Distribution and Binding Kinetics of Ciprofloxacin and Ofloxacin in the Hindlimb of the Rat

A. Sanchez-Navarro,[1,3] A. C. Casquero-Dorado,[1] and M. Weiss[2]

Received December 20, 1998; accepted January 23, 1999

KEY WORDS: quinolones; pharmacokinetics; permeability; tissue binding; hindlimb.

INTRODUCTION

The quinolones ciprofloxacin and ofloxacin penetrate relatively well into most tissues as reflected by steady-state distribution volumes of 2–5 l/kg for ciprofloxacin and 1–3 l/kg for ofloxacin reported for humans (1,2). They also show a good capacity to cross cell membranes, accessing into the erythrocytes and alveolar macrophages among other body cells (3). However, nothing is known about the kinetics of binding to tissues as for example skeletal muscle.

The kinetics of distribution processes may influence the time course and extent of pharmacological and/or toxicological effects of those drugs which highly bind to tissues that constitute the target organ or the toxicological phase. However, in pharmacokinetics characterization of drug, distribution is mostly limited to the steady-state situation, i.e., to the steady-state distribution volume at the whole body level or more specifically to the equilibrium distribution coefficient for a particular organ/tissue.

Isolation and perfusion of organs provides an excellent methodology to study the dynamics of drug distribution in particular tissues of interest from a kinetic, toxicological and/or pharmacological point of view. Kinetic analysis of drug and indicator outflow curves using appropriate mathematical models allows one to estimate parameters of binding dynamics of drugs to tissue constituents. The isolated hindlimb preparation includes muscle, bone, skin and fat, which constitute over 75% of the total body weight. It has been widely used by physiologists as an experimental model to study the permeability of the capillary bed (4) and other physiological processes in muscle (5–7). In recent years, this preparation has been addressed as an experimental model of drug distribution by several authors (8–11), and more realistic models of the intravascular and intratissue distribution processes of drugs have been applied to rat hindlimb data (12,13).

The aim of the present work was to determine the permeation and binding kinetics of ciprofloxacin and ofloxacin using the isolated hindlimb of the rat as the experimental approach and water as an indicator of the steady-state distribution space of the unbound drug. The underlying mathematical model allows not only the estimation of distribution spaces separated by a permeability barrier but also the binding kinetics within the tissue or cellular space. This information should be also useful for the development of a whole body kinetic model since the tissues of the hindlimb constitute the major body components determining drug distribution.

METHODS

Experimental Methodology

The experiments were carried out using 3 months old female Wistar rats (mean body weight = 166.10 ± 17.84 g) in compliance with "Principles of Laboratory Animal Care". The animals were maintained with water ad libitum for 12 h prior to the surgical procedure, which is a modification of the experimental method described by Ruderman (6).

Briefly, it consists of isolating the left hindlimb of the anaesthetized rat (sodium pentobarbital, 40 mg/kg, i.p. route) by placing and tying ligatures in all vessels emerging and flowing into the abdominal aorta and inferior vena cava, respectively; isolation and ligation of the internal-iliac vessels, the inferior epigastric vessels and the pudendal vessels is also necessary to restrict the perfusion to the hindlimb. Additional ligatures are placed around the contralateral iliac vessels and the lowest joint of the perfused limb in order to exclude the paw from the perfused circuit.

After ligation, a cannulae is first inserted into the inferior vena cava, pulled down until the bifurcation of the common iliac vessels and tied; then, another cannulae is placed into the abdominal Aorta at the same position as the former one and fixed by tying. Immediately the artificial perfusion is started using a modified Tyrode medium. Cannulation of the artery after the vein reduces the ischaemic period to 2–4 s and avoids reperfusion injury in the preparation. The animal was then overdosed with pentobarbitone administered as an intrathoracic injection. After 20 min of stabilisation period a dose of drug (450 μg of ciprofloxacin, 900 μg of ofloxacin) or tritiated water (10 μCi) is injected through the cannulae in the aorta as a bolus injection and outflow sampling begins at programmed times, using a fraction collector connected to the cannulae in the vena cava. Samples were collected for 20 min at different frequency intervals. Five experiment were carried out for each drug and tritiated water, respectively.

Additional experiments were carried out in the same experimental conditions as describe above, but in absence of the hindlimb to estimate the impulse response of the catheter for correction of the drug and indicator outflow curves.

Tritiated water was determined by liquid scintillation counting. Standard solutions were prepared in the same medium as used for hindlimb perfusion, at a concentration range of 1×10^{-3} to 3 μCi/ml. 5 ml of scintillation cocktail (Ready Protein^{+TM}) were added to 0.5 ml of sample or standard solution, the mixture was shaken for 15 s and stored in scintillation vials for measuring (2 min).

Quantification of ciprofloxacin and ofloxacin in outflow perfusate samples is carried out by a HPLC, ion paired and

[1] Department of Pharmacy, University of Salamanca, 37007 Salamanca, Spain.

[2] Section of Pharmacokinetics, Department of Pharmacology, Martin Luther University Halle-Wittenberg, 06097 Halle, Germany.

[3] To whom correspondence should be addressed. (e-mail: asn@gugu.usal.es)

0724-8741/99/0400-0587$16.00/0

reverse phase technique, widely described for these drugs. Two standard solutions were prepared with Sorensen buffer (pH = 7.4) for each quinolone at concentration ranges of 1–0.01 μg/ml and 1–50 μg/ml.

The internal standard method was used, with ciprofloxacin as internal standard for ofloxacin samples and ofloxacin as internal standard for ciprofloxacin samples. Standard solutions and samples underwent the same procedure: 50 μl of internal standard solution and 100 μl of triclhoroacetic acid solution (20%) were added to 100 μl of sample or standard; the mixture was vigorously shaken for 15 s and centrifuged at 3500 r.p.m. for 5 min, then 100 μl of the supernatant were injected in the cromatograph for analytical determination. Using these experimental conditions the analytical technique proved to have a detection limit of 0.01 μg/ml for both drugs and the intra and inter-day coefficients of variation showed values lower than 5% and 6% for ciprofloxacin and ofloxacin, respectively.

Modeling Analysis

A diagrammatic description of the pharmacokinetic model is shown in Fig. 1. The model is build out of a plasma or vascular compartment considered as a well-stirred space (volume V_P) and a tissue space which consists of two compartments, one for the unbound drug (tissue water space V_{Tu}) and one for the drug bound to tissue constituents. The drug is transported through V_P by perfusate flow Q, penetrates from V_P to V_{Tu} across a permeability barrier (permeation clearance CL_{PT}) and its binding to tissue constituents is described by binding/unbinding rate constants k_{on}/k_{off}. Note that permeation clearance is determined by the permeability-surface product PS of the capillary wall and the fraction unbound in perfusate f_{Pu}, $CL_{PT} = f_{Pu}PS$.

The following differential equations result from the model:

$$V_P dC_{out}/dt = Q(C_{in} - C_{out}) - CL_{PT}(C_{out} - C_{Tu}) \quad (1)$$

$$V_{Tu} dC_{Tu}/dt = CL_{PT}(C_{out} - C_{Tu}) - k_{on}V_{Tu}C_{Tu} + k_{off}V_{Tb}C_{Tb} \quad (2)$$

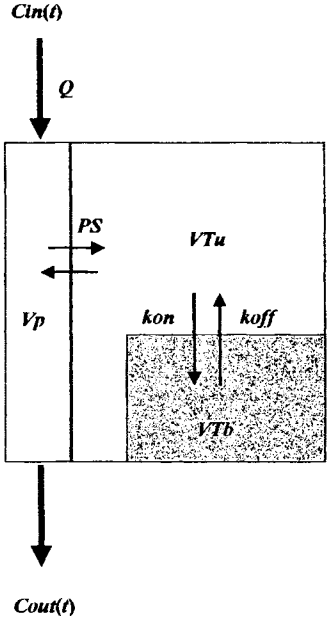

Fig. 1. Model for quinolone distribution in the isolated perfused hindlimb of the rat.

$$V_{Tb} dC_{Tb}/dt = k_{on}V_{Tu}C_{Tu} - k_{off}V_{Tb}C_{Tb} \quad (3)$$

As in previous modeling approaches (12,13) the model is formulated in the Laplace domain resulting in the following equation for the transformed outflow profile:

$$\hat{C}_{out}(s) = \frac{\hat{C}_{in}(s)\,Q}{V_p s + Q + CL_{PT} - \dfrac{CL_{PT}^2}{V_{Tu}s + CL_{PT} + k_{on}V_{Tu} - \dfrac{k_{on}\,k_{off}V_{Tu}}{s + k_{off}}}} \quad (4)$$

This approach is useful to separate out the effects of the catheters on the impulse-response profiles. The catheter response was adequately fitted by a sum of two inverse Gaussian functions

$$\hat{C}_{in}(s) = \frac{D}{Q}[p\,\hat{f}_1(s) + (1 - p)\,\hat{f}_2(s)] \quad (5)$$

and

$$\hat{f}_i(s) = exp\left\{\frac{1}{CV_i^2} - \left[\frac{MT_i}{CV_i^2/2}\left(s + \frac{1}{2MT_iCV_i^2}\right)\right]^{1/2}\right\} \quad (6)$$

where the mean MT_i and relative dispersion $CV_i^2 (i = 1, 2)$ are empirical parameters and the mean input time (catheter transit time) is obtained as $MTT_{in} = pMT_1 + (1 - p)\,MT_2$. Note that the same function was used previously as an empirical model for the transit time density of intravascular markers (12). The outflow curve is then obtained by numerical inverse Laplace transformation of Eq. 4 as

$$C_{out}(t) = L^{-1}\{\hat{C}_{out}(s)\} \quad (7)$$

According to the above model the total water distribution volume V_w (as an estimate of the anatomical distribution volume of the drug) is given by $V_w = V_P + V_{Tu}$. In additional experiments the volume V_w is determined independently using water as the reference compound. The steady-state distribution volume of the drug in the organ is the sum of the intravascular volume V_P and the apparent tissue distribution volume $V_T = T_{Tb} + V_{Tu}$, whereby the latter is dependent on the degree of tissue binding

$$V_{ss} = V_p + V_{Tu}(1 + k_{on}/k_{off}) \quad (8)$$

and the equilibrium distribution coefficient of the drug in the organ can be calculated as V_{ss}/V_w in accordance with the conventional definition of the distribution coefficient in terms of steady-state distribution volume per tissue weight.

Function 7 (substituting Eqs. 4, 5 and 6) was fitted to the data applying numerical inverse Laplace transformation using the nonlinear regression program package SCIENTIST (Micro-Math Scientific Software, Salt Lake City, USA) and weighting the data according to $1/y_{obs}^2$. The approach differs from models applied previously to analyze drug distribution in the rat hindlimb (12,13) in so far that the vascular space was regarded as a well-mixed compartment and no vascular reference was used. This simplifying assumption appears justified because of the rate limiting role of drug input to the organ due to the relatively delayed catheter response (MTT_{in} = 0.4 min).

Statistical moments ($M_n = \int_0^\infty t^n C(t)dt$) were estimated by numerical integration up to the last time point, using the slope value of the last linear phase of the curve for the extrapolation to infinity. The volume of distribution V_{ss} is then obtained as product of mean transit time ($MTT = M_1/M_0$) and flow rate (Q). The relative dispersion of transit times is defined as $CV^2 = VTT/MTT^2 = M_2M_0/M_1^2 - 1$ where VTT denotes the variance of the transit time distribution. The parameters were calculated after correcting MTT and VTT for the catheter effects.

Statistical analysis was performed with Student's t test with $P \leq 0.05$ as the minimal level of significance.

RESULTS

Figure 2 shows typical outflow curves of ciprofloxacin and ofloxacin after bolus injection into the isolated perfused hindlimb. All data were successfully described by Eq. 4 with a coefficient of determination > 0.99 which indicates a good fit. The approximate coefficients of variation of the individual parameter estimates were less than 30%. The rate-limiting effect of the catheter response (Eq. 5) is obvious by the delayed increasing part of the curve; the fit indicates that the well-mixed vascular compartment assumption is not in contradiction to the data. (Note that MTT_{in} of 0.4 min is in the same order of magnitude as the mean vascular transit time, $V_P/Q = 0.5$ and 0.7 min, for ciprofloxacin and ofloxacin, respectively). The same equation was used as an empirical model to describe the outflow profiles of water to estimate V_w without giving any interpretation to the other model parameters. Table I summarizes, for ciprofloxacin and ofloxacin, the estimated model parameters (V_p, V_{Tu}, k_{on}, k_{off}, and CL_{PT}) together with the derived parameters (V_{ss}/V_w) and the recovery of the drugs (up to the last sampling point). The sum of the distribution volumes V_P and V_{Tu} (denoted by V_w) for both ciprofloxacin (17.78 ± 8.64 ml) and ofloxacin (20.93 ± 1.22 ml) are well in accordance with the total water distribution volume (18.26 ± 11.35 ml) estimated separately in the hindlimb. The steady-state distribution volumes of 56.8 ± 32.04 ml and 43.0 ± 9.5 ml of the drugs for ciprofloxacin and ofloxacin, respectively, were not statistically different. The same holds for the corresponding equilibrium distribution coefficients. From the curve moments calculated by numerical integration mean transit times MTT of 8.71 ± 0.22 min and 9.44 ± 2.17 min (corresponding to V_{ss} of 26.13 and 28.32 ml) were obtained for ciprofloxacin and ofloxacin, respectively. From the mean transit time of water (4.80 ± 0.94 min) a mean steady-state distribution volume of 14.40 ml was calculated. Note that these steady-state distribution volumes, estimated model-independent, are smaller than the corresponding values derived from the model parameters. The relative dispersion of transit times CV^2 for ciprofloxacin, ofloxacin, and water, respectively, are 3.9 ± 0.8, 3.2 ± 0.6 and 2.0 ± 0.8. No significant difference was detected between the CL_{PT} values of 4.53 ± 1.18 ml/min and 6.42 ± 1.05 ml/min estimated for ciprofloxacin and ofloxacin, respectively. The kinetics of binding to tissue constituents is characterized by binding and unbinding rate constants with ratios of 0.53/0.21 and 0.98/1.80 for ciprofloxacin and ofloxacin, respectively.

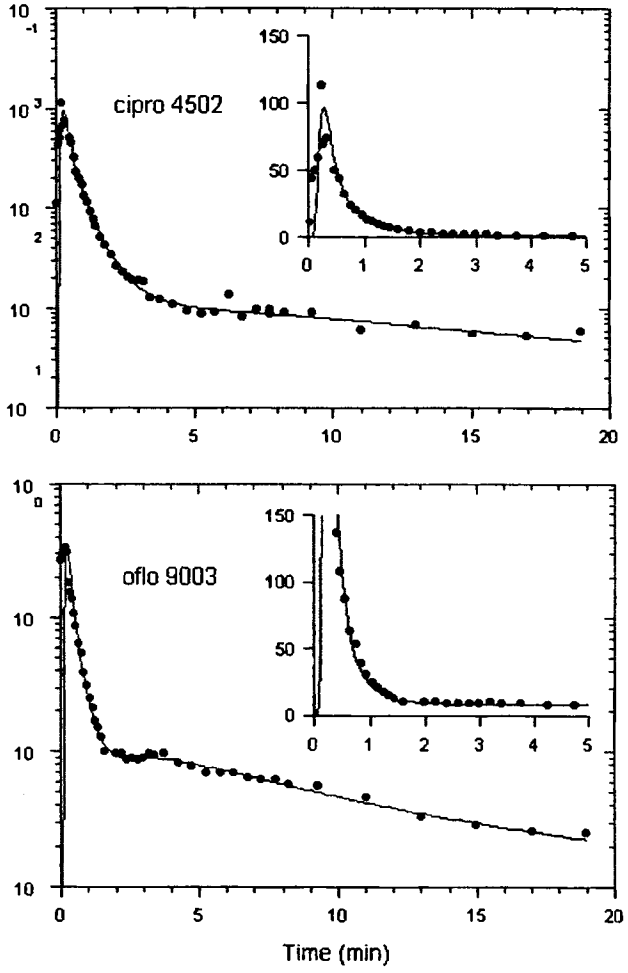

Fig. 2. Typical outflow data (•) for ciprofloxacin and ofloxacin after bolus injection in the perfused rat hindlimb and the theoretical curves (—) of the organ model (Eqs. 4 and 7) corresponding to the parameter estimates.

DISCUSSION

The pharmacological response to chemotherapeutic agents depends on their access and permanence at the site of infection. Since one of the advantages of quinolones in antimicrobial therapy is their ability to penetrate into the tissue phase, modeling of tissue distribution is of special interest for these drugs. Because the distribution processes should be thoroughly characterized when the pharmacological and/or toxicological effect is related to distribution in a particular organ or body tissue, we present a model analysis of quinolone kinetics in the rat hindlimb.

The advantage of the experimental and modeling approach is that besides steady-state tissue partition and the blood-tissue permeation barrier, also the binding kinetics of the drug within the tissue phase is analyzed.

In our study, no statistically significant difference was observed between the distribution coefficients of ciprofloxacin and ofloxacin (3.65 ± 1.31 vs. 2.18 ± 0.53). Also the values of the permeation clearences estimated for ciprofloxacin and ofloxacin (4.53 vs. 6.42 ml/min), which were not statistically different, suggest for both drugs an intermediate situation, which is located between flow- and permeability-limited penetration kinetics. The kinetic behaviour of both quinolones can not be explained without the assumption of a slow binding process to tissue constituents after instantaneous distribution

Table I. Model Parameters Estimated for Ciprofloxacin (Cipro) and Ofloxacin (Oflo) in the Isolated Perfused Hindlimb of the Rat

	V_P (ml)	V_{Tu} (ml)	CL_{PT} (ml/min)	k_{on} (min^{-1})	k_{off} (min^{-1})	V_{ss}/V_w	Recovery (%)
Cipro							
1	1,64	11,06	3,48	0,49	0,18	3,74	51,1
2	1,39	5,36	3,28	0,95	0,22	5,34	43,1
3	1,37	22,99	4,98	0,48	0,14	4,32	60,0
4	2,02	14,88	4,73	0,20	0,22	1,88	43,9
5	1,65	26,49	6,16	0,54	0,27	2,97	67,2
Mean	1,61	16,16	4,53	0,53	0,21	3,65	53,1
SD	0,27	8,63	1,18	0,27	0,05	1,31	10,5
Oflo							
1	1,99	19,48	5,03	0,23	0,18	2,25	74,4
2	1,68	18,97	6,26	2,76	6,41	1,43	66,7
3	2,47	16,91	7,49	0,56	0,33	2,67	81,9
4	2,25	19,97	6,89	0,39	0,28	2,37	73,2
Mean	2,10	18,83	6,42	0,98	1,80	2,18	74,0
SD	0,34	1,35	1,05	1,19	3,07	0,53	6,2

Note: Recovery was calculated as the $(AUC_{0-20\ min}\ Q)/D$ ratio.

across the tissue water space. (Alternative models were tested but failed to describe the data.) The time constants of the binding and unbinding process (1.9 and 4.8 min for ciprofloxacin) are higher than the time constant characterizing the permeation process into tissue (0.4 min for ciprofloxacin).

One main argument for the validity of our model (i.e., the existence of a permeability barrier and non-instantaneous tissue binding) is the finding that the estimated total water space V_w defined by the model (17.78 and 20.93 ml for ciprofloxacin and ofloxacin) is in reasonable agreement with the independent measurement using water as indicator (14.4 ml). The latter is in accordance with the value of 17.50 ml obtained in the rat hindlimb for $Q = 4$ ml/min (13) and higher than that of 10.73 ml reported by Heatherington and Rowland (9). Furthermore, the estimates of the plasma volume V_p (1.61 and 2.10 ml) are in accordance with values of the vascular volume in the isloated hindlimb of the rat estimated by Wu *et al.* (8) (1.5 ml) and slightly higher than the reported values of Heatherington and Rowland (9) (1.03 ml) and Weiss et al (13) (0.73 ml) for a flow rate of 4 ml/min.

Comparing the above modeling results with those of the model-independent moment analysis, lower distribution volumes but similar distribution coefficients (of about 2 for both drugs) are obtained. However, the model estimates appear to be more reliable in view of the way data error and curve extrapolation may affect the moment estimates. Analysis of CV^2 values gives information about mixing/distribution dynamics of solutes in the system and it has been established that CV^2 values > 1 (in the present context) implies the existence of peripheral compartments or non-instantaneous distribution equilibrium (14). Accordingly, ciprofloxacin and ofloxacin, with CV^2 values of 3.9 and 3.2 respectively, distribute non-instantaneously in the hindlimb as already shown by the modeling analysis. Water, in contrast, equilibrates faster showing a CV^2 of 2.0 (in accordance with the value of 1.58–1.94 reported for the rat hindlimb, (8)). The fact that also CV^2 for water exceeds 1 can be explained, for example, by its non-instantaneous intravascular distribution due to the complexity of the hindlimb microcirculatory network, i.e., the marked regional heterogeneity within muscle blood flow (15).

The results of this study underline the importance of a detailed analysis of distribution dynamics of drugs, both in terms of the experimental method and the used modeling approach. Such results are difficult to obtain at the whole body level, and without detailed model one can not separate the respective effects of permeation and binding kinetics. With the present approach one can distinguish between free and bound drug in the tissue, which is of interest when only the free drug is active. The organ distribution kinetics of ciprofloxacin and ofloxacin is not only determined by the permeation process but also by the non- instantaneous distribution tissue distribution due to relatively slow binding (unbinding) to extravascular structures.

ACKNOWLEDGMENTS

The authors acknowledge to Martin Luther University (Halle-Wittenberg) (German Academic Exchange Service) for supporting this work.

REFERENCES

1. S. Allard, M. Kinzig, G. Boivin, F. Sörgel, and M. LeBel. Intravenous ciprofloxacin disposition in obesity. *Clin. Pharmacol. Ther.* **54**:368–373 (1993).
2. J. H. Yuk, C. H. Nightingale, R. Quintiliani, and K. R. Sweeney. Bioavailability and pharmacokinetics of ofloxacin in healthy volunteers. *Antimicrob. Agents Chemother.* **35**:384–386 (1991).
3. P. Tega-Isavadharm, D. Keeratithakul, G. Watt, W. H. Kyle, and M. D. Edstein. Measurement of ciprofloxacin in human plasma, whole blood and erythrocites by high-performance liquid chromatography. *Ther. Drug Monit.* **13**:263–267 (1991).
4. B. Rippe and B. Haraldsson. Capillary permeability in rat hindquarters as determined by estimation of capillary reflection coefficients. *Acta Physiol. Scand.* **127**:289–303 (1986).
5. B. Haraldsson and B. Rippe. Trancapillary clearance of albumin in rat skeletal muscle monitored by external detection. Effects of alteration in capillary surface area. *Acta Physiol. Scand.* **132**: 495–504 (1988).
6. N. H. Ruderman, C. R. S. Houghton, and R. Hems. Evaluation of the isolated perfused rat hindquarters for the study of muscle metabolism. *Biochem. J.* **124**:639–651 (1971).
7. J. Gorski, D. A. Hood, and R. L. Terjung. Blood flow distribution in tissues of perfused rat hindlimb preparations. *Am. J. Physiol.* **250**:E441–E448 (1986).

8. Z. Wu, L. P. Rivory, and M. S. Roberts. Physiological pharmacokinetics of solutes in the isolated perfused rat hindlimb: characterization of the physiology with changing perfusate flow, protein content and temperature using statistical moment analysis. *J. Pharmacokin. Biopharm.* **21**:653–688 (1993).
9. A. C. Heatherington and M. Rowland. Estimation of reference spaces in the perfused rat hindlimb. *Eur. J. Pharm. Sci.* **2**:261–270 (1994).
10. A. Sanchez-Navarro. A theoretical approach to the estimation of tissue flows using tritiated water as indicator. *J. Pharm. Sci.* **84**:643–646 (1995).
11. A. C. Casquero-Dorado and A. Sanchez-Navarro. The isolated hindlimb preparation as a drug distribution model. *Meth. Find. Exp. Clin. Pharmacol.* **18**:453–458 (1996).
12. M. Weiss and M. S. Roberts. Tissue distribution kinetics as determinant of transit time dispersion of drugs in organs: Application of a stochastic model to the rat hindlimb. *J. Pharmacokin. Biopharm.* **24**:173–196 (1996).
13. M. Weiss, A. Koester, Z. Wu, and M. S. Roberts. Distribution kinetics of diazepam, lidocaine and antipyrine in the isolated perfused rat hindlimb. *Pharm. Res.* **14**:1640–1643 (1997)
14. M. Weiss. Distribution kinetics in the body and single organs: moment analysis. In D. Z. D'Argenio (ed.), *Advanced methods of pharmacokinetic and pharmacodynamic systems analysis, Vol. 2*, Plenum Press, New York, 1995, pp. 89–100.
15. P. O. Iversen, M. Standa, and G. Nicolaysen. Marked regional heterogeneity an blood flow within a single skeletal muscle at rest and during exercise. *Acta. Physiol. Scand.* **136**:17–28 (1989).