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Early Arteriolar and Capillary Changes in Streptozotocin-Induced Diabetic Rats and Intraperitoneal Hyperglycaemic Rats

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Summary. Comparison of intestinal arteriolar characteristics in vivo were made in normal rats, normal rats injected intraperitoneally twice daily with either 0.15 mmol/l saline or 16.6 mmol/l glucose in 0.15 mmol/l saline (total value 5% of body weight), and in streptozotocin treated rats (plasma glucose > 22.2 mmol/l). Each regimen was continued for 4–5 weeks before the study. Interperitoneal injection of glucose increased the plasma glucose concentration by 0.8–1.6 mmol/l for 2–2.5 h. The microvascular characteristics of normal and saline injected rats were identical. Results obtained in glucose-treated and diabetic animals were very similar and included: 1) resting arteriolar vasodilation, 2) subnormal dilation at removal of all vascular control with adenosine, 3) total arteriolar vessel wall, including smooth muscle, cross-sectional area and wall to lumen ratio one-third to one-half less than normal, and 4) capillary separation distances increased above normal by 40%–50%. The results indicate that the morphological and functional changes in intestinal arterioles and capillaries found in diabetic rats after 4–5 weeks can be reproduced in the intestinal tissues of a normal animal exposed to intermittent increases in intraperitoneal glucose concentration.

Key words: Diabetes, hyperglycaemia, microvasculature, microangiopathy.

Determination of the relative roles of hyperglycaemia as opposed to a disturbance of insulin in the generation of a morphologically and functionally impaired microvasculature in diabetes mellitus is difficult. Even though West et al. [19] and others [12, 17] have reported a weak correlation between retinopathy and nephropathy and hyperglycaemia, one or more mechanisms better correlated to hyperglycaemia and microvascular anomalies could cause both conditions. We [4] and others [1] have shown previously that hyperglycaemia in hyper- and hypoinsulinaemic models of diabetes is associated with a common form of microvascular disturbance. However, these data [1, 4] and those obtained in studies of excessive dietary glucose intake [11, 13, 14] which indicated a correlation of glucose levels and vascular disturbances, can all be criticized because overall metabolism is deranged and the general health of the whole animal is impaired. In this context, a better model to study the influence of hyperglycaemia on the vasculature would be a healthy, normoglycaemic and normoinsulinaemic animal which has a limited portion of the vasculature exposed to hyperglycaemia. This can be achieved by daily intraperitoneal injections of a hyperglycaemic physiological solution followed by analysis in vivo of intestinal microvascular morphology and function. Comparisons of microvascular disturbances in this model were therefore made to those in streptozotocin-induced diabetic rats.

Methods

Male rats aged 18–20 weeks, and from the same litters and colony were used for all studies. Animals were made diabetic with a single IV injection of streptozotocin (STZ) (65 mg/kg). Within 3 days after injection, all STZ-treated rats had a plasma glucose concentration in excess of 14 mmol/l. Other groups of non-diabetic rats were either only handled or received twice daily IP injections (equivalent to 5% of body weight) of either physiological saline (Normosol-R, Abbott Laboratories, Chicago, USA) or 16.6 mmol/l glucose in physiological saline solution. All solutions were isotonic. Animals were carefully observed in order to detect any signs of peritoneal trauma or infection. Two of 26 animals which received injections did develop signs of trauma and were destroyed; all others were normal at the time of surgery. Both IP injected and diabetic rats were studied 4–5 weeks after commencement of the experiment. Normal rats which received no treatment other than handling were

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kept in the same environmental conditions as the other animals and studied at an age similar to that of the other groups. All animals were anaesthetized with a saline solution of 10% urethane and 2% chloralose (0.6 ml/100 g, IP).

The intestinal muscle and submucosal microvasculatures were prepared by previously described techniques [3]. The tissue was bathed with a bicarbonate buffered physiological solution [2] held at 37 °C. The pO_2, pCO_2 and pH of the fluid in contact with the tissues was 40–42 mmHg, 42–45 mmHg and pH 7.37–7.39, respectively. These conditions closely reflect the gaseous and pH properties of the intra-abdominal fluid [2]. All preparations were allowed to equilibrate for approximately 45 min after the end of surgical procedures. If the mean arterial pressure during the experiment decreased by more than 15 mmHg, the experiment was terminated.

The vessels were observed with a Nikon SKE microscope system and all optical images were video-displayed and recorded for later analysis. The magnification at the face of a video monitor was calculated with the use of a stage micrometer marked in 10 and 100 μm units. The magnification range used was × 420–860 and was known for each vessel. The vessel dimensions were measured on the video monitor with a ruler and transformed to microns by an appropriate calibration factor.

Vessel diameters were measured at 10 s intervals during still frame replay of video recordings. The average diameter was computed for a minimum period of 60 s. The thickness of the vessel wall for the average diameter was calculated from multiple determinations of the vessel wall thickness and cross-sectional area at different diameters. Arterioles were selected at random on the basis of visual clarity and their branching order, to be subsequently defined, was ascertained. In order to abolish transiently all vascular control so that the passive diameter of a given vessel could be measured, a bathing fluid containing 10^{-4} mmol/l adenosine was flushed over the tissue. Tissue exposure to adenosine had no influence on mean arterial blood pressure. After the adenosine exposure ceased, the vessels returned to their previous diameter within 3 min. In order to measure intercapillary distances, a constant suffusion of adenosine was used and the distances between all the adjacent capillaries (wall-to-wall) perfused by the same arteriole in the muscle layer were recorded. For a given arteriole, all capillaries lie in the same tissue plane and the measured separation distance need not be corrected for different depths of the capillaries in tissue. In addition, the capillaries lay almost parallel to each other in the muscle tissue.

All data are expressed as mean ± SEM based on the mean of data of a given type recorded in each animal. In this way, each animal makes the same contribution to the data population. Values of $p < 0.05$ were chosen to indicate significant differences from means obtained in normal animals. P values were determined with Duncan's new multiple range test [18]. The data analysis was based on successful studies of nine STZ-treated, nine glucose-treated and 12 normal or saline treated rats.

Complete descriptions of the intestinal microvasculature have been described previously [9]. In brief, the largest arteriole to enter the tissue is termed the first order arteriole and subsequent branches are numbered consecutively. Five branch orders of arterioles exist and all branch orders can be readily observed except the fourth order arterioles. The fourth order arterioles rise through the tissue parallel to the optical axis of the microscope and are seen 'end-on'. This substantially impairs their observation.

Results

Normal animals and animals that received saline or glucose injections had a basal plasma glucose concentration of 5–6.6 mmol/l. Saline injection did not have a significant influence on plasma glucose. However, glucose injection increased the systemic plasma glucose from 5.58 ± 0.19 mmol/l (mean ± SEM) at rest to 6.91 ± 0.75 mmol/l during the second hour after injection. During the third hour after injection, the plasma glucose concentration was not different from the initial resting concentration. Rats treated with STZ had a basal plasma glucose of 26.8 ± 0.82 mmol/l during the 4–5 weeks following the STZ injection.

Glucose and saline injections had no appreciable influence on body weight during the period of study. In contrast, the STZ diabetic animals were emaciated and body weight gain during the period of study was less than half of normal. The mean arterial blood pressure in all animal groups during the experiments was 95–120 mmHg and no differences in mean arterial blood pressures between groups were noted.

Arteriolar Diameter Characteristics

The inner diameters at control and during passive conditions and the percentage of control diameters when passive are presented in Table 1. There were no significant differences between normal and saline injected animals and therefore results from these two groups were combined.

With the exception of the large first order arterioles, the other arterioles of diabetic and glucose-treated rats when at control conditions were significantly dilated relative to similar types of normal vessels ($p < 0.05$). Following removal of all vascular control with adenosine, the passive diameter of comparable types of arterioles was not significantly different ($p > 0.05$). Thus, the arterioles of diabetic and glucose-treated rats were dilated at rest but had a normal passive diameter when fully relaxed. As a consequence, the percentage of control diameters of passive vessels in diabetic and glucose-treated rats were smaller than normal in almost all cases (Table 1).

Vessel Wall Characteristics

Histological studies in vitro and observation in vivo indicated that a continuous, unicellular coat of vascular smooth muscle cells was present for first, second and third order arterioles. Data for these vessels are presented in Table 2.

The vessel wall thickness, wall area and wall to lumen ratio for all arterioles in diabetic and glucose-treated rats at rest were significantly smaller than normal in more than half of the groups studied ($p < 0.05$). Vessel wall area was measured by subtracting the area based on the inner vessel diameter of the vessel from the area based on the total outer diameter of the ves-

Table 1. Inner arteriolar diameters at rest and passive states for normal, streptozotocin-diabetic (STZ) and glucose injected rats

Arteriolar order	Rat experimental group	No. of vessels measured	No. of animals	Control inner diameter (μm)	Passive inner diameter (μm)	% of control diameter
First	Normal	9	12	71.0 ± 5.1	77.4 ± 7.9	124 ± 3
	STZ-diabetic	31	9	68.5 ± 3.9	81.4 ± 1.6	120 ± 3
	Glucose injected	25	9	70.1 ± 4.4	80.7 ± 3.9	120 ± 4
Second	Normal	14	12	17.7 ± 0.9	31.9 ± 2.5	174 ± 14
	STZ-diabetic	26	9	21.3 ± 1.5[a]	32.9 ± 2.1	155 ± 7[a]
	Glucose injected	34	9	22.1 ± 2.5[a]	33.5 ± 1.3	163 ± 12
Third	Normal	14	12	9.3 ± 0.7	16.2 ± 1.1	184 ± 25
	STZ-diabetic	24	9	11.1 ± 0.8[a]	16.3 ± 0.9	152 ± 14[a]
	Glucose injected	26	9	10.2 ± 0.3[a]	15.9 ± 0.6	152 ± 7[a]
Fifth	Normal[1]	11	12	7.0 ± 0.7	9.5 ± 1.5	120 ± 2
	STZ-diabetic	23	9	8.3 ± 0.2[a]	8.9 ± 0.2	109 ± 1[a]
	Glucose injected	22	9	8.4 ± 0.2[a]	9.3 ± 0.3	109 ± 1[a]

Results expressed as mean ± SEM. Passive inner diameter refers to the diameter after adenosine treatment
[a] Significantly different from normal rats ($p < 0.05$)
[1] Vessel wall characteristics not measured for fourth order arterioles

Table 2. Arteriolar wall characteristics at rest in normal, streptozotocin-diabetic (STZ), and glucose injected rats

Arteriolar order	Rat experimental group	Wall thickness (μm)	Wall area (μm²)	Wall to diameter ratio
First	Normal	16.1 ± 0.7	4477 ± 80	0.24 ± 0.03
	STZ	11.2 ± 0.4[a]	2887 ± 219[a]	0.17 ± 0.01[a]
	Glucose injected	12.6 ± 1.1[a]	3474 ± 337[a]	0.20 ± 0.02[a]
Second	Normal	17.7 ± 0.1	817 ± 101	0.42 ± 0.01
	STZ	6.0 ± 0.4[a]	540 ± 62[a]	0.29 ± 0.02[a]
	Glucose injected	6.7 ± 0.5	695 ± 74	0.35 ± 0.03
Third	Normal	6.0 ± 0.3	357 ± 57	0.67 ± 0.02
	STZ	5.3 ± 0.3	272 ± 25[a]	0.49 ± 0.03
	Glucose injected	5.8 ± 0.2	311 ± 24	0.58 ± 0.03[a]

Results expressed as mean ± SEM. Appropriate vessel diameter at rest and number of vessels and animals as in Table 1. Same vessels studied as in Table 1
[a] Significantly different from normal ($p < 0.05$)

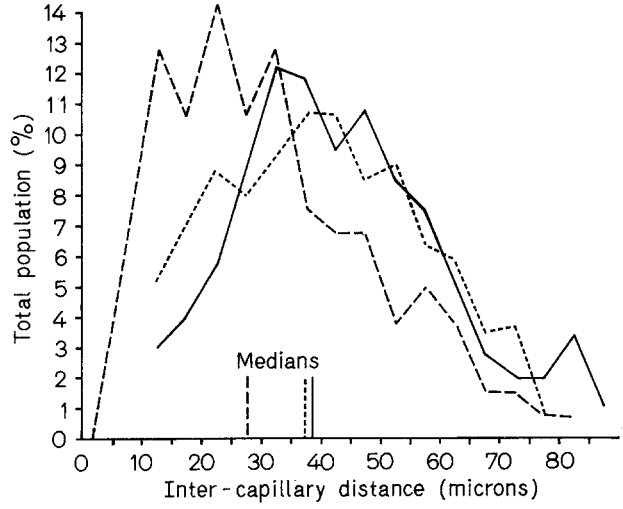

Fig. 1. Distribution of inter-capillary distances during totally passive conditions for normal (----), glucose-treated (——) and streptozotocin diabetic rats (------). The mean ± SEM separation distances were 33.8 ± 1.4 μ for normal, 44.6 ± 1.1 μ for glucose injected and 41.3 ± 0.9 μ for STZ-diabetic rats. Median distances are presented by vertical lines along the abscissa. Number of capillary separation distances measured in each animal type: 132 (normal), 296 (glucose-injected) and 375 (STZ-diabetic)

sel, including the vessel wall. If the vessel wall thickness was not equal on both sides of the vessel, a calculation was not made.

Capillary Separation Distances

To ensure an adequate evaluation of the minimum capillary separation distances, these measurements were made in totally passive vasculatures. The distributions of intercapillary separation distances are shown in Figure 1. The mean intercapillary distance from capillary wall to capillary wall was 33.8 ± 1.4 μ in normal rats compared with 44.6 ± 1.1 μ ($p < 0.01$) and 41.3 ± 0.9 μ ($p < 0.01$) in glucose and STZ-treated rats. From the histographical distribution of intercapillary distances, it was evident that the distribu-

tions in glucose and STZ-treated animals were very similar. In addition, the distributions of the distances in glucose-treated and diabetic rats were higher than in normal rats.

Discussion

The major finding of this study was that intermittent intraperitoneal hyperglycaemia in normoglycaemic rats caused a group of intestinal microvascular disturbances qualitatively and, in the majority of instances, quantitatively similar to those in the early stages of chemically induced diabetes of equal duration. Therefore, excessive exposure to glucose can be a causative factor in the precipitation of vascular disturbances similar in form to those in the diabetic animal. This does not imply that glucose itself caused the microvascular disturbances or that intraperitoneal glucose injection is a model of diabetes mellitus. However, the results are consistent with the hypothesis that excessive glucose has the potential to initiate mechanisms related to early vascular disturbances of diabetes.

The study indicated that the early stages of diabetes and injections of glucose in the rat are associated with microvascular dilation at rest, as shown in Table 1. The clinical finding in man is that some degree of vasodilation occurs in the very early stages of diabetes [7, 8]. Longer duration of diabetes [5, 6] causes constriction of the arterioles of diabetic mice and the vessels cannot be made to dilate to a normal passive diameter as the vessel wall becomes progressively thinner. Ditzel et al. [7, 8] have reported from long term observations in man that vasoconstriction develops progressively and some form of vessel wall atrophy occurs [8], as would appear to be the case for rodents [5, 6]. As shown in Table 2, a one-fourth to one-third decrease in vessel cross-sectional wall area and vessel lumen to wall thickness ratio occurred in diabetic and glucose-treated animals four weeks after onset. Therefore, the degeneration of arteriolar wall materials, such as smooth muscle, apparently begins early in the diabetic process, although there is a possibility that the arteriolar basement membrane hypertrophies as occurs in capillaries.

One of the major indices of impending microvascular disturbances after four weeks of hyperglycemia was the decreased number of capillaries in the tissue, as indicated by increased intercapillary distances (Fig. 1) in both diabetic and glucose-treated rats. Capillary rarefaction is characteristic of longer duration diabetes in animals [5, 6] and may be related to the enzymatic anomalies and loss of pericytes of the diabetic capillary described by Agren et al. [1]. Katz and Janjan [15] have found the forearm capillary filtration coefficient at rest and during tonic arm exercise to be nearly one-half normal in stable, non-insulin dependent diabetic men. In view of the general increase in capillary permeability during diabetes [10, 17], a decline in capillary filtration coefficient may represent a loss of functional capillaries in man as has been demonstrated in rodents.

The existence of a qualitatively and nearly quantitatively similar set of microvascular disturbances in actual diabetic rats and normal rats subjected to intermittent intraperitoneal hyperglycaemia provides several indications as to the prerequisites for the development of diabetic-like microvascular disturbances. The abdominal fluid glucose concentration was 16.6 mmol/l immediately after injection and decreased to below 11 mmol/l by the beginning of the third hour after injection. In effect, the animal had an intra-abdominal hyperglycaemia between 11–16.6 mmol/l twice daily for a total duration of about 4 h each day. In contrast, the STZ-treated animals maintained a plasma hyperglycaemia in excess of 22 mmol/l and their microvascular disturbances were only marginally worse than those of glucose injected animals. This indicates two important points. First, abdominal fluid glucose concentrations of 16.6 mmol/l and somewhat lower precipitated the full development of intestinal microvascular anomalies. Second, the duration of hyperglycaemia each day can be relatively short and still activate the development of vascular disturbances.

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