How does dipyridamole elevate extracellular adenosine concentration?

Predictions from a three-compartment model of adenosine formation and inactivation

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INTRODUCTION

Adenosine appears to be an important mediator of blood-flow autoregulation to correct an imbalance between ATP synthesis and degradation (Berne et al., 1983). The ability of adenosine to dilate the coronary arterioles, thus increasing energy supply, but to constrict the renal artery, thus decreasing metabolic load, is consistent with this general hypothesis (Newby, 1984). Dipyridamole, dilazep and hexobendine are likewise coronary dilators (Kubler et al., 1970; Nott, 1970; McInnes & Parratt, 1969; Buyinski et al., 1972) whereas dipyridamole is a renal vasodilator (Arend et al., 1985). Since both the coronary dilator and renal constrictor effects of dipyridamole are reversed by adenosine receptor antagonists (Afonso, 1970; Arend et al., 1985) it has been concluded that dipyridamole acts by potentiating the effects of endogenously-generated adenosine. Dipyridamole, dilazep and hexobendine are, at vasoactive concentrations, inhibitors of the unspecific symmetric nucleoside transporter (Kubler et al., 1970; Turnheim et al., 1978; Paterson et al., 1984). They are, however, approximately equipotent in inhibiting influx and efflux of adenosine (Kubler et al., 1970; Kukovetz & Poch, 1971/2; Degenring et al., 1976; Schutz et al., 1981; Dobson & Schrader, 1984; Belloni et al., 1985; Meghji et al., 1985). There is controversy, therefore, as to whether and how they can increase the concentration of adenosine at extracellular receptor sites on coronary vascular smooth muscle.

To investigate this further, mathematical models were constructed according to which adenosine is formed in the cytoplasm of a group of cells, rises in the extracellular space via the symmetric nucleoside transporter and is inactivated in the adenosine forming cells and after rate-limiting transport into other cell-types. Dipyridamole increases the \( K_m \) and \( V_{max} \) of the transporter symmetrically with respect to influx and efflux. 2. Models incorporating differing degrees of compartmentation are used to predict intracellular and extracellular adenosine concentration as a function of dipyridamole concentration and adenosine formation rate. 3. The vasodilator action of dipyridamole is explained since it is predicted to elevate interstitial fluid adenosine concentrations at all rates of adenosine formation provided that washout of the interstitial compartment is restricted.

models are used to predict the influence of nucleoside transport inhibition and of adenosine formation rate on the steady-state concentration of adenosine in the adenosine-forming cells and in the extracellular (interstitial) compartment.

METHODS

The derivation of equations is given in the Appendix. Predictions of the model were computed with the aid of an Apple IIE (Apple Computer, Cupertino, CA, U.S.A.) microcomputer.

Values of parameters used in the illustrative examples

The value used for the \( K_m \) for adenosine of the nucleoside transporter (0.1 mM) is that determined for rat, mouse and human cells (Lum et al., 1979;
Plagemann & Wohlheuter, 1980; Paterson et al., 1984). In rat cells the inhibition by dipyridamole is mixed with $K_s = 3 \mu M$ and $K_i = 12 \mu M$ (Plagemann & Wohlheuter, 1980). In other species, including humans, the inhibition may be competitive with a $K_i$ in the range 10–300 nM (Hammond et al., 1981; Paterson et al., 1980; Jarvis et al., 1982; Plagemann & Wohlheuter, 1984); a value of 0.1 $\mu M$ is used. Dipyridamole concentrations found in plasma during pharmacological responses to the drug (0.1–10 $\mu M$: Sollevi et al., 1984; Pedersen, 1979) are modelled. The value for the $V_{max}$ of the human erythrocyte nucleoside transporter at 37 °C (approx. 100 pmol/s per ml of cell water: Paterson et al., 1984) is used. This translates to approx. 4 $\mu M$/min per g wet wt. which is arbitrarily divided into two equal pools (i.e., $U = V = 2 \mu M$/min per g wet wt.). A $K_m$ value of 25 $\mu M$ for adenosine deaminase and a $K_m$ value of 1 $\mu M$ for adenosine kinase are considered (Arch & Newsholme, 1978). Rates of adenosine formation of 2 nmol/min per g wet wt. in the normoxic state (Achterburg & De Jong, 1984) and 20 nmol/min per g wet wt. in the fully-stimulated state (Newby et al., 1983; Edlund et al., 1983) are modelled. Flow rates of perfused heart preparations from 2 to 20 ml/min per g wet wt. are used to model washout (Degenring et al., 1976; Frick & Lowenstein, 1976). In the legends to the Figures, 1 unit of enzyme activity = 1 nmol/min.

RESULTS

Model 1: complete cellular compartmentation of adenosine forming and inactivating pathways

The simplest three-compartment model is obtained if no metabolism of adenosine to inosine or rephosphorylation to AMP occurs in the adenosine-forming cells (see Fig. 1). This yields an equation (see the Appendix) for the extracellular adenosine concentration:

$$S_o = K_m(1 + I/K_i)/(V/\nu I - 1)$$

from which competitive inhibition of the transporter by increasing concentrations of dipyridamole is predicted to cause a proportional increase in $S_o$ (Fig. 2). Non-competitive inhibition is also predicted to elevate $S_o$ (see Appendix eqn. 5 and Fig. 2). To understand these predictions it should be noted that the model also predicts increases in adenosine concentration inside the adenosine-forming cells (Fig. 2). In fact the rates of transport of adenosine out of and into the adenosine-forming cells are independent of dipyridamole concentration; the increases in intracellular and extracellular adenosine concentration exactly compensating for the changes in $K_m$ and $V_{max}$. The ratio of predicted intracellular to extracellular adenosine concentration is dictated (under the range of conditions modelled) largely by the proportion of total nucleoside transporter activity which is in the adenosine-forming cells (see Appendix eqn. 4). When this is 50%, the predicted adenosine concentration in the adenosine-forming cells is approximately twice that in the interstitial compartment (Fig. 2).

The model also predicts a linear rise in interstitial adenosine concentration as the rate of adenosine formation increases provided that this is much less than the $V_{max}$ of the nucleotide transporter.

Model 2: metabolism of adenosine inside the adenosine-forming cells

Increasing activities of adenosine deaminase or adenosine kinase inside the adenosine-forming cells reduce both the concentration of adenosine predicted in the absence of dipyridamole and dampen the rise due to nucleoside-transport inhibition (Fig. 3). The effect of adenosine kinase is more dramatic owing to its low $K_m$. The model predicts, none-the-less, that interstitial adenosine concentration is always increased by dipyridamole. This conclusion is independent of the values chosen for the inhibitor constants which do, however, dictate the concentration range over which a maximal effect is obtained. The reason for this increase is, again, the predicted rise in the adenosine concentration in the cytosol of the adenosine-forming cells. Indeed, the relationship between intracellular and interstitial adenosine concentration is the same in models 1 and 2. The difference between them is that the increase in adenosine concentration in the adenosine-forming cells no longer completely compensates for the inhibition of nucleoside efflux.

The factor by which dipyridamole is predicted to increase interstitial adenosine concentration is reduced as the proportion of total nucleoside transporter which is located in the adenosine-inactivating cells is reduced, for example from 50% to 20% (Fig. 3a, broken line). This increases the predicted extracellular adenosine concentration in the absence of nucleoside transport inhibitor from 0.086 $\mu M$ to 0.200 $\mu M$ but reduces the stimulation caused by a saturating concentration of dipyridamole from 6.24-fold to 4.59-fold. In the absence of any adenosine-inactivating cells, the model predicts no increase in extracellular adenosine concentration on adding dipyridamole. A similar pattern is produced by reducing the total activity ascribed to the transporter by 2-fold (Fig. 3a, dotted line). This decreases the predicted basal extracellular adenosine concentration to 0.152 $\mu M$ but reduces the stimulation by dipyridamole to 3.89-fold.
Dipyridamole elevates interstitial adenosine

Fig. 3. Influence of nucleoside transport inhibition on predicted adenosine concentrations allowing for metabolism in the adenosine-forming cells

Concentrations of adenosine in the extracellular space and inside the adenosine-forming cells (solid lines) were computed according to model 2 (see the Appendix) with \( n = 2 \) units/g, \( U = V = 2000 \) units/g, \( K_m = 100 \) \( \mu M \), \( K_I = 0.1 \) \( \mu M \) and \( T = 20, 40, 60 \) or 80 units/g as indicated. (a) \( K = 25 \) \( \mu M \) modellling adenosine deaminase; (b) \( K_T = 1 \) \( \mu M \) modelling adenosine kinase. The dotted line shows the concentrations when \( U = 1000, V = 1000 \) and \( T = 40 \) units/g. The broken line shows the extracellular concentration when \( U = 3200, V = 800 \) and \( T = 40 \) units/g.

If the transporter is ascribed a very low activity, inactivation after transport becomes insignificant compared with metabolism in the adenosine-forming cells and dipyridamole again fails to elevate adenosine concentration.

The presence of metabolizing enzymes in the adenosine-forming cells also reduces the extent to which interstitial adenosine concentration rises in response to an increase in adenosine formation rate (Fig. 4). This effect is progressively overcome at higher rates especially when the metabolizing enzymes become saturated. The increase is greater when the proportion of total nucleoside transporter activity in the adenosine-inactivating cells is reduced (Fig. 4, broken line).

Model 3: metabolism by both adenosine kinase and adenosine deaminase

To account precisely for both enzymes would require the solution by numerical methods of cubic equations (see the Appendix). At physiological concentrations of adenosine, however, well below the \( K_m \) of adenosine deaminase and that of the transporter, approximate solutions can be obtained algebraically. Using estimates of the activities of the transporter, the kinase and the deaminase which might be expected in myocardial tissue, interstitial adenosine concentrations in the low \( \mu M \) range are predicted at basal levels of adenosine formation (approx. 2 nmol/min per g). These may be elevated to the \( \mu M \) range at adenosine formation rates of 20 nmol/min per g, corresponding to extreme hypoxia (Fig. 5).

Dipyridamole is predicted to elevate the interstitial adenosine concentration at all adenosine formation rates (Fig. 5). Greater-fold elevations are obtained, however, at higher rates of adenosine formation. Indeed, if the rate of adenosine formation exceeds the \( V_{\text{max}} \) of the metabolizing enzymes, both models 2 and 3 reduce to a form similar to model 1.

Model 4: washout from the interstitial compartment

Washout of adenosine from the interstitial space introduces a term into the equation for nucleoside removal which is independent of dipyridamole concentration but linearly dependent on the interstitial fluid adenosine concentration (see the Appendix). Incorporation of this term into a simplified form of model 2 allows one to assess the effects of washout on predicted adenosine concentrations. Strikingly, the adenosine concentrations inside the adenosine-forming cells are not greatly influenced by washout (Fig. 6a). However, the interstitial adenosine concentration may be either elevated at low concentrations of dipyridamole and low rates of washout or reduced at high concentrations of dipyridamole and high rates of washout (Fig. 6b). The predicted concentration of dipyridamole which optimally elevates adenosine concentration is proportional to the value of the inhibitor constant used in the model, although the shape of the curves is not otherwise altered.
The rate of washout of adenosine, since it is proportional to the interstitial adenosine concentration, will show a similar dependence on dipyridamole concentration and on flow rate.

**DISCUSSION**

Relevance of the models to the physiological processes of adenosine formation, transport and inactivation

Cellular compartmentation of adenosine formation and inactivation has been demonstrated in the heart (Schrader & Gerlach, 1976; Schrader et al., 1977; Nees & Gerlach, 1983). Thus during ischaemia or increased work-load adenosine formation takes place in the cytoplasm of the cardiac myocytes (Schrader et al., 1981; Schutz et al., 1981) probably by the action of a cytoplasmic 5'-nucleotidase (Worku & Newby, 1983; Lowenstein et al., 1983). Using neonatal-rat heart cells in culture, Meghji et al. (1985) confirmed the cytoplasmic location of adenosine formation and demonstrated unequivocally that adenosine release occurs via the symmetric nucleoside transporter. Vascular smooth muscle and endothelial cells may be important sites of adenosine inactivation (Pearson et al., 1978) and indeed preferential incorporation of infused adenosine into these cell types occurs in the heart (Nees & Gerlach, 1983; Bassingthwaithe et al., 1985). As pointed out by Plagemann & Wohlheuter (1980), adenosine inactivation by cells not actively producing adenosine will at low nucleoside concentrations be limited by transport. This assumption can be tested mathematically (see the Appendix) and is valid particularly in the presence of transport inhibitors (Fig. 7). The vascular smooth muscle adenosine receptors are located in an interstitial compartment between the cardiac myocytes and the smooth muscle and endothelial cells, and washout from this compartment may be restricted by the endothelial barrier (Bassingthwaithe et al., 1985). Thus the mathematical models are based on the known features of adenosine formation, transport and inactivation and given reasonable estimates of the input parameters they predict interstitial fluid adenosine concentrations in the vasoactive range.

Precise comparison of the predictions of the models with experimental data must await the determination of the activities and distribution of the adenosine transporter and of the adenosine-metabolizing enzymes in the different cell types of the heart and other adenosine-sensitive tissues. Absolute rather than net rates of adenosine formation must also be measured.

**Implications for the action of dipyridamole**

The vasodilator action of dipyridamole has been cited as evidence both for and against the adenosine hypothesis of coronary blood-flow autoregulation. Kubler et al. (1970) argued that since dipyridamole inhibited the outflow of adenosine from buffer-perfused dog hearts it must reduce adenosine efflux from cardiac
Fig. 7. Is transport rate-limiting for inactivation of adenosine?

The thesis that nucleoside transport would be rate-limiting for nucleoside inactivation was tested by using values of parameters in human erythrocytes, $V = 4000$ units/g, $K_m = 100 \mu M$, $K_I = 0.1 \mu M$, $K_e = 25 \mu M$, $T_i = 630$ units/g, $K_f = 1 \mu M$, $T_e = 42$ units/g and fixed extracellular adenosine concentrations of 1, 11 and 21 $\mu M$ as indicated. The ratio of extracellular to intracellular adenosine concentration was approx. 2.5 in the absence of transport inhibitors and much greater in their presence, indicating that nucleoside transport would be rate-limiting for inactivation of adenosine particularly in the presence of transport inhibitors.

In terms of concentration, no restriction is placed on the size of the interstitial pool of adenosine which may, therefore, be small compared with the cytosolic pool. As predicted by model 4, release of adenosine into perfusates of tissues may be either increased (Wiener et al., 1983), decreased or unaltered by nucleoside-transport inhibitors, depending on the concentration of transport inhibitor used and on the efficiency of washout of the various interstitial compartments which communicate with the vascular lumen. The only essential conditions for the coronary vasodilator effect of dipyridamole are, according to these models, that there should exist cell types close to the vascular smooth muscle adenosine receptor that inactivate but do not readily form adenosine during hypoxia or ischaemia and that the receptors must be located in an interstitial compartment, the washout from which is restricted. These predictions must now be tested further.

REFERENCES


APPENDIX

DEFINITION OF SYMBOLS

As illustrated in Fig. 1:

\[ U = V_{\text{max}} \]

of transport in adenosine-forming cells;

\[ U^I = U/(1 + I/K_i^I) \]

in the case of non-competitive inhibition;

\[ V = V_{\text{max}} \]

of transport in adenosine-inactivating cells;

\[ V^I = V/(1 + I/K_i^I) \]

in the case of non-competitive inhibition;

\[ K_m = K_m \]

of the transporter;

\[ K_i^I = K_m(1 + I/K_i^I) \]

in the case of competitive inhibition;

\[ K_i^E = \text{competitive inhibitor constant of transporter} \]

\[ K_i^N = \text{non-competitive inhibitor constant of transporter} \]

\[ K_i^T = \text{constant of} \]

and \[ T_i^N = K_m \]

and \[ V_{\text{max}} \]

of the \[ n \]

enzyme inactivating adenosine:

\[ \nu_I = \text{rate of adenosine formation} \]

\[ \nu_I = \text{rate of influx of extracellular adenosine} \]

\[ I = \text{concentration of transport inhibitors} \]

\[ S_o = \text{extracellular fluid concentration of adenosine} \]

\[ S_I = \text{concentration of adenosine in the adenosine-forming cells} \]

\[ S_I^N = \text{concentration of adenosine in the adenosine-inactivating cells} \]

DERIVATION OF EQUATIONS

From the assumption of steady state:

\[ \nu_{\text{formation}} = \nu_{\text{inactivation after transport}} + \nu_{\text{metabolism}} \]

(1)

assuming that inactivation is rate-limited by transport and that all enzymic reactions obey Michaelis-Menten kinetics:

\[ \nu_I = \frac{S_o V^I}{K_m + S_o} + \frac{S_I T_i}{K_i^I + S_i} + \frac{S_I T_i^N}{K_i^N + S_i} \]

etc.

(2)

From constancy of extracellular adenosine concentration:

\[ S_o V^I + S_I U^I = S_I U^I \]

\[ K_m + S_o + K_i^I + S_i + K_i^N + S_i \]

Therefore:

\[ S_o = \frac{K_m^I}{(U^I + V^I)(1 + K_i^I/S_o) - 1} \]

(3)

Model 1: no metabolism within adenosine-forming cells

Assumption: \( \nu_{\text{metabolism}} = 0 \)

Then from eqn. (2):

\[ S_o = \frac{K_m^I}{(U^I/V^I)(1/I - 1)} \]

(5)

Substituting in eqn. (4) gives:

\[ S_I = \frac{K_m^I}{R/I - 1} \]

(6)

where:

\[ R = U^I V^I/(U^I + V^I) \]

Rate of efflux of adenosine:

\[ \frac{S_I U^I}{K_m^I + S_o} = \nu_I(1 + U^I/V^I) \]

(7)

Rate of influx of adenosine:

\[ \frac{S_o U^I}{K_m^I + S_o} = \nu_I U^I/V^I \]

(8)

Model 2: one metabolizing enzyme in adenosine-forming cells

Assumption: \( \nu_{\text{metabolism}} = \frac{S_I T}{K^T + S_I^N} \)

Substituting \( S_o \) from (4) into (2) gives:

\[ \nu_I = \frac{S_I R}{K_m^I + S_I} + \frac{S_I T}{K^T + S_I} \]

(9)

Therefore:

\[ S_o[I(T - R) + S_I[(I - T)K_m^I + (I - R)K^T]]+ \nu_I K_m^I T^I = 0 \]

(10)

\( S_I \) is the positive root of eqn. (10); \( S_o \) is obtained from eqn. (4).

Model 3: simplified system with two metabolizing enzymes in adenosine-forming cells

Assumptions: (i) for transport \( S_I < K_m^I \), \( S_o < K_m^I \);

(ii) for metabolism \( S_I < K^T \).

Substituting \( S_o \) from (4) into (2) gives:

\[ \nu_I = \frac{S_I R}{K_m^I + S_I} + \frac{S_I T}{K^T + S_I} \]

(11)

Therefore:

\[ S_o(RK^T + K_m^T T^I) + S_o(RK^T K^T + T, K_m^I K^T + T, K_m^I K^T + T) \]

\[ + T, K_m^I K^T - v_I K_m^I K^T - v_I K_m^I K^T = 0 \]

(12)

\( S_I \) is the positive root of eqn. (12); \( S_o \) is obtained from eqn. (4).
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Model 4: simplified system incorporating washout of adenosine from the extracellular space

Assumptions: (i) \( v_{\text{washout}} = CS_o \), where \( C \) is constant;
(ii) for transport \( S_o \ll K_m^o \);
(iii) for metabolism \( S_i \ll K_r^m \).

Eqn. (1) becomes:

\[
v_{\text{formation}} = v_{\text{inactivation}} + v_{\text{metabolism}} + v_{\text{washout}}
\]

Eqn. (3) becomes:

\[
\frac{S_o(U^1 + V^1)}{K_m^o} + CS_o = \frac{S_i U^1}{K_m^o + S_i}
\]

Therefore:

\[
S_i = \frac{K_m^o}{(U^1 + V^1 + CK_m^o) \cdot S_o - 1}
\]

Let:

\[
R = U^1 + V^1 + CK_m^o
\]

\[
\sigma = \frac{K_m^o}{S_o}
\]

Substituting in eqn. (2) gives:

\[
v_i = \frac{V^1}{\sigma} + \frac{K_m^o RT}{(\sigma U - R)K_r^o} + \frac{CK_m^o}{\sigma}
\]

\[
\sigma^2(U^1 K_r^o v_i) - \sigma[RK_r^o v_i + K_r^o U^1 (V^1 + CK_m^o) - K_m^o RT] + (V^1 + CK_m^o)K_r^o R = 0
\]

\( \sigma \) is the positive root of eqn. (10), from which follows \( S_o \) and from eqn. (4a) \( S_i \).

Is transport rate-limiting for inactivation?

Considering entry, exit and metabolism of adenosine in the cells which inactive adenosine:

\[
v_{\text{influx}} = v_{\text{efflux}} + v_{\text{metabolism}}
\]

At a fixed extracellular adenosine concentration, \( S_o \):

\[
v_i = \frac{S_o V^1}{K_m^o + S_o} = \frac{S_i V^1}{K_m^o + S_i + K_r^o + S_i + \ldots}
\]

Considering both adenosine kinase \( (T_r, K_r^o) \) and adenosine deaminase, for which \( S_i \ll K_r^m \) and simplifying so that \( S_i \ll K_m^o \):

\[
v_i = \frac{S_i V^1}{K_m^o + S_i + S_i + \frac{S_i T_r}{K_r^o + S_i + \frac{S_i T_r}{K_r^o}}}
\]

Therefore:

\[
S_i^2(V^1 K_r^o T_r + T_r K_m^o) + S_i(V^1 K_r^o T_r + T_r K_m^o K_r^o + T_r K_m^o K_r^o - T_r K_m^o K_r^o - v_i K_m^o K_r^o) - v_i K_m^o K_r^o K_r^o = 0
\]

\( S_i \) is the positive root of eqn. (18); if \( S_i \ll S_o \), transport is rate-limiting for metabolism.

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