JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1992, 43, 3, 209—218

M. STANDA· P. O. IVERSEN, T. FLATEBØ & G. NICOLAYSEN

REGIONAL HETEROGENEITY OF MYOCARDIAL BLOOD FLOW WITHIN THE RABBIT LEFT VENTRICLE DURING HAEMORRHAGIC HYPOTENSION

Institute of Physiology, School of Medicine, Wroclaw, Poland* and Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, Norway.

Several studies have reported an extensive regional heterogeneity in myocardial blood flow. The reported coefficients of variation for regional myocardial perfusion range from about 0.2 to 0.4 in normotensive animals. The spatial distribution of myocardial perfusion during haemorrhagic hypotension seems not to have been assessed.

The goal of the present study was to determine the regional heterogeneity in myocardial blood flow within the rabbit left ventricle during normal conditions and after haemorrhagic hypotension.

Radioactive microspheres were infused into the left ventricle in barbiturate anaethetized rabbits over either 30 or 120 sec.

The haemorrhagic hypotension was induced by bleeding, so that mean arterial blood pressure was reduced to about 50% of control. The left ventricles were divided into samples of about 0.025 g each. Regional heterogeneity in the blood flow was expressed as the coefficient of variation corrected for the Poisspn distribution of microspheres (CVc). The CVc was 0.37 ± 0.09 (mean $\pm$ SD) during control and 0.41 ± 0.11 after bleeding, the CVc obtained after bleeding being somewhat higher than during control ($P < 0.05$). We obtained a high correlation coefficient (τ about 0.68) between regional perfusion values at control and after bleeding which indicates a stable perfusion pattern within the myocardium.

We conclude that the regional distribution of coronary blood flow within the left ventricle is markedly heterogenous during control condition and that this pattern is not changed during haemorrhagic hypotension.

Key words: *Haemorrhage, microspheres, myocardial blood flow, regulation.*

INTRODUCTION

Several papers have reported a marked regional heterogeneity in blood flow on a non-microvascular level, i.e. at the level of larger arterioles or smaller arteries, within the left ventricle (1—3) as well as within single skeletal muscles

(4,5). The presence of this uneven perfusion pattern has been throughly confirmed with several independent flow tracers such as antipyrine (6), iododesmethylimipramine (7) and microspheres (7).

Recently, Coggins et al. (8) found an extensive regional variation in the left ventricular vasodilator reserve during reduced coronary arterial perfusion pressure (range 30 to 70 mmHg) in the normovolaemic dog.

The effect of haemorrhagic hypotension on coronary resistance has been described by several investigators as reviewed by Schadt and Ludbrook (9). The resistance in the coronary vascular bed has been reported to increase (10), decrease (11), or remain unchanged (12) following hypotension caused by bleeding. Vatner et al. (10) studied the effects of haemorrhage on regional blood flow and vascular resistence in dogs and baboons using the Doppler ultrasonic technique. They observed a reduction of coronary blood flow to about 39% of control concomitant with an increase of vascular resistance to about 127% of control during moderate hypotension (reduction in mean arterial blood pressure with about 20—30 mmHg). Neutze et al. (11) found in bled rabbits (blood loss 28 ml/kg body wt) an increase in the fraction of cardiac output delivered to the heart by about 150% of control and decrease of vascular resistance to about 69% of control. They used microspheres as a tracer to determine the blood flow (11). Forsyth et al. (12) studied coronary blood flow during haemorrhage using the microsphere technique in unanaesthetized monkeys. They did not find significant changes in the coronary blood flow after a 30% or 50% blood loss compared to the normovolaemic group. The above-mentioned authors (10—12) did not look at the spatial distribution of blood flow within left ventricle.

The aim of our study was to determine the spatial distribution of blood flow within the left ventricle during haemorrhagic hypotension in the rabbit. Any increase in the regional heterogeneity could increase the possibility of severe local hypoxia even if local coronary flow is not markedly reduced.

MATERIALS AND METHODS

The protocol was accepted by the Animal Experimentation Committee.

Surgery

We used 6 rabbits of either sex (mixed breed, 3.85 ± 0.25 kg, mean $\pm$ SD) which were anaesthetized with pentobarbitone, 30 mg/kg body wt iv. Additional anaesthesia was given when needed.

All rabbits were tracheotomized and had an endotracheal tube inserted. The rabbits were allowed to ventilate spontaneously.

A cannula was inserted into a central ear artery to obtain blood for analysis of PO_2, PCO_2 and continuous mean arterial blood pressure recording. The rabbits were heparinized (2000 IU/kg body wt iv) prior to the infusion of microspheres.

The catheter (PE 50) used for microsphere infusion as well as for checking left ventricular pressure, was inserted into the left ventricle via one right carotid artery under blood pressure control.

In our experiments we also placed a catheter (PE50) into one femoral artery for withdrawal of blood in order to collect reference samples and to bleed the animals. The exact postions of the catheters were controlled by dissection of the rabbits *post mortem.*

A temperature probe was placed about 10 cm inside the recturn.

Blood flow tracers

Microspheres (diam. 15—16.5μm, NEM, Boston, Ma., USA) labelled with either ^{51}Cr, ^{103}Ru, ^{141}Ce or ^{46}Sc were used (random order).

The technique for preparation of microspheres as a blood flow tracer was esentially that of Iversen et al. (5). The microsphere suspensionss was infused (3 ml/min) over either 30 or 120 sec. Approximately 1.1×10^6 microspheres were given per infusion.

The haemorrhagic hypotension was induced by leeding (4 ml/min) via the catheter in he femoral artery until mean arterial blood pressure was reduced by aboud 50% of pre-bleeding level. The time between end of bleeding and start of microsphere infusion was always 30 min. The infusions of microspheres were done in the following sequence: In each of two rabbits we gafe one control infusion lasting 120 sec. We then bled the animals before another 120-sec infusion was given. In another two rabbits we gave two control infusions, first a 30-sec infusions, then a 10-min interval before a 120-sec infusion. T he same pattern of infusions was used after bleeding. In another rabbit we used two 120-sec infusions separated by a 10-min interval before and after bleeding of the animal. In the latter 3 animals a total of 4 infusions were thus given to each animal.

In a separate rabbit one PE 50 catheter was inserted into the left ventricle and one PE 50 catheter was put the left atrium via small thoracotomy before the chest was closed. We could then compare the results of regional myocardial perfusion obtained with simultaneous microsphere infusions into left atrim and into left ventricle (infusion time 30 sec).

The withdrawal of blood reference samples (4 ml/min) was started 30 sec prior to the infusion of microspheres and was maintained until 30 sec after the infusion was completed.

Preparation of samples

Each experiment was terminated by giving the rabbit an overdose of pentobarbitone followed by saturated KCl (iv).

The heart was dissected out and fixed for 24h in a phosphate buffered 4% formaldehyde solution (pH 7.4) to facilitate cutting of the heart muscle. The free walls of the right venticle, left and right atrium and epicardial fat were removed. The left ventricle was divided into five to six equal slices vertical to the basis-apex axis. Each slice was then divided into twelve segments. Each myocardial segment was futher divided into an endocardial, a mid-myocardial and an epicardial region. In this way the total number of examined regions was 880 in the 6 rabbits. The samples weighed 0.026 ± 0.009 g (mean $\pm$ SD).

Calculation

The radioactivity for each isotope corrected for background and istotope cross-over was calculated for each individual sample. The number of microspheres per g tissue was determined for each sample as well as the mean number of microspheres per g for each whole left ventricular muscle. The ratio between these latter values provided for each sample a relative microsphere density value. The lowest number of microspheres deposited in any sample was 290.

Myocardial blood flow (MBF, ml/100 g $\times$ min) was calculated as:

$$\text{MBF} = \text{Cm} \times 100 \times \text{RBF/CR}$$

where Cm denotes the counts per gram of myocardium, RFB is the reference blood flow (rate of withdrawal of blood through reference catheter) and CR is the total counts in the reference blood sample.

We calculated the experimental coefficient of variation (CV) for microsphere distribution which was obtined with each microsphere infusion in each left ventricular muscle.These CV values were corrected for the Poisson distribution of the microspheres to yield the corrected coefficient of variation (CVc) as outlined by Iversen et al. (5).

Statistics

All values are given as a mean $\pm$ SD unless otherwise stated. Correlation analysis was based on the non-parametric Kendalls Rank correlation coefficient (τ). We used the Student t-test (two-tailed) for analyses of differences. Significance was established at a level of 5%.

RESULTS

Figure 1 shows the frequency distribution of the relative microsphere densities within the left ventricle (LV) at normal condition and after bleeding in one rabbit based on 30-sec infusions of microspheres. First, there was a marked heterogeneity in regional microspheres densities both during the control condition and after bleeding. Second, the perfusion distribution obtained after bleeding was not appreciably different from that obtained during the normotensive state. Similar distributions were obtained in the other rabbits irrespective of the duration of the microsphere infusions.

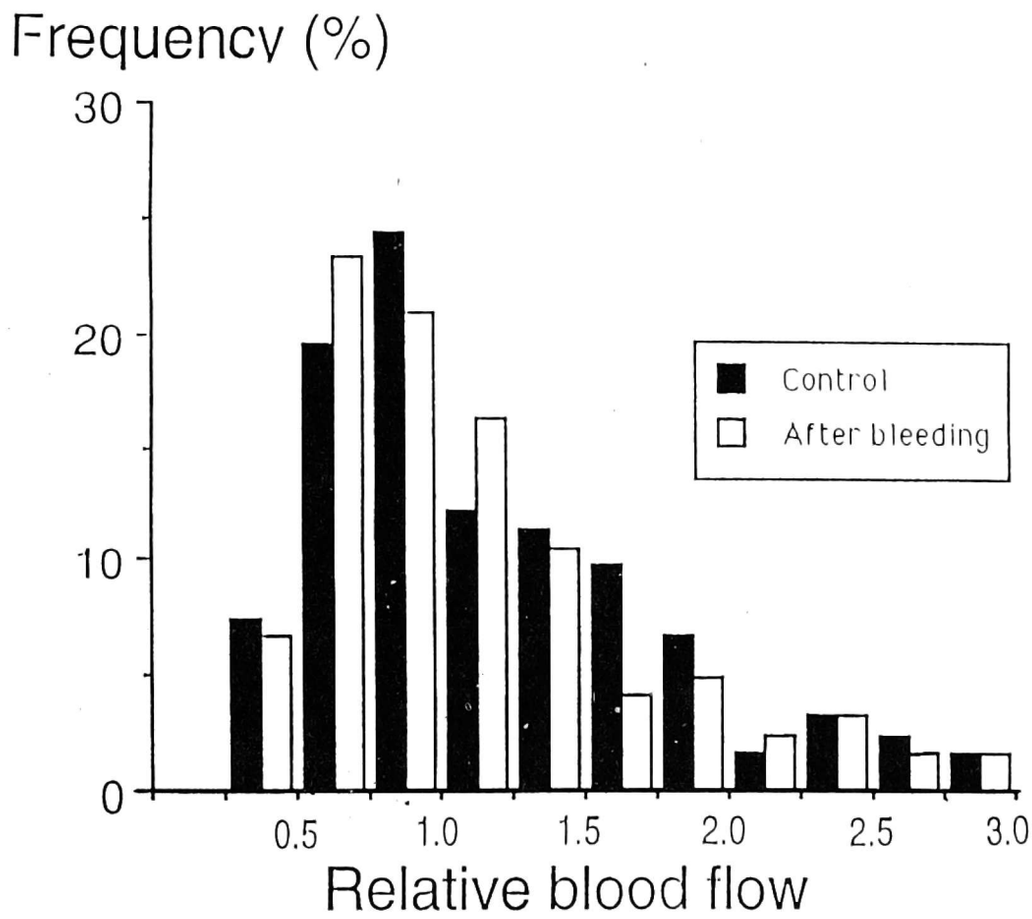

Figure 1 Frequency distributions of relative blood flow obtained at control (closed columns) and after bleeding (open columns). Results from 30 sec. infusions into one rabbit.

The mean arterial blood pressures in the control group and in the bleed one were: 110 ± 15 mmHg and 50 ± 12.5 mmHg, respectively. Similar changes were obtained for the left ventricular pressure. We observed no changes in mean arterial blood pressure during infusion of microspheres suspensions. The arterial values for PO_2 and PCO_2 ranged from 10.1 to 13.0 kPa and from 5.0 to 5.4 kPa, respectively (sampled frequentyl). The body temperature was the same both in the control and in the hypotensive state.

Table 1. Ninety-five per cent confidence intervals for the lower and upper quartiles of relative microsphere densities at control and after bleedong.

Before bleeding	Lower quartil	Upper quartil
Infusion time 30 sec. n=4 N=2	0.71—0.82	1.20—1.50
Infusion time 120 sec. n=6 N=3	0.72—0.83	1.17—1.49
After bleeding	**Lower quartil**	**Upper quartil**
Infusion time 30 sec. n=4 N=2	0.71—0.78	1.20—1.43
Infusion time 120 sec. n=6 N=3	0.70—0.82	1.22—1.45

n — number of infusion
N — number of animals

In Table 1 we have presented the 95% confidence intervals for the lower and upper quartiles for the relative microsphere densities for the three infusion periods during control and after haemorrhage. The quartile values for the relative microsphere density distributions obtained after bleeding were not different ($P > 0.05$) from the corresponding values in the control LV muscles.

Table 2. The coefficient of variation (CVc) for regional blood flow for different infusion periods during normal conditions and after bleeding.

Infusion time	30 sec.	120 sec.
CVc (control)	0.39 ± 0.09	0.36 ± 0.08
n	4	6
CVc (after bleeding)	0.42 ± 0.12	0.40 ± 0.11
n	4	6

Values are mean $\pm$ SD, while n-denotes the number of infusions.

For each LV muscle the corrected coefficients of variation (CVc) for the distribution of microspheres were calculated. Table 2 presents the CVc obtained during different infusion periods before and after bleeding. These rather high CVc values point to a marked regional heterogeneity in myocardial blood flow, both during the normotensive and the hypotensive state. We observed a slight increase ($P < 0.05$) in variability after bleeding independent of the infusion duration (Table 2).

We also calculated the correlation coefficient for the paired perfusion values obtained at control and after bleeding based on the all individual samples. We found a positive correlation coefficient ranging from 0.61 to 0.76 indicating stability in regional perfusion. Also the paired regional blood flows

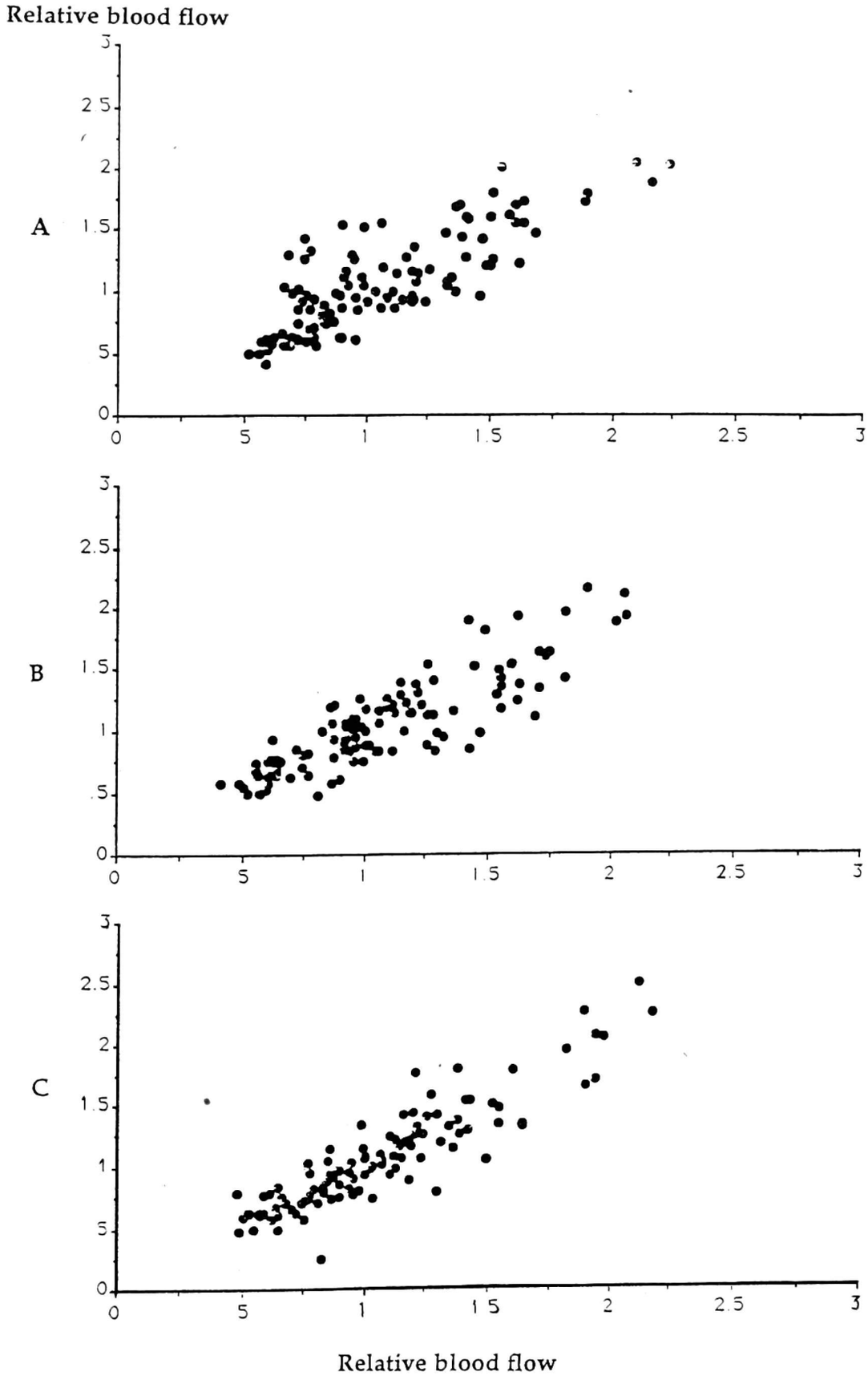

Figure 2. Scatterplots of paired values for regional myocardial relative blood flow from one rabbit. Each dot represents one myocardial region (n = 109).

A: Control vs. control (before bleeding); relative bood flow obtained with a 120-sec infusion period on the abscissa; relative blood flow obtained with a 30-sec infusion period on the ordinate. $\tau = 0.61$.

B: Control vs. hypotension; relative blood flow obtained during control with a 30-sec infusion period on the abscissa, relative blood flow (hypotension) obtained with a 120-sec infusion period on the ordinate. $\tau = 0.67$.

C: Hypotension vs. hypotension; relative blood flow obtained with a 30-sec infusion period on the abscissa, reative blood flow obtained with a 120-sec infusion period on the ordinate. $\tau = 0.73$.

in the experiments in which two microspheres infusions were given prior to bleeding were positively correlated (τ range: 0.68 to 0.73, Fig. 2).

The estimated myocardial blood flows to the left ventricle were 74.5 ± 9.6 and 84.2 ± 11.0 ml/100g × min in the control group and after haemorrhage, respectively ($P < 0.05$).

The experiment with microspheres simultaneously infused into the left ventricle and left atrium confirmed that infusion into the left ventricle is adequate and provide accurate and reliable measurments of regional myocardial blood flow. The correlation coefficient for paired values of regional blood flow based on microspheres infusions into the left atrium and into the left ventricle, was high ($\tau = 0.99$).

DISCUSION

Methological considerations

The microspheres as tracers for regional coronary blood flow determinations have been positively by several invesitigators (6,7). The left atrium has been most frequently used as the place to infuse microspheres, thereby ensuring sufficient mixing of microspheres both in the left atrium and in the left ventricle. However, infusing microspheres into the left ventricle requires less surgical manipulations and gives less cardiac distrurbances. In the rat, infusions of microspheres into the left ventricle have been reported to give reliable (13) but also unreliable data (14) for regional coronary perfusion. Our observation of a high correlation ($r = 0.99$) between paired values of myocardial blood flow (simultaneously infusions into the left atrium and into the left ventricle) support the usefulness of microsphere infusions into left ventricle for determination of regional coronary perfusion in the rabbit.

Regional myocardial blood flow

Changes in the myocardial blood flow as well as in the coronary vascular resistance after bleeding have been observed by many investigators (9—12).

In our experiments we noticed an increase of myocardial blood flow and a decrease in vascular resistance to about 62% of the control level. The discrepancies which have been reported (see Introduction) regarding the response of the coronary perfusion to severe bleeding, may be explained by species differences. It may also be due to technical difficulties (different techniques for blood flow determination) and also by anaesthesia which may distort the haemodynamic response to acute hypovolvemia, although the effect and its magnitude vary with the anaesthetic agent and species (9). Evidently, it seems to be important that these investigators (10—12) used different levels of hypotension.

Schadt et al. (9) suggested the pesence of two sets of cardiovascular sensors which may play an important role int he response to acute haemorrhagic hypotension: The baroreceptors and the cardiopulmonary receptors. They included not only the cardiac vegetative nervous system, but also circulating substances such as catecholamines, vaspressin and angiotensin. On the basis of the present data, we are not able to differentiate between nervous and hormonal regulatory mechanisms initiated by the bleeding. It is noteworthy that both Angelakos (15) and Verhoeven et al. (16) found a marked regional variation in distribution of catecholamines within the dog left ventricle during normotensive conditions.

We did not find any differences between the control and haemorrhagic condition with regard to the regional perfusion pattern. Neither did we obtain any differencies related to the infusion durations (30 versus 120 sec). Neither the quartile values for microsphere densities nor analysis of the coefficients of variation revealed any statistical difference between the control and bled condition. Even more, the obtained correlation coefficients for paired blood flow values either between the control and the haemorrhagic condition or between two control or two post bleeding infusions were high: 0.68, 0.69 and 0.72 (mean), respectively and pointed to a marked stability in regional coronary perfusion. We thus found that the marked disturbances in the neuro-endocrie system that occur in response to a heavy bleeding (9), did not cause any changes in the regional myocardial blood flow heterogeneity.

Our findings support the observations in dogs by Coggins et al. (8) who concluded that during reduced myocardial perfusion pressures, a marked spatial heterogeneity in blood flow persisted even though the endogenous blood flow reserve was exahausted in some myocardial regions. Thus, the marked regional heterogeneity in coronary blood flow during hypotension may cause local hypoxia and thereby hamper cardiac function even if local blood flow is unaltered.

Acknowledgements: The financial support from Langfeldts Fundation and Anders Jahres Fundation for he Promotion of Science is gratefully appreciated.

REFERENCES

1. Austin Jr, RA, Aldea GS, Coggins DL, Flynn AE. Hoffman IE. Profound spatial heterogeneity of coronary reserve. *Circ Res* 1990; 67:319—331.
2. Marcus ML, Kerber RE, Erhardt JC, Flasetti HL, Davis DM, Abboud FM. Spatial and temporal heterogeneity of the left ventricular perfusion in awake dogs. *Am Heart J* 1977; 94:748—754.
3. King RB, Bassingthwaighte JB, Hales JRS, Rowell LB. Stability of heterogeneity of myocardial blood flow in normal awake baboons. *Circ Res* 1985; 57:285—295.

4. Pendergast DR, Krasney JA, Ellis A McDonald B, Marconi C, Cerretelli P. Cardiac output and muscle blood flow in exercising dogs. *Respir Physiol* 1985; 61:317—326.
5. Iversen PO, Standa M and Nicolaysen G. Marked regional distribution of blood flow within a single skeletal muscle in the rabbit. *Acta Physiol Scand* 1989; 136:17—28.
6. Yipintsoi T, Dobbs WA Jr, Scanlon PD, Knopp TJ, Bassingthwaighte JB. Regional distribution of diffusible tracers and carbonized microspheres in the left ventricle of isolated dog hearts. *Circ Res* 1973; 33:573—587.
7. Bassingthwaghte JB, Malone MA, Moffett TC et al. Validity of microsphere depositions for regional myocardial flows. *Am J Physiol* 1987; 253:H184—H193.
8. Coggins DL, Flynn AE, Austin Jr RE et al. Nonuniform loss of regional flow reserve during myocardial ischemia in the dogs. *Circ Res* 1990; 67:253—264.
9. Schadt JC, Ludbrook J. Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammmals. *Am J Physiol* 1991; 260: H305—H318.
10. Vatner SF. Effects of hemorrhage on regional blood flow distribution in the dogs and primates. *J Clin Invest* 1974; 54:225—235.
11. Neutze JM, Wyler F, Rudolph AM. Changes in distribution of cardiac output after hemorrhage in rabbits. *Am J Physiol* 1968; 215:857—864.
12. Forsyth RP, Hoffbrand BJ, Melmon KL. Redistribution of cardiac output during hemorrhage in the unanesthetized monkey. *Circ Res* 1970; 27:311—320.
13. Schrock GD, Krahmer RL, Fergurson JL. Coronary flow by left atrial and left ventricular microspheres injection in the rat. *Am J Physiol* 1990; 259:H653—H638.
14. Wicker PA, Healy BP. Variability of coronary blood flow measurments with microspheres in the rat: role of injection site and sphere number. *Cardiovasc Res* 1989; 23:443—452.
15. Angelakos ET. Regional distribution of catecholamines in the dog heart. *Circ Res* 1965; 16:39—44.
16. Verhoeven L, Gerlock T, Weiss HR. Relationship between myocardial tissue norepinephrine and coronary flow heterogeneity. *Microvasc Res* 1991; 41:289—298.

R e c e i v e d: December 30, 1991
A c c e p t e d: May 18, 1992

Authors' address: M. Standa, Department of Physiology, School of Medicine, ul. Chałubińskiego 10, 50—368 Wrocław, Poland.