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A Contrasting effect of the diabetic state upon the contractile responses of aortic preparations from the rat and rabbit

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1 Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (65 mg kg^{-1}). Rabbits were rendered diabetic by injecting alloxan (100 mg kg^{-1}) into the lateral ear vein. Diabetes was confirmed by a significant elevation of serum glucose in both species 8 weeks after injection.

2 The maximum contraction to noradrenaline (NA), 5-hydroxytryptamine (5-HT) and KCl was markedly diminished in thoracic aortic rings (AR) from diabetic rats with no change in the EC_{50} of the agonists. There were no differences in the contractile properties of AR from diabetic rabbits to NA, 5-HT or KCl. Diabetes did not alter the responsiveness of AR from the rat to angiotensin II (AII). However, AR from diabetic rabbits displayed a decreased maximal contraction and an increased EC_{50} to AII.

3 The magnitude of the acetylcholine-induced relaxation to precontracted AR was not different between diabetic and control rats and rabbits.

4 The contractile responses of AR to NA, 5-HT and KCl were depressed in diabetic rats, regardless of the control tissue to which they were compared. The decrease in maximal contraction to NA, 5-HT and KCl seen in diabetic animals was prevented by insulin replacement.

5 The results demonstrated that while both rats and rabbits exhibited a similar degree of hyperglycemia after treatment with a diabetogenic agent, aortic preparations from the rabbit are not affected in the same way as the aorta from the diabetic rat when exposed to NA, 5-HT and KCl. This feature may be related to the marked differences between the extent of sympathetic innervation of the aorta in the rabbit and rat. Furthermore, the decrease in maximal contraction in rat aorta was non-specific with respect to agonists since it could also be demonstrated with KCl. Therefore, it follows that the diabetic state may affect processes responsible for contraction beyond the level of receptor activation.

Introduction

It is well established that the vascular disease is a complicating feature in human diabetes mellitus (Christlieb, 1973). Moreover, the pathological and physiological changes associated with the diabetic state are complex. Rodents treated with diabetogenic agents (e.g., alloxan or streptozotocin) having specific cytotoxic effects on pancreatic β-cells (Kennedy & Lukens, 1944; Rakieton *et al.*, 1963) exhibit symptoms typical of diabetes mellitus. It follows that the use of the animal models of the disease may offer insights into the nature and progression of vascular disease in diabetes mellitus.

As a generalization, the results of experiments in which vascular smooth muscle contractility have been examined in animals made diabetic chemically fall into two categories. First, in preparations of aorta from diabetic rodents it has been found that diabetes is associated either with a decreased maximal response with no change in sensitivity (Sullivan & Sparks, 1978; Pfaffman *et al.*, 1982) or a decreased maximal response with a decrease in sensitivity (Turlapaty *et al.*, 1980). The latter studies showing a decreased vascular responsiveness are in agreement with clinical findings demonstrating that, within a diabetic population, one

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can observe blunted vascular responses to catecholamines (Cryer *et al.*, 1978).

In contrast to the above observations, an increased maximal response to noradrenaline in aortic preparations from diabetic rats has been demonstrated (Owen & Carrier, 1979; 1980; Scarborough & Carrier 1983; 1984). Furthermore, another research group has shown an increased senstivity to agonists in diabetic rat aorta (MacLeod & McNeill, 1982). An increased sensitivity of the diabetic rat caudal artery, thought to reflect a supersensitivity in the face of deranged sympathetic neuronal mechanisms (Ramanadham *et al.*, 1984), also has been found. Diabetic neuropathy and altered peripheral nerve function are features of the diabetic state, and there is evidence of an enhanced vascular responsiveness to noradrenaline in subjects with peripheral neuropathies (Moorhouse *et al.*, 1966).

In the present study we examined the effect of the diabetic state upon the contractile responses of the rabbit and rat aorta. The choice of tissue was based on the fact that the rabbit aorta has considerable sympathetic innervation in contrast to the rat aorta which has only a sparse innervation (Head *et al.*, 1982). Through a comparison of the contractile responses of the two tissues, we sought to isolate those features of the diabetic state that exclusively and directly impinge upon vascular smooth muscle cell function from those that may also include an effect secondary to an influence upon sympathetic neuronal mechanisms. As the study progressed, it became apparent that the rat, but not the rabbit, aorta exhibited marked changes in responsiveness as a result of the induction of diabetes in this species. Accordingly, we characterized the nature of the changes and their relationship to the altered metabolic state.

Methods

Animals

Male Sprague-Dawley rats weighing 250–275 g and male New Zealand White rabbits weighing 2–3 kg were used. The experimental procedures described in this manuscript were approved by the West Virginia University Animal Care and Use Committee. All animals were allowed free access to water and to a standard laboratory diet (Wayne, Lab-Blox) except when indicated.

Induction of diabetes and control animals

(1) *Rabbits* Rabbits were fasted for between 18 and 24 h before induction of diabetes. Rabbits were rendered diabetic by injecting alloxan (100 mg kg^{-1}) into the lateral ear vein. In preliminary experiments we established that injection of streptozotocin in the concentration range 50 to 150 mg kg^{-1} intravenously did not induce diabetes in the rabbit. A similar observation was described earlier by Lazar *et al.* (1968). Diabetes in the rabbit was characterized by a marked increased in serum glucose and a failure of diabetic rabbits to show an increase in body weight when compared with age-matched, non-diabetic rabbits (Table 1).

(2) *Rats* Rats were fasted for 24 h before a single intraperitoneal injection of streptozotocin 65 mg kg^{-1}. The streptozotocin was dissolved in critrate-saline buffer (0.02 M, pH 4.5) just before administration. Control rats were injected with the citrate-saline vehicle alone. Diabetic and non-diabetic rats were further divided as described below.

Table 1 Body weight, aortic weight and serum glucose values of diabetic rabbits, rats and nondiabetic control animals

Species	*Treatment*	*Aorta wet weight* (mg)	*Initial weight pre-fast*	*Weight at death*	*Serum glucose* (mg dl^{-1})
Rabbit	Age-matched control	14.15 ± 0.41	3.02 ± 0.21 kg	4.11 ± 0.10[b] kg	128.03 ± 5.91[c]
	Diabetic	13.70 ± 0.68	2.95 ± 0.20 kg	2.97 ± 0.31 kg	556.97 ± 49.5
Rat-group I	Age-matched control	2.85 ± 0.42[a]	280.0 ± 12.7 g	423.0 ± 26.6[c] g	102.9 ± 22.3[c]
	Intermediate weight control	2.30 ± 0.71		251.5 ± 20.9[a] g	112.5 ± 14.4[c]
	Food-restricted control	2.15 ± 0.40	277.0 ± 10.5 g	198.5 ± 14.4 g	106.5 ± 17.5[c]
	Diabetic	1.78 ± 0.34	267.3 ± 17.3 g	182.7 ± 14.1 g	609.2 ± 97.8
Rat-group II	Age-matched control	2.90 ± 0.39[a]	298.8 ± 17.1 g	433.5 ± 15.3[c] g	105.9 ± 27.6[c]
	Insulin replacement	3.14 ± 0.48[a]	296.7 ± 10.5 g	416.3 ± 32.4[c] g	247.1 ± 21.6[c]
	Diabetic	1.82 ± 0.27	297.8 ± 11.7 g	192.4 ± 35.4 g	738.4 ± 108.9

Values shown are the mean ± s.e.mean for 6 experiments with rabbits and means ± s.d. for 8–9 experiments with rats. Insulin replacement consisted of administering insulin (PZI, 1 u 100 g^{-1} body wt. day^{-1}) to diabetic rats [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ with respect to values for diabetic animals.

Group I Rats within this group comprised diabetic rats (i.e., streptozotocin-injected), age-matched control rats (i.e., vehicle-injected), age-matched control rats given limited access to food, and non-age matched control rats whose weight at the time of death was intermediate between those of the diabetic rats and their age-matched controls. A summary of the results obtained in individual control groups and the diabetic group are shown in Table 1. It should be noted that only rats injected with the diabetogenic agent showed an elevation in serum glucose (Table 1).

Group II Animals within this group consisted of streptozotocin treated rats, age-matched control rats, and diabetic rats given insulin replacement. The latter animals were initially rendered diabetic (streptozotocin; 65 mg kg^{-1} i.p.) and then one week after the streptozotocin injection those animals exhibiting glucosuria were placed on a regimen of daily subcutaneous injections of protamine zinc insulin (PZI, 1 u 100 g^{-1} body weight). Throughout an 8 week period urine glucose tests were conducted biweekly and the insulin dosages adjusted to an average of 4.0 u day^{-1} in order to maintain glucose-free urine levels. A summary of the animal body weights and serum glucose values of rats within this group is shown in Table 1. It should be noted that insulin replacement in diabetic rats prevented the loss of body weight seen in the diabetic rats (Table 1). Furthermore, insulin replacement which was discontinued 24 h before killing the rats resulted in serum glucose levels that were only 2 to 3 fold greater than those existing in non-

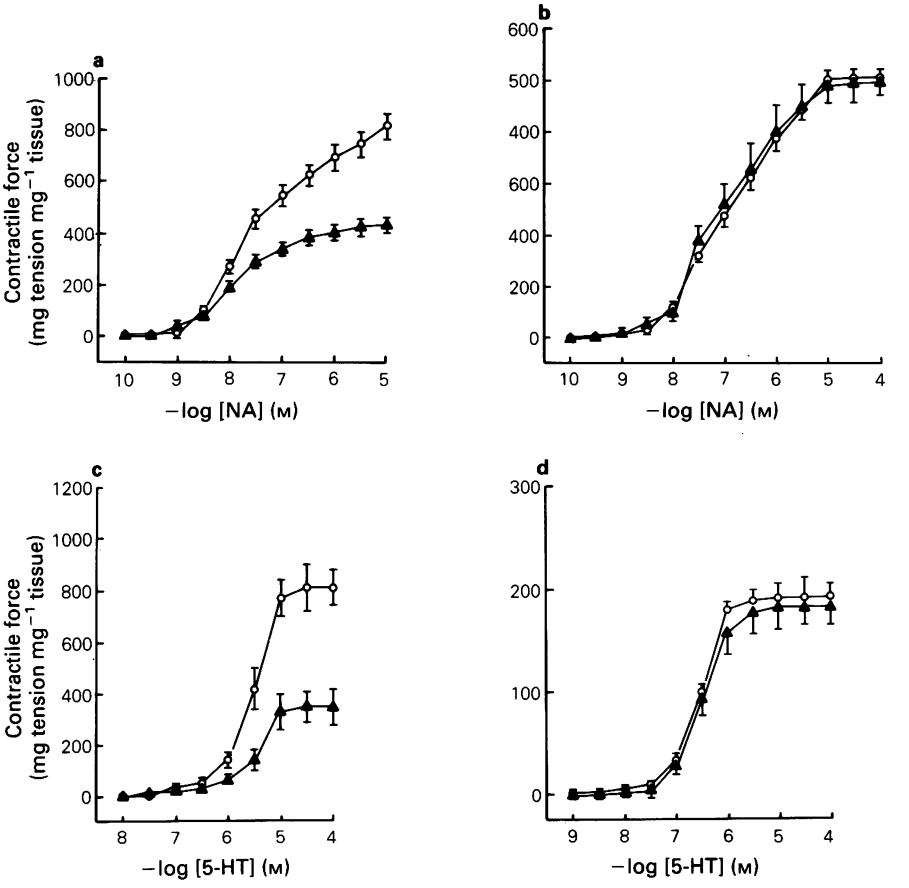

Figure 1 Concentration-response curves for contractions to noradrenaline (NA) and 5-hydroxytryptamine (5-HT) of aortic rings from age-matched control (○) and 8 week diabetic (▲) rats and rabbits. (a and b) Concentration-response curves for NA in rat and rabbit aortae, respectively. (c and d) Concentration-response curves for 5-HT in rat and rabbit aortae, respectively. Values represent the mean of six to eight experiments and vertical lines indicate the s.e.mean.

diabetic rats and markedly less ($P < 0.05$) than the 7 fold increase in serum glucose seen in the plasma of diabetic rats (Table 1).

Preparation of tissues

A diabetic animal, as well as its appropriate control, was used in every experiment. Eight weeks after induction of diabetes, rats were killed by decapitation while rabbits were killed by stunning followed by exsanguination. The blood was collected in ice-chilled tubes and the serum separated and analyzed for glucose concentration using the ABTS method of Bergmeyer & Bernt (1974). A section of thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated Krebs solution. Aortae were cleaned of loosely adhering fat and connective tissue and cut into rings of 3 mm length. Care was taken to ensure that the endothelial layer was not damaged during tissue preparation. Rings were then suspended in a 10 ml organ bath containing a modified Krebs-bicarbonate solution of the following

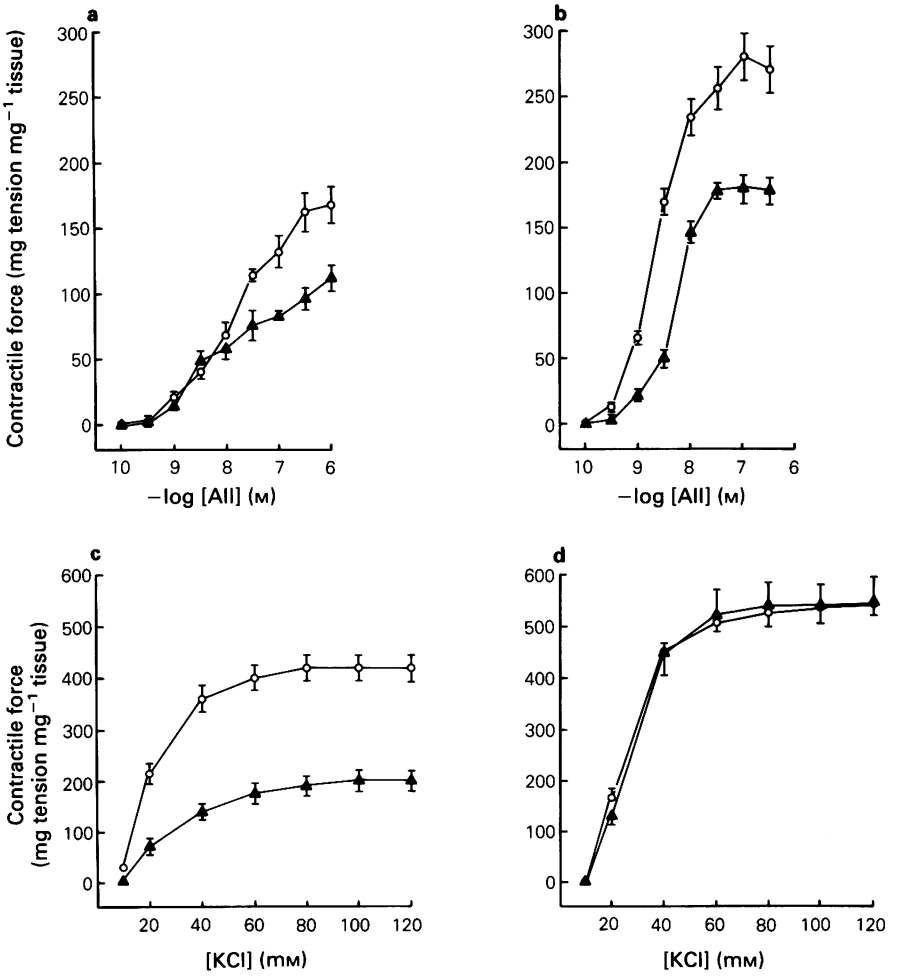

Figure 2 Concentration-response curves for contractions to angiotensin II (AII) and KCl of aortic rings from age-matched control (○), and 8 week diabetic (▲) rats and rabbits. (a and b) Concentration-response curves for AII in rat and rabbit aortae, respectively. (c and d) Concentration-response curves for KCl in rat and rabbit aortae, respectively. Values represent the mean of six to nine experiments with the s.e.mean indicated by vertical lines.

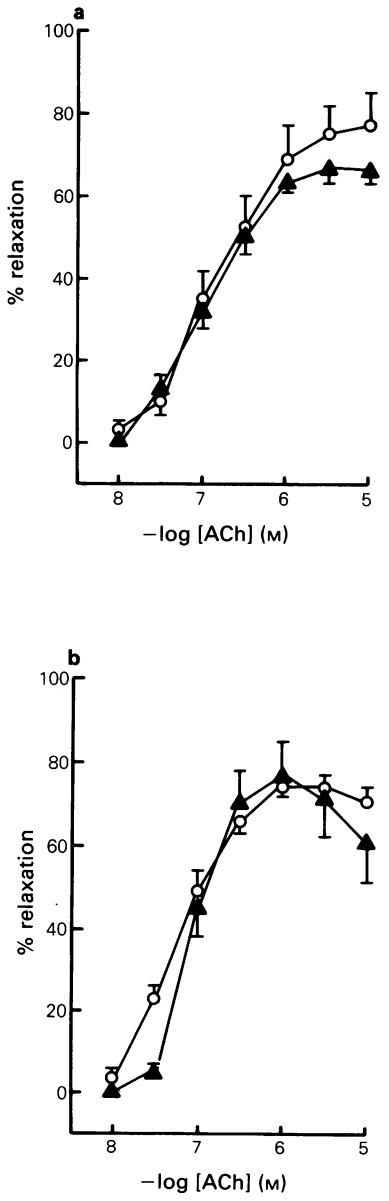

Figure 3 Concentration-response curves for acetylcholine (ACh) showing the endothelium-dependent relaxation of aortic rings from age-matched control (○) and 8 week diabetic (▲) rats (a) and rabbits (b). Rings were precontracted with a submaximal concentration of noradrenaline (3×10^{-7} M) and responses obtained by the cumulative addition of ACh (10^{-8}–10^{-5} M). Values represent the mean of six separate experiments with the s.e.mean indicated by vertical lines.

composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 10, pH 7.4. The Krebs solution was continously gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. Tissues were equilibrated for 90 min under a resting tension of 2 g (rat and rabbit). These conditions were previously determined to be optimal for tissue responsiveness to agonists. Preparations were washed with fresh oxygenated Krebs solution every 20 min. Isometric contractions were measured with a Grass FT.03 force-displacement transducer and recorded on a Grass model 79 polygraph.

Concentration-response curves

After the initial equilibration period, the tissues were exposed to a maximally effective concentration of one of the agonists to ensure stabilization of the preparations. Drugs were removed from the chamber by several washes with Krebs solution and tension was allowed to return to baseline. Tissues were allowed to equilibrate for 1 h after each exposure to an agonist to ensure complete washout of agonist and to minimize the possibility of receptor desensitization.

Concentration-response curves to noradrenaline (NA), 5-hydroxytryptamine (5-HT) and potassium chloride (KCl) were obtained by a cumulative increase in the total concentration of agonist. Each tissue was exposed to all drugs and the order of drug application was also randomized. Each drug addition was made after the contractile response to the previous concentration had reached a maximum. For concentration-response curves obtained for 5-HT, the selective α_1-adrenoceptor antagonist prazosin (10^{-6}M) was added to the organ bath 30 min before the addition of the agonist. Concentration-response curves to angiotensin II (AII) were obtained by non-cumulative increases in total concentration of the drug, i.e., each concentration was left in contact with the tissue until a plateau response was reached. The tissues were then washed for 30 min before the next addition of AII in order to avoid tachyphylaxis.

For relaxation studies, submaximal tone (50–70% of maximum) was first induced with NA. Relaxation was then produced by the cumulative addition of acetylcholine (10^{-8}–10^{-5} M) to the precontracted tissues.

Statistical analysis

Contractile force was expressed as mg of tension developed per mg of tissue wet weight to account for the differences in cross-sectional area of the ring preparations. EC_{50} values were determined by the method of Fleming *et al.*, (1972). Where appropriate, statistical differences were compared by use of analysis of variance or Student's *t* test for unpaired observa-

Table 2 EC_{50} and maximum contractile force of various vasoactive agents in aortae from control and diabetic rabbits

Agonist	*EC_{50}[1] (95% confidence interval)*		*Maximum contractile force[2]* (mg tension mg^{-1} tissue)	
	Control	*Diabetic*	*Control*	*Diabetic*
NA	131.0 (86.50–208.0)	124.2 (63.09–244.1)	508 ± 16.8	498 ± 36.7
AII	2.351[a] (1.902–2.914)	5.401 (4.653–6.261)	283 ± 22.0[a]	184 ± 10.7
5-HT	302.7 (266.9–343.2)	304.3 (236.9–396.5)	193 ± 11.9	182 ± 20.3
KCl	27.50 (26.05–28.95)	26.71 (25.64–28.66)	543.7 27.8	543.0 ± 46.7

[1] Values indicate the geometric mean of 6 determinations in nM (except for KCl, mM).
[2] Values indicate the mean ± s.e.mean of 6 determinations.
[a] $P < 0.05$ with respect to values from diabetic animals. Abbreviations used: NA, noradrenaline; AII, angiotensin II; 5-HT, 5-hydroxytryptamine; KCl, potassium chloride.

tions. The Student Neuman-Keuls test was employed only if the analysis of variance gave a P value of < 0.05.

Drugs

(−)-Noradrenaline bitartrate, 5-hydroxytryptamine HCl, Val[5]-angiotensin II, acetylcholine chloride, alloxan and streptozotocin were purchased from Sigma Chemical Co. (St. Louis, MO). Prazosin HCl was obtained by Pfizer Inc. (Groton, CT).

Results

Aortic contracile responses in diabetic rats and rabbits

The contractile rsponses of aortic rings from diabetic rats, diabetic rabbits, and their age-matched controls are shown in Figures 1 and 2. There were no significant differences in contractile responses, either maximum contractile force or EC_{50} values, of aortic rings between diabetic and age-matched rabbits to either NA, 5-HT or KCl (Figures 1 and 2, Table 2). In contrast, the maximum contractions generated by aortic rings from diabetic rats to NA, 5-HT, and KCl were markedly smaller than those generated by corresponding control tissues from age-matched rats (Figures 1 and 2, Table 3). However, there were no differences between the EC_{50} values for individual agonists in tissues from diabetic and age-matched control rats (Table 4).

The effect of the diabetic state upon the responses of aortic preparations from the rat and rabbit to AII differed somewhat from the pattern seen above for NA, 5-HT and KCl. Responses of aortic rings from diabetic rats to AII were characterized by a slight, although not significant, reduction in the maximal force of contraction (Figure 2). It also should be noted that the maximal force of contraction of rat aortic

Table 3 Maximum contractile force for agonists in aortae from control and diabetic rats

Group	*NA*	*5-HT*	*AII*	*KCl*
I				
Age-matched control	843 ± 113.9[b]	804 ± 157[b]	168 ± 36.3	430 ± 53.4[b]
Intermediate weight control	1337 ± 136.2[c]	1200 ± 159.5[c]	872 ± 130.3[c]	ND
Food-restricted control	951 ± 133.7[b]	1363 ± 171.5[c]	367 ± 59.3[b]	435 ± 99.7[b]
Diabetic	433 ± 88.2	356 ± 126.9	112 ± 26.5	192 ± 61.9
II				
Age-matched control	594 ± 44.7[a]	473 ± 75.4[b]	205 ± 62.7	419 ± 63.4[b]
Insulin replacement	519 ± 79.6[a]	462 ± 73.5[b]	172 ± 155.2	470 ± 97.0[b]
Diabetic	309 ± 58.8	241 ± 105.0	148 ± 74.7	200 ± 61.8

Each value represents the mean ± s.d. mg tension mg^{-1} of tissue of 6 to 9 animals.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ with respect to diabetic animals. ND = not done. For other abbreviations see legend of Table 2.

Table 4 EC_{50} values for various vasoactive agents in aortae from control and diabetic rats

Group	*Noradrenaline* (nM)	*5-hydroxytryptamine* (nM)	*Angiotensin II* (nM)	*Potassium Chloride* (mM)
I				
Age-matched control	14.39 (9.95–20.80)	3.52 (1.20–4.90)	17.14 (12.16–24.15)	21.41 (14.92–26.54)
Intermediate weight control	8.26 (4.62–14.75)	4.23 (2.70–6.50)	10.26 (6.39–16.53)	ND
Food-restricted control	11.67 (9.12–14.93)	1.31 (1.01–3.80)	7.75 (3.01–19.99)	24.12 (14.33–29.62)
Diabetic	10.50 (7.26–15.22)	3.80 (3.00–4.70)	7.94 (4.80–13.15)	27.66 (21.91–33.06)
II				
Age-matched control	24.60 (21.44–28.20)	2.91 (2.14–4.46)	7.59 (2.14–26.92)	20.84 (18.12–23.56)
Insulin replacement	22.93 (12.31–42.74)	2.45 (1.69–4.55)	14.51 (7.08–29.53)	21.09 (18.89–23.39)
Diabetic	21.94 (12.01–38.93)	2.95 (1.79–4.90)	–8.32 (6.03–11.51)	29.00 (23.34–34.63)

[1] Values indicate the geometric mean and 95% confidence interval of 6 to 9 animals. Analysis of variance revealed no significant differences between the EC_{50} values. ND = not determined.

tissue to AII was considerably smaller than that produced by NA, 5-HT or KCl. After 8 weeks of diabetes in the rabbit, the preparations of aortic rings displayed a decreased sensitivity and decreased maximal contractile force to the peptide AII (Figure 2, Table 2).

Acetylcholine-mediated relaxation in diabetic rats and rabbits

The acetylcholine-mediated relaxation of aortic rings precontracted with NA is shown in Figure 3. Acetylcholine produced a dose-dependent relaxation of aortic rings from rats and rabbits. The diabetic state had no effect upon the pattern of relaxation in either species (Figure 3).

Aortic contractile responses in diabetic rats and a series of control rats

The contractile responses of aortic rings from diabetic rats were compared to the responses from a series of control animals using NA, 5-HT, AII and KCl as agonists. As indicated in the Methods section, three separate groups of control rats were utilized: an age-matched group, a food-restricted group, and a non-age-matched group whose weight was intermediate between that of the diabetic and the age-matched control rats. It is apparent from Figure 4 that the maximal force of contraction produced by NA, 5-HT and KCl in aortic preparations from diabetic rats is markedly diminished regardless of the type of control rats that served as a tissue source. In contrast, contractile responses of diabetic rat aortae to AII were markedly reduced only when compared to either the intermediate-weight or food-restricted control animals. There were no differences in responses to AII between age-matched control and diabetic rats.

Table 3 shows that the maximum contractile force generated by NA, 5-HT, KCl and AII was significantly lower in aortae from diabetic rats; NA: 50%, 55% and 65%, when compared to the age-matched, food-restricted, and intermediate-weight controls, respectively; 5-HT: 55%, 70% and 74%, when compared to the age-matched, intermediate-weight and food-restricted controls, respectively; KCl: 53% and 54%, when compared to the age-matched and food-restricted controls, respectively; AII: 70% and 87%, when compared to the food-restricted and intermediate-weight controls, respectively.

Table 4 illustrates the EC_{50} values for NA, 5-HT, AII and KCl from the age-matched, intermediate-weight, and food-restricted controls and from diabetic rat aortae. It was observed that there were no differences in the EC_{50} values between control and diabetic rat aortic preparations for any of the agonists tested.

Comparison of aortic contractile responses between age-matched control, insulin-treated diabetic and untreated diabetic rats

Figure 5 illustrates the contractile responses of aortic rings to NA, 5-HT, AII and KCl from control, insulin-treated diabetic and untreated diabetic rats. Treatment of diabetic animals with insulin prevented the diabetes-induced depression of contractile responses

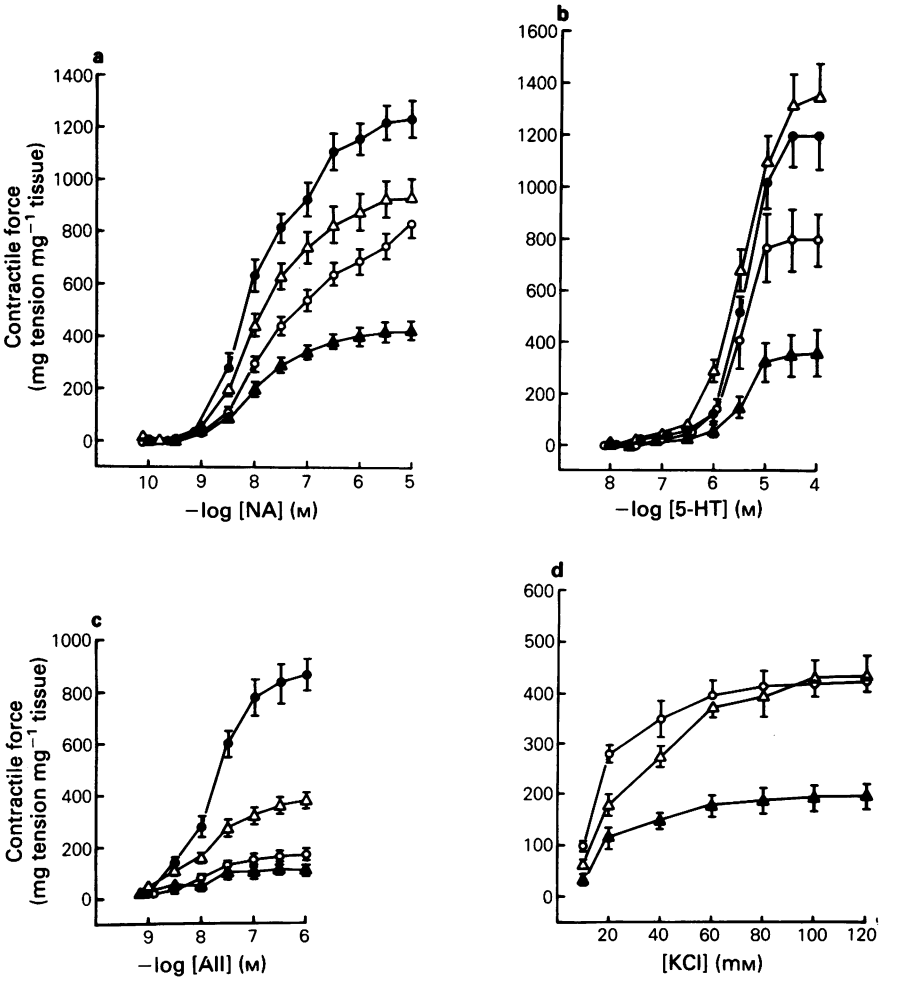

Figure 4 Concentration-response curves for contractions to noradrenaline (NA; a), 5-hydroxytryptamine (5-HT, b), angiotensin II (AII, c) and KCl (d) of aortic rings from age-matched controls (○), intermediated weight control (●), food-restricted control (△) and 8 week diabetic (▲) rats. The age-matched control group used in this figure is the same group used in Figure 1 and is illustrated again in this figure for purposes of clarity. Values represent the mean of six to nine experiments with the s.e.mean indicated by vertical lines.

to NA, 5-HT and KCl (Table 3). In contrast, contractile responses to AII (Figure 5c and Table 3) were not different between control, insulin-treated or untreated diabetic animals. When the EC_{50} values for contractions to NA, 5-HT, AII and KCl were examined (Table 4), it was observed that there were no differences in the EC_{50} values between control, insulin-treated and untreated diabetic rat and aortic preparations.

Discussion

The results of the present study suggest that chemically-induced diabetes has an effect upon the contractile properties of the rat, but not the rabbit, aorta. The latter conclusion is based upon the observation that while both rats and rabbits exhibited a similar degree of hyperglycemia after treatment with a

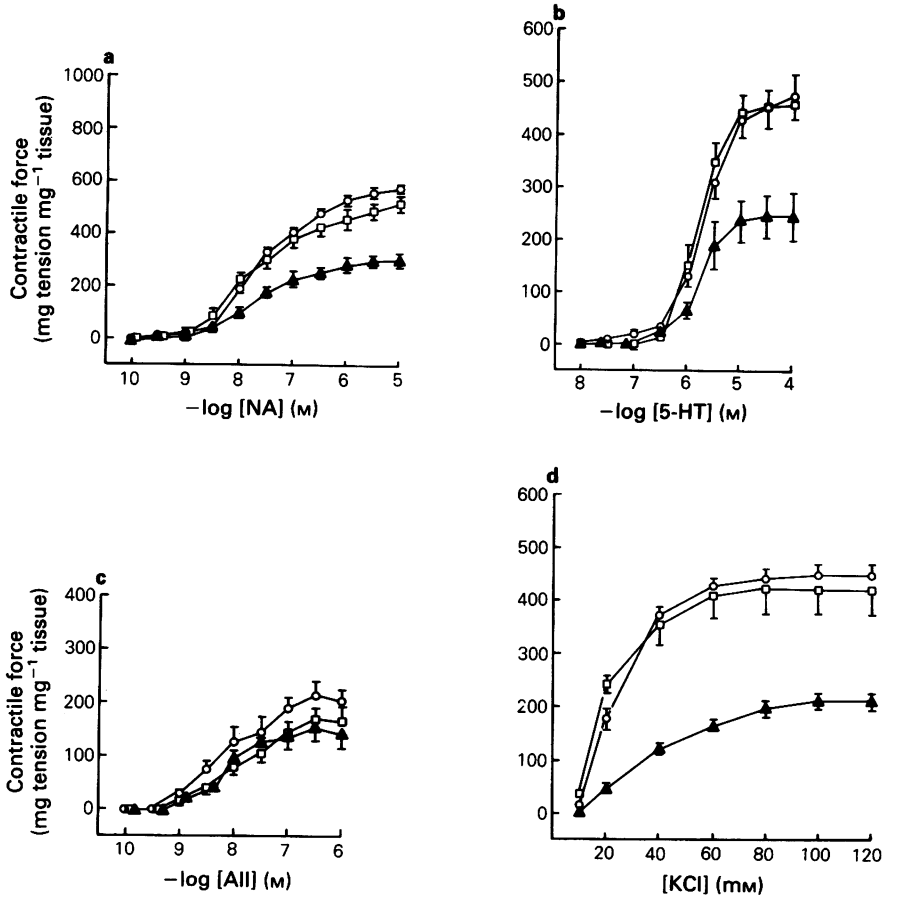

Figure 5 Concentration-response curves for contractions to noradrenaline (NA; a), 5-hydroxytryptamine (5-HT, b), angiotensin II (AII, c) and KCl (d) of aortic rings from age-matched control (○), insulin-treated diabetic (□) and untreated diabetic (▲) rats. Values represent the mean of six to nine experiments with the s.e.mean indicated by vertical lines.

diabetogenic agent, aortic preparations from the rat, but not the rabbit, exhibited a marked depression in maximal contractile force to NA, 5-HT and KCl.

Two considerations are of importance in this comparison. First, as indicated in the Methods section, rabbits were rendered diabetic with the diabetogenic agent alloxan since administration of streptozotocin failed to induce hyperglycemia in rabbits. It could be argued that the decreased contractile force seen in the aortic preparation from the rat was a feature restricted to streptozotocin and not alloxan, and therefore, this effect was independent of the hyperglycemic state. This conclusion seems unlikely for two reasons. First, we demonstrated that reversal, or near reversal, of the hyperglycemic state was associated with a restoration of the depressed contractile responses in aortic preparations from streptozotocin-treated rats. In other words, the alterations in contractile properties were linked to the metabolic imbalance. Further support for this view comes from previous studies that also have shown decreased responses to vasoactive substances in aortic preparations from the alloxan-treated rat (Turlapaty *et al.*, 1980).

Our results clearly demonstrate that the aorta from the diabetic rabbit is not affected in the same way as the aorta from the diabetic rat when exposed to NA, 5-HT and KCl. It was quite interesting that angiotensin II unmasked a decreased contractile response in both

the diabetic rat and rabbit aorta. In view of the fact that responses of the rabbit aorta from diabetic rabbits to KCl, NA and 5-HT were similar to the corresponding responses of control tissues, we would suggest that diabetes in the rabbit may have an effect unique to angiotensin II. It should be remembered that the responses of vascular tissue to angiotensin II can often be modified by prior exposure to the peptide. It is not known, however, if diabetes in the rabbit is associated with an increase in circulating concentrations of angiotensin II.

Several features of the influence of the diabetic state upon responses of the rat aorta are evident from this investigation. Firstly, diabetes reduced the maximum contraction of the preparations to NA, 5-HT, angiotensin II and KCl without changing the sensitivity of the preparations to the agonists. A similar observation was described earlier by Pfaffman *et al.* (1982). Secondly, diabetes has a dramatic effect upon body weight and, as pointed out earlier by Cohen & Berkowitz (1974), the contractile properties of the rat aorta are in part dependent upon the smooth muscle mass of the preparation. With this in mind, we used a variety of control groups of rats including age-matched (but not weight matched) rats, food-restricted rats, and a group of rats not age-matched but with body weights intermediate between those of diabetic rats and their age-matched controls. Body weights were lower, in the food-restricted and intermediate weight controls, than in the age-matched controls. Aortic weights were numerically lower in these modified control groups, but the differences were not significant. However, regardless of the source of control tissues, aortic preparations from diabetic rats consistently displayed a decreased maximal force of contraction. Maximum contractile responses of the food-restricted and intermediate weight controls were greater than the responses of the age-matched control tissues, even though aortic wet weights were not different between these groups. Therefore, we argue that the decreased contractile responses in tissues from diabetic rats is not attributable to an inappropriate source of control tissues, i.e., only the use of the age-matched rats.

Thirdly, we demonstrated that the acetylcholine-induced, endothelial cell-mediated relaxation was not modified in either diabetic rats or rabbits. The latter observation suggests that the decreased maximal force of contraction in aortic preparations from diabetic rats was not due to a concurrent enhancement of vascular smooth muscle relaxation mechanisms. However, our analysis does not preclude there being slight differences in the absolute degree of ACh-mediated relaxation in tissues from diabetic rats. Such differences in relaxation would be a consequence of the contraction of tissues from diabetic animals being somewhat less than those from control animals.

Finally, we established that the diabetes-induced inhibition of rat aortic smooth muscle contractile responses to vasoactive agonists and potassium ions was reversed with insulin replacement. The latter observation is in accord with the findings of Pfaffman *et al.* (1980, 1982), who found that the diminished responses to phenylephrine and KCl in aortic preparations from diabetic rats were reversed with insulin treatment. Collectively, our results suggest that chemically-induced diabetes in the rat, but not in the rabbit, is associated with a decreased ability of the aorta to contract to vasoactive agonists. This feature is non-specific with respect to agonists and can be reversed with insulin replacement.

Our findings, when viewed against accounts in the literature of similar investigations, reinforce areas of agreement and highlight puzzling areas of difference. For example, it was shown previously that rat aortic ring preparations as well as the perfused coronary artery display a decreased responsiveness to 5-HT (Owen & Carrier 1979; Longhurst, Goto, Stitzel & Head, unpublished observations). Our present findings are in accord with this view. Likewise, there is general agreement that vascular responses to potassium ion are attenuated in the diabetic rat as shown for the rat aorta (Pfaffman *et al.*, 1980; Ramanadham *et al.*, 1984), perfused mesenteric vascular bed (Longhurst & Head, 1985) and the caudal artery (Ramanadham *et al.*, 1984). In contrast, responses of vascular tissue from diabetic rats to exogenous NA fail to adhere to a consistent pattern. In previous studies Pfaffman *et al.* (1980), Ramanadham *et al.* (1984) and Fortes *et al.* (1983) provided evidence for either a decreased sensitivity or a decreased force of contraction of the aorta from the diabetic rat. Our present findings are consistent with this view. In contrast, Owen & Carrier (1979) and Scarborough & Carrier (1983) have provided equally compelling evidence for an increase in contractile force in the aorta from the diabetic rat. The reasons for this discrepancy are unknown but may relate to the observation of Fortes *et al.* (1983), that the presence of a functionally intact endothelium in necessary to see the reduced responses of the aorta from the diabetic rat. Obviously, attention should be drawn to the integrity of endothelial structures in studies involving exogenous NA and, to a lesser extent, potassium ion and 5-HT.

By way of summary, there are two key findings from our present study. Firstly, the decreased maximal contraction of aortic tissue from diabetic rats is a feature that is not apparent in the diabetic rabbit. It follows that the diabetic rabbit provides a useful control to determine why diabetes is associated with decreased contractile responses in the rat. Secondly, the reasons for the decreased maximal responses in aortic tissue from diabetic rats deserves comment. Since the deficit is non-specific with respect to agonists

and can be demonstrated with potassium ions, it follows that the diabetic state may affect processes responsible for contraction that occur beyond the level of recepetor activation. The latter may include an alteration in the utilization of Ca^{2+} (Bose & Stephens, 1977; Hartshorne *et al.*, 1977; Pfaffman *et al.*, 1980; 1982) or a derangement in smooth muscle cell metabolism (Clements *et al.*, 1969; Winegrad *et al.*, 1972; Arnquist, 1977). It should be noted that the rabbit aorta has an extensive sympathetic innervation wheras the rat aorta is essentially devoid of sympathetic innervation (Head *et al.*, 1982). Since changes in the contractile properties of the aorta from diabetic rats were greater than those of the highly innervated rat caudal artery (Ramanadham *et al.*, 1984) or mesenteric artery (Longhurst & Head, 1985), it follows that the extent of sympathetic innervation may affect the degree to which one can demonstrate the effect of the diabetic state upon individual vascular beds.

Of course, the above suggestions are speculative, but, when viewed from the standpoint of the progressive development of neuropathies in the diabetic state, they do provide a stimulus for further investigation.

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