Altered adrenergic responses of the coronary arterial bed in alloxan-diabetic dogs

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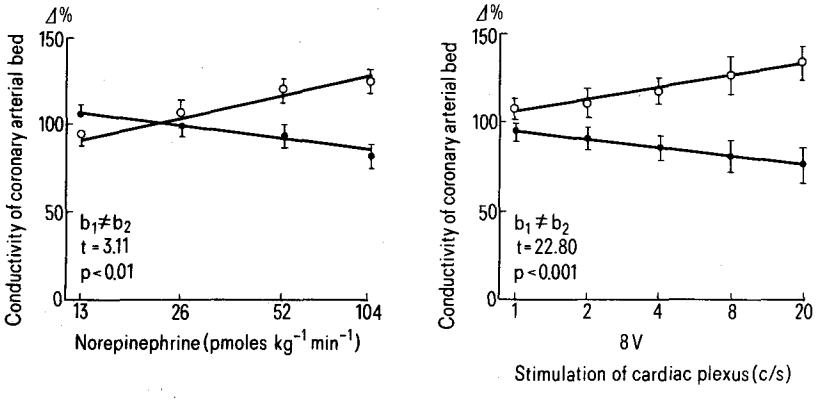
Summary. The conductivity of the coronary arterial bed increased in healthy and decreased in alloxan-diabetic dogs in response to intracoronary administration of norepinephrine or sympathetic stimulation. This suggests a modification of adrenergic receptor sensitivity in diabetes.

Ischaemic heart diseases are more frequent and serious in patients with diabetes mellitus[1] than in nondiabetics. It is well known that metabolic alterations may influence both the structure[2] and the adrenergic receptor sensitivity[3] of the vascular wall. Therefore, the conductivity and morphology of the coronary arterial bed were studied in alloxan-diabetic dogs.

Methods. Healthy mongrel dogs of both sexes, weighing 16-26 kg, 2-3 years of age were selected for the study. All animals received the same diet consisting of 25% protein, 60% carbohydrate and 15% fat. At the beginning of the experiment urine samples collected over 24 h were tested for glucose[4] and acetone[5]. Fasting venous blood samples were also taken for glucose[4] and urea nitrogen[6]. The plasma disappearance rate of glucose[7] was determined by i.v. challenge using 6 mmoles/kg glucose in the unanesthetized state. After determination of the basal values 12 dogs were made diabetic using alloxan tetrahydrate (Merck) 280 mmoles/kg i.v. 12 dogs served as controls. The haemodynamic investigation was performed 3 months after the induction of diabetes. Before pentobarbital (Nembutal, Serva, 133 mmoles/kg) anesthesia, all chemical variables were redetermined. The left anterior descending coronary artery was exposed and its blood flow was measured by an electromagnetic flowmeter (Godard-Statham, SP 2202). Arterial blood pressure was determined in the right carotid artery with a Statham gauge (p23Db). In 6 healthy and 6 alloxandiabetic dogs a polyethylene cannula was inserted through a collateral branch into the coronary artery. After recording of the basal values on a multiscriptor (9400 Hellige) 13-26-52-104 pmoles/kg/min norepinephrine (Norepinephrine bitartrate, Burroughs Wellcome) were infused till the steady state was reached. In the remaining 6 healthy and 6 alloxan-diabetic animals the cardiac plexus was exposed and electrically stimulated (Multistim 13G04, Disa) with 5 min long trains of square pulses of 1.0 msec duration, 8 V amplitude and increasing frequency (1-2-4-8-20 c/sec). In this way an overall effect of sympathetic intervention is described for the conductivitiy of the coronary arterial bed, characterized by the ratio of flow and pressure. Paraffin sections of the left coronary artery wall and myocardium were used for histological study, after fixation in formalin or Carnoy fixative. The sections were stained for PAS-reaction, with Gömöri's silver stain, and partly exposed to digestion by diastase. Frozen sections were stained for lipids with Sudan III or Sudan black. The results were evaluated statistically using Student's paired and unpaired t-tests and regression analysis.

Results and discussion. The plasma disappearance rate of glucose and body weight decreased, while fasting plasma glucose level and urinary glucose excretion increased, after alloxan treatment. No acetone was excreted. Blood urea nitrogen, arterial blood pressure and coronary blood flow did not differ significantly from those of healthy animals (table).

The conductivity of the coronary arterial bed increased in healthy and decreased in alloxan-diabetic dogs under intracoronary infusion of norepinephrine and under the electrical stimulation of the cardiac plexus (fig.). An increase in cardiac work is not likely to be responsible for these observations, because there were no differences in the mean arterial blood pressure, heart rate and 'double product' characteristic of cardiac work calculated from heart rate and peak systolic pressure[8] between the 2 groups.



Correlation between the percent change of conductivity in coronary arterial bed and the dosage of intracoronary administered norepinephrine or the increasing frequency of cardiac plexus stimulation. The open circles represent the mean values of 6 metabolically healthy dogs and the solid circles those of alloxan-diabetic animals.

Metabolic and haemodynamic state of control and alloxan-diabetic dogs

	Plasma disappearance rate of glucose (μmole/min)	Plasma glucose (mmole/l)	Glucose excretion (mmole/day)	Body weight (kg)	Mean arterial blood pressure (kPa)	Coronary blood flow (ml/min)
Control (n = 6)	12.5 ± 1.0	5.7 ± 0.7	0 ± 0	16.2 ± 2.0	14.0 ± 0.7	42.3 ± 10.8
Before alloxan (n = 6)	13.2 ± 1.0	5.2 ± 0.4	0 ± 0	14.3 ± 1.0	-	-
After alloxan (n = 6)	7.0 ± 0.5[a]	14.2 ± 0.8[a]	125 ± 10[a]	12.8 ± 0.7[a]	15.0 ± 1.4	50.0 ± 11.7

Data are expressed as mean ± SEM. Significance, referred to values obtained before alloxan treatment is indicated by: [a] $p < 0.001$.

Histologically no alteration could be found. Hence an early change in adrenergic vasomotor regulation of diabetic coronary arteries could be demonstrated. The adrenergic receptor is supposed to be an entity which can be of alpha of beta character[9], its sensitivity changing according to the metabolic and endocrinological state[3,10]. Recently it was suggested that not only the compliance of the vessel wall[11] is decreased in diabetes mellitus but also the relation of the adrenergic receptors to the vascular smooth muscle cells[12]. Besides the dominance of metabolic autoregulation, adrenergic vasomotor regulation may be important in coronary blood flow alterations[13-15]. The present observation may explain why ischaemic heart diseases are more frequent and pronounced in diabetic than in healthy individuals.

1 A.S. Krolewski, A. Czyzyk, D. Janecko and J. Kopczynsky, Acta med. pol. *18*, 213 (1977).
2 G. Pogátsa, M. Bihari-Varga and Gy. Szinay, Acta diabet. lat. *16*, 129 (1979).
3 G. Kunos, Br. J. Pharmac. *59*, 177 (1977).
4 A. Hyvärinen and E.A. Nikkilä, Clinica chim. Acta *7*, 140 (1962).
5 W. Neuweiler, Klin. Wschr. *12*, 869 (1933).
6 J.J. Coulomba and L. Favreau, Clin. Chem. *9*, 102 (1963).
7 V. Conard, J.R.M. Franckson, P.A. Bastenie, J. Kerstens and L. Kovács, Archs Int. Pharmacodyn. *93*, 277 (1953).
8 J. Scheuer and S. Penpargkul, in: Clinical cardiology, p.50. Eds J.T. Willerson and C.A. Sanders. Grune and Stratton, New York, San Francisco, London 1977.
9 G. Kunos and M. Szentiványi, Nature, Lond. *217*, 1077 (1968).
10 G. Stene-Larsen and B.K. Helle, Life Sci. *23*, 2681 (1978).
11 H.C. Pillsbury, W. Hung, M.C. Kyle and E.D. Freis, Am. Heart J. *87*, 783 (1974).
12 S. Sullivan and H.V. Sparks, Am. J. Physiol. *236*, H301 (1979).
13 A. Juhász-Nagy and M. Szentiványi, Am. J. Physiol. *200*, 125 (1961).
14 E.O. Feigl, Circulation Res. *37*, 88 (1975).
15 D.E. Mohrman and E.O. Feigl, Circulation Res. *42*, 79 (1978).

Influence of hypoxia on contractility and calcium uptake in rabbit aorta

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Summary. The influence of hypoxia on noradrenaline (NA)-induced contractions and ^{45}Ca uptake has been studied on isolated rabbit aortae. Hypoxia significantly decreased the contractility of aortic strips. NA stimulation resulted in increased or decreased ^{45}Ca uptake by normoxic or hypoxic specimens, respectively. Relating ^{45}Ca movement with mechanical activity, the results suggest that decrease in Ca^{++} uptake may be a mechanism for hypoxic relaxation of aortic smooth muscle.

Numerous studies have shown that the contractility of isolated systemic vessels depends on the oxygen tension of the medium[2-5]. The mechanism of action is, however, still not clear. Since vascular smooth muscle relaxants may produce their effects by lowering the concentration of activator Ca^{++}, the present study was designed to investigate the relationship between hypoxic relaxation and Ca supply in the rabbit aorta. The study is based on the observations that isolated systemic vessels from a given site respond similarly to hypoxia and to withdrawal of extracellular Ca^{++}, suggesting a common mechanism of action[6,7]. A preliminary communication of part of this work has been published[8].

Methods. Male New Zealand rabbits (1.5-3.0 kg) were killed by a blow on the neck. Aortae were isolated and freed of connective tissues. For contraction experiments, helical strips were prepared. Strips were suspended in 5 ml organ baths containing physiological salt solution (PSS) of the following composition (mM): NaCl, 119.0; KCl, 4.7 KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 14.9; glucose, 11.5. Temperature was maintained at 37 °C. The medium was equilibrated with 16% O_2, 5% CO_2, 79% N_2 gas mixture ($PO_2 = 110$ mm Hg). Hypoxia was induced by switching to a 95% N_2, 5% CO_2 mixture ($PO_2 < 14$ mm Hg). pH was 7.4 throughout. Strips were given an initial load of 1 g; isotonic contractions were recorded. An equilibration period of 90 min was allowed. Except in cumulative dose-response tests, strips were contracted using 1×10^{-6} M NA.

Cellular ^{45}Ca uptake was determined by the lanthanum method[11]: Aortae were cut into rings weighing about 20 mg and allowed to equilibrate in the PSS. Thereafter, the rings were transferred to ^{45}Ca loading solution containing a sp. act. of 0.2 μCi/ml $CaCl_2$ for 30 min or in other experiments, 80 min. The loading solution was either normoxic or hypoxic and contained 1×10^{-6} M NA when added. ^{45}Ca uptake was terminated by transfer to 10 mM $LaCl_3$ PSS for 5 min to block $^{45}Ca^{++}$ exchange between the inside and outside of the cells. (In $LaCl_3$ PSS, Hepes buffer was used in place of HCO_3^-; SO_4^{2-} and PO_4^{3-} were replaced by

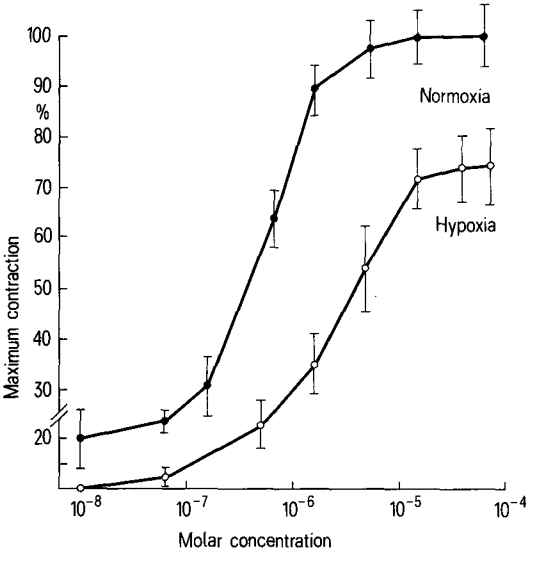

Figure 1. Noradrenaline dose-response curves in rabbit aortic strips under normoxic (●) and hypoxic (○) conditions.