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BRIEF REVIEWS

The Role of Adenosine in the Regulation of Coronary Blood Flow

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THE coronary vasculature is influenced by physical (arterial pressure, extravascular compression, hematocrit), neural, and chemical factors, and although there is considerable interplay among these various factors, the predominant one is chemical. Furthermore, the chemical factors of greatest importance are those that take origin from the cardiac parenchymal tissue and serve as a link between myocardial oxygen needs and supply. One of the principal contenders that can serve as a mediator of coronary blood flow (CBF) regulation in response to cardiac metabolic activity is the nucleoside, adenosine (Berne, 1963).

Criteria for Adenosine as a Mediator of Metabolic Regulation of CBF

For adenosine, or any other vasoactive substance, to play a key role in the metabolic regulation of CBF, certain criteria must be met. (1) The substance must be a potent dilator of the coronary resistance vessels. (2) There must be an endogenous source of the mediator. (3) The substance should have access to the arterioles and be present under basal physiological conditions. (4) The concentration reached in the interstitial fluid (ISF) must be capable of eliciting vasodilation, and there should be a close relationship between the ISF concentration and CBF (dose-response relationship). (5) The time course of oxygen deficit (either decreased oxygen supply or increased oxygen demand) should parallel the increment in CBF. (6) The physiological effect at different concentrations of the endogenous mediator should be mimicked by exogenous administration of the substance. (7) Agents that potentiate or attenuate the action of administered mediator should elicit a similar effect on endogenously liberated mediator. (8) A direct cause-and-effect relationship should be established under all physiological and pathophysiological conditions between change in CBF and adenosine release.

Currently, many but not all of these criteria are fulfilled with respect to the hypothesis that adenosine is indeed the primary mediator in the regulation of CBF. Adenosine is a very active vasodilator. When injected intraarterially in the isolated perfused heart, it elicits marked coronary dilation from $10^{-7}$ to $10^{-5}$M and maximum dilation at $10^{-5}$M concentrations (Schrader et al., 1977a). Even in the blood-perfused heart, adenosine produces marked coronary dilation at concentrations less than those reached with ischemia, despite rapid inactivation by the cellular elements in the blood (Rubio et al., 1969). Furthermore, strips of dog coronary arteries caused to contract with norepinephrine or KCl, relax when adenosine ($10^{-6}$ M) is added to the tissue bath and the nucleoside does not exhibit tachyphylaxis (Herlihy et al., 1976).

The source of adenosine is myocardial ATP and possibly to some extent via cyclic AMP (Schrader and Gerlach, 1976). However, little if any change is observed in tissue ATP levels because small and immeasurable decreases in tissue ATP can result in a several-fold increment in adenosine, when oxygen supply is inadequate for cardiac needs (Rubio et al., 1974). It has been reported that ATP, which also is a potent vasodilator, is released from isolated adult rat heart cells subjected to hypoxia (Forrester and Williams, 1977) and isolated perfused guinea pig heart (Paddle and Burnstock, 1974). However, studies in our laboratory have failed to reveal release of ATP from skeletal muscle (Bockman et al., 1975) or into cardiac effluents (Jacob and Berne, 1961), even when the tissues have been subjected to intense, unphysiological conditions, such as contraction during ischemia. Actually, it is virtually impossible to make accurate measurements of ATP in venous blood since the nucleotide is rapidly degraded in blood and also can be released from the cellular elements of the blood (Bockman et al.,
The enzyme that produces adenosine from ATP is 5'-nucleotidase. This enzyme, which is thought to be a membrane bound or an "ectoenzyme" is histochemically localized at the myocardial cell margins (sarcosomes, intercalated discs, transverse tubules, and flattened sarcoplasmatic reticulum) (Rostgaard and Behnke, 1965; Rubio et al., 1973). However, 5'-nucleotidase may also exist within the external cell membrane, the latter is the primary site of dephosphorylation of AMP, and adenosine is released into the ISF where it can reach and dilate arterioles without being inactivated by intracellularly located adenosine deaminase. Some of the adenosine in the ISF crosses the capillary wall where a significant fraction is degraded to inosine and hypoxanthine (Rubio et al., 1972), whereas a large fraction of the adenosine in the ISF is taken up by the myocardial cells, reincorporated into the myocardial adenine nucleotides (Wiedmeier et al., 1972). Adenosine enters the cells by facilitated diffusion, and at low extracellular concentrations (<3 μM) it is primarily phosphorylated to AMP rather than deaminated to inosine, presumably because of the lower Kₘ of adenosine kinase than of adenosine deaminase for the nucleoside (Schrader et al., 1972; Olsson et al., 1972).

Unfortunately, actual levels of adenosine in ISF cannot be measured directly, and also cannot be determined from assay of cardiac lymph because of the degradative enzymes in the cellular elements of lymph, and the prolonged contact with these enzymes as a result of the slow lymph flow (Berne, unpublished observations; Belloni et al., 1977). Calculations of ISF adenosine concentrations based on estimates of the size of the ISF compartment, and pericardial fluid and coronary sinus blood levels of adenosine have been made in the dog, and suggest that there is normally a low concentration of adenosine in the ISF during basal conditions (Rubio and Berne, 1969), and that this increases to levels capable of inducing maximal coronary arteriolar dilation after brief periods of ischemia (Rubio et al., 1969). However, from measurements of adenosine levels in the oxygenated dog heart (Olsson et al., 1978a) and in the isolated perfused guinea pig heart (Schrader and Gerlach, 1976), it was concluded that if all the adenosine were restricted to the ISF, the concentration would be great enough to produce maximal coronary dilation. Existence of an intracellular pool of adenosine in cardiac cells has been suggested (Schrader and Gerlach, 1976; Olsson et al., 1978a; Frick and Lowenstein, 1978). This pool could be in the form of S-adenosylhomocysteine (Schrader and Schütz, 1979), but it is difficult to understand how this source of adenosine could result in the release of free adenosine into the ISF without the nucleoside being subjected to deamination by the ubiquitous cytosolic adenosine deaminase. One would have to postulate a protective compartment and conveyor of adenosine from the cytosol to the exterior of the myocardial cell. Furthermore, experiments with red cell ghosts (Schrader et al., 1972) and cultured embryonic chick heart cells (Mustafa et al., 1975) indicate that free adenosine is not present within the cells. These disparate results have not been reconciled, and tissue adenosine levels probably do not accurately reflect ISF adenosine concentrations. It is possible that a large fraction of the adenosine is bound to membrane or intracellular protein and is not vasoactive. Studies on dispersed liver cells indicate that there is an adenosine fraction associated with the cells that is not destroyed by incubation of the cells with adenosine deaminase in the medium (Belloni et al., 1980).

With as little as 5 seconds of ischemia, myocardial adenosine levels increase almost 3-fold (Berne et al., 1971; Olsson, 1970), and with progressive hypoxia the adenosine content of the myocardium of the isolated heart, as well as the adenosine released by the heart, correlates well with the increment in coronary flow (Rubio et al., 1974). Furthermore, during reactive hyperemia following 5- and 15-second coronary occlusions, there is a close correlation between the CBF following release of the occlusion and the myocardial adenosine levels (Olsson et al., 1978b). Hence, the time course of the oxygen deficit is reflected in the myocardial adenosine levels. Moreover, in isolated perfused hearts, adenosine infusion shows a dose-response relationship that closely resembles that observed with induced endogenous adenosine release (Schrader et al., 1977a).

The methylxanthines, theophylline and aminophylline, attenuate the vasodilator effects of intraarterially administered adenosine and act as competitive inhibitors of the nucleoside (Bünger et al., 1975). Some investigators have also observed attenuation of reactive hyperemia after the administration of either theophylline or aminophylline (Juhran et al., 1971; Curnish et al., 1972), whereas most investigators have observed either no effect or minimal attenuation (Bittar and Pauly, 1971; Juhran and Dietmann, 1970). One group (Merrill et al., 1978) observed that a reduction in perfusion fluid pH potentiated the vasodilator response to adenosine and proposed that theophylline’s inability to attenuate reactive hyperemia regularly might be related to enhanced adenosine-induced dilation caused by decreased tissue pH. The effect of a reduction in pH may in fact augment coronary
vasodilation by increasing myocardial release of adenosine, since a decrease in pH to 7.1 or 6.8 induced by elevation of Pco2 or by HCl in the isolated perfused rabbit heart, significantly increased cardiac adenosine levels and coronary flow (Mustafa and Mansour, 1980).

There are also discrepant results with respect to the potentiating effect of dipyridamole during reactive hyperemia (Miura et al., 1967; Bittar and Pauly, 1970; Juhran et al., 1971). This drug blocks adenosine uptake by cells and thus prevents intracellular metabolism, and hence, disappearance of adenosine, when both dipyridamole and adenosine are administered intraarterially. However, dipyridamole and lidoflazine (a drug with a similar mode of action) have often failed to potentiate reactive hyperemia (Bittar and Pauly, 1970; Bittar and Pauly, 1971; Juhran et al., 1971). In brief, the results with these attenuating and potentiating drugs are controversial and inconclusive, and considerably more work is necessary if these discordant results are to be reconciled.

It is of interest, however, that adenosine deaminase of small molecular size that is capable of crossing the capillary membrane reduces reactive hyperemia in the dog heart by 40% when administered intraarterially (Olsson, personal communication). This latter observation, as well as many of those cited above, suggests a cause and effect relationship between myocardial adenosine formation and CBF. Nevertheless, absolute proof of this relationship is still lacking.

**Adenosine Production with Increased Cardiac Performance**

Some additional support is given the adenosine hypothesis by recent studies on cardiac adenosine formation and release under conditions of increased cardiac work. Most of the early studies dealt with inadequate myocardial oxygen supply, and it was originally proposed that hypoxia was the only stimulus for adenosine production by the heart (Berne, 1963; Rubio et al., 1969). However, increased cardiac pressure work produced by constriction of the aorta in the rat elicited a significant increment in the myocardial adenosine levels (Foley et al., 1978). In these experiments, the animals were not hypoxic, and CBF was undoubtedly enhanced by the high coronary perfusion pressure as well as by a metabolic mechanism. Whether there was some degree of subendocardial ischemia as a result of the increased afterload was not determined.

Aortic constriction also was performed in the open-chest dog and produced an inverse relationship between coronary vascular resistance and the log of the myocardial adenosine content (McKenzie et al., in press). Associated with these changes were significant increases in CBF, myocardial oxygen consumption, peak left ventricular pressure, and peak dP/dt. However, there was no significant increase in lactate content of the myocardium or decrease in coronary sinus blood pH during aortic constriction, suggesting that the hearts were not hypoxic. In these studies (McKenzie et al., in press), a gradient for adenosine across the free wall of the left ventricle did not occur with aortic constriction. However, administration of isoproterenol changed the endocardial-to-epicardial ratio for adenosine from 0.96 to 2.58 as a result of a reduction in coronary perfusion pressure (blood pressure) in the face of an increase in myocardial oxygen demand. A similar change in the transmural gradient for adenosine occurred in the dog heart subjected to a 50% constriction of the left coronary artery, but was not observed in the normal dog heart (Foley et al., 1979). Hence, there is no evidence to support the hypothesis that the reduced resistance (and vasodilator reserve) of the endocardial vessels that compensates for the greater extravascular resistance and results in equal endocardial and epicardial blood flow is attributable to adenosine. However, it is quite possible that the relatively slow methods of myocardial sampling and inactivation of degradative enzymes mask detection of small differences in the endocardial and epicardial adenosine levels. Obviously, improved methodology is needed, especially since changes in myocardial adenosine levels can occur with extreme rapidity (Berne et al., 1971; Olsson et al., 1970; Thompson et al., 1980).

In another study in the open-chest dog it has been demonstrated that adenosine release parallels cardiac metabolic activity when the latter is increased by stimulation of the stellate ganglia (Miller et al., 1979). In these experiments, 40 ml of Krebs-Henseleit solution were introduced into the pericardial sac via a silastic tube, removed after 4.5-minute contact with the epicardial surface of the heart, and analyzed for adenosine and its degradation products. Control experiments indicated that adenosine levels in the pericardial infusates (although considerably lower than those of the epicardium) served as an accurate index of adenosine production and release by the epicardium. Furthermore, the volume and the contact time of the infusate with the cardiac surface in these experiments did not alter the rate of adenosine release during control or experimental periods. There was a high rate of adenosine turnover between tissue and infusate, as measured with 14C-labeled adenosine, but enzymes that metabolize adenosine were not leached out of the myocardium. With graded stellate ganglia stimulation, there was excellent correlation among CBF, myocardial oxygen consumption, and adenosine release into the pericardial infusate under conditions in which arterial blood was fully saturated with oxygen. Lactate concentration in myocardium and pericardial infusates increased (possibly in part due to increased arterial lactate levels), but there was no significant change in lactate extraction by the heart with stellate ganglia stimulation. Nor was there a significant change in coronary sinus blood Po2 during periods of experimentally increased cardiac ac-
tivity. Hence, there was no evidence for myocardial hypoxia. Also, the levels of prostaglandin E in the infusate were unchanged with alterations of cardiac metabolic rate.

With a similar technique for assessment of adenosine release, studies were performed in the trained unanesthetized dog (Watkinson et al., 1979). In these experiments, the tube connected to the pericardial sac contained wires for recording of the electrocardiogram and was exteriorized posteriorly and covered by a cloth jacket around the dog’s thorax. After complete recovery from the operation, the pericardial infusate was introduced and removed for analysis during control periods (rest), treadmill exercise, and recovery (1 hour after cessation of exercise). Adenosine levels in control and recovery samples were equal, but there was a 2- to 3-fold increase in the adenosine concentration of the infusate collected during exercise. More recent studies of exercise in the dog revealed about a 2-fold increase in heart rate, cardiac output, CBF and myocardial oxygen consumption associated with a decrease in coronary vascular resistance and a 3-fold increase in myocardial adenosine levels after 10 minutes of treadmill exercise at 5 mph and a grade of 20% (McKenzie et al., 1980). These observations strongly support the concept that adenosine is released under physiological conditions in the absence of hypoxia.

The studies with aortic constriction in the rat and dog, and stellate ganglia stimulation and treadmill exercise in the dog, indicate a close relationship between myocardial metabolic activity and CBF. However, they deal with steady state conditions, and if there is a very tight linkage between CBF and cardiac metabolism, and if adenosine serves the role of mediator, then one might expect to observe changes in myocardial adenosine levels during a single cardiac cycle. Therefore, experiments were conducted on the isolated perfused guinea pig heart in which heart rate was reduced to 40–60 beats per minute by crushing the S-A node, substituting Sr2+ for a major fraction of the Ca2+ in the perfusion fluid, so as to prevent adenosine loss, and if there is a very tight linkage between CBF and cardiac performance, and since ADP is a more potent inhibitor of 5'-nucleotidase than is ATP (Burger and Lowenstein, 1970; Sullivan and Alpers, 1971), increments of ADP at the expense of ATP cannot account for enhanced 5'-nucleotidase activity. Therefore, attention has been focused on Pcr. In vitro studies with purified 5'-nucleotidase from snake venom or from crude enzyme prepared from cardiac tissue indicate that Pcr is a potent inhibitor or with enhanced oxygen usage could represent the sum of the changes taking place within single cardiac cycles. A summary of the production, action, and fate of adenosine in the heart is schematically shown in Figure 1.

**Regulation of Adenosine Formation**

As mentioned earlier in this review, adenosine is formed from 5'-AMP by dephosphorylation catalyzed by the enzyme 5'-nucleotidase, which is located at the cardiac cell margins. This enzyme is about 100 times more active in vitro than in vivo, indicating that it is greatly inhibited in the intact heart (Olason et al., 1973). A number of substances inhibit 5'-nucleotidase, such as ATP, ADP, and phosphocreatine (Pcr). Since changes in ATP and ADP are essentially undetectable with alterations in cardiac performance, and since ADP is a more potent inhibitor of 5'-nucleotidase than is ATP (Burger and Lowenstein, 1970; Sullivan and Alpers, 1971), increments of ADP at the expense of ATP cannot account for enhanced 5'-nucleotidase activity. Therefore, attention has been focused on Pcr. In vitro studies with purified 5'-nucleotidase from snake venom or from crude enzyme prepared from cardiac tissue indicate that Pcr is a potent inhibitor

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**Figure 1** Schematic diagram of two myocardial cells, an arteriole and a capillary, to illustrate the formation, action, and fate of adenosine in the heart. Adenosine formed by 5'-nucleotidase at the cell margins can enter the interstitial fluid where it can induce arteriolar dilation, pass through the pericytes and capillary endothelial cells where it is in large part degraded to inosine and hypoxanthine by adenosine deaminase and nucleoside phosphorylase. Further degradation occurs in the erythrocytes by these enzymes. A large fraction of the adenosine is reincorporated into myocardial nucleotides via the salvage pathway, namely by direct phosphorylation catalyzed by adenosine kinase or by synthesis from hypoxanthine. Only a small fraction of the cardiac adenosine nucleotides is derived by de novo synthesis from small molecule precursors (Zimmer et al., 1973). (Reproduced by permission from Berne and Rubio, 1979.)
of the enzyme (Rubio et al., 1979). However, small amounts of free Mg^2+ (ca 0.4 mm) are capable of reversing the inhibition exerted by Pcr. Extrapolation of these observations to the in vivo situation suggest that with increased myocardial metabolism 5'-nucleotidase activity is increased by a combination of three factors. (1) The most important is probably an increase in free Mg^2+ that was originally chelated to ATP and is released with enhanced ATP use (the small decrease in the large total cellular pool of ATP is undetectable by present methods). (2) Phosphorylation of ADP by Pcr which results in a reduction of the 5'-nucleotidase inhibitor, Pcr. (3) A small increase in AMP (via the myokinase reaction), the substrate for 5'-nucleotidase in the formation of adenosine. This hypothesis for the regulation of 5'-nucleotidase activity, which requires validation in the in vivo situation, is presented schematically in Figure 2.

**Mechanism of Action of Adenosine**

How adenosine elicits relaxation of the coronary vascular smooth muscle is still unknown. One possible mechanism is via production of an increase in vascular smooth muscle cyclic AMP which has been reported to be associated with vascular smooth muscle relaxation (Triner et al., 1971). In brain slices, adenosine elicits a significant increase in cyclic AMP (Sattin and Rall, 1970), but in strips of media of hog carotid arteries or dog coronary arteries, an increase in cyclic AMP could be produced only at pharmacological concentrations of adenosine (Herlihy et al., 1976). Furthermore, adenosine failed to increase cyclic AMP levels in dispersed smooth muscle cells from rat aorta (Nickols and Brooker, personal communication). Hence, it does not seem likely that this relaxing effect of adenosine on coronary arterioles is via an increment in cyclic AMP.

A more likely possibility is that adenosine blocks calcium uptake by the vascular smooth muscle cells, or that it interferes with calcium utilization in the contractile machinery of the tissue. Studies on skeletal muscle have indicated that adenosine inhibits caffeine-induced rigor which is attributed to excess Ca^{2+} (DeGubareff and Sleator, 1965; Berne et al., 1976). Furthermore, in superfused guinea pig atrium in which the fast sodium channels were blocked with tetrodotoxin or by partial depolarization with K^+, and in which the slow inward Ca^{2+} current was evident when the tissue was electrically stimulated in the presence of norepinephrine, the addition of adenosine (10^{-6}M) to the bath abolished the induced action potential (Schrader et al., 1974). Destruction of the adenosine by addition of adenosine deaminase promptly restored the slow action potential. Finally, in guinea pig atria, where the overshoot of the slow action potential has a slope of 30 mV per decade change in Ca^{2+}, adenosine produced a downward displacement of the curve without changing its slope (Belardinelli et al., 1979). This could be caused by a decrease in the number of Ca^{2+} channels or a decrease in their permeability to Ca^{2+}. We have carried out 45Ca^{2+} flux studies with media strips of hog carotid and dog coronary arteries and with dispersed vascular smooth muscle cells in the presence and absence of physiological concentrations of adenosine (10^{-6}M), but the results to date have been inconclusive. Therefore, although the evidence is suggestive of an adenosine effect via blockage of Ca^{2+} uptake and/or intracellular interference, the mechanism of action of the nucleoside on vascular smooth muscle remains unclear.

It appears that the vasodilator effect of adenosine is initiated by attachment to an adenosine receptor on the coronary myocytes. High molecular weight conjugates of adenosine and AMP that pass through the coronary vascular bed intact fail to enter the myocardial cells, are not inactivated by adenosine deaminase, and are antagonized by theophylline—produce coronary dilation comparable to equimolar concentrations of adenosine or AMP (Olsson et al., 1976; Schrader et al., 1977c). Furthermore, adenosine-induced dilation of the coronary resistance vessels is not affected by adrenergic blocking agents, indicating that the adenosine receptor is separate from the adrenergic receptors.
Interaction of Adenosine with Other Agents

To what extent adenosine acts alone in altering coronary vascular resistance, and to what extent other factors play a significant role, either independently or in conjunction with adenosine, is still obscure. As noted earlier in this review, an increase in hydrogen ion concentration has been found to potentiate the vasodilator action of adenosine (Merrill et al., 1978), probably by increasing the rate of adenosine production by the myocardium (Mustafa and Mansour, 1980). Hence, under conditions of inadequate myocardial oxygenation and acidosis, more adenosine is released by the heart, which results in a greater increase in CBF and restoration of the oxygen balance of the myocardium. Strong evidence for participation of $P_0_2$ per se, potassium, calcium, inorganic phosphate, or increase in osmolarity in the regulation of CBF is lacking.

Adenosine also inhibits isoproterenol-induced increases in cardiac contractility (dP/dt) and cyclic AMP, as well as inhibiting adenylate cyclase activity in a membrane fraction of guinea pig heart (Schrader et al., 1977b), and shows similar effects in the isolated perfused rat heart in addition to an inhibition of cyclic AMP-dependent protein kinase and glycogen phosphorylase (Dobson, 1980). Adenosine release is also increased by catecholamines (Katori and Berne, 1966; Wiedmeier and Spell, 1977; McKenzie et al., 1980a, in press), presumably by their enhancement of myocardial metabolism. Hence, adenosine, in addition to increasing CBF, may aid in achieving oxygen balance by opposing the increased contractility and hence the additional oxygen needs of the heart that result from catecholamines or cardiac sympathetic nerve stimulation.

In summary, adenosine fulfills almost all of the criteria for a mediator of metabolic regulation of the coronary circulation and appears to function as the agent that can rapidly adjust CBF to changing oxygen requirements of the heart under different physiological and pathophysiological conditions. However, there are certain problems that still need resolution, such as, (1) whether free and/or bound adenosine is normally in the myocardial cell, and if so, to what extent it arises from $5'\text{-nucleotidase}$ and from $\text{S-adenosylhomocysteine}$, (2) the reason for the different effects of dipyridamole and theophylline (and like compounds) on exogenous and endogenous adenosine, (3) the true ISF concentration of adenosine under different physiological conditions, (4) elucidation of the mechanism of action of adenosine, (5) the means whereby adenosine production by the myocardium is controlled, and (6) definitive evidence for a direct cause-and-effect relationship between cardiac adenosine release and CBF.

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