the free energy change of ATP hydrolysis under these circumstances (about 20%) should be taken into account also. This would minimize the difference between the two percentages, suggesting that the economics of force production may in fact be quite similar.

In the muscles stimulated at 1.0 Hz, resting force rose towards the end of the anoxic period to 19% of the peak force produced in oxygen at 0.2 Hz stimulation. To compare the energetic cost of the production of resting force with that of twitch force the use of mean force instead of developed force is more convenient. If we take twitch duration at 0.2 Hz to be 2 s, average force at 0.2 Hz is about 20% of peak force. The ATP turnover at the end of the 40-min period in muscles stimulated at 1.0 Hz is very low, 1.3% of that in the oxygenated muscles stimulated at 0.2 Hz. This indicates that in the anoxic 1.0 Hz-stimulated muscles the rise of passive force to 19% of the twitch force produced in oxygen does indeed require little energy, which suggests that cross-bridge cycling and calcium cycling occur at a very low rate.

In conclusion this study shows that, in anoxia, when ATP formation is limited the energy demand is still an important determinant of the rate of ATP hydrolysis. Furthermore, it is found that the economics of force production does not change very much when twitch force drops to a low level in anoxia. However, the increase in resting force is not accompanied by an increase in ATP turnover and occurs when the rate of ATP hydrolysis is very low. This suggests that under these conditions force production is related to the formation of rigor bridges.

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Adenosine is a sensitive oxygen sensor in the heart

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Summary. Cardiac adenosine is formed both by an oxygen-sensitive (AMP→adenosine) and by an oxygen-insensitive (S-adenosylhomocysteine → adenosine) pathway. The phasic adenosine release during β-adrenergic stimulation with isoproterenol is closely linked to coronary venous P_{O_2} (isolated heart) and can be almost fully prevented when diastolic aortic pressure is maintained constant (heart in situ). During pressure autoregulation the transmural gradient of free adenosine is only increased when the autoregulatory reserve is exhausted. The critical P_{O_2} below which adenosine formation is enhanced was found to be 3 mm Hg (isolated cardiomyocytes). Collectively, these data indicate that the formation of adenosine is not primarily coupled to the energy expenditure of the heart but to the supply/demand ratio for oxygen.

Key words. Coronary blood flow; energy metabolism; hypoxia, P_{O_2}; β-adrenergic stimulation; autoregulation; S-adenosylhomocysteine.

Regulation of coronary flow is in essence the regulation of cardiac energy metabolism. Taking a purely regulatory point of view, two principal alternatives by which coronary flow may be adjusted to cardiac metabolic demand are conceivable:

A) Any mismatch between oxygen supply and oxygen demand is linked to the production of vasodilatory metabolites. Such a P_{O_2}-dependent metabolite may be adenosine, a degradative product of ATP which is thought to increase coronary flow and to bring the oxygen supply/demand ratio back to a new equilibrium[3]. Essential features of this model are:

1) the vasodilator concentration inversely reflects tissue oxygen tension and 2) at least a minor degree of hypoxia is required. This local vascular control is not perfect so

that some error signal (adenosine) accumulates in proportion to the diminished tissue oxygen tension.

B) A stoichiometry exists between turnover of ATP as reflected by cardiac oxygen consumption and the formation of a vasodilator[9]. In the case of adenosine this could involve a hypothetical metabolic sequence: cyclic AMP produced for example, in response to β-adrenergic stimulation, is not only responsible for the stimulation of cardiac mechanical work but is also, via AMP, a substrate for adenosine formation[2].

For the discussion to follow it is important to note that model A is a feed-back controlled system whereas model B can be described as a feed-forward regulation. In a feed-back controlled system any increase in oxygen supply via the coronary circulation should diminish the production of vasoactive adenosine. This is not the case when model B applies.

In the past, our laboratory has provided several lines of evidence that tissue oxygenation is the major determinant for the production of adenosine by the heart[1,2,6]. The initial observation was made in the isolated perfused guinea pig heart in which an increase in coronary perfusion pressure (increased oxygen supply) substantially increased the adenosine production following β-adrenergic stimulation[2]. The phasic release of adenosine observed under these conditions[16] is closely linked to coronary venous P_{O_2} (unpublished observation). Similarly, when cardiac work was increased by changes in afterload, the increase in oxygen consumption and coronary flow was not associated with an enhanced adenosine production[1].

It might be argued that the clear dissociation between adenosine formation and cardiac energetics might be due to borderline oxygenation of the isolated heart perfused with a saline medium of low oxygen carrying capacity. We therefore reinvestigated this question recently using a blood-perfused dog heart in situ. As in the isolated heart, coronary adenosine release was phasic with β-adrenergic stimulation, and was not linked to myocardial oxygen consumption in the hemodynamic steady state. More importantly, however, prevention of the isoproterenol-induced fall in diastolic aortic pressure almost fully prevented cardiac release of adenosine, while myocardial oxygen consumption was even further enhanced[8]. The implication of these latter findings is that metabolic regulation of coronary flow by adenosine requires changes in tissue P_{O_2} provided adenosine plays an important regulatory role. On the other hand these findings substantiate the view that adenosine is a sensitive oxygen sensor in the heart.

Within the cardiomyocyte there are two major metabolic reactions which participate in the formation of adenosine. One is the transmethylation pathway (fig. 1) which is an essentially oxygen-insensitive metabolic route[7,10]. The pathway via AMP, however, is highly oxygen-sensitive. Formation of adenosine by this latter

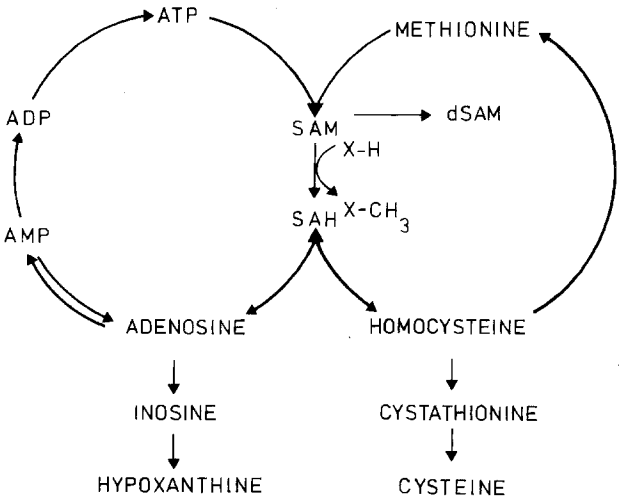

Figure 1. Schematic outline of the principal pathways involved in the metabolism of adenosine.

pathway can be explained by either an elevated substrate concentration of AMP, or deinhibition of cytosolic 5'-nucleotidase, or both[11]. Comparing the tissue content of high-energy phosphates including adenosine with the degree of tissue oxygenation it is important to recall that the free cytosolic concentration of most purine compounds is much lower than the measured total tissue content[4,5]. In the table, data on the tissue content of ATP, ADP, AMP and adenosine are compiled together with the calculated free cytosolic concentration of each purine compound. With the exception of ATP the free concentrations of ADP, AMP and adenosine are considerably lower than the total tissue measurements. This is due to intracellular protein binding, presumably of actin in the case of ADP, and S-adenosylhomocysteine-hydrolase in the case of adenosine[5,15]. One important consequence of adenosine being protein-bound is that changes in the concentration of free adenosine are likely to be greatly underestimated by measurements of the total tissue content of this purine.

In order to evaluate further the relationship between adenosine and tissue oxygenation we have recently elaborated a new method that permits the measurement of free cytosolic adenosine in cardiac tissue[5,6]. This method is based on the kinetic properties of SAH-hydrolase catalyzing the reversible reaction: (S-adenosyl-homocysteine (SAH)⇄adenosine + L-homocysteine. In the well-

Bound and free levels of various purine compounds in the guinea pig heart

	Tissue content (nmoles/g)	Cytosol. concentration (μM)
ATP	4540	6450 +
ADP	920	22 +
AMP	120	0.08 +
Adenosine	2	0.08 *

+ Bünger and Soboll[4], * Deussen et al.[5]

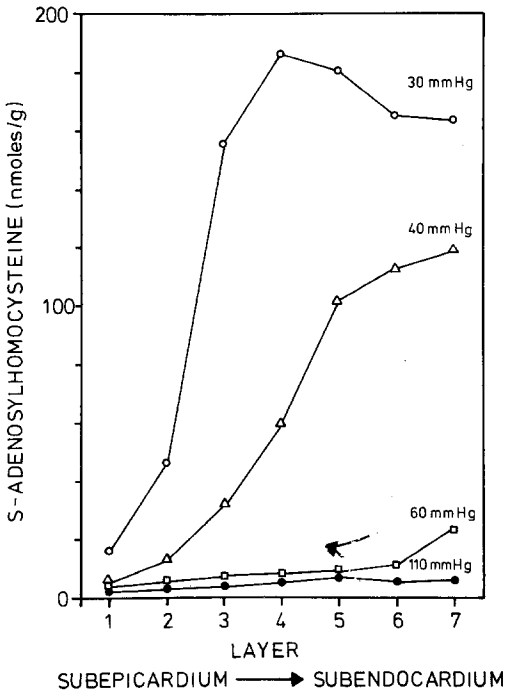

Figure 2. Transmural gradient of SAH, an index of free cytosolic adenosine, at different coronary perfusion pressures[6].

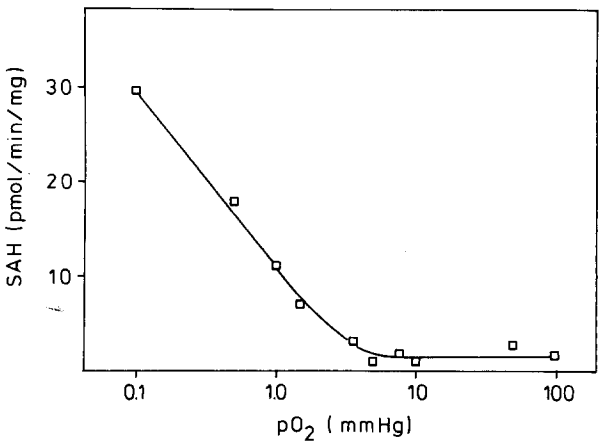

Figure 3. Relationship between SAH-content of isolated rat cardiomyocytes and medium P_{O_2} maintained constant with an oxystat system. HEPES-buffered medium contained 1 mM $CaCl_2$, 2 mM pyruvate, 5.5 mM deoxyglucose, 0.2 mM l-homocysteine thiolactone, 5μM EHNA and 2% albumin (pH 7.3), time of incubation: 30 min.

oxygenated heart this reaction proceeds in the hydrolytic direction because the concentration of adenosine and L-homocysteine is only low due to rapid metabolism. However, in the presence of an elevated concentration of L-homocysteine this reaction is reversed and SAH is formed. In the presence of a saturating concentration of L-homocysteine (200 μM) the rate of conversion to SAH only depends on the free cytosolic concentration of adenosine. SAH is not further metabolized and also does not penetrate cellular membranes. Thus, the rate of SAH-formation directly reflects free tissue adenosine.

Using the SAH-technique for measuring cardiac adenosine we have evaluated the relationship between a reduced coronary perfusion pressure and the transmural level of adenosine. As shown in figure 2, transmural SAH levels remained fairly unaltered in the pressure range of 110–60 mm Hg. This is the well-known autoregulatory range in which coronary resistance is altered to maintain coronary blood flow constant despite changes in perfusion pressure. Only the subendocardial SAH slightly increased when perfusion pressure was maintained at 60 mm Hg. Further lowering of the perfusion pressure to 40 and 35 mm Hg considerably elevated the subendocardial SAH and steepened the transmural gradient. These changes are most pronounced at a perfusion pressure of 35 mm Hg. These data clearly demonstrate that adenosine is only formed by the heart at an accelerated rate when the autoregulatory reserve is exhausted and the oxygen supply becomes limiting.

What is the degree of coronary underperfusion or the critical P_{O_2} which must be reached in order to compromise cardiac energy metabolism and to elicit the formation of a vasodilatory metabolite such as adenosine? We have recently studied this question using the isolated cardiomyocyte as a model. Metabolically stable cardiomyocytes from the rat were incubated in a gas-tight incubation chamber which allowed the ambient P_{O_2} to be maintained constant at any preselected value[12]. This was achieved by a computer-controlled feed-back loop by which an oxygen-containing medium was infused into the incubation vessel in amounts matching cellular oxygen consumption, thereby maintaining P_{O_2} constant. In experiments similar to those shown in figure 2 we measured changes in adenosine production via the accumulation of SAH. From the data given in figure 2 it can be seen that in the P_{O_2}-range from 100 mm Hg to about 3 mm Hg basal SAH production is unaltered. Below 3 mm Hg SAH progressively increased, reflecting changes in the free adenosine concentration. Microelectrode measurements of tissue P_{O_2} carried out some years ago in blood-perfused dog hearts suggest that the frequency distribution of P_{O_2} is such that values between 0–5 mm Hg occur most frequently[14].

Taken together, our findings provide evidence that energy expenditure as such is not the critical parameter which links production of adenosine to cardiac energy metabolism. Formation of adenosine is closely coupled with tissue oxygenation. A mismatch between oxygen supply and demand is the major stimulus. Thus, adenosine may be useful in future studies as a sensitive metabolic marker sensing impaired tissue oxygenation.

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Endothelial cells as part of a vascular oxygen-sensing system: Hypoxia-induced release of autacoids

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Summary. Higher developed organisms are equipped with many central and local control mechanisms, which enable an adequate blood and oxygen supply to tissues over a wide range of demands. Global adaptive responses include changes in the circulatory and ventilatory system as well as increases in the oxygen carrying capacity of the blood. At the level of the specialized organs there exist additional control systems for the regulation of local blood flow. Most systems make use of highly specialized cells which are able to sense the oxygen partial pressure of the transport medium, blood, and within the tissues. In the past years, it has been shown that the vascular endothelium lining the entire circulatory system can actively modulate the vascular tone and platelet functions by the release of autacoids, among them prostacyclin and endothelium-derived nitric oxide (EDRF). Recent experiments demonstrate that the release of EDRF is P_{O_2}-dependent, which suggests that endothelial cells may act as functional local oxygen sensors within the vascular system.
Key words. Prostacyclin; EDRF; P_{O_2}; calcium; vascular oxygen sensitivity.

Introduction

It is of vital importance for the integrity and the function of cells that they receive a continuous supply of oxygen. It is therefore not surprising that the circulatory system makes use of various central and local control mechanisms which enable an adequate tissue oxygen supply to be maintained over a wide range of oxygen demands. In general, an increased oxygen consumption of organs, e.g. during exercise, as well as a reduced oxygen content of the transport medium, blood, leads by local vasodilation to an increase in regional blood flow. This vasomotor response is often combined with an increase in cardiac output and an augmented vascular resistance in other organs, which results in a redistribution of blood flow towards the hypoxic organ. This coordinated response to hypoxia reflects the function of effective mechanisms controlling tissue oxygen supply. An integral part of these control systems must be cells which directly or indirectly sense changes of oxygen tension of the blood or within the tissue and respond by the generation of signals which induce central or local responses of the circulatory system.