

Role of Rho-kinase in early diabetic coronary vasoconstriction

The coronary circulation is vulnerable to diabetic complications independent of atherosclerosis. Chronic hyperglycemia causes angiopathy. This leads eventually to congestive heart failure. Abnormal coronary blood flow regulation develops in disease states when the factors that maintain muscular arteries open, vasodilators or relaxing factors, are not produced in sufficient quantities by the lining of cells in the lumen of the vessels called the endothelium to counterbalance an increase in constrictor factors. We have shown that basal endothelium dependent vasodilation is maintained in early diabetes (type-1), but focal stenoses and segmental constrictions occur when prostacyclin and nitric oxide (NO) production are prevented [1]. Thus the diabetic coronary circulation is vulnerable to ischemia when dilator bioavailability is reduced. The RhoA/Rho-kinase (ROCK) pathway is implicated in hypertension, angina and vasospasm [2].

Our objective was to determine if an increase in ROCK mediated constriction plays a role in the onset of coronary vascular dysfunction in type-1 diabetes. In this study we utilized microangiography with synchrotron radiation from the beamline **BL28B2** as the X-ray source to investigate the constrictor tone present in the *in vivo* coronary circulation of Sprague-Dawley rats from the microvessels to large arteries [3]. Briefly, male rats were made diabetic with streptozotocin (65 mg/kg) and three weeks later we surgically prepared them under anaesthesia for closed chest imaging at physiological heart rates. Cine-angiograms were recorded during drug administrations for ~3 seconds as an iodine contrast agent was infused through a cannula inserted into the aorta via the right carotid artery. Up to four branching orders of arterial vessels were visualized in diabetic and control rats (internal diameter 40-300 μm), including the microvessels that are the main source of vascular resistance (Fig. 1). Vessel diameter and vessel number were quantified and compared during various stimulations with vasodilators (including acetylcholine, ACh), following complete inhibition of prostacyclin and NO production (combined blockade) and subsequent to administration of the ROCK inhibitor fasudil (10 mg/kg).

Diabetic rats had significantly elevated blood glucose and lower heart rates, but did not differ in baseline coronary vessel calibre across the first 3 arterial branching segments (Fig. 1). Pronounced dilation in response to ACh infusion was found in both groups in the presence of NO and prostacyclin,

but widespread segmental constrictions (white arrows, Fig. 1(g)) in their absence in diabetic rats. ROCK inhibition during nitric oxide synthase and cyclooxygenase blockade restored or dilated vessel calibre relative to baseline in diabetic rats (Fig. 1(h)). A novel finding in this study is the *in vivo* identification of ROCK as a likely mediator of localised constrictions in diabetic hearts. This is supported by the fact that acute ROCK inhibition greatly reduced the incidence of segmental constrictions in second and third

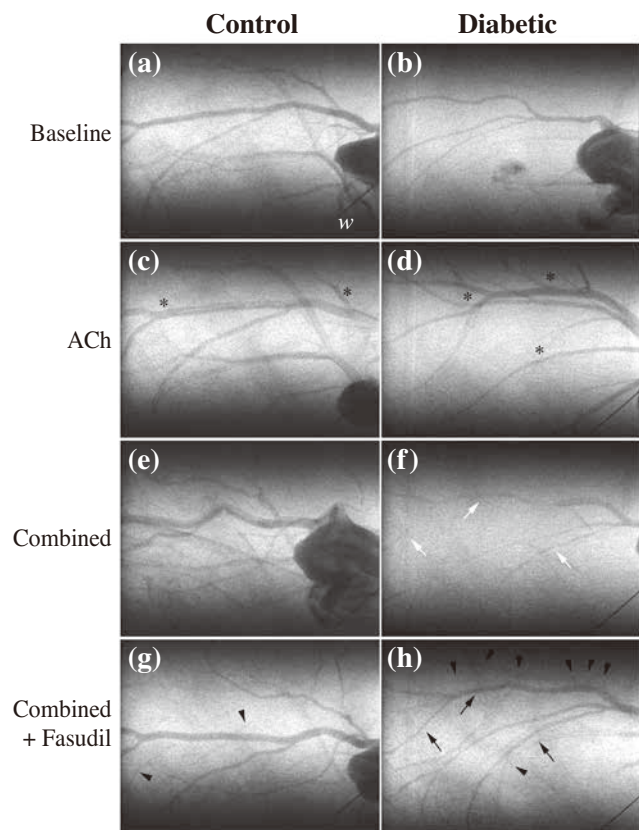


Fig. 1. Representative coronary angiograms in control and diabetic rats [3]. Baseline vehicle lactate infusion (a & e), ACh infusion (b & f: 3.0 $\mu\text{g}/\text{kg}/\text{min}$), following administration of L-NAME and meclofenamate (combined: c & g) and subsequent fasudil treatment (d & h: 10 mg/kg intravenous). (a & e) Baseline response to lactate infusion. (b & f) Control and diabetic, large vessels maintained vessel diameter greater than baseline (black asterisks). (c) Control, vessel diameter maintained. (g) Diabetic segmental vasoconstriction (white arrows). (d) Fasudil response in control, vessel diameter maintained. (h) Vasodilation of medium and small arteries (black arrows). w: 50 μm tungsten wire.

order vessels following combined blockade (Fig. 2). Furthermore, there was a trend towards increased ROCK2 activation (Fig. 3). Based on nitrotyrosine staining, oxidative stress was not significantly elevated in early diabetic rats. Since there was neither an increase in perivascular fibrosis nor an increase in intimal thickening of coronary arteries (confirmed by immunohistochemistry) we attribute the increase in vasoconstriction after blockade to functional upregulation of Rho-kinase activity, rather than structural remodeling of the heart.

These findings demonstrate that an increase in vasoconstrictor tone mediated by Rho-kinase influences coronary perfusion in the early stages of diabetes, and is therefore likely to contribute to vulnerability to ischemia when vasodilator bioavailability becomes diminished in later stages. Notably, ROCK-mediated vasoconstriction was not a consequence of increased oxidative stress, inferring that it was most likely mediated by increased activation of myosin light chain phosphatase in the arterial smooth muscle cells. Moreover, this microangiography approach demonstrates that fasudil treatment might improve the diabetic coronary circulation in the long term. Since this study was published a clinical small trial has now shown that indeed, chronic treatment with fasudil also improves cardiac dysfunction in patients with advanced diabetes [4].

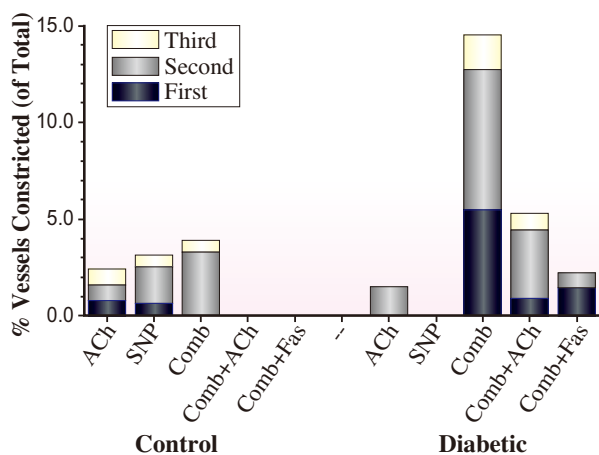


Fig. 2. Occurrence of segmental constrictions in control and diabetic rats in relation to treatment and arterial branching order. Segmental constrictions (most of segment length constricted relative to baseline) are expressed as a percentage of total visible vessels. Control, n=7 and diabetic, n=8.

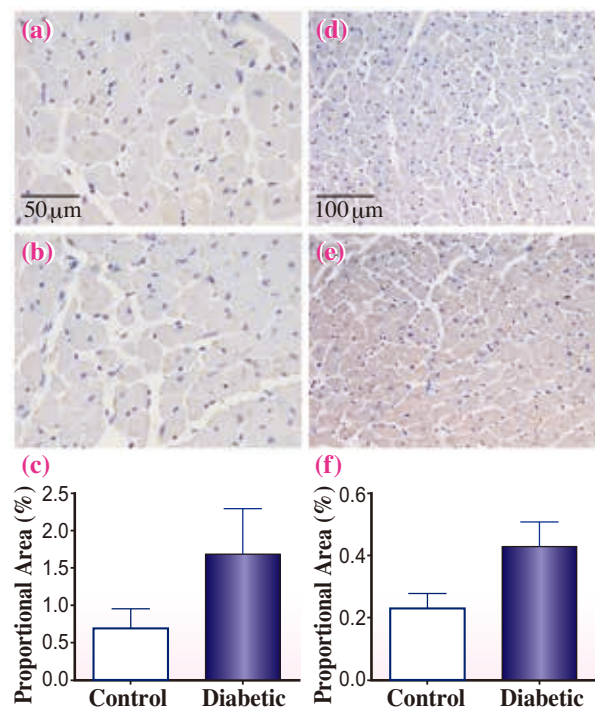


Fig. 3. Myocardial ROCK1 and ROCK2 immunohistochemistry in control and diabetic rats. ROCK1 expression (c) in control (a) and diabetic (b) rats (40x objective). ROCK2 expression (f) in control (d) and diabetic (e) rats (20x objective). ROCK1 expression was not significantly different between control and diabetic groups, although there was a trend towards increased ROCK1 expression in diabetic rodents. ROCK2 expression was borderline significantly increased ($P < 0.053$) in diabetic compared to control animals. Control, n=6 and diabetic, n=7. Values expressed as mean \pm SEM.

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