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ROLE OF VASCULAR REACTIVITY AND COLLAGEN BIOSYNTHESIS IN THE DEPRESSOR EFFECT OF STREPTOZOTOCIN IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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BY

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ABSTRACT

Diabetes decreases systolic arterial pressure in the spontaneously hypertensive rat (SHR). Two possible mechanisms, a decrease in vascular collagen biosynthesis and an altered vascular reactivity, were investigated. Collagen biosynthesis, as indicated by prolyl hydroxylase activity, was reduced in the aorta in the diabetic SHR to a greater extent than the diabetic Wistar-Kyoto rat (WKY). Prolyl hydroxylase activity was also reduced, but to a lesser extent, in the aorta of food restricted and hypothyroid SHR and WKY rats. Streptozotocin (STZ)-induced diabetes increased the sensitivity of the mesenteric artery of both strains to methoxamine only. The response of the mesenteric artery of the SHR was increased, though not significantly, to norepinephrine and methoxamine. Therefore, it appears that the depressor effect of STZ in the SHR is not associated with a reduction in vascular reactivity. The results suggest that a reduction in collagen synthesis may play a role in the depressor effect of STZ in the SHR. The reduction in collagen biosynthesis in STZ-induced diabetes may in part be due to the altered nutritional state and a reduction in thyroid hormones associated with diabetes.

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INTRODUCTION

Essential hypertension is a disease of elevated blood pressure of no known cause characterized by an elevated total peripheral resistance (TPR) (Davidman and Opsahl, 1984). Many factors, including an increase in vascular reactivity and an altered vascular structure, have been proposed to be involved in the elevated TPR (Mulvany, 1983). The spontaneously hypertensive rat (SHR) is a widely used animal model for human essential hypertension. Elevated blood pressure in the SHR is, like human essential hypertension, characterized by an elevated TPR and a normal cardiac output (Smith and Hutchins, 1979).

Hypertension is one of many cardiovascular complications associated with diabetes mellitus and occurs in from 40 to 80 percent of diabetics (Christlieb, 1973). In contrast, diabetes in the SHR has a depressor effect, with the blood pressure of the SHR approaching that of normotensive controls (Somani et al, 1979; Cavaliere et al, 1980). In normotensive rats, the effect of STZ diabetes has been inconsistent with pressor and depressor effects reported (Somani et al, 1979; Kohler et al, 1980; Jackson and Carrier, 1983). This difference in the effect on blood pressure of normotensive rats and SHR could be due in part to differences in the effects of STZ diabetes on the vasculature.

STZ diabetes could alter vascular structure by altering collagen synthesis and content. Resistance vessels in the SHR are characterized by medial hypertrophy and a reduced lumen, due to smooth muscle cell hypertrophy, hyperplasia and an increase in connective tissue components such as collagen (Mulvany et al, 1978; Warshaw et al, 1979; Olivetti et

al, 1982). Collagen synthesis and content is increased in the aorta and mesenteric artery of the SHR (Ooshima et al, 1974; Iwatsuki et al, 1977; Ehrhart and Ferrario, 1981). In an aging study, Newman and Langner (1978) found these changes to occur only after the establishment of elevated blood pressure. STZ diabetes in normotensive rats decreases collagen synthesis in the aorta (Schneir et al, 1979). The effect of STZ diabetes in the SHR on vascular collagen synthesis and content is unknown.

STZ diabetes could also alter vascular reactivity to various vasoactive agents. Vascular reactivity is defined in terms of sensitivity and contractility. Sensitivity is inversely related to the agonist concentration required to produce half-maximal response. Contractility is related to the smooth muscle force generating capacity and is the maximum response which can be developed with a given agonist. The effects of diabetes on vascular reactivity of normotensive rats is influenced by the duration of diabetes. In general, aorta from STZ-diabetic (4 to 12 week duration), normotensive rats are hyperresponsive to norepinephrine (NE) with no change in sensitivity (Owen and Carrier, 1979; Owen and Carrier, 1980; Scarborough and Carrier, 1983; Scarborough and Carrier, 1984b; MacLeod, 1985), hyposensitive and hyporesponsive to serotonin (5HT) (Owen and Carrier, 1979), and hyporesponsive to KCl (Pfaffman, 1980; Pfaffman et al, 1980; Pfaffman et al, 1982). Owen and Carrier (1979) also report a reduced but not significant decrease in response to KCL. Jackson and Carrier (1981) report no change in sensitivity or response to NE of the mesenteric artery from 4 week STZ diabetic normotensive rats. Diabetes of longer duration (≥3 months) results in an increase in sensitivity and response to NE in the mesenteric artery of the normotensive rat (Jackson and Carrier, 1981;

MacLeod, 1985).

In the nondiabetic SHR, there is a vessel-dependent response to vasoactive agents. Sensitivity of aorta from the SHR to NE and KCl is not different from that of normotensive rats (Shibata et al, 1973; Laher and Triggle, 1984). However, maximum response to NE, 5HT, and KCl is reduced in aorta from SHR (Spector, 1969; Shibata, 1973). In contrast, the mesenteric artery from the SHR, in perfusion and isolated tissue studies, is supersensitive and hyperresponsive to the above agents (Haeusler and Haefely, 1970; Haeusler and Finch, 1972; Bhattacharya et al, 1977; Mulvany et al, 1980; Asano et al, 1984).

The effect of diabetes on the vascular reactivity of the SHR is unknown. The purpose of this study is to investigate whether a change either in vascular reactivity or in prolyl hydroxylase (PH) activity, which is a marker of collagen synthesis, are possible mechanisms for the hypotensive effect of STZ diabetes in the SHR. The working hypotheses of the study are as follows: 1) Diabetes depresses the reactivity of mesenteric arteries from SHR without affecting the reactivity of mesenteric arteries from WKY and 2) Diabetes reduces collagen synthesis in the aorta from SHR as indicated by a decrease in PH activity, but, does not affect PH activity of WKY aorta. It is assumed that a change in collagen synthesis will indicate an altered collagen metabolism in general. A reduction either in vascular reactivity or in collagen biosynthesis will suggest that STZ diabetes has a depressor effect in the SHR at least in part by interfering with vascular reactivity or collagen metabolism.

METHODS

I. Animals. Male, age matched spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) were obtained from Charles River Laboratories Wilmington, Mass. The rats were housed collectively by strain and maintained on normal laboratory rat chow. The rats were fed and given water ad libitum.

II. Experimental Procedures

A. Induction of diabetes. Experimental diabetes was chemically induced by streptozotocin (STZ) (Sigma Chemical Company) in 14 to 16 week old rats. The rats received a single tail vein injection of STZ (50 mg/kg) dissolved in citrate buffer (pH 4.5). Control rats were injected with the vehicle. The rats were diabetic for 8 - 9 weeks before sacrifice.

B. Induction of Hypothyroidism. Hypothyroidism was chemically induced by methimazole (Sigma Chemical Company) in 14 week old rats. Methimazole was added to the drinking water at a concentration of 0.01% (10 mg/100 mls). The rats were maintained on methimazole for 8 - 9 weeks before sacrifice.

C. Protocol for Food Restriction. Rats, 14 to 16 weeks old, were placed on a food restricted diet. The rats were housed individually and given 1 - 2 pellets (10 grams) of rat chow per day. The rats were given water ad libitum. The rats were maintained on the food restricted diet for 8 - 9 weeks before sacrifice.

D. Blood Pressure and Metabolism Studies. Weekly blood pressure measurements and metabolism studies were determined for each rat. Blood

pressure was measured by the indirect tail cuff method. Food intake, water intake, and urine production were measured by housing the rats individually in metabolism cages for 24 hours. Blood pressure and metabolism studies were performed on control and experimental rats before and during the appropriate treatment.

E. Isolation of Blood Vessels. Eight weeks after the induction of diabetes, or after the induction of hypothyroidism or food restriction, control and experimental rats were sacrificed by cervical dislocation. The heart was removed and the pooled blood in the chest cavity was collected and stored temporarily in a refrigerator. The mesenteric artery was isolated and a 2 cm piece was removed and placed in 100 ml of oxygenated (95% O_2 - 5% CO_2) Krebs-Henseliet buffer of the following composition (mM): NaCl (113), KCL (4.7), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $MgSO_4$ (1.2), Glucose (11.5), $NaHCO_3$ (19). The aorta was also removed, cleaned and rinsed in buffer, quick frozen in liquid nitrogen and stored at -70°C until assayed for collagen.

Fat was carefully trimmed from the mesenteric artery so as to not stretch or damage the vessel. A 3 - 5 mm ring was cut and placed on two platinum hooks. Rings were used instead of helical strips because the former is relatively more intact, and more effectively measures the contraction of circular muscles which control vessel diameter (Moulds, 1983). The vessel was placed in a jacketed tissue chamber containing 100 ml oxygenated KH buffer maintained at 37°C. The bottom platinum hook was secured in the tissue chamber and the top hook was attached to a Grass FT03 force-displacement transducer. One gram of tension was placed on the vessel, and it was allowed to equilibrate for 90 minutes. The buffer was changed every thirty minutes throughout the experiment.

F. Protocol for Agonist Addition. The vasoactive agents employed were norepinephrine (NE), 5-hydroxytryptamine (5HT), and methoxamine (MOX). Cocaine (10^{-6} M) was added to the buffer to prevent reuptake of norepinephrine (Webb and Vanhoutte, 1981). Developed tension responses were obtained over a range of 10^{-9} M to 10^{-4} M for NE and over a range of 10^{-7} M to 10^{-4} M for 5HT and methoxamine. The maximum response to a single addition of 100 mM KCl was also determined, in order to confirm tissue responsiveness. At the completion of each experiment, the tissues were lightly blotted and weighed.

G. Serum Analysis. The collected blood was centrifuged at 1400 x g for thirty minutes. The serum was separated and stored at -70°C until analysis. The serum was analyzed for glucose, insulin and thyroxine (T_4). Glucose concentration was determined by Stanbio's OT-V Direct Glucose Test Kit. Insulin concentration was determined using Amersham's Radioimmunoassay (RIA) Kit. Intraassay variation was determined to be 11.3%. T_4 was measured using Amersham's T_4 RIA Kit. Intraassay variation was determined to be 9.1%.

H. Prolyl Hydroxylase Activity. The rate of collagen synthesis of the aorta was measured indirectly by prolyl hydroxylase activity. Prolyl hydroxylase activity was measured by the tritium release assay (Hutton et al, 1966). Aortas were homogenized in 1 ml of 0.05M Tris - HCl buffer (pH 7.4) containing 0.25M Sucrose, 10^{-5}M EDTA, and 10^{-5}M dithiothreitol, using a ground glass homogenizer. The homogenate was centrifuged at 15,000g for 15 minutes and the supernatant was saved for PH assay. An aliquot (200 μl) of the homogenate was incubated with substrate and cofactors for 30 minutes at 30°C. The reaction was stopped by addition of 100 ul 50% TCA. The titrated water was collected by distillation and counted.

I. Data Presentation. Vascular reactivity was measured as sensitivity and as maximum response. Sensitivity of the tissue to each agonist was determined by calculation of pD_2. The pD_2 is defined as $-\log ED_{50}$. The ED_{50} is the effective dose needed to produce 50% of the maximum response. The maximum response was defined as maximum tension developed (mg tension/mg wet tissue weight) at the maximally effective agonist concentration (Mulvany, 1983).

J. Statistical Analysis. Where multiple means were present, the results were compared by a one-way ANOVA using an Apple II statistical computer program. Results with significant F values were further analyzed for significance by a Newman-Keuls Test (Winer, 1971). Single comparisons of means were made using the unpaired Student's t test. Significance was restricted to $p<0.05$.

RESULTS

I. General Observations. Metabolism cage measurements showed that the diabetic rats of both strains exhibited polyuria and polydipsia (Table I). Only WKY diabetic rats were polyphagic. The urine of the diabetic rat (either strain) exhibited a higher glucose content (>2000 mg/dl: Ames Diastix) than did the urine of control rats (<100 mg/dl). The diabetic rats had apparently less skeletal muscle mass and fat deposits than did the controls.

II. Serum Analysis

A. Glucose concentration. Serum glucose levels were significantly elevated following induction of diabetes in the SHR and WKY (Table II). Food restriction and hypothyroidism had no effect on serum glucose levels in either strain.

B. Insulin Concentration. Diabetes significantly decreased serum insulin levels in the SHR and WKY (Table II). Food restriction and hypothyroidism significantly decreased serum insulin levels in the WKY only.

C. Serum T_4 Concentration. STZ diabetes and food restriction produced hypothyroidism in both strains as indicated by lower T_4 values (Table II). Hypothyroidism, induced by methimazole, significantly lowered T_4 concentrations in both strains.

III. Blood Pressure Measurements. The SAP of nondiabetic SHR was elevated compared to that of the nondiabetic WKY group at all time points (Table III). The blood pressure of the diabetic SHR declined steadily relative to the nondiabetic SHR group becoming significant by

the sixth week (Table III). Blood pressure of the diabetic WKY rats was not significantly different from blood pressure of the control WKY. Food restriction had no effect on blood pressure in either strain. Blood pressure of the hypothyroid rats was unobtainable by the tail cuff method.

IV. Prolyl Hydroxylase Activity. Diabetes significantly decreased prolyl hydroxylase activity in the aorta from the SHR when expressed per mg wet tissue weight and per mg protein (Table IV). PH activity per mg wet tissue weight in the aorta of the diabetic SHR decreased 87 per cent relative to the nondiabetic SHR. PH activity per mg protein in the aorta of the diabetic SHR decreased 70 percent when compared to the nondiabetic SHR. Only PH activity per mg wet tissue weight was significantly reduced in the diabetic WKY rat when compared to the nondiabetic WKY, with a decrease of 57 per cent. Food restriction and hypothyroidism had similar effects as diabetes on the PH activity in the aorta of the SHR and WKY rat (Tables IV, V). However, the decrease in PH activity due to food restriction and hypothyroidism in the SHR was only approximately 30 to 38 per cent when compared to the control SHR. The decrease in PH activity per mg wet tissue weight due to food restriction and hypothyroidism in the WKY was only approximately 26 to 38 per cent when compared to the control WKY.

V. Sensitivity of Mesenteric Artery. Diabetes and food restriction significantly increased the sensitivity of the mesenteric artery from both strains to MOX (Table VI). However, neither diabetes, food restriction nor hypothyroidism altered the sensitivity (pD_2) of the mesenteric artery from either strain to NE when compared to mesenteric arteries of the control rats of the same strain. Food restriction significantly increased the sensitivity of the mesenteric artery of both

strains to 5HT. Hypothyroidism significantly decreased the sensitivity of the mesenteric artery of both strains to 5HT.

VI. Maximum Response of the Mesenteric Artery. Diabetes, hypothyroidism and food restriction had no effect on the maximum response of the mesenteric artery from the SHR to NE, 5HT, MOX, or KCL (Table VII). The maximum response of the mesenteric artery from the hypothyroid WKY rat to 5HT and KCl was significantly decreased. Diabetes, hypothyroidism and food restriction had no effect on the maximum response of the mesenteric artery from the WKY rat to NE and MOX.

TABLE 1.--Effect of STZ-induced Diabetes (8 weeks) on Body Weight, Food Intake, Water Intake and Urine Output.

	n	Body Weight (g)	Food Intake (g/24 hr)	Water Intake (mls/24 hr)	Urine Output (mls/24 hr)
SHR					
Control	10	325 ±15	23.1 ±2.2	23.0 ±13.4	9.5 ± 4.4
Diabetic	8	194 ±41*	27.9 ±6.6	55.0 ±24.2*	40.0 ±23.3*
WKY					
Control	8	356 ±23	24.1 ±1.0	21.3 ±11.9	13.1 ± 5.9
Diabetic	7	293 ±22*	41.6 ±3.4*	143.0 ±30.9*	122.9 ±25.0*

Note: Each value represents the mean ± S.D.

* Denotes a significant difference when compared to age-matched control of the same strain ($p<0.05$).

TABLE 2.--Effect of STZ-induced Diabetes (8 weeks) and of Food Restriction and Hypothyroidism on Serum Glucose, Insulin and T_4 of SHR and WKY Rats.

		Glucose mg/dl	Insulin μU/ml	T_4 μg/dl
SHR	n			
Control	13	133 ± 19	36.8 ±17.7	7.2 ± 1.1
Diabetic	11	472 ±121*	19.5 ± 6.5*	3.6 ± 1.3*
Food Restricted	9	128 ± 16	24.0 ± 5.6	5.0 ± 1.3*
Hypothyroid	7	143 ± 25	30.2 ±13.4	2.2 ± 0.3*
WKY	n			
Control	13	158 ± 23	59.9 ±25.8	7.6 ± 1.6
Diabetic	10	517 ± 83*	26.0 ± 6.5*	4.5 ± 0.6*
Food Restricted	10	167 ± 56	24.0 ± 6.4*	5.6 ± 0.9*
Hypothyroid	7	150 ± 27	34.6 ±13.5*	2.5 ± 0.2*

Note: Each value represents the mean ± S.D.

* Denotes a significant difference when compared to age-matched control of the same strain ($p<0.05$).

TABLE 3.--Effect of STZ-induced Diabetes (8 weeks) and Food Restriction on Systolic Arterial Pressure (SAP) of SHR and WKY Rats.

	Week:[a]	0	2	4	6	8
SHR						
Control	SAP(mm Hg)	179 ±20	185 ±19	185 ±19	200 ±21	206 ±26
	n =	8	8	8	8	8
Diabetic	SAP(mm Hg)	175 ±29	184 ±37	171 ±37	165 ±24*	152 ±33*
	n =	11	11	12	10	7
Food	SAP(mm Hg)	189 ±28	183 ±18	207 ±18	204 ±20	203 ±20
Restricted	n =	10	10	9	10	8
WKY						
Control	SAP(mm Hg)	135 ± 9	142 ±14	147 ±18	145 ±13	152 ±14
	n =	7	8	8	8	8
Diabetic	SAP(mm Hg)	132 ±12	143 ±14	132 ±13	134 ±14	138 ±14
	n =	8	9	9	9	9
Food	SAP(mm Hg)	147 ±18	152 ±12	153 ±21	162 ±20	156 ±18
Restricted	n =	10	10	9	10	9

Note: Each value represents mean blood pressure ± S.D.

[a] Designates number of weeks after STZ injection, start of methimazole or start of food restriction.

* Denotes a significant difference when compared to age-matched control of the same strain ($p<0.05$).

TABLE 4.--Effect of STZ-induced Diabetes (8 weeks) and Food Restriction on Prolyl Hydroxylase Activity in the Aorta of the SHR and WKY Rat.

	n	CPM/mg wet weight	CPM/mg protein
SHR			
Control	5	149 ± 11	4437 ± 699
Diabetic	5	19 ± 11*	1340 ± 583*
Food Restricted	5	93 ± 21*	3126 ± 1220*
WKY			
Control	4	90 ± 12	3581 ± 1128
Diabetic	4	39 ± 6*	2319 ± 404
Food Restricted	5	67 ± 11*	2965 ± 1079

Note: Each value represents the mean ± S.D.

* Denotes a significant difference when compared to the age-matched control of the same strain ($p<0.05$).

TABLE 5.--Effect of Hypothyroidism on Prolyl Hydroxylase Activity in the Aorta of the SHR and WKY Rat.

		CPM/mg wet weight	CPM/mg protein
SHR			
Control	4	102 ± 20	2914 ± 167
Hypothyroid	7	64 ± 8*	2028 ± 298*
WKY			
Control	4	47 ± 14	1966 ± 703
Hypothyroid	7	29 ± 11*	1239 ± 482

Note: Each value represents the mean ± S.D.

* Denotes a significant difference when compared to the age-matched control of the same strain ($p<0.05$).

TABLE 6.--Effect of STZ-induced Diabetes (8 weeks) and of Food Restriction and Hypothyroidism on Sensitivity (pD_2) of the Mesenteric Artery of the SHR and WKY Rat.

		pD_2		
		NE	5HT	MOX
SHR	n			
Control	10	6.62 ±0.26	6.25 ±0.10**	5.21 ±0.17**
Diabetic	8	6.57 ±0.29	6.24 ±0.14	5.53 ±0.24*
Food Restricted	8	6.61 ±0.40	6.54 ±0.17*	5.48 ±0.21*
Hypothyroid	7	6.76 ±0.34	6.07 ±0.10*	5.08 ±0.11
WKY	n			
Control	8	6.66 ±0.36	5.97 ±0.09	4.98 ±0.19
Diabetic	7	6.53 ±0.26	6.07 ±0.05	5.39 ±0.12*
Food Restricted	7	6.68 ±0.35	6.20 ±0.10*	5.56 ±0.12*
Hypothyroid	7	6.42 ±0.09	5.77 ±0.12*	4.87 ±0.16

Note: Each value represents the mean ± S.D.

* Denotes a significant difference when compared with age-matched control rats of the same strain ($p<0.05$).

** Denotes a significant difference when compared with age-matched WKY control rats ($p<0.05$).

TABLE 7.--Effect of STZ-induced Diabetes (8 weeks) and of Food Restriction and Hypothyroidism on Responsiveness of the Mesenteric Artery of the SHR and WKY Rat.

		Maximum change in tension (mg/mgwet wt)			
		NE (10^{-4} M)	5HT (10^{-4} M)	MOX (10^{-4} M)	KCl (100mM)
SHR	n				
Control	10	868 ±372**	1057 ±384	926 ±402	566 ±258
Diabetic	8	1267 ±395	1309 ±587	1267 ±527	681 ±246
Food Restricted	8	1027 ±144	1308 ±262	1237 ±402	711 ±282
Hypothyroid	7	930 ±267	1022 ±305	930 ±320	589 ±129
WKY	n				
Control	8	1405 ±499	1389 ±592	1392 ±697	740 ±439
Diabetic	7	1512 ±601	1566 ±678	1698 ±748	876 ±434
Food Restricted	7	1331 ±416	1503 ±537	1598 ±471	791 ±222
Hypothyroid	7	1201 ±512	770 ±322*	783 ±225	324 ±118*

Note: Each value represents the mean ± S.D.

* Denotes a significant difference when compared with age-matched control rats of the same strain ($p<0.05$).

** Denotes a significant difference when compared with age-matched WKY control rats ($p<0.05$).

DISCUSSION

The spontaneously hypertensive rat (SHR), an animal model for human essential hypertension, exhibits an elevated blood pressure characterized by an increased total peripheral resistance (TPR) (Smith and Hutchins, 1979). This increased TPR implies differences in the vasculature of the SHR compared to normotensive rats. Changes could include structural, functional and membrane differences.

Essential hypertension is a complication of diabetes mellitus (Christlieb, 1973). However, experimental diabetes, induced by either STZ or alloxan, in the SHR has a depressor effect (Table III; Somani et al, 1979; Cavaliere et al, 1980). In contrast, STZ diabetes in the WKY strain had no effect on SAP (Table III). In the present study STZ diabetic SHR were hypotensive, relative to the nondiabetic SHR group by the sixth week after the injection of STZ. The depressor effect might be associated with diabetes-induced alterations in vascular reactivity or collagen biosynthesis.

Structurally, resistance vessels in the SHR are characterized by medial hypertrophy and a reduced lumen, due to smooth muscle cell hypertrophy, hyperplasia, and an increase in connective tissue components such as collagen (Mulvany et al, 1978; Warshaw et al, 1979; Olivetti et al, 1982). It appears that structural changes are a consequence and not a cause of the elevated pressure. Mulvany et al (1980) found no medial hypertrophy in prehypertensive SHR (6 weeks) but did observe medial hypertrophy in hypertensive SHR (12 and 24 weeks). Prehypertensive SHR, treated with captopril, remained normotensive and did not exhibit medial hypertrophy when compared to WKY (Henrich et al, 1980). Little is known

about the effect of STZ diabetes on the smooth muscle cell. In the aorta, no histological differences or difference in wall thickness were observed for control, STZ diabetic and insulin-treated diabetic normotensive rats (Pfaffman et al, 1982).

Other structural components such as connective tissue (collagen) are increased in the aorta and mesenteric artery of the SHR (Ooshima et al, 1974; Iwatsuki et al, 1977). Connective tissue is essential to the strength, structure, and integrity of tissues. Procollagen is the precursor of collagen consisting of three polypeptide chains in a triple helix. The chains have a high content of glycine, proline, hydroxyproline and lysine (Gilligan and Spector, 1984). The triple helix is stabilized by the hydroxylation of specific prolines by the action of prolyl hydroxylase (Hutton et al, 1966). The reaction involves a direct displacement of the hydrogen atom on carbon four with the released hydrogen equilibrating with water. Carboxyprotease and lysyl oxidase are then involved in the conversion of procollagen into collagen and collagen into fibrils by crosslinking through lysyl or hydroxylysyl amino acids, respectively.

STZ diabetes decreases collagen biosynthesis, as indicated by a decrease in PH activity (Table IV) in the SHR and WKY. This agrees with a previous study in the normotensive rat (Schneir et al, 1979). The results suggests that alteration of collagen synthesis by STZ diabetes could be responsible for the depressor effect in the SHR. However, during the genesis of hypertension in the SHR, increases in collagen synthesis and content occurs only after the establishment of elevated blood pressure (Newman and Langner, 1978). Whether the observed decrease in collagen synthesis in the STZ diabetic SHR occurs before or after the drop in blood pressure is open to speculation. Caloric

deprivation and hypothyroidism also decrease PH activity in the SHR and WKY, but, neither can fully explain the effect of STZ diabetes (Table IV, V).

The above structural changes in the nondiabetic SHR appear to be adaptive changes to the elevated blood pressure. Therefore, some other mechanisms must be involved in the elevation of blood pressure in the SHR. Vascular smooth muscle function has been extensively investigated as a possible mechanism and is altered in the SHR when compared to normotensive rats. One such altered function is the vascular reactivity to various vasoactive agents. The mesenteric artery from the SHR, in perfusion and isolated tissue studies, is supersensitive and hyperresponsive to NE, 5HT, and KCl (Haeusler and Haefely, 1970; Haeusler and Finch, 1972; Bhattacharya et al, 1977; Mulvany et al, 1980; Asano et al, 1984). Results showing increased sensitivity to NE in prehypertensive SHR suggest that the altered vascular reactivity occurs prior to the development of elevated blood pressure (Lais and Brody, 1978; Mulvany et al, 1980).

Evidence indicates that the altered vascular reactivity in the SHR may be related to a vascular membrane abnormality. Calcium sensitivity of vessels from SHR is increased (Mulvany and Nyborg, 1980; Nilsson and Mulvany, 1981). In isolated tissue studies, as calcium concentration is increased, the maximum response of mesenteric vessels from prehypertensive and hypertensive SHR to a fixed concentration of NE is increased. Mulvany and Nyborg (1980) observed no difference in calcium sensitivity to a KCl response. NE and KCL interact with different calcium channels and the results indicate that the NE activated calcium channels in the SHR are different from those of the WKY (Nilsson and Mulvany, 1981). The observed calcium sensitivity is also found in the visceral smooth

muscle of the SHR (Altman et al, 1977) and in the mesenteric artery of denervated SHR (Mulvany et al, 1981). Therefore, the increase in calcium sensitivity of SHR smooth muscle is not restricted to the vasculature and thus is probably not a consequence of increased blood pressure or neurogenically mediated.

Other calcium properties of vascular smooth muscle appear to be altered in the SHR. Calcium binding has a stabilizing effect on the membrane and inhibits contraction. Calcium binding of the mesenteric artery of the SHR has been reported to be decreased (Webb and Bohr, 1981) or the same as normotensive rats (Kwan and Daniel, 1981). If vessels of the SHR do have a decrease in calcium binding, then the membranes may be less stable making them more susceptible to contraction. An ATP-dependent calcium transport has been identified in rat mesenteric arteries (Daniel et al, 1982) and is altered in the SHR. ATP-dependent calcium accumulation is decreased in mesenteric arteries of young and adult SHR (Kwan et al, 1979; Kwan, 1985).

Permeability of other ions such as Na^+ and K^+ of vascular smooth muscle of SHR is also increased (Jones, 1973). Increased passive movement of Na^+ and K^+ should lead to a less negative membrane potential, but membrane potential in the SHR is similar to normotensive rats. Hermsmeyer et al (1976) observed that the electrogenic Na^+ - K^+ pump activity is increased in the SHR to offset the increased passive permeability. They also proposed that the greater response to NE in the SHR is the result of this increased Na^+ - K^+ pump activity.

In the present study, alpha-adrenoreceptor reactivity of the nondiabetic SHR is altered compared to the nondiabetic WKY. The sensitivity of the mesenteric artery to NE is not altered in the nondiabetic SHR, while the responsiveness is significantly reduced when

compared to the nondiabetic WKY (Table VI, VII). Alpha-adrenoreceptors, pharmacologically classified as α_1 and α_2 (Bethelsen and Pettinger, 1977; Godfraind et al, 1982), have been located in the rat mesenteric artery (Agrawal et al, 1984). Radioligand binding studies indicate that the α_1 subtype is predominant in this tissue (Colucci et al, 1980). The increase in sensitivity to MOX, an α_1 agonist, and the absence of any change in sensitivity to NE, a nonselective α_1 and α_2 agonist suggests a change in the affinity of the α_1-receptors for MOX, even though direct evidence is not available (Table VI). The results of the reactivity to NE disagree with the previous observations (Bhattacharya et al, 1977; Mulvany et al, 1980; Asano et al, 1984). Since there is an increase in the sympathetic nerve activity in the SHR (Judy et al, 1979), the decrease in postjunctional reactivity to NE could be the result of a compensatory mechanism. An increase in sympathetic nervous system tone might be expected to result in down regulation of alpha-adrenoreceptors. This seems unlikely since the above studies did not see this. The difference between this study and the above studies may simply be due to the method of tissue preparation. The above studies used either a mesenteric bed preparation or a helical strip of the mesenteric artery. A mesenteric bed preparation involves a mixture of vessel types. If a difference in responsiveness is observed, its impossible to tell which vessel type is contributing to the observed difference in responsiveness. Helical strips destroy the integrity of the vessel and are not as physiologically representative as rings (Moulds, 1983). In this study, using an isolated artery ring, the mesenteric artery of the nondiabetic SHR has a similar reactivity to NE as previously reported for the aorta (Spector, 1969; Shibata et al, 1973; Laher and Triggle, 1984).

It appears that diabetes may alter α-adrenoreceptor response in

the SHR. Maximum response to NE and MOX is increased, although not significantly, in the diabetic SHR as compared to the nondiabetic SHR (Table VII). This increase not being significant may be due to the high variability observed. The increased response to NE and MOX suggests an altered α_1-activity in the mesenteric artery of the diabetic SHR. Activity may be altered by a change in the binding properties or number of α_1-adrenoreceptors or in the sequence of events leading to contraction after receptor activation. A lack of change in NE sensitivity (pD_2, Table VI) in the diabetic SHR (vs nondiabetic SHR) suggests that α_1-adrenoreceptor number is not altered. Therefore, the altered NE response is the result of a change in the events after receptor activation. Recent observations indicate that a membrane abnormality may be involved. Both α_1 and α_2 induced contractions are biphasic and involve intracellular and extracellular calcium (Scarborough and Carrier, 1984a). Diabetes increases the sensitivity of the aorta to calcium and increases the influx of extracellular calcium due to α_2 activation in the aorta of normotensive rats (Owen and Carrier, 1980; Scarborough and Carrier, 1984b). Even though no direct evidence is available, Jackson and Carrier (1981) proposed that the increase in responsiveness in the mesenteric artery of STZ diabetic normotensive rat may be due to an increase in calcium utilization by the smooth muscle cell of the mesenteric artery. If calcium influx due to α_1 activation and calcium utilization is increased in the diabetic SHR, one would expect an increase in the maximum response to NE and MOX in the diabetic SHR. The increase in response to NE and MOX, even though not significant, in the mesenteric artery of the diabetic SHR indicates that an increase in calcium influx and calcium utilization may be occurring.

Diabetes increases the sensitivity of the mesenteric artery of the

SHR to MOX (Table VI) suggesting a change in affinity of the α_1 receptors for MOX even though no direct evidence is presented. Therefore, in regards to MOX, the increase in maximum response to MOX in the diabetic SHR may be the result of an increase in affinity and a change in the events after α_1 receptor activation, such that the response to MOX is similar to the response to NE.

Diabetes does not alter NE sensitivity or response of the mesenteric artery of the WKY (Table VI, VII), supporting recent observations in short term diabetes (Jackson and Carrier, 1981). In the same study, Jackson and Carrier observed mesenteric arteries from long term diabetic rats to be supersensitive and hyperresponsive to NE. Therefore, it appears that the sensitivity and response of the mesenteric artery from diabetic normotensive rats to NE is dependent upon the duration of diabetes. The sensitivity of the mesenteric artery of the diabetic WKY to MOX is similar to that of the diabetic SHR (Table VI), suggesting a change in affinity of α_1 receptors for MOX.

The mesenteric artery of the nondiabetic SHR is supersensitive to 5HT supporting previous results (Table VI; Haeusler and Finch, 1972). No change in response to KCl was present, in contrast to earlier studies (Table VII; Haeusler and Finch, 1972; Asano et al, 1984). Diabetes has no effect on the sensitivity or response of the mesenteric artery of either strain to 5HT and KCl (Table VI, VII).

The contributory effect of a reduction in thyroid hormones on the increase in response of the mesenteric artery of the diabetic SHR to NE was investigated. The results indicate that hypothyroidism did not alter the sensitivity or responsiveness of the mesenteric artery of either strain to NE, supporting a recent study (Table VI, VII; Ishac and Pennefather, 1983). Therefore, it appears that a reduction in thyroid hor-

mones is not a factor in the observed increase in response in the diabetic SHR.

Hypothyroidism alters the sensitivity of the mesenteric artery of both strains to 5HT (Table VI). In the WKY, the decrease in sensitivity was accompanied by a decrease in response (Table VII). In the WKY, a decrease in response to KCl was also observed. KCl contractions involve the influx of calcium through voltage dependent channels (Mulvany and Nyborg, 1980). Whether the observed decrease is the result of hypothyroidism affecting the influx of calcium through these channels is unknown.

Since STZ-induced diabetes is accompanied by an altered nutritional state (Madia et al, 1979) and caloric restriction reduced blood pressure in the SHR (Young et al, 1978), a food restricted control was included. Food restriction increased the sensitivity of the mesenteric artery of both strains to 5HT and MOX, but had no effect on responsiveness (Table VI, VII). Since the sensitivity to MOX was increased in both the diabetic and food restricted rats, the effect of diabetes on sensitivity to MOX may be related in part to the altered nutritional state rather than to the lack of insulin or the hypoglycemia. Food restriction did not affect the sensitivity or responsiveness of the mesenteric artery of either strain to NE or KCl, which agrees with a recent study using normotensive rats (Longhurst and Head, 1985).

Other systems which regulate blood pressure include the sympathetic nervous system and the renal system. The role of each in hypertension in unknown. Judy et al (1979) observed an increase in sympathetic nerve activity (SNA) in young and adult SHR. By crossbreeding SHR and WKY to produce a range of blood pressures, SNA decreased in direct proportion to the decrease in blood pressure. This suggests that

the sympathetic nervous system plays a major role in the development and management of hypertension in the SHR. Sympathetic denervation by pithing reduced blood pressure in the SHR to normotensive levels (Albrecht et al, 1975). Arterial pressure is a combination of peripheral resistance and cardiac output. When Albrecht et al measured peripheral resistance they found it to still be increased in the pithed SHR. Equal blood pressure in the pithed SHR was the result of an equal decrease in cardiac output. Denervation by immunosympathectomy in newborn SHR produced a reduced (vs SHR controls) but still higher (vs denervated normotensives) blood pressure (Folkow et al, 1972). These results suggest that an increased SNA may be partially involved in the initiation and maintenance of the increased blood pressure in the SHR. The role of the sympathetic nervous system in diabetes is unknown. Abnormalities of the peripheral nerves, characterized by a decrease in conduction velocity, has been demonstrated in the STZ diabetic normotensive rat (Thomas et al, 1981). Whether this diabetic neuropathy occurs in the STZ diabetic SHR is unknown. If it does occur, it may produce an effect similar to that of sympathetic denervation and may be a contributory factor in the depressor effect of STZ in the SHR.

Altered renal function may also be involved in the initiation and maintenance of elevated blood pressure in the SHR. Renal blood flow and glomerular filtration appear to be normal indicating an elevated renal vascular resistance (Arendshorst and Beierwaltes, 1979). The elevated renal vascular resistance is the result of both an increase in sympathetic nerve activity and a structural abnormality (Arendshorst and Beierwaltes, 1979; Collis and Vanhoutte, 1977). The kidney also produces pressor substances which circulate in the blood. Inconsistent results on plasma renin activity in the SHR have been reported (Sen et

al, 1972; Forman and Mulrow, 1974; Bagby et al, 1979). Renin is involved in the formation of angiotensin II and captopril, which inhibits this formation of angiotensin II, lowers blood pressure in the SHR (Koike et al, 1980). These results indicate that circulating pressor substances may play a role in the elevated blood pressure in the SHR.

STZ diabetes produces early renal changes characterized by an increase in glomerular basement membrane, glomerular filtration rate and plasma renin activity (Carney et al, 1979; Kohler et al, 1980). These changes indicate that diabetes may increase blood pressure. Therefore, it seems unlikely that renal changes are playing a role in the depressor effect of STZ in the SHR.

SUMMARY

1. STZ-induced diabetes has a depressor effect in the SHR. Blood pressure in the WKY is not affected by STZ-induced diabetes. These results suggest differences in the vasculature of the two strains.

2. STZ-induced diabetes alters collagen biosynthesis, as indicated by a decrease in PH activity, of both strains. The effect is greater in the SHR suggesting that the depressor effect of STZ-induced diabetes may be related to a reduction in collagen synthesis. Food restriction and hypothyroidism also reduced PH activity, but to a lesser extent than did STZ-induced diabetes suggesting that the decrease in collagen biosynthesis in diabetes may be explained in part by the altered nutritional status and the reduction in thyroid hormones associated with diabetes.

3. STZ-induced diabetes increases, though not significantly, the response of the mesenteric artery of the SHR to NE and MOX suggesting an altered α_1-adrenoreceptor response. Since no change in sensitivity to NE was observed, the results suggests that STZ-induced diabetes alters the series of events after receptor activation that lead to contraction. STZ-induced diabetes alters calcium influx due to α_2 activation in the aorta of normotensive rats. Whether the same effect is occurring in the SHR, with respect to α_1 activation is unknown.

4. The above effects of diabetes on arterial reactivity are not well correlated with the depressor effect of STZ in the SHR. The effects of STZ-induced diabetes on arterial response to adrenoreceptor agonists in the SHR might be expected to produce a pressor response, not the observed depressor response. Therefore, it appears that the depressor effect of STZ in the SHR is not related to the altered vascular reactivity.

BIOLOGICAL RELEVANCE

1. The initial intent of this research project was to develop a diabetic, hypertensive animal model. Since STZ-induced diabetes reduced blood pressure in the SHR, the original intent was not accomplished. Other diabetic animals and other hypertensive animal models and combinations thereof need to be investigated.

2. A decrease in collagen biosynthesis, as indicated by a decrease in PH activity, was well correlated with the decrease in blood pressure in the diabetic SHR. The results indicate that reducing collagen biosynthesis and content may be an effective treatment of hypertension. It may be possible to treat hypertension clinically with drugs that reduce collagen biosynthesis and content. At present, some antihypertensives have been shown to reduce blood pressure and collagen biosynthesis and content in rats (Gilligan and Spector, 1984).

APPENDIX

TABLE 8.--Prolyl Hydroxylase Distillation Recovery.[a]

Sample	CPM	% Recovery
Blank	20.3	-
Undistilled	223.1	-
First Replicate	215.6	96.6[b]
Second Replicate	208.5	93.5[b]
Third Replicate	221.6	99.3[b]
		X = 96.5 ±2.9

[a] Prolyl hydroxylase distillation step was carried out using a reaction mix spiked with tritiated water. Results indicate good recovery and excellent repeatability. Recovery values validate that the assay is working in my hands.

[b] [CPM(replicate) / CPM(undistilled sample)] x 100

TABLE 9.--Lowry Protein Assay. Test for Interference of Tissue Homogenate.

Standard μg BSA/100 μl	Abs(750nm)[a]	Expected Abs (750nm)[b]	Actual Abs (750nm)[c]	% Difference[d]
5	0.065	0.407	0.349	-14
10	0.111	0.453	0.435	- 4
20	0.201	0.543	0.516	- 5
30	0.313	0.655	0.629	- 4
40	0.448	0.790	0.750	- 5
50	0.549	0.891	0.880	- 1
Tissue Homogenate (20μl aliquot)	0.342	-	-	-

[a] Observed absorbance of standards and tissue homogenate sample.

[b] Expected absorbance value = observed absorbance + 0.342 i.e. for the 5μl standard expected Abs of 0.407 = 0.065 + 0.342.

[c] Actual absorbance observed for standards spiked with 20μl of tissue homogenate.

[d] % difference between actual absorbance and expected absorbance.These results indicate that the reaction mixture has negligible effect on the assay, therefore, the Lowry assay is valid under my sample conditions.

TABLE 10.-Effect of STZ-induced Diabetes on Body Weight of the SHR and WKY Rat. Raw Data.

SHR	Body Weight[a] (g)	SHR	Body Weight[a] (g)
Control		Diabetic	
1	303	1	211
2	325	2	261
3	346	3	169
4	311	4	174
5	334	5	164
6	335	6	247
7	315	7	158
8	344	8	167
9	324		
10	313		
WKY	Body Weight[a] (g)	WKY	Body Weight[a] (g)
Control		Diabetic	
1	368	1	316
2	333	2	262
3	362	3	266
4	330	4	319
5	326	5	293
6	361	6	304
7	389	7	291
8	375		

[a]Body weight as recorded using an animal balance. Weekly body weights were recorded. Reported values are body weights at time of sacrifice.

TABLE 11.-Effect of STZ-induced Diabetes on the Food Consumption of the SHR and WKY Rat. Raw Data.

SHR Control	Food Intake[a] (g/24hr)	SHR Diabetic	Food Intake[a] (g/24hr)
1	23	1	35
2	24	2	30
3	22	3	23
4	20	4	22
5	26	5	26
6	27	6	40
7	22	7	24
8	21	8	23
9	22		
10	24		
WKY Control	**Food Intake[a] (g/24hr)**	**WKY Diabetic**	**Food Intake[a] (g/24hr)**
1	25	1	39
2	24	2	40
3	24	3	39
4	22	4	42
5	25	5	44
6	24	6	48
7	25	7	39
8	24		

[a]Rats were housed individually in metabolism cages and given 100 grams of food. Twenty-four hours later rats were removed from metabolism cages and the amount of food consumed was recorded. Values reported are the last measurement before sacrifice.

TABLE 12.--Effect of STZ-induced Diabetes on the Water Consumption of the SHR and WKY Rat. Raw Data.

SHR Control	Water Intake[a](mls/24 hr)	SHR Diabetic	Water Intake[a](mls/24 hr)
1	35	1	70
2	15	2	60
3	35	3	45
4	40	4	30
5	40	5	40
6	20	6	105
7	20	7	35
8	10	8	55
9	5		
10	10		

WKY Control	Water Intake[a](mls/24 hr)	WKY Diabetic	Water Intake[a](mls/24 hr)
1	15	1	140
2	30	2	110
3	25	3	125
4	20	4	155
5	45	5	200
6	10	6	155
7	10	7	115
8	75		

[a]Rats were housed individually in metabolism cages and given 600 mls of water. After 24 hrs the rats were removed from the cages and the water consumption was recorded. Values reported are the last measurements before sacrifice.

TABLE 13.--Effect of STZ-induced Diabetes on the Urine Production of the SHR and WKY Rat. Raw Data.

SHR	Urine Output[a] (mls/24hr)	SHR	Urine Output[a] (mls/24hr)
Control		Diabetic	
1	10	1	40
2	10	2	40
3	10	3	20
4	10	4	20
5	5	5	20
6	5	6	90
7	5	7	40
8	10	8	50
9	10		
10	20		
WKY	Urine Output[a] (mls/24hr)	WKY	Urine Output[a] (mls/24hr)
Control		Diabetic	
1	5	1	120
2	10	2	90
3	10	3	110
4	10	4	140
5	20	5	160
6	10	6	140
7	20	7	100
8	20		

[a]Rats were housed individually in metabolism cages. Urine was collected for 24 hrs and the amount recorded. Values reported are the last measurements before sacrifice.

TABLE 14.--Effect of STZ-induced Diabetes and of Food Restriction and Hypothyroidism on Serum Glucose of the SHR and WKY Rat. Raw Data.

Standards (mg/dl)	Absorbance @ 500nm
50	0.041
100	0.078
200	0.172
300	0.237
400	0.327
500	0.421

	Absorbance @ 500nm	Glucose[a] mg/dl		Absorbance @ 500nm	Glucose[a] mg/dl
SHR			SHR		
Control			Diabetic[b]		
1	0.121	145	1	0.073	438
2	0.075	90	2	0.045	270
3	0.110	132	3	0.059	354
4	0.128	154	4	0.068	408
5	0.144	173	5	0.078	468
6	0.111	133	6	0.078	468
7	0.108	130	7	0.079	474
8	0.111	133	8	0.121	726
9	0.098	118	9	0.102	612
10	0.108	130	10	0.087	522
11	0.104	125	11	0.075	450
12	0.117	140			
13	0.103	124			

TABLE 14 Continued

Food Restricted			Hypothyroid		
1	0.119	143	1	0.123	148
2	0.105	126	2	0.107	128
3	0.120	144	3	0.093	112
4	0.109	131	4	0.106	127
5	0.085	102	5	0.110	132
6	0.083	100	6	0.138	166
7	0.109	131	7	0.154	185
8	0.116	139			
9	0.111	133			
WKY			WKY		
Control			Diabetic[b]		
1	0.143	172	1	0.078	468
2	0.145	174	2	0.083	498
3	0.115	138	3	0.068	408
4	0.122	146	4	0.086	516
5	0.142	170	5	0.094	564
6	0.123	148	6	0.096	576
7	0.138	166	7	0.115	690
8	0.121	145	8	0.075	450
9	0.105	126	9	0.074	444
10	0.118	142	10	0.092	552
11	0.172	206			
12	0.156	187			
13	0.117	140			

TABLE 14 Continued

Food Restricted			Hypothyroid		
1	0.094	113	1	0.099	119
2	0.083	100	2	0.120	144
3	0.141	169	3	0.115	138
4	0.133	160	4	0.148	178
5	0.182	218	5	0.158	190
6	0.177	212	6	0.102	122
7	0.185	222	7	0.131	157
8	0.124	149			
9	0.070	84			
10	0.201	241			

[a] Glucose of rat serum determined by using Stanbio's OT-V Direct Glucose Test Kit. Standard absorbance values were plotted versus glucose concentration and best line was drawn. Sample glucose values were determined from the sample absorbance values and the above standard curve.

[b] Serum from diabetic rats diluted 1:5, therefore glucose concentration from standard curve multiplied by 5.

TABLE 15.--Effect of STZ-induced Diabetes and of Food Restriction and Hypothyroidism on Serum Insulin of the SHR and WKY Rat. Raw Data.

Assay 1.	Insulin Standards[a] (μU/ml)	CPM
	0	7882
	10	7078
	20	7239
	40	6031
	80	4549
	160	3504

SHR	CPM	Insulin[a] (μU/ml)
Control		
1	5085	67.1
2	6483	31.9
3	7118	22.8
4	7345	20.2
5	7222	21.6
6	6599	30.0
Diabetic		
1	7161	22.3
2	6323	34.8
3	7391	19.7
4	7151	22.4
5	7237	21.4
6	7434	19.3
7	7321	20.4
Food Restricted		
1	6511	31.4
2	6817	26.7

TABLE 15 Continued

3	6529	31.1
4	7677	16.9
5	6945	25.0
WKY	CPM	Insulin[a] (μU/ml)
Control		
1	4486	92.9
2	5307	59.6
3	6625	29.6
4	5207	62.9
5	5129	65.5
6	5739	47.4
Diabetic		
1	6730	28.0
2	7038	23.8
3	6894	25.7
4	6231	36.5
5	6322	34.8
6	6896	25.6
Food Restricted		
1	7002	24.2
2	6059	40.0
3	7044	23.4
4	6630	29.5
5	7033	23.8

TABLE 15 Continued

Assay 2.	Insulin Standards[a] (μU/ml)	CPM
	0	19733
	10	18636
	20	17232
	40	14374
	80	10795
	160	8018

SHR	CPM	Insulin[a] (μU/ml)
Control		
7	14608	32.2
8	13595	41.8
9	11030	80.8
10	14592	32.3
11	14444	33.6
12	14694	31.5
13	14587	32.3
Diabetic		
8	18554	11.7
9	16953	17.6
10	18572	11.6
11	18164	12.9
Food Restricted		
6	16047	22.2
7	16975	17.5
8	14806	30.6
9	16318	20.7

TABLE 15 Continued

Hypothyroid		
1	14534	32.8
2	16129	21.8
3	16747	18.6
4	12578	54.2
5	16339	20.6
6	13627	41.4
7	16069	22.1
WKY	CPM	Insulin[a] (μU/ml)
Control		
7	13986	37.8
8	10445	93.9
9	14938	29.6
10	9936	107.0
11	14792	30.7
12	13228	45.9
13	12385	57.0
Diabetic		
7	17369	15.8
8	15329	26.7
9	15418	26.1
10	17040	17.2
Food Restricted		
6	17289	16.1
7	16526	19.6
8	16515	19.7

TABLE 15 Continued

9	16230	21.2
10	16313	20.7
Hypothyroid		
1	17971	22.7
2	12331	57.8
3	14640	31.9
4	13234	45.8
5	14753	31.0

[a] Serum insulin determined using Amersham's Insulin RIA Kit (Arlington Heights, Il.) Standard CPM values plotted versus log insulin concentration and best line drawn. Sample insulin values determined from sample CPM values and the above standard curve.

TABLE 16.--Effect of STZ-induced Diabetes and of Food Restriction and Hypothyroidism on Serum Thyroxine (T_4) of SHR and WKY Rat. Raw Data.

Assay 1.	T_4 Standards[a] (μg/dl)	CPM
	0	12897
	2.3	9889
	5.8	6547
	11.8	5255
	24.6	3155

SHR	CPM	T_4[a] (μg/dl)
Control		
1	6852	6.8
2	6285	8.2
3	6314	8.1
4	6860	6.8
5	5962	9.1
6	6622	7.3
Diabetic		
1	8534	3.9
2	10258	2.2
3	8180	4.4
4	9006	3.3
5	9879	2.5
6	9693	2.6
Food Restricted		
1	6510	7.6
2	7342	5.8
3	7672	5.2
4	9204	3.1

TABLE 16 Continued

	5	8266	4.2
WKY		CPM	T_4[a] (μg/dl)
Control			
	1	5649	10.1
	2	6505	7.6
	3	5970	9.1
	4	6225	8.4
	5	6723	7.1
Diabetic			
	1	7970	4.7
	2	8351	4.1
	3	7790	5.0
	4	8543	3.1
	5	7819	4.1
Food Restricted			
	1	7740	5.0
	2	7120	6.2
	3	8742	3.6
	4	7563	5.4
	5	7775	5.2

Assay 2.	T_4 Standards[a] (μg/dl)	CPM
	0	13856
	2.3	10348
	5.8	7423
	11.8	5230
	24.6	3527

TABLE 16 Continued

SHR	CPM	T_4[a] (μg/dl)
Control		
7	8290	4.8
8	7001	7.1
9	7466	6.2
10	6984	7.2
Diabetic		
7	7053	7.0
Hypothyroid		
1	10447	2.4
2	10175	2.6
3	10891	2.1
4	11514	1.7
5	10632	2.3
6	10935	2.1
7	10752	2.2
WKY	CPM	T_4[a] (μg/dl)
Control		
6	8072	5.1
7	7300	6.5
8	7349	6.4
9	7820	5.5
10	7218	6.7
Diabetic		
6	8000	5.2

TABLE 16 Continued

Hypothyroid		
1	10313	2.5
2	10340	2.5
3	10318	2.5
4	10497	2.4
5	10464	2.4
6	7820	5.5
7	10823	2.1
Assay 3.	T_4 Standards[a] (μg/dl)	CPM
	0	5804
	2.3	4527
	5.8	3140
	11.8	2189
	24.6	1324
SHR	CPM	T_4[a] (μg/dl)
Control		
11	3031	6.7
12	2988	6.9
13	2720	8.4
Diabetic		
8	3737	4.1
9	4077	3.2
10	4052	3.2
Food Restricted		
6	3751	4.0
7	3469	4.9

TABLE 16 Continued

8	3550	4.6
9	3028	6.7
WKY	CPM	T_4[a] (μg/dl)
Control		
11	2751	8.2
12	2551	9.5
13	2663	8.7
Diabetic		
7	3554	4.6
8	3601	4.5
9	3501	4.8
10	3572	4.6
Food Restricted		
6	2908	7.3
7	3205	5.9
8	3196	6.0
9	3206	5.9
10	3157	6.1

[a] Serum thyroxine determine using Amersham's T_4 RIA Kit (Arlington Heights, Il.). Standard CPM values plotted versus log T_4 concentration and best line drawn. Sample T_4 values determined using sample CPM value and the above standard curve.

TABLE 17.--Effect of STZ-induced Diabetes and Food Restriction on Systolic Arterial Pressure (SAP) of the SHR and WKY Rat. Raw Data.

Week:[a]	0	2	4	6	8
SHR			SAP[b] (mm Hg)		
Control					
1	150	156	180	161	190
2	202	193	203	199	215
3	185	182	190	210	234
4	-	213	147	222	-
5	170	-	176	196	171
6	215	206	218	233	248
7	170	194	189	188	216
8	180	170	168	-	194
9	160	163	193	193	176
Diabetic					
1	148	223	254	190	170
2	199	215	224	213	173
3	163	253	185	158	-
4	144	203	155	139	-
5	-	164	118	146	-
6	174	-	148	140	136
7	235	141	130	158	-
8	176	205	164	139	143
9	184	153	197	183	-
10	168	208	170	180	211
11	162	129	144	-	118
12	213	160	160	-	110

TABLE 17 Continued

Food Restricted					
1	189	170	-	179	171
2	191	179	219	200	195
3	185	195	199	208	195
4	185	-	200	193	223
5	245	202	204	201	213
6	-	178	168	185	-
7	173	220	-	225	-
8	160	184	239	249	239
9	153	173	216	186	183
10	234	156	215	-	200
11	170	168	199	215	212
WKY			SAP[b] (mm Hg)		
Control					
1	115	138	142	149	148
2	144	161	-	160	143
3	143	164	164	158	181
4	135	125	140	156	150
5	-	131	120	126	-
6	138	-	161	126	130
7	137	141	126	134	153
8	-	126	175	149	148
9	133	148	150	-	159
Diabetic					
1	129	170	128	135	131
2	145	141	-	161	176
3	129	140	130	120	134

TABLE 17 Continued

4	-	161	131	115	-
5	144	139	131	143	130
6	126	-	166	116	125
7	108	129	123	144	143
8	-	147	120	136	134
9	144	119	121	134	140
10	129	145	136	-	128
Food Restricted					
1	180	163	-	161	139
2	154	143	166	162	145
3	136	134	139	131	139
4	135	-	176	173	171
5	143	153	159	161	129
6	-	164	158	123	-
7	145	144	106	175	155
8	140	165	178	-	171
9	118	158	143	168	168
10	175	130	148	195	184
11	145	163	-	169	-

[a] Number of weeks after induction of diabetes or start of food restriction.

[b] SAP determined using the indirect tail cuff method. Briefly, the rats were placed in restraints in a warming box (30^{o}) for about 10 minutes. The cuff was placed on the tail and pressure was applied until no pulse was detected on the recorder. Pressure was released slowly to baseline. The first reappearance of the pulse was marked and recorded as the SAP.

TABLE 18.--Effect of STZ-induced Diabetes and of Food Restriction and Hypothyroidism on Prolyl Hydroxylase Activity in the Aorta of the SHR and WKY Rat. Raw Data.

Assay 1.	Tissue Weight mg	CPM[a]	CPM/mg tissue[b]	CPM/mg protein[c]
SHR				
Control				
1	47.3	1385	146	3617
2	37.7	1242	165	5010
3	45.0	1298	144	4898
4	40.5	1093	135	3729
5	33.1	1012	153	4986
Diabetic				
1	32.4	163	25	1548
2	49.4	68	7	628
3	41.1	74	9	827
4	46.0	187	20	1735
5	42.4	275	32	1964
Food Restricted				
1	40.2	1023	127	2982
2	45.9	673	73	2022
3	48.2	779	81	2940
4	30.6	587	96	5193
5	50.5	884	88	2491
WKY				
Control				
1	33.5	676	101	2941
2	24.8	454	92	4637
3	41.2	774	94	4422

TABLE 18 Continued

4	37.4	553	74	2323
Diabetic				
1	49.2	328	33	2297
2	27.8	220	40	2314
3	28.9	202	35	1838
4	38.8	353	46	2827
Food Restricted				
1	25.7	420	82	1728
2	20.8	240	58	2664
3	20.7	223	54	2349
4	26.3	381	73	3632
5	55.7	748	67	4451
Assay 2.	Tissue	CPM[a]	CPM/mg tissue[b]	CPM/mg protein[c]
SHR	Weight mg			
Control				
6	49.9	890	89	2871
7	25.8	564	109	3133
8	28.2	464	82	2730
9	43.9	1110	126	2920
Hypothyroid				
1	27.8	382	69	2181
2	33.7	466	69	2218
3	45.6	556	61	1764
4	43.0	593	69	2325
5	33.3	348	52	1512
6	40.6	464	57	1974
7	44.2	654	74	2324

TABLE 18 Continued

WKY				
Control				
5	38.7	357	46	1878
6	18.0	159	44	1676
7	20.6	128	31	1342
8	35.6	460	65	2966
Hypothyroid				
1	37.4	338	45	1649
2	36.7	226	31	1253
3	36.7	276	38	1723
4	20.4	77	19	644
5	36.4	261	36	1683
6	20.4	61	15	581
7	40.5	166	20	1143

[a] Prolyl hydroxylase activity determined by the method of Hutton et al, 1966. Briefly, the aorta was homogenized in 1 ml buffer containing sucrose (0.25M), tris (0.05M), EDTA (10^{-5}M), DL-dithiothreitol (10^{-5}M), and triton-100 in a ground glass homogenizer. Tissue homogenate was centrifuged at 15,000 x g for 15 minutes. An aliquot (200 μl) of supernatant was placed in a 10 x 75 mm tube with 800 μl of reaction mixture containing tris, ascorbic acid, iron, bovine serum albumin, catalase, α-ketogluterate, and ^{3}H-proline. The tubes were incubated in a 30^{o}C water bath for 30 minutes. At the end of thirty minutes, 100 μl of 50% trichloroacetic acid was added to each tube to stop the reaction. The tritiated water formed during the incubation was collected by vacuum distillation. An aliquot (800 μl) of distillate was counted in a liquid scintillation counter for ten minutes and the CPMs recorded.

[b] calculation is (CPM x 5)÷ tissue weight (mg)

[c] calculation is (CPM x 5)÷ protein (mg) per ml of supernatant

TABLE 19.--Lowry Protein Assay. Raw Data.

Assay 1.

BSA Standards[a] μg	Absorbance[b] @750nm	Protein[c] (mg)	Protein[d] (mg)/ml
2	0.032	-	-
5	0.078	-	-
10	0.142	-	-
20	0.255	-	-
30	0.351	-	-
40	0.432	-	-
50	0.488	-	-
SHR			
Control			
1	0.438	0.0383	1.915
2	0.318	0.0248	1.240
3	0.382	0.0265	1.325
4	0.381	0.0293	1.465
5	0.326	0.0203	1.015
Diabetic			
1	0.200	0.0105	0.525
2	0.173	0.0108	0.540
3	0.150	0.0090	0.450
4	0.174	0.0108	0.540
5	0.235	0.0140	0.700
Food Restricted			
1	0.443	0.0343	1.715
2	0.411	0.0333	1.665
3	0.383	0.0265	1.325
4	0.180	0.0113	0.565

TABLE 19 Continued

5	0.429	0.0355	1.775
WKY			
Control			
1	0.320	0.0230	1.150
2	0.206	0.0098	0.490
3	0.279	0.0175	0.875
4	0.362	0.0238	1.190
Diabetic			
1	0.223	0.0143	0.715
2	0.172	0.0095	0.475
3	0.184	0.0110	0.550
4	0.221	0.0125	0.625
Food Restricted			
1	0.359	0.0243	1.215
2	0.155	0.0090	0.450
3	0.178	0.0095	0.475
4	0.180	0.0105	0.525
5	0.275	0.0168	0.840

Assay 2.

BSA Standards[a] μg	Absorbance[b]@750nm	Protein[c](mg)	Protein[d](mg/ml)
5	0.084	-	-
10	0.156	-	-
20	0.269	-	-
30	0.380	-	-
40	0.455	-	-
50	0.529	-	-

TABLE 19 Continued

SHR			
Control			
6	0.388	0.0310	1.550
7	0.251	0.0180	0.900
8	0.236	0.0170	0.850
9	0.447	0.0380	1.900
Hypothyroid			
1	0.247	0.0175	0.875
2	0.284	0.0210	1.050
3	0.394	0.0315	1.575
4	0.340	0.0255	1.275
5	0.307	0.0230	1.150
6	0.310	0.0235	1.175
7	0.346	0.0270	1.350
WKY			
Control			
5	0.265	0.0190	0.950
6	0.145	0.0095	0.475
7	0.145	0.0095	0.475
8	0.220	0.0155	0.775
Hypothyroid			
1	0.277	0.0205	1.025
2	0.249	0.0180	0.900
3	0.228	0.0160	0.800
4	0.180	0.0120	0.600
5	0.221	0.0155	0.775
6	0.154	0.0105	0.525
7	0.209	0.0145	0.725

[a] Standard curve determined for each assay. A bovine serum albumin stock (1 mg/ml) was used and appropiate aliquots removed to give the listed BSA concentration. Each aliquot was diluted to 100 μl with 1N NaOH.

[b] Protein determined by the method of Lowry et al, 1952. Briefly, an aliquot (20 μl) of the supernatant of the tissue homogenate from the prolyl hydroxylase assay was placed in 10 x 75 mm tubes. The aliquots were diluted to 100 μl with 1N NaOH. To each standard and sample tube

TABLE 19 Continued

1 ml of solution D (49.0 mls 2% Na_2CO_3, 0.5 ml 2% Na Tartrate, 0.5 ml 1% $CuSO_4$) was added. After waiting 20 minutes, 100 μl of 1N Folin Phenol Reagent was added to all standard and sample tubes. The tubes were mixed. After 40 minutes, the absorbance of each tube @ 750nm was read.

[c] Determined from the standard curve using the absorbance values.

[d] Calculation is protein (mg) x 50.

TABLE 20.--Effect of STZ-induced Diabetes and of Food Restriction and Hypothyroidism on the Sensitivity (pD_2) of the Mesenteric Artery of the SHR and WKY Rat. Raw Data.

	pD_2[a]		
Agonist:	Ne	5HT	MOX
SHR			
Control			
1	6.44	6.17	5.20
2	6.73	6.35	5.52
3	6.73	6.30	5.44
4	6.30	6.30	5.00
5	7.00	6.25	5.24
6	6.35	6.05	4.98
7	6.78	6.30	5.22
8	7.05	6.13	5.04
9	6.43	6.30	5.26
10	6.43	6.37	5.20
Diabetic			
1	6.72	6.05	5.35
2	6.98	6.42	5.62
3	6.50	6.28	6.02
4	6.40	6.22	5.50
5	6.94	6.45	5.76
6	6.65	6.14	5.30
7	6.25	6.30	5.35
8	6.10	6.05	5.35

TABLE 20 Continued

Food Restricted			
1	7.15	6.50	5.75
2	7.10	6.86	5.82
3	6.58	6.50	5.66
4	-	6.56	5.38
5	6.84	6.75	5.32
6	6.28	6.38	5.34
7	6.30	6.40	5.28
8	6.05	6.38	5.30
Hypothyroid			
1	6.38	6.02	5.02
2	7.50	6.02	4.95
3	6.90	6.14	5.15
4	6.70	6.16	5.15
5	6.58	6.20	5.08
6	6.68	6.02	4.95
7	6.56	5.90	5.28
WKY			
Control			
1	6.00	5.88	5.00
2	7.18	6.00	4.85
3	7.06	5.88	4.85
4	6.35	6.02	4.95
5	6.82	5.88	4.75
6	6.56	6.05	4.90
7	6.75	5.90	5.20
8	6.52	6.15	5.35

TABLE 20 Continued

Diabetic			
1	6.34	6.02	5.15
2	6.60	6.08	5.42
3	6.65	6.12	5.36
4	6.65	6.18	5.52
5	6.35	6.05	5.35
6	6.14	6.04	5.38
7	6.98	6.02	5.55
Food Restricted			
1	7.10	6.05	5.55
2	6.45	6.22	5.50
3	7.35	6.25	5.84
4	6.55	6.35	5.45
5	6.45	6.28	5.58
6	6.40	6.18	5.52
7	6.45	6.10	5.45
Hypothyroid			
1	6.55	5.96	4.68
2	6.38	5.70	4.94
3	6.50	5.86	4.95
4	6.40	5.80	4.95
5	6.50	5.78	5.00
6	6.54	5.74	5.00
7	6.30	5.54	4.55

[a] Determined from the -log ED_{50}. ED_{50} is the agonist dose needed to produce 50% of the maximum response. Dose response curves were determined using the following procedure. The mesenteric artery was

TABLE 20 Continued

isolated and a 2 cm piece was removed and placed in 100 ml of oxygenated (95% O_2 - 5% CO_2) Krebs-Henseliet buffer of the following composition (mM): NaCl (113), KCL (4.7), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $MgSO_4$ (1.2), Glucose (11.5), $NaHCO_3$ (19). Fat was carefully trimmed from the mesenteric artery so as to not stretch or damage the vessel. A 3 - 5 mm ring was cut and placed on two platinum hooks. The vessel was placed in a jacketed tissue chamber containing 100 ml oxygenated KH buffer maintained at 37°C. The bottom platinum hook was secured in the tissue chamber and the top hook was attached to a Grass FT03 force-displacement transducer. One gram of tension was placed on the vessel, and it was allowed to equilibrate for 90 minutes. The buffer was changed every thirty minutes throughout the experiment. The vasoactive agents employed were norepinephrine (NE), 5-hydroxytryptamine (5HT), and methoxamine (MOX). Cocaine (10^{-6} M) was added to the buffer to prevent reuptake of norepinephrine (Webb and Vanhoutte, 1981). Developed tension responses were obtained over a range of 10^{-9} M to 10^{-4} M for NE and over a range of 10^{-7} M to 10^{-4} M for 5HT and methoxamine. A sixty minute period with frequent buffer changes was employed between agonist dose response curves.

TABLE 21.--Effect of STZ-induced Diabetes and of Food Restriction and Hypothyroidism on Responsiveness of the Mesenteric Artery of the SHR and WKY Rat. Raw Data.

		Maximum Change in Tension[a] (mg)			
	Tissue Weight (mg)	NE 10^{-4}M	5HT 10^{-4}M	MOX 10^{-4}M	KCL 100 mM
SHR					
Control					
1	0.98	530	650	550	500
2	1.01	650	890	690	290
3	0.98	470	640	510	330
4	1.47	700	750	550	400
5	0.34	450	500	400	200
6	0.53	610	640	480	250
7	0.55	700	760	730	480
8	0.57	700	920	860	500
9	0.86	600	780	700	420
Diabetic					
1	1.05	1550	1600	1300	700
2	0.90	700	1000	730	400
3	0.61	660	760	710	350
4	0.62	820	900	1000	600
5	0.55	400	200	300	250
6	0.49	850	1100	1010	500
7	0.45	800	800	820	400
8	0.93	1150	700	820	400

TABLE 21 Continued

Food Restricted					
1	1.00	1000	900	430	200
2	1.35	1320	1500	1600	1050
3	0.60	520	800	880	650
4	0.46	450	580	500	200
5	0.60	540	750	870	450
6	0.78	780	1000	820	500
7	0.57	690	900	840	510
8	0.72	920	1260	1260	650
Hypothyroid					
1	0.66	810	860	720	430
2	0.84	530	550	430	300
3	0.97	580	610	600	500
4	0.87	680	770	750	460
5	0.65	710	810	760	270
6	0.73	690	740	660	350
7	0.68	840	950	920	550
WKY					
Control					
1	0.43	600	750	820	570
2	0.60	530	500	430	200
3	0.52	580	460	510	250
4	0.58	650	730	660	300
5	0.54	670	530	490	260
6	0.52	590	500	410	180
7	0.22	500	500	500	300
8	0.40	830	870	970	430

TABLE 21 Continued

Diabetic					
1	1.12	600	570	630	520
2	0.93	930	1040	1210	690
3	0.53	770	780	720	320
4	0.54	1150	1400	1490	750
5	0.79	1100	1190	1280	550
6	0.57	1180	1240	1430	910
7	0.37	740	590	660	200
Food Restricted					
1	0.88	600	550	650	450
2	0.40	730	850	700	400
3	0.58	950	1150	1250	620
4	0.49	850	910	1000	400
5	0.71	880	1150	1130	500
6	0.69	750	830	930	360
7	0.80	890	920	1250	730
Hypothyroid					
1	0.37	650	370	390	180
2	0.89	540	220	330	150
3	0.47	620	500	400	180
4	0.82	840	580	730	210
5	0.66	480	330	440	190
6	0.78	710	580	550	180
7	0.53	1010	600	500	240

[a] Maximum change in tension reported is the response observed for the agonist concentration listed. Dose response curves were determined using the following procedure. The mesenteric artery was isolated and

TABLE 21 Continued

a 2 cm piece was removed and placed in 100 ml of oxygenated (95% O_2 - 5% CO_2) Krebs-Henseliet buffer of the following composition (mM): NaCl (113), KCL (4.7), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $MgSO_4$ (1.2), Glucose (11.5), $NaHCO_3$ (19). Fat was carefully trimmed from the mesenteric artery so as to not stretch or damage the vessel. A 3 - 5 mm ring was cut and placed on two platinum hooks. The vessel was placed in a jacketed tissue chamber containing 100 ml oxygenated KH buffer maintained at 37°C. The bottom platinum hook was secured in the tissue chamber and the top hook was attached to a Grass FT03 force-displacement transducer. One gram of tension was placed on the vessel, and it was allowed to equilibrate for 90 minutes. The buffer was changed every thirty minutes throughout the experiment. The vasoactive agents employed were norepinephrine (NE), 5-hydroxytryptamine (5HT), and methoxamine (MOX). Cocaine (10^{-6} M) was added to the buffer to prevent reuptake of norepinephrine (Webb and Vanhoutte, 1981). Developed tension responses were obtained over a range of 10^{-9} M to 10^{-4} M for NE and over a range of 10^{-7} M to 10^{-4} M for 5HT and methoxamine. Developed tension response was also obtained for KCl (100 mM). A sixty minute period with frequent buffer changes was employed between agonist dose response curves.

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