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The Effects of Type-1 and Type-2 Diabetes on Endothelium-Dependent Relaxation in Rat Aorta

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ALTAN, V. M., C. KARASU AND A. ÖZÜARI. *The effects of Type-1 and Type-2 diabetes on endothelium-dependent relaxation in rat aorta.* PHARMACOL BIOCHEM BEHAV **33**(3) 519–522, 1989.—Diabetes mellitus is known to produce alterations in vascular reactivity. In the present study we have examined the effects of endothelium-dependent and endothelium-independent relaxing substances on thoracic aorta from insulin-dependent (Type-1) and noninsulin-dependent (Type-2) diabetic rats and their appropriate controls. Endothelium-dependent relaxations produced by acetylcholine and histamine in aortic rings precontracted with noradrenaline were significantly increased in insulin-dependent diabetic vessels. In contrast, the relaxations elicited by those agents were significantly attenuated in noninsulin-dependent diabetic aorta preparations. On the other hand, the relaxations induced by sodium nitroprusside (an endothelium-independent relaxant agent) in both types of diabetic preparations were comparable to those in control vessels. The results indicate that insulin-dependent and noninsulin-dependent diabetes lead to specific alterations of the endothelium-dependent relaxation of rat aorta.

Type-1 diabetes Type-2 diabetes EDRF Rat aorta

SINCE the original observations of Furchgott and Zawadzki (8) demonstrating that the vasodilator action of acetylcholine on rabbit aorta was mediated indirectly by release from the endothelial cells of a relaxant substance, there has been considerable interest in the function of vascular endothelium (9,19). The property of endothelium-dependent relaxation of blood vessel has been shown to be impaired in several vascular disease states (11,12). However, the results obtained with studies on endothelium-dependent relaxation in diabetic rats are inconclusive.

On the other hand, diabetics are known to develop numerous thrombotic, atherosclerotic and cardiovascular complications (3, 10, 13). Moreover, no data are yet available for noninsulin-dependent diabetic animals. Therefore, the purpose of the present study was to characterize alterations in the relaxing effect of acetylcholine and histamine in thoracic aorta from insulin-dependent and noninsulin-dependent diabetic rats.

METHOD

Insulin-dependent diabetes was chemically induced in 7-week-old male albino rats by a single tail vein injection of alloxan monohydrate (50 mg/kg) dissolved in physiological saline. Age-matched control rats were injected with the same physiological saline solution alone. All rats had free access to food and water until they were killed.

Noninsulin-dependent diabetes was induced according to the method described by Weir *et al.* (21). The only difference was the chemical used to produce diabetes. Two-day-old male albino rat pups were injected intraperitoneally with 100 mg/kg alloxan monohydrate. Age-matched control rats received physiological saline solution alone. At 4 days of age, blood was collected by cardiac puncture and blood glucose was determined by glucose oxidase enzymatic assay (Sigma).

Rats who demonstrated blood glucose levels of 200 mg/dl or above at 4 days of age were selected as the noninsulin-dependent diabetic group. The rats were subsequently weaned at 24 days of age and allowed free access to food and water until they were killed. The blood glucose levels of those rats then remained at approximately normal levels up to about 6–7 weeks of age. Subsequently, chronic hyperglycemia developed with blood glucose concentrations ranging between 195–262 mg/dl at 11–12 weeks of age. Serum insulin levels were determined by standard radioimmunoassay techniques using a commercial kit available from Diagnostic Products Corporation.

Insulin-dependent diabetic rats and their age-matched controls were killed by a blow on the head after a lapse of 5–6 weeks. Noninsulin-dependent diabetic rats and their age-matched controls were killed after a lapse of 11–12 weeks. Thus, both groups of diabetic rats and their age-matched controls were 11–12 weeks of age on the day of sacrifice.

The thoracic aorta was then rapidly removed and placed into an oxygenated (95% O_2 and 5% CO_2) physiological salt solution (37°C, pH 7.4), of the following composition (mM): NaCl 118.2, KCl 4.7, $MgCl_2$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, and glucose 10.0. The aorta was cleaned of fat and connective tissue and cut into rings 3 mm long. Special care was taken to avoid contact with the luminal surface to preserve the endothelial cells. In some experiments, the luminal surface of the preparation was rubbed to remove the endothelium. The rings were mounted in a 10-ml organ bath for isometric contractile force recordings by

TABLE 1

GENERAL CHARACTERISTICS OF INSULIN-DEPENDENT DIABETIC RATS AND THEIR AGE-MATCHED CONTROLS

	Control n = 18	Insulin-Dependent Diabetic n = 21
Body weight (g)	214 ± 8.60	170 ± 9.30*
Blood glucose (mg/dl)	121 ± 4.80	392 ± 11.00*
Serum insulin (μU/ml)	28.2 ± 2.30	12.8 ± 2.41*
The contraction of aorta in response to $2 \cdot 10^{-7}$ M noradrenaline (g)	1.76 ± 0.12	1.86 ± 0.16

Values are means ± SEM; n = number of animals. *Statistically different from controls ($p<0.05$).

TABLE 2

GENERAL CHARACTERISTICS OF NONINSULIN-DEPENDENT DIABETIC RATS AND THEIR AGE-MATCHED CONTROLS

	Control n = 20	Noninsulin-Dependent Diabetic n = 20
Body weight (g)	210 ± 7.50	221 ± 6.20
Blood glucose (mg/dl)	118 ± 5.80	230 ± 9.50*
Serum insulin (μU/ml)	30.1 ± 3.20	19.8 ± 6.40*
The contraction of aorta in response to $2 \cdot 10^{-7}$ M noradrenaline (g)	1.74 ± 0.33	1.90 ± 0.41

Values are means ± SEM; n = number of animals. *Statistically different from controls ($p<0.05$).

means of Ugo Basile 7006 isometric force transducer and a Ugo Basile 7050 microdynamometer. The thoracic aorta preparations were allowed to equilibrate 90 minutes under an optimal resting tension of 1.0 g. During the equilibration period the tissue bathing solution was changed every 30 minutes. The preparations were contracted with noradrenaline ($2 \cdot 10^{-7}$ M). After the contraction reached a plateau, concentration-response relationships for acetylcholine, histamine and sodium nitroprusside were obtained by adding one of those agents to the bath in a cumulative manner.

Relaxant effects of acetylcholine, histamine and sodium nitroprusside were expressed as percent of the maximal contraction obtained by noradrenaline. Statistical significance between groups was evaluated by Student's *t*-test.

RESULTS AND DISCUSSION

In the present study, 24 to 48 hours after alloxan treatment, rats demonstrated some of the characteristics of insulin-dependent diabetes, namely polyphagia, polyuria and stable hyperglycemia. The body weight of insulin-dependent diabetic rats was significantly less than age-matched control animals 5–6 weeks after alloxan treatment (Table 1). Furthermore, blood glucose determinations showed a significant hyperglycemia relative to control animals. The results shown in Table 1 also indicate that the serum levels of insulin were significantly reduced in diabetic rats when compared with controls.

On the other hand, alloxan treatment (IP) of two-day-old neonatal rats resulted in a diabetic state similar with many aspects of noninsulin-dependent diabetes mellitus. The general features of these rats and their appropriate controls are listed in Table 2. No significant change was observed in weight gain profiles of diabetic rats when compared with controls. In addition, diabetic rats demonstrated decreased serum insulin levels relative to controls, but complete insulin deficiency was not observed. Blood glucose concentrations of these rats, however, were found to be significantly higher than those of control rats.

Acetylcholine and histamine elicited concentration-dependent relaxations of the precontracted aortas from control and insulin-dependent diabetic rats (Fig. 1A, B). These responses were, however, significantly increased in insulin-dependent diabetic rat aortas (Fig. 1A). When aortas were denuded mechanically, the acetylcholine- and histamine-induced relaxation was abolished in both control and diabetic aorta preparations (data not shown).

On the other hand, the relaxations elicited by acetylcholine and histamine were strongly supressed in noninsulin-dependent diabetic aorta preparations compared with those from controls (Fig. 2A, B). In addition, both acetylcholine- and histamine-induced relaxations were abolished in both control and noninsulin-dependent diabetic rats when aortas were denuded mechanically (data not shown). There was no significant difference in the concentration-dependent relaxation produced by sodium nitroprusside in both insulin-dependent and noninsulin-dependent diabetic and their appropriate control preparations (Fig. 1C; Fig. 2C).

In 1980, Furchgott and Zawadzki demonstrated that acetylcholine can relax rabbit aorta indirectly via the release of an endothelium-derived relaxant factor (EDRF) (8). There is a general agreement that endothelium-dependent relaxation is associated with a rise in the cyclic GMP content of the smooth muscle cells (15,17). Although the nature of this factor is still under investigation, recent reports suggests that EDRF is nitric oxide (NO). Like EDRF, NO is a powerful vasodilator that acts directly on the smooth muscle (9,17). Functional and structural vascular aberrations are evident in patients and experimental models of diabetes (4,5). Moreover, abnormal reactivity of vascular smooth muscle to various vasoactive agents has been suggested as the principle cause of diabetes-induced vascular complications (1, 2, 5, 18). Thus, it was of interest to determine whether endothelium-dependent relaxation was altered in both insulin-dependent and noninsulin-dependent diabetic rats.

The major findings of our study are that the endothelium-dependent relaxations in response to acetylcholine and histamine are significantly increased in aortas from insulin-dependent diabetic rats, whereas the relaxant responses to both of those agents are strongly depressed in the aortas from noninsulin-dependent diabetic rats. On the other hand, dose-dependent relaxations produced by sodium nitroprusside, an endothelium-independent substance (17), in aortas from both insulin-dependent and noninsulin-dependent diabetic rats showed no shift and no depression of maximal relaxation capacity (Figs. 1 and 2). Our results thus strongly suggest that there exists characteristic alterations of the relaxation responses to endothelium-dependent substances rather than a generalized altered relaxing capacity of diabetic smooth muscle.

As it is well known, macrovascular disease is a significant cause of morbidity and mortality in patients with diabetes. Many studies have demonstrated that a substantial portion of morbidity and mortality associated with both insulin-dependent diabetes and noninsulin-dependent diabetes is due to large vessel atherosclero-

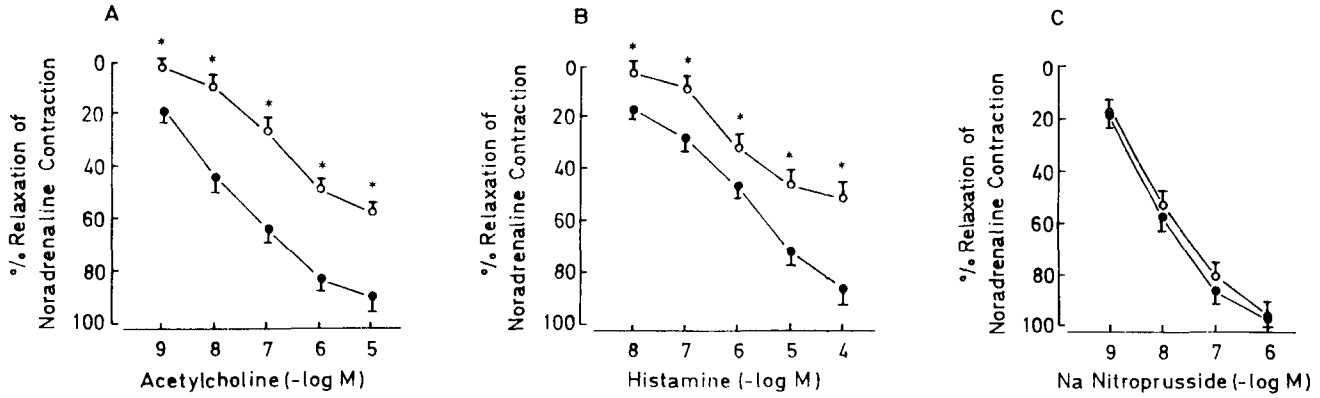

FIG. 1. Relaxant response of aorta to acetylcholine (A), histamine (B) and sodium nitroprusside (C) in insulin-dependent diabetic (solid circle) and control (open circle) rats. Each point represents the mean of 16–20 experiments ± SEM. *$p<0.05$, **$p<0.01$.

sis (3, 10, 13). Recent reports have demonstrated depressed endothelium-mediated vasodilator responses in various vascular disease states including atherosclerosis (11,12). Furthermore, diabetes mellitus is known to promote endothelial cell injury (2,4). Thus, enhanced endothelium-dependent relaxation in response to acetylcholine and histamine that we observed in aortas from insulin-dependent diabetic rats is somewhat surprising. As a matter of fact, many of previous studies, in contrast to our results, revealed diminished endothelium-dependent relaxations in diabetic rats (6, 14, 16). However, Oyama *et al.* reported that there was no morphological difference in the surface of the endothelial layer between the diabetic and the control aorta preparations (16). Therefore, these authors suggested that depressed endothelium-dependent relaxation in the diabetic aorta was not due to morphological derangement of endothelial layer. On the other hand, White and Carrier reported that endothelium-dependent relaxations of rat mesenteric arteries caused by histamine were enhanced in arteries from diabetic rats (22). Fortes *et al.* demonstrated that precontracted aorta from normal and diabetic animals were equally relaxed by acetylcholine and histamine, provided the endothelium was left intact (7). More recently, Wakabayashi *et al.* observed that endothelium-dependent relaxation did not differ between control and diabetic rats (20). These results suggested that the work on endothelium-dependent relaxation in diabetic rats is still inconclusive. On the other hand, no data are yet available for noninsulin-dependent diabetic rats. In contrast to our results with insulin-dependent diabetic rats, we found impaired relaxation of the aorta from rats with noninsulin-dependent diabetes in response to endothelium-dependent relaxation substances. There exist a number of possible explanations for this discrepancy including altered production, release, transport and destruction of EDRF in both types of diabetic states. Alterations in acetylcholine and histamine receptor density on endothelium cells and ability of smooth muscle to respond to EDRF, the muscle levels of cyclic GMP which initiates the process of relaxation in insulin-dependent and noninsulin-dependent diabetic states, might be the reasons as well. These should be elucidated in detail in further experiments.

In conclusion, although the underlying mechanisms remain obscure at present, our results suggest that the sequence of events involved in endothelium-dependent relaxation is markedly enhanced in insulin-dependent diabetes, but markedly depressed in noninsulin-dependent diabetes.

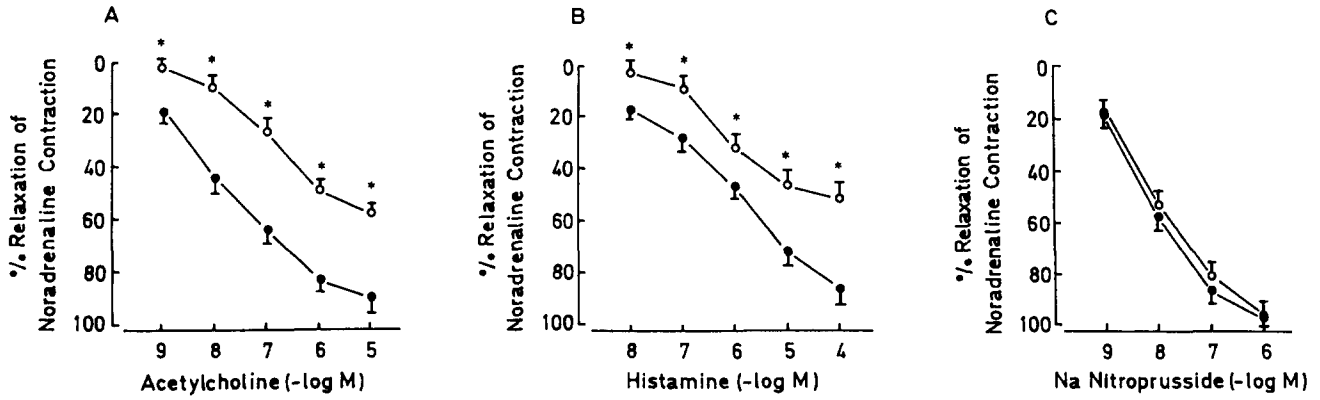

FIG. 2. Relaxant response of aorta to acetylcholine (A), histamine (B) and sodium nitroprusside (C) in noninsulin-dependent diabetic (solid circle) and control (open circle) rats. Each point represents the mean of 16–20 experiments ± SEM. *$p<0.05$, **$p<0.01$.

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