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REVIEW ARTICLE

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Metabolic flux rates of adenosine in the heart

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Abstract The quantitatively most important source of adenosine under well-oxygenated conditions is 5′-AMP hydrolyzed by cytosolic 5′-nucleotidase N-I. Hydrolysis of *S*-adenosylhomocysteine and extracellular dephosphorylation of 5′-AMP further contribute to total production. More than 90% of the total production occur intracellularly under well-oxygenated conditions. Besides cardiomyocytes, endothelial cells and smooth muscle contribute significantly to total cardiac adenosine production. Rapid enzymatic conversion of adenosine is provided by adenosine kinase and adenosine deaminase, keeping the cytosolic adenosine concentration in the nanomolar range. Due to the high intracellular rates of adenosine rephosphorylation and deamination the cytosolic is normally below the extracellular adenosine concentration, making the cytosol to a sink rather than a source of adenosine. It is for this reason that blockers of membrane transport enhance the plasma adenosine concentration. With increasing catabolism of adenine nucleotides the rate of intracellular adenosine production exceeds the rate of adenosine deamination and rephosphorylation. Thus, this condition will result in a concentration gradient from intra- to extracellular. Thence, membrane transport blockers would be expected to increase the intracellular adenosine concentration. A considerable insecurity on the importance of experimental data results from species differences of purine metabolism. Cardiac adenosine metabolism has recently been described in quantitative terms using mathematical model analysis. This analysis tool may prove useful in future when (1) clarifying the importance of various regulatory actions described for the different pathways of adenosine metabolism, (2) making quantitative comparisons of different experimental models possible and (3) deepening the insight from experimental data.

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Introduction

Adenosine metabolism is highly complex and rapid in mammalian tissues. The truth of this simple statement was not foreseen when studies of adenosine metabolism were started more than 35 years ago (Conway and Cooke 1939; Kerr and Seraidarian 1945; Kutscher and Sarreither 1948; Lee 1957; Gerlach et al. 1961; Berne 1963; Imai et al. 1964; Richman and Wyborny 1964; Brady and O'Donovan 1965; Baer et al. 1966; Deuticke et al. 1966). Originally, the adenosine concentrations were assumed to be static and furthermore the complexity of compartmentalization of adenosine metabolism was unknown. Therefore, several studies reported in the literature may need a considerable re-interpretation today due to important conceptional changes. By continuous research a clearer and more detailed quantitative picture of adenosine metabolism has emerged recently. This progress was largely due to the evolution of new experimental approaches. Earlier insight into adenosine metabolism was largely based on measurement of total purine content in cells or tissues, total tissue release, and assessment of in vitro K_m- and V_{max}-values. More recently enzyme inhibitor-based experiments on intact cells, isolated organs and in vivo hearts have proven very useful to improve our concepts. Such studies have revealed important semiquantitative insights into the metabolic flux rates of adenosine under (near) physiological conditions. Lately, application of comprehensive mathematical models of adenosine metabolism and transport to experimental data (Kroll et al. 1992; Kroll and Stepp 1996; Stepp et al. 1996; Decking et al. 1997b; Deussen et al. 1999; Schwartz et al. 1999) have provided true quantitative estimates of the various flux rates. It is the purpose of this overview to summarize this recent progress and to allude to in vitro measurements of K_m- and V_{max}-values where this is important for the general understanding. The most comprehensive data set is

available now for heart tissue on which this communication is focussed. Extensive previous reviews of adenosine metabolism are found in Schrader (1981), Sparks and Bardenheuer (1986), Belardinelli et al. (1989), Olsson and Pearson (1990), Deussen (1995), Thorn and Jarvis (1996) and Moriwaki et al. (1999).

Overview of adenosine metabolism

Enzymes catalysing the production of adenosine exist in the cytosol and in extracellular regions (Fig. 1). Cytosolic sources of adenosine are 5′-AMP (Schutz et al. 1981; Newby et al. 1985; Darvish et al. 1993) and *S*-adenosylhomocysteine (De la Haba and Cantoni 1959; Schutz et al. 1981). Cytosolic 5′-nucleotidase has been localized using immunolocalization (Darvish et al. 1993; Sala-Newby et al. 1999) or cell fractionation experiments. The latter approach has indicated that cytosol, lysosomes and plasma membrane were particularly rich of 5′-nucleotidase activity, while mitochondria and sarcoplasmic reticulum were devoid of activity (Kiviluoma et al. 1990). Under well-oxygenated conditions metabolism of AMP may proceed mainly through IMP to inosine rather than via adenosine. This is indicated by measurement of enzyme activities of cardiomyocytes isolated from the rat heart (Zoref-Shani et al. 1988) and the effects of inhibition of adenosine deaminase on cardiac release of adenosine and inosine (Deussen et al. 1989, 1999; Kroll et al. 1993). With increasing ATP degradation dephosphorylation of AMP to adenosine may gain relatively more importance (Achterberg et al. 1985b; Zoref-Shani et al. 1988; Deussen et al. 1989; Chen and Gueron 1996). However, the preference of both routes of metabolism is dependent on the metabolic state during which deenergization is induced (Altschuld et al. 1987; Chen and Gueron 1996; Hohl 1999). Recently, factors like intracellular adenosine, PKC activation and cAMP have been shown to exert modulatory effects on AMP deaminase activity (Hu et al. 1993; Chen and Gueron 1996; Hohl 1999).

An extracellular source of adenosine is provided by 5′-AMP degraded by action of ecto-5′-nucleotidase (Frick and Lowenstein 1978; Schutz et al. 1981; Bowditch et al. 1985; Dieckhoff et al. 1986; Darvish et al. 1996). This enzyme is bound to the cell membrane via a GPI-anchor (Panagia et al. 1981), the catalytic site facing the extracellular region (Fleetwood et al. 1989; Meghji et al. 1992, 1995). In heart tissue ecto-5′-nucleotidase activity exhibits striking species differences (Meghji et al. 1988b). In addition to the activities of 5′-nucleotidases, alkaline phosphatases have been associated with cytosolic and membrane fractions from cardiac muscle (Schutz et al. 1981; Bowditch et al. 1985). However, although the enzyme activities of alkaline phosphatases may be in the range of those of 5′-nucleotidase activities, their contribution to adenosine production may usually be small as the K_m-values of alkaline phosphatases are high. Recently ATP diphosphohydrolase activity was associated with sarcolemmal membrane from rat heart (Menezes de Oliviera et al. 1997). The quantitative importance of this pathway is unclear. Besides nucleotidase and phosphatase activities ecto-ADP kinase activity was reported for pig endothelial and smooth muscle cells (Pearson et al. 1980) as well as a human fibroblast cell line (Boyle et al. 1989). This enzyme activity may significantly interact with the extracellular nucleotide dephosphorylation cascade.

Metabolism of adenosine is brought about by adenosine deaminase and adenosine kinase. While adenosine kinase activity has been described to be confined to the cytosolic region (Schutz et al. 1981), different localizations of adenosine deaminase activity were reported. Besides a cytosolic fraction a membrane-bound ecto-enzyme (Schrader et al. 1987, 1994; Meghji et al. 1988a; Schrader and West 1990; Aran et al. 1991; Martin et al. 1995a) as well as a plasma adenosine deaminase (Storch et al. 1981; Donald et al. 1986; Ungerer et al. 1992; Kopff et al. 1997) were described.

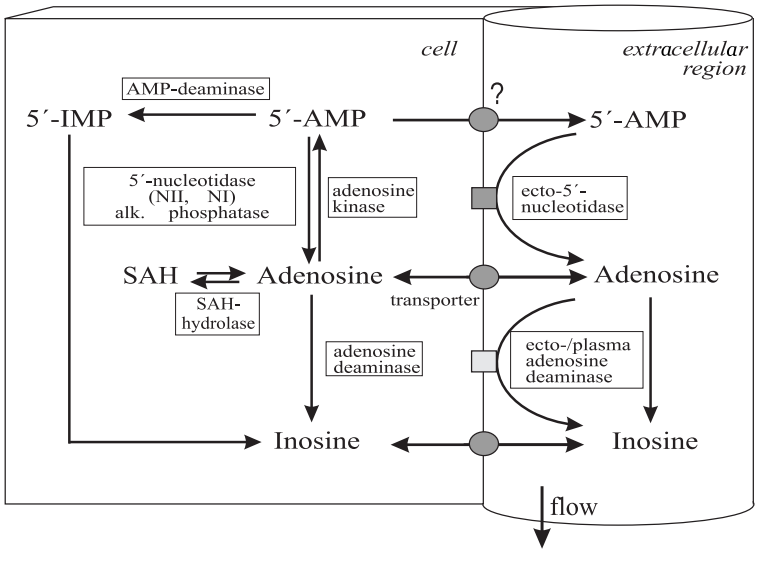

Fig. 1 Pathways and enzymes of adenosine metabolism. The *question mark* for membrane nucleotide transport indicates that the identity of the transport mechanism is still unsettled

Total production rate

The adenosine production rate has been estimated in experiments in which specific and potent blockers of adenosine kinase (e.g. iodotubericidine, 5′-amino-5′-deoxyadenosine) and adenosine deaminase (e.g. erythro-9-hydroxynonyl-adenine) have been applied. These inhibitors have no effect on the activity of cytosolic 5′-nucleotidase (Kroll et al. 1993). As adenosine kinase and adenosine deaminase provide the only routes of adenosine removal, adenosine release from isolated cells or organs under this condition provides a minimum estimate of the total adenosine production rate. This approach rests on the assumption that a steady-state adenosine concentration is reached during enzyme inhibition and that membrane transport is not rate-limiting for adenosine release.

The approach was used in isolated cells (polymorphonuclear leukocytes; Newby and Holmquist 1981; Newby et al. 1983; cardiomyocytes; Smolenski et al. 1998; endothelial cells; Deussen et al. 1993; Smolenski 1994; Smolenski et al. 1998; smooth muscle cells; Mattig 1997) and isolated perfused hearts from guinea pig (Ely et al. 1992; Kroll et al. 1993; Deussen et al. 1999) and rat (Meghji et al. 1988b; Lorbar et al. 1999). The general result was that during simultaneous inhibition of adenosine kinase and adenosine deaminase, adenosine release increased several-fold above that obtained under control conditions, indicating that cellular or tissue adenosine turnover must be several-fold higher than control release rate. Minimal adenosine production rates deduced from these experiments were 2.3–3.5 nmol min^{-1} g^{-1} (wwt) in guinea pig heart (Ely et al. 1992; Kroll et al. 1993; Deussen et al. 1999) and 6.4–7.7 nmol min^{-1} g^{-1} (wwt) in rat heart (Lorbar et al. 1999). These production rates exceed the respective adenosine release rates 9- to 43-fold, indicating that tissue turnover of this nucleoside must be very high and close to the production rate under well-oxygenated conditions. The conclusions drawn from experiments using adenosine release data are in agreement with data of tissue SAH accumulation during homocysteine infusion (Deussen et al. 1988a) and membrane transport block of adenosine (Deussen et al. 1999). Total adenosine production is largely increased during energy deprivation. During normothermic (37°C) ischemia total adenosine production rate was estimated 470 nmol min^{-1} g^{-1} and 410 nmol min^{-1} g^{-1} in rat and pigeon myocardium, respectively (Meghji et al. 1988b). In rat myocardium this corresponds to a 61- to 73-fold increase of the total production rate as compared with well-oxygenated conditions.

Estimates of the different sources of adenosine

On the basis of phosphocellulose chromatography two forms of cytosolic 5′-nucleotidase have been documented (Truong et al. 1988). Both enzymes also exist in human heart (Tavenier et al. 1995; Skladanowski et al. 1996). The two forms termed N-I and N-II differ with respect to substrate specificity. N-I prefers AMP over IMP (Naito and Lowenstein 1981; Truong et al. 1988), while N-II (Itoh et al. 1986) prefers IMP. N-I isolated from rat and rabbit heart has a pH optimum around 7.5, is activated by Mg^{2+} (Naito and Lowenstein 1981; Yamazaki et al. 1991) and by ADP, but not ATP (Yamazaki et al. 1989, 1991). These characteristics are shared with the enzyme isolated to apparent homogeneity from dog heart (Darvish and Metting 1993). The molecular mass of this enzyme was estimated to be 166 kDa. The enzyme may exist in a tetrameric structure and exhibits sigmoidal saturation kinetics with respect to the AMP concentration in the absence of ADP. An AMP-selective 5′-nucleotidase has also been isolated from pigeon heart (Newby 1988). This enzyme was reported to be stimulated by ADP plus ATP, while inhibited by nucleoside monophosphates and inorganic phosphate. The enzyme termed N-II isolated from rat heart is activated by ATP as well as ADP, and inhibited by inorganic phosphate (Itoh et al. 1986). Like N-I it is activated by Mg^{2+} (Yamazaki et al. 1991). Recently inhibitors of N-I with convincing selectivity have been developed (Garvey et al. 1998). In isolated rat myocytes the selective inhibitor 5-ethynyl-2′,3′-dideoxyuridine reduced AMP hydrolysis to adenosine by 76% (Garvey and Prus 1999). Besides the differing substrate preference of N-I vs. N-II this result indicates that N-I may be the enzyme largely responsible for the production of adenosine from AMP. In accordance with this interpretation are the effects of 5′-deoxy-5′-isobutythioadenosine, an inhibitor of N-II. Presence of this inhibitor did not largely decrease adenosine accumulation in rat cardiomyocytes (Meghji et al. 1993). However, some quantitative differences may exist between species. While rat cardiomyocytes contain similar activities of N-I and N-II, human myocardium exhibits an approximately 60% higher activity of N-II as compared with N-I (Skladanowski et al. 1996).

In contrast to cytosolic N-I, ecto-5′-nucleotidase is inhibited by ADP and ATP (Naito and Lowenstein 1985; Darvish et al. 1996). Mg^{2+} is an allosteric effector of the enzyme (Mallet et al. 1996) which is powerfully inhibited by the ADP analogue α,β-methylene-adenosinediphosphate (Naito and Lowenstein 1985; Yamazaki et al. 1991; Deussen et al. 1993; Darvish et al. 1996; Mallet et al. 1996). By use of immunohistochemistry the enzyme distribution has been studied in the cardiovascular system. It was found that significant animal species differences exist with respect to the cellular localization of the enzyme (Borgers and Thone 1992). Pericytes as well as fibroblasts have been reported to stain particularly positive for ecto-5′-nucleotidase (Borgers and Thone 1992; Mlodzik et al. 1995). Results on endothelial cells show a high degree of variability. Ecto-5′-nucleotidase was localized on endothelial cells from resistance arterioles, while for capillary endothelial cells only a weak immunoreaction was reported (Werner and Schunke 1989; Borgers and Thone 1992; Mlodzik et al. 1995). On endothelial cells from lymph vessels, however, rich enzymatic activity has been documented (Werner and Schunke 1989; Kato 1990). De-

spite only a weak association of membrane 5′-nucleotidase associated with capillary endothelial cells, the enzyme has been reported to be localized primarily on the abluminal membrane and associated vesicles (Roberts and Sandra 1993). This polarized distribution is changed upon introduction of cells to tissue culture. On the other hand, total ecto-5′-nucleotidase activity of endothelial cells has been documented to persist during cell cultivation (Nees et al. 1981). Furthermore, ecto-5′-nucleotidase has been reported on smooth muscle cells (Dieckhoff et al. 1986; Nitahara et al. 1995) where it may be localized with caveolae (Kittel and Bacsy 1994). On cardiomyocytes the enzyme has been documented only in rat heart (Bowditch et al. 1985; Borgers and Thone 1992; Aleksiuk et al. 1993) and most recently in mouse heart (Fretes et al. 1999).

In agreement with the immunolocalization studies a highly inhomogeneous activity of ecto-5′-nucleotidase is reported from biochemical enzyme activity measurements. The relation of cytosolic vs. ecto-5′-nucleotidase activity differs largely in cardiac tissue with respect to the animal species studied (Newby et al. 1987; Meghji et al. 1988b). While interspecies differences of the cytosolic enzyme activity are found to be smaller, huge differences exist with respect to the activity of ecto-5′-nucleotidase. Rat and guinea pig myocardium exhibit ecto-5′-nucleotidase activities of 4900 nmol min^{-1} g^{-1} and 3600 nmol min^{-1} g^{-1} (Newby 1987). In both species ecto-5′-nucleotidase exceeds activity of cytosolic 5′-nucleotidases approximately tenfold. Although total 5′-nucleotidase activity of human myocardium was determined to be roughly half that of rat myocardium, the overall distribution of enzymatic activity is similar with the membrane fraction exceeding the soluble fraction 5.5-fold (Kochan et al. 1994; Tavenier et al. 1995).

A large body of evidence supporting the existence of functional ecto-5′-nucleotidase activity is provided by isolated heart and cell experiments in which adenine nucleotides were applied and the formation of adenosine was measured. When 5′-AMP is infused into isolated hearts (Fleetwood et al. 1989; Borst and Schrader 1991; Mallet et al. 1996; Sato et al. 1997; Obata and Yamanaka 1999), endothelial cells (Pearson et al. 1978, 1980; Gordon et al. 1986; Bonitati et al. 1993; Deussen et al. 1993; Meghji et al. 1995) or vascular smooth muscle cells (Pearson et al. 1978, 1980; Gordon et al. 1989), the nucleotide is rapidly hydrolyzed to adenosine. Ecto-5′-nucleotidase represents the final and irreversible catalytic step in the extracellular dephosphorylation cascade of adenine nucleotides. There is evidence for complex interaction of the substrates and products of this dephosphorylation cascade (Gordon et al. 1986, 1989; Meghji et al. 1992, 1995).

Despite earlier pioneering work on the release of adenine nucleotides from endothelial and smooth muscle cells (Pearson and Gordon 1979), blood cells (Coade and Pearson 1989; Morabito et al. 1998) or isolated perfused heart (Imai et al. 1989; Borst and Schrader 1991), the quantitative importance of ecto-5′-nucleotidase in contributing to native cardiac adenosine production has been successfully addressed only recently (Deussen et al. 1999). From a total adenosine production of approximately 2.3 nmol min^{-1} g^{-1} only 8% could be attributed to extracellular production most likely via ecto-5′-nucleotidase in guinea pig heart during well-oxygenated conditions. Experiments carried out on cultured aortic endothelial cells (Deussen et al. 1993; Mattig and Deussen 2000) and coronary smooth muscle cells (Mattig and Deussen 2000) have also provided evidence for continuous extracellular adenosine production via ecto-5′-nucleotidase. The contribution of this pathway to total cell adenosine production was estimated to be 30% and 24% in endothelial and smooth muscle cells, respectively.

Besides hydrolysis of 5′-AMP, adenosine may be produced from *S*-adenosylhomocysteine (SAH) by action of *S*-adenosylhomocysteine hydrolase (De la Haba and Cantoni 1959; Ueland 1982). Under physiological conditions this enzyme exclusively present in the cytosolic cell fraction (Schutz et al. 1981) favors the production of adenosine and homocysteine (Schrader et al. 1981; Ueland 1982) because both reaction products are rapidly metabolized and therefore cytosolic concentrations are low (Deussen et al. 1988b). The contribution of this pathway has been estimated in experiments in which the SAH pool has been prelabeled (Lloyd et al. 1988) or accumulation of SAH was measured during blockage of SAH-hydrolase (Helland and Ueland 1983; Deussen et al. 1989; Kroll et al. 1993; Wagner et al. 1994; Loncar et al. 1997). Results obtained with both approaches agree that there is continuous production of adenosine from SAH under physiological conditions. There is, however, some disagreement on the quantity of this flux rate. The labeling experiment suggested a flux rate around 1200 pmol min^{-1} g^{-1} for guinea pig heart, while the blocker experiments indicate a flux rate in the range of 160 pmol min^{-1} g^{-1} for the same species (Deussen et al. 1989; Kroll et al. 1993). The latter estimate is probably more realistic given the total tissue adenosine production rate of 2.3–3.5 nmol min^{-1} g^{-1} in the guinea pig heart (Ely et al. 1992; Kroll et al. 1993; Deussen et al. 1999) and the pronounced effects of blockers of cytosolic 5′-nucleotidase on AMP degradation determined in rat cardiomyocytes (Garvey and Prus 1999). For dog heart net SAH-hydrolysis is only 33% of that found for guinea pig heart (Loncar et al. 1997) in agreement with a lower in vitro enzyme activity of SAH-hydrolase in canine as compared with guinea pig myocardium (Deussen and Schrader 1991). Another study conducted on rabbit cardiomyocytes suggested that SAH hydrolysis is not an important pathway of total adenosine production (Wagner et al. 1994). Thus, in conclusion the hydrolysis of SAH may provide a rather small fraction of steady-state adenosine production in the heart. This flux rate may differ significantly between animal species, which corresponds to large interspecies differences of myocardial enzyme activity (Schrader et al. 1981). Due to the lack of alternative metabolic pathways flux rate through the SAH pathway is a measure of the actual transmethylation rate. Cardiac transmethylation rate and subsequent hydrolysis of SAH are independent of tissue oxygenation

(Deussen et al. 1989). This is in contrast to total adenosine production in the heart which largely increases as the oxygen supply-to-demand ratio decreases (Bardenheuer and Schrader 1986; Deussen et al. 1991). A quantitatively negligible contribution of SAH to adenosine under ischemic or hypoxic conditions has been concluded from several independent studies (Achterberg et al. 1985a; Deussen et al. 1989; Wagner et al. 1994).

Metabolism of adenosine

Cytosolic adenosine kinase effectively phosphorylates cytosolic adenosine to 5′-AMP. As the K_m-value of adenosine kinase is approximately 1 μM (Schutz et al. 1981) and the free cytosolic adenosine concentration was estimated in a range of 0.01–0.1 μM under physiological conditions (Deussen et al. 1988b; Kroll et al. 1992; Deussen et al. 1999), the enzyme operates usually below its K_m-value. Only with hypoxia or ischemia a cytosolic adenosine concentration may result that saturates the enzyme. Adenosine deaminase exhibits a K_m-value considerably higher than that of adenosine kinase (Schutz et al. 1981). This makes the deamination reaction especially suited to effectively meet the large rate of adenosine production during conditions of impaired oxygenation.

While adenosine kinase activity is confined to the cytosolic region, differences may exist with respect to localization of adenosine deaminase activity. In guinea pig heart adenosine deaminase activity has been demonstrated exclusively in the cytosolic fraction (Schutz et al. 1981), whereas in rat heart and pig lung the enzyme activity is in part extracellular (Hellewell and Pearson 1983; Meghji et al. 1988a). For rabbit heart an association of membrane adenosine deaminase with an adenosine deaminase binding protein has been suggested (Schrader et al. 1979; Schrader and West 1990). Adenosine deaminase activity is also found on the surface of blood cells (Aran et al. 1991) and in blood plasma (Morisaki et al. 1985; Niedzwicki and Abernethy 1991). The two isoforms of plasma adenosine deaminase termed ADA1 and ADA2 exhibit distinct susceptibility to different enzyme inhibitors (Niedzwicki and Abernethy 1991). Our own, as yet unpublished experiments indicate that the plasma activity of adenosine deaminase is below 1% of that measured in packed red cells from healthy volunteers. Thus, the physiological significance of plasma adenosine deaminase needs to be clarified. Furthermore, the origin of plasma adenosine deaminase under physiological conditions needs further investigation (Jackson et al. 1996).

Compartmentalization: cell species

A considerable extent of species-related differences (Jarvis et al. 1982; Moser et al. 1989; Parkinson and Clanachan 1991; Borgers and Thone 1992; Moriwaki et al. 1999) partly obscures general rules of compartmentalization of adenosine metabolism and transport. Hence, the following rules should be applied with great caution. Ecto-5′-nucleotidase was found species-independently on pericytes and endothelial cells from resistance arterioles (Dendorfer et al. 1987; Borgers and Thone 1992). Only in rat heart ecto-5′-nucleotidase activity was also found to be associated with cardiomyocytes (Nees and Dendorfer 1991; Borgers and Thone 1992). Besides cardiomyocytes, smooth muscle and endothelial cells, sympathetic nerve terminals were suggested as an additional source of adenine nucleotide release in guinea pig heart (Imai et al. 1989). SAH-hydrolase activity may be higher in endothelial cells as compared with cardiomyocytes (Mistry and Drummond 1986; Kochan et al. 1994) although species differences may exist (Borst et al. 1992). Adenosine deaminase activity was reported to be largely localized in the endothelial cell region (Nees and Dendorfer 1991; Kochan et al. 1994) as was purine nucleoside phosphorylase (Nees et al. 1981; Nees and Dendorfer 1991; Borgers and Thone 1992; Kochan et al. 1994). The heterogeneous distribution of the various enzyme activities may significantly affect the concentration gradients of adenosine within the tissue.

Highly active adenosine production pathways are present in cardiomyocytes, endothelial cells as well as smooth muscle cells. When expressed per microliter of cytosolic volume, aortic endothelial cells and coronary smooth muscle cells from pig have total adenosine production rates of 7.5 pmol min^{-1} μl^{-1} and 12.3 pmol min^{-1} μl^{-1} (Mattig 1997), respectively. As each of both cell species contributes approximately 2.5% to the water space of cardiac tissue (Mall et al. 1982; Anversa et al. 1983), contributions to adenosine production of approximately 150 pmol min^{-1} and 250 pmol min^{-1} per gram heart mass, respectively, may be estimated. Thus, both cell species may roughly contribute 10% of total tissue adenosine production rate under well-oxygenated conditions (see above). This calculation does not take possible animal species differences into account. In another study adenosine content of rat cardiomyocytes and endothelial cells was determined to be similar after 3-h block of adenosine kinase and adenosine deaminase (Smolenski et al. 1998). This may suggest similar adenosine production rates in both cell species. However, as membrane transport was not inhibited in these experiments, true production rates cannot be derived from these measurements.

Using prelabelling of the endothelial adenine nucleotide pool with [^{3}H]adenosine (Nees et al. 1985; Nees and Dendorfer 1991), several studies have aimed to estimate the contribution of the endothelial cells to total cardiac adenosine release with the effluent perfusate (Deussen et al. 1986; Becker and Gerlach 1987; Kroll et al. 1987; Raatikainen et al. 1994). These studies indicate that the largest fraction of adenosine release was derived from an unlabeled pool, most likely the cardiomyocyte. A quantitative assessment of the contribution of endothelial cells (Becker and Gerlach 1987; Kroll et al. 1987) suggested that 14% and 25% of total venous adenosine release, respectively, originated from endothelial cells.

Compartmentalization: intracellular vs. extracellular

The available literature provides ample evidence for intra- and extracellular adenosine production and metabolism. Hence, it is important to understand the quantitative contributions of intra- and extracellular regions to total adenosine production to assess the prevailing net concentration gradients across cell membranes. Measurement of adenosine release from cells and tissues provides an index of the extracellular or intravascular adenosine concentration. The SAH-technique (Deussen et al. 1988b) on the other hand has been used as an index of the free cytosolic adenosine concentration (Deussen et al. 1988a, 1991, 1999; Kroll and Martin 1994; Decking et al. 1997b). In combination with detailed mathematical model analysis (Kroll et al. 1992; Deussen et al. 1999) experimental data from isolated guinea pig heart experiments indicate that the major site of adenosine production is intracellular (92%) under well-oxygenated conditions. Simultaneously, intracellular metabolism occurs at a rate higher than that of intracellular production mainly because of effective adenosine rephosphorylation to 5′-AMP (Arch and Newsholme 1978; Fisher and Newsholme 1984; Kroll et al. 1993). In conjunction with continuous extracellular adenosine production via ecto-5′-nucleotidase this results in a cytosolic adenosine concentration below the extracellular concentration and a transmembranous concentration gradient that is directed to the cytosol (Deussen et al. 1999). This result is in contrast to our intuition that a substrate concentration is greatest at the site of highest production.

The transmembranous concentration gradient from extra- to intracellular is in good agreement with the result of blockade of the transporter under physiological conditions. Dipyridamole which acts specifically on the nucleoside carrier at a concentration of 1 μM (Klabunde 1983; Jarvis 1986) was frequently demonstrated to increase the plasma adenosine concentration (Sollevi and Fredholm 1981; Sollevi et al. 1984; German et al. 1989; Moser et al. 1989; Yoneyama and Power 1992; Hegedus et al. 1997; Saito et al. 1999). This effect is most likely brought about by preventing access of adenosine to its site of metabolism which is largely intracellular (Deussen et al. 1999; Mattig and Deussen 2000). Accumulation of adenosine in blood plasma can be prevented by EDTA (Gewirtz et al. 1987) which reduces ecto-nucleotidase activities (Harkness et al. 1984; Yegutkin and Burnstock 1999). This concept requires adenine nucleotide release from cells which may potentially occur via the p-glycoprotein (Hauser et al. 1998), CFTR (Reisin et al. 1994; Schwiebert et al. 1995) or via cAMP release (Dubey et al. 1996).

In heart tissues the transmembranous adenosine transport is facilitated by an equilibrative, sodium-independent nucleoside carrier (Conant and Jarvis 1994; Thorn and Jarvis 1996). Experimental evidence in agreement with the assumption of a symmetric transport has been provided (Meghji et al. 1985, 1988c), although a nonsymmetric membrane transport was postulated by others (Buchwald et al. 1987). A recent quantitative mathematical model analysis has indicated that experimental results can be explained without assumption of nonsymmetric transport (Deussen et al. 1999). Autoradiographic studies show that the carrier is heterogeneously distributed in guinea pig heart, reflecting the vascular branching pattern (Parkinson and Clanachan 1989a), while myocardial distribution is rather homogenous in rat (Parkinson and Clanachan 1989b). Furthermore, in guinea pig heart different carrier subtypes on endothelial cells vs. cardiomyocytes have been suspected (Parkinson and Clanachan 1991). The importance of these differences for myocardial concentration gradients of adenosine are not well understood to date.

Regulation of adenosine metabolism

Numerous investigations indicate that enzyme activities and flux rates of adenosine metabolism may not be handled as constants, but that there is short- and long-term regulation. Stimuli including physical exercise (Pierce et al. 1989; Langfort et al. 1996; Delgado et al. 1999), pressure loading (Panagia et al. 1986; Czarnowski et al. 1996), estradiol (Dubey et al. 2000), thyroxine (Daly et al. 1986; Smolenski et al. 1995), dietary lipid composition (Baracca et al. 1994), alpha-adrenergic stimulation (Kitakaze et al. 1995; Sato et al. 1997) as well as hypoxia and ischemia (Bak and Ingwall 1994; Minamino et al. 1995; Kitakaze et al. 1996; Gustafson and Kroll 1998) were studied. Also, age-related changes were investigated (Awad and Chattopadhyay 1983; Wang et al. 1987; De Jong et al. 1990; Torii and Ito 1990; Grosso et al. 1992; Lorbar et al. 1999). Presently available evidence of acute effects on specific pathways is summarized here.

Cytosolic 5′-nucleotidase

Increases of Mg^{2+} and ADP may enhance the activity of cytosolic 5′-nucleotidase (N-I; Naito and Lowenstein 1981; Yamazaki et al. 1989, 1991; Darvish and Metting 1993), conditions which may result during impaired tissue oxygenation from degradation of ATP. However, adenosine production at a maximal rate occurs only for a short period after onset of ischemia (Meghji et al. 1988b). Recent in vivo studies suggest that 5′-nucleotidase may become inhibited during conditions of ischemia or hypoxia (Bak and Ingwall 1998; Gustafson and Kroll 1998). It has been proposed that an inhibitory action of cellular acidosis may overcome the activating effects of Mg^{2+} and ADP during cardiac ischemia (Bak and Ingwall 1994, 1998). While 5′-nucleotidase activity may be high during short periods of ischemia, it was suggested to be depressed with sustained or repetitive ischemia (Gustafson and Kroll 1998). However, it needs to be said that the evidence for 5′-nucleotidase inhibition during ischemia is still incomplete as Bak and Ingwall (1998) did not assess net adenosine production, and Gustafson and Kroll (1998) did not take AMP deaminase activity into account.

Ecto-5′-nucleotidase

The contribution of this pathway to total adenosine production of endothelial and smooth muscle cells, and global heart has been demonstrated recently (Deussen et al. 1993, 1999; Mattig and Deussen 2000). Two previous studies suggest that extracellular cardiac adenosine production may be enhanced under conditions of beta-adrenergic stimulation and hypoxic perfusion (Headrick et al. 1992, 1996). The mechanism by which activity of ecto-5′-nucleotidase may be regulated during ischemia has recently been addressed. Alpha$_1$-adrenoceptor stimulation may result in activation of PKC which may activate ecto-5′-nucleotidase activity (Kitakaze et al. 1995; Node et al. 1997a; Sato et al. 1997; Obata and Yamanaka 1999). Other effective stimuli of PKC which also resulted in activation of ecto-5′-nucleotidase were PMA (Node et al. 1997b), monophosphoryllipid A (Przyklenk et al. 1996), ischemia/hypoxia (Minamino et al. 1995; Kitakaze et al. 1996), and adenosine A_1-receptor stimulation (Downey and Cohen 1997; Iliodromitis et al. 1998). Of particular importance may be that adenosine is generated in high concentrations during ischemia/hypoxia and may therefore represent a molecular link by which this condition stimulates PKC. Activation of ecto-5′-nucleotidase from PKC does not require protein synthesis (Kitakaze et al. 1995). Thus, externalization of some compartmentalized enzyme is a possible mechanism. Conflicting results have been reported on the interaction of NO with ecto-5′-nucleotidase activity. While NO-donors and 8-bromo-cGMP enhanced ecto-5′-nucleotidase activity in the rat heart in vivo (Obata et al. 1998), inhibitors of NO-synthesis enhanced the enzyme activity via PKC in a cGMP-independent manner in cultured human coronary endothelial cells (Minamino et al. 1997).

SAH-hydrolase

Beta-adrenergic stimulation was recently reported to inhibit SAH-hydrolase by a calcium-dependent mechanism (Suarez and Chagoya de Sanchez 1997). The thermodynamic equilibrium of the kidney enzyme was shifted toward SAH synthesis during elevated phosphate concentrations (Kloor et al. 1998). It is expected that mechanisms that augment cellular transmethylation reactions will also enhance the production of adenosine by this pathway.

Adenosine kinase

This enzyme interacts closely with cytosolic and ecto-5′-nucleotidase to control the free cytosolic adenosine concentration (Kroll et al. 1993). Recent experiments conducted on the isolated perfused guinea pig heart indicate that during reduced tissue oxygenation the free cytosolic adenosine concentration increased more than the free cytosolic AMP concentration (Decking et al. 1997a). It has been suspected that this may have been brought about by a decrease of adenosine kinase activity (Decking et al. 1997b). The mechanism by which this effect is mediated is unclear. One possible explanation may be inhibition of adenosine kinase by a rise in inorganic cellular phosphate (Gorman et al. 1997) which is increased during ATP depletion. H_2O_2 which may be of importance during ischemia-reperfusion (Richter et al. 1995; Kristian and Siesjo 1998) was also shown to reduce adenosine kinase activity (Griesmacher et al. 1993).

Adenosine deaminase

Cardiac adenosine deaminase activity was reported to change in response to endurance exercise (Langfort et al. 1996), during enhanced left ventricular afterload (Czarnowski et al. 1996), and during development (Wang et al. 1987). The mechanism(s) by which these changes are caused remain to be determined. Adenosine deaminase activity associated with the membrane of blood cells may change during occurrence of lymphoma (Martin et al. 1995b; Mesarosova et al. 1995) and several inflammatory reactions (Maeda et al. 1992; Kroegel and Antony 1997). Under these circumstances also changes of the plasma adenosine deaminase isoenzyme pattern have been reported (Tsuboi et al. 1995; Ungerer et al. 1996). The mechanism of induction as well as the pathophysiological role of these changes are not well understood to date.

Regulation of the interstitial adenosine concentration

As described above the major site of adenosine production under well-oxygenated conditions is intracellular and provided by the activity of cytosolic 5′-nucleotidase NI. However, because there is continuous extracellular adenosine production at a lower rate and highly active intracellular rephosphorylation of adenosine, there is net cellular uptake of adenosine under physiological conditions (Fig. 2). This scenario may be profoundly altered during cellular hypoxia which due to net catabolism of adenine nucleotides results in an increased intracellular adenosine concentration (Deussen et al. 1988a, 1988b; Decking et al. 1997b). As a consequence the flux rates through the adenosine kinase and the adenosine deaminase pathways increase. As the K_m- and V_{max}-values of both enzymes differ largely, the relative increases of these flux rates depend on the extent of change of the cytosolic adenosine concentration. In general, the more the cytosolic adenosine concentration increases, the more adenosine will undergo deamination relative to phosphorylation. This redistribution of metabolic fluxes is even augmented by the fact that adenosine kinase may become inhibited under this condition (Decking et al. 1997a, 1997b). During tissue hypoxia the transmembranous adenosine concentration gradient reverses and net cellular adenosine release results. Thus, adenosine membrane transport inhibition results in a decrease of cardiac adenosine release during

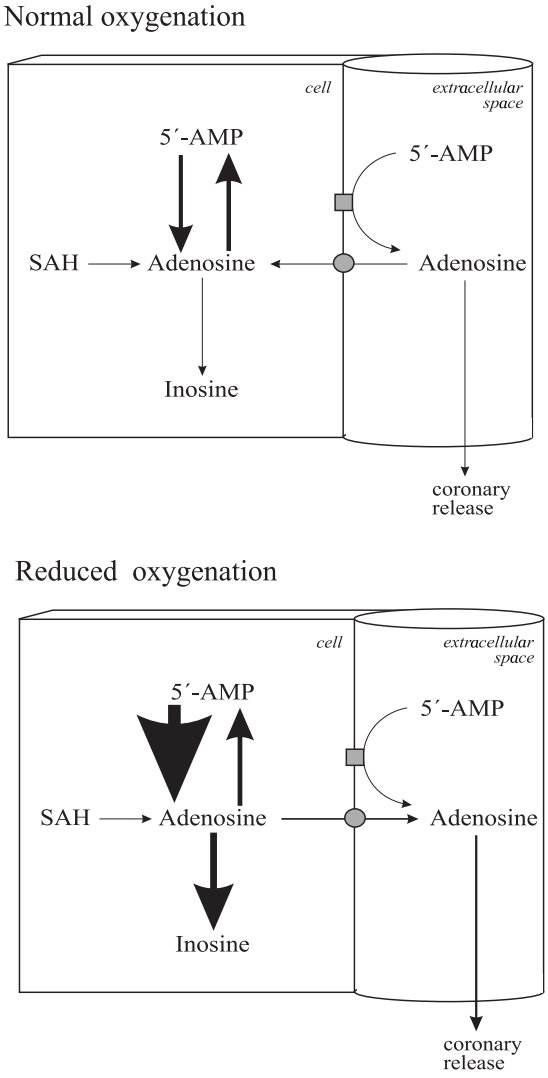

Fig. 2 Flux rate schemes of adenosine metabolism in cardiac tissue for normal and reduced oxygenation (equivalent to 95% and 40% oxygen equilibration of coronary perfusate in the isolated guinea pig heart). The *arrow sizes* are scaled to each other representing the different net flux rates (after Kroll et al. 1992; Decking et al. 1997b; Deussen et al. 1999)

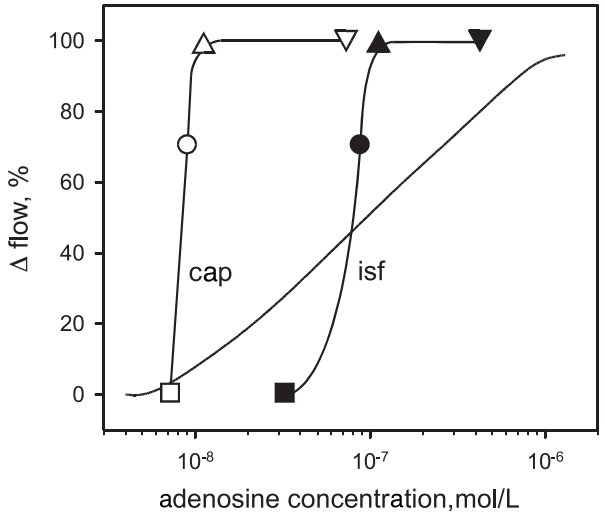

Fig. 3 Adenosine concentration-coronary flow relationship in the guinea pig heart. The steep concentration-effect relationships shown for the capillary (*cap*) and the interstitial fluid (*isf*) regions were obtained by mathematical model analysis using experimental measurements of coronary venous adenosine release and coronary flow. *Symbols* represent measurements (after Deussen et al. 1999). The concentration-flow relationship with the more gradual slope represents the relationship for a direct comparison of measured venous effluent adenosine and coronary flow (after Bardenheuer and Schrader 1986). The figure emphasizes the potential of mathematical model analysis to obtain realistic concentration-effect relationships

reduced tissue oxygenation (Schutz et al. 1981). Extracellular adenosine production may also increase during hypoxia (Headrick et al. 1992). However, a quantitative figure for extracellular AMP and adenosine under this condition has not been obtained to date.

Increased concentrations of adenosine may also occur under conditions of adrenergic stimulation. However, it is unlikely that in the intact heart a decrease of myocardial oxygenation and consecutively increased net ATP catabolism result under this condition (Balaban and Heineman 1989; Tune et al. 2000), providing an increased adenosine production. Rather, ATP is released as a co-transmitter of noradrenaline (Sneddon et al. 1999) or an increased cAMP level provides the substrate for extracellular adenosine production via 5′-AMP. However, these possibilities need further consideration and experimental testing.

Adenosine acts by binding to cell surface receptors. Of particular importance for predicting possible biological effects is the finding that the concentration-effect relationship of adenosine may be rather steep (Fig. 3). Almost full effectiveness with regard to coronary flow enhancement may be obtained by blocking cellular uptake of adenosine produced in the extracellular region (Deussen et al. 1999). Thus, it is not required to utilize the full potential of total tissue adenosine production, but modulation of the fractional production at the site of receptor localization seems to be an attractive alternative option. Besides inhibition of cellular adenosine uptake, further options include activation of ecto-5′-nucleotidase, enhancement of precursor release (cAMP, ATP) or inhibition of ecto- or plasma adenosine deaminase. The quantitative assessment of the different pathways of adenosine metabolism in conjunction with mathematical model analysis may help in predicting the possible outcome of such interventions.

Future directions

The thorough investigation of cardiac adenosine metabolism and transport over more than three decades has provided a quantitative picture of the rapid turnover of this nucleoside under steady-state conditions. Based on detailed experimental results and application of mathematical model analysis physiologically realistic estimates of metabolic flux rates, regional adenosine concentrations,

and transmembranous and tissue concentration gradients are beginning to emerge (Kroll et al. 1992; Kroll and Stepp 1996; Stepp et al. 1996; Decking et al. 1997b; Deussen et al. 1999; Schwartz et al. 1999). A sigificant problem in adenosine metabolism research arises from large species differences (van Belle et al. 1985). In medicine the ultimate importance of experimental results is measured against the applicability to human physiology. Thus, more detailed measurements in cardiovascular tissues from man should be encouraged. Recently, there is accumulating evidence for regulation of enzyme activities involved in adenosine metabolism. However, the underlying signal transduction pathways need a more detailed and quantitative investigation to establish true causal relationships. Also, the impact of enzyme activity changes on the regulation of adenosine flux rates and the local adenosine concentration need to be clarified. It seems feasible that recently developed mathematical models can be extended to link individual molecular steps of the signal transduction pathway and relate them on the enzyme level to metabolic activities which determine substrate concentrations on cellular and tissue levels. In a further step this approach might permit to determine local substrate concentration – receptor interactions in a true quantitative manner.

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