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.

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Influence of hypoxia on contractility and calcium uptake in rabbit aorta

A. B. Ebeigbe[1]

Institute of Physiology, University of Glasgow, Glasgow G12 8QQ (Scotland), 6 October 1981

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

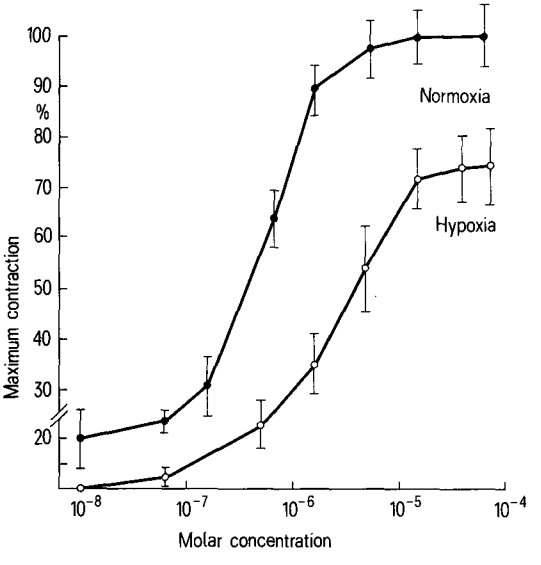

Figure 1. Noradrenaline dose-response curves in rabbit aortic strips under normoxic (●) and hypoxic (○) conditions.

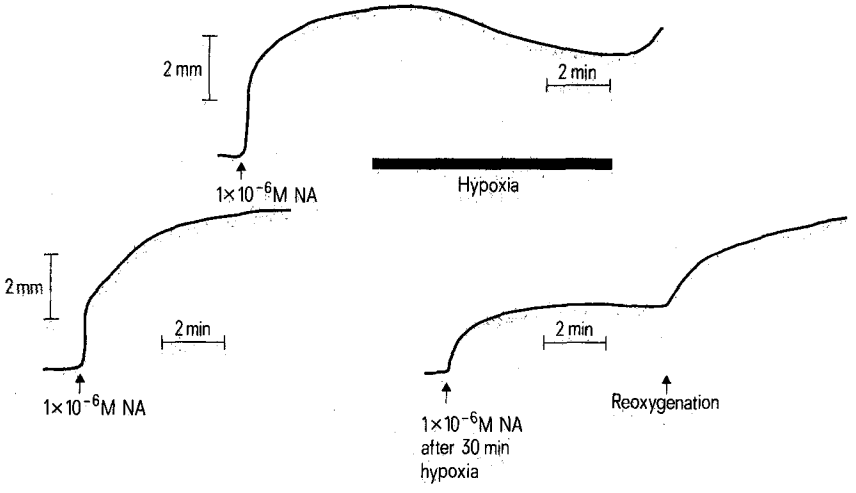

Figure 2. Hypoxic relaxation of NA contraction in a rabbit aortic strip (upper trace). Lower traces show the NA contraction in another strip before (left) and after (right) exposure to 30 min hypoxia.

Cl^-; pH was adjusted to 7.4; solutions were not gassed). The aortic rings were then transferred to a Ca-free, 10 mM $LaCl_3$ PSS for 55 min to expel extracellularly-bound $^{45}Ca^{++}$. Rings were then blotted, weighed, and solubilized with soluene 350 at 50 °C overnight. ^{45}Ca activity was determined by a Packard liquid scintillation counter. Values quoted are means ± SEM; n-values denote number of experiments. Differences between means were assessed using Student's t-test.

Results. Dose-response tests were carried out on 13 strips by cumulatively increasing the dose of NA at 2-min intervals. The procedure was repeated during hypoxia, after preliminarily exposing the strips to hypoxia of 10-min duration. Hypoxia caused a shift to the right of the NA dose-response curve (fig. 1). The threshold and ED_{50} concentrations for contractions were increased significantly and there was a reduction in maximum contraction.

The effect of hypoxia on NA contraction was studied in 2 ways: a) Hypoxia during NA contraction: When contraction produced by 1×10^{-6} M NA was stable, hypoxia was induced and maintained for 30 min. All strips (n = 9) relaxed during hypoxia and contracted again on reoxygenation (fig. 2). Magnitude of relaxation (expressed as percentage of initial contraction) was 39.5 ± 4.3%.

b) NA contraction during hypoxia: In 7 strips, hypoxia was induced and maintained for 30 min. Thereafter, 1×10^{-6}M NA was applied, and the magnitude of the contraction was compared with the normoxic control response (fig. 2). NA contraction was reduced by 45.7 ± 6.3% in hypoxia.

^{45}Ca uptake was determined in unstimulated normoxic specimens, and NA-stimulated normoxic and hypoxic specimens. In specimens exposed for 30 min to ^{45}Ca loading solution, uptake by NA-stimulated normoxic specimens (n = 10) was significantly ($p < 0.01$) higher than by unstimulated normoxic controls (fig. 3). Hypoxia reduced NA-stimulated uptake (n = 9) significantly ($p < 0.01$).

In additional experiments (n = 2), specimens exposed for 80 min to ^{45}Ca loading solution, were treated following the protocol shown in figure 4: Aortic rings were exposed to normoxic loading solution for 80 min (A). Thereafter, specimens were exposed to NA, ^{45}Ca-labelled normoxic solution for 30 min (B); then made hypoxic for 30 min (C), followed by 15 min reoxygenation (D). ^{45}Ca uptake determined on specimens at the various stages of this protocol (A, B, C, D) is shown. NA-stimulated uptake was decreased by hypoxia, but restored by reoxygenation; the results, however, require further confirmation in view of the small number of experiments.

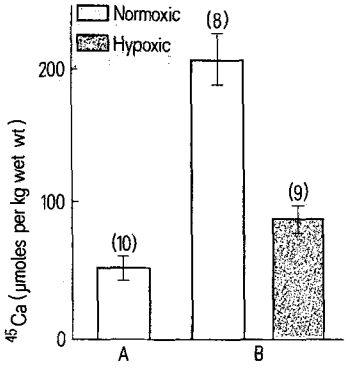

Figure 3. ^{45}Ca uptake by aortic rings (30 min loading): *A*, unstimulated normoxic specimens and *B*, NA-stimulated normoxic and hypoxic specimens (n).

Discussion. The present results are in agreement with other reports[2,3,7,9,10] that contractility of isolated rabbit aortic smooth muscle is dependent on the oxygen tension of the medium. The results suggest that hypoxia-induced depression of aortic contractility is associated with decreased Ca uptake.

The validity of the present ^{45}Ca uptake results is based on the criteria proposed by Van Breemen et al.[11]. However, it should be noted that uptake values in this study may have been underestimated by the use of Hepes buffer, which was recently reported to inhibit contractility and ^{45}Ca uptake in vascular smooth muscle[12]. However, quantitatively similar results have been observed using HCO_3^- buffer (Ebeigbe, unpublished).

Calcium required for vascular smooth muscle contraction arises from 2 main sources; influx from the extracellular compartment, and release from intracellular stores[13–16]. Hypoxia may thus decrease vascular smooth muscle contractility by affecting either influx or intracellular Ca release/sequestration. In this study, NA-stimulated uptake was found to be significantly higher than in unstimulated controls (figs 3, 4) indicating a net gain. The reduced NA-stimulated ^{45}Ca uptake by hypoxic specimens is compatible with the observed reduction in mechanical activity.

It may be argued that labelling of exchangeable Ca^{++} might have been incomplete after 30 min loading; thus hypoxia might be affecting the rate of exchange of labelled

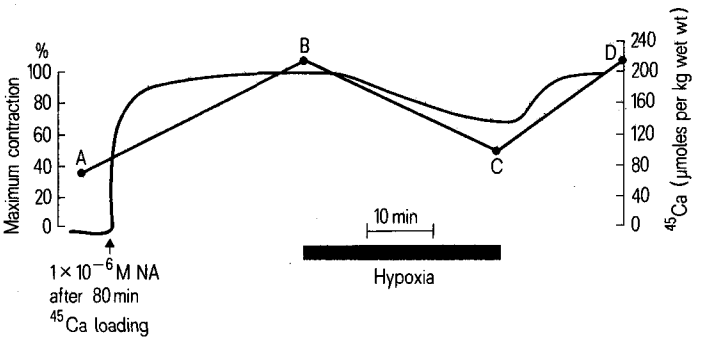

Figure 4. Relationship between ^{45}Ca uptake and typical mechanical activity of NA-stimulated rabbit aortic strips exposed to normoxic and hypoxic conditions. Each point is a mean of 7 determinations, n = 2.

for unlabelled Ca^{++}, rather than a real uptake. This possibility was however ruled out in experiments employing a preliminary loading period of 80 min. This duration was considered to be sufficient to allow complete labelling of all exchangeable Ca^{++}[17]. Using this procedure, NA-stimulated uptake was still observed to be less in hypoxia.

In this study, a net increase in ^{45}Ca uptake was observed on NA addition, as reported by other workers[18,19]. Hypoxic relaxation was associated with reduced uptake (fig. 4, C); this effect was, however, reversed by reoxygenation (fig. 4, D). Although no efflux studies were carried out, the reduced uptake in hypoxia (fig. 4, C) may be taken to reflect a loss of Ca^{++} accumulated during normoxic NA stimulation.

In conclusion, these results suggest that hypoxia depresses NA-induced contraction of rabbit aortic smooth muscle by interfering with Ca^{++} uptake.

1 Present address: Department of Physiology, College of Medical Sciences, University of Benin, Benin City, Nigeria. The author acknowledges facilities at the Wellcome Surgical Research Institute, University of Glasgow, U.K. Much of this work was in collaboration with Drs Sheila Jennett and J.D. Pickard.
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Silver labeling of vascular basement membranes in streptozotocin diabetic mice

P. Naeser and J. Rastad[1]

Department of Ophthalmology and Department of Anatomy, University of Uppsala, S-750 14 Uppsala (Sweden), 30 October 1981

Summary. Diabetic and nondiabetic control mice were given silver by ingestion. The glomerular basement membrane was labeled with silver granules and the labeling was marked in the diabetic animals. The retinal capillaries failed to incorporate silver both in normal and diabetic animals.

The microangiopathy of diabetes mellitus affects the capillaries throughout the body. A prominent morphological change of the disease is the thickening of the basement membranes of the capillary walls[2]. The turn-over of these basement membranes has previously been investigated by silver labeling in vivo[3-6]. The aim of the present report was to compare the silver labeling of the vascular basement membranes of the renal glomeruli and the retinal capillaries in diabetic and nondiabetic mice.

Material and methods. 8 lean mice of the obese-hyperglycemic strain (gene symbol *ob*) were used[7]. At the age of 2 months, 4 of the animals were made diabetic by a single i.v. injection of streptozotocin (120 mg/kg b.wt). 4 control mice were treated with saline only. During the next 2 months all the mice were given free access to pelleted food and drinking water containing 0.25% silver nitrate. The animals were killed by decapitation and the retinas and sections from the kidneys were fixed for 30 min at 4 °C by immersion in a mixture of 2% glutaraldehyde and 1% formaldehyde in 0.15 M sodium cacodylate buffer at pH 7.4. The tissues were osmified, dehydrated and plastic embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate.

At the time of sacrifice, the serum glucose level of each animal was determined by a glucose oxidase method[8].

Results. The average serum glucose level was significantly higher ($p < 0.001$) in the diabetic mice (20.3 ± 1.2 mM) than in the controls (8.9 ± 0.9 mM). The diabetic animals were also lighter (28.3 ± 0.8 g) than the normal controls (30.5 ± 0.9 g).