Biol Res 28: 165-171 (1995)

Relative participation of adenosine and endothelium derived mediators in coronary reactive hyperemia in the dog

PILAR MACHO, RAUL DOMENECH
and the late MARIO PENNA

Departamento de Medicina Experimental, División Oriente,
Facultad de Medicina, Universidad de Chile, Santiago, Chile

The metabolites that mediate coronary reactive hyperemia have not been definitely identified. Although adenosine and endothelium derived substances seem to be involved, their relative contributions have not been defined yet. In the canine coronary circulation, we studied the relative participation of adenosine, nitric oxide and prostacyclin in reactive hyperemia, by measuring the changes produced by interfering with the synthesis or action of these metabolites. The dose-response curve for flow changes vs intracoronary administration of adenosine was displaced to the right after the inhibition of nitric oxide synthesis with N-omega-nitro-L-arginine, revealing that nitric oxide release partly mediates the vasodilator action of adenosine. The inhibition of PGI-2 synthesis with indomethacin did not modify reactive hyperemia. Interference with adenosine action, by administration of adenosine deaminase plus theophylline, decreased reactive hyperemia by 31.0 ± 4.0% ($p < 0.001$). Inhibition of nitric oxide synthesis decreased reactive hyperemia by a larger ($p < 0.005$) magnitude, 41.0 ± 3.9% ($p < 0.001$), revealing the existence of other stimuli for nitric oxide release in reactive hyperemia besides adenosine. Simultaneous inhibition of nitric oxide and PGI-2 syntheses and of adenosine action reduced reactive hyperemia, but the effect was not additive, reaching 49.5 ± 4.5% of control. Since nitric oxide and adenosine are the most important mediators in reactive hyperemia so far described, our results suggest that other metabolites, acting directly or through mediators other than adenosine or nitric oxide, are responsible for about 50% of coronary reactive hyperemia.

Key terms: *adenosine, coronary vessels, endothelium, nitric oxide, N-omega-nitro-L-arginine, prostacyclin, reactive hyperemia.*

INTRODUCTION

Myocardial reactive hyperemia is a well known vasodilator response to a brief occlusion of a coronary artery that allows for the "payment" of the flow "debt" established during the occlusion. It is generally considered as manifestation of a local metabolic process that matches flow to the metabolic demand of the myocardium (Rubio *et al*, 1969; Olsson *et al*, 1978). Many substances have been proposed as mediators. Adenosine, in particular, seems to be important in this regard (Saito *et al*, 1981), but it does not seem to be the only one mediator. It is not clear either whether adenosine vasodilator effect during the hyperemic period is a direct one or is in turn mediated by other

Correspondence to: Pilar Macho, Departamento de Medicina Experimental, División Oriente, Facultad de Medicina, Universidad de Chile, Casilla 16038, Santiago 9, Chile. Fax: (56-2)-274-1628.

substances, as for example those produced in the endothelium, particularly nitric oxide (NO) (Nees *et al*, 1987). The present study, performed in the canine coronary circulation, was intended to analyze the relative participation of adenosine and the most important vasodilator substances so far described as released by the endothelium, *i.e.* prostacyclin and NO. In addition, we analyzed the participation of NO as mediator of adenosine effect.

METHODS

Mongrel domestic dogs, weighing between 15 and 20 kg, were anesthetized with sodium pentobarbitone 30 mg/kg iv. The lungs were mechanically ventilated through an endotracheal tube and the thorax opened through the fifth left intercostal space. Blood gases and pH were monitored (Radiometer BMS 3Mk2 blood microsystem and Radiometer OSM2 hemoxymeter) and kept within constant values during the experiments.

Aortic pressure and heart rate were kept constant, to avoid changes in the oxygen consumption of the heart that may influence coronary vascular resistance. Aortic pressure was maintained at 100 Torr by connecting the systemic circulation to a large reservoir with fresh heparinized homologous blood, thermoregulated to 37° C and leveled to the desired pressure. Heart rate was kept constant at 120 cycles/min by blocking atrioventricular conduction (Steiner and Kovalik, 1968) and electrically stimulating the outflow tract of the right ventricle. Pressures in the root of the aorta and within the left ventricle were measured through catheters connected to extracorporeal electronic transducers (Statham P23 Db). Coronary blood flow was measured with an electromagnetic flow transducer, placed proximally on the circumflex coronary artery, and connected to a Nihon Kohden flowmeter. A thin catheter was implanted into the circumflex artery, distal to the flow probe, to administer drugs. Coronary flow, aortic and ventricular pressures were recorded on a Nihon Kohden physiograph.

Reactive hyperemia was induced by occluding the circumflex coronary artery during 30 s with an arterial clamp between the flow probe and the intraarterial catheter. Reactive hyperemia was expressed as the repayment ratio, *i.e.*, the ratio of the blood volume in excess of control during hyperemia (repayment volume) to the blood volume that did not enter the coronary artery during the occlusion (debt volume). Repayment volume was calculated by time integration of flow in excess of control during hyperemia; debt volume, by the product of control coronary flow times the occlusion period (Olsson, 1975). Since aortic pressure was kept constant during the experimental procedure, changes in flow represent reciprocal changes in coronary vascular resistance.

The repayment ratio to 30 s coronary occlusion was measured before and after performing the following maneuvers:

1. Inhibition of prostacyclin synthesis by iv administration of indomethacin (7 mg/kg), performed in 11 experiments in 11 dogs.
2. Inhibition of adenosine action by simultaneous administration of theophylline (5 mg/kg, iv) to block adenosine receptors, and adenosine deaminase (4.5 μg/kg/min, intracoronary infusion) to inactivate adenosine. This was performed in 10 experiments in other 10 dogs.
3. Inhibition of NO synthesis by iv administration of N-omega-nitro-L-arginine (NONLA) (15 mg/kg), performed in 12 experiments in other 12 dogs.
4. Simultaneous performance of the three maneuvers mentioned above, in 13 experiments in other 13 dogs.

The capacity of the endothelium to synthesize NO and the endothelial indemnity were tested by measuring the changes in coronary flow produced by the intracoronary administration of a bolus of acetylcholine (5.5 nmol/kg), diluted in saline in a volume of 0.5 ml. This was performed in all animals before and after any of the blockades that we made. The vascular smooth muscle indemnity was assessed by the change in coronary flow to the intracoronary infusion of sodium nitroprusside (2 μg/kg/min for 5 min) before and after the administration of any blocker.

To test the hypothesis that the vasodilator effect of adenosine is mediated partly through NO release, in 7 additional dogs, we obtained dose-response curves of coronary flow *vs* adenosine, by intracoronary infusion of adenosine at increasing concentrations from 18 to 190 μg/min, delivered at constant infusion flows of 0.3 ml/min. The infusion for each dose was maintained up to the appearance of a plateau, which occurred within 5 min of starting the infusion of any dose. This infusion was performed before and after blocking NO synthesis with NONLA. Although the maximal vasodilator effect of adenosine may manifest after 20-45 min of infusion (L'Abbate *et al*, 1981), we consider unnecessary to compute this delayed response for the purpose of analyzing the effect of NO synthesis inhibition on the vasodilator effect of adenosine during a short period of time, as reactive hyperemia is. Accordingly, the vasodilator response was computed after only 5 min of adenosine infusion, a time at which it has attained almost a plateau at any infusion dose.

Adenosine, acetylcholine chloride, adenosine deaminase from calf intestinal mucosa, theophylline, indomethacin and N-omega-nitro-L-arginine were obtained from Sigma Chemical Co. Sodium nitroprusside was obtained from F Hoffmann-La Roche, SA.

Results were expressed as means ± SEMs, and absolute changes were analyzed by paired Student's *t* tests or by one way analysis of variance (ANOVA) for multiple comparisons of means, followed by Bonferroni's *t* test, whichever appropriate (Zar, 1984).

This investigation conforms to the Guiding Principles in the Care and Use of Laboratory Animals, endorsed by the American Physiological Society.

RESULTS

The design of the present experiments prevented changes in heart rate and aortic pressure during the different maneuvers performed. Therefore, the changes in coronary flow observed during reactive hyperemia correspond to reciprocal changes in coronary vascular resistance, and were not influenced by modifications of the mechanical variables that determine myocardial metabolism.

The basal repayment flow ratios were 2.95 ± 0.23 before indomethacin, 3.00 ± 0.17 before interference with adenosine action, 2.99 ± 0.29 before inhibiting NO synthesis and 2.86 ± 0.29 before performing all the above maneuvers together.

Figure 1 shows the effects of the different maneuvers on the repayment ratio. The inhibition of the synthesis of prostacyclin did not modify basal flow, resistance nor the repayment ratio. The interference of adenosine action, by administration of adenosine deaminase plus theophylline, did not change basal flow or resistance, but decreased by 31.0 ± 4.0% the repayment ratio ($p < 0.001$). After the administration of NONLA, the vasodilator response to acetylcholine was decreased by at least 90%, the control coronary vascular resistance increased by 26 ± 6%, but the vasodilator response to nitroprusside did not change. Therefore, NO synthesis was almost completely abolished, but the vascular smooth muscle response was preserved. The inhibition of NO synthesis with NONLA decreased by 41.0 ± 3.9% ($p < 0.001$) the repayment ratio, an effect larger than that produced by interference with adenosine action ($p < 0.02$). Simultaneous interference with adenosine action plus inhibition of NO and prostacyclin syntheses produced an apparent larger decrease of the repayment ratio (49.5 ± 4.5%) than that produced by the sole inhibition of NO synthesis, but the difference between both conditions was not statistically significant.

Figure 2 shows the effect of NONLA on the coronary flow response to the intracoronary infusion of increasing doses of adenosine in 7 dogs. NONLA displaced the dose-response curve to the right (ANOVA, $p < 0.05$), decreasing flow changes by 40.4 ± 2.8% for the different doses of adenosine.

DISCUSSION

According to our results, the reactive hyperemic response of the canine coronary

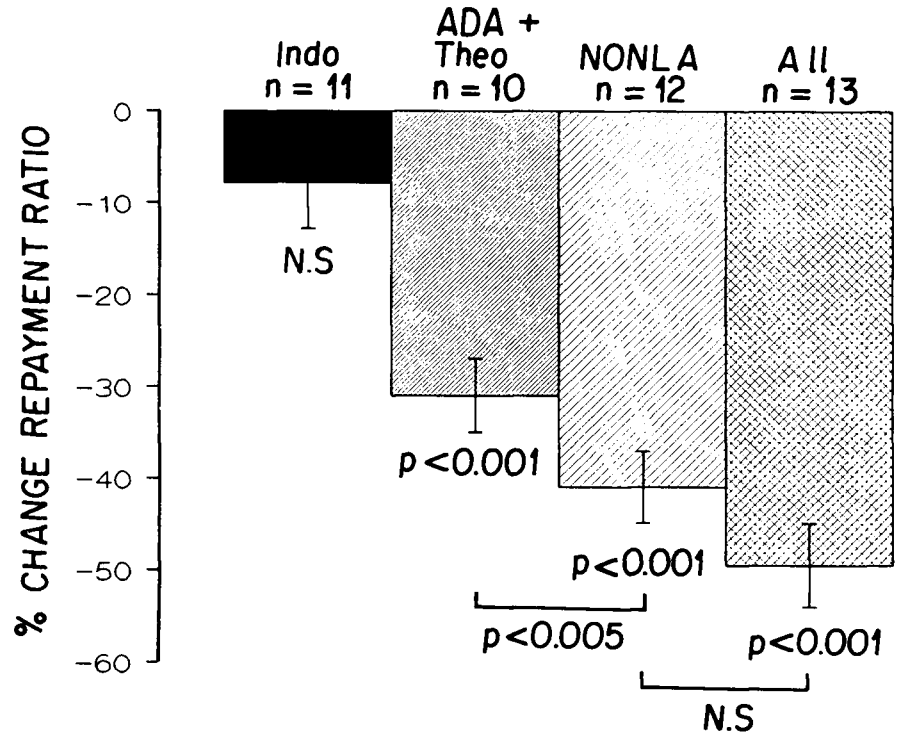

Fig 1. Effects of different inhibitors on reactive hyperemia, assessed by the repayment ratio (see text). Indomethacin (Indo), adenosine deaminase plus theophylline (ADA + Theo), N-omega-nitro-L-arginine (NONLA) and all inhibitors given simultaneously (All). Bars represent changes in percentage (means ± SEM's).

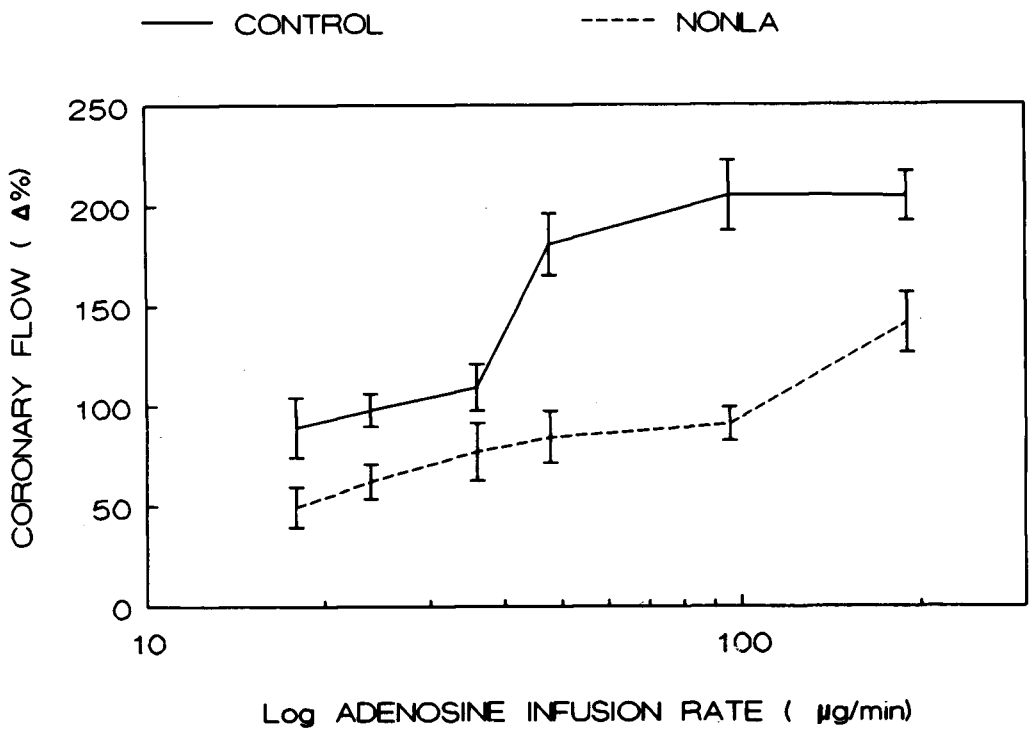

Fig 2. Changes in percentage of coronary blood flow induced by increasing doses of adenosine, administered before and during NO inhibition by N-omega-nitro-L-arginine (NONLA). Dots and vertical lines represents means ± SEM's obtained in 7 experiments performed in 7 dogs.

vessels is mediated to a large extent by adenosine and NO, with no significant participation of prostacyclin. This lack of participation of prostacyclin in reactive hyperemia in our experiments agrees with the results of other authors (Hintze and Kaley, 1977), that reported no changes in the repayment ratio of the canine coronary circulation after inhibition of prostaglandin synthesis with indomethacin or meclofenamate. On the other hand, this is at variance with the reduction of reactive hyperemia observed in rabbit skeletal muscle (Koller and Kaley, 1990) and in rat cremaster muscle (Messina *et al*, 1977) after inhibition of prostaglandin synthesis. Thus, Messina *et al* (1977) found that cyclooxygenase blockade inhibits by 50 to 52% the reactive hyperemia to 15-60 s arteriole occlusion of the rat cremaster muscle. Besides, this effect was not affected by inhibition of NO synthesis (Wolin *et al*, 1990). Differences in species and vascular beds probably account for these differences in results.

It has been shown that adenosine is a mediator in reactive hyperemia (Rubio *et al*, 1969; Olsson *et al*, 1978; Saito *et al*, 1981). Saito *et al* (1981) showed that the intracoronary administration of adenosine deaminase at the same infusion rate that we used reduces the repayment ratio by 30-39%, a result of similar magnitude to ours. This infusion rate produces a concentration of the enzyme in myocardial interstitium large enough to deaminase the adenosine formed during the ischemic interval to levels below the threshold for coronary vasoactivity, which is probably negligible during the enzyme infusion (Saito *et al*, 1981). Therefore, adenosine is an important mediator in reactive hyperemia, but only accounts for less than 50% of the response. This is in agreement with the quantitative test for the participation of adenosine in coronary reactive hyperemia in the dog, reported by Olsson *et al* (1978).

The inhibition of NO synthesis decreased the repayment ratio by a larger magnitude than that obtained by preventing the adenosine effect. Since coronary blood flow decreased by 26% with NO synthesis blockade, reactive hyperemias performed after the blockade started from a basal flow lower than those performed before the blockade. This should increase the percent changes in flow ratio if the inhibition of NO synthesis would had no effect in reactive hyperemia (Ward *et al*, 1993) since the coronary reserve for dilation increases when starting from a constricted vessel. However our results show that reactive hyperemia was decreased by effect of this blockade and, therefore, the effect of NO synthesis inhibition is presumably underestimated.

With regard to the inhibition of NO synthesis, our results are similar to those obtained by Kostic and Schrader (1992) in the Guinea pig heart and by us in previous experiments performed in the dog heart (Domenech *et al*, 1993). These experiments demonstrate an important participation of NO in reactive hyperemia. The decrease in the flow response to intracoronary infusion of adenosine when NO synthesis was inhibited, as we observed, supports the hypothesis that, at least in part, the release of NO is mediated by activation of adenosine receptors in the endothelium (Nees *et al*, 1987). If all the NO released were due to the activation of endothelial receptors by adenosine, and adenosine produced vasodilation by this indirect mechanism plus a direct effect on smooth muscle, then the inhibition of NO synthesis should have produced a smaller decrease in reactive hyperemia than the inhibition of adenosine action. However, according to our results, the inhibition of NO synthesis produced a larger decrease of reactive hyperemia, suggesting that NO release is triggered by other stimuli besides adenosine. These other stimuli may be related to myocardial metabolic alteration during the ischemic period as, for example, liberation of ATP from myocardial cells (Borst and Schrader, 1981) or other yet undetermined metabolites. However, the possibility also exists that NO release could be mediated by hemodynamic changes in the luminal surface of the vessels at the time of occlusion release, such as the sudden increase in wall shear and normal stresses secondary to the reestablishment of flow and pressure, respectively (Kuo *et al*, 1990; La Montagne *et al*, 1992). If this should be true, then part of the hyperemic response could be

mediated by a mechanism not related to the supply/demand ratio for oxygen of the myocardial cell. Such a response could explain the "overpayment" of the flow debt observed in reactive hyperemia as revealed by the maintenance of a higher than normal coronary venous oxygen saturation during the hyperemia, while oxygen consumption has already returned to its control value (Ruiter *et al*, 1977). This peculiar behavior of reactive hyperemia is in opposition to the observation that during coronary autoregulation, a process related to the myocardial supply/demand ratio, flow is inversely related to venous coronary oxygen partial pressure (Dole and Nuno, 1986).

An alternative explanation for the greater decrease in reactive hyperemia produced by inhibiting NO synthesis, as compared to interference with adenosine action, is NO overproduction when adenosine synthesis is inhibited. This hypothesis is suggested by the results of Kostic and Schrader (1992), who showed that the inhibition of NO synthesis enhances the production of adenosine. The opposite effect has not been studied, but is feasible that it could occur, because a balance between several vasoactive substances has been postulated in the metabolic regulation of coronary flow (Berne, 1964).

If the direct vasodilation produced by adenosine (not mediated through NO) were of significant magnitude, then the simultaneous interference with adenosine action and inhibition of NO synthesis should have produced a larger effect than the sole blockade of NO synthesis. However, the effect was similar in both circumstances. This could be interpreted as if most of the vasodilator effect of adenosine during reactive hyperemia would be mediated by NO release. Nevertheless, since the simultaneous blockade of the actions of the three substances tested did not decrease reactive hyperemia by more than 50%, additional metabolites should mediate this reaction and a change in the production of these mediators may have occurred after simultaneous inhibition of NO and prostacyclin syntheses and adenosine action. Such an effect would underestimate the absolute and relative participations of NO and adenosine. This assumed but probable effect, suggested by the experiments of Kostic and Schrader (1992), as explained above, makes it difficult to reach a quantitative conclusion. Nevertheless, it seems that NO release plays a substantial role in reactive hyperemia. In this regard, Chlopicki and Gryglewski (1993) showed that NO is the major mediator in reactive hyperemia evoked by brief (1-5 s) coronary occlusions in the Guinea pig heart, accounting for 91 to 100% of the response observed. However, the participation of adenosine as a possible activator of NO release was not studied by these authors (Chlopicki and Gryglewski, 1993).

We can not assess, with the present results, the participation of a myogenic response in our experiments. However, the participation of this mechanism in coronary reactive hyperemia is limited to the first 600 ms of the response after the occlusion (Dubé *et al*, 1991) and presumably the reverse effect may occur during the first milliseconds after releasing the occlusion. This, perhaps, would limit the maximal vasodilation attained during hyperemia. Furthermore, a probable mediation of the endothelium in the myogenic response is not definitely established (Kaley *et al*, 1991; Van Bavel *et al*, 1991).

In conclusion, our results reveal that about 50% of the reactive hyperemic response of the canine myocardium is mediated by adenosine and NO. Nitric oxide is released in part by adenosine and in part by other mechanisms not yet defined. Prostacyclin does not seem to participate in this response. However there still remains a large fraction of the reactive hyperemic response to be explained. The activation of unknown metabolites mediating this fraction, during the pharmacological inhibition of the known ones, may in part compensate the hyperemic response underestimating the effect of the known mediators.

ACKNOWLEDGEMENTS

This work was supported by grant 1940296 from FONDECYT and grant M-3229 from DTI, Universidad de Chile. We gratefully acknowledge the skillful technical assistance of Mr Juan-Carlos Fuenzalida.

REFERENCES

BORST MM, SCHRADER J (1981) Adenine nucleotide release from isolated perfused guinea pig hearts and extracellular formation of adenosine. Circ Res 68: 797-806

BERNE RM (1964) Regulation of coronary flow. Physiol Rev 44: 1-29

CHLOPICKI S, GRYGLEWSKI RJ (1993) Nitric oxide is a major mediator in reactive hyperaemia evoked by a brief coronary occlusion in the guinea pig heart. Eur J Pharmacol 241: 117-120

DOLE WP, NUNO DW (1986) Myocardial oxygen tension determines the degree and pressure range of coronary autoregulation. Circ Res 59: 202-215

DOMENECH RJ, MACHO P, PENNA M, HUIDOBRO-TORO JP, THUMALA A (1993) Role of endothelium-derived relaxing factor on coronary blood flow regulation in the dog. Eur J Pharmacol 238: 53-58

DUBE GP, BEMIS KG, GREENFIELD JC (1991) Distinction between metabolic and myogenic mechanisms of coronary hyperemic response to brief diastolic occlusion. Circ Res 68: 1313-1321

HINTZE TH, KALEY G (1977) Prostaglandins and the control of blood flow in the canine myocardium. Circ Res 40: 313-320

KALEY G, KOLLER A, MESSINA EJ, WOLIN MS (1991) Role of endothelium-derived vasoactive factors in the control of the microcirculation. In: RUBANYI GM (ed) Cardiovascular Significance of Endothelium-Derived Vasoactive Factors. New York: Futura Publishing Co. pp 179-195

KOLLER A, KALEY G (1990) Role of endothelium in reactive dilation of skeletal muscle arterioles. Am J Physiol 259: H1313-H1316

KOSTIC MM, SCHRADER J (1992) Role of nitric oxide in reactive hyperemia of guinea pig heart. Circ Res 70: 208-212

KUO L, DAVIS J, CHILIAN WM (1990) Endothelium-dependent flow induced dilatation of isolated coronary arterioles. Am J Physiol 28: H1063-H1070

L'ABBATE AP, CAMICI P, TRIVELLA MG, PELOSI G, DAVIES GJ, BALLESTRA AM (1981) Time dependent response of coronary flow to prolonged adenosine infusion: doubling of peak reactive hyperaemic flow. Cardiovasc Res 15: 282-286

LA MONTAGNE D, POHL V, BUSSE R (1992) Mechanical deformation of vessel wall and shear stress determining the basal release of endothelium -derived relaxing factor in the intact coronary vascular bed. Circ Res 70: 123-130

MESSINA EJ, WEINER R, KALEY G (1977) Arteriolar reactive hyperemia: Modification by inhibitors of prostaglandin synthesis. Am J Physiol 232: H571-H575

NEES S, DES ROSIERS C, BÖCK M (1987) Adenosine receptors at the coronary endothelium: functional implications. In: GERLACH E, BECKER BF (eds) Topics and Perspectives in Adenosine Research. Berlin, Heidelberg: Springer-Verlag. pp 454-467

OLSSON RA (1975) Myocardial reactive hyperemia. Circ Res 37:263-270

OLSSON RA, SNOW JA, GENTORY MK (1978) Adenosine metabolism in canine coronary reactive hyperemia. Circ Res 42: 358-363

RUBIO R, BERNE RM, KATORI M (1969) Release of adenosine in reactive hyperemia of the dog heart. Am J Physiol 216: 56-62

RUITER JH, SPAAN JAE, LAIR JD (1977) Transient oxygen uptake during myocardial reactive hyperemia in the dog. Am J Physiol 232: H437-H440

SAITO D, STEINHART CR, NIXON DG, OLSSON RA (1981) Intracoronary adenosine deaminase reduces myocardial reactive hyperemia. Circ Res 49: 1262-1267

STEINER C, KOVALIC ATW (1968) A simple technique for production of chronic complete heart block in dogs. J Appl Physiol 25:631-633

VAN BAVEL E, GIEZEMAN MJMM, SPAAN JAE (1991) Arteriolar mechanics and control of flow. In: SPAAN JAE (ed) Coronary Blood Flow Mechanics, Distribution and Control. The Netherlands: Klawer Academic Publishers. pp 193-218

WARD ME, MAGDER SA, HUSSAIN SNA (1993) Role of endothelium-derived relaxing factor in reactive hyperemia in canine diaphragm. J Appl Physiol 74: 1606-1612

WOLIN MS, RODENBURG JM, MESSINA EJ, KALEY G (1990) Similarities in the pharmacological modulation to H_2O_2 in rat skeletal muscle arterioles: Effects of probes for endothelium-derived mediators. J Pharmacol Exp Ther 253: 508-512

ZAR JH (1984) Biostatistical Analysis. New Jersey: Prentice Hall.