Microcirculation of the Heart

Theoretical and Clinical Problems

Edited by
H. Tillmanns W. Kübler H. Zebe

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With 177 Figures

Springer-Verlag
Berlin Heidelberg New York 1982

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ISBN 3-540-11346-0 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-11346-0 Springer-Verlag New York Heidelberg Berlin

Library of Congress Cataloging in Publication Data. Main entry under title: Microcirculation of the heart. Based on a symposium held in Heidelberg in January, 1980, and sponsored by the Microcirculation Working Group of the European Society of Cardiology. Bibliography: p. Includes index. 1. Heart–Blood-vessels–Diseases–Congresses. 2. Heart–Blood-vessels–Congresses. 3. Microcirculation–Congresses. I. Tillmanns, H. (Harald), 1943 II. Kübler, W. (Wolfgang), 1934 III. Zebe, H. (Horst), 1939-IV. European Society of Cardiology. Microcirculation Working Group. [DNLM: 1. Microcirculation-Congresses. 2. Coronary circulation-Physiopathology-Congresses. 3. Myocardial disease-Physiopathology-Congresses. WG 300 M626 1980]
RC681.A2M5 616.1'23 82-684
ISBN 0-387-11346-0 AACR2

Printed in Germany

Druck- und Bindearbeiten: Beltz Offsetdruckerei, Hemsbach/Bergstr.
2119/3140-5 4 3 2 1 0

Preface

Microcirculation is a rather new field which has been of predominant interest to basic scientists, linking together technical, hemodynamic, and biochemical aspects.

The findings elaborated, however, are not only of theoretical interest, but bear in addition great clinical implications. In clinical cardiology this became quite evident by the use of tracers in order to study myocardial perfusion and by the description of certain clinical entities – such as angina with normal coronary arteries – which are best explained by „disturbed microcirculation".

With respect to this new developing theoretical and clinical field of cardiac microcirculation it was the aim of the Microcirculation Working Group of the European Society of Cardiology to have a symposium on which all different but clinically relevant aspects of cardiac microcirculation will be covered. This symposium, held in Heidelberg in January 1980, was planned not only for the exchange of concepts and ideas, but was expected to be in addition partially a teaching session; the basic scientists should be directed toward a better understanding of the clinical problems, and the clinicians should learn more about the basic mechanisms regulating substrate and ion exchange in such an important organ as the heart, and furthermore the theoretical limitations of some of the diagnostic and therapeutical procedures should be taught.

Without a lot of help we would never have succeeded in organizing the symposium and editing its results. The program was made by a scientific committee, consisting of Dr. Grant de Lee from Oxford, my co-chairman of the Microcirculation Working Group, Prof. Dr. E. Gerlach, Munich, and Priv.-Doz. Dr. H. Tillmanns as well as Priv.-Doz. Dr. H. Zebe, both from Heidelberg.

Due to the generous financial support of Pharma-Schwarz/Monheim the symposium could take place in a very enjoyable environment. For editing the results we have to thank in addition the Springer Company – especially Dr. Graf-Baumann.

It is hoped that the Heidelberg symposium will be followed by a series of events in order to stimulate a fruitful discussion between clinical and basic scientists interested in different fields of cardiac hemodynamics and metabolism. This was the basic concept of the Heidelberg meeting.

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Part A

Physiological, Biochemical and Morphological Aspects

1. The Arterial Wall – Transport Properties

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Introduction

In this symposium concerned with the microcirculation of the heart, it is not, we believe, inappropriate to consider the mass transport of the arterial wall. This is firstly because this transport is thought to play a role in the development of atherosclerosis and, secondly, because it appears that it depends on the wall microcirculation, including the lymphatics.

The emphasis of the present paper is on arterial wall macromolecule transport under steady conditions which can be expected to be obtained in vivo. Our initial approach to this complex problem has been to use the relatively inert material serum albumin as the transported species and to work with the rabbit common carotid artery perfused in situ. The in situ arterial preparation [7, 10] was used, firstly because it has been found possible to cannulate and perfuse arteries in situ without damaging the intima [3] or disturbing the adventitia – previous studies have shown that the vasa vasorum contribute to arterial wall mass transport [1, 11] – and secondly because it affords good control of experimental parameters under essentially physiological conditions.

Our findings support the postulate [5, 16, 18] that there is net transport of macromolecules across the arterial wall. They provide, moreover, some understanding of the relevant mechanisms, including the role of the media and of the adventitial circulation. We report in addition some preliminary results we have obtained on the influence of vasoactive materials on the uptake of albumin by the media of segments of this artery when incubated in vitro.

Methods

The rabbit common carotid artery was studied when (1) perfused in situ with plasma containing radioactively labelled albumin; (2) perfused as in (1) and simultaneously subjected to gentle irrigation of its outer surface with a solution containing label at the same concentration as in the lumen; and (3) perfused as in (1) after deliberately damaging the intima. Excised segments of the artery were also incubated in vitro in labelled plasma. Label uptake was assessed both by determining whole wall radioactivity and by studying the distribution of activity through the thickness of the wall using the technique of sequential frozen sectioning parallel to the intima. The quantity of diffusible native protein in the wall was also determined, and light and transmission electron microscopic studies were undertaken.

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^{125}I albumin was used in the whole wall uptake studies, and in order to achieve the required levels of tissue radioactivity, ^{131}I albumin was used in the frozen section work. Radioactive iodide was removed from the tracer by dialysis or ultrafiltration. Its concentration was shown to remain low in all the uptake studies.

The perfusion apparatus is illustrated diagrammatically in Fig. 1. Fresh rabbit plasma flowed steadily through the cannulated common carotid artery at physiological levels of luminal hydrostatic pressure and temperature. A slow plasma flow rate (4 ml min^{-1}) was

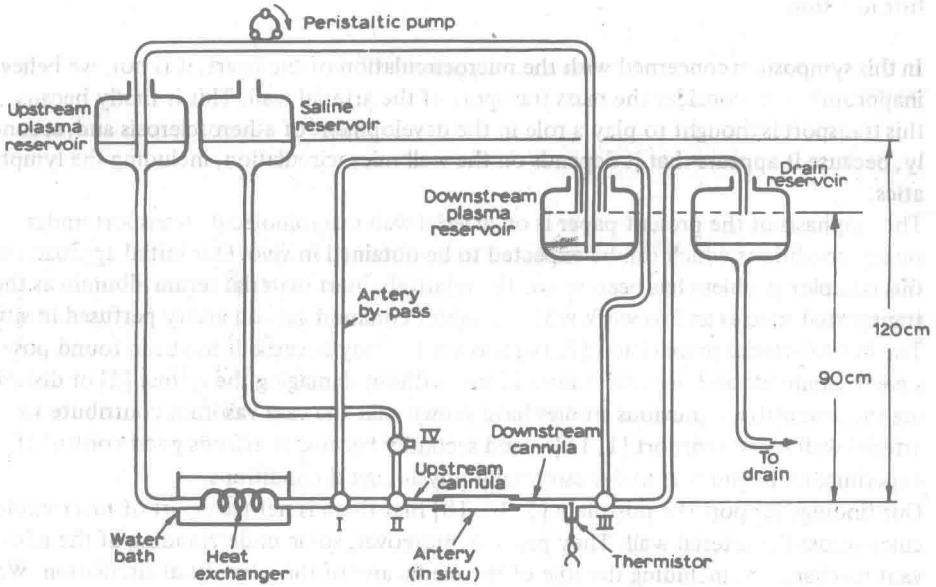

Fig. 1. Perfusion apparatus

used in order to minimize any effect of wall shear stress on label uptake [6, 11]. Pressure pulsations in the artery, due to the pump, were less than 0.01 of the mean luminal pressure.

Cannulation of the artery was achieved with minimal disturbance to it, except over short lengths at its proximal and distal ends and with only slight disturbance of the luminal hydrostatic pressure [9]. Because the animals remained alive throughout the experiments and there was no interference with the branches of the cannulated artery, it was necessary for the luminal pressure to exceed 80-100 cm H_2O in order to prevent blood from the animals' own circulations flowing into the perfusion system. In most experiments, 5-10 ml per hour of labelled plasma flowed from the perfusion apparatus into the animals' circulations.

In eight in situ perfused artery experiments, label at the same concentration as in the lumen, but contained in plasma diluted with an equal volume of Tyrode's solution so as to resemble lymph, was gently run over the outer surface of the vessel throughout the experiment. These studies involved no additional surgery.

In ten experiments, arteries were perfused in situ for periods ranging from 20-90 min, flushed with saline solution and then damaged at their intimal surface by slowly sliding a cannula along the lumen. The cannula was withdrawn to its original location, resecured, and perfusion with the same labelled plasma was resumed for 15-20 min.

At the conclusion of the in situ studies, the arteries were briefly flushed with saline solution at a luminal pressure of 100 cm H_2O in order to remove label both from the lumen and the vasa vasorum. The animal was then killed with an overdose of anaesthetic, and the artery was quickly excised. End portions of the vessels which might have been damaged and loose adventitia were discarded, and the segments were then blotted and weighed, and their radioactivity was determined.

In the in vitro studies the vessels, after excision, were freed of loose connective tissue, divided into 0.5-cm long segments and stored in plasma for up to 1 h pending their use. In short term (30 s-3 h) studies, the segments were incubated for a predetermined time in labelled plasma, rinsed, blotted, weighed and subjected to determination of their radioactivity. In long term (overnight) studies, they were incubated in labelled plasma in dialysis sacs suspended in buffer at 4°C.

The distribution of radioactivity through the thickness of the artery wall was determined both in in situ perfused and in vitro incubated segments by freezing them and sectioning them parallel to the lumen in a manner similar to that adopted by others [5]. After the tissue was frozen onto prepared cutting blocks, its edges were trimmed to remove any overhang, the projected surface area was determined, and 20 μm sections were cut through the wall. The sections were dried, weighed to check the accuracy of sectioning and to estimate the thickness of the first section, and their radioactivity was determined.

In all the studies, wall uptake was defined as a tissue/plasma activity ratio, C_T/C_P, that is cpm g^{-1} wet tissue/cpm g^{-1} plasma (assuming unit density). Plasma label concentration remained constant throughout the in situ and the in vitro studies.

The diffusible native protein in three arteries was determined. The vessels were excised and incubated for 36 h in 0.9% saline solution at 4°C. The extract was then dialysed against 0.9% saline solution at 4°C to remove low molecular weight material, and the protein was determined by Lowry's method. It was related to wet tissue weight.

Results

Light microscopy, dye exclusion studies with Evans blue added to the perfusing plasma [4], and transmission electron microscopy did not reveal damage in the in situ perfused vessels. Sliding a cannula along the lumen caused endothelial damage (Fig. 2). The whole wall label uptake was determined for 18 arteries perfused in situ for periods ranging from 1 to 90 min. There was no evidence that uptake varied with time. The average value of C_T/C_P was 0.0067, SEM 0.0009.

For sectioned arteries perfused in situ, the C_T/C_P value of each section was plotted against scaled distance from the lumen in order to produce an activity profile. There was considerable scatter, particularly affecting the adventitia. The average profile for

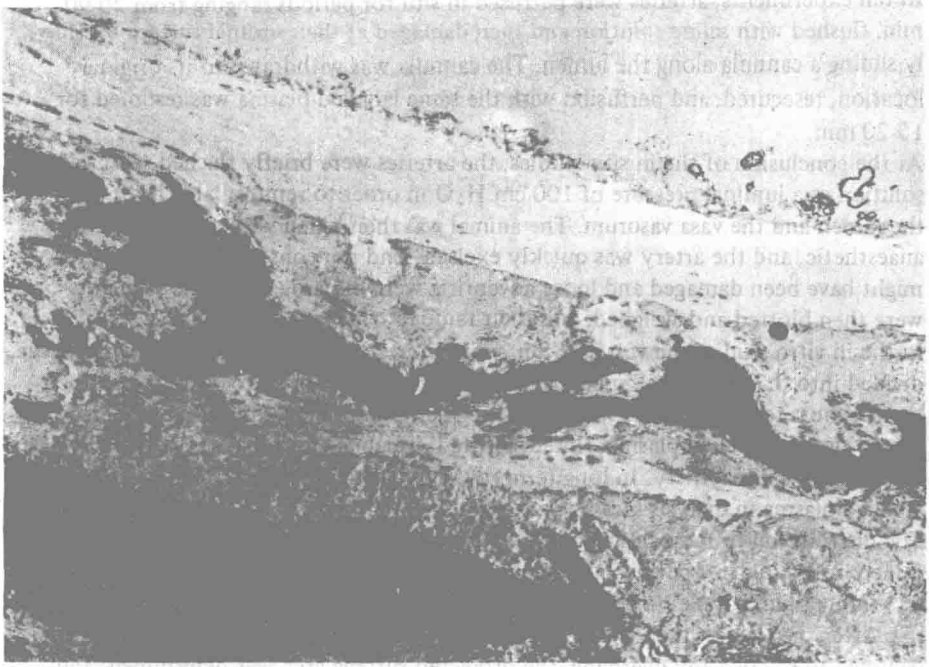

Fig. 2. Transmission electron micrograph of artery after cannula damage to the endothelium

each perfusion time has been plotted in Fig. 3. Mean medial and adventitial C_T/C_P values were calculated, and these are shown as a function of perfusion time in Fig. 4. The mean medial value increases with time, and an apparently steady value of 0.0095, SEM 0.0013 (n = 11) is reached at about 30 min. No firm conclusion can be drawn about the adventitia because of the large scatter.

Mean medial and adventitial values were similarly calculated from the C_T/C_P profiles obtained from arteries perfused in situ while simultaneously exposed to label at their outer surface. The experiments were of 30 and 60 min duration, and there was no significant difference between either the medial or adventitial values at these two times. The average medial and adventitial values were respectively 0.032, SEM 0.0032 and 0.22, SEM 0.034 (eight arteries).

The average whole wall C_T/C_P for ten arteries perfused in situ and subjected to intimal damage was 0.42, SEM 0.0096. C_T/C_P was determined for the whole wall in 20 segments incubated for varying periods of time in labelled plasma in vitro. In addition, the total wall C_T/C_P was calculated for 57 segments similarly incubated and then subjected to frozen sectioning by summing the section values. The average values at different incubation times are shown in Fig. 5. C_T/C_P rises with time and reaches an apparently steady value of 0.22 within about 30 min. Whole wall values are also shown for segments incubated in dialysis sacs overnight. The average value is 0.19.

The average C_T/C_P profiles for segments incubated in vitro for periods ranging from 10 min to 3 h are shown in Fig. 6. The time-dependent behaviour of the mean medial and adventitial values of C_T/C_P for these vessels is shown in Fig. 7. For the adventitia,