Impact of Insulin or Tolbutamide Treatment on ^{14}C-Arachidonic Acid Conversion to Prostacyclin and/or Thromboxane in Lungs, Aortas, and Platelets of Streptozotocin-induced Diabetic Rats

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SUMMARY

Diabetes is associated with a dramatic increase in the risk of thrombotic and atherosclerotic disease. The underlying factors responsible for this predisposition as well as the mechanisms involved have yet to be elucidated. Two endogenous substances, prostacyclin (PGI_2) and thromboxane (TXA_2), have recently been shown to possess significant vascular and thrombotic activity and are known to be altered in atherosclerosis, thrombotic conditions, and diabetes. We determined the conversion of ^{14}C-arachidonic acid (AA) to PGI_2 and TXA_2 by lungs, aortas, and platelets obtained from chemically induced diabetic rats. In addition, we investigated the ability of insulin or tolbutamide to reverse these changes. Streptozotocin (STZ)-injected rats developed blood glucose levels 2–4 times that seen in normoglycemic controls. Intact perfused lungs isolated from rats beginning 7 days after STZ treatment synthesized 22–30% less PGI_2 from ^{14}C-AA. The ratio of PGI_2/TXA_2 was decreased in the diabetic rat lungs and was inversely proportional to plasma glucose levels at the time of death. Platelet TXA_2 generation was increased 67% above control in diabetic rats while aortic PGI_2 generation was decreased 28% below normoglycemic controls. Ten-day treatment with NPH insulin 20 U/kg s.c. in STZ-pretreated rats lowered plasma glucose toward normoglycemia more effectively than tolbutamide 200 mg/kg orally. Partial correction of the decreased pulmonary PGI_2/TXA_2 ratio seen in diabetic rats was produced by insulin and tolbutamide in proportion to their ability to lower blood glucose. At the doses employed, insulin caused aortic PGI_2 and platelet TXA_2 generation from ^{14}C-AA to approach that seen in normoglycemic controls more effectively than tolbutamide. An inverse correlation with plasma glucose levels was observed for pulmonary PGI_2/TXA_2 ratios as well as aortic PGI_2 generation, $r = -0.72$ and -0.74, respectively. Platelet TXA_2 generation correlated positively with plasma glucose, $r = 0.69$. This study indicates that metabolism of ^{14}C-AA by pulmonary tissue, platelets, and aortas is altered by experimentally induced diabetes. Furthermore, these findings suggest that such alterations can be partially reversed by regulation of the diabetic condition. DIABETES 32:846–851, September 1983.

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Diabetes mellitus afflicts over five million Americans. Of all deaths among diabetics, 70% are associated with some type of cardiovascular disease.[1] Diabetics have numerous thrombotic complications including increased platelet aggregation,[2] abnormal platelet adhesiveness,[3] as well as elevated β-thromboglobulin[4] and plasma factor 4.[5] These abnormalities of the various clotting constituents may contribute to the elevation in atherosclerosis among diabetics.[6]

Prostaglandins have recently been reported by Moncada and co-workers[7] to possess extremely potent thrombotic and cardiovascular properties; alterations in several of these compounds have been observed in diabetic patients or in animal models of diabetes. The synthesis of prostacyclin (PGI_2), an inhibitor of platelet aggregation, is decreased in arteries from human diabetics.[8] An increase in the generation and release of thromboxane (TXA_2), a potent promoter of platelet aggregation, has been observed in platelets from diabetic Wistar rats[9] and diabetic patients.[2]

The lung, which consists of numerous cell types, has the capacity to synthesize PGI_2 and TXA_2 as well as PGE_2 and $PGF_{2\alpha}$. Perfused lungs isolated from rats 14 days after streptozotocin (STZ) showed a 30% decrease in PGI_2 synthesis with no change in TXA_2 generation.[10] Moreover, the pulmonary ratio of PGI_2/TXA_2 produced from ^{14}C-arachidonic acid (AA) was inversely correlated with plasma glucose levels. A decrease in vascular or pulmonary PGI_2 in association with elevated platelet TXA_2 secondary to hyperglycemia may promote localized platelet hyperaggregability and therefore enhance atherosclerotic and thrombotic development.

The impact of hypoglycemic agents on AA metabolism in diabetics or animal models of diabetes has not been well documented. Harrison and associates[11] have shown that chronic (8 days) but not acute (1 day) insulin treatment partially normalizes aortic PGI_2 generation in STZ-diabetic rats. No study of insulin's impact on AA metabolism in other tissues of diabetics, such as platelets or lungs, has been reported. Also, no determination of tolbutamide's role, a common, orally active hypoglycemic agent, in altering AA metabolism, has been shown for aortas, platelets, or lungs. This article describes the alterations that experimentally induced diabetes produces in the conversion of AA to prostacyclin and/or thromboxane by various tissues and the relative capabilities of insulin and tolbutamide to reverse these alterations.

MATERIALS AND METHODS

Materials. Prostaglandin standards for TLC were obtained from The Upjohn Company (Kalamazoo, Michigan). ^{14}C-AA (58.4 mCi/mmol) from Amersham Corp. (Arlington, Illinois), 4-bromomethyl-7-methoxycoumarin from Regis Chemical (Morton Grove, Illinois), NPH insulin (Eli Lilly and Company, Indianapolis, Indiana), tolbutamide (Upjohn), and Whatman LK5D TLC plates (Clifton, New Jersey) were used. LC grade ethyl acetate and acetone were purchased from MCB (Cincinnati, Ohio). STZ, unlabeled AA, and plasma glucose-oxidase assay kits were obtained from Sigma (St. Louis, Missouri). Keto-Diastix (Ames Laboratories, Elkhart, Indiana) were used to determine glucosuria. The fast glycosylated hemoglobin column chromatography method from Isolab (Akron, Ohio) was used to quantitate the severity of diabetes.

Induction and evaluation of diabetes. Male Sprague-Dawley albino rats (Harlan Industries, Indianapolis, Indiana) weighing 300–375 g were housed in Bioclean rooms at a constant ambient temperature (73°–75°F) on a 12-h light cycle with free access to Purina Rat Chow and water. Animals were given a minimum 10-day acclimatization period before initiation of any experimental procedures. Diabetes was induced with a 50-mg/kg intravenous dose of STZ in 0.1 M citrate buffer pH 4.5; controls received an equal volume of the citrate buffer vehicle. STZ-injected animals exhibited glucosuria within 24 h and were considered diabetic if urinary glucose levels exceeded 300 mg/dl. Glycosylated hemoglobin and plasma glucose levels were quantitated in control and diabetic rats.

Insulin or tolbutamide treatment. Rats were divided into six groups 21 days after injection with the citrate buffer vehicle or STZ. Groups 1 and 2 were citrate buffer controls, given s.c. saline or oral 0.25% methylcellulose, respectively, and were designated "controls." Groups 3 and 4 were STZ-treated, given s.c. saline or oral 0.25% methylcellulose, respectively, and were designated "diabetic-vehicle." Group 5 was STZ-treated, given NPH insulin 20 U/kg s.c., and designated as "diabetic-insulin." Group 6 was STZ-treated, given 200 mg/kg tolbutamide (suspended in 0.25% methylcellulose) orally, and designated "diabetic-tolbutamide." All vehicle or hypoglycemic treatments were given in the late afternoon to precede nocturnal food intake. Dosing was once daily for 10 days. Preliminary experiments indicated that s.c. saline and oral methylcellulose treatments resulted in identical amounts of aortic PGI_2 and platelet TXA_2 generation from ^{14}C-AA. Therefore, "controls" consisted of equal numbers of rats from groups 1 and 2. "Diabetic-vehicles" consisted of equal numbers of animals from groups 3 and 4. All rats were killed in the morning 31 days after STZ.

^{14}C-AA metabolism in isolated perfused lungs. Rats were selected in a sequence to randomize any diurnal variation in AA metabolism, then lightly anesthetized with ether, given 500 IU of heparin i.p., and laparotomized. Blood was collected from the dorsal aorta for preparation of washed platelets as described below.

The isolated perfused lung procedure developed by Niemier and Bingham[12] for rabbits was adapted for rats as briefly described previously.[13] Trachea, heart, and lungs were excised immediately after blood collection and quickly rinsed in physiologic saline (0.9%). The trachea and pulmonary artery were cannulated and inserted into a water-jacketed artificial thorax maintained at 37°C. Lungs were gently perfused until cleared of blood with 30 ml of Krebs-Ringer solution containing 4.5% bovine serum albumin at a flow of 10 ml/min. The initial perfusate was discarded and replaced with 30 ml of fresh recirculating perfusate maintained between pH 7.35 and 7.45 by balancing a slow glucose-bicarbonate infusion into the perfusate against the air-CO_2 ventilating gas. Lungs were ventilated to a 4-ml tidal volume at a rate of 50 inspirations/min with a warmed humidifed air-CO_2 mixture at atmospheric pressure. An alternating negative pressure between −2 and −6 cm of water was continually applied to the thorax with a respirator to provide pulmonary ventilation.

Following a 10-min stabilization period, 0.33 μmol of AA carrying 1 μCi ^{14}C-AA (58.4 mCi/mmol) in 500 μl of Krebs-Ringer was administered over a 20-s period into the perfusate entering the lung. One-milliliter aliquots of perfusate were collected at 3, 5, 10, and 15 min after AA administration and added immediately to tubes containing 50 μl of 1 N HCl, 100 mg NaCl, and 4 ml ethyl acetate.

AA metabolism was examined in perfused lungs isolated from rats 1, 3, 4, 14, and 30 days after STZ injection. AA metabolism in isolated perfused lungs was also measured in diabetic rats following 10 days of treatment with insulin or tolbutamide.

AA metabolism in aortas. The thoracic aorta was excised, rinsed in ice-cold phosphate buffer (0.04 M, pH 7.4), and all excess connective tissue removed. A 1-cm strip of aorta was cut into rings and placed in 0.5 ml phosphate buffer containing 5.5 mM glucose. The aortic rings were incubated for 15 min at 37°C with 0.2 μCi of ^{14}C-AA. Reactions were terminated by acidifying with 1 N HCl to pH 3. To quantitate AA metabolism, 450 μl of the incubant was extracted with ethyl acetate. The aortic rings were rinsed, dried in a desicator, and aortic PGI_2 production expressed as pmol/mg dry aortic tissue.

AA metabolism in platelets. A 9-ml sample of blood was collected from the dorsal aorta into a syringe containing 1 ml of 3.8% sodium citrate. Platelet-rich plasma (PRP) was obtained by centrifuging the blood at 200 × *g* for 10 min at room temperature. The PRP was respun at 1200 × *g* for 10 min to harvest the platelet pellet. Platelets were resuspended in phosphate buffer (0.04 M, pH 7.4) containing 5.5 mM glucose and adjusted to 6×10^8 platelets/ml using a ZBI Coulter Counter (Coulter Electronics, Hialeah, Florida). One

TABLE 1
Pulmonary PGI_2/TXA_2 ratios* after streptozotocin† injection

	Day			
	1	7	14	30
Control‡	2.2 ± 0.26	1.9 ± 0.22	2.1 ± 0.18	1.8 ± 0.15
Diabetic	1.8 ± 0.84	1.3 ± 0.16§	1.1 ± 0.12§	1.3 ± 0.20§

*Values represent the mean ± SEM (N = 6) and were determined as described in MATERIALS AND METHODS.
†Streptozotocin was administered i.v. in 0.1 M citrate buffer pH 4.5.
‡Control animals received an equal volume of the citrate buffer vehicle.
§Significantly different from control ($P < 0.05$).

milliliter of platelet suspension was incubated for 2 min at 26°C with 25 μl of a solution containing 0.05 μCi ^{14}C-AA and 2.5 μg unlabeled AA. Incubations were terminated by acidification to pH 3.

Assay of AA metabolites. Arachidonic acid metabolites were extracted and isolated by TLC. The method utilizes a quantitative adaptation of the procedure of Turk et al.[14] that we have described previously.[10,13] Samples were extracted 3 times with 4 ml of ethyl acetate and the organic layer was dried under a stream of nitrogen. The residue was combined with 2 μg of authentic PGE_2, $PGF_{2\alpha}$, TXB_2, and 6-keto-$PGF_{1\alpha}$ standards before derivatization with 4-bromo-methyl-7-methoxycoumarin.[14] Sixty microliters of acetone was added to the dried derivatives and 40 μl spotted on Whatman LK5D TLC plates that were subsequently developed in a chloroform:methanol (100:5) system. Bands containing prostaglandins were identified under fluorescent light, scraped into scintillation vials, and the radioactivity quantitated as disintegrations per minute using quench curves. Rf values were 0.15, 0.18, 0.28, 0.33, and 0.79 for 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, TXB_2, PGE_2, and AA, respectively.

Statistical analysis. Differences in the generation of aortic PGI_2 or platelet TXA_2 between control, diabetic-vehicle, diabetic-tolbutamide, and diabetic-insulin groups were compared using a Student Newman-Keuls test and considered significant at $P < 0.05$. The pulmonary ratio of PGI_2/TXA_2 was calculated from the levels of 6-keto-$PGF_{1\alpha}$, the stable end product of PGI_2, and TXB_2, the stable end product of TXA_2, for control and diabetic groups 15 min after AA addition. The ratios of PGI_2/TXA_2 were plotted against plasma glucose levels at the time of death and a linear regression performed.

RESULTS

Blood was collected 1, 7, 14, and 30 days following administration of STZ; glucose concentrations ranged from 393 ± 15 to 712 ± 10 mg/dl (mean ± SEM, N = 6).

It has recently been suggested that a diminished ratio of PGI_2/TXA_2 may be associated with development of atherosclerosis and thrombotic conditions.[15] Consequently, the impact of STZ-induced diabetes on ^{14}C-AA metabolism in isolated perfused lungs was assessed as the ratio of PGI_2/TXA_2 15 min after the addition of ^{14}C-AA. The diabetic animals had a diminished pulmonary PGI_2/TXA_2 ratio beginning 3 days after STZ injection (Table 1). The diminished PGI_2/TXA_2 ratio was due primarily to decreases in PGI_2, which were depressed 27–35%, rather than to increases in TXA_2, which were elevated 0–5%. The difference between control and diabetic PGI_2/TXA_2 ratios was statistically significant ($P < 0.05$) beginning 7 days after STZ injection and continuing throughout the 30-day study. More importantly, the diminished pulmonary PGI_2/TXA_2 ratios observed in the diabetic group were inversely correlated with plasma glucose levels at the time of death (Figure 1).

To determine the hypoglycemic efficacy of the insulin and tolbutamide doses used in this study, plasma glucose concentrations were measured at 2–4-h intervals over a 16-h period from 7 p.m. to 9 a.m. Controls, diabetic-vehicle, diabetic-tolbutamide, and diabetic-insulin groups displayed plasma glucose levels that varied from 50 ± 1 to 118 ± 6, 385 ± 14 to 621 ± 6, 240 ± 10 to 369 ± 12, and 92 ± 9 to 208 ± 25 mg/dl, respectively (mean ± SEM, N = 6); glycosylated hemoglobin was 3.3 ± 0.1%, 5.6 ± 0.7%, 4.5 ± 0.2%, and 5.2 ± 0.5%, respectively (mean ± SEM, N = 6).

The impact of 10-day insulin or tolbutamide treatment on AA metabolism was determined in aortas, platelets, and isolated perfused lungs. As shown in Figure 2A, aortic PGI_2 generation was diminished 28% in diabetic-vehicle rats as compared with normoglycemic controls ($P < 0.05$). Insulin or tolbutamide treatment for 10 days reversed the diminished aortic PGI_2 generation seen in the diabetic rats up to a level not statistically different from the normoglycemic control group.

Platelet TXA_2 generation was increased 67% in the diabetic-vehicle rats ($P < 0.05$) when compared with controls, as shown in Figure 2B. After 10 days of insulin treatment, this elevation was reversed and not statistically different from control. Tolbutamide treatment, on the other hand, did not lower platelet TXA_2 when compared with diabetic-vehicle animals.

Correlation coefficients were calculated for plasma glucose levels at death versus pulmonary PGI_2/TXA_2 ratios, aortic PGI_2 generation, and platelet TXA_2 production. An inverse correlation between pulmonary PGI_2/TXA_2 ratios and plasma glucose levels was observed ($r = -0.72$, $P < 0.05$, Figure 3). A similar inverse relationship was obtained between aortic PGI_2 production and plasma glucose levels ($r = -0.74$, $P < 0.05$) while a positive correlation was observed between platelet TXA_2 and plasma glucose ($r = 0.69$, $P < 0.05$, Figure 4).

DISCUSSION

In these studies, diabetes was induced by STZ administration, which decreases insulin secretion by damaging the

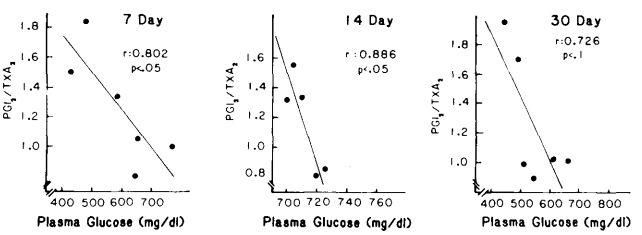

FIGURE 1. Relationships of pulmonary PGI_2/TXA_2 ratios to plasma glucose levels 7, 14, and 30 days following 50 mg/kg streptozotocin i.v. PGI_2/TXA_2 ratios were determined as described in MATERIALS AND METHODS.

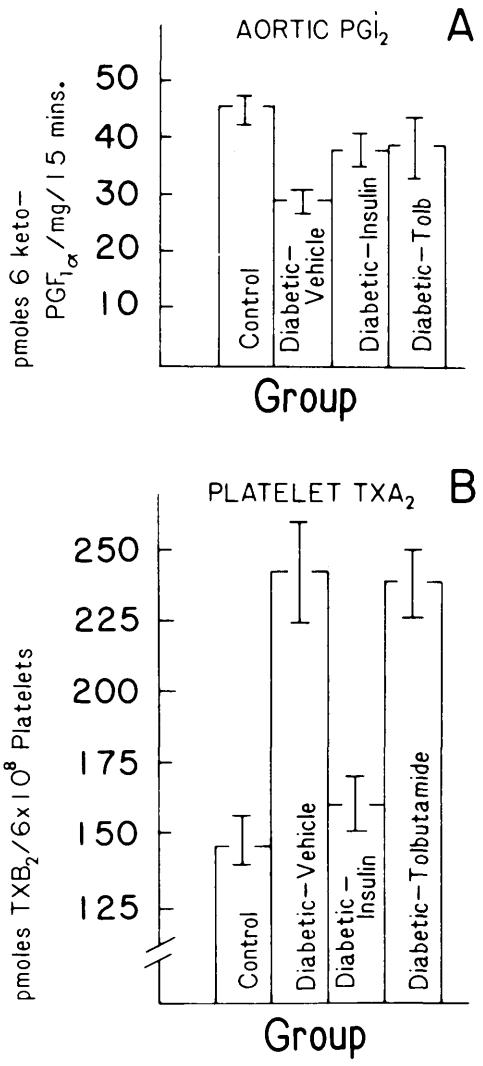

FIGURE 2. A: PGI_2 generation from ^{14}C-AA (measured as 6-keto $PGF_{1\alpha}$, the stable end product of PGI_2 decomposition) in aortic tissue of control, diabetic-vehicle, diabetic-insulin, and diabetic-tolbutamide groups. Diabetic-vehicle is significantly different ($P < 0.05$) from control, diabetic-insulin, and diabetic-tolbutamide. B: TXA_2 generation from ^{14}C-AA (measured as TXB_2, the stable end product of TXA_2 decomposition) in platelets from control, diabetic-vehicle, diabetic-insulin, and diabetic-tolbutamide rats. Diabetic-vehicle and diabetic-tolbutamide were statistically different ($P < 0.05$) from control and diabetic-insulin groups. Controls were given 0.1 M citrate buffer pH 4.5 i.v. After 21 days, half were given saline s.c. and the other half 0.25% methylcellulose orally once daily for 10 days. Diabetic-vehicle rats were given 50 mg/kg STZ i.v. in 0.1 M citrate buffer pH 4.5. After 21 days, half were given saline s.c.; the other half were given 0.25% methylcellulose orally once daily for 10 days. Diabetic-insulin rats were made diabetic using the same dose of STZ. Twenty-one days later, they were given insulin 20 U/kg s.c. daily for 10 days. Diabetic-tolbutamide animals were given orally 200 mg/kg tolbutamide suspended in 0.25% methylcellulose daily for 10 days. All values represent the mean ± SEM (N = 6).

pancreas.[16] This form of induction of hyperglycemia is a commonly used model for type I diabetes or for type II diabetes due to deficient insulin secretion. It is not an adequate model for some forms of type II diabetes in which insulin levels are elevated, and hyperglycemia is due to receptor or postreceptor abnormalities.[17]

Streptozotocin treatment evoked a chronic fourfold increase in blood glucose levels beginning 24 h after injection

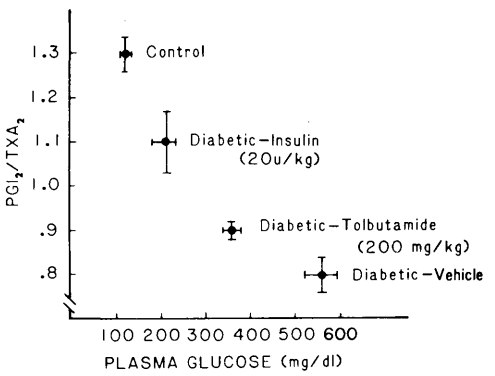

FIGURE 3. Relationship of pulmonary PGI_2/TXA_2 ratios to plasma glucose levels of control, diabetic-vehicle, diabetic-insulin, and diabetic-tolbutamide groups 15 min after ^{14}C-AA addition to isolated perfused lungs ($r = -0.72$). All values represent the mean ± SEM (N = 6). Treatments were as described in the legend to Figure 2.

and continuing throughout the 30-day study. Pulmonary AA metabolism, measured as the ratio of PGI_2 to TXA_2 since both substances are synthesized by the lung, was not altered until 7 days after STZ injection. In addition, lowering plasma glucose levels with insulin or tolbutamide tended to reverse the depression in pulmonary PGI_2/TXA_2 ratios seen in the diabetic rats. These two observations indicate that the changes occurring in pulmonary prostaglandin generation during the 30-day study can probably be attributed to the progression of the diabetic state rather than any direct immediate effect on pulmonary AA metabolism by the STZ. These alterations in pulmonary AA metabolism extend and confirm our original observation that depressed pulmonary PGI_2/TXA_2 ratios occur in rats made diabetic for 14 days.[10]

AA metabolism was also measured in aortas and platelets 30 days after STZ treatment in order to compare the impact of experimentally induced diabetes on prostaglandin generation in these tissues. A 28% reduction in aortic PGI_2, as well as a 67% increase in platelet TXA_2 synthesis, occurred in the diabetic group. These results agree with data from studies utilizing humans or animal models of diabetes in which aortic PGI_2 decreased from 15% to 40%[9,18,19] and platelet TXA_2 increased from 15% to 60%.[8,9,19]

In the United States, atherosclerotic disease is a prevalent complication among diabetic persons, accounting for nearly

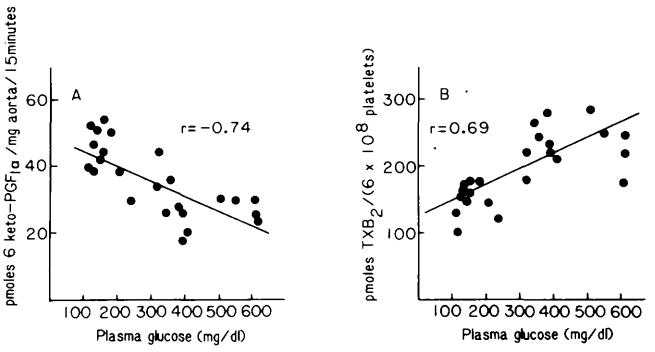

FIGURE 4. Relationship of (A) aortic PGI_2 ($r = -0.74$, $P < 0.05$) and (B) platelet TXA_2 ($r = 0.69$, $P < 0.05$) to plasma glucose levels at time of death in control, diabetic-vehicle, diabetic-insulin, and diabetic-tolbutamide groups. Treatments were as described in the legend to Figure 2.

70% of all deaths. Diabetic individuals frequently have platelet hyperaggregability[2] as well as a shortened platelet survival time:[20] An interaction of platelet aggregates with blood vessel walls has been postulated as an early step in the development of atherosclerotic plaque formation.[21] The altered platelet and aortic AA metabolism, coupled with the diminished pulmonary PGI_2/TXA_2 ratios that we observed, may partially account for the platelet hyperaggregability and thrombotic complications present in people with diabetes and contribute to the progression of atherosclerosis.

Ordinarily, a diabetic patient takes an insulin or tolbutamide dose in the morning preceding the rise in plasma glucose that occurs after breakfast. Rats are nocturnal with their greatest food intake and activity in the evening. Consequently, animals in this study were dosed with insulin or tolbutamide in the late afternoon to parallel the diabetic patient's treatment pattern.

Insulin treatment considerably reversed the alterations in pulmonary PGI_2/TXA_2 ratios seen in the diabetic group and returned the aortic PGI_2 and platelet TXA_2 values to levels indistinguishable from those obtained in control rats. The aortic PGI_2 data are in agreement with those reported by Harrison et al.,[11] who demonstrated that 8 days of treatment with insulin in diabetic rats increased aortic PGI_2 production to levels indistinguishable from those seen in control rats. One-day insulin treatment, however, was insufficient to correct aortic PGI_2 synthesis.[22]

Tolbutamide treatment (200 mg/kg orally) was unable to return plasma glucose levels to normal but improved them considerably over that seen in the vehicle-treated diabetic rats. This improvement in blood glucose was sufficient to cause a minor improvement in pulmonary PGI_2/TXA_2 ratios and return aortic PGI_2 generation to normal. The treatment was completely ineffective at correcting the excess platelet TXA_2 synthesis seen in the diabetic rats. These results imply either that platelets are more sensitive to poor diabetes control than vascular tissue or that tolbutamide produces some alterations in the handling of AA independent of its ability to lower plasma glucose. Alternatively, the duration of tolbutamide treatment may have been insufficient for the resulting correction of plasma glucose to adequately influence platelet AA metabolism. It is known that recovery from administration of the irreversible cyclooxygenase inhibitor aspirin is more rapid in the vasculature than in platelets, which are unable to synthesize new protein.[23] Unlike aortic tissue, mature, circulating platelets have no protein turnover, and therefore changes in net platelet enzyme activity require new platelet generation.

These results also imply that treatment with low doses of irreversible cyclooxygenase inhibitors (i.e., aspirin) may be a desirable addition to oral hypoglycemic therapy. The oral hypoglycemic serves to correct elevated blood glucose and increase vascular PGI_2 synthesis, while the irreversible cyclooxygenase inhibitor decreases platelet TXA_2 production.

A primary role of insulin is to control circulating glucose levels. The impact of glucose concentration on AA metabolism is not well understood. Tannenbaum and co-workers[24] have described a decrease in renal PGE_2 and $PGF_{2\alpha}$ as a function of increasing glucose concentrations. Glucose may not be the only factor influencing altered AA metabolism in diabetes, however. Diabetic individuals have lower ratios of high-density lipoproteins (HDL) to low-density lipoproteins (LDL).[25] This imbalance may also have a direct impact on AA metabolism since Szczeklik and associates[26] reported LDL decreased aortic PGI_2 generation, while HDL had no effect.

Hyperinsulinemia has also been reported as a risk factor for atherosclerosis.[27] Insulin has been shown to decrease aortic PGI_2 generation after direct addition to aortic tissue.[28] The deleterious effect of hyperinsulinemia, as compared with the advantageous effect of low insulin treatment we observed, suggests a possible bimodal relationship between the level of circulating insulin and aortic PGI_2 generation.

If, as these studies suggest, improvement in the balance of PGI_2 and TXA_2 in lungs, aortas, and platelets is accomplished by better regulation of plasma glucose, and if the overall balance of PGI_2 and TXA_2 is important in the development of atherosclerosis and other vascular diseases, then better regulation of the diabetic condition should increase longevity and reduce the vascular complications of diabetes.

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REFERENCES

[1] Marble, A.: Late complications of diabetes. A continuing challenge. Diabetologia 1976; 12:193–99.

[2] Halushka, P. V., Mayfield, R., Wohltmann, H. J., Rogers, R. C., Goldberg, A. K., McCoy, S. A., Loadholt, C. B., and Colwell, J. A.: Increased platelet AA metabolism in diabetes mellitus. Diabetes 1981; 30 (Suppl. 2):44–48.

[3] Kwaan, H. C., Colwell, J. A., Cruz, S., Suwanwela, N., and Dobbie, J. G.: Increased platelet aggregation in diabetes mellitus. J. Lab. Clin. Med. 1972; 80:236–46.

[4] Borsey, D. Q., Dawes, J., Fraser, D. M., Prowse, C. V., Elton, R. A., and Clarke, B. F.: Plasma B-thromboglobulin in diabetes mellitus. Diabetologia 1980; 18:353–57.

[5] Betteridge, D. J., Zahaui, J., Jones, N. A., Shine, B., Kakkar, V. V., and Galton, D. J.: Platelet function in diabetes mellitus in relationship to complications, glycosylated haemoglobin and serum lipoproteins. Eur. J. Clin. Invest. 1981; 11:273–77.

[6] Steiner, G.: Diabetes and atherosclerosis. Diabetes 1981; 30 (Suppl. 2):1–7.

[7] Moncada, S., Gryglewski, R. J., Buntings, S., and Vane, J. R.: An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 1976; 263:663–65.

[8] Johnson, M., Harrison, H. E., Raferty, A. T., and Elder, J. B.: Vascular prostacyclin may be reduced in diabetes in man. Lancet 1979; 1:325–26.

[9] Subbiah, M. T. R., and Deitemeyer, D.: Altered synthesis of prostaglandins in platelet and aorta from spontaneously diabetic Wistar rats. Biochem. Med. 1980; 23:231–35.

[10] Lubawy, W. C., and Valentovic, M.: Streptozocin induced diabetes decreases formation of prostacyclin from arachidonic acid in intact rat lungs. Biochem. Med. 1982; 28:290–97.

[11] Harrison, H. E., Reece, A. H., and Johnson, M.: Effect of insulin treatment on prostacyclin in experimental diabetes. Diabetologia 1980; 18:65–68.

[12] Niemier, R. W., and Bingham, E.: An isolated perfused lung system for metabolic studies. Life Sci. 1972; 11:807.

[13] Valentovic, M., Gairola, C., and Lubawy, W.: Lung, aorta and platelet metabolism of ^{14}C-arachidonic acid in vitamin E-deficient rats. Prostaglandins 1982; 24:215–24.

[14] Turk, J., Weiss, S. J., Davis, J., and Needleman, P.: Fluorescent derivatives of prostaglandins and thromboxanes for liquid chromatography. Prostaglandins 1978; 16:291–309.

[15] Moncada, S., and Vane, J. R.: Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. Pharmacol Rev. 1979; 30:293–331.

[16] Rerup, C. W.: Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev. 1970; 22:485–518.

[17] Robbins, S., Angell, M., and Kumar, V.: Genetic Diseases in Basic Pathology. Philadelphia, W. B. Saunders, 1981:133–47.

[18] El Tahir, K. E. H., Williams, K. I., and Betteridge, D. J.: The effect of experimental diabetes on prostacyclin production by tissues from pregnant rats. Prost. Leuk. Med. 1982; 8:429–35.

[19] Gerrard, J. M., Stuart, M. J., Rao, G. H., Steffes, M. W., Mouer, S. M., Brown, D. M., and White, J. G.: Alteration in the balance of prostaglandin and thromboxane synthesis in diabetic rats. J. Lab. Clin. Med. 1980; 95:950–58.

[20] Paton, R. C.: Platelet survival in diabetes mellitus using an aspirin technique. Thromb. Res. 1979; 15:793–802.

[21] Moncada, S.: Prostacyclin and arterial wall biology. Arteriosclerosis 1982; 2:193–207.

[22] Harrison, H. E., Reece, A. H., and Johnson, M.: Decreased vascular prostacyclin in experimental diabetes. Life Sci. 1978; 23:351–56.

[23] Moncada, S.: Biological importance of prostacyclin. Br. J. Pharmacol. 1982; 76:3–31.

[24] Tannenbaum, J., Sweetman, B. J., Nies, A. S., Aulsebrook, K., and Oates, J. A.: The effect of glucose on the synthesis of prostaglandins by the renal papilla of the rat in vitro. Prostaglandins 1979; 17:337–50.

[25] Dornan, T. L., Carter, R. D., Bron, A. J., Turner, R. C., and Munn, J. I.: Low density lipoprotein cholesterol: an association with the severity of diabetic retinopathy. Diabetologia 1982; 22:167–70.

[26] Szczeklik, A., Gryglewski, R. J., Domagala, B., Zmuda, A., Hartwich, J., Wozny, E., Grzywacz, M., Madej, J., and Gryglewska, T.: Serum lipoproteins, lipid peroxides and prostacyclin biosynthesis in patients with coronary heart disease. Prostaglandins 1981; 22:795–807.

[27] Stout, R. W.: Diabetes and atherosclerosis—the role of insulin. Diabetologia 1979; 16:141–50.

[28] Lasche, E. M., and Larson, R. E.: Interaction of insulin and prostacyclin production in the rat. Diabetes 1982; 31:454–58.