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# Vascular reactivity in diabetes mellitus: role of the endothelial cell

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**1** The response to vasoactive agents of microvessels *in situ* and large arteries *in vitro* was compared in normal and alloxan-diabetic rats.

**2** Noradrenaline was equally effective in evoking a constrictor response of mesenteric microvessels in normal and diabetic animals.

**3** The constrictor response to a standard amount of noradrenaline in such vessels was fully antagonized by acetylcholine or papaverine, the minimum effective doses being equivalent in normal and diabetic animals. In contrast, the minimum doses of histamine or bradykinin, effective in normal animals, had to be increased about 20 fold to be active in diabetic animals.

**4** Increased osmolarity of extracellular fluid caused a significant and equivalent increase in latency of the vasoconstrictor response of microvessels to noradrenaline in normal and diabetic animals.

**5** Concentration-effect curves, constructed from the response of isolated aortae to noradrenaline, were similar in normal and diabetic animals, provided the endothelium was removed. Diabetes only affected preparations in which the endothelium was left intact. In these, the median effective concentrations of noradrenaline were greatly increased in comparison with normal values.

**6** Precontracted aortae from normal and diabetic animals were equally relaxed by acetylcholine and histamine, provided the endothelium was left intact. Loss of the relaxant response of the preparations in all groups of animals was observed following removal of endothelial cells.

**7** It is suggested that different mechanisms may be involved in the effects of vasodilator agents on large arteries *in vitro* or small vessels *in situ*. Histamine and bradykinin which are potent permeability-increasing factors, may antagonize the vasoconstrictor response of microvessels to noradrenaline through an action on endothelial cells with increased vascular permeability and temporary changes in composition of extracellular fluid. The reactive process of endothelial cells to permeability factors was affected by diabetes mellitus. However, the response of microvessels to acetylcholine and papaverine which are devoid of permeability-increasing properties, was not influenced by diabetes.

## Introduction

The integrity of microcirculatory responses to vasoactive agents depends partly on the availability of insulin. Functional changes in the behaviour of microvessels are observed in diabetes mellitus (Altura, Halevy & Turlapaty, 1979; Garcia Leme, 1981). Inflammatory responses are greatly reduced in animals rendered diabetic by the administration of alloxan or by subtotal pancreatectomy. This inhibition is reversed by previous injection of insulin and is not related to the glycaemic levels of the animals (Garcia Leme, Hamamura, Migliorini & Leite, 1973). In addition, systemic vessels of diabetic animals challenged with permeability factors such as histamine or 5-hydroxytryptamine show less labelling by intravenously injected colloidal carbon particles than do vessels of normal animals. Insulin given beforehand prevents this and also potentiates, leakage of carbon induced by permeability factors in normal animals (Llorach, Böhm & Garcia Leme, 1976). Electron microscopic studies of vessels submitted to the action of permeability factors reveal that interendothelial openings in venules are easily found in normal animals whereas they are rarely observed in diabetic animals, thus suggesting a role for insulin as modulator of endothelial cell functions (Garcia Leme, Böhm, Migliorini & Souza, 1974). An acute effect of

insulin on endothelial cells of the microcirculation, including a change in the kinetics of the vesicular transport pathway has been described (Osterby, Gundersen & Christensen, 1978). Despite the fact that noradrenaline evokes a vasoconstrictor response of mesenteric microvessels *in situ*, the latency and nature of which is analogous in normal and diabetic animals, histamine and bradykinin are capable of antagonizing this response in normal but not in diabetic animals, when minimal effective doses are employed. In contrast, acetylcholine acting as antagonist to noradrenaline, is equally effective in both groups of animals. The altered responses to histamine and bradykinin are not associated with hyperglycaemia since fasting renders the diabetic animals normoglycaemic and yet does not restore the reactivity of microvessels. Previous administration of insulin to diabetic animals corrects the impaired responses. Accordingly, histamine and bradykinin must antagonize the vasoconstrictor effect of noradrenaline in microvessels through a different mechanism from acetylcholine. Local and temporary changes in composition of the interstitial fluid resulting from the effect of permeability-increasing factors might be relevant factors to explain such differences (Fortes, Garcia Leme & Scivoletto, 1983).

There is evidence, however, to show that endothelial cells play an obligatory role in the relaxation *in vitro* of arterial smooth muscle by acetylcholine, bradykinin or histamine. The mechanism which applies in the case of large arteries depends on the release of a substance that causes relaxation of the vascular smooth muscle (Furchgott & Zawadzki, 1980; Chand & Altura, 1981a,b,c; Van de Voorde & Leusen, 1982).

The present experiments were undertaken to compare, in normal and diabetic conditions, the response to vasoactive agents of microcirculatory vessels *in situ* and large arteries *in vitro*. This was done in an attempt to investigate further the influence of short and long-term diabetes mellitus on the functional behaviour of blood vessels and the mechanisms through which diabetes interferes with their reactivity.

## Methods

Male Wistar rats were used for studies on mesenteric microvessels *in situ*; their thoracic aortae were isolated and fragments tested *in vitro* for the capacity to respond to drugs. The experimental groups comprised diabetic animals and matching controls. Diabetes was produced by the injection of alloxan, either 10 days or 6 months beforehand. The animals weighed between 100–150 g when the experiments started.

### *Procedures with mesenteric microvessels*

The mesentery was exteriorized and arranged for microscopic observation *in situ* according to the method of Zweifach (1948) with slight modifications. The animals, under chloral hydrate anaesthesia (400–450 mg $kg^{-1}$, s.c.), were kept on a special board which included a transparent plate on which the tissue to be transilluminated was placed. The mesentery was kept moist and warmed by irrigating the tissue intermittently with warmed (37°C) Ringer-Locke solution (pH 7.2–7.4) containing 1% gelatin. The composition of the solution was (mM): NaCl 154, KCl 5.6, $CaCl_2 2H_2O$ 2, $NaHCO_3$ 6 and glucose 5. After selecting a suitable microscopic field observed under a magnification of 100 ×, the preparation was standardized on the basis of the response to 0.01 ml of a 3 μg $ml^{-1}$ solution of noradrenaline topically applied. The response was characterized by the complete cessation of blood flow within 12 to 25 s in at least one vessel of the microscopic field. The experiments were designed to evaluate the antagonistic effect on this response of acetylcholine, papaverine, histamine or bradykinin, topically applied in a standard volume of 0.01 ml. In another series of experiments, the osmolarity of the control Ringer-Locke solution (300 mosmol $l^{-1}$) was increased to 340 or 500 mosmol $l^{-1}$ by the addition of sucrose and the effect of hyperosmolarity on the response to noradrenaline tested. Osmolarity of the solutions was checked by the freezing-point depression method (Fiske osmometer). Noradrenaline was added 15 to 30 s after the addition of the antagonist drugs, or after a 5 min interval of contact of the preparation with the hypertonic solutions topically applied. A complete series of tests comprised the following steps, with a 3 min interval between each step: (a) application of noradrenaline and determination of latency time to response in the presence of the control Ringer-Locke solution (300 mosmol $l^{-1}$); (b) addition of the agent to be tested as antagonist or substitution of the control Ringer-Locke solution for an equivalent volume of a hypertonic solution, followed by application of noradrenaline; any effects were followed up to 90 s; (c) addition of the standard dose of noradrenaline to test recovery. Drugs and/or the hypertonic solutions were removed between each step by washing out with the control Ringer-Locke solution. An antagonistic effect was assumed to occur when the latency to interruption of blood flow following noradrenaline application was significantly increased in comparison with values obtained in the absence of antagonist. A given section of the vascular bed was tested only once and no more than 3 series were performed on a single animal. Drugs added to the preparation were dissolved in Ringer-Locke sol-

ution, the osmolarity of which varied according to the type of experiment. Doses are given in terms of the salt.

*Procedures with isolated preparations of thoracic aorta*

Descending thoracic aortae were excised, and trimmed free of adhering fat and connective tissue. Two transverse rings of the same artery, each about 4 mm in length, were cut and mounted at optimal length for isometric tension recording in organ chambers, according to Furchgott & Zawadzki (1980). One ring served as control while in the other the endothelium was mechanically removed by gently rubbing the luminal surface with a small cylindric piece of artificial sponge attached to a thread to permit insertion through the lumen. Two hooks of stainless steel wire bent in a modified L-shape were used to mount each ring. The short straight portion of each hook passed through the lumen of the ring. The lower hook was attached to the base of the organ chamber, the upper to a strain gauge. The preparations were mounted in organ baths containing 10 ml Krebs-Henseleit solution, the composition of which was (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.1, $KH_2PO_4$ 1.1, ascorbic acid 0.11, EDTA 0.03 and glucose 11. The bathing fluid, kept at 37°C, was saturated with a gas mixture of 95% $O_2$ and 5% $CO_2$. The preparations were allowed to equilibrate for at least 1 h under a resting tension of 1 g which was maintained throughout. Tension was recorded by a F-60 microdisplacement myograph, and displayed on a physiograph. Cumulative concentration-effect curves were constructed from the response of the tissue to noradrenaline. At the end of the experiment, effective doses of acetylcholine were added to test the presence or loss of the relaxant response of the preparation and, therefore, the efficacy of the procedure for removal of the endothelium. In another group of experiments, responses of arteries precontracted with noradrenaline to cumulative concentrations of histamine and acetylcholine were recorded. Drugs were dissolved in Krebs-Henseleit solution and doses are given as the final molar concentration of the salt in the organ bath.

*Determination of blood pressure*

The method for determining the systolic blood pressure of unanaesthetized rats was a slight modification of that described by Williams, Harrison & Grollman (1939). The apparatus consisted of a warming box, a suitable holder for the animals, a compression cuff, and a plethysmograph filled with water connected to a water manometer. The animals were placed for 10 min in the warming box kept at 40°C. On removal of the box the animals were placed in the holder and the tail was inserted through a hole into the cuff and the plethysmograph. The pressure cuff was inflated well above the usual systolic level, the air slowly released to allow flow of blood into the tail and the pressure determined by transmission of tail volume changes to the water manometer.

*Production of alloxan diabetes*

The animals were fasted for 18 h, with water *ad libitum*, then injected intravenously with 40 mg $kg^{-1}$ alloxan. The effect of the drug was assessed by determination of blood sugar levels as described by King & Garner (1947). Animals with glycaemia above 200 mg 100 $ml^{-1}$ were used. This comprised two groups which were injected with alloxan 10 days or 6 months before. Matching controls were kept in analogous conditions during the same time intervals and their blood sugar levels then estimated.

*Drugs*

The following were used: acetylcholine chloride (Sigma); alloxan hydrate (Carlo Erba); bradykinin triacetate (Sigma); histamine hydrochloride (Carlo Erba); (−)-noradrenaline bitartarate (Sigma); papaverine hydrochloride (Sigma) and sucrose (Reagen).

*Statistical analysis*

Results were compared by analysis of variance, $P < 0.05$ being taken as statistically significant. To test differences among means the Q method was used (Snedecor & Cochran, 1974).

## Results

*Studies on mesenteric microvessels* in situ

*Response to noradrenaline and the effect of previous addition of acetylcholine, papaverine, histamine or bradykinin in short-term diabetes mellitus* Animals in this group were rendered diabetic by the injection of alloxan 10 days before. Noradrenaline (NA) in a dose of 0.03 μg topically applied to mesenteric microvessels, induced in either normal or alloxan-diabetic rats a response characterized by the complete interruption of blood flow in at least one vessel of the microscopic field. This response was evoked, in both groups of animals, within 12 to 25 s of drug addition. Acetylcholine (ACh) and papaverine topically applied to the preparation 30 s beforehand in doses of 3 μg and 1 μg, respectively, antagonized the

**Table 1** Mesenteric microvessels: influence of acetylcholine and papaverine on latency of the vasoconstrictor response to noradrenaline in normal and alloxan-diabetic rats

| | *Latency (s) of response to noradrenaline (0.03 μg)*[a] | | | | *Glycaemia* |
|---|---|---|---|---|---|
| | *Acetylcholine* (3 μg) | | *Papaverine* (1 μg) | | (mg %) |
| *Animals* | Absent | Present | Absent | Present | |
| Diabetic (10 days)[c] | 17.8 ± 1.9 ($n = 7$) | > 90[b] | 16.7 ± 1.9 ($n = 7$) | > 90 | 260–420 |
| Matching controls | 18.0 ± 1.6 ($n = 7$) | > 90 | 16.8 ± 1.8 ($n = 6$) | > 90 | 75–105 |

[a] The response was characterized by the complete interruption of blood flow in at least one vessel of the microscopic field. Acetylcholine and papaverine were applied 30 s before noradrenaline. Doses of the antagonists were the minimum effective doses to prevent consistently the standard amount of noradrenaline from producing its vasoconstrictor action. Drugs were applied topically. Results are mean ± s.e.mean. Number of microscopic fields tested is indicated in parentheses.
[b] No vasoconstrictor effect was observed up to 90 s after the application of noradrenaline; drugs were then washed out.
[c] Time after alloxan injection.

vasoconstrictor response to NA in both normal and diabetic animals. In these circumstances, no vasoconstrictor response was observed up to 90 s after the application of NA. The doses employed were the minimum effective doses capable of antagonizing consistently the response to the standard amount of NA (Table 1). In contrast, the minimum effective doses of histamine (0.03 μg) and bradykinin (0.03 μg) capable of consistently antagonizing the response to NA in normal animals had to be increased about 20 fold to block the response of diabetic rats to the vasoconstrictor agent in the subsequent 90 s interval. Doses of 0.1 and 0.3 μg of both histamine and bradykinin, topically applied to the preparation, 30 s and 15 s before NA, respectively, only caused an increase in latency of response to the vasoconstrictor agent (Table 2).

*Response to noradrenaline and the effect of previous addition of acetylcholine or histamine in long-term diabetes mellitus* Animals in this group were rendered diabetic by the injection of alloxan 6 months beforehand. Due to the similarities between the effects of ACh and papaverine, or of histamine

**Table 2** Mesenteric microvessels: influence of histamine and bradykinin on latency of the vasoconstrictor response to noradrenaline in normal and alloxan-diabetic rats

| | *Latency(s) of response to noradrenaline (0.03 μg)*[a] | | | | | *Glycaemia* |
|---|---|---|---|---|---|---|
| | | | *Histamine* | | | (mg %) |
| *Animals* | Absent | 0.03 μg | 0.10 μg | 0.30 μg | 0.60 μg | |
| Diabetic (10 days)[c] | 15.5 ± 1.1 ($n = 15$) | 18.1 ± 2.2 ($n = 15$) | 26.3 ± 3.7* ($n = 13$) | 38.0 ± 5.3* ($n = 7$) | > 90[b] ($n = 7$) | 230–450 |
| Matching controls | 17.0 ± 1.2 ($n = 6$) | > 90 ($n = 6$) | — | — | — | 80–110 |
| | | | *Bradykinin* | | | |
| | Absent | 0.03 μg | 0.10 μg | 0.30 μg | 0.60 μg | |
| Diabetic (10 days) | 16.4 ± 1.5 ($n = 7$) | 20.0 ± 1.8 ($n = 7$) | 28.7 ± 2.4* ($n = 6$) | 35.5 ± 4.8* ($n = 5$) | > 90 ($n = 7$) | 300–470 |
| Matching controls | 18.0 ± 1.5 ($n = 5$) | > 90 ($n = 5$) | — | — | — | 70–105 |

[a] The response was characterized by the complete interruption of blood flow in at least one vessel of the microscopic field. Histamine or bradykinin were applied 30 s or 15 s before noradrenaline, respectively. The smaller doses of the antagonists were the minimum effective doses to prevent consistently the standard amount of noradrenaline from producing its vasoconstrictor action in normal animals. Drugs were applied topically. Results are mean ± s.e.mean. Number of microscopic fields tested is indicated in parentheses.
[b] No vasoconstrictor effect was observed up to 90 s after the application of noradrenaline; drugs were then washed out.
[c] Time after alloxan injection.
* $P < 0.05$ in comparison with values in the absence of histamine or bradykinin.

**Table 3** Mesenteric microvessels: influence of histamine and acetylcholine on latency of the vasoconstrictor response to noradrenaline in normal and alloxan-treated (6 months) diabetic rats

| | *Latency(s) of response to noradrenaline (0.03 μg)*[a] | | | | | *Glycaemia* |
|---|---|---|---|---|---|---|
| | | | *Histamine* | | | (mg %) |
| *Animals* | Absent | 0.03 μg | 0.10 μg | 0.30 μg | 0.60 μg | |
| Diabetic (6 months)[c] | 14.8 ± 0.9 ($n = 20$) | 17.0 ± 1.8 ($n = 12$) | 19.0 ± 3.1 ($n = 12$) | 22.2 ± 3.0* ($n = 11$) | 27.0 ± 4.0* ($n = 6$) | 405–440 |
| Matching controls | 18.0 ± 1.8 ($n = 6$) | > 90[b] ($n = 6$) | — | — | — | 80–105 |
| | | | *Acetylcholine* | | | |
| | | Absent | | Present (3 μg) | | |
| Diabetic (6 months) | | 17.4 ± 1.7 | ($n = 6$) | > 90 | | 405–440 |
| Matching controls | | 20.3 ± 1.4 | ($n = 6$) | > 90 | | 80–105 |

[a] The response was characterized by the complete interruption of blood flow in at least one vessel of the microscopic field. Acetylcholine and histamine were applied 30 s before noradrenaline. The smaller dose of histamine was the minimum effective dose to prevent consistently the standard amount of noradrenaline from producing its vasoconstrictor action in normal animals. Acetylcholine was employed in the minimum effective dose. Drugs were applied topically. Results are mean ± s.e.mean. Number of microscopic fields tested is indicated in parentheses.

[b] No vasoconstrictor effect was observed up to 90 s after the application of noradrenaline; drugs were then washed out.

[c] Months after alloxan injection.

* $P < 0.05$ in comparison with values in the absence of histamine.

and bradykinin in the preceding series of experiments, the present studies were restricted to the investigation of the effects of ACh and histamine as representatives of each group of drugs. ACh remained fully active as an antagonist of the vasoconstrictor response of alloxan-treated (6 months) diabetic animals to NA, whereas histamine was now even less active than it was in rats with short-term diabetes mellitus. Doses of histamine about 20 fold higher than the minimum effective dose, as referred to above, were not capable of blocking the vasoconstrictor response to NA, despite the increase in latency to this response (Table 3).

*Response to noradrenaline and the effect of hyperosmolarity* The effect of increasing the osmolarity of the control Ringer–Locke solution (300 mosm $l^{-1}$), employed to keep the mesentery moist and warmed, on the latency of the response to NA was evaluated (Table 4). Significant and equivalent increases in comparison with control responses, were noted in normal and alloxan-treated (10 days) diabetic animals, after a 5 min interval of contact of the preparation with hypertonic solutions (340 and (500 mosmol $l^{-1}$). Topical application of histamine (0.03 μg) or bradykinin (0.03 μg) to the mesentery of normal animals, in addition to the hyperosmotic (500 mosmol $l^{-1}$) solution, caused complete blockade of the vasoconstrictor response to NA in the subsequent 90 s interval. In these circumstances, the preparation was kept in contact with the hypertonic solution for 5 min, then histamine or bradykinin was added 30 s or 15 s before NA, respectively. However, in diabetic animals histamine and bradykinin did not produce any further increase in latency of response to NA, when similarly applied. This was observed, despite the fact that the doses used were the minimum effective doses capable of consistently antagonizing the response of normal animals to the vasoconstrictor agent.

*Studies on isolated preparations of thoracic aortae*

*Concentration-effect curves to noradrenaline* Rats with short or long-term duration of experimental diabetes mellitus (i.e. 10 days or 6 months after alloxan) and matching controls were used. Animals with short-term diabetes were divided into two groups, according to their plasma glucose levels: one group in which glycaemia ranged between 250–350 mg %, and another in which glucose levels were above 450 mg %. In all groups of animals cumulative concentration-effect curves to NA were simultaneously constructed from the response of two pieces of the same artery, one in which the endothelium was left intact, and the other in which the endothelium had been removed. In preparations without endothelium similar results were observed for normal and diabetic animals, irrespective of the duration of the disease and of the individual plasma glucose levels. Threshold and median effective concentrations ($EC_{50}$) were significantly lower, whereas

**Table 4** Mesenteric microvessels: influence of the osmolarity of the bathing fluid (Ringer-Locke solution) on latency of the vasoconstrictor response to noradrenaline in normal and alloxan-diabetic rats

| | *Latency(s) of response to noradrenaline (0.03 μg)*[a] *at* | | | | *Glycaemia* |
|---|---|---|---|---|---|
| *Animals* | *300 mosmol l*$^{-1}$ | *340 mosmol l*$^{-1}$ | *500 mosmol l*$^{-1}$ | *500 mosmol l*$^{-1}$ *+ Histamine (0.03 μg)* | (mg%) |
| Diabetic (10 days)[b] | 16.8 ± 2.5 | 22.3 ± 1.4* | 28.7 ± 2.7* | 30.1 ± 3.4** | 280–500 |
| | (*n* = 10) | (*n* = 6) | (*n* = 9) | (*n* = 10) | |
| Matching controls | 16.0 ± 1.7 | 27.0 ± 2.4* | 35.8 ± 3.7* | > 90[c] | 70–110 |
| | *300 mosmol l*$^{-1}$ | *340 mosmol l*$^{-1}$ | *500 mosmol l*$^{-1}$ | *500 mosmol l*$^{-1}$ *+ Bradykinin (0.03 μg)* | |
| Diabetic (10 days) | 14.1 ± 1.5 | 25.4 ± 1.9* | 29.0 ± 4.3* | 38.4 ± 5.1** | 210–540 |
| | (*n* = 14) | (*n* = 8) | (*n* = 7) | (*n* = 8) | |
| Matching controls | 18.5 ± 2.8 | 29.0 ± 2.1* | 36.1 ± 3.4* | > 90 | 70–110 |
| | (*n* = 6) | (*n* = 8) | (*n* = 10) | (*n* = 6) | |

[a] The response was characterized by the complete interruption of blood flow in at least one vessel of the microscopic field. The preparation was left in contact with isotonic (300 mosmol l$^{-1}$) or hypertonic Ringer-Locke solution for 5 min, then the drugs were added topically. Hypertonic solutions were prepared by the addition of sucrose. Histamine or bradykin were applied 30 s or 15 s before noradrenaline, respectively, and were dissolved in the corresponding hypertonic solution. Results are mean ± s.e.mean. Number of microscopic fields tested is indicated in parentheses. Doses of histamine and bradykinin were the minimum effective doses to prevent consistently the standard amount of noradrenaline from producing its vasoconstrictor action in normal animals.

[b] Days after alloxan injection.

[c] No vasoconstrictor effect was observed up to 90 s after the application of noradrenaline; drugs were then washed out.

* $P < 0.05$ in comparison with corresponding control value at 300 mosmol l$^{-1}$.

** $P > 0.05$ in comparison with corresponding value at 500 mosmol l$^{-1}$.

maximal responses were significantly higher than in preparations with an intact endothelium. Diabetes only affected preparations in which the endothelium was left intact. In these, $EC_{50}$s were markedly increased in comparison with normal values. Results are shown in Figure 1 and Table 5.

*Relaxation by acetylcholine and histamine* Aortae from either normal or diabetic animals precontracted with NA ($10^{-7}$ M) were similarly relaxed by ACh or histamine, provided the endothelium was left intact. Loss of the relaxant response of the preparations from all groups of animals was observed after rubbing the inner surface to remove endothelial cells. Animals with short or long-term duration diabetes, as above, were used. Similar results were obtained with preparations from both groups. Glycaemia ranged between 320–460 mg % in short-term diabetes, and between 290–450 mg % in long-term diabetes. Figure 2 illustrates a typical experiment. Figure 3 summarizes results obtained with preparations from both groups of diabetic animals and their matching controls.

*Biological parameters in short and long-term diabetes mellitus* Table 6 shows age, body weight, plasma glucose levels and blood pressure values in alloxan-treated (10 days and 6 months) diabetic rats and matching controls. Loss of weight was observed in both groups of diabetic animals in comparison with corresponding controls. Blood pressure values remained within the normal range in all animals tested.

## Discussion

Evidence has accumulated showing that diabetes mellitus influences vascular reactivity to drugs. We now suggest that this might be due to a functional alteration of the endothelial cell, particularly evident when microvessels are tested. Though noradrenaline was equally effective in normal and diabetic animals as a vasoconstrictor agent, and was fully antagonized by acetylcholine and papaverine in both groups of animals, histamine and bradykinin exhibited different properties when normal and diabetic animals were considered. The minimum dose of histamine or bradykinin, employed to prevent consistently a standard amount of noradrenaline from producing its vasoconstrictor action on mesenteric microvessels of normal animals, had to be increased about 20 fold in diabetic animals. Intermediate doses of histamine or bradykinin increased the latency of the vasoconstrictor response in these animals. The defect was detected in the early stages of the induced disease, i.e. 10 days after the injection of alloxan, and was aggravated in long-term diabetes mellitus, i.e. 6 months

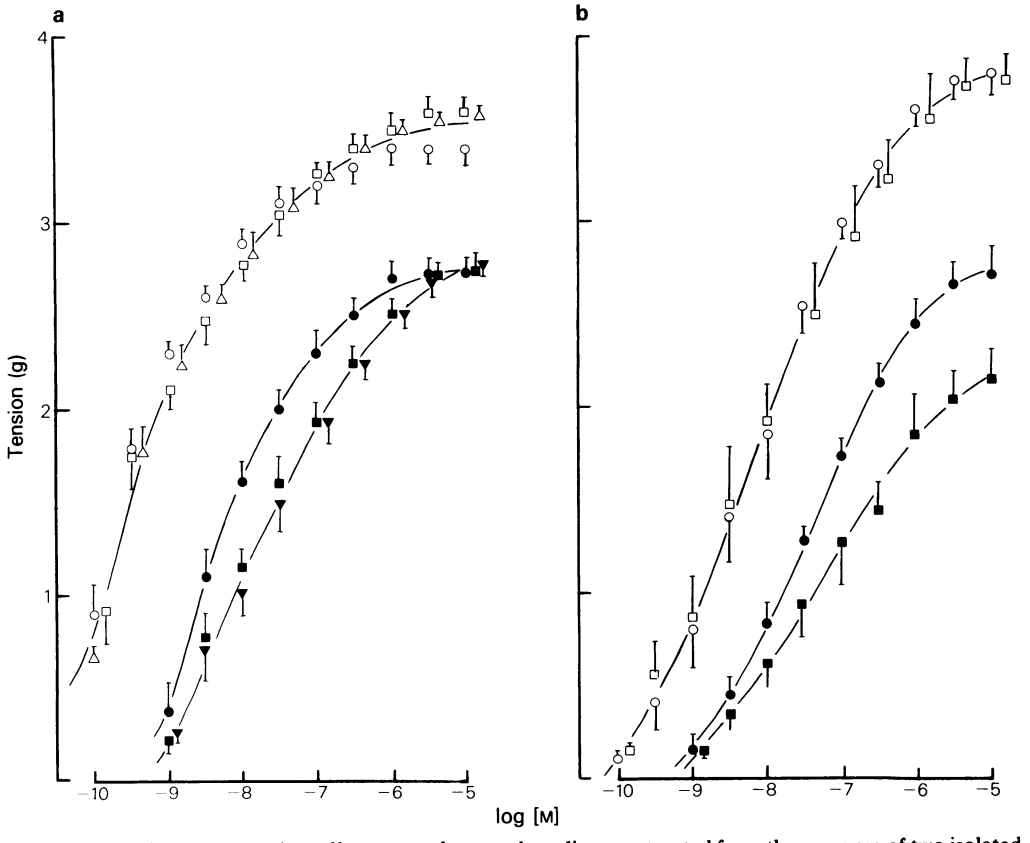


**Figure 1** Cumulative concentration-effect curves for noradrenaline constructed from the response of two isolated transverse rings of the same aorta. In one ring the endothelium was left intact (closed symbols), in the other it was mechanically removed prior to testing (open symbols). In (a) preparations were obtained from animals injected with alloxan 10 days before and from corresponding controls. (○) and (●): controls; (□) and (■): diabetic animals (glycaemia: 250–350 mg %); (△) and (▲): diabetic animals (glycaemia: > 450 mg %). In (b) preparations were obtained from animals injected with alloxan 6 months beforehand and from corresponding controls. (○) and (●): controls; (□) and (■): diabetic animals (glycaemia 290–440 mg %). Each point represents the mean value of 7 determinations. Vertical bars show s.e.mean.

**Table 5** Median effective concentration ($EC_{50}$) values obtained from the response of isolated thoracic aortae to noradrenaline

| *Animals* | $EC_{50}$ (nM)[a] *Intact endothelium* | *Without endothelium* |
|---|---|---|
| Diabetic (10 days)[b] | 25.10* | 0.34** |
| (glycaemia: 250–350 mg %) | (13.50–46.80) | (0.12–1.00) |
| Diabetic (10 days) | 26.90* | 0.35** |
| (glycaemia: > 450 mg %) | (14.30–50.40) | (0.23–0.51) |
| Matching controls | 4.80 | 0.28** |
| (glycaemia: 70–110 mg %) | (3.05–7.40) | (0.20–0.40) |

[a] Estimated from concentration-effect curves constructed from the response of two transverse rings of the same artery. One ring served as control while in the other the endothelium was mechanically removed. Results are mean geometric values of 7 determinations in each group. Figures in parentheses indicate 95% confidence intervals.
[b] Days after alloxan injection.
* $P < 0.05$ in comparison with corresponding value in matching control.
** $P < 0.05$ in comparison with corresponding values in preparations with intact endothelium.

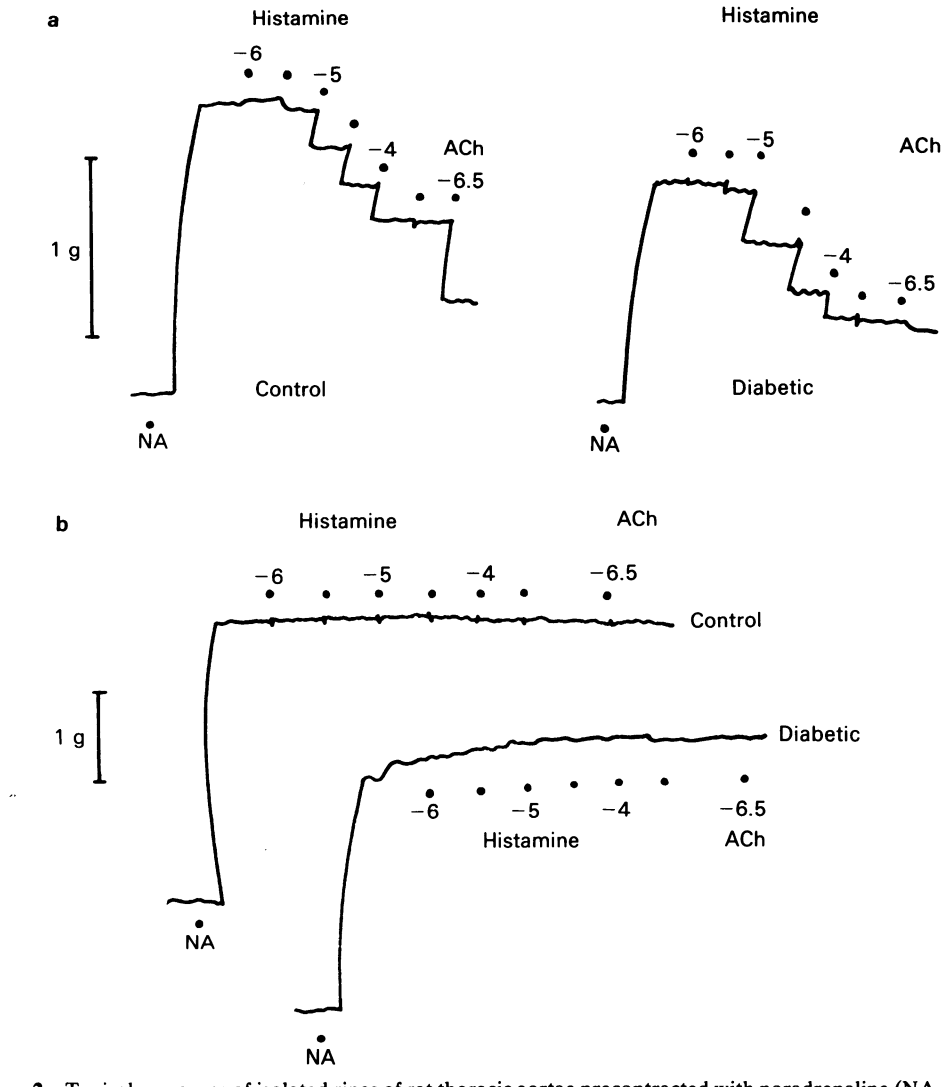


**Figure 2** Typical responses of isolated rings of rat thoracic aortae precontracted with noradrenaline (NA, $10^{-7}$ M) to histamine and acetylcholine (ACh). Doses are expressed as the logarithm of the final molar concentration of the salts in the organ bath. Preparations were obtained from normal and alloxan-treated (10 days) diabetic animals. In (a) the endothelium was left intact. In (b) it was mechanically removed prior to testing. Loss of the relaxant response was observed in (b).

after the injection of alloxan. In contrast, the minimum effective doses of acetylcholine and papaverine were found to be equivalent in normal and diabetic animals. Though papaverine was not tested, acetylcholine remained fully active as an antagonist to the vasoconstrictor action of noradrenaline 6 months after the injection of alloxan.

Histamine and bradykinin are potent permeability-increasing agents in most species (Wilhelm, 1973), whereas acetylcholine and papaverine are practically devoid of such an action. We propose that histamine and bradykinin might antagonize the vasoconstrictor response of microvessels to noradrenaline through an action on lining endothelial cells resulting in interendothelial gaps, and increased vascular permeability with temporary changes in composition of extracellular fluid. Changes in local steady-state conditions, if only from the osmotic point of view, might be the main factor responsible for the antagonistic effect of histamine and bradykinin toward the vasoconstrictor response to noradrenaline. Microscopic studies show that the

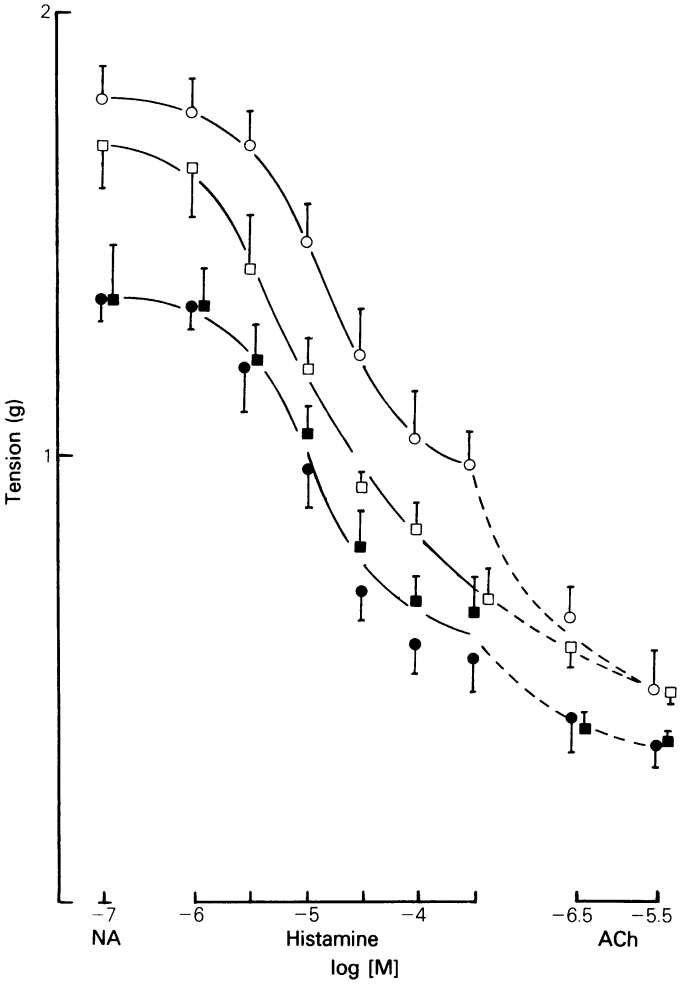


**Figure 3** Concentration-dependent relaxation by histamine (continuous lines) and acetylcholine (dashed lines) of isolated rings of rat thoracic aortae precontracted with $10^{-7}$ M noradrenaline and with intact endothelium. The preparations still responded to acetylcholine after maximal relaxation by histamine. (●): Diabetic animals injected with alloxan 10 days beforehand (glycaemia: 320–460 mg%); (○): matching controls; (■): diabetic animals injected with alloxan 6 months before (glycaemia: 290–450 mg%); (□): matching controls. Each point represents the mean value of 6 to 8 determinations. Vertical bars show s.e.mean.

**Table 6** Biological parameters of normal and alloxan-diabetic animals: alloxan was injected either 10 days or 6 months beforehand

| *Animals* | *Age* (days) | *Body weight* (g) | *Glycaemia* (mg%) | *Blood pressure* (mmHg) |
|---|---|---|---|---|
| Diabetic (10 days) ($n = 8$) | 40–50 | 110–140 | 230–410 | 100–120 |
| Matching controls ($n = 8$) | 40–50 | 140–150 | 80–110 | 90–110 |
| Diabetic (6 months) ($n = 7$) | 240–250 | 160–250 | 290–440 | 100–115 |
| Matching controls ($n = 6$) | 240–250 | 380–440 | 90–100 | 100–105 |

direct application of hypertonic solutions to cerebral (Wahl, Kuschinsky, Bosse & Thurau, 1973) and muscle vessels (Gray, 1971) causes their relaxation. Depression of the excitation induced by noradrenaline on helical strips from veins is observed on increasing the osmolarity of the bathing fluid (McGrath & Shepherd, 1976). Furthermore, in microvessel beds of the cheek pouch and cremaster muscle of the hamster, arteriolar dilatation is produced by suffusing the tissues with hyperosmotic solutions. The dilatation requires between 2 and 5 min to reach full effect (Duling & Staples, 1976). A significant increase in latency of the vasoconstrictor response to noradrenaline, in comparison with control responses, was observed in microvessels of either normal or diabetic animals in the present experiments, following contact of the preparation with hypertonic solutions. Histamine and bradykinin, however, even when applied in combination with hyperosmotic solutions did not further increase the latency of the response to noradrenaline in diabetic animals. If histamine and bradykinin are acting primarily on endothelial cells to antagonize the vasoconstrictor effect of the catecholamine, and if diabetes impairs a related endothelial cell function, one would not expect to be able to produce a summation of effects by exposing microvessels of diabetic animals to a combined action of permeability factors and hyperosmotic solutions. Temporary changes in composition of extracellular fluid, as apparently occur when microvessels respond to permeability factors, might, therefore, affect the reactivity of such vessels to constrictor agents.

Acetylcholine, bradykinin and histamine, however, evoke relaxation of large arteries *in vitro* by similar mechanisms which involve the release of a substance by the endothelial cell (Furchgott & Zawadzki, 1980; Chand & Altura, 1981a; Van de Voorde & Leusen, 1982). It was relevant, therefore, to test the response of large arteries from normal and diabetic animals to these agents. Precontracted aortae from both groups of animals were equally relaxed by acetylcholine and histamine, provided the endothelium was left intact. Loss of the relaxant response of the preparations was observed after removal of the endothelium. In addition, similar concentration-effect curves to noradrenaline were obtained in preparations without endothelium from normal and diabetic animals, thus suggesting that diabetes mellitus did not affect the reactivity of the vascular smooth muscle. Both threshold and median effective concentrations of noradrenaline, determined in preparations without endothelium, from both normal and diabetic animals, were lower and the maximal responses were higher than those estimated in preparations in which the endothelium was left intact. This seemed to indicate that lining endothelial cells of large arteries *in vitro* can hinder the full expression of noradrenaline effects. The situation is aggravated in diabetic animals, as shown by the much higher values obtained for median effective concentrations of noradrenaline in comparison with control values. Diabetes mellitus, therefore, apparently provoked a functional alteration of the endothelial cell in such vessels which, nevertheless, is unrelated to its relaxant properties, since acetylcholine and histamine were still capable of relaxing precontracted aortae from diabetic animals in which the endothelium was left intact. On the other hand, diabetes interfered with the action of histamine and bradykinin as antagonists to the vasoconstrictor effect of noradrenaline in microvessels. It is possible, therefore, that different mechanisms are involved when one considers the effect of vasodilator agents acting on small vessels *in situ* or large arteries *in vitro*. Despite the fact that the endothelial cell is involved in both circumstances, the intimate mechanisms might be of a different nature, at least when permeability-increasing factors are tested.

The relative lack of insulin that occurs in diabetes mellitus, seems to be a relevant causative factor in the alteration of the response of the endothelial cell to permeability factors. First, because previous administration of the hormone to diabetic animals corrects the impaired responses to histamine and bradykinin. Second, because a similar condition of impaired response to these agents is produced in normal animals by the intravenous injection of 2-deoxyglucose, the acute effects of which are the result of intracellular glucopaenia, secondary to inhibition of glucose utilization. Acetylcholine remains fully active in this situation (Fortes *et al.* 1983). Increased vascular permeability, which leads to local and temporary changes in composition of extra-vascular fluid, is generally associated with the partial disconnection of endothelial cells along the intercellular junctions, resulting in the formation of gaps through which intravascular materials exude (Majno & Palade, 1961). This is a reactive process occurring under the influence of appropriate stimuli (for instance, the presence of permeability-increasing factors), and depends on the availability of insulin, the lack of which leads to impaired responses of the cells.

There is a variety of large and small vessel morphological abnormalities which are prominent in diabetes mellitus although not essentially indistinguishable from those found in other pathological states affecting the vascular system. The degree of vascular disease does not appear to be proportional to the alterations in carbohydrate metabolism but correlates best with the duration of the disease. (Williams & Porte, 1974). No changes in arterial blood pressure were observed in the present series in short or long-term diabetes mellitus, which suggests that

the functional alterations reported here are not closely associated with conditions necessary for abnormal blood pressure levels.

In conclusion, diabetes mellitus in its early stages caused impaired responses of microvessels to histamine and bradykinin, acting as antagonists to the vasoconstrictor effect of noradrenaline. Apparently this resulted from an alteration of endothelial cell function due to a relative lack of insulin. The present findings might explain why permeability-increasing factors are less active in diabetic animals, and consequently why decreased inflammatory reactions are observed in these animals.

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## References

ALTURA, B.M., HALEVY, S. & TURLAPATY, P.D.M.V. (1979). Vascular smooth muscle in diabetes and its influence on the reactivity of blood vessels. *Adv. Microcirc.*, **8**, 118–150.

CHAND, N. & ALTURA, B.M. (1981a). Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells: role in lung vascular diseases. *Science*, **213**, 1376–1379.

CHAND, N. & ALTURA, B.M. (1981b). Inhibition of endothelial cell-dependent relaxations to acetylcholine and bradykinin by lypoxygenase inhibitors in canine isolated renal arteries. *Microcirculation*, **1**, 211–223.

CHAND, N. & ALTURA, B.M. (1981c). Endothelial cells and relaxation of vascular smooth muscle cells: possible relevance to lypoxygenases and their significance in vascular diseases. *Microcirculation*, **1**, 297–317.

DULING, B.R. & STAPLES, E. (1976). Microvascular effects of hypertonic solutions in the hamster. *Microvasc. Res.*, **11**, 51–56.

FORTES, Z.B., GARCIA LEME, J. & SCIVOLETTO, R. (1983). Influence of diabetes on the reactivity of mesenteric microvessels to histamine, bradykinin and acetylcholine. *Br. J. Pharmac.*, **78**, 39–48.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

GARCIA LEME, J. (1981). Regulatory mechanisms in inflammation: New aspects of autopharmacology. *Gen. Pharmac.*, **12**, 15–24.

GARCIA LEME, J., BÖHM, G.M., MIGLIORINI, R.H. & SOUZA, M.Z.A. (1974). Possible participation of insulin in the control of vascular permeability. *Eur. J. Pharmac.*, **29**, 298–306.

GARCIA LEME, J., HAMAMURA, L., MIGLIORINI, R.H. & LEITE, M.P. (1973). Influence of diabetes upon the inflammatory response of the rat. A pharmacological analysis. *Eur. J. Pharmac.*, **23**, 74–81.

GRAY, S.H. (1971). Effect of hypertonicity on vascular dimensions in skeletal muscle. *Microvasc. Res.*, **3**, 117–124.

KING, E.J. & GARNER, R.J. (1947). Colorimetric determination of glucose. *J. clin. Pathol.*, **1**, 30–33.

LLORACH, M.A.S., BÖHM, G.M. & GARCIA LEME, J. (1976). Decreased vascular reactions to permeability factors in experimental diabetes. *Br. J. exp. Pathol.*, **57**, 747–754.

MAJNO, G. & PALADE, G.E. (1961). Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: An electron microscopic study. *J. biophys. biochem. Cytol.*, **11**, 571–605.

McGRATH, M.A. & SHEPHERD, J.T. (1976). Hyperosmolarity: effects on nerves and smooth muscle of cutaneous veins. *Amer. J. Physiol.*, **231**, 141–147.

OSTERBY, R., GUNDERSEN, H.J. & CHRISTENSEN, N.J. (1978). The acute effect of insulin on capillary endothelial cells. *Diabetes*, **27**, 745–749.

SNEDECOR, G.W. & COCHRAN, W.G. (1974). *Statistical Methods*, 6th ed. Ames, Iowa: State University Press.

VAN de VOORDE, J. & LEUSEN, I. (1982). Vascular endothelium and the relaxation effect of histamine on aorta of the rat. *Archs int. Pharmacodyn.*, **256**, 329–330.

WAHL, M., KUSCHINSKY, W., BOSSE, O. & THURAU, K. (1973). Dependency of pial arterial and arteriolar diameter on perivascular osmolarity in the cat. *Circulation Res.*, **32**, 162–169.

WILHELM, D.L. (1973). Chemical mediators. In *The Inflammatory Process*, 2nd. ed., vol. II. ed. Zweifach, B.W., Grant, L., McCluskey, R.T., pp. 251–301. New York and London: Academic Press.

WILLIAMS, JR., J.R., HARRISON, T.R. & GROLLMAN, A. (1939). A simple method for determining the systolic blood pressure of unanesthetized rat. *J. clin. Invest.*, **18**, 373–376.

WILLIAMS, R.H. & PORTE, JR., D. (1974). The pancreas. In *Textbook of Endocrinology*, 5th ed., ed. Williams, R.H. pp. 502–626. Philadelphia, London, Toronto: W.B. Saunders.

ZWEIFACH, B.W. (1948). Indirect methods for regional blood flow. I. Microscopic observation of circulation in rat mesoappendix and dog omentum. Use in study of vasotropic substances. *Meth. med. Res.*, **1**, 131–138.

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