The role of adenosine in regulation of cardiac and skeletal muscle blood flow during exercise: an overview

I P Goonewardene[1]

The Ceylon Journal of Medical Science 1997; 40: 43-49

Summary

Adenosine (ADO) is known to have vasodilatory properties, and is produced endogenously during increased activity in many tissues including skeletal and cardiac muscle. The vasodilatory action of ADO was first reported by Drury and Szent-Gyorgy in 1929, but it was in 1963 that Berne put forward his adenosine hypothesis without real evidence that ADO may be an important mediator of metabolic regulation of blood flow during exercise. Subsequently, several groups of investigators attempted to determine the role and contribution of ADO in coronary active hyperaemia and exercise hyperaemia in skeletal muscle by using in vitro and in vivo studies on different species of animals and subsequently in clinical use.

By using both biochemical methods (such as high performance liquid chromatography) and pharmacological methods (such as methylxanthines, dipyridamole and adenosine deaminase), the role of ADO in exercise vasodilation was investigated in several species of animals. Using a specific pharmacological tool, adenosine deaminase, it has now been established that ADO contributes and mediates sustained exercise vasodilation in skeletal and cardiac muscle. ADO was found to be more important in the mediation of exercise induced sustained coronary active hyperaemia than in active hyperaemia in skeletal muscle. However, it appears unlikely that ADO plays any important role in the initiation of active hyperaemia in the skeletal or cardiac muscle.

As a result of these extensive studies on the actions of ADO, the emerging roles of ADO have now been identified, and has been used therapeutically in patients in the diagnosis and treatment of supraventricular tachycardias, and will soon be available for cardiac imaging. As a result of its coronary vasodilator and other properties, in the future ADO is likely to be available for use as a protective agent in acute myocardial infarction and reperfusion injury.

Introduction

More than a hundred years ago, Gaskell (1) first observed that blood flow increased several hundred percent from resting levels during muscular exercise. The mechanism of this exercise hyperaemia has always been controversial. Current evidence suggests that myogenic and local metabolic factors may be involved in causing relaxation of the vascular smooth muscle. The contribution of one such factor, adenosine (ADO), to sustained exercise vasodilatation in skeletal muscle and myocardium is presented in this review.

Adenosine hypothesis

The vasodilatory action of ADO was first reported by Drury and Szand-Gyorgy (2) in 1929, but it was in 1963, that Berne (3) put forward his hypothesis without real evidence, that ADO may be an important mediator of the metabolic regulation of blood flow. This hypothesis was first tested in skeletal and cardiac muscle under ischaemic and non ischaemic conditions (4). ADO appeared in cardiac but not in skeletal muscle following ischaemia. They concluded that although ischaemia was required to demonstrate the release of ADO in cardiac muscle, its appearance was consistant with the hypothesis that under physiological conditions, quantities of ADO undetectable by their analytical methods may play a role in the regulation of coronary blood flow. However, for the skeletal muscle, they rejected the adenosine hypothesis.

1. Senior Lecturer, Department of Physiology, Faculty of Medicine, Talagolla Road, Ragama, Sri Lanka.

1. Biochemical methods used to test the adenosine hypothesis

Subsequent improvements in analytical techniques enabled several groups of investigators to detect ADO in muscle tissue, and also in venous blood draining skeletal and cardiac muscle during increased activity under restricted as well as under free flow conditions (5,6). However, only the work of Ballard *et al* in 1987 (7) attempted to assess the contribution of this released ADO to active vasodilatation by using high performance liquid chromatography (HPLC). They found that ADO contributed to about 40% during sustained vasodilatation. Their assessment of the contribution of ADO however, might have been an under estimation since the venous ADO concentration may not have truely reflected the actual interstitial ADO levels due to the endothelial cell barrier (8), and also due to some loss of ADO during its passage up to the venous sampling port and in subsequent analysis. However, using HPLC, a good correlation has been observed between the release of ADO from skeletal muscle and the intensity of muscle contractions in gracilis muscles of anaesthetized dogs (9).

2. Pharmacological methods used to test the adenosine hypothesis

2.1 Use of methylxanthines

2.1.1. Use of methylxanthines in skeletal muscle

Because of the above criticisms of biochemical methods, pharmacological methods such as methylxanthines (theophylline and aminophylline), which are ADO receptor blockers, have been used to test the adenosine hypothesis in the skeletal muscle under both constant and free flow conditions (10). However, these have led to controversial results, probably due to their direct effects on blood vessels (11), and a lack of adequate concentrations to block the ADO effect on blood vessels.

By using adenosine deaminase (ADA) and theophylline, Proctor (10) observed that these agents reduced arteriolar dilatation by 22-40% during 2,5 and 10 Hz contractions of the hamster cremaster muscle under free flow condition. More recently, Poucher *et al* (12) in 1990 observed that free-flow exercise hyperaemia induced by 1 Hz stimulation of isolated gracilis muscles in anaesthetized cats for 20 min was significantly attenuated by 8-phenyl theophylline and concluded that ADO contribute about 40% to free-flow exercise hyperaemia.

The use of methylxanthines in the above investigations had led to conflicting results due to the direct vasodilating effects of these agents (11), and the inability to block the dilatory effect of ADO in states of acidosis (14). It would seem therfore, that methylxanthines are not suitable for determination of the contribution of ADO in active hyperaemia.

2.1.2. Use of methylxanthines in the heart

In the heart also, several groups of investigations demonstrated that the methylxanthines could block the vascular adenosine receptors (14,15). Afronso and O'Brien in 1971 (16) found that aminophylline attenuated 50-80% of the coronary vasodilator action of exogenous ADO administered intravenously or directly into the coronary artery in dogs but it did not inhibit coronary vasodilation induced by hypoxia (16).

Randall and Jones (17) in 1985 were able to demonstrate that aminophylline could actually attenuate pacing induced coronary hyperaemia although the same result could not be obtained on using isoproterenol instead of pacing (14). Their failure to demonstrate a role of ADO in isoproterenol induced coronary active hyperaemia was attributed to the manner in which cardiac activity was increased. The use of atrial pacing seems to be a better and a more physiological method than the use of adrenergic drugs to study the role of ADO in active hyperaemia, since these adrenergic drugs have direct effects on the alpha and beta receptors on coronary vessels (18). Thus the use of methylxanthines as pharmacological agents to study the role of ADO in reactive and active hyperaemia have led to conflicting results.

2.2 Use of dipyridamole

2.2.1. The use of dipyridamole in skeletal muscle

Another group of pharmacological agents, which have been used to test the adenosine hypothesis in both skeletal muscle and myocardium are the drugs, which inhibit the cellular uptake of ADO in tissues, thereby potentiating the ADO effect. Dipyridamole is such an ADO action potentiator (19). If ADO functions as a vasodilating metabolite in contracting muscle, then dipyridamole could be expected to potentiate the active hyperaemia. Therefore, dipyridamole has been used by several groups of investigators (11,19), who observed a potentiation of vasodilatation, but only under certain experimental conditions such as contractions during constant low flow perfusion (11) and during maximal tetanic contractions (19,20). However, under each of these conditions, blood flow and hence oxygen delivery was either experimentally restricted or mechanically impaired and could not be considered to be physiological. Later Klabunde and colleagues (19,20) observed that dipyridamole potentiated the post tetanic hyperaemia by about 50-70% following free-flow 40 Hz tetanic contractions of 10 s duration in the gracilis muscle in anaesthetized dogs. They concluded that the changes in post tetanic hyperaema and recovery times produced by dipyridamole were consistant with the hypothesis that ADO contributed to it. Their findings also contradict their earlier results, which could be explained by the fact that the measurement of tissue ADO content did not represent the interstitial ADO (21).

Lately, Laughlim and colleagues in 1989 (22) examined the contribution of ADO to 2 min of treadmill induced moderate (11.2 km/hr) and maximal (17.6 km/hr) exercise hyperaemia in conscious miniature swine. They observed that dipyridamole potentiated the blood flow to all muscles during maximal exercise, but it potentiated the blood flow only to the gracilis muscles (contains slow twitch oxidative fibres) during moderate exercise. Since ADO was shown to play a role in active hyperaemia during maximal exercise, they suggested that the muscle blood flow might have been restricted in this condition, because the cardiorespiratory oxygen transport could have reached maximal. However, their results have demonstrated that oxygen utilization of the muscles increased with exercise intensity. Furthermore, Laughlim *et al* used radio labelled microsphere technique to measure the blood flow, which is not as accurate as electromagnetic flowmetry. More recently, it has been observed that specific adenosine receptor antagonist 1,3, dipropyl 8-p-sulpho-phenylxanthine (DPSPX; 10^{-5}M) attenuated the muscle exercise hyperaemia in anaesthetized rabbits, and dipyridamole induced potentiation of exercise hyperaemia was reversed by DPSPX (23).

The species difference, the use of conscious and anaethetized animals, and the methods by which the exercise were induced could also have contributed to the differences in results reported (20,22,23).

2.2.2. Use of dipyridamole in the heart

Dipyridamole has also been used to establish the role of ADO in coronary active hyperaemia. Knabb and colleagues in 1983 (24) used dipyridamole and demonstrated significant positive correlation between coronary blood flow and pericardial fluid ADO concentrations during coronary active hyperaemia in anaesthetized dogs and their results suggested that interstitial ADO levels were increased by the actions of dipyridamole.

2.3. Use of adenosine deaminase

2.3.1. The use of adenosine deaminase in skeletal muscle

Adenosine deaminase (ADA) preparations have been used by different groups of investigators to study the role of ADO in exercise hyperaemia in skeletal muscle (10,25,26). ADA is considered a reliable investigative agent for this purpose, as it specifically metabolises ADO into vasoinactive inosine (27).

Other investigators also studied the effect of continuous superfusion of ADA on the microcirculation of the hamster cremaster

muscle during twitch contractions and were able to demonstrate a significant attenuation (20-25%) of exercise vasodilatation during ADO infusion (10,25). The role of ADO in active hyperaemia in skeletal muscle has also been examined in conscious rats during normal locomotor activity by using ADA (26). Since they used radiolabelled microspheres to measure the blood flow, the control and test observations were not carried out on the same animal. Therefore, the results of investigations in which ADA has been used have not led to unequivocal evidence for a contribution of ADO to muscle active hyperaemia because of a number of limitations in the experimental protocols and methods.

2.3.2. The use of adenosine deaminase in the heart

ADA has also been used by several groups of investigators to examine the role of ADO in the regulation of coronary blood flow under different conditions, such as reactive hyperaemia, hypoxic hyperaemia and active hyperaemia (6,8,28,29). This agent was used for the first time by Saito *et al* in 1981, who were able to reduce the reactive hyperaemia by 30-39% following 5-30s occlusions of the left circumflex coronary artery in anaesthetized dogs (28).

Gewirtz and colleagues in 1986 (29) observed that intracoronary administration of ADA ($10 U kg^{-1} min^{-1}$) did not change the basal coronary blood flow in anaesthetized pigs, and concluded that ADO was not important in the regulation of arteriolar tone under basal conditions in the normal coronary circulation. Their findings were in agreement with that of Kroll and Feigl (31), who in 1985 used intracoronary ADA (107 U min^{-1} in dogs weighing between 25-35 kg), and could not find any difference in the basal coronary blood flow in the presence and absence of ADA solution. They concluded therefore, that ADO was unimportant in controlling coronary blood flow in unstressed hearts of anaesthetized dogs.

Several groups of investigators also used ADA to examine the contribution of ADO in coronary hyperaemia (29,30). Using a dose of 10 U kg^{-1} min^{-1} of ADA, Gewirtz *et al* (30) observed, in intact paced hearts of anaesthetized pigs, ADO contributed about 30% to the flow response at the first minute of stimulation of the heart, but not to the sustained flow response at the 10 min of isoproteronol infusion. However, these conditions cannot be considered as ideal to test the contribution of ADO in active hyperaemia, because of the beta 2 agonist properties of isoproterenol on the coronary blood vessels, which would have caused some coronary vasodilation (32). By using ADA (5 U kg^{-1} min^{-1}), Bache *et al* (29) in 1988 failed to demonstrate a role of ADO in mediating coronary vasodilatation during treadmill exercise in chronically instrumented dogs.

These conflicting results could have been due to the reasons given below: the use of different species of animals such as pigs (30) and dogs (29); the different methods used for increasing cardiac activity such as exercise (29), isoproterenol (30) and norepinephrine (18), the use of anaesthetized animals (30) and conscious animals (29).

Therefore, at attempt was made to determine the exact contribution of ADO to exercise vasodilatation in the skeletal muscle and denervated heart in the same animal species (dogs) by using ADA (33,34,35). This investigation was the first detailed study carried out to determine the contribution of ADO to exercise vasodilatation in a relatively less active tissue (skeletal muscle) and in a highly active tissue (cardiac muscle) under various flow conditions by using ADA.

Vascularly and neurally isolated gracilis muscles were perfused at constant high flow (99% of maximum of maximum free flow), and electrical stimulation of the nerve (4 Hz, 6v, 0.2ms) supplying the muscle for 3-5 min resulted in twitch contractions of the muscle and a fall in arterial perfusion pressure by about 40% due to exercise vasodilatation (34). However, ADO did not mediate the initiation of exercise vasodilatation.

Isolated gracilis muscles when electrically stimulated by 40 Hz (6v. 0.2ms) to produce tetanic contractions, experienced post tetanic

hyperaemia, which in the presence of ADA was attenuated by about 30%, thus confirming a significant role of ADO in the mediation of post tetanic hyperaemia under free-flow condition (33). However, ADA did not attenuate the initial hyperaemia response observed at 10s after the withdrawal of the tetanic stimulus (33).

Twitch contractions of free flow perfused gracilis muscles resulted in an attenuation of the free-flow hyperaemia response by about 25% (33). ADA did not attenuate the initiation of exercise hyperaemia (10s after commencement of muscle stimulation).

The contribution of ADO to coronary hyperaemia was also assessed by these workers (35,36). The anterior descending or the circumflex branch of the left coronary artery of the dog was cannulated and the heart was perfused via an extracorporeal circuit with blood from the left subclavian artery. ADA siginficantly attenuated the increase in vascular conductance during sustained coronary active hyperaemia induced by atrial pacing, and ADO appears to be much more important in the metabolic regulation of blood flow to the myocardium than to the skeletal muscle (36).

3. Possible clinical applications of adenosine research

More recently, these beneficial effects of ADO have been applied successfully in clinical use (37,38). Adenosine has been found to be not only a regulator of coronary blood flow, but is also a regulator of cardiac rhythm, and is useful in the diagnosis and treatment of paroxysmal supraventricular tachycardias (37,38). In addition, ADO will soon be available for cardiac imaging and could be used as a protective agent against acute myocardial infarction and reperfusion injury as has been shown in animal models (37).

Conclusions

It is well established that ADO is a potent vasodilator, and plays an important role in the regulation of blood flow in the skeletal muscle and myocardium during exercise under varying conditons. It appears to play a more important role in the mediation of sustained coronary functional hyperaemia than in the sustained skeletal muscle exercise hyperaemia. However, it appears unlikely that ADO plays any important role in the initiation of active hyperaemia in the skeletal or cardiac muscle. Due to its vasodilator and other effects, ADO is likely to be used in cardiac imaging as well as a cardioprotective agent against myocardial infarction and reperfusion injury in clincal practice in the near future.

References

1. Gaskell W H. On the changes of the blood stream in muscle through stimulation of their nerves. Journal of Anatomy & Physiology 1877; 11: 360-402.
2. Drury A N, Szent-Gyorgyi A. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart Journal of Physiology (London) 1929-1930; 68: 213-236.
3. Berne R M. Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. American Journal of Physiology 1963; 204: 317-322.
4. Imai S, Riley A, Berne R M. Effect of ischaemia on adenine nucleotides in cardiac and skeletal muscle. Circulation Research 1964; 15, 443-450.
5. Dewitt D F, Wangler R D, Thompson C I, Sparks H V Jr. Phasic release of adenosine during steady state metabolic stimulation in the isolated guinea pig heart. Circulation Research 1983; 53: 636-643.
6. Ballard H J, Cotterrell D, Karim F. Analysis of submicromolar concentrations of adenosine in plasma using reversed phase high performance liquid chromatography, Journal of Pharmaceutical and Biomedical Analysis 1986; 4: 207-219.
7. Ballard H J, Cotterrell D, Karim F. Appearance of adenosine in venous blood from the contracting gracilis muscles and its role in vasodilation in the dog. Journal of Physiology (London) 1987; 387: 401-413.

8. Gorman M W, Bassingthwaighte J B, Olsson R A, Sparks H V. Endothelial cell uptake in adenosine in canine skeletal muscle. American Journal of Physiology 1986; 250: H482-H489.

9. Achike F I, Ballard H J. Influence of stimulation parameters as the release of adenosine, lactate and CO_2 from contracting dog gracilis muscle. Journal of Physiology (London) 1993; 463: 107-121.

10. Proctor K G. Reduction of contraction induced arteriolar vasodilation by adenosine deaminase or theophylline. American Journal of Physiology 1984; 247: H195-H205.

11. Honig C R, Frierson J L. Role of adenosine in exercise vasodilation in dog gracilis muscle. American Journal of Physiology 1980; 238: H703-H715.

12. Poucher S M, Nowell C G, Collis M G. The role of adenosine in exercise hyperaemia of the gracilis muscle anaesthetized cats. Journal of Physiology (London) 1990; 427: 19-29.

13. Afonso S. Inhibition of coronary vasodilation action of dipyridamole and adenosine by aminophylline in the dog. Circulation Research 1970; 26: 743-752.

14. Jones C E, Hurst T W, Randall J R. Effect of aminophylline on coronary functional hyperaemia and myocardial adenosine. American Journal of Physiology 1982; 243: H480-487.

15. Afonso S, O'Brien G S. Mechanism of enhancement of adenosine action by dipyridamole and lidoflazine. Archives of International Pharmacodynamics 1971; 194: 181-196.

16. Afonso S, Ansfield T J, Berndt T B, Rowe G G. Coronary vasodilator responses to hypoxia before and after amonophylline. Journal of Physiology (London) 1970; 221: 589-599.

17. Randall J R, Jones C E. Adenosine antagonist aminophylline attenuates pacing induced coronary functional hyperaemia. American Journal of Physiology 1985; 248: H1-H7.

18. Mohrmann D E, Feigal O. Competition between sympathetic vasoconstriction and metabolic vasodilation in the canine coronary circulation. Circulation Research 1978; 42: 79-86.

19. Kille J M, Klabunde R E. Adenosine as a mediator of post contraction hyperaemia in dog gracilis muscle. American Journal of Physiology 1984; 246: H274-282.

20. Klabunde R E. Attenuation of reactive and active hyperaemia by sympathetic stimulation in dog gracilis muscle. American Journal of Physiology 1986; 251: H1183-1187.

21. Klabunde R E. Conditions for dipyridamole potentiation of skeletal muscle active hyperaemia. American Journal of Physiology 1986; 250: H62-67.

22. Laughlim H M, Klabunde R E, Delp M D, Armstrong R B. Effects of dipyridamole on muscle blood flow in exercising miniature swine. American Journal of Physiology 1989; 257: H1507-1515.

23. Persson M G, Ohlen A, Lindban L, Hedquist P, Gustafsson L E. Role of adenosine in functional hyperaemia in skeletal muscle as indicated by pharmacological tools. Archives of Pharmacology 1991; 343: 52-57.

24. Knabb R M, Ely S W, Bacchus A N, Rubio R, Berne R M. Consistant parallel relationships among myocardial oxygen consumption, coronary blood flow and pericardial infusate adenosine concentration with various interventions and ß blockade in the dog. Circulation Research 1983; 53: 33-41.

25. Proctor K G, Duling B R. Adenosine and free flow functional hyperaemia in striated muscle. American Journal of Physiology 1982; 242: H688-697.

26. Klabunde R E, Laughlim H M, Armstrong R B. Systemic adenosine deaminase administration does not reduce active hyperaemia in running rats. Journal of Applied Physiology 1988; 64: 108-114.

27. Olsson R A. Intra-arterial adenosine deaminase: a tool for assessing physiolgic functions of adenosine. Topics and perspectives in adenosine research 1987; 1: 438-444.

28. Saito D, Steinhart C R, Nixon D G, Olsson R A. Intracoronary adenosine deaminase reduces canine myocardial reactive hyperaemia. Circulation Research 1981; 49: 1262-1267.

29. Bache J J, Dai X Z, Schwartz J S, Homans D C. Role of adenosine in coronary vasodilation during exercise. Circulation Research 1988; 62: 846-853.

30. Gewirtz H, Olsson R A, Most A S. Role of adenosine in mediating myocardial blood flow response to isoproterenol: observations in closed chest, sedated, domestic swine. Cardiovascular Research 1986; 20: 504-511.

31. Kroll K, Feigl E O. Adenosine is unimportant in controlling coronary blood flow in unstressed dog hearts. American Journal of Physiology 1985; 249: H1176-1187.

32. Pitt B, Elliot E C, Gregg D E. Aderenergic receptor activity in the coronary arteries of the unanaesthetized dog. Circulation Research 1967; 26: 75-84.

33. Goonewardene I P, Karim F. Effect of adenosine deaminase on free-flow post tetanic hyperaemia in anaesthetized dogs. Journal of Physiology (London), 1989; 416:598p.

34. Goonewardene I P, Karim F. Attenuation of exercise vasodilatation by adenosine deaminase in anaethestized dogs. Journal of Physiology (London) 1991; 442: 65-79.

35. Karim F, Goonewardene I P. The effect of adenosine deaminase on pacing induced coronary functional hyperaemia in denervated hearts of anaesthetized dogs. Journal of Physiology (London) 1991; 438:134p.

36. Karim F, Goonewardene I P. Adenosine and sustained coronary functional hyperaemia in anaesthetized dogs. Journal of Physiology (London) 1996; 490: 793-803.

37. Teplitz C. The cardiovascular effects of adenosine: clinical use and nursing implications. Progress in Cardiovascular Nursing 1993; 8: 20-28.

38. Pelleg A. Adenosine in the heart: its emerging roles. Hospital Practice 1993; 28: 71-74.