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Influence of diabetes on the reactivity of mesenteric microvessels to histamine, bradykinin and acetylcholine

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1 Noradrenaline (NA) evoked a vasoconstrictor response in rat mesenteric microvessels *in situ*, the latency and nature of which was analogous in normal and alloxan-diabetic animals.

2 Histamine and bradykinin (Bk) were capable of antagonizing the response to NA in normal but not in diabetic animals. In contrast, acetylcholine (ACh) was equally effective as an antagonist to NA in both groups of animals.

3 The altered responses to histamine and Bk were not associated with hyperglycaemia since fasting rendered the diabetic animals normoglycaemic and yet did not restore the reactivity of microvessels. Previous administration of insulin to diabetic animals corrected the impaired responses to histamine and Bk.

4 A similar condition of impaired responses to histamine and Bk was produced in normal animals by the intravenous injection of 2-deoxyglucose although ACh remained fully active.

5 Apparently, the functional changes observed in the response to histamine or Bk, as antagonists of the vasoconstrictor reaction to NA, were not associated with a defective response of all smooth muscle. First, because ACh remained active in diabetic animals, and, second, because extravascular smooth muscles obtained from either normal or diabetic rats were equally relaxed by histamine or Bk *in vitro*.

6 It is suggested that histamine and Bk antagonized the vasoconstrictor response of microvessels to NA through an action on lining endothelial cells resulting in increased vascular permeability and hyperosmolarity of extracellular fluids.

7 The process depended on the availability of insulin, and, therefore, might be affected by intracellular glucopaenia as occurring in diabetes.

8 Intracellular glucopaenia markedly affected other structures. Reduced atria rates were observed in diabetes, despite the fact that the isolated preparation responded normally to NA, ACh or tyramine. Partial substitution of glucose in the bathing fluid by 2-deoxyglucose or addition of NaF to the organ bath evoked similar changes in atria from normal animals.

9 ACh which has little effect on vascular permeability must exert its vasodilator effects through mechanisms which are different from those influenced by the biochemical changes occurring in diabetes.

Introduction

Vascular reactivity and the functional properties of blood vessels are altered in diabetes mellitus. Microscopic investigation shows that systemic blood vessels of diabetic rats, challenged with permeability factors (histamine, 5-hydroxytryptamine (5-HT)) show less labelling by intravenously injected colloidal carbon particles than do vessels of normal animals; insulin, given beforehand, corrects this. In addition, electron microscopic studies of subdermal vessels submitted to the action of permeability factors, reveals that endothelial openings in venules are easily found in normal animals, whereas they are rarely observed in diabetic animals, thus suggesting an effect of insulin at this level (Garcia Leme, Böhm, Migliorini & Souza, 1974; Llorach, Böhm & Garcia Leme, 1976). Minimum concentrations of insulin may be required

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for the normal function of endothelial cells (Osterby, Gundersen & Christensen, 1978). Due to alterations in the reactivity of peripheral blood vessels, inflammatory oedema in the rat's paw after the local injection of a variety of irritants is markedly reduced when the animals are rendered diabetic by the administration of alloxan or by subtotal pancreatectomy. Diabetes, however, does not affect the store or release of endogenous vasoactive agents of importance for the development of inflammatory responses, nor is the accompanying hyperglycaemia a relevant circumstance (Garcia Leme, Hamamura, Migliorini & Leite, 1973).

These facts suggest that insulin may modulate the responses of blood vessels to endogenous vasoactive agents.

The present experiments were, therefore, undertaken, to investigate the influence of experimentally induced diabetes mellitus on the reactivity of mesenteric microvessels to histamine, bradykinin and acetylcholine. In addition, other preparations (right atrium, intestinal smooth muscle) were examined to verify whether the alteration in blood vessel function occurring in diabetes is also observed in nonvascular structures.

Methods

Male Wistar rats, 100–150 g body weight, were used. Each experimental group comprised at least 5 animals which were allocated as follows: (i) alloxan-diabetic rats; (ii) alloxan-diabetic rats subcutaneously injected with insulin; (iii) alloxan-diabetic rats fasted for 12–15 h; (iv) normal rats receiving 2-deoxyglucose, intravenously; (v) matching controls for each of the preceding groups. The animals were used for studies on mesenteric microvessels *in situ*, or their right atrium and duodenum isolated and tested *in vitro* for the capacity to respond to drugs.

Procedures with mesenteric microvessels

The mesentery was exteriorized and arranged for microscopic observation *in situ* according to the method of Zweifach (1948), slightly modified. The animals, under chloral hydrate anaesthesia (400–450 mg/kg, subcutaneously), were kept on a special board which included a transparent plate on which the tissue to be trans-illuminated 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 at a magnification of 100 ×, the preparation was standardized on the basis of the response to 0.03 ml of a 1 μg/ml 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 histamine, bradykinin or acetylcholine, topically applied in a standard volume of 0.01 ml. Noradrenaline was added either 30 s after the application of histamine or acetylcholine, or 15 s after the addition of bradykinin. An antagonistic effect was assumed to occur when interruption of blood flow was not observed in the subsequent 90 s interval. A complete series of tests comprised the following steps, performed at 3 min intervals: (a) application of noradrenaline and timed determination of latency to observe a response; (b) addition of the agent to be tested as antagonist, followed by application of noradrenaline; any drug effects were followed up to 90 s; (c) addition of the standard dose of noradrenaline to test recovery. Drugs were removed between each step by washing out with the warmed Ringer-Locke solution. 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 solution and doses are given in terms of the salt.

Isolated right atrium

The right atrium, resected under anaesthesia as above, was mounted in an organ bath containing 15 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 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 preparation was allowed to equilibrate for at least 1 h under a resting tension of 1 g which was maintained throughout. The frequency of the spontaneously beating atrium at equilibrium is referred to as the resting rate of the preparation. Frequencies, expressed in beats per min, were recorded by an F-50 microdisplacement miograph, and displayed on a physiograph. Cumulative concentration-effect curves were constructed from the response of the tissue to noradrenaline, acetylcholine, tyramine or sodium fluoride. In another group of experiments, after the equilibration period, glucose was substituted for varying proportions of 2-deoxyglucose in the Krebs-Henseleit solution, and the rate of the preparation then determined at equilibrium. A few experiments were performed to estimate atrial rates after a 20 min interval of continuous contact of the preparation with a fixed dose of propranolol or atropine. Drugs were dissolved in Krebs-Henseleit sol-

ution and doses are given as the final molar concentration of the salt in the organ bath.

Isolated duodenum

The animals were anaesthetized as above and the duodenum removed and mounted in an organ bath containing 20 ml Ringer-Locke solution saturated with a gas mixture of 95% O_2 and 5% CO_2. The preparation was kept at 28°C and maintained throughout under a resting tension of 4 g. After a stabilization period of 45 min, noncumulative doses of histamine or bradykinin were added to the organ bath. Responses, expressed as relaxation in cm/g tissue (wet weight), were recorded by an F-50 microdisplacement miograph and displayed on a physiograph. Drugs were dissolved in Ringer-Locke solution and doses are given as the final molar concentration of the salt in the organ bath.

Production of alloxan diabetes

The animals were fasted for 18 h, with water *ad libitum*, then injected intravenously with 40 mg/kg alloxan. The effect of the drug was assessed by determining blood sugar levels as described by King & Garner (1947). Animals with glycaemia above 200 mg/100 ml were used. These comprised several groups which were injected with alloxan either 1, 3, 6, 10, 30 or 45 days 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); atropine sulphate (E. Merck); bradykinin triacetate (Sigma); 2-deoxy-D-glucose (Sigma); histamine hydrochloride (Carlo Erba); insulin NPH (Lilly); (−)-noradrenaline bitartarate (Sigma); propranolol hydrochloride (Sigma); sodium fluoride (Baker); tyramine hydrochloride (E. Merck).

Statistical analysis

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

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

	Latency (s) of response to noradrenaline (0.03 μg)[a]				
	Histamine (0.03 μg)		*Bradykinin* (0.03 μg)		*Glycaemia*
Animals	Absent	Present	Absent	Present	(mg %)
Normal	17.0 ± 3.9 ($n=6$)	>90[b]	17.7 ± 1.6 ($n=6$)	>90	66–99
Diabetic (3 days)[c]	12.7 ± 2.2 ($n=7$)	15.3 ± 2.2	16.9 ± 1.8 ($n=10$)	19.1 ± 2.0	209–440
Normal	18.0 ± 3.3 ($n=6$)	>90	18.1 ± 1.6 ($n=7$)	>90	75–102
Diabetic (6 days)	12.0 ± 1.0 ($n=7$)	14.3 ± 2.8	16.9 ± 2.0 ($n=9$)	15.3 ± 2.9	243–453
Normal	21.9 ± 1.6 ($n=8$)	>90	17.7 ± 1.7 ($n=9$)	>90	70–87
Diabetic (10 days)	17.8 ± 1.8 ($n=7$)	24.7 ± 3.0	16.3 ± 0.9 ($n=6$)	23.8 ± 4.4	226–440
Normal	19.8 ± 4.6 ($n=5$)	>90	16.9 ± 2.0 ($n=9$)	>90	66–101
Diabetic (30 days)	17.9 ± 1.6 ($n=9$)	20.2 ± 2.0	14.3 ± 1.8 ($n=7$)	16.1 ± 1.9	216–438

(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 or 15 s, respectively, before noradrenaline. All drugs were applied topically. Results are mean ± s.e.mean. Number of microscopic fields tested is indicated in parentheses. Unless antagonism occurred (latency > 90 s), values in each group were not significantly different in the absence or presence of histamine or bradykin.

(b) No vasconstrictor effect was observed up to 90 s after the application of noradrenaline; drugs were then washed out.

(c) Days after alloxan injection.

Results

Studies on mesenteric microvessels in situ

Response to noradrenaline and the effect of previous addition of histamine or bradykinin 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. In normal animals, either histamine (0.03 μg) or bradykinin (Bk) (0.03 μg) topically applied to the preparation 30 or 15 s respectively beforehand, antagonized the vasoconstrictor response to NA. The doses employed were the minimum effective doses capable of consistently antagonizing the response to the standard amount of NA added and the intervals between drug additions the optimal, in each case, to produce the antagonism. The blockade was readily reversible on washout. In contrast, the same doses of histamine or Bk were ineffective in antagonizing the response to NA in alloxan-diabetic rats, irrespective of the duration of pretreatment with alloxan (3, 6, 10 or 30 days before). Longer intervals were not tested. A few animals which received alloxan but did not develop a diabetic state behaved as controls. Results are summarized in Table 1.

Response to noradrenaline and the effect of previous addition of acetylcholine In contrast, acetylcholine (ACh) (3 μg) topically applied to the preparation 30 s before the addition of NA, antagonized the vasoconstrictor effect of the catecholamine not only in normal but also in alloxan-diabetic animals (10 days pretreatment). The dose of ACh employed was the minimum effective dose to antagonize consistently the vasoconstrictor response to NA in the present conditions. After ACh had been removed from the preparation by washing the microvessel response to NA returned. Results are presented in Table 2.

The effect of insulin on the response of diabetic animals to vasoactive agents Alloxan-diabetic animals were subcutaneously injected with 4 i.u. insulin NPH. Insulin was suspended in 0.1 ml of inert mineral oil for slow absorption. Four hours later, normal blood sugar levels were measured in these animals, and the response of mesenteric microvessels to NA tested in the absence or presence of histamine or Bk, under the conditions referred to previously. Histamine and Bk, which were ineffective in antagonizing the vasoconstrictor response of diabetic rats to NA, in the present circumstance prevented the catecholamine from producing its effect, i.e., pretreatment with insulin restored the antagonism between histamine or Bk, and NA (Table 3).

The effect of fasting on the response to vasoactive agents Normal and alloxan-diabetic rats were fasted for 12–15 h; then the microvessels were tested for their capacity to respond to NA in the absence or presence of histamine or Bk. Fasting reduced glycaemic levels of diabetic animals to normal values and slightly decreased such levels in controls. Despite normal blood sugar levels, the microvessels of diabetic rats still behaved as in hyperglycaemic animals, i.e., normal responses to NA were observed which were not antagonized by histamine or Bk (Table 4). Hyperglycaemia, *per se*, therefore, did not seem to be the causative factor for the impaired microvessel responses of diabetic rats to histamine or Bk.

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

Animals	*Latency (s) of response to noradrenaline* (0.03 μg)[a] *Acetylcholine* (3 μg) Absent	Present	*Glycaemia* (mg %)
Normal	18.0 ± 3.6 ($n = 7$)	>90[b]	60–98
Diabetic (10 days)[c]	16.8 ± 1.9 ($n = 11$)	>90	207–368

(a) The response was characterized by the complete interruption of blood flow in at least one vessel of the microscopic field. Acetylcholine was applied 30 s before noradrenaline. 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) Days after alloxan injection.

Table 3 Mesenteric microvessels: influence of histamine and bradykinin on latency of the vasoconstrictor response to noradrenaline in normal rats and in alloxan-diabetic rats pretreated with insulin

	Latency (s) of response to noradrenaline (0.03 μg)[a]				
	Histamine (0.03 μg)		*Bradykinin* (0.03 μg)		*Glycaemia*
Animals	Absent	Present	Absent	Present	(mg %)
Normal	21.9 ± 1.6 ($n = 8$)	>90[b]	17.7 ± 1.7 ($n > 9$)	>90	70–87
Diabetic + insulin[c] (10 days)[d]	18.8 ± 2.0 ($n = 12$)	>90	12.4 ± 1.5 ($n = 9$)	>90	233–420 (before insulin) 60–110 (after insulin)

(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 or 15 s, respectively, before noradrenaline. All 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) Insulin NPH, 4 i.u. in oil, was given subcutaneously 4 h before testing.
(d) Days after alloxan injection.

The effect of 2-deoxyglucose on the response to vasoactive agents Normal animals were injected with 200 mg/kg 2-deoxyglucose (2DG) by the intravenous route and 3 h later the responses of the mesenteric microvessels to NA were tested in the absence or presence of either histamine, Bk or ACh. Whereas ACh prevented NA from producing its vasoconstrictor effect in these animals, histamine or Bk were ineffective in antagonizing this action. Accordingly, the microvessels of normal animals previously given 2DG behaved as those of diabetic animals (Table 5).

Studies on the isolated right atrium

Influence of diabetes, insulin administration and fasting on resting rates Resting rates of spontaneously beating atria were consistently lower, compared to normals, in preparations obtained from alloxan-diabetic animals. Decreased resting rates were observed in 60% of the preparations from animals receiving alloxan 24 h before, and in 100% of the preparations from animals given alloxan 3 to 45 days before. Longer intervals were not tested. In a few animals which were injected with alloxan but did not develop a diabetic state, normal frequencies were observed. Pretreatment of diabetic animals with a single dose of 4 i.u. insulin NPH, in oil, by the subcutaneous route 4 h before testing, was ineffective in restoring the decreased atrial rates to normal. Fasting of diabetic animals for 12–15 h resulted in normal glycaemic values without any improvement in atrial frequencies. Rather, a further reduction was observed. Normal animals fasted for an equivalent period showed normal rates. Results are summarized in Figure 1.

Table 4 Mesenteric microvessels: influence of histamine and bradykinin on latency of the vasoconstrictor response to noradrenaline in normal and alloxan-diabetic rats fasted for 12–15 h

	Latency (s) of response to noradrenaline (0.03 μg)[a]				
	Histamine (0.03 μg)		*Bradykinin* (0.03 μg)		*Glycaemia* (mg %)
Animals	Absent	Present	Absent	Present	Before/after fasting
Normal	20.0 ± 1.4 ($n = 7$)	>90[b]	17.8 ± 1.5 ($n = 5$)	>90	59–110/47–68
Diabetic (10 days)[c]	19.7 ± 1.9 ($n = 6$)	19.3 ± 2.5	16.1 ± 1.6 ($n = 13$)	20.2 ± 2.5	230–450/60–110

(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 or 15 s, respectively, before noradrenaline. All drugs were topically applied. Results are mean ± s.e.mean. Number of microscopic fields tested is indicated in parentheses. Unless antagonism occurred (latency > 90 s), values in each group were not significantly different in the absence or presence of histamine or bradykinin.
(b) No vasoconstrictor effect was observed up to 90 s after the application of noradrenaline; drugs were then washed out.
(c) Days after alloxan injection.

Table 5 Mesenteric microvessels: influence of histamine, bradykinin and acetylcholine on latency of the vasoconstrictor response to noradrenaline in normal rats given 200 mg/kg 2-deoxyglucose, intravenously, 3 h beforehand

Latency (s) of response to noradrenaline (0.03 μg)[a]					
Histamine (0.03 μg)		*Bradykinin* (0.03 μg)		*Acetylcholine* (3 μg)	
Absent	Present	Absent	Present	Absent	Present
24.0 ± 1.6	29.5 ± 1.8	17.1 ± 1.8	15.6 ± 1.4	15.1 ± 2.2	> 90[b]
($n = 9$)		($n = 8$)		($n = 7$)	

(a) The response was characterized by the complete interruption of blood flow in at least one vessel of the microscopic field. Histamine or acetylcholine were applied 30 s before noradrenaline; bradykinin, 15 s before. All drugs were applied topically. Results are mean ± s.e.mean. Number of microscopic fields tested is indicated in parentheses. Values were not significantly different in absence or presence of histamine and bradykinin.
(b) No vasoconstrictor effect was observed up to 90 s after the application of noradrenaline; drugs were then washed out.

Effect of drugs acting on neuroeffector sites Concentration-effect curves (Figure 2) constructed from the response of isolated atria to added NA, tyramine and ACh were not significantly different in preparations from normal or diabetic rats (alloxan 10 days before). Atria from both groups of animals incubated in the presence of 10^{-6} M atropine, or 3×10^{-8} M propranolol, showed no changes in resting rates for at least 60 min. The doses of atropine and propranolol employed were previously shown to produce a shift to the right of about 1 log unit in the concentration-effect curve constructed from the response of the tissue to NA or ACh, respectively.

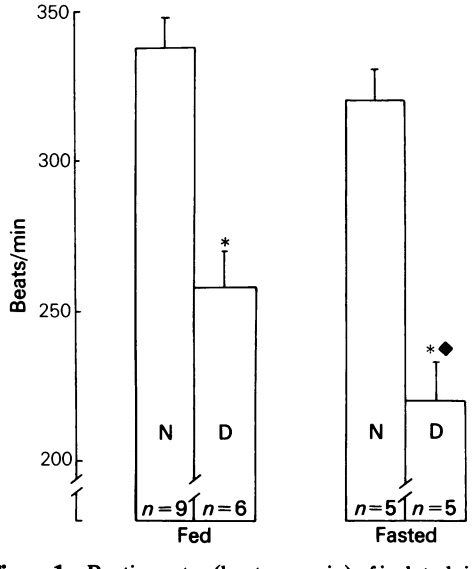


Figure 1 Resting rates (beats per min) of isolated right atria from normal (N) and alloxan-diabetic (D) rats, either fed or fasted. Animals were fasted for 12–15 h with water *ad libitum*. Glycaemia ranged between 226–400 mg% and 60–100 mg% in fed and fasted diabetic rats, respectively; and between 70–87 mg% and 50–70 mg% in fed and fasted normal rats, respectively. Results are mean of n (number of) preparations; vertical lines are s.e.means.
*$P < 0.05$ in comparison with corresponding normal values.
◆ $P < 0.05$ in comparison with values in the group of fed diabetic animals.

Effect of 2-deoxyglucose When 4/5 of the total amount of glucose in the bathing fluid was substituted for 2DG, the rate frequency of atria from nondiabetic animals was reduced, in about 40 min, to levels observed in atria from diabetic animals (Figure 3). Lower concentrations of 2DG (2/3 or 1/3) evoked less marked changes in the same period.

Effect of sodium fluoride A negative chronotropic effect was observed (Figure 4) after the addition of sodium fluoride to the bathing fluid in which atria from normal animals were immersed. The normal concentration of glucose was unaltered.

Studies on the isolated duodenum

Concentration-dependent relaxation induced by histamine or Bk on the isolated duodenum from either normal or alloxan-diabetic rats was equivalent in both groups of animals (as indicated in Table 6). Diabetes resulted from the injection of alloxan 10 days beforehand.

Discussion

Marked differences in the functional behaviour of mesenteric microvessels were observed between normal and diabetic rats. Despite the fact that noradrenaline evoked a vasoconstrictor response the latency and nature of which was analogous in both groups of animals, histamine and bradykinin were capable of antagonizing this response in normal but not in diabetic animals. In contrast, acetylcholine acting as

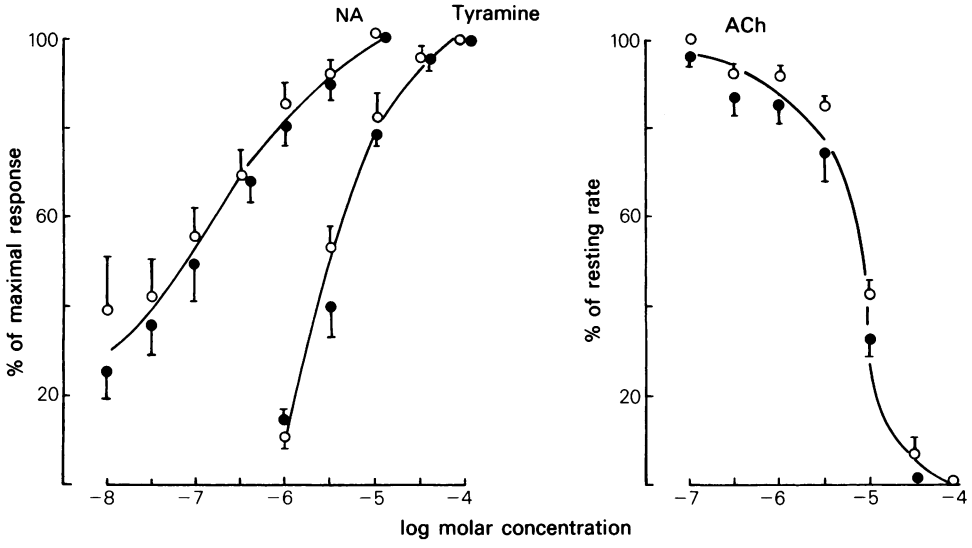
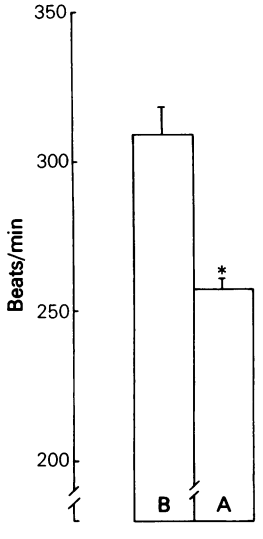


Figure 2 Cumulative concentration-response curves to the positive chronotropic effect of noradrenaline (NA) and tyramine, and to the negative chronotropic effect of acetylcholine (ACh). Each point represents the mean value of six determinations on isolated right atria from normal (○) or alloxan-diabetic (●) rats. Vertical bars show s.e.mean. Alloxan was injected 10 days beforehand. Glycaemia ranged between 228–440 mg% and 60–86 mg% in diabetic and normal animals, respectively. On ordinate scale for NA and tyramine, frequency (beats per min) is expressed as percentage of maximal response; on ordinate scale for ACh, frequency (beats per min) is expressed as percentage of the resting rate.



Fire 3 Frequency (beats per min) of isolated right atria from normal rats. The spontaneously activity of the preparations was recorded before (B) and 40 min after (A) substitution of 4/5 glucose by an equivalent amount (moles) of 2-deoxyglucose in the bathing fluid. Results are the mean value of seven determinations. Vertical bars show s.e.mean.
*$P<0.05$ in comparison with values obtained before the addition of 2-deoxyglucose.

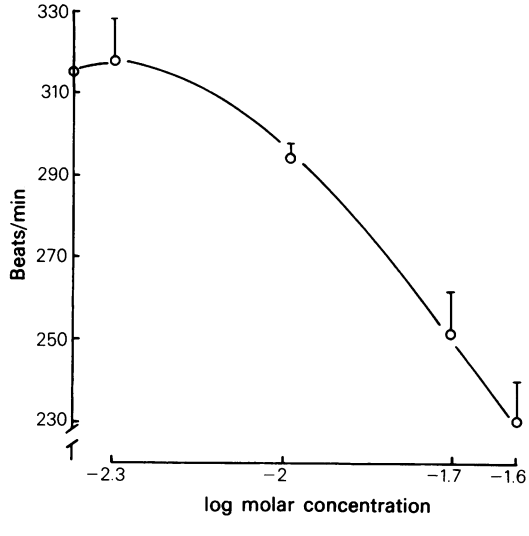


Figure 4 Cumulative concentration-response curve to the negative chronotropic effect of sodium fluoride. Each point represents the mean value of six determinations on isolated right atria from normal animals. Vertical bars show s.e.mean. Ordinate scale: frequency expressed as beats per min.

Table 6 Isolated duodenum: relaxation induced by histamine and bradykinin on preparations from normal and alloxan-diabetic rats

	Relaxation (cm/g tissue)[a]			*Glycaemia* (mg %)
Histamine (10^{-8} M)[b]	5	10	20	
Normal	4.4 ± 1.2 ($n = 6$)	5.4 ± 0.8 ($n = 7$)	6.9 ± 0.9 ($n = 7$)	75–100
Diabetic (10 days)[c]	4.0 ± 0.6 ($n = 9$)	4.8 ± 0.4 ($n = 11$)	5.3 ± 0.4 ($n = 11$)	244–356
Bradykinin (10^{-8} M)	10	20	40	
Normal	4.6 ± 0.4 ($n = 11$)	5.1 ± 0.4 ($n = 11$)	5.0 ± 0.5 ($n = 10$)	60–90
Diabetic (10 days)	3.7 ± 0.9 ($n = 7$)	4.1 ± 0.8 ($n = 7$)	4.2 ± 1.0 ($n = 6$)	230–320

(a) Results are mean ± s.e.mean; n = number of preparations. No significant differences were observed between corresponding values in diabetic and matching controls.
(b) Doses are cited as the final molar concentration of the salt in the organ bath.
(c) Days after alloxan injection.

an antagonist to noreadrenaline, was equally effective in control and diabetic rats. Minimum effective doses of the three antagonists were employed which consistently prevented the standard amount of noradrenaline, added to the preparation, from producing its vasoconstrictor action in normal animals. Though the technique employed did not allow the characterization of the type of vessel(s) involved, it was possible to recognize that diabetes, even in its early stages, interfered with a particular component in microvessels sensitive to histamine and bradykinin, but not to acetylcholine.

The altered responses to histamine and bradykinin were observed in animals injected with alloxan 3 to 30 days before, and were corrected by previous administration of insulin. The functional changes could not be associated with hperglycaemia, since fasting rendered the animals normoglycaemic and yet did not restore the reactivity of microvessels to such agents.

A similar condition of impaired responses to histamine and bradykinin was evoked in normal animals by the intravenous administration of 2-deoxyglucose. Acetylcholine, however, remained fully active in these circumstances. The acute effects of 2-deoxyglucose are the result of cellular glucopaenia secondary to inhibition of glucose utilization. The analogue prevents the penetration of glucose into the cell, and the phosphorylated metabolite 2-deoxyglucose-6-phosphate is apparently responsible for the inhibition of glucose permeability (Sols & Crane, 1954; Long & Thomson, 1955; Wick, Drury, Nakado & Wolfe, 1957; Kipnis & Cori, 1959). Inhibition of the anaphylactic reaction of rats to dextran and ovomucoid is observed after pretreatment of the animals with 2-deoxyglucose (Goth, 1959). In addition, the integrity of the microcirculatory response to noxious stimuli is dependent, at least partially, on the availability of insulin. This is indicated by the finding that experimental diabetes resulting from subtotal pancreatectomy or alloxan administration leads to decreased inflammatory responses to chemical and physical stimuli and to reduced vascular permeability reactions to intradermally injected histamine, bradykinin or 5-HT. These defects were corrected by insulin administration (Garcia Leme *et al.* 1973; 1974; Llorach *et al.* 1976). It is plausible, therefore, that insulin is involved with the reactivity of microvessels and that its action ultimately reflects the adequate utilization of glucose by the reacting structures.

Histamine and bradykinin cause increased permeability, whereas acetylcholine does not. Increased vascular permeability 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). Active contraction of endothelial cells is considered to be an acceptable explanation of this (Majno, Shea & Leventhal, 1969; Becker & Hardy, 1973; Gabbiani, Badonnel & Rona, 1975). Bradykinin produces potent relaxation of canine isolated intrapulmonary and renal arteries contracted by phenylephrine, provided the endothelium is left intact. Selective, mechanical destruction of the endothelium transforms the vasodilator activity of bradykinin to either contraction or to no response at all (Chand & Altura, 1981). Relaxation of isolated preparations of rabbit thoracic aorta and other blood vessels by acetylcholine requires the presence of endothelial cells. Acetylcholine acting on muscarinic receptors of these cells,

stimulates release of a substance(s) that causes relaxation of the vascular smooth muscle (Furchgott & Zawadzki, 1980).

The present observations suggest that the relaxation of microvessels *in situ*, induced by histamine and bradykinin, might also require the intervention of endothelial cells. The process apparently depends on the availability of insulin since it was affected by diabetes. Probably the hormone-modulated contraction of endothelial cells in such vessels, with increase in vascular permeability and exudation of macromolecules, is a major event. As pointed out by Vanhoutte (1978), changes in composition of extracellular fluid, if only from the osmotic point of view, may provide an acceptable basis for vasodilatation, because hyperosmolarity inhibits vascular smooth muscle reactivity (Mellander & Lundvall, 1971; Haddy & Scott, 1975; McGrath & Shepherd, 1976). Accordingly, histamine and bradykinin might antagonize the vasoconstrictor response to noradrenaline through an increase in vascular permeability, from which osmotic changes of the extracellular fluid resulted. In contrast, acetylcholine would exert vasodilator effects through different mechanisms. Since it has little effect on vascular permeability, and its action was not influenced by the diabetic state of the animals, an active contraction of endothelial cells is a rather improbable mechanism.

The possibility that histamine and bradykinin might antagonize the vasoconstrictor response to noradrenaline independently of a direct relaxation of vascular smooth muscle, and that contraction of the endothelial cells rather than the response of the muscle might be the component altered in diabetes, led us to investigate the relaxant properties of histamine and bradykinin on non-vascular smooth muscle in normal and diabetic animals. Equivalent concentration-dependent relaxation was induced by both substances in the isolated duodenum from normal and diabetic rats. This finding gave additional support for the suggestion that changes in the relaxant effects of histamine or bradykinin on smooth muscle need not necessarily occur in diabetes.

In addition, it seemed relevant to estimate, in the present conditions, the extent to which diabetes would interfere with the functional integrity of cells endowed with a high degree of specialization, so as to compare it with the functional changes occurring in microvessels. Decreased resting rates were detected in spontaneously beating right atria from diabetic animals. This could not be related to hyperglycaemia, since fasting of the animals resulted in normal glycaemic values without improvement of atrial activity; on the contrary, fasting further reduced rates. Pretreatment of the animals with an effective dose of insulin, as tested in the experiments with microvessels, did not restore frequency of the preparation to normal values. Normal responses of the preparation to noradrenaline, acetycholine or tyramine were observed, thus indicating that neuroreffector sites or storage and release of endogenous mediators (catecholamines) were not affected during the early stages of diabetes. The incubation of the preparation with effective doses of either atropine or propranolol, without significant changes in atrial frequencies, revealed that increased spontaneous release of acetylcholine or decreased spontaneous release of catecholamines were not relevant factors in the reduced frequencies resulting from diabetes. In contrast, when glucose in the bathing fluid was partially substituted for 2-deoxyglucose, normal atrial rates were reduced to levels comparable to those observed in diabetes. Furthermore, the addition of increasing concentrations of sodium fluoride to the organ bath, gradually reduced normal atrial frequencies. In both circumstances impaired glucolytic pathways are expected to occur. The glucose analogue, 2-deoxyglucose, prevents the penetration of glucose into the cells, and sodium fluoride inhibits enolases which transform phosphoenolpyruvate into pyruvate.

The diabetic heart shows the expected diminution in the rates of glucose uptake, glucolysis and glucose oxidation (Randle, 1969). Cellular glucopaenia might, therefore, be a relevant factor affecting not only atrial activity but also contractile functions of endothelial cells in microvessels. The mechanisms involved in the former circumstances, however, must be more sensitive to changes in intracellular glucose, since they were not corrected by the pretreatment of the animals with insulin.

In conclusion, the mechanisms involved in the relaxation of microvessels may vary depending on the nature of the 'vasodilator' agent employed. Histamine and bradykinin seemed to antagonize the vasoconstriction evoked by noradrenaline through the contraction of endothelial cells. The resulting increase in vascular permeability might change osmolarity in the vicinity of the vessels, thus leading to relaxation of the vascular structures. Apparently this process is insulin-dependent and is affected by diabetes. Acetylcholine, which is devoid of vascular permeability activity must exert its vasodilator effect through different mechanisms which are insensitive to the acute biochemical changes occurring in the early stages of diabetes.

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