ON THE OXYGEN CONSUMPTION OF INTACT VESSEL WALL SEGMENTS AND INTIMA-MEDIA PREPARATIONS OF THE RABBIT CAROTID ARTERY

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1. Introduction

Our knowledge about vessel wall metabolism is based predominantly on in vitro incubation measurements of vascular tissue. There are only a few in situ experiments which evaluate oxygen consumption by measuring tissue oxygen tension using standard values for diffusion and solubility coefficients [1,2]. Since the smooth muscle cell is considered to be the primary metabolically active cell, attention has been focused on the tunica media. The rather wide range of reported values of oxygen consumption have been attributed to the different contractile states of the smooth muscle cells [3,4]. Although the vascular smooth muscle cell is the predominant cell type, the vessel wall is far from being homogenous and there is evidence that the morphological integrity of the different cell layers of the vessel wall is a prerequisite for in situ respiration. An intact endothelial cell layer may be important in the metabolism of aortic intima-media preparations [5].

Here we have investigated the oxygen consumption of intima-media preparations, adventitia layers, as well as whole vessel wall segments of the rabbit carotid artery. The results indicate that oxygen consumption of the media myocytes is strongly dependent on the integrity of an adventitio-medial junction. Removal of the adventitial layer reduces the respiration by >50% and also changes the permeability of the cell membrane, as indicated by an increased succinate oxidation.

2. Materials and methods

New Zealand rabbits (male, 2.5–3.0 kg) were anaesthetized with pentobarbital. The common carotid arteries were rapidly dissected and placed into a preparation chamber which was continuously perfused with 37°C gas-equilibrated (5% CO_2, 21% O_2, 74% N_2) Tyrode solution of the following composition (in mM): NaCl 120.0, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.0, $NaHCO_3$ 23.0, NaH_2PO_4 1.2, EDTA 0.01. The vessels were placed around a steel support bar, carefully trimmed of fat and connective tissue and dissected into portions of ~4 mm. In some samples, the adventitia was carefully removed without injuring the tunica media. Under an operation microscope the adventitia was held at one edge of the vessel with micro-forceps and peeled off in one piece with the help of blunt dissection scissors. The media itself was not touched using this technique so that mechanical damage of the media myocytes was avoided. The dry weight of the intima-media cylinders and the adventitial tissue pieces were comparable (0.5–0.8 mg). Dry weight averaged 25% of wet weight.

Oxygen consumption of samples was measured polarographically in a water-jacketted microchamber (300 μl) at 37°C by means of a Clark-type oxygen-electrode [6]. Incubation medium was the same gas-equilibrated Tyrode solution, to which 100 mg penicillin/l and 300 mg streptomycin/l was added to reduce bacterial contamination. After incubation, the tissue samples were lyophilized, weighed and analyzed for their DNA content according to [7].

3. Results and discussion

The oxygen consumption of intact arterial segments, intima-media preparations and adventitial tissue is shown in fig.1. In relation to the DNA content of the tissue (table 1), the oxygen consumption of

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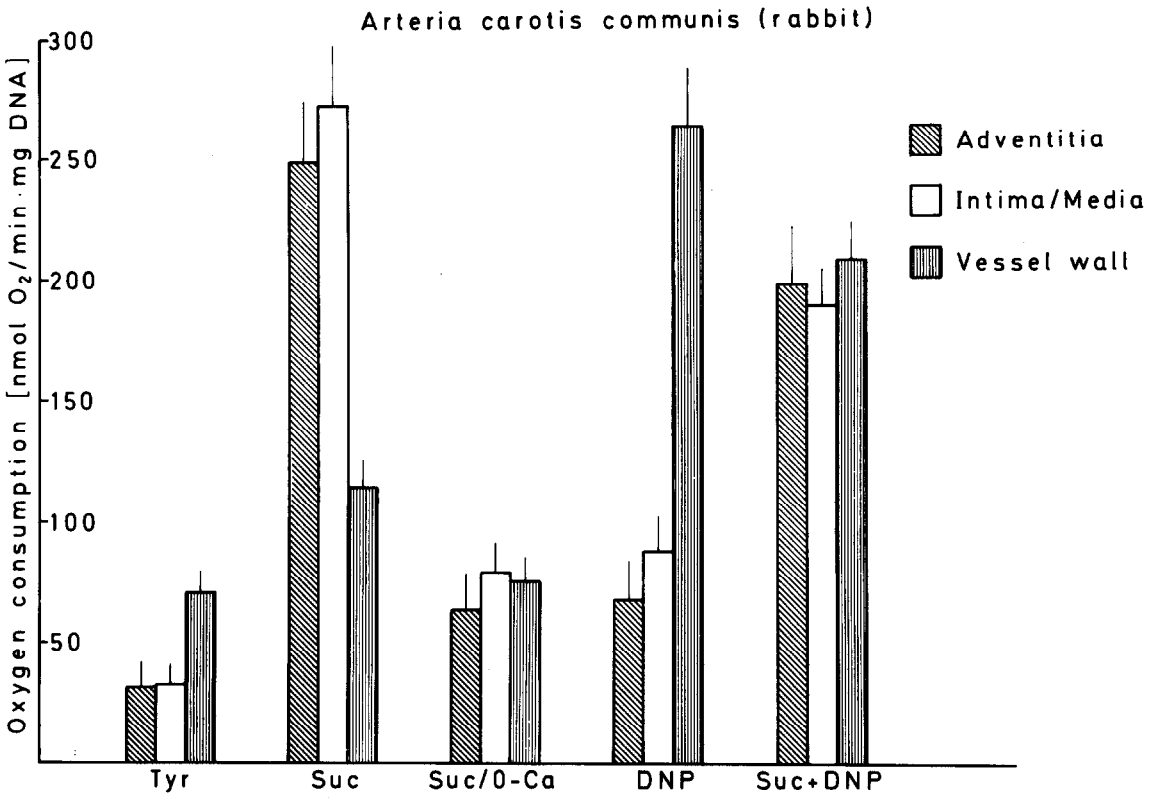

Fig.1. Oxygen consumption of adventitial tissue, intima-media preparations and vessel wall segments of the rabbit carotid artery in different incubation media. Tyr refers to Tyrode solution without exogenous substrate, Suc to Tyrode solution with 4×10^{-3} M succinate, Suc/O-Ca to Ca^{2+}-free Tyrode solution with 4×10^{-3} M succinate, DNP to Tyrode solution with 10^{-4} M 2,4-dinitrophenol, Suc + DNP to Tyrode solution with 4×10^{-3} M succinate + 10^{-4} M 2,4-dinitrophenol. The results are expressed as the means ± SE (n = 10).

the intima-media and adventitia is equal (~32 nmol $O_2 \cdot min^{-1} \cdot mg\ DNA^{-1}$) and ~50% of that of the intact vessel wall (~71 nmol $O_2 \cdot min^{-1} \cdot mg\ DNA^{-1}$). This contrasts with [8] where similar values of oxygen consumption with rat aortic rings and intima-media preparations were reported. In agreement with [8] we found that the addition of exogenous substrates like glucose, β-hydroxybutyrate, glutamine or

Table 1
DNA content of tissue samples of the rabbit carotid artery (means ± SE, n = 12)

Sample	μg DNA/mg dry wt
Vessel wall	9.6 ± 0.9
Intima-media	11.2 ± 1.1
Adventitia	8.1 ± 1.2

n-butyrylcarnitine did not increase the oxygen consumption significantly, which suggests that endogenous substrates are preferentially oxidized by vascular smooth muscles under in vitro conditions.

In intact artery segments respiration increases 4-fold in response to 2,4-dinitrophenol (DNP) (to ~263 nmol $O_2 \cdot min^{-1} \cdot mg\ DNA^{-1}$) indicating that mitochondria are tightly coupled and that the smooth muscle cells are supplied with sufficient substrate. In intima-media preparations and adventitial tissue the increase in oxygen consumption in response to DNP was comparatively small (2.5-fold). This distinct reduction of metabolic capacity must either be due to an altered mitochondrial function or to a loss of enzymatic activity and/or substrate intermediates. Since preparation was very carefully performed by peeling off the adventitia without touching the tunica media, mechanical damage of the media myocytes

can be ruled out. We assume that the destruction of the inner sympathetic plexus on the adventitio-medial junction causes changes in the membrane permeability of the smooth muscle cells.

This is confirmed by the large increase in oxygen uptake of the intima-media preparations and adventitial tissue after incubation with succinate. Succinate stimulates the oxygen consumption of isolated mitochondria, but does not readily cross intact cell membranes [9]. When the plasma membrane is damaged, succinate freely enters the cell and is oxidized by the mitochondria to fumarate and malate, which diffuse out of the cell [10]. Stimulation of oxygen uptake by succinate has therefore been used as an index of cellular damage in liver and heart preparations [11–13]. Compared with the 8-fold increase of oxygen consumption in intima-media preparations and adventitial tissue, the level of succinate oxidation was negligible in intact arterial wall segments. These results are paralleled by those in [10] where perfused liver preparations did not convert exogenous succinate to malate while isolated liver cells oxidized succinate to a large extent.

In view of a changed permeability of the cell membrane, the low oxygen consumption of the intima-media preparations is easily explained. Endogenous substrate intermediates, co-factors and even enzymes may diffuse out of the cell, effecting a decreased metabolic capacity.

In agreement with [14], succinate stimulation was abolished in Ca^{2+}-free media. The entry of succinate into mitochondria seems to be dependent on the presence of Ca^{2+}, as demonstrated in liver slices and isolated hepatocytes [15,16]. Uncoupling of the oxidative phosphorylation reduces exogenous succinate oxidation [17]. Our results with intact artery wall segments show, furthermore, that succinate oxidation is able to reduce uncoupled respiration of endogenous substrates, indicating a competition of NADH- and flavoprotein-linked substrates for electron carriers.

The results show that removal of the tunica adventitia of the carotid artery of the rabbit changes the oxygen consumption of the smooth muscle cells of the tunica media. Moreover, the increased succinate oxidation indicates a change in membrane permeability of the myocytes. An intact inner sympathetic plexus seems to be essential, not only for the physiological substrate degradation, but also for the metabolic homeostasis of the vascular smooth muscle cell. This must be taken into account when metabolic capacities obtained from in vitro incubation measurements of intima-media preparations are extrapolated for the metabolic activity of the vascular smooth muscles in the intact vessel wall or in vivo.

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