	403 ± 25 Φ	903 ± 61 Φ	229 ± 41	4.04 ± 0.70	2.24 ± 0.10 Φ
Diabetic	11	340 ± 24*	796 ± 83	217 ± 40	3.75 ± 0.59	2.36 ± 0.15
Diabetic + T ₃	7	304 ± 40*	890 ± 85	283 ± 83	3.34 ± 0.81	2.97 ± 0.47**
Diabetic + I	7	431 ± 38	961 ± 115	254 ± 73	3.97 ± 0.87	2.23 ± 0.08
Nondiabetic + T ₃	7	383 ± 26	1076 ± 151	309 ± 90	3.60 ± 0.52	2.84 ± 0.64**
<u>SD</u>						
Nondiabetic	9	633 ± 69 Φ	1991 ± 83 Φ	309 ± 59 Φ	3.62 ± 0.58	1.73 ± 0.11 Φ

Values are means ± SD, obtained 8 weeks after initiation of diabetes or treatment from animals used in Studies 2 and 3. T₃, triiodothyronine; I, protamine zinc insulin.

* Significantly different from the nondiabetic group (within strain; p < 0.05).

**Significantly different from the nondiabetic and diabetic groups (within strain; p < 0.05).

Φ Significantly different from nondiabetic groups (between strains; p < 0.05).

TABLE 4. Left Ventricular ODC activity in nondiabetic, diabetic and treated SHR, WKY and SD rats.

Strain Group	LEFT VENTRICULAR ODC ACTIVITY (pmol 14 C $_2$ /hr/mg protein)					
	STUDY 1	n	STUDY 2	n	STUDY 3	n
<u>SHR</u>						
Nondiabetic	123	3	100 \pm 16	8	66 \pm 13	4
Diabetic	35	2	36 \pm 22*	5	24 \pm 10*	3
Diabetic + T ₃			71 \pm 27**	6		
Diabetic + I			48 \pm 14*	9		
Nondiabetic + T ₃			97 \pm 18	6		
<u>WKY</u>						
Nondiabetic	99	2	87 \pm 23	8	61 \pm 13	4
Diabetic	20	4	49 \pm 20*	7	24 \pm 13*	4
Diabetic + T ₃					42 \pm 14**	7
Diabetic + I					52 \pm 20	7
Nondiabetic + T ₃					76 \pm 28	7
<u>SD</u>						
Nondiabetic					66 \pm 14	9

Values are means \pm SD obtained 8 weeks after initiation of diabetes or treatments. T₃, triiodothyronine; I, protamine zinc insulin.

* Significantly different from the nondiabetic group (within strain; p < 0.05).

** Significantly different from the nondiabetic and diabetic groups (within strain; p < 0.05).

Figure 1. Ornithine decarboxylase (ODC) activity of nondiabetic and diabetic SHR left ventricle, at various substrate concentrations. Inset: Lineweaver-Burk Plot of left ventricular ODC activity.

Kinetics of Left Ventricular

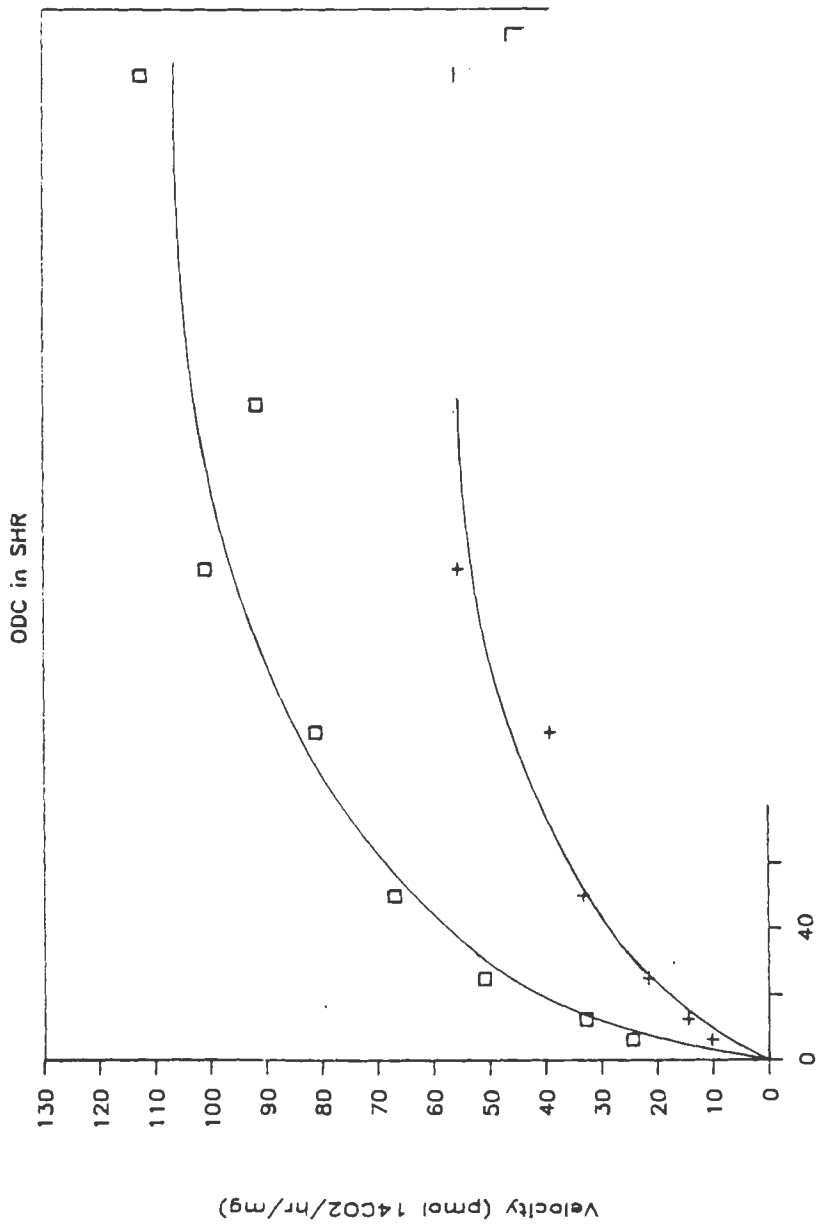


TABLE 5. Left ventricular ODC kinetics in nondiabetic and diabetic SHR, WKY and SD rats.

<u>Strain</u> Group	n	K _m (μ M)	V _{max} (pmol ¹⁴ CO ₂ /hr/mg prot.)
<u>SHR</u>			
Nondiabetic	4	26.8 \pm 9.9	111.4 \pm 21.5
Diabetic	3	23.7 \pm 5.7	51.2 \pm 8.8*
<u>WKY</u>			
Nondiabetic	4	26.0 \pm 9.6	118.5 \pm 12.7
Diabetic	4	31.6 \pm 7.1	61.0 \pm 13.2*

Values are means \pm SEM, obtained 8 weeks after initiation of diabetes or treatments.

* Significantly different from the nondiabetic group (within strain; p < 0.05).

DISCUSSION

The first two related hypotheses of the proposed study, that ODC activity of nondiabetic, hypertrophic spontaneously hypertensive rat (SHR) left ventricle would be higher than that of the nonhypertensive rat strains, and that diabetes would exert a selective effect in the SHR, were not supported by the results. The rationale for the hypotheses was derived, in part, from the assumption that ODC activity could be associated with both the development and maintenance of left ventricular hypertrophy in a variety of experimental models (3, 4, 10, 13, 17, 30, 40, 54, 60). SHR exhibit a relatively slow progressive increase in arterial pressure and left ventricular mass (52, 80, 84), characterized by a thickening of the left ventricular wall (left ventricular hypertrophy). Thus, this model of hypertrophy is similar to the cardiac alterations seen in essential (idiopathic) hypertension in humans (77, 80).

Diabetes affects a number of variables which conceivably might influence the course of hypertrophy development in the left ventricle. One proposed etiologic factor in the development of hypertensive hypertrophy in the SHR model is an increase in either the activity of, or the myocardial responsiveness to, sympathoadrenal influences (1, 83). Catecholamines, the mediators of sympathetic activation, stimulate myocardial ODC activity and induce left ventricular hypertrophy (4, 9, 22, 30, 76). One consequence of untreated diabetes is interference with sympathetic activation of cardiac muscle, characterized in part by a reduction in the density

of beta adrenoceptors (16, 24, 79). Another proposed stimulus of cardiac ODC activity is physical stress on the ventricular wall (7). Left ventricular hypertrophy results in part from elevated wall stress secondary to abnormally increased total vascular resistance (80). Previous studies from this laboratory and others have shown that diabetes reduces arterial pressure of SHR (15, 58, 70), and thus would likely relieve the pressure load on the left ventricle. The depressor effect of diabetes in SHR was confirmed in the present study (Table 2). The earlier studies also showed that diabetes caused a relatively more pronounced reduction in whole heart and left ventricular mass in SHR than it did in the WKY strain, associated with an inconsistent reversal of left ventricular hypertrophy (15, 58).

The only available study of ODC activity in nondiabetic SHR myocardium was reported by Ruskoaho et al (60). They observed that left ventricular (LV) ODC activity was elevated in the SHR, relative to that of the WKY, only in young animals during the development of left ventricular hypertrophy. However, after left ventricular hypertrophy was established, beginning at about 20 weeks of age, the ODC activities of SHR and nonhypertensive WKY rat left ventricle were not different. The results of this study (Table 4), confirm those of Ruskoaho et al. (60). At 23 weeks of age, the activity of LV ODC in SHR was similar to that of the WKY and the more outbred SD rat. The results also show that the effect of diabetes on ODC activity was approximately equal in magnitude in the hypertrophic SHR and nonhypertrophic WKY left ventricle (Table 4). Thus, hypertrophy did not seem to predispose the left ventricle to the influences of

diabetes on LV ODC activity. These results are not consistent with a role of increased ODC activity in the maintenance of left ventricular hypertrophy in the SHR.

Although diabetes did not exert a selective effect on hypertrophic myocardial ODC activity, it did exhibit a preferential action on the left ventricle versus the right in both the SHR and WKY rat strains (Table 4 and Table 7, Appendix). The data confirm and extend previous results from other laboratories, which showed that untreated diabetes depressed myocardial ODC activity after 8 days and 4 weeks (14, 25, 69). However, these earlier studies did not distinguish between left and right ventricular ODC activity. The present results also seem to show that the apparent differences in ODC activity between left and right ventricles, present in nondiabetic animals, disappear after imposing chronic, untreated diabetes. The difference in workload between left and right ventricle is much greater than the incremental increase in workload imposed on the left ventricle by hypertension (26). Even acute elevations in workload can stimulate ODC activity (7). In general, the data support the concept that myocardial ODC activity may be regulated by mechanical and hormonal influences.

Stimulation of myocardial ODC activity by a variety of stimuli has been shown to precede the development of hypertrophic responses, and suggests that polyamine synthesis may be an important regulatory component of cardiac hypertrophy (9, 10, 17, 22, 30, 39, 40, 45, 60, 62). It is now well established that virtually all stimulatory or trophic hormones cause an increase in ODC activity in their

appropriate target tissues (2). Although, the mechanism of ODC regulation has not been well defined, several have been proposed.

First, as mentioned earlier, an increase in ODC activity in various tissues may be the result of an increase in the amount of enzyme protein which, in turn, results from either a decrease in degradation or an increase in synthesis of the enzyme, or both (61). This mechanism is supported by an observed accumulation of ODC-mRNA in mouse kidney which had been induced by androgens (31, 64, 68). Such regulation by alteration in the rate of enzyme protein synthesis or degradation is unusual in mammalian cells. Regulation is usually accomplished by posttranslational modification (47), such as phosphorylation, thiol modification, and related mechanisms. In order for rapid changes to occur by the former mechanism, the protein must turn over rapidly. The bulk of the available data indicates that ODC has a half-life of approximately 10-20 minutes (40, 65, 67). Insulin has been shown to increase ODC activity in various cell cultures (5). This increase is due to the formation of new ODC mRNA and a subsequent rise in ODC protein (5). Mallette and Exton (38) observed an increase in liver ODC activity during ex vivo perfusion with a pharmacological dose of insulin. Finally, Conover (14) and Sochor (69) both found that insulin could prevent the decrease in cardiac ODC activity due to diabetes. However, it should be noted that the insulin-treated animals in these two studies were profoundly hypoglycemic. Insulin-induced hypoglycemia can markedly increase sympathoadrenal activity, which can increase cardiac ODC activity. Consequently, the prevention of the diabetic decrease in cardiac ODC activity may be a direct or indirect effect of insulin.

Second, some hormonal influences may affect the affinity of ODC for its substrate. Lau and Slotkin proposed that increases in cardiac ODC due to acute isoproterenol or T_3 treatment resulted in the appearance of a form of the enzyme with an increased affinity for ornithine (33, 35). This suggests that the stimulation of cardiac ODC occurs through a K_m shift in the absence of any increase in the number of ODC molecules. Millan (41) reported that both the K_m and V_{max} of ODC were decreased in neonatal rat heart following hypertonic saline injection. No changes were found in liver and other tissues, thus supporting the hypothesis that myocardial ODC may be under unique regulatory control. However, Flamigni et al. (19) observed a marked increase in V_{max} , with no change in the K_m , of myocardial ODC from rats treated with isoproterenol. Thus, the exact role of changing affinity states as a point of regulation remains unclear.

Third, regulation of the enzyme may involve the expression of more than one isoform. Flamigni et al. (18, 19) also showed that two chromatographically distinct forms of ODC exist in the rat heart. Separable forms of ODC have been detected in other tissues (56), and do not seem to differ in their affinity for ornithine (42), but do differ with respect to their half-lives (49). Both forms are increased after isoproterenol treatment to the same extent, and thus the two forms cannot reasonably explain the isoproterenol induction of ODC (19). However, the analytical methods used do not eliminate the possibility of purification artifacts due to the high level of pyridoxal phosphate used during the extraction (48). When analysed by gel electrophoresis, no heterogeneity of ODC protein was observed with respect to size, but two forms were detected which varied

slightly in charge (65). The possible role of ODC isoforms in the hormonal regulation of the enzyme is not known.

Finally, ODC antizymes may be targets of regulation. Canellakis has demonstrated the existence of a non-competitive protein inhibitor of ODC (antizyme) in various cell types, which is induced by polyamines (11). Recent evidence supports the existence of an antizyme molecule in the rat heart treated with putrescine and indicates that a significant amount of ODC occurs in an inactive, complexed form (20). The absolute and relative levels of ODC protein, as well as activity, may vary with different conditions or stimuli. The antizyme appears to have a half-life comparable to ODC and thus could constitute a sensitive modulator of enzyme activity (27). Also, the decline in ODC protein after exposure to exogenous polyamines has been shown to occur more rapidly than the fall when protein synthesis is blocked by cycloheximide (11). This observation has led to the suggestion that another physiological role of antizyme may its involvement in the initial step of degradation of the ODC protein (48), perhaps by making ODC more susceptible to proteolytic cleavage (27). The development of specific antibodies and RIA techniques has permitted more thorough investigation of the mechanism of rapid ODC induction. No evidence has been found for the regulation of ODC enzyme protein by post-translational modifications or by changes in the content of activating or inhibitory factors. Seely and Pegg (65, 66) found an excellent correlation between the amount of ODC protein and the enzymic activity in induced rat liver and kidney. ODC activity declines very quickly in cells in response to exogenous polyamines (11) possibly through the induction of the

antizyme, and appears to be due to an increased degradation rate and a decreased rate of synthesis. However, there is no change in the content of ODC-mRNA, suggesting that the translation of ODC-mRNA may be affected by the polyamine content. In some instances the increased synthesis of ODC protein in response to acute treatment with ODC inhibitors is also not accompanied by any change in the mRNA content (50). Thus, it seems that overall regulation of ODC protein occurs both at the level of transcription of the ODC gene and at the level of translation of the ODC-mRNA.

The presents results, which demonstrated that diabetes caused a decrease in the maximum velocity without affecting the apparent K_m of ODC for ornithine (Figure 1 inset, and Table 5), are not inconsistent with mechanisms 1, 3, and 4 as discussed above. They do tend to rule out, however, those which invoke an alteration in the affinity of the enzyme for its substrate (mechanism 2 above). This is consistent with some preliminary results which show a decrease in the total activity of the enzyme in the cytosol and particulate fraction (Table 8 Appendix). The present results also imply that diabetes causes a shift in the subcellular localization of the enzyme (Table 8, Appendix), as indicated by the ratio of ODC activity in the pellet versus the supernatant fractions. Obviously, these experiments need to be repeated in order to obtain a more definitive characterization. One explanation for this phenomenon may be an increase in the amount of antizyme-ODC complex. The ODC antizyme is a non-competitive protein inhibitor of ODC, which would decrease the velocity of the decarboxylase reaction, without affecting the K_m (27). The design of

our experiments cannot define the role of antizyme in the diabetic decrease of LV ODC activity.

Experimental diabetes also decreases absorption in the intestinal tract (8). Starvation of nondiabetic rats has been shown to depress ODC activity in normotensive hearts to the same extent as diabetes does (14). However, decreased sympathetic activity as well as β receptor concentration during fasting could contribute to the reduction of cardiac ODC activity (79). Also, serum T_3 and insulin levels are both reduced during fasting and may contribute to the decrease in cardiac ODC activity (79). Consequently the overall influence of malabsorption secondary to diabetes remains unclear.

Another possible explanation of the decrease of LV ODC due to diabetes may be due to changes in cellular ornithine concentration, which may have resulted in alterations in the rate of myocardial synthesis of ODC protein (8). Ornithine is formed from arginine by the enzyme arginase, and thus changes in ornithine concentration may be secondary to alterations in arginine content. Conceivably, a fraction of the ornithine substrate might originate from myocardial arginase in addition to the arginine obtained from the serum. Ornithine is primarily manufactured in the liver and is an essential part of the urea cycle. Ornithine concentration in human plasma ranges between 30 and 50 μM (71), which is close to the apparent K_m of ODC in the left ventricle (Table 5). It is also known that human red blood cells contain arginase and can secrete ornithine in surprisingly high concentrations into the plasma (82). Finally, Brosnan et al. (8) showed that ornithine concentration actually increases in the liver during severe experimental diabetes. We

carried out preliminary experiments which demonstrated minimal arginase activity in the heart (Table 9, Appendix), confirming previous results (28). Thus, any role of myocardial arginase in the effects of diabetes on cardiac ODC activity seems highly unlikely.

Insulin treatment prevented the decrease in LV ODC activity in diabetic WKY, but did not in the SHR strain (Table 4). Although insulin prevented the serum alterations of diabetes in the WKY, it did not reverse them in the diabetic SHR (Table 1). However, insulin restored SAP and HR in the SHR (Table 2) and partially or fully restored BW, LVW, and RVW (Table 3). The ineffectiveness of insulin in diabetic SHR may be due to inappropriate therapy, leading to poor metabolic control. These animals were sacrificed 15-20 hours after their last injection. Conover et al. (14) and Sochor et al. (69) both found that insulin could prevent the decrease in cardiac ODC activity due to diabetes. However, as mentioned previously, it should be noted that the insulin-treated animals in these two studies were profoundly hypoglycemic. Insulin-induced hypoglycemia can markedly increase sympathoadrenal activity, which can increase cardiac ODC activity. Consequently, the prevention of the diabetic decrease of cardiac ODC activity in these two studies may be a direct or indirect effect of insulin. Hu et al. (29) have described a temporal increase of liver ODC activity in response to pharmacological doses of insulin. Unfortunately, they did not show any time points after 6 hours. Later time points would better reveal the effectiveness of insulin therapy on tissue ODC activity in diabetes. The enzyme's very short half-life may require constant stimulation in order to maintain normal levels in the myocardium.

This treatment group is presently being repeated with the aim of better control of the hyperglycemia in the diabetic SHR.

Induction of ODC activity by different growth factors on various cultured cells seems to require a minimum concentration of specific ornithine decarboxylase-inducing amino acids, such as asparagine, in the medium (57). Growth factors, including insulin, appear to act synergistically with the inducing amino acid (57). Insulin has also been shown to increase amino acid transport in cultured embryonic chick heart cells (63). Thus, the inability of insulin to restore LV ODC activity in diabetic SHR may also have been related, in part, to decreased plasma or tissue concentrations of specific ODC-inducing amino acids during diabetes.

T_3 treatment of diabetic SHR and WKY restored serum T_3 levels (Table 1) and partially prevented the decline in LV ODC activity (Table 4). It also prevented the depressions in HR and SAP of SHR (Table 2). Hypothyroidism, which is associated with diabetes (53), has been shown to decrease cardiac ODC activity (13), whereas injections of T_3 stimulate it (12, 13, 34, 45, 46, 54). Raymondjean et al. (54) found that T_3 induced a biphasic response of cardiac ODC activity, with peaks at 4 and 21 hours that were inhibited by cycloheximide, indicating that T_3 increased the synthesis of new ODC molecules. The trough of this response could be explained by the complexing of ODC to antizyme, since there was an accumulation of ODC molecules that "...were catalytically inactive but immunologically reactive..." (54).

In summary, the results of this study show that chronic, untreated diabetes depresses ODC activity in the left ventricle, but

not the right ventricle, of the rat heart. This effect of diabetes is: 1) Not influenced by preexisting left ventricular hypertrophy in the SHR model of hypertension; 2) Reversible by insulin treatment which confers good metabolic control; 3) Partially reversible by T_3 treatment; and 4) Not associated with any apparent change in the affinity of the enzyme for its substrate. The results support the hypothesis that both insulin and thyroid hormone, along with mechanical stress on the ventricle, are important regulators of myocardial ODC activity. They also suggest that neither ODC activity nor its possible regulation by these factors is uniquely influenced by established left ventricular hypertrophy.

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APPENDIX. RESULTS ANCILLARY TO MANUSCRIPT

Figure 2a,b. Myocardial Ornithine Decarboxylase activity is typically higher in weanlings than in mature adult. Thus, characterization of the enzyme was initially carried out in 21 to 35 day old Sprague Dawley (SD) rat hearts. Figure 3a depicts the Michaelis-Menten kinetics of myocardial ODC activity over 2 hours, obtained by isotopically diluting (1-¹⁴C) L-ornithine with unlabelled L-ornithine to the desired concentration. The double reciprocal plot of weanling SD cardiac ODC activity is shown in Figure 3b. The K_m is 35 uM and V_{max} is 710 pmol ¹⁴CO₂ generated/hr/mg protein, obtained by linear regression analysis.

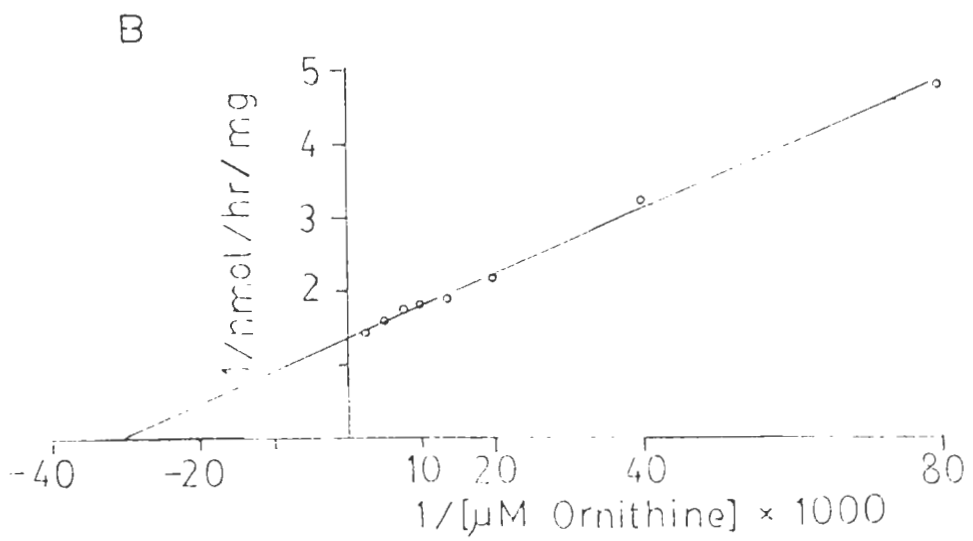
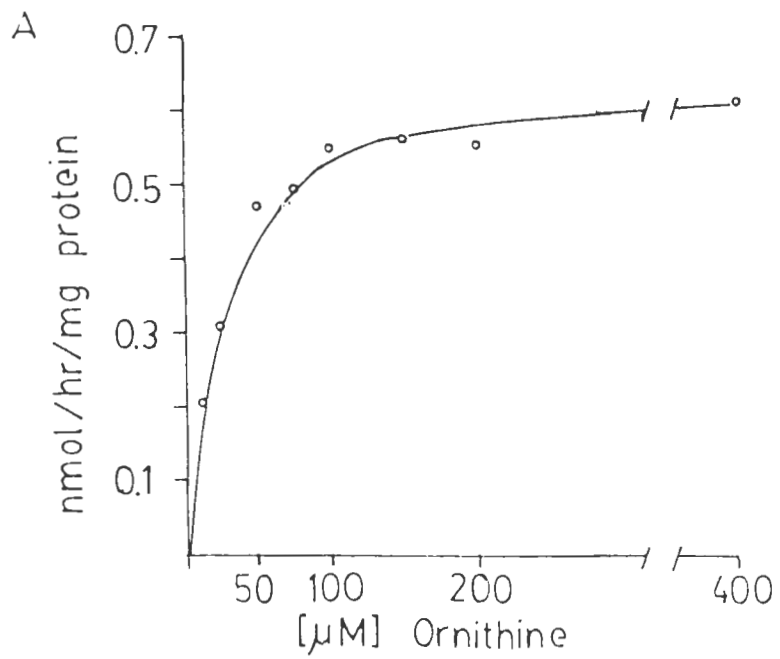


Figure 3. Effect of increasing concentrations of enzyme supernatant at a fixed ornithine concentration of 25 μ M and time of 2 hours on ODC activity in weanling SD rat hearts.

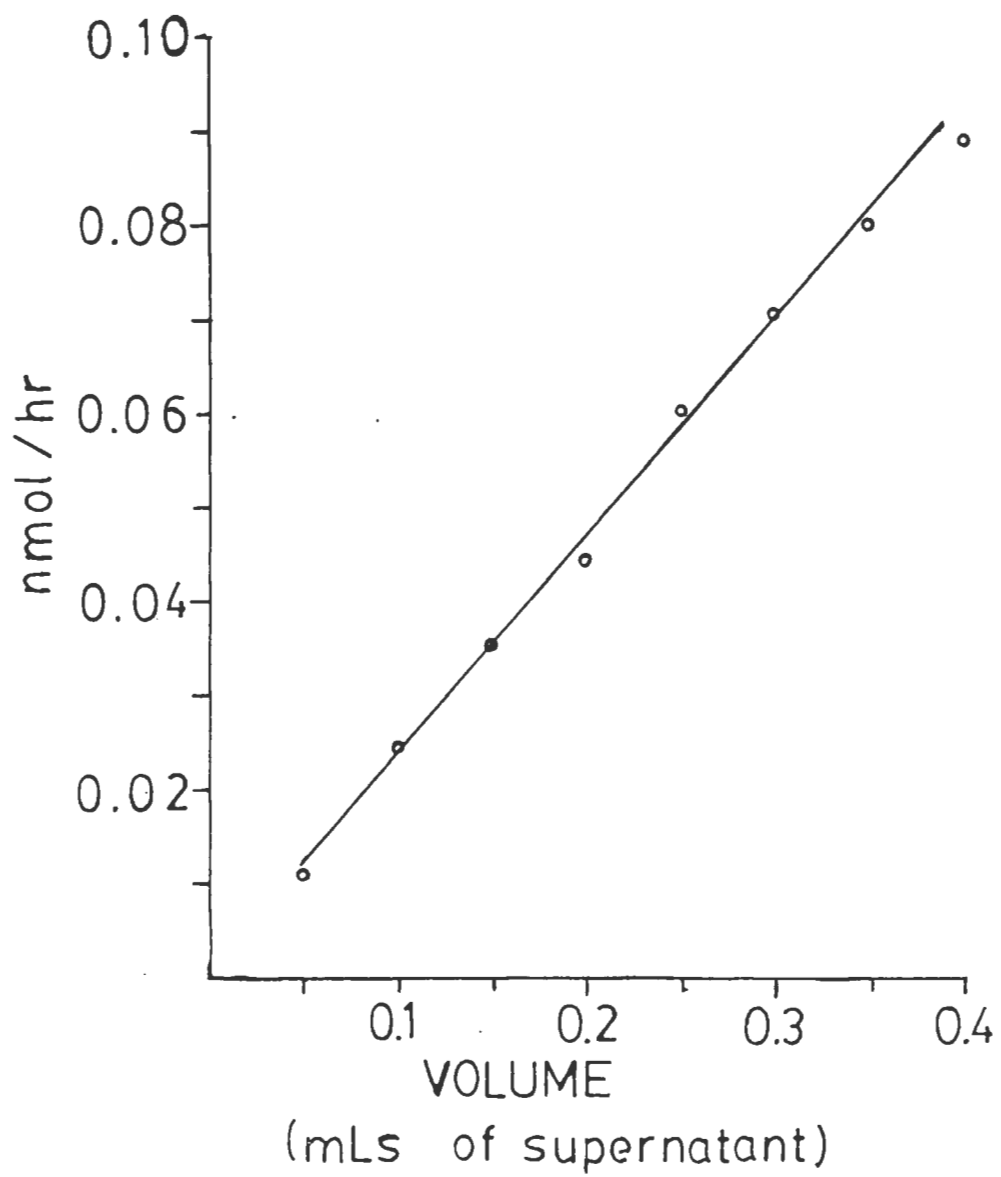


Figure 4. Verification of linearity with time of myocardial ODC activity at a fixed enzyme supernatant volume (0.3 mL) and substrate concentration (50uM).

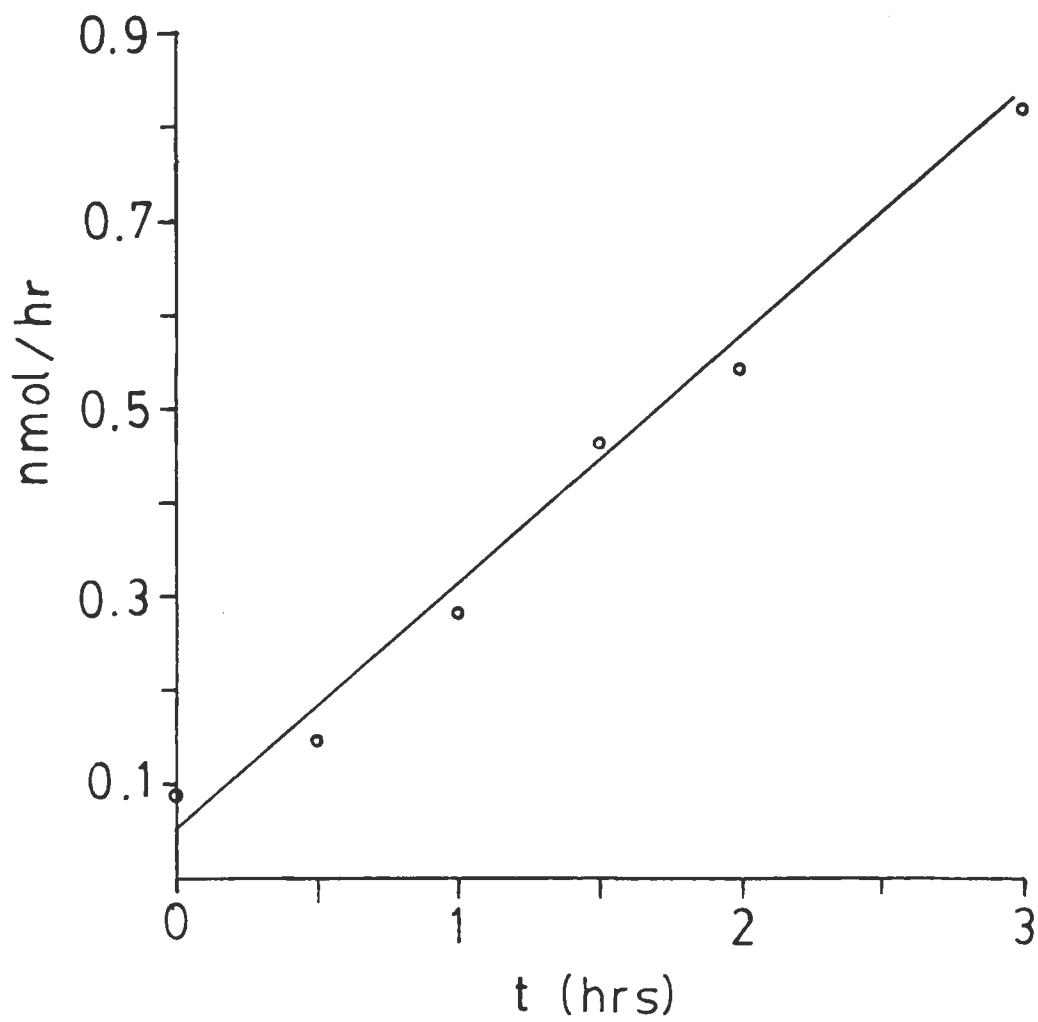


Figure 5. Verification of recovery of generated $^{14}\text{CO}_2$ from incubation media. $^{14}\text{C-NaHCO}_3$ was added to correct volume and concentration of incubation constituents (see Methods). Vials were acidified and allowed to incubate for the indicated times. The results indicate that 96% of the $^{14}\text{CO}_2$ was recovered in one hour and 100% recovery was obtained at four hours.

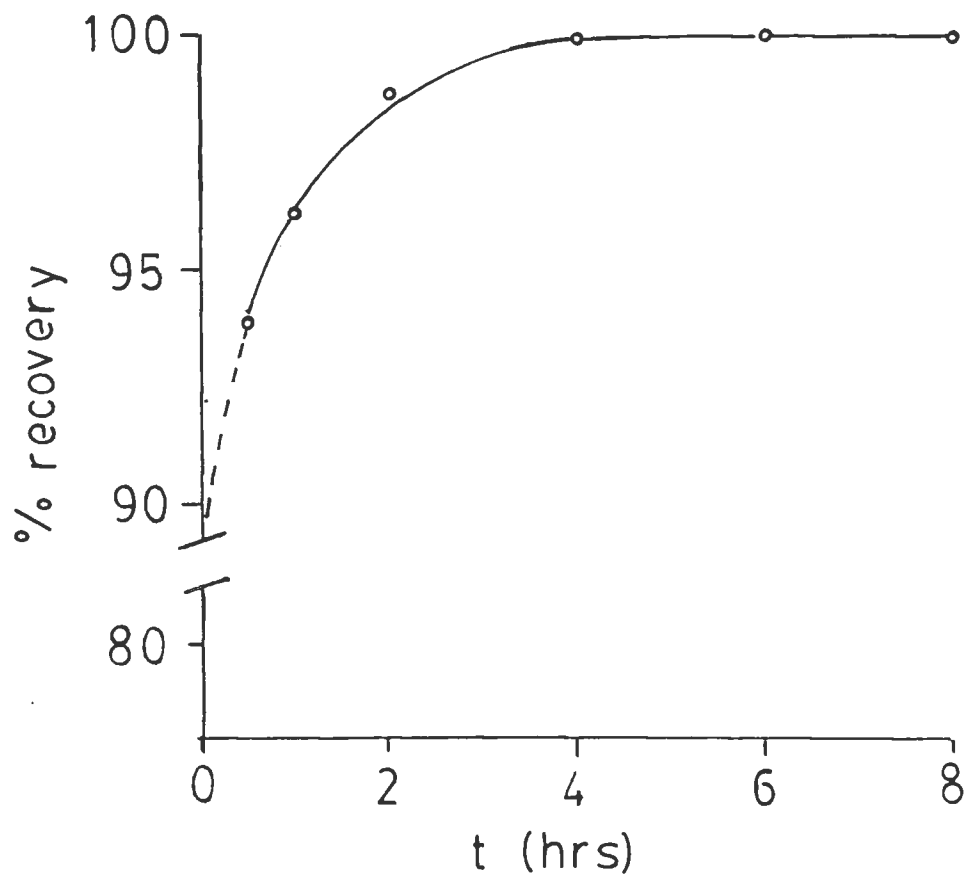


TABLE 6. Left ventricular ornithine decarboxylase (ODC) in nondiabetic, diabetic and treated SHR, WKY and SD.

Strain Group	Left Ventricular ODC Activity (pmol ^{14}C CO ₂ /hr/mg protein)					
	STUDY 1	n	STUDY 2	n	STUDY 3	n
	[Ornithine] 400 μM		200 μM		200 μM	
<u>SHR</u>						
Nondiabetic	204	3	178 \pm 42	8	112 \pm 16	4
Diabetic	90	2	89 \pm 73*	5	53 \pm 7*	3
Diabetic + T ₃			143 \pm 47**	6		
Diabetic + I			121 \pm 27*	9		
Nondiabetic + T ₃			176 \pm 35	6		
<u>WKY</u>						
Nondiabetic	187	2	168 \pm 44	8	113 \pm 7	4
Diabetic	46	4	107 \pm 33*	7	55 \pm 24*	4
Diabetic + T ₃					76 \pm 40**	7
Diabetic + I					90 \pm 45	7
Nondiabetic + T ₃					134 \pm 60	7
<u>SD</u>						
Nondiabetic					111 \pm 41	9

Values are means \pm SD obtained 8 weeks after initiation of diabetes or treatments. T₃, triiodothyronine; I, protamine zinc insulin.

* Significantly different from the nondiabetic group (within strain), p < 0.05.

** Significantly different from the nondiabetic and diabetic groups (within strain), p < 0.05.

TABLE 7. Right Ventricular ODC activity in nondiabetic, diabetic and treated SHR, WKY and SD rats.

Strain Group	n	pmol ¹⁴ RV ODC Activity CO ₂ generated/hr/mg protein
<u>SHR</u>		
Nondiabetic	9	40 ± 25
Diabetic	8	29 ± 23
Diabetic + T ₃	6	46 ± 26
Diabetic + I	8	32 ± 14
Nondiabetic + T ₃	7	30 ± 15
<u>WKY</u>		
Nondiabetic	8	35 ± 18
Diabetic	8	26 ± 15
Diabetic + T ₃	6	30 ± 12
Diabetic + I	7	43 ± 30
Nondiabetic + T ₃	7	51 ± 25
<u>SD</u>		
Nondiabetic	3	56 ± 27

Values are means ± SD obtained 8 weeks after initiation of diabetes or treatments from animals used in Studies 2 and 3. T₃, triiodothyronine; I, protamine zinc insulin.
 ODC units: pmol ¹⁴CO₂ generated/hr/mg protein, using 25 μM ornithine.

TABLE 8. Subcellular localization of ODC activity in nondiabetic and diabetic SHR and WKY.

Strain Group	n	Pellet	Supernatant	Total	<u>Pellet</u> Supernatant
<u>SHR</u>					
Nondiabetic	2	121	66	187	1.83
Diabetic	2	82	21	103	3.91
<u>WKY</u>					
Nondiabetic	2	65	57	122	1.14
Diabetic	2	84	36	120	2.33

Values are means of ODC activity from Supernatant of 5% homogenate as described previously in Methods or from reconstitution of Pellet with 25 mM HEPES. ODC units; pmol $^{14}\text{CO}_2$ generated/hr/mg protein, using 25 mM Ornithine.

TABLE 9. Arginase activity in liver and heart of adult male Sprague Dawley rats.

Tissue	n	Arginase activity
Liver		
Fresh	4	255 ± 48
Frozen	4	281 ± 31
Heart		
Fresh	5	0.25 ± 0.05
Frozen	5	0.21 ± 0.08

Values are means ± SD for Arginase activity determined by the detection of urea generation.

Arginase units: umoles of urea formed/min/g tissue weight

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