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Blockade of Thromboxane Influences Does Not Affect Renal Blood Flow Deficit in Anesthetized Diabetic Rats[1,2,3]

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ABSTRACT

Studies were conducted to determine whether reduced renal blood flow (RBF) exhibited by rats with uncontrolled, streptozotocin (STZ)-induced diabetes is attributable to diabetes-associated, enhanced renal vasoconstrictor influence of endogenous thromboxane (TX)A_2. Rats which were injected with STZ after pretreatment with 3-O-methyl glucose (3OMG), an agent which prevents STZ-induced hyperglycemia, were also studied. Basal values of total RBF (RBF; $ml \cdot min^{-1} \cdot gKw^{-1}$; electromagnetic flow probe), systemic arterial pressure (BP; mm Hg) and renal vascular resistance (RVR; $BP \cdot RBF^{-1}$) in pentobarbital-anesthetized rats during a control period were 5.9 ± 0.3 ($P < .01$ vs. CR), 115 ± 3 and 20.3 ± 1.0 ($P < .01$ vs. CR) for STZR (n=15), and 8.4 ± 0.4, 123 ± 3 and 15.1 ± 0.8 for age-matched control rats (CR; n=15), respectively. Basal values of RBF, BP and RVR in 3OMG pretreated STZR were identical to CR. In preparations shown capable of renal vasodilatation, OKY 1581 (1 mg/kg, i.v. followed by 0.4 mg/kg·min infusion) abolished arachidonate-induced TXA_2 synthesis, but did not alter basal BP, RBF or RVR in either STZR or CR (n=4/group). Similarly, i.r.a. infusion of SQ29548 (100 ng/ml RBF) abolished renal vasoconstriction induced by a TX/prostaglandin endoperoxide mimic, U46619, but had no discernable affect on RVR in either STZR (n=8) or CR (n=8). The data indicates that TXA_2 does not participate in the elevated basal RVR of STZR which are associated with the diabetic state.

Key Words: Streptozotocin-induced diabetes, renal hemodynamics, 3-O-methyl glucose, OKY-1581, SQ29548

Abbreviations: AA, arachidonic acid, ACh, acetylcholine chloride, ANOVA, analysis of variance, BP, mean systemic arterial pressure, CR, control rat (age-matched with respect to treated rats and injected with streptozotocin vehicle), GFR, glomerular filtration rate, gKw, gram kidney weight, INDO, indomethacin, I.R.A., intrarenal arterial, MCh, methacholine chloride, OKY-1581, Sodium (E)-3-[4-(3-pyridylmethyl) Phenyl]-2-methyl acrylate, PG, prostaglandin, RBF, total renal blood flow (expressed per gram kidney weight), RPF, renal plasma flow, RVR, renal vascular resistance (BP/RBF), SQ29548, [1S-[1α, 2β (5Z), 3β, 4α]]-7-[3 [(phenylamino) carbonyl] hydrazino] methyl]-7-oxabicyclo [2.2.1] hept-2-yl]-

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5-heptenoic acid, STZ, streptozotocin, STZR, rat which received i.v. injection of STZ, TLC, thin layer chromatography, 3OMG, 3-O-methyl glucose, 3OMG/STZR, rat which received intraperitoneal injection of 1 mmole 3OMG just before injection of streptozotocin, TX, thromboxane, U46619, 9, 11-methanoepoxy PGH_2

INTRODUCTION

Glomerular filtration rate (GFR) and renal plasma flow (RPF) are increased (Christiansen *et al.*, 1981; Mogensen 1971) in association with increased kidney size (Christiansen *et al.*, 1981) in early insulin-dependent human diabetes. However, this initial hyperfunction is followed by renal failure, one of the most common terminal events in severe diabetes. As summarized in recent review (O'Donnell *et al.*, 1988), previous animal studies have indicated divergent effects of experimental diabetes on renal hemodynamics, depending on the presence or absence of insulin treatment, *i.e.* severity of diabetes. In rats with uncontrolled, experimental diabetes, single nephron glomerular plasma flow (SNGPF) and single nephron glomerular filtration rate (SNGFR) decreased as a consequence of increased glomerular arteriolar resistance (Hostetter *et al.*, 1981; Michels *et al.*, 1981). Using direct, continuous measurements of total RBF in the *in situ*, autoperfused kidney, we also found that anesthetized STZ-induced diabetic rats exhibited elevated total RVR compared to age-matched control rats (Ha and Dunham, 1987).

The mechanism(s) contributing to the pathogenesis of diabetic renal hemodynamics are poorly understood. Our previous study (Ha and Dunham, 1987) suggested that not only renal vascular structural changes associated with diabetes but also undefined humoral factors or abnormal interaction of formed blood elements with vessel walls might account for elevated RVR in STZR. Among endogenous vasoactive agents, eicosanoids can modulate renal hemodynamics. Thus, an imbalance in the production of renal vasoconstrictor (TXA_2) and vasodilator (PGE_2, PGI_2) eicosanoids may be associated with the development of renal vascular complications as well as the platelet hyperaggregability attending diabetes.

Numerous *in vitro* studies have demonstrated altered tissue synthesis of certain eicosanoids in association with diabetes. As reviewed by Brown *et al.* (1982), increased TXA_2 production by platelet as well as decreased vessel wall production of PGI_2 occurs in association with diabetes. The urinary excretion of PGE_2, TXB_2 and 6-keto-$PGF_{1\alpha}$ might reflect diabetes-induced alteration in renal arachidonic acid metabolism. Whereas TXB_2 excretion is enhanced in response to furosemide in diabetic subjects (Wilson and Tan, 1985) and basal excretion of TXB_2 is elevated in STZR, urinary excretion of PGE_2 is decreased in STZR (Quilley and McGiff, 1985, 1986). An increased TXA_2/PGI_2 ratio in the diabetic kidney and/or alterations in the renal reactivity to these eicosanoids, might contribute to the observed elevation of RVR in uncontrolled, experimental diabetes; however, the functional significance of the changes in eicosanoid levels/excretion that have been reported in association with diabetes is not established.

The objective of the present study was to determine whether the elevated basal RVR observed in STZR is attributable to an enhanced renal vasoconstrictor influence of endogenous TXA_2. An additional objective was to determine whether elevated RVR in STZR might be a consequence of the direct toxic effect of STZ rather than the STZ-induced diabetic state. This purpose was examined by measuring mean BP and RBF in STZR and rats which were injected with STZ after pretreatment with 3-O-methyl glucose (3OMG), an agent which has been shown to prevent the elevation in serum glucose caused by STZ (Ganda *et al.*, 1976).

MATERIALS AND METHODS

Induction of experimental diabetes mellitus

Male, Wistar rats were obtained (Charles River, Kingston, NY) and kept one to two weeks for acclimatization. Diabetes was induced by a single i.v. injection in the lateral tail vein of 50 mg/kg STZ (The Upjohn Co., Kalamazoo, MI) dis-

solved in pH 4.5 citrate buffer immediately before injection. Age-matched, six-week-old control rats (CR) from the same shipment received citrate buffer.

In a separate, control group of five rats, 1 mmole of 3-O-methyl glucose (3OMG; Sigma Chemical Co., St. Louis, MO) was administered just prior to STZ injection (3OMG-pretreated STZR). All STZR, 3OMG-pretreated STZR, and CR were given 3 ml of 10% glucose by gavage at 4 and 10 hours after injection, in order to prevent hypoglycemic shock due to initial, massive release of insulin by STZ (Rerup, 1970). Induction of the diabetic state was verified by estimating urine glucose with commercial enzymatic test strips. Before each experiment, a blood sample was collected for enzymatic measurement of plasma glucose.

Surgical preparation and measurements of total renal blood flow (RBF)

At four weeks after treatment, fifteen rats from both STZR and CR groups were prepared by a modification (Ha and Dunham, 1987) of the method of Dunham and Vince (1986) for measurements of basal values of mean arterial blood pressure (BP) and RBF and determination of the involvement of enhanced renal vasoconstrictor influence of endogenous TXA_2 on the elevated basal RVR observed in STZR. Briefly, the rats were anesthetized with 50 mg pentobarbital sodium/kg initially plus i.v. maintenance infusion of 30 and 21 mg/kg·hr during and after surgery, respectively. A tracheal tube was inserted to maintain a patent airway. Body temperature was maintained at 37.5-38℃ with a heating lamp. The bladder was catheterized (PE 200) to allow free urine flow. Drugs and fluids (pentobarbital-saline: rat serum; 2:1, v/v; 180 μl/kg·min during surgery followed by 120 μl/kg·min thereafter) for volume maintenance were infused *via* a cannulated jugular vein (PE 20) and the renal artery (see below). Mean BP was monitored from a cannulated carotid artery (PE 50). The left kidney was approached *via* a retroperitoneal incision thus avoiding the stress caused by laparotomy. RBF was monitored continuously with a precalibrated, noncannulating, electromagnetic flow transducer (2 or 2.5 mm circumference; Carolina Medical Electronics, Inc., King, NC) placed on the left renal artery. The RBF zero baseline was verified by brief occlusion of the renal artery distal to the probe after stabilization of the preparations and the conclusion of each experiment. For intrarenal arterial (i.r.a.) administration of drugs (Protocol 2: SQ29548 experiments), the left renal artery was catheterized by inserting a 33 gage needle at the origin of the renal artery according to the method described by Fine *et al.* (1974). The needle was advanced into the renal artery until the tip lay near the probe and was attached to a catheter system connected to a Harvard infusion pump. Heparinized saline (5 U/ml) was continuously infused at 15 μl/min to maintain patency of the catheter.

Seven STZR, five 3OMG-pretreated STZR, and four CR were used for measurements of basal values of BP and RBF.

BP and RBF transducer voltage were amplified and recorded on a Beckman Dynograph, digitized with a Metrabyte A/D converter (4 samples/sec), and the data was stored and processed with an IBM PC utilizing a program that derives RVR from BP and RBF. After each experiment, the left kidney was excised, decapsulated, cut in half and weighed after blotting.

Drug administration and experimental protocols

Basal Hemodynamics-A minimum of fifteen min was allowed to elapse after renal artery occlusion before experimental protocols were begun. Basal hemodynamic parameters were then measured for thirty minutes.

Determination of Possible, Increased Influence of Endogenous TXA_2 on Basal RVR in STZR-To determine whether endogenous TXA_2 exerts enhanced renal vasoconstrictor influences on basal RVR in STZR, OKY-1581, a specific TXA_2 synthetase inhibitor (Miyamoto *et al.*, 1980), or SQ29548, a TXA_2 antagonist (Ogletree *et al.*, 1985), were administered after measurement of basal hemodynamics parameters. First of all, a time control study was conducted using both CR and STZR (n=3 rats/group) in order to evaluate the stability of measured parameters. Hemodynamic measurements were conducted for 30 min, vehicle (saline) was injected, and measurements continued for an additional 60 minutes.

In protocol 1 (OKY-1581 experiments), BP and RBF were measured in four rats from both STZR and CR for 30 min after administration of OKY-1581 (1 mg/kg initially followed by 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$ throughout the treatment period) into a low-dead volume, latex injection port interposed within the jugular vein catheter. After thirty min-

utes, indomethacin (INDO; 2 mg/kg) was administered and BP and RBF were observed for an additional thirty minutes. Inasmuch as the onset of cyclooxygenase inhibition by INDO is known to be time-dependent, the INDO- treatment period was considered to begin fifteen minutes after INDO injection. Methacholine Cl (60 to 180 ng, i. v. bolus doses) was injected to verify the capacity of individual preparations to exhibit renal vasodilatation after OKY-1581 and INDO treatments.

To determine the efficacy of OKY-1581, in three experiments arterial blood samples were drawn from the carotid arterial catheter before (control samples), 30 minutes after the loading and maintenance doses of OKY-1581 and again thirty min after terminating i.v. infusion of OKY-1581 to anesthetized rats which were surgically prepared for blood flow measurements. The samples (0.9 ml) were withdrawn into a plastic syringe containing 0.1 ml of 3.2% sodium citrate and immediately added into a plastic tube containing a saline solution of cold carrier-(final concentration 9.9×10^{-5} M) and [^{14}C (U)]-sodium arachidonate (ca. 0.18 μ Ci/sample, final concentration 6.5×10^{-7} M). After 10 minutes incubation (37℃) with mixing, 0.1 ml each of $CaCl_2$ (50 mM) and thrombin (0.1 U/μl) were added, and the samples were incubated for 1 hour. The serum was separated by centrifugation at 3000 g (Sorval SS4 centrifuge), acidified with 0. 2 ml of 2.3 M formic acid and extracted twice with 2 volumes of ethylacetate. The ethylacetate extracts were applied with authentic eicosanoid and arachidonic acid standards to Whatman LK5D silica gel thin-layer chromatography (TLC) plates. The TLC plates were developed (2 times) at room temperature with chloroform: ethylacetate: methanol: acetic acid: water (70: 30: 8: 1: 0.5; v/v). The relative mobilities of the lipid standards and radioactive products were ascertained by staining with I_2 and by scanning with a Berthold radiochromatogram scanner. Quantitaion of the [^{14}C]-labelled lipids was done by liquid scintillation counting of silica zones scraped from the TLC plates as described previously (Engstrom and Dunham, 1982).

In protocol 2 experiments, SQ29548 (100 ng/ml of RBF) was administered i.r.a. as described above to eight rats from STZR and CR. SQ29548 was infused continuously during the thirty minute observation period, because preliminary studies indicated this agent has a short half-life. The efficacy of the SQ29548 dose employed was verified by determining the ability of SQ29548 to antagonize renal vasoconstriction induced by i.r.a. administration of the stable TX/prostaglandin endoperoxide mimic, U46619, in each SQ29548-treated rat. The dosage regimen for U46619 employed in these experiments varied among rats; two doses were chosen which caused less than 50% decrements in the level of RBF existent prior to U46619 administration. Three CR and five STZR received i.r.a. bolus injection of U46619 (200 to 900 ng). The remaining rats received i.r.a. infusion of U46619 (25-200 ng/ml of RBF; 30 sec). Intrarenal arterial administration of ACh (50 to 100 ng/ml of RBF) was used to verify the capacity of individual preparations to exhibit renal vasodilatation.

Drugs and preparation of solutions

OKY-1581 (Sodium (E)-3-[4-(3-pyridylmethyl) phenyl]-2-methyl acrylate), obtained from ONO Pharmaceutical Co. (Osaka, Japan), was prepared in normal saline. Doses are expressed in terms of the salt. SQ29548 ([1S-[1α, 2β (5Z), 3β, 4α]]-7-[3-[(phenylamino) carbonyl] hydrazino] methyl]-7-oxabicyclo 2.2.1] hepta-2-yl]-5-heptenoic acid) was obtained from E. R Squibb and Sons (Princeton, NJ) and dissolved in Krebs solution. U46619 (9, 11-methanoepoxy PGH_2; The Upjohn Co.) was stored at -20℃ as 2. 5 mg/ml solutions in ethanol. For use, aliquots of this solution were mixed with one equivalent of Na_2CO_3 and diluted with saline to 25μg/ml. Unlabelled arachidonic acid (Nucheck Prep, Elysian, MN) and [^{14}C (U)]-arachidonate (New England Nuclear) were prepared as sodium salts with Na_2CO_3 and dissolved in saline. Sodium salts of indomethacin (INDO; Sigma Chemical Co., St. Louis, MO) was prepared using Na_2CO_3 as described by Sakr and Dunham (1982), and dosage are expressed in terms of the free acids. Methacholine Cl (MCh; Sigma), thrombin (Parke-Davis, Morris Plains, NJ), acetylcholine Cl (ACh; Sigma) were dissolved in saline. Pentobarbital (Ganes Chemicals; Pennsville, NJ) were used for anesthesia.

Analysis of data

The data collected include BP (mm Hg) and RBF (ml·min^{-1}·gKw^{-1}). RVR was calculated as BP·RBF^{-1}. Data is reported as means±SE. Reported basal values of BP, RBF and RVR were

obtained from the average of six computer-derived averages of values sampled over sequential five min intervals. Data from control and STZ-treated groups were analyzed using two-tailed, Student's t tests for comparison of means of BP, RBF and RVR. One-way analysis of variance (ANOVA) was employed to compare these parameters among STZR, 3OMG-pretreated STZR and CR. Regression analysis (Wallenstein, 1980) was used to evaluate the effects of time as well as OKY-1581, SQ29548 and INDO on basal BP, RBF and RVR. Statistical calculations were performed using Statfast software (StatSoft, Tulsa, OK). A P value <0.05 was used as the criterion for statistically significant differences.

RESULTS

Chronic effects of streptozotocin treatment

Rats treated with STZ which exhibit urine glucose concentrations >2000 mg% as determined by semiquantitative assay at the time of experiments were studied. These rats failed to gain weight as rapidly as CR and exhibited polydipsia and polyuria. At the time of the experiments (four weeks after induction of diabetes) STZR had significantly lower body weights, higher kidney weights and higher plasma glucose levels than CR. Values of these parameters for rats used in this study are shown in Table 1. Body weight, kidney weight and plasma glucose levels in rats protected from STZ-induced diabetes by pretreatment with 3OMG prior to STZ injection were similar to those of CR as summarized in Table 2.

Table 1. Comparison of STZR[a] and CR[b] used in renal hemodynamic studies

	STZR (15)[c]	CR (15)
Body Weight, g	268±10[d]**	319±11
Kidney Weight, g	1.48±0.07**	1.18±0.05
Serum Glucose, mg%	592±36 (4)**	189±16 (3)
Basal[e] Hemodynamic Values:		
Mean Arterial Pressure, mm Hg	115±3	123±3
Renal Blood Flow ml · min^{-1} · gKw^{-1}	5.9±0.3**	8.4±0.4
Renal Vascular Resistance mm Hg · ml^{-1} · min · gKw	20.3±1.0**	15.1±0.8

a,b: STZR = Streptozotocin-treated rats (50 mg/kg); CR = Control rats. Values shown were obtained 4 weeks after treatment (10-11 week-old rats).
c: Numbers in parentheses refer to number of rats.
d: Values shown are means±S.E.
e: Basal values represent measurements taken every 5 minutes ($\bar{\bar{X}}$, 7 values/rat) in anesthetized rats.
**: $p<0.01$; Student's t-test

Table 2. Comparison of STZR[a], CR[b] and 3OMG/STZR[c] used in renal hemodynamic studies

	STZR (7)[d]	CR (4)	3OMG/STZR (5)
Body Weight, g	279±8[e]**	341±17	358±17
Kidney Weight, g	1.50±0.08**	1.28±0.07	1.28±0.04
Serum Glucose, mg%	725±68**	234±24	243±11
Basal[f] Hemodynamic Values:			
Mean Arterial Pressure, mm Hg	127±8	114±2	123±10
Renal Blood Flow ml · min^{-1} · gKw^{-1}	6.2±0.7**	9.3±0.2	9.6±1.0
Renal Vascular Resistance mm Hg · ml^{-1} · min · gKw	21.7±2.6**	12.3±0.1	13.1±2.3

a,b,c: STZR = Streptozotocin-treated rats (50 mg/kg); CR = Control rats; 3OMG/STZR = 3-O-methyl glucose (1 mmole/rat) pretreated STZR. Values shown were obtained 4 weeks after treatment (10-11 week-old rats).
d: Numbers in parentheses refer to number of rats.
e: Values shown are means±S.E.
f: Basal values represent measurements taken every 5 minutes ($\bar{\bar{x}}$; 7 values/rat) in anesthetized rats.
**: $p<0.01$; significantly different compared to CR or 3OMG/STZR, one-way ANOVA

Table 3. Statistics describing the relationship between time and RVR
Each equation was derived based on the data presented in Figure 1.

	STZR			CR		
	Equation	r	P	Equation	r	P
Time Control Experiments						
Period 1	$y=-0.02x+22.95$	0.22	0.341	$y=-0.11x+16.62$	0.12	0.622
Period 2	$y=0.03x+20.87$	0.03	0.865	$y=0.12x+15.92$	0.10	0.681
Period 3	$y=-0.29x+21.26$	0.48	0.082	$y=-0.46x+16.34$	0.46	0.081
OKY-1581 Experiments						
Basal Period	$y=0.14x+21.39$	0.07	0.718	$y=-0.04x+13.23$	0.02	0.871
OKY-1581	$y=-0.24x+22.59$	0.15	0.452	$y=0.05x+12.46$	0.04	0.832
INDO	$y=-0.59x+21.91$	0.22	0.360	$y=-0.13x+12.95$	0.08	0.721
SQ 29548 Experiments						
Basal Period	$y=-0.01x+18.58$	0.01	0.925	$y=-0.09x+15.88$	0.09	0.532
SQ 29548	$y=-0.24x+19.12$	0.14	0.315	$y=-0.06x+16.51$	0.07	0.638

RVR in anesthetized STZR, 3OMG-pretreated STZR and CR

Basal RBF and RVR-Hemodynamic data obtained from rats used for the present study were also summarized in Table 1. The mean arterial pressure of pentobarbital-anesthetized STZR prepared for RBF measurements was similar to that of CR, but RBF was significantly lower (31%) and RVR was significantly higher (33%) in diabetic STZR than in CR (Table 1).

Values for BP, RBF and RVR in 3OMG-pretreated STZR were similar to the respective values in CR, but RBF and RVR values in these rats were significantly different from the corresponding values in STZR (Table 2).

Lack of Influence of OKY-1581 and SQ29548 on Basal Renal Hemodynamics in STZR and CR-The basal values of BP and RBF from rats utilized in time control experiments were 122 ± 6 and 5.6 ± 0.6 for STZR ($n=3$) and 118 ± 13 and 7.4 ± 1.1 for CR ($n=3$), respectively. Based on the values of RVR exhibited by STZR and CR in the time control study (as shown in top panel of Figure 1), our preparation for measurement of RBF was relatively stable over the period of study. Regression analysis (Table 3) did not reveal a statistically significant correlation between time and basal values of RVR obtained during the first-, second- and third- thirty minute observation period in either STZR or CR, thus mean values of RVR for each control period were determined from average basal values of RVR for individual rats that were obtained by averaging data gathered during six consecutive, five minute periods. Mean values of RVR (mm Hg·ml^{-1}·min·gKw) for the first-, second- and third- thirty minute periods were 22.1 ± 1.3, 20.9 ± 1.2 and 20.3 ± 0.2 for STZR and 16.2 ± 1.2, 16.4 ± 1.2 and 15.0 ± 0.6 for CR, respectively, STZR exhibited significantly higher ($P=0.03$) RVR than CR during the first- thirty minute period.

OKY-1581 had no discernable effect on RVR in either STZR or CR ($n=4$/group; middle panel of Figure 1). Regression analysis did not reveal a statistically significant correlation between time and values of RVR obtained during OKY treatment period in either STZR ($P=0.45$) or CR ($P=0.83$). During the control (basal) and OKY-1581 treatment periods, mean values of RVR were 22.0 ± 2.2 and 21.6 ± 1.8 for STZR and 13.0 ± 1.9 and 12.7 ± 1.1 for CR, respectively. Neither BP nor RBF was changed significantly compared to the control period. During the control and OKY-1581 treatment periods, mean BP and RBF values were 116 ± 9 *vs.* 116 ± 7 and 5.4 ± 0.3 *vs.* 5.5 ± 0.5 for STZR and 125 ± 7 *vs.* 126 ± 4 and 10.0 ± 0.9 *vs.* 10.3 ± 1.0 for CR, respectively. Even a dosage regimen lower than that used in these experiments, *i.e.* 0.5 mg OKY-1581/kg loading dose followed by 0.1 mg/kg·min continuous infusion, was sufficient to significantly reduce TXA_2 synthesis (Figure 2), as evaluated by assessing the ability of whole blood to synthesize TXA_2 form radiolabeled arachidonate during clotting. TXB_2 was measured by radio thin layer chromatographic methods as an index of TXA_2 synthesis. TXA_2 synthesis by

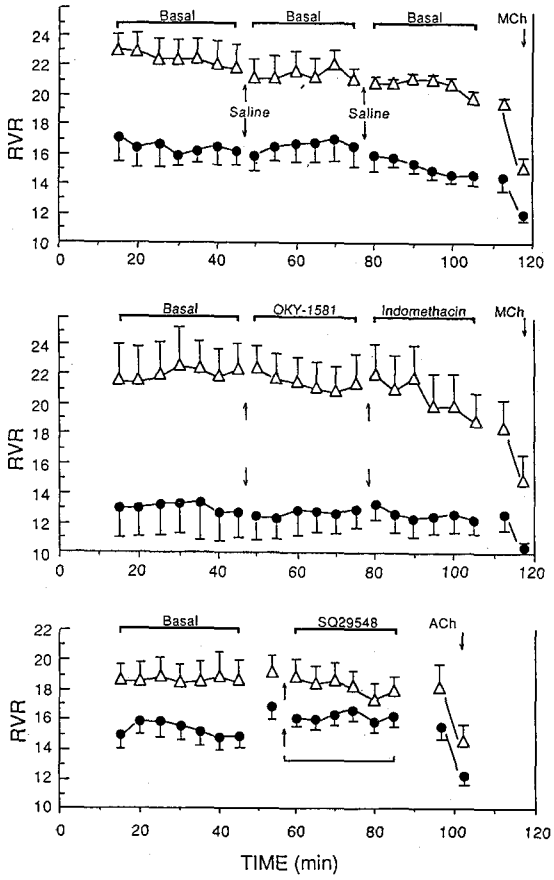

Fig. 1. Renal vascular resistance (RVR; RVR=mm Hg · ml^{-1} · min · g) before and during drug intervention in streptozotocin-treated (STZR; -Δ-) and control (CR; -●-) rats. Values plotted are means±S.E.

The *top panel* (time control experiments) summarizes basal RVR values taken every five minutes, starting fifteen minutes after renal artery occlusion for verification of the zero-RBF baseline. Saline (vehicle control for agents administered in the lower panels) was injected at the times denoted by the arrows. The symbols at the far right show RVR just before and at the time of peak effect following i.v. injection of 180 ng methacholine Cl (MCh).

The *middle panel* summarizes basal levels of RVR in a conrol period (denoted *Basal*, left seven symbols), following i.v. injection of OKY-1581 (1 mg/kg+maintenance infusion of 0.4 mg/kg · min after at the time shown by the arrow) and following i.v. injection of indomethacin (2 mg/kg). Verification of the preparation's ability to exhibit renal vasodilatation in response to MCh is shown at the right-hand side of the panel.

The *bottom panel* summarizes basal levels of RVR in a control period (left seven symbols) and during the continuous intrarenal arterial infusion (denoted by the bracket) of SQ 29548 in a quantity which established a concentration of 100 ng SQ 29548/ml renal arterial blood. The RVR values shown between the control and SQ 29548 periods were taken following two challenge, i.r.a. injections of U46619, which caused an increase in basal RVR in CR. The symbols at the far right show renal vascular responsiveness of STZR and CR to i.r.a. infusion of acetylcholine Cl (ACh; 100 ng/ml RBF).

blood from OKY-treated rats was reduced to 25± 8% (n=3) of the amount recovered in control experiments.

RVR in the third observation period following INDO treatment (middle panel of Figure 1) tended to be reduced compared to the earlier periods, but this tendency was not different that seen in the time-control experiments. Administration of INDO following the OKY-1581 period did not affect a significant change in the RVR of either STZR or CR as determined by regression analysis of values obtained from fifteen-to thirty-minutes after i.v. injection of INDO (Table 3).

Similarly, intraarterially administered SQ29548 did not alter basal BP, RBF, or RVR significantly (bottom panel of Figure 1) over a 30 min observation period in either STZR (n=8) or CR (n=8). During the control period, BP, RBF and RVR values were 114±5, 6.2±0.4 and 18.7± 1.2 for STZR and 125±4, 8.1±0.4 and 15.8±0.9 for CR, respectively. The mean values for each parameter exhibited by these subgroups are comparable to the characteristics of STZR and CR as presented in Table 1. Eventhough the basal RBF of STZR studied SQ29548 experiments was significantly lower ($p<.01$) than CR, the basal RVR was not significantly different ($P=0.08$) from that of CR. During SQ29548 infusion, the mean values of BP, RBF and RVR were 111±4, 6.3±0.4 and 17. 6±1.2 for STZR and 118±5, 7.3±0.3 and 16.2±

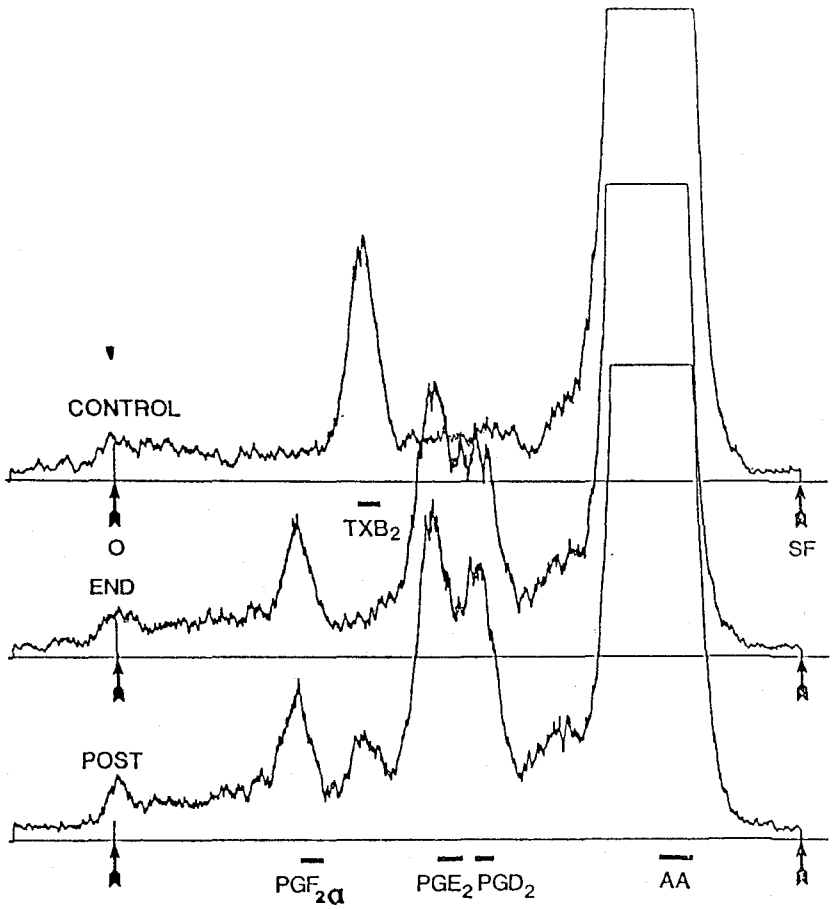

Fig. 2. Photograph of thin-layer radiochromatogram of acidic lipid extracts of rat serum obtained following incubation (10 min) of citrated whole blood with [^{14}C(U)]-arachidonic acid (AA) and subsequent stimulation with Ca^{+2} and thrombin. The relative mobilities of AA and eicosanoid standards are shown by the bars just below the radioactivity traces.

Top tracing-The sample of arterial blood was taken prior to OKY-1581 treatment (*control* sample).

Middle tracing-The sample of arterial blood (*end* sample) was taken at the end of the OKY-1581 treatment period.

Bottom tracing-The sample of arterial blood (*post* sample) was taken thirty min after termination of OKY -1581 infusion.

1.2 for CR, respectively. RVR of CR was increased from 15.8±0.9 to 16.8±0.8 after administration of two doses of U46619 that were injected at the end of the control observation period to allow subsequent verification of SQ29548's efficacy in individual preparations. The new level of RVR in CR was not altered during SQ29548 infusion based on the regression anaysis shown in Table 3. The dose of SQ29548 employed in this study was sufficient to block renal vasoconstriction elicited by the TX/ prostaglandin endoperoxide mimic, U46619, as presented in Figure 3. There was considerable variability in U46619-induced renal vasoconstriction among rats employed in this series, for unknown reasons. The two, arbitrarily chosen, doses of U46619 that did not decrease RBF by more than 50% when given after the control period were repeated thirty minute after initiating SQ29548 infusion. For data analysis, these doses were grouped and termed low and high doses.

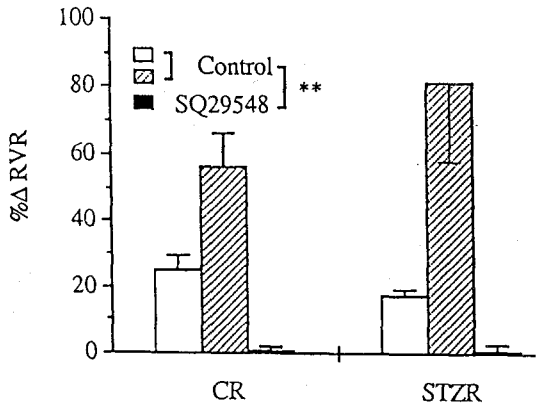

Fig. 3. Summary of effects of i.r.a. infusions of SQ 29548 (100 ng/ml RBF) on renal vasoconstriction caused by the *high* dose of U 46619 in anesthetized STZR and CR (filled columns; right-hand column of each group). U 46619 doses varied among rats; two doses which did not decrease renal blood flow by more than 50 % were arbitrarily chosen (25 to 200 ng U46619/ml RBF for infusion; 200 to 900 ng boluses). Open and hatched columns represent renal vasoconstriction induced by *low* and *high* doses of U 46619, respectively, before SQ 29548 treatment. Values plotted are means±S. E. of changes in RVR expressed as % changes from the levels of RVR existent just prior to drug injection. The mean values of basal BP (mm Hg), RBF (ml · min^{-1} · g^{-1}) and RVR (mm Hg · ml^{-1} · min · g) were 114±3, 6.4± 0.4 and 18.7±1.1 for STZR (n=8) and 119± 4, 7.5±0.2 and 16.0±0.6 for CR (n=8), respectively. Vertical lines indicate S.E. **$p<$ 0.01.

All the preparations employed in protocols 1 and 2 were shown to be capable of active renal vasodilatation (Figure 1). STZR and CR exhibited comparable responsiveness to i.v. bolus injections of MCh (60 to 180 ng/kg) in the experiments in which the effects of OKY-1581 and INDO were examined. During the peak response to the 180 ng i.v. bolus injection of MCh, RVR decreased by 19. 6±2.9% and 19.8±3.7% in STZR and CR, respectively. Similarly, i.r.a. infusions (30 sec) resulting in 50 and 100 ng ACh/ml RBF elicited renal vasodilatation in both STZR and CR in which the effect of SQ29548 was examined. During the peak response to 100 ng ACh/ml of RBF, RVR decreased by 20.6±4.2% and 22.3±2.4% in STZR and CR, respectively.

DISCUSSION

Pentobarbital-anesthetized STZR with uncontrolled diabetes were found to have similar blood pressure but lower total RBF, and higher RVR than age-matched CR as presented in Table 1. Basal RBF values in CR on a gram kidney weight basis were within the range of published values (Arendshorst *et al.*, 1975). The deficit of RBF in STZR is in agreement with clearance studies (Hostetter *et al.*, 1981) which showed reduced RPF in the presence of uncontrolled (non-insulin treated) diabetes.

In view of the renal toxicity (Sadoff, 1970) and acute effects (Carney *et al.*, 1979) of STZ on renal function, it must be considered whether the reduced RBF and elevated RVR exhibited by STZR represent renal hemodynamic changes attributable to the diabetic state or to direct nephrotoxic effects of STZ. In the present studies employing 3OMG, which has been shown to prevent STZ-induced hyperglycemia (Ganda *et al.*, 1976), 3OMG-pretreated STZR did not differ from CR with respect to body and kidney weight, plasma glucose levels, BP and RBF (Table 2). Thus the results provide evidence that reduced RBF in STZR is likely attributable to STZ-induced diabetic state rather than to direct effects of STZ.

The elevated basal RVR exhibited by diabetic STZR cannot be explained by a myogenic autoregulatory response to increased systemic arterial pressure, since the BP of STZR was decreased compared to that of CR rather than increased under the condition of our experiments. Although there have been numerous reports concerning the occurrence of mild hypertension in awake diabetic rats, others have found, as in ours, that BP is not increased in anesthetized STZR that are prepared for renal hemodynamic studies (Hostetter *et al.*, 1981). Anesthesia likely prevented demonstration of elevated BP, if present, in our studies.

Renal cyclooxygenase-derived metabolites of arachidonic acid (AA) have been proposed to affect both normal and abnormal renal function, including the regulation of RBF (Schlondorff and Ardaillou, 1986). Our experiments were designed to assess the functional (*i.e.*, with respect to renal hemodynamics) importance of the possible alterations in renal arachidonate metabolism that have been shown by numerous investigators to be associated with diabetes, based on measurements of

PGs and TX production by isolated tissue (Brown *et al.*, 1982; Quilley and McGiff, 1986 and references therein).

OKY-1581, a specific thromboxane synthetase inhibitor (Miyamoto *et al.*, 1980), was employed to determine whether enhanced renal TXA_2 synthesis (and/or TXA_2 production by non-renal tissues) contribute to elevated RVR in STZR. Since little information was available concerning the *in vivo* efficacy of this agent in the rat when this study was initiated, we evaluated its ability to reduce TXA_2 synthesis by assessing TXA_2 generation in clotting whole blood removed from rats treated with OKY-1581, as shown in Figure 2. Provided that this test adequately reflects the *in vivo* efficacy of OKY-1581 for inhibition of TXA_2 synthesis, the dosing regimen employed in the RBF experiments should have been sufficient to greatly reduce TXA_2 synthesis that may have been elevated in association with diabetes. The middle panel of Figure 1 shows that OKY-1581 did not alter basal RVR in either STZR or CR. This data suggests that endogenous TXA_2 does not contribute to elevated RVR in diabetic rats, but several caveats regarding the failure of OKY-1581 to affect renal hemodynamics in the present study must be considered.

The negative results with OKY-1581 might reflect a lack of specific action on TXA_2 synthetase by this agent, *e.g.* concomitant, partial inhibition of PGI_2 synthesis (Smith and Jubiz, 1981). Concomitant inhibition of PGI_2 synthesis might prevent functional expression of effective TXA_2 synthetase antagonism if "shunting" of endoperoxide metabolism to PGI_2 is needed to uncover the functional effect of a lack of TXA_2 synthesis. OKY-1581 might lack efficacy in the renal vasculature as shown with another thromboxane synthesis inhibitor (Patrignani *et al.*, 1984). However, OKY-1581 can cause changes in renal hemodynamics that have been interpretated as indicative of TXA_2-mediated vasoconstriction in two models of renal injury (Lianos *et al.*, 1983; Ichikawa *et al.*, 1985). In these studies, the dose of OKY-1581 employed was lower than that used in the present study. Another reservation concerning the use of OKY-1581 is that a possible accumulation of vasoconstrictor endoperoxide PGs upon blockade of the TXA_2 synthesis could prevent the expected renal vasodilatation in STZR. It is clear from our results with U46619 (protocol 2) that endoperoxide PGs would be expected to reduce RBF in the diabetic rat, in agreement with previous findings in spontaneously hypertensive and normotensive rats (Dunham and Sakr, 1983). INDO, given after OKY-1581 treatment, did not significantly alter RVR either STZR or CR suggesting that buildup of PG endoperoxide or less production of PGI_2 in STZR compared to CR after the inhibition of TXA_2 synthesis did not account for the lack of OKY-1581 influence on basal RVR in STZR. The dosage regimen for INDO employed in the present study had been shown to inhibit renal PG synthesis in rats (Roman *et al.*, 1978). Jensen *et al.* (1986) reported that administration of INDO in a dose which did not cause any significant changes in GFR in age-matched CR, decreased GFR and increased arteriolar resistance in diabetic STZR prepared for micropuncture measurements. INDO's effect on arteriolar resistance in their study, in contrast to our results, is likely attributable to differences in the preparation, *i.e.*, their use of laparotomy, a longer duration of diabetes (4 weeks in the present study *vs.* 3 months) and insulin treatment. Numerous studies have confirmed the findings (Piper and Vane, 1971; Satoh and Zimmerman, 1976) that trauma, such as laparotomy, activates PG synthesis and thus allows expression of their influence in preparation which otherwise synthesize PGs to a small degree.

It is unlikely that above mentioned reservations explain the lack of OKY-1581 on RVR, inasmuch as SQ29548, a TXA_2 receptor antagonist (Ogletree *et al.*, 1985), also did not affect BP or RBF in preparations capable of active renal vasodilatation (bottom panel of Figure 1). Our dosage regimen for SQ29548 nearly abolished U46619-induced renal vasoconstriction as summarized in Figure 3. The results with SQ29548 further suggest that, if enhanced TXB_2 excretion represents elevated renal TXA_2 synthesis in diabetic STZR, as reported by Quilley and McGiff (1985, 1986), the quantity of this eicosanoid is not sufficient to account for the elevated basal RVR in diabetic rats.

In summary, autoperfused kidneys of anesthetized STZR exhibited a deficit in basal RBF and an increase in RVR relative to age-matched control rats. The increase in RVR in rats with STZ-induced diabetes is not attributable to elevated BP. Further, as shown by experiments utilizing 3OMG, these renal hemodynamic changes are not attributable to direct effects of STZ. It is unlikely that endogenous TX influences exert a significant role *in vivo* with respect to the elevated RVR associated with diabetes in the rat. However, the influence of

eicosanoids derived from other pathways of AA metabolism, *e.g.* the leukotrienes, might be altered in diabetes.

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=국문초록=

마취된 당뇨 흰쥐의 신혈류량 감소에 관여하는 기전 : 내인성 쓰롬복산계의 무관성

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하헌주 • 얼웨인던함

본 연구는 스트렙토조토신 투여에의해 유도된 인슐린 의존적 당뇨 흰쥐에서 관찰되는 신혈류량의 감소가 내인성 혈관 수축계의 하나인 쓰롬복산의 영향이 증가된 때문인지를 규명하고자 하였다. 펜토바비탈을 이용하여 마취를 유도한 후 주어진 시간동안 아무런 약리학적 자극이 없는 상태에서 신동맥의 혈류량 ($ml \cdot min^{-1} \cdot gKw^{-1}$), 혈압 (mm Hg) 및 그때의 신동맥 저항 (혈압/신동맥의 혈류량)을 관찰하였다. 그 각각에 대응하는 값은 당뇨쥐에서는 5.9±0.3($p<0.01$, 대조군과 비교), 115±3 및 20.3±1.0($p<0.01$, 대조군과 비교)이었고, 연령 대조군에서는 각각 8.4±0.4, 123±3 및 15.1±0.8이었다. 스트렙토조토신에 의한 고혈당 유도를 방지한다고 알려진 3-O-메칠 글루코즈로 전처리한 후에 스트렙토조토신을 투여한 흰쥐에서 관찰되는 혈압 및 신동맥의 혈류량은 연령 대조군의 값과 동일하였다. 신동맥이 확장될 수 있음이 확인된 상태에서, 쓰롬복산의 합성을 저해할 수 있는 용량의 OKY-1581 (1 mg/kg, i.v.에 뒤이은 0.4 mg/kg·min 지속적 투여)는 대조군 ($n=4$)뿐 아니라 실험군($n=4$) 흰쥐의 혈압, 신혈류량 및 신동맥의 저항을 변화시키지 않았다. 마찬가지로 쓰롬복산/프로스타글란딘 엔도펄옥사이드 효현제인 U46619에 의한 신동맥 수축을 저해할 수 있는 용량의 쓰롬복산 수용체에대한 길항제인 SQ29548 (100 ng/ml 신혈류량)을 신동맥으로 투여했을 때에도, 관찰되는 신혈류역학에 아무런 변화가 없었다($n=8$ 각 군). OKY-1581 투여후에 사이클로옥시게나아제의 활성을 저해하는 약물인 인도메타신(2 mg/kg)을 투여했을 때에도 관찰되는 신동맥의 저항은 대조군에서 뿐만 아니라 실험군인 당뇨쥐에서도 변화가 없었다. 따라서 본 연구 결과는 스트렙토조토신 투여후 관찰되는 신동맥의 저항 증가는 약물의 신장에대한 직접적인 독성이 아닌, 유도된 당뇨에 기인함을 제시하였고, 이러한 신동맥 저항 증가는 고조된 내인성 혈관 수축계의 하나인 쓰롬복산의 영향이 아님을 시사하고 있다.