A model for adenosine transport and metabolism

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1. A model is presented for adenosine transport and metabolism in different steady states. The model considers steady-state equations for metabolic enzymes based on information from the literature on their kinetic behaviour. 2. Assuming that extracellular adenosine and inosine are translocated by three transporters, we have devised rate equations for these nucleoside transporters which are valid when both nucleosides are present. Since the Na^+ -independent transporter can either incorporate nucleosides into the cell or release them, various conditions have been simulated in which inosine was either incorporated or released. 3. Control analyses are reported which show that the fluxes towards intracellular adenine nucleosides are controlled by ecto-5'-nucleotidase in some circumstances and by the nucleoside transporters in others. The nucleoside transporter is responsible for five fluxes (two Na^+ dependent adenosine transport mechanisms, a Na^+ -dependent inosine transport, a Na^+ -independent adenosine transport and a Na^+ -independent inosine influx or efflux) but the control is not always positive for all these fluxes. The control patterns of these five fluxes indicate that, in the presence of extracellular adenosine and inosine, the intracellular metabolism of adenine derivatives would be highly dependent on the extracellular and intracellular concentrations of both nucleosides, on the ectoenzymes (5'-nucleotidase and adenosine deaminase) and on the transporter. 4. Predictions of the model were examined. The results indicate that a change in one independent variable (extracellular AMP concentration) makes the system evolve towards a new steady state which is far from the initial one and has a different control pattern. In contrast, simulation of inhibition of the carriers produces only slight modification of the fluxes since the concentrations of the metabolites change to counteract the effect. Thus, for instance, a 50 % inhibition of the three carriers does not affect the flux towards intracellular adenine nucleotides. Finally, our model has confirmed that the evolution of the concentration of extracellular adenosine, when an increase in extracellular AMP is produced, agrees with the behaviour expected for a neurohormone.

INTRODUCTION

Although the key role of adenosine as a regulator of many physiological processes is now accepted, there has been considerable controversy about the origin of extracellular adenosine and its mechanisms of disappearance. Extracellular adenosine may appear by direct release from cells or by degradation of released adenine nucleotides, mainly ATP. On the other hand, disappearance (inactivation) was assumed to be by uptake, although from recent evidence [1,2] it is becoming clear that conversion of adenosine into inosine may be due to the ectoenzymic behaviour of adenosine deaminase. ('Transport' denotes the transfer of unmodified substrate across the cell membrane as mediated by a selective carrier. 'Uptake' denotes the total intracellular accumulation of compounds derived from the extracellular precursor, regardless of metabolic conversions.)

As a whole the appearance and disappearance of extracellular adenosine is a dynamic process which cannot be isolated from intracellular purine metabolism. Adenosine, once it enters the cell by mediated transport, can follow two distinct pathways as indicated in Fig. 1. It can be phosphorylated by adenosine kinase or it can be deaminated by intracellular adenosine deaminase. The proportion of nucleoside phosphorylated or deaminated depends upon the K_m of the kinase (in the micromolar range) and deaminase (K_m ten times higher than that of adenosine kinase) and the relative proportion of these two enzymes.

In addition, although there exists a Na^+ -dependent adenosine transporter which does not translocate inosine, extracellular inosine produced by ecto-adenosine deaminase can be transported by other Na^+ -independent and Na^+ -dependent carriers, which also translocate adenosine [3]. Inosine can follow the degradative pathway to uric acid or be converted into IMP via hypoxanthine. It has even been demonstrated that inosine

functions in the non-glycolytic pig erythrocytes as an '*in vivo*' energy substrate [4,5].

The aim of this paper is to gain insight into the mechanisms of extracellular and intracellular adenosine metabolism in different circumstances. To achieve this goal, we put forward an elaborate model which would seem to be sufficiently detailed to form the basis for realistic tests of the kinetic behaviour and control properties of the reaction system under conditions that are physiologically relevant.

THE MODEL

Two systems were studied, those described in Figs 1(a) and 1(b). The main difference between them is the direction of Na^+ -independent inosine flux across the cell membrane. Thus in Fig. 1(b) there is a net inosine efflux which, to maintain the steady-state, needs a flux of extracellular catabolism towards hypoxanthine. The destination of this extracellular hypoxanthine (incorporation by cells or elimination via plasma) was not studied in this paper. Also the whole study of intracellular purine metabolism was outside the scope of this paper. Notwithstanding, suitable arrows indicating fluxes towards compounds that are not considered as internal metabolites have been included as indicated in Fig. 1.

The first product to be considered is extracellular AMP which must result from breakdown of extracellular ATP and ADP. The final intracellular products were: (i) AMP with an exit (v_{18}) which reflects anabolic flux towards ADP and ATP, (ii) inosine with a withdrawal (v_9) which reflects catabolic flux towards hypoxanthine and (iii) IMP with a withdrawal (v_{17}) which reflects interconnecting flux towards GMP. Also in the case of Fig. 1(b) extracellular inosine should be considered a final product with a withdrawal (v_{18}) reflecting catabolic flux towards extracellular hypoxanthine.

Fig. 1. Diagram of the transport and metabolism of adenosine

Reactions are catalysed by the enzymes (or processes): E_1 , external 5'-nucleotidase (EC 3.1.3.5); E_2 , external adenosine deaminase (EC 3.5.4.4); E_3 , Na^+ -independent adenosine and inosine transporter; E_4 , Na^+ -dependent adenosine and inosine transporter; E_5 , Na^+ -dependent adenosine transporter; E_6 , internal adenosine deaminase (EC 3.5.4.4); E_7 , internal inosine withdrawal; E_8 , internal adenosine kinase (EC 2.7.1.20); E_9 , internal 5'-nucleotidase (EC 3.1.3.5); E_{10} , internal AMP deaminase (EC 3.5.4.6); E_{11} , internal adenylosuccinate synthase (EC 6.3.4.4); E_{12} , internal adenylosuccinate lyase (EC 4.3.2.2); E_{13} , internal AMP kinase (EC 2.7.4.3); E_{14} , internal IMP-GMP conversion; E_{15} , external inosine withdrawal. Reaction rates are indicated as fluxes in the text and they are known by the corresponding number: from 1 to 14 in system A_1 and A_2 and from 1 to 15 in system B_1 and B_2 . (a) The scheme corresponds to systems A_1 and A_2 (for further details see the text). (b) The scheme corresponds to systems B_1 and B_2 (for further details see the text).

In the case of a single enzyme catalysing two distinct reactions, the experimental V ratio for the different substrates has been maintained. For instance, in the case of internal 5'-nucleotidase, the ratio $V_{\text{AMP}}/V_{\text{IMP}}$ was equal to 1.61 which is the experimental ratio given by Mallol [6]. With this ratio, with the fluxes given in Figs. 1(a) and 1(b) and fixing $[\text{AMP}]_i$, $[\text{IMP}]_i$ is automatically fixed. Using the same criterion $[\text{inosine}]_i$ is automatically fixed when, for the transporters, the $V_{\text{inosine}}/V_{\text{adenosine}}$ ratio (0.68) is fixed along with $[\text{adenosine}]_i$, $[\text{inosine}]_i$ and $[\text{adenosine}]_o$.

Individual reaction mechanisms considered

The variation with time of the concentration of intermediate compounds is governed by the operation of enzyme-catalysed reactions within the pathway and by fluxes external to it. The formulation of the problem starts by considering rate equations in terms of mechanisms of the reactions catalysed by enzyme E_{14} (Fig. 1a) or E_{15} (Fig. 1b). Order numbers for rate equations (fluxes) corresponding to each reaction are indicated in Figs. 1(a) and 1(b). Maximum velocities for each enzyme were calculated so as to achieve the respective steady-state conditions (constant

fluxes and intermediate concentrations) displayed in Figs. 1(a) and 1(b). Maximum velocities for the system of Fig. 1(b) (case B_1) are indicated in the Appendix. Rate equations and all parameter values detailed in the Appendix have been chosen (or calculated) so as to be representative of the operation in rat kidney.

Concentrations and fluxes considered

The plasma concentrations of adenine nucleotides remain somewhat elusive [7,8]. Interstitial purine concentrations in individual organs could differ greatly from the systemic circulating concentrations and still remain difficult to assess [8]. Moreover, intracellular concentrations of these compounds vary from tissue to tissue. On the other hand, in the only report in which extracellular and intracellular adenosine concentrations are given, the data are not consistent with an adenosine influx. Thus, in rat striatum, Hagberg *et al.* [9] have found $1.9 \mu\text{M}$ -adenosine in the interstitial fluid whereas the intracellular concentration was $4.4 \mu\text{M}$; in such conditions adenosine release should occur. In our opinion the concentrations reported are indicative but not mandatory, since the concentrations of purine

compounds vary greatly with the treatment given to the animal before the determination. In summary, we have analysed the data taken from the literature [4,7–19] and have chosen for our model the intermediate concentrations that best agree with the low micromolar range of rat kidney. In order to increase the range of validity of our model, two different conditions in which extracellular adenosine concentration varies greatly were chosen. In cases A₁ and B₁ [adenosine]_o was relatively low (0.1 μM) whereas in cases A₂ and B₂ [adenosine]_o was higher (6.3 μM) (see below). Thus cases A₁ and B₁ may represent a basal situation in normoxic conditions whereas cases A₂ and B₂ could resemble an hypoxic situation. For other data, such as adenylosuccinate concentration, which are not readily obtainable we have selected figures from our previous work [20]. On the other hand, cellular [IMP] was automatically fixed when fixing the [AMP] (see above). Also the concentration of inosine was dependent on the activity of the nucleoside transporter since we maintained a fixed ratio of adenosine/inosine incorporation (see above).

With respect to the fluxes, the same kind of consideration as was made for concentrations applies. Fluxes are very dependent on the type of cell and the condition chosen (normoxic, hypoxic, etc). Thus, from a standard flux 1 (v_1) equal to 100 nmol/time unit we selected the remaining with the following conditions: (i) the anabolic flux is higher than the catabolic flux (60 versus 40; 60 towards ADP and ATP and 40 corresponding to IMP plus inosine withdrawal), (ii) the flux through the transporters is higher than that through ecto-adenosine deaminase (90 versus 10 considering that the relative importance of the extracellular degradation of adenosine remains obscure), (iii) adenosine is mainly transported by the Na⁺-dependent rather than the Na⁺-independent carrier (80 versus 20) [21], (iv) AMP catabolism proceeds mainly by deamination rather than by dephosphorylation [22], (v) intracellular adenosine at low concentrations is preferentially converted into AMP rather than deaminated, (vi) recirculation flux through the purine nucleotide cycle in cells other than muscle is relatively low. From these premises and assuming that withdrawal fluxes are net fluxes irrespective of the number of reactions actually involved, fluxes were calculated to maintain steady-state conditions. The set chosen is indicated below and appears in Fig. 1.

System A; case A₁ (Fig. 1a). For this system, fluxes were (nmol/time unit): $v_1 = 100$, $v_2 = 10$, $v_3 = 18$, $v_4 = 3.5$, $v_5 = 45$, $v_6 = 6.5$, $v_7 = 27$, $v_8 = 8$, $v_9 = 30$, $v_{10} = 83.5$, $v_{11} = 1.5$, $v_{12} = 12$, $v_{13} = 30$, $v_{14} = 8$, $v_{15} = 8$, $v_{16} = 60$, $v_{17} = 10$ (see Fig. 1a).

The concentrations of intermediates selected were: [adenosine]_o = 0.11 μM; [inosine]_o = 0.11 μM; [inosine]_i = 89 nM; [AMP]_i = 3.8 μM; [adenosine]_i = 11 nM; [adenylosuccinate]_i = 0.53 μM; [IMP]_i = 0.225 mM; [Na⁺]_o = 152 mM; [Na⁺]_i = 14 mM.

System A; case A₂ (Fig. 1a). With respect to system A, case A₁, the main modification was [adenosine]_o = 6.31 μM. To maintain the direction and magnitude of nucleoside transport, other concentrations were changed: [inosine]_o = 14.68 μM; [inosine]_i = 3 μM. The remaining concentrations, including [adenosine]_i, were as in case A₁.

System B; case B₁ (Fig. 1b). In this system, there was an external pathway of inosine degradation. Thus the flux of inosine across the membrane (v_4) was in the direction of inosine efflux. To maintain the steady state, inosine was released from the cell. For this system, fluxes were the same as above except for $v_9 = 10$ nmol/time unit and $v_{18} = 20$ nmol/time unit (See Fig. 1b).

Simulation results were also obtained for two cases with different external adenosine concentrations. Thus the concentrations that change with respect to the previous systems were: [adenosine]_o = 0.11 μM; [inosine]_o = 91 nM; [inosine]_i = 0.1 μM.

System B; case B₂ (Fig. 1b). With respect to system B, case B₁,

the main modification was [adenosine]_o = 6.3 μM. To maintain the direction and magnitude of nucleoside transport, other concentrations were changed: [inosine]_o = 3.7 μM; [inosine]_i = 13.8 μM.

Calculation of control coefficients

For each steady-state condition, flux control coefficients were calculated. Flux control coefficients were defined by Kacser & Burns [23] as the relative change in flux that is caused by a relative change in enzyme concentration, i.e. the coefficient of a given flux with respect to a given enzyme (E_i) is: $C_{E_i}^J = d \ln(\text{Flux}) / d \ln([E_i])$. These coefficients are defined for a given steady state. Steady states were imposed for the conditions given in each case which correspond to the situations displayed in Fig. 1. By fixing fluxes and intermediate concentrations, the suitable maximum velocity for each enzyme was calculated to maintain the steady state. Coefficients were calculated from individual rate equations using the matrix algebra developed by Cascante *et al.* [24]. It should be noted that, in the model of Figs 1(a) and 1(b), the summation theorem for flux control coefficients is valid, i.e. the sum of coefficients for a given flux is 1 ($\sum C_{E_i}^J = 1$) [23]. Thus the enzyme with greatest incidence in the flux is that which has the greatest value of $C_{E_i}^J$.

Dynamic predictions of the model

All dynamic calculations were performed on the original steady state defined in Fig. 1(b) and with the concentrations for case B₁. For dynamic simulations, differential equations were numerically integrated with a Fortran 77 program based on the Runge-Kutta fifth-order method as described by Franco & Canela [20,25].

The system of differential equations employed was:

$$d[\text{adenosine}]_o/dt = v_1 - v_2 - v_3 - v_5 - v_7 \quad (1)$$

$$d[\text{inosine}]_o/dt = v_2 - v_4 - v_6 \quad (2)$$

$$d[\text{AMP}]_i/dt = v_{10} + v_{15} - v_{11} - v_{13} - v_{16} \quad (3)$$

$$d[\text{adenosine}]_i/dt = v_3 + v_5 + v_7 + v_{11} - v_8 - v_{10} \quad (4)$$

$$d[\text{inosine}]_i/dt = v_4 + v_6 + v_8 + v_{12} - v_9 \quad (5)$$

$$d[\text{adenylosuccinate}]_i/dt = v_{14} - v_{15} \quad (6)$$

$$d[\text{IMP}]_i/dt = v_{13} - v_{12} - v_{14} - v_{17} \quad (7)$$

For compounds that traverse the plasma membrane, it is obvious that the differential equations are valid, assuming that the total volume in both compartments is the same. The extracellular fluid is, in volume, less than the intracellular one (20 compared with 15). However, considering that the extracellular fluid is not static, i.e. it has dynamic characteristics, and that the nucleus occupies a relatively high intracellular volume, we considered that the equivalence of volumes was a fair assumption.

For the model of Fig. 1(b), eqns. (2) and (5) were modified accordingly by considering v_{18} , and by considering the direction of flux v_4 .

$$d[\text{inosine}]_o/dt = v_2 + v_4 - v_6 - v_{18} \quad (8)$$

$$d[\text{inosine}]_i/dt = v_6 + v_8 + v_{12} - v_4 - v_9 \quad (9)$$

RESULTS

Distribution of the control in the steady state: case A₁ (Fig. 1a)

Under the conditions given in Fig. 1(a) (case A₁, see above) the control coefficients of the different final catabolic and anabolic fluxes with respect to all enzymes were calculated. The control coefficients of adenosine and inosine flux across the membrane were also considered. The results are shown in Table 1.

The flux through the first step (flux 1), AMP_o → adenosine_o, is only controlled by ecto-5'-nucleotidase. The control of flux 9 (inosine removal) is shared by different enzymes, although the

Table 1. Control pattern for system A₁ (Fig. 1a)

The flux control coefficients for the different rate equations (indicated as v_i in Fig. 1) are presented.

	Flux 1	Flux 3	Flux 5	Flux 7	Flux 4	Flux 6	Flux 9	Flux 16	Flux 17
E ₁	1.00	1.00	1.10	0.85	0.91	1.10	1.20	1.10	0.19
E ₂	≈ 0	-0.11	-0.10	-0.08	1.70	0.48	0.22	-0.11	-0.02
E ₃	≈ 0	0.80	-0.19	-0.15	-0.09	-0.24	-0.05	0.02	≈ 0
E ₄	≈ 0	-0.51	0.54	-0.38	-2.20	0.50	-0.11	0.05	0.01
E ₅	≈ 0	-0.30	-0.28	0.77	-0.51	-0.15	-0.07	0.03	0.01
E ₆	≈ 0	0.01	≈ 0	≈ 0	-0.23	0.12	0.19	-0.09	-0.02
E ₇	≈ 0	≈ 0	≈ 0	≈ 0	1.10	-0.61	≈ 0	≈ 0	≈ 0
E ₈	≈ 0	0.07	-0.02	≈ 0	0.17	-0.14	-0.20	0.10	0.02
E ₉	≈ 0	≈ 0	≈ 0	≈ 0	-0.18	0.10	0.16	-0.07	-0.08
E ₁₀	≈ 0	≈ 0	≈ 0	≈ 0	-0.46	0.25	0.40	-0.22	0.14
E ₁₁	≈ 0	≈ 0	≈ 0	≈ 0	0.12	-0.07	-0.11	0.06	-0.04
E ₁₂	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0
E ₁₃	≈ 0	≈ 0	≈ 0	≈ 0	0.46	-0.25	-0.40	0.22	-0.14
E ₁₄	≈ 0	≈ 0	≈ 0	≈ 0	0.23	-0.12	-0.20	-0.06	0.93

control coefficient of E₁ is higher than 1. Since the summation theorem is valid, this indicates that negative control coefficients (for E₃, E₄, E₅, E₈, E₁₁, E₁₃ and E₁₄) exist.

The most interesting physiological result concerns the control of flux from AMP towards ADP and ATP (flux 16) and of adenosine (flux 3, flux 5 and flux 7) and inosine (flux 4 and flux 6) transport. Flux 16 (AMP → ATP) is controlled by ecto-5'-nucleotidase. The other enzymes have less control. Adenosine incorporation by the specific Na⁺-dependent transporter is also controlled by ecto-5'-nucleotidase ($C_E^J = 0.85$) but also by the Na⁺-dependent transporter itself ($C_E^J = 0.77$); interestingly, the unspecific Na⁺-dependent transporter as well as the Na⁺-independent transporter have negative control coefficients. Similarly, adenosine incorporation through the unspecific Na⁺-dependent transporter is controlled by ecto-5'-nucleotidase ($C_E^J = 1.00$) and by the transporter itself ($C_E^J = 0.80$), whereas transporter and the Na⁺-independent transporter having negative flux control coefficients. Adenosine incorporation by the Na⁺-independent transporter is controlled by ecto-5'-nucleotidase ($C_E^J = 1.00$) and by the transporter itself ($C_E^J = 0.80$), whereas the Na⁺-dependent transporters have negative control coefficients. Ecto-adenosine deaminase which exerts a negative control plays a much smaller role in flux control. Inosine incorporation mediated by the Na⁺-independent carrier (flux 4) is mainly controlled by ecto-adenosine deaminase ($C_E^J = 1.70$), although ecto-5'-nucleotidase ($C_E^J = 0.91$) and intracellular inosine withdrawal also have high control coefficients. It should be noted that the Na⁺-dependent carrier has a strong but negative value of the control coefficient for this flux 4 ($C_E^J = -2.20$). Inosine flux through the Na⁺-dependent component is controlled by ecto-5'-nucleotidase and to a similar extent by the transporter itself and by both, ecto and intracellular adenosine deaminase. In this case the effect of the other transporter in this flux 6 is smaller. These results show that the control exerted by ecto-5'-nucleotidase on adenosine uptake is very important in the simulated conditions.

Control analysis in the steady state: case B₁ (Fig. 1b)

In the situation of Fig. 1(b) (case B₁, see above) the control distribution is nearly identical with respect to the fluxes that are common in Figs. 1(a) and 1(b) (Table 2). The main difference is found when inosine release and incorporation are analysed (flux 4 and flux 6 respectively). Inosine release is interestingly controlled by the enzyme that converts AMP into di- and tri-

nucleotides of adenine and also by intracellular adenosine deaminase, IMP degradation and adenosine kinase. In this case, ecto-5'-nucleotidase has a strong negative control coefficient of ($C_E^J = -1.20$). Inosine incorporation mediated by the Na⁺-dependent component is positively and strongly controlled by ecto-5'-nucleotidase ($C_E^J = 1.20$) and its own transporter ($C_E^J = 0.87$). The other enzymes or carriers have small or negative control coefficients for this flux 6. Finally flux 18, which is the new flux in the situation of Fig. 1(b), is controlled by all enzymes, but again C_E^J is very high for ecto-5'-nucleotidase ($C_E^J = 1.20$). These results confirm that in these conditions ecto-5'-nucleotidase seems to be a key enzyme for regulating the extracellular and intracellular metabolism of adenosine.

Further control analysis in the steady states: cases A₂ and B₂ (Fig. 1)

The control distribution is dependent on the concentration of internal metabolites (see for instance Ref. 26). This does not mean that the control depends on these concentrations, since they are the dependent variables of the system. If we maintain fluxes constant, the concentrations of internal metabolites automatically fix the enzyme concentrations or activities. Then it is these varied enzyme concentrations that produce changes in the control pattern. To analyse the behaviour of the system further, we modified some concentrations as indicated above.

The study of system A₂, in which there are three concentrations modified with respect to system A₁, indicates some variations in the control distribution of adenosine and inosine incorporation whereas the remaining fluxes have similar control patterns (Table 3). Thus adenosine incorporation by the Na⁺-independent carrier is mainly controlled by its own transporter ($C_E^J = 0.99$) and afterwards by ecto-5'-nucleotidase ($C_E^J = 0.61$). In contrast, adenosine incorporation by the unspecific Na⁺-dependent transporter is now more controlled by ecto-5'-nucleotidase and less by its own transporter. In the new situation, the control of adenosine incorporation via the specific Na⁺-dependent transporter is mainly exercised by its own transporter ($C_E^J = 0.97$) whereas in the former situation (see above) it was the ecto-5'-nucleotidase that had the greatest control. Inosine incorporation via the Na⁺-independent transporter (flux 4) is again controlled by ecto-adenosine deaminase and by intracellular inosine withdrawal and less by ecto-5'-nucleotidase. In this case its own transporter has an appreciable flux control coefficient ($C_E^J = 0.53$). The distribution of the control for inosine incorporation through the

Table 2. Control pattern for system B₁ (Fig. 1b)

The flux control coefficients for the different rate equations (indicated as v_i in Fig. 1) are presented.

	Flux 1	Flux 3	Flux 5	Flux 7	Flux 4	Flux 6	Flux 9	Flux 16	Flux 17	Flux 18
E ₁	1.00	1.00	1.10	0.85	-1.20	1.20	1.20	1.10	0.19	1.20
E ₂	≈ 0	-0.11	-0.11	-0.09	0.17	0.23	0.19	-0.11	-0.02	0.23
E ₃	≈ 0	0.80	-0.19	-0.15	-0.09	-0.02	-0.10	0.02	≈ 0	-0.02
E ₄	≈ 0	-0.51	0.54	-0.38	-0.46	0.87	-0.07	0.05	0.01	-0.13
E ₅	≈ 0	-0.31	-0.28	0.77	-0.05	-0.07	-0.06	0.03	0.01	-0.07
E ₆	≈ 0	0.01	≈ 0	≈ 0	-0.31	0.19	0.20	-0.09	-0.02	0.19
E ₇	≈ 0	≈ 0	≈ 0	≈ 0	0.52	-0.33	0.65	≈ 0	≈ 0	-0.33
E ₈	≈ 0	0.07	-0.02	≈ 0	0.30	-0.20	-0.21	0.10	0.02	-0.20
E ₉	≈ 0	≈ 0	≈ 0	≈ 0	-0.26	0.16	0.17	-0.07	-0.08	0.16
E ₁₀	≈ 0	≈ 0	≈ 0	≈ 0	-0.63	0.39	0.42	-0.22	0.14	0.39
E ₁₁	≈ 0	≈ 0	≈ 0	≈ 0	0.17	-0.10	-0.11	0.06	-0.04	-0.10
E ₁₂	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0
E ₁₃	≈ 0	≈ 0	≈ 0	≈ 0	0.63	-0.39	-0.42	0.22	-0.14	-0.39
E ₁₄	≈ 0	≈ 0	≈ 0	≈ 0	0.32	-0.20	-0.21	-0.06	0.93	-0.20
E ₁₅	≈ 0	≈ 0	≈ 0	≈ 0	-0.10	-0.69	-0.61	≈ 0	≈ 0	0.31

Table 3. Control pattern for system A₂ (Fig. 1a)

The flux control coefficients for the different rate equations (indicated as v_i in Fig. 1) are presented.

	Flux 1	Flux 3	Flux 5	Flux 7	Flux 4	Flux 6	Flux 9	Flux 16	Flux 17
E ₁	0.97	0.61	1.60	0.12	0.19	1.60	1.10	1.00	0.19
E ₂	≈ 0	-0.34	-0.04	≈ 0	0.81	0.81	0.19	-0.09	-0.02
E ₃	0.01	0.99	-0.33	-0.03	0.53	-0.57	-0.04	0.03	0.01
E ₄	0.01	-0.10	0.21	-0.06	-0.95	-0.22	-0.10	0.07	0.01
E ₅	0.01	-0.17	-0.43	0.97	-0.18	-0.39	-0.07	0.04	0.01
E ₆	≈ 0	≈ 0	≈ 0	≈ 0	-0.10	0.04	0.19	-0.09	-0.02
E ₇	≈ 0	0.01	-0.01	≈ 0	0.52	-0.23	0.02	-0.01	≈ 0
E ₈	≈ 0	0.01	≈ 0	≈ 0	0.10	-0.05	-0.19	0.09	0.02
E ₉	≈ 0	≈ 0	≈ 0	≈ 0	-0.09	0.04	0.16	-0.07	-0.08
E ₁₀	≈ 0	≈ 0	≈ 0	≈ 0	-0.21	0.09	0.39	-0.22	0.14
E ₁₁	≈ 0	≈ 0	≈ 0	≈ 0	0.06	-0.02	-0.10	0.06	-0.04
E ₁₂	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0
E ₁₃	≈ 0	≈ 0	≈ 0	≈ 0	0.21	-0.09	-0.39	0.22	-0.14
E ₁₄	≈ 0	≈ 0	≈ 0	≈ 0	0.10	-0.05	-0.20	-0.06	0.93

Na⁺-dependent transporter (flux 6) is similar to that in the previous situation, although the absolute values are different (compare Tables 1 and 3). It should be noted, however, that its own transporter has a negative value for the flux coefficient which indicates that a relative increase in the number of carrier molecules provokes a decrease in inosine influx via either the Na⁺-dependent or Na⁺-independent carrier.

For system B₂, in which three concentrations are modified with respect to system B₁, control coefficients for the common fluxes are similar. Some differences with respect to system B₁ are found for flux corresponding to adenosine incorporation via the specific transporter (see Table 4 and compare with Table 2). This flux (flux 7) is now almost completely controlled by its own transporter ($C_E^7 = 0.97$) and much less by ecto-5'-nucleotidase ($C_E^7 = 0.11$).

Dynamic behaviour of the system

All dynamic simulations were performed over the original steady state indicated in system B, case B₁ (see Fig. 1b and simulation procedure). These conditions were selected since the concentrations [adenosine]_o = 0.11 μM and [adenosine]_i =

0.011 μM are closer to those of the interstitial fluid in a basal (normoxic) condition than the concentrations of case B₂.

When we simulated a fall in the influx from the first product (AMP_o) in Fig. 1(b) (case B₁), all fluxes and intermediate concentrations decayed. Whereas in Fig. 1(b) (case B₂) the addition of drainage fluxes ($v_8 + v_{14} + v_{15}$) was less than the anabolic flux (v_{13}) (40 versus 60 nmol/time unit), we found that, under the new conditions, when there was a decrease in extracellular AMP at time $t = 5$ (i.e. very soon), the addition of drainage fluxes (22.5 nmol/time unit) exceeded the anabolic flux (6.9 nmol/time unit) (see Fig. 2). These new conditions cannot easily be extrapolated to physiological conditions. However, we can be sure that in the absence of an extracellular nucleotide pool, adenosine release is likely to occur. Moreover, this release can be quantitatively important when the intracellular AMP concentration rises, for instance as a result of hypoxic conditions.

The opposite situation appears when the concentration of extracellular AMP increases. This was simulated by fixing the AMP concentration at 0.1 mM under the conditions of Fig. 1(b). The system evolved towards a new steady state which was slowly reached as a result of the continuous but slow increase in

Table 4. Control pattern for system B₂ (Fig. 1*b*)

The flux control coefficients for the different rate equations (indicated as v_i in Fig. 1) are presented.

	Flux 1	Flux 3	Flux 5	Flux 7	Flux 4	Flux 6	Flux 9	Flux 16	Flux 17	Flux 18
E ₁	0.98	0.92	1.50	0.11	-0.87	1.00	1.40	1.00	0.19	1.00
E ₂	≈ 0	-0.12	-0.13	-0.01	0.07	0.29	0.06	-0.10	-0.02	0.28
E ₃	≈ 0	0.76	-0.23	-0.02	-0.50	0.24	-0.53	0.02	≈ 0	0.22
E ₄	0.01	-0.35	0.32	-0.05	-0.20	0.65	0.25	0.09	0.02	-0.34
E ₅	0.01	-0.23	-0.40	0.97	≈ 0	-0.13	0.02	0.05	0.01	-0.13
E ₆	≈ 0	0.02	≈ 0	≈ 0	-0.19	0.12	0.31	-0.09	-0.02	0.11
E ₇	≈ 0	-0.03	0.01	≈ 0	0.32	-0.20	0.47	-0.01	≈ 0	-0.19
E ₈	≈ 0	-0.02	≈ 0	≈ 0	0.19	-0.12	-0.31	0.09	0.02	-0.11
E ₉	≈ 0	0.01	≈ 0	≈ 0	-0.16	0.10	0.26	-0.06	-0.08	0.10
E ₁₀	≈ 0	0.03	-0.01	≈ 0	-0.39	0.24	0.64	-0.21	0.14	0.23
E ₁₁	≈ 0	-0.01	≈ 0	≈ 0	0.10	-0.06	-0.17	0.06	-0.04	-0.06
E ₁₂	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0
E ₁₃	≈ 0	-0.03	0.01	≈ 0	0.39	-0.24	-0.63	0.21	-0.14	-0.23
E ₁₄	≈ 0	-0.02	≈ 0	≈ 0	0.19	-0.12	-0.32	-0.06	0.93	-0.12
E ₁₅	≈ 0	0.05	-0.03	≈ 0	0.04	-0.79	-0.40	-0.01	≈ 0	0.24

Fig. 2. Dynamic behaviour of the system when [AMP]₀ is fixed at 1 μM

The original steady state at zero time corresponds to system B₁ (see Fig. 1*b*). (a) —, [adenosine]₀; ----, [inosine]₀; ····, [inosine]₀. (b) —, [adenosine]₀. (c) —, [AMP]₀. (d) —, [adenylosuccinate]₀.

intracellular IMP (Fig. 3). The increase in intracellular AMP was rapid at first but then continued to increase as it was being converted into IMP by AMP deaminase.

The fluxes in the new steady state are given in Table 5. By comparing fluxes, it is evident that flux 1 (ecto-5'-nucleotidase) and flux 2 (ecto-adenosine deaminase) are now very high compared with those in Fig. 1*b*). This in turn produces a marked decrease (in comparative terms) in adenosine incorporation and a marked increase in the drainage efflux from extracellular inosine (v_{18}).

In this new steady state, the control analysis was performed. The results are shown in Table 6 where, in comparison with the control pattern of the original steady state displayed in Table 2, it is noted that the situation is quite different. This becomes evident when each individual flux is analysed. However, in general, the most interesting results concern the change in control distribution of the nucleoside flux through the membrane and the flux towards intracellular adenine nucleotides. For instance,

ecto-5'-nucleotidase now markedly diminishes its control with respect to specific Na⁺-independent adenosine transport and extracellular cleavage but retains the control over flux 5. Interestingly, the flux control coefficients of ecto-5'-nucleotidase with respect to fluxes 4 and 7 are negative. Overall, under these new conditions, an increase in ecto-5'-nucleotidase molecules would lead to a decrease in adenosine uptake via the specific Na⁺-dependent transporter and an increase in adenosine influx via the Na⁺-dependent carrier. It should also be noted that adenosine kinase now controls adenosine entry via the specific Na⁺-dependent carrier.

Finally, inhibition of the nucleoside transporter was simulated. This was achieved by decreasing the maximum velocity of the transporters by 50% in the equations (v_3 , v_4 , v_5 , v_6 and v_7) without modifying the remaining conditions of Fig. 1*b*) (case B₁). The concentrations and fluxes in the new steady state are indicated in Table 5. As expected, extracellular concentrations of adenosine and inosine rise whereas the intracellular

Fig. 3. Dynamic behaviour of the system when $[AMP_o]$ is fixed at 0.1 mM

The original steady state at zero time corresponds to system B_1 (see Fig. 1b). (a) —, $[adenosine_o]$; ----, $[inosine_o]$; ····, $[inosine_i]$. (b) —, $[adenosine_i]$. (c) —, $[AMP_i]$; ----, $[IMP_i]$. (d) —, $[adenylosuccinate_i]$.

Table 5. New steady states obtained after perturbing system B_1

The intermediate concentrations and the fluxes in the new steady states obtained after perturbing the initial steady state of system B_1 are shown. The values for fluxes are given in mol/time unit.

	Initial steady state $[AMP_o] = 10^{-7}$ M	Modifications		
		$[AMP_o] = 10^{-12}$ M	$[AMP_o] = 10^{-4}$ M	Transporter inhibition
v_1	1.00×10^{-7}	1.00×10^{-12}	1.90×10^{-5}	1.00×10^{-7}
v_2	1.00×10^{-8}	9.45×10^{-14}	1.62×10^{-6}	1.85×10^{-8}
v_3	1.80×10^{-8}	1.71×10^{-13}	5.86×10^{-7}	1.75×10^{-8}
v_4	5.50×10^{-8}	1.35×10^{-13}	5.18×10^{-6}	1.02×10^{-8}
v_5	4.50×10^{-8}	4.23×10^{-13}	1.67×10^{-5}	4.20×10^{-8}
v_6	4.50×10^{-8}	5.63×10^{-14}	1.91×10^{-6}	4.02×10^{-9}
v_7	2.70×10^{-8}	3.16×10^{-13}	1.00×10^{-7}	2.19×10^{-8}
v_8	8.00×10^{-9}	8.21×10^{-14}	6.39×10^{-6}	7.24×10^{-9}
v_9	1.00×10^{-8}	8.70×10^{-14}	3.13×10^{-6}	1.22×10^{-8}
v_{10}	8.35×10^{-8}	8.76×10^{-13}	1.10×10^{-5}	7.57×10^{-8}
v_{11}	1.50×10^{-9}	4.82×10^{-14}	2.76×10^{-9}	1.53×10^{-9}
v_{12}	1.20×10^{-8}	8.33×10^{-14}	1.70×10^{-8}	1.12×10^{-8}
v_{13}	3.00×10^{-8}	3.21×10^{-13}	5.63×10^{-7}	2.75×10^{-8}
v_{14}	8.00×10^{-9}	8.96×10^{-14}	9.91×10^{-9}	7.69×10^{-9}
v_{15}	8.00×10^{-9}	9.33×10^{-13}	9.91×10^{-9}	7.69×10^{-9}
v_{16}	6.00×10^{-8}	6.02×10^{-13}	1.05×10^{-5}	5.44×10^{-8}
v_{17}	1.00×10^{-8}	4.42×10^{-13}	1.05×10^{-8}	9.90×10^{-9}
v_{18}	2.00×10^{-8}	1.73×10^{-13}	4.89×10^{-6}	2.47×10^{-8}
$[Adenosine_o]$ (M)	1.10×10^{-7}	1.04×10^{-12}	4.56×10^{-5}	2.05×10^{-7}
$[Inosine_o]$ (M)	9.12×10^{-9}	7.90×10^{-13}	2.89×10^{-5}	1.13×10^{-7}
$[Adenosine_i]$ (M)	1.10×10^{-8}	1.13×10^{-13}	2.06×10^{-5}	9.95×10^{-9}
$[Inosine_i]$ (M)	1.00×10^{-7}	8.69×10^{-13}	4.62×10^{-5}	1.23×10^{-7}
$[AMP_i]$ (M)	3.80×10^{-6}	3.81×10^{-11}	8.22×10^{-4}	3.44×10^{-6}
$[IMP_i]$ (M)	2.25×10^{-4}	4.88×10^{-10}	3.76×10^{-2}	1.86×10^{-4}
$[Adenylosuccinate_i]$ (M)	5.30×10^{-7}	5.86×10^{-12}	6.65×10^{-7}	5.08×10^{-7}

concentrations of AMP, IMP, adenylosuccinate and adenosine diminish. In contrast, the intracellular concentration of inosine increases due to the inhibition of inosine efflux. Notwithstanding this, fluxes are not very much altered with respect to the original ones, only those corresponding to inosine transport (see Table 5). Thus adenosine transport is nearly identical in magnitude since

the decrease in carrier activity was counteracted by a twofold increase in the extracellular adenosine concentration. The same can be noted for the remaining fluxes and it can be concluded that the flux towards intracellular adenine nucleotides (v_{16}) is only slightly affected by the shortfall of adenosine entry. It should, however, be noted that, under the new conditions,

Table 6. Control pattern for system obtained after perturbing system B₁

Control analyses were performed in the new steady-state obtained after perturbing the system as indicated in Fig. 3. The conditions of the new steady state are given in Table 5. The control coefficients for the different rate equations (indicated as v_i in Fig. 1) are presented.

	Flux 1	Flux 3	Flux 5	Flux 7	Flux 4	Flux 6	Flux 9	Flux 16	Flux 17	Flux 18
E ₁	0.95	0.08	2.10	-0.39	-0.40	0.73	1.40	1.10	≈ 0	0.69
E ₂	≈ 0	-0.04	-0.17	0.03	0.05	0.34	0.08	-0.09	≈ 0	0.26
E ₃	≈ 0	0.46	-0.12	-0.05	-0.34	0.21	-0.29	≈ 0	≈ 0	0.15
E ₄	0.01	-0.18	0.35	-0.30	-0.11	0.66	0.27	0.07	≈ 0	-0.28
E ₅	0.01	-0.20	-0.36	0.83	0.08	-0.14	0.08	0.04	≈ 0	-0.11
E ₆	≈ 0	0.22	-0.09	0.04	-0.28	0.17	0.21	-0.07	≈ 0	0.13
E ₇	≈ 0	-0.21	0.04	0.04	0.36	-0.23	0.57	-0.03	≈ 0	-0.19
E ₈	0.01	0.38	-0.40	0.56	0.05	-0.12	-0.95	0.23	≈ 0	-0.15
E ₉	≈ 0	0.26	-0.06	-0.03	-0.41	0.26	0.46	-0.14	≈ 0	0.21
E ₁₀	≈ 0	0.03	-0.01	0.01	-0.04	0.02	0.03	-0.01	≈ 0	0.02
E ₁₁	≈ 0	-0.01	≈ 0	≈ 0	0.01	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0
E ₁₂	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0
E ₁₃	≈ 0	0.13	-0.14	0.20	0.02	-0.04	-0.34	0.08	≈ 0	-0.05
E ₁₄	≈ 0	0.01	-0.02	0.03	0.02	-0.02	-0.07	-0.15	1.00	-0.01
E ₁₅	≈ 0	0.07	-0.06	0.01	-0.02	-0.82	-0.45	-0.04	≈ 0	0.35

Fig. 4. Dynamic behaviour of the system when [AMP]_o is increased to 0.1 mM and allowed to vary (from 0.1 mM to 0.1 μM)

The original steady state at zero time corresponds to system B₁ (see Fig. 1*b*). (a) —, [adenosine]_o; ----, [inosine]_o. (b) —, [adenosine]_i; ----, [inosine]_i. (c) —, [AMP]_i; ----, [IMP]_i; ····, [AMP]_o. (d) —, [adenylosuccinate]_i.

inosine efflux is achieved by an exquisite balance between intracellular (123 nM) and extracellular (113 nM) inosine concentrations.

Dynamic simulation of an extracellular AMP peak

To simulate a transient increase in extracellular AMP concentration, we (i) chose the conditions of Fig. 1*b*) (case B₁), (ii) elevated the AMP concentration to 0.1 mM and (iii) left the AMP concentration free to vary from 0.1 mM to the initial concentration of the steady state of Fig. 1*b*) (0.1 μM). Thus it is obvious that the final steady state will be the same as the initial one. The aim of this simulation was to achieve a momentary increase in the extracellular adenosine concentration. This kind of behaviour is likely to occur in the case of ATP release. Burnstock [27] indicated that ATP interacts directly in purinergic P₂ receptors and indirectly in purinergic P₁ receptors after conversion into adenosine.

The results are presented in Fig. 4 where it is shown that

extracellular adenosine increases transiently as do the remaining compounds except intracellular IMP and adenylosuccinate, which, during the time of the simulation, accumulated continuously. It should be noted, however, that the peak of adenosine is sharper than the peaks shown for the other compounds. The peak of adenosine starts immediately the signal (increase in extracellular AMP) starts and does not last for long after the signal disappears. In this simulation the signal, i.e. AMP concentration, goes from 0.1 mM to 0.1 μM in 10 arbitrary time units which is also the time in which the adenosine maximum is reached. The adenosine concentration returns to the baseline in less than 20 time units.

DISCUSSION

In the model described here we attempt to understand adenosine transport and metabolism. Studies were performed assuming an inosine influx (Fig. 1*a*) or an inosine efflux (Fig. 1*b*).

The extracellular metabolism of adenine nucleotides and nucleosides is a complex process which involves an increasing number of ectoenzymes. The results presented in this paper confirm the complexity of the process which also has important consequences for intracellular purine metabolism.

Although not complete, the model employed in our simulations is able to predict some interesting trends of the whole process. First of all, the main controlling step for the formation of extracellular adenosine and intracellular adenine nucleotides is ecto-5'-nucleotidase and occasionally the nucleoside transporter. The values of flux control coefficients of adenosine kinase and adenosine deaminase were relatively low in the simulated conditions shown in Figs. 1(a) and 1(b). This somehow confirms the recent ^{32}P n.m.r. spectroscopy data of Headrick & Willis [28] which are consistent with an important role for 5'-nucleotidase in the formation of extracellular adenosine. It should be noted that, by studying the purine metabolism of the cell as a whole, the role of ecto-5'-nucleotidase in the production of adenosine from ATP (for instance, in hypoxic conditions) is subject to controversy [29–33]. On the other hand, our results do not support a key role for adenosine kinase, which has been intuitively assigned to be the regulator of the intracellular concentration of adenosine, of the adenosine transport and of the flux towards adenine nucleotides. The role of adenosine kinase appears to be important only in very specific conditions such as those produced by an increased concentration of extracellular AMP (see the Results section and Tables 5 and 6).

From our results we suggest a key role for the nucleoside carriers that deal with adenosine and inosine in the whole process for two reasons: first, because of the relative importance of the carriers in the control of the main fluxes and, second, because of their kinetic behaviour. From the equations we have derived, it appears that the flux of a given nucleoside can sometimes be against a chemical gradient. For the Na^+ -dependent transporter this is explained by the Na^+ gradient, whereas for the Na^+ -independent transporter the explanation comes from an antiport effect of adenosine and inosine. Thus, in the absence of the Na^+ -dependent transporter, we have seen an efflux of inosine against the gradient with an adenosine influx down its concentration gradient (results not shown). When there is a sharp inward gradient for adenosine (because [adenosine]_i is very low), inosine efflux mediated by the Na^+ -independent carrier is more likely to occur. At the molecular level this is a consequence of the dynamic steady state for a reversible process. This kinetic peculiarity also explains the strange pattern of flux control for inosine transport (v_4) through the Na^+ -independent transporter. Thus the negative control exerted by the transporter itself can only be explained by assuming that an increase in the number of carrier molecules serves to increase the flux of adenosine through the membrane, whereas the inosine flux diminishes. It is surprising that this should occur for all the situations simulated using the data of Fig. 1(b) ($C_{E_3}^J$ is negative for flux 4 in Tables 1, 2, 4 and 6). Obviously, by suitably changing the extracellular and intracellular concentrations of the nucleosides the opposite effect could be achieved, i.e. positive flux control coefficients for inosine flux and negative coefficients for adenosine flux could have been encountered.

As demonstrated by the results presented, the control distribution is dependent on the conditions chosen. To improve our simulation conditions, the relative activities (V) of all the intracellular and extracellular enzymes involved as well as of the nucleoside transporters should be known for a given type of cell. Instead of this, we can obtain a satisfactory representation of the system if we know the concentration of the intracellular and extracellular metabolites. Meghji *et al.* [33] have measured the activities of ecto- and cytosolic 5'-nucleotidase, adenosine kinase,

adenosine deaminase and AMP deaminase in ventricular myocardium from man, rats, rabbits, guinea pigs, pigeons and turtles. They have found striking variations in ecto-5'-nucleotidase activity among these species as well as a high variable cytochemical distribution of ecto-5'-nucleotidase. These results confirm the complexity of the pathway and the importance of ecto-5'-nucleotidase in the whole process. The distribution of 5'-nucleotidase, adenosine kinase and adenosine deaminase, which is very dependent on the species and the tissue considered [34,35], also supports the view that adenosine metabolism is very variable and is different for each type of cell considered. Moreover, the very interesting results on intracellular purine metabolism obtained by van den Berghe and co-workers [36,37] indicate that for a single cell (the erythrocyte) there is extreme versatility in the ATP-catabolic pathways. In our opinion, and on the basis of the results presented here, computer simulation is a very useful and powerful tool for analysing different possibilities that are not evident when experimental data are analysed. In particular, we can predict that (i) the control distribution is highly dependent on the extracellular and intracellular concentration of adenosine and (ii) there are catabolic ectoenzymes that control intracellular anabolic pathways. Thus more experimental effort should be directed to this area.

Finally it should be noted that our model can mimic the behaviour of adenosine as a hormone or neuromodulator coming from extracellular adenine nucleotides. When the suitable signal (in this case an increase in extracellular AMP concentration) is emitted, the extracellular adenosine concentration immediately reaches a maximum and returns to basal values in a short period of time. This is the behaviour expected for a hormone or a neuromodulator acting in the interstitial space on adenosine receptors of the cellular surface. A rapid increase in concentration is required to activate the receptor and a rapid decrease is required to deactivate the receptor. This latter effect is achieved by means of adenosine deaminase and the nucleoside transporters. Obviously the simulation results obtained confirm the hypothesis of Burnstock [27] that extracellular adenine nucleotides can activate adenosine receptors if they have first been converted into adenosine by ecto-ATPases, nucleoside diphosphatases and 5'-nucleotidase. The appearance of adenosine from adenine nucleotide precursors in the extracellular space is also beneficial in hypoxic conditions in which depletion of ATP stores occurs. Our results highlight this fact since it is demonstrated that extracellular adenosine is transported and anabolized towards the synthesis of adenine nucleotides. It has been clearly established in tissues subjected to hypoxia, such as the kidney, that extracellular adenosine obtained from the breakdown of exogenously added AMP may be preferentially transported into renal cells to be resynthesized into cellular AMP and ATP [38].

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APPENDIX

Rate equations and other parameters for the model of adenosine transport and metabolism

Ecto 5'-nucleotidase (EC 3.1.3.5)

The enzyme catalyses the irreversible dephosphorylation of external AMP [1] following a Michaelian rate equation. External adenosine is a competitive inhibitor of the 5'-nucleotidase. Constants are $K_{m(\text{AMP})} = 24 \mu\text{M}$ [1], $K_{i(\text{adenosine})} = 0.4 \text{ mM}$ [2] and V_1 for the conditions of Fig. 1(b) (case B₁) is $24 \mu\text{mol/time unit}$.

Ecto-adenosine deaminase (EC 3.5.4.4)

Adenosine deaminase catalyses the irreversible deamination of adenosine to inosine. The rate equation for v_2 is Michaelian with inosine as a competitive inhibitor. Constants are $K_{m(\text{adenosine})} = 45 \mu\text{M}$, $K_{i(\text{inosine})} = 52 \mu\text{M}$ [3] and V_2 for the conditions of Fig. 1(b) (case B₁) is $4.1 \mu\text{mol/time unit}$.

Nucleoside transporter

Assuming that adenosine and inosine are translocated by the same transporters, we have developed rate equations for the mechanisms of the three transporters shown in Scheme 1. These transporters are (i) an Na⁺-independent nucleoside transporter, which translocates adenosine and inosine (20%), (ii) a Na⁺-dependent nucleoside transporter, which translocates adenosine and inosine (50%), and (iii) an Na⁺-dependent adenosine transporter (20%) [4].

Na⁺-independent nucleoside transporter. Adenosine and inosine are both translocated by the sodium-independent transporter. The rate for adenosine transport (adenosine_o → adenosine_i) is given by:

$$v_3 = \frac{\text{NUM}_{\text{Adenosine}}}{\text{DENOM}}$$

The rate for inosine transport (inosine_o → inosine_i) is given by:

$$v_4 = \frac{\text{NUM}_{\text{Inosine}}}{\text{DENOM}}$$

Scheme 1. Mechanism for the three nucleoside transporters considered

The subscript o denotes outside and the subscript i inside the cell. T denotes the transporter, A adenosine and I inosine. (a) Na⁺-independent adenosine and inosine transporter. (b) Na⁺-dependent adenosine and inosine transporter. (c) Na⁺-dependent adenosine transporter.

where

$$\text{NUM}_{\text{adenosine}} = V_3[\text{adenosine}_o] + \frac{V_3[\text{adenosine}_o][\text{inosine}_i]}{K_{\text{Ado-i}}} - V_3[\text{adenosine}_i] - \frac{V_3[\text{adenosine}_i][\text{inosine}_o]}{K_{\text{Ado-i}}}$$

$$\text{NUM}_{\text{inosine}} = V_4[\text{inosine}_o] + \frac{V_4[\text{inosine}_o][\text{adenosine}_i]}{K_{\text{Ado-i}}} - V_4[\text{inosine}_i] - \frac{V_4[\text{inosine}_i][\text{adenosine}_o]}{K_{\text{Ado-i}}}$$

and

$$\text{DENOM} =$$

$$K_{\text{Ino-i}} + [\text{inosine}_i] + (K_{\text{Ino-i}}/K_{\text{Ino-o}})[\text{inosine}_o] + (K_{\text{Ino-i}}/K_{\text{Ado-i}})[\text{adenosine}_i] + (K_{\text{Ino-i}}/K_{\text{Ado-o}})[\text{adenosine}_o] + (K_{\text{Ino-i}}/K_{\text{Ino-o/Ino-i}})[\text{inosine}_o][\text{inosine}_i] \\ + (K_{\text{Ino-i}}/K_{\text{Ado-i/Ino-o}})[\text{inosine}_o][\text{adenosine}_i] + (K_{\text{Ino-i}}/K_{\text{Ado-o/Ado-i}})[\text{adenosine}_o][\text{adenosine}_i] + (K_{\text{Ino-i}}/K_{\text{Ado-o/Ino-i}})[\text{adenosine}_o][\text{inosine}_i]$$

Constants were obtained from Franco *et al.* [5]; where $K_{\text{Ado-i}}$ and $K_{\text{Ado-o}}$ were assumed equal to K_m for adenosine and $K_{\text{Ino-i}}$ and $K_{\text{Ino-o}}$ equal to K_1 for inosine: $K_{\text{Ino-o}} = 12 \mu\text{M}$, $K_{\text{Ino-i}} = 12 \mu\text{M}$, $K_{\text{Ado-i}} = 8 \mu\text{M}$, $K_{\text{Ado-o}} = 8 \mu\text{M}$, $K_{\text{Ino-o/Ino-i}} = K_{\text{Ino-o}}K_{\text{Ino-i}} = 144 \mu\text{M}^2$, $K_{\text{Ado-i/Ino-o}} = K_{\text{Ado-i}}K_{\text{Ino-o}} = 96 \mu\text{M}^2$, $K_{\text{Ado-o/Ado-i}} = K_{\text{Ado-o}}K_{\text{Ado-i}} = 64 \mu\text{M}^2$, $K_{\text{Ado-o/Ino-i}} = K_{\text{Ado-o}}K_{\text{Ino-i}} = 96 \mu\text{M}^2$. V_3 and V_4 for the conditions of Fig. 1(b) (case B₁) are respectively 2.2 and 20.2 $\mu\text{mol}/\text{time unit}$.

Na⁺-dependent nucleoside transporter. Adenosine and inosine are both translocated by the Na⁺-dependent transporter. The rate for adenosine transport (adenosine_o → adenosine_i) is given by:

$$v_5 = \frac{\text{NUM}_{\text{Adenosine}}}{\text{DENOM}}$$

The rate for inosine transport (inosine_o → inosine_i) is given by:

$$v_6 = \frac{\text{NUM}_{\text{Inosine}}}{\text{DENOM}}$$

where

$$\text{NUM}_{\text{Adenosine}} = V_5[\text{adenosine}_o][\text{Na}_i^+] - V_5[\text{adenosine}_i][\text{Na}_o^+]$$

$$\text{NUM}_{\text{Inosine}} = V_6[\text{inosine}_o][\text{Na}_i^+] - V_6[\text{inosine}_i][\text{Na}_o^+]$$

and

$$\text{DENOM} = K_1 + K_2[\text{adenosine}_o] \\ + K_3[\text{inosine}_o] + K_4[\text{adenosine}_i] \\ + K_5[\text{inosine}_i]$$

From the experimental values obtained by Franco *et al.* [5] were calculated: $K_1 = 1.89$, $K_2 = 1.53$, $K_3 = 0.65$, $K_4 = 1.53$ and $K_5 = 0.65$. V_5 and V_6 for the conditions of Fig. 1(b) (case B₁) are respectively 3.9 and 0.4 $\mu\text{mol}/\text{time unit}$.

Na⁺-dependent adenosine transporter. The rate for adenosine transport (adenosine_o → adenosine_i) is given by:

$$v_7 = \frac{\text{NUM}_{\text{Adenosine}}}{\text{DENOM}}$$

where

$$\text{NUM}_{\text{adenosine}} = V_7[\text{adenosine}_o][\text{Na}_o] - V_7[\text{adenosine}_i][\text{Na}_i]$$

and

$$\text{DENOM} = K_{\text{Ado-i}} + K_{\text{Na-o}}[\text{adenosine}_o] \\ + K_{\text{Ado-o}}[\text{Na}_o] + [\text{adenosine}_i] \\ + K_{\text{Ado-i}}[\text{Na}_i] + [\text{adenosine}_o][\text{Na}_o] \\ + K_{\text{Na-o}}[\text{adenosine}_i][\text{Na}_i] \\ + [\text{adenosine}_i][\text{Na}_i] + [\text{adenosine}_i][\text{Na}_o] \\ + (1/K_{\text{Na-o}})[\text{adenosine}_o][\text{Na}_o][\text{Na}_i] \\ + (1/K_{\text{Na-i}})[\text{adenosine}_i][\text{Na}_o][\text{Na}_i]$$

Constants were obtained from LeHir & Dubach [4]; where $K_{\text{Ado-i}}$ and $K_{\text{Ado-o}}$ were assumed equal to K_m for adenosine and $K_{\text{Na-i}}$ and $K_{\text{Na-o}}$ equal to K_m for sodium: $K_{\text{Ado-o}} = 1.1 \mu\text{M}$, $K_{\text{Ado-i}} = 1.1 \mu\text{M}$

[4], $K_{\text{Na-o}} = 10 \text{ mM}$, $K_{\text{Na-i}} = 10 \text{ mM}$ [6]. V_7 for the conditions of Fig. 1(b) (case B₁) was 0.4 $\mu\text{mol}/\text{time unit}$.

Internal adenosine deaminase (EC 3.5.4.4)

Deamination of adenosine inside the vesicles follows the same equation as that outside the vesicles. Inosine is a competitive inhibitor. Constants are identical with those for external adenosine deaminase. V_8 for the conditions of Fig. 1(b) (case B₁) is 32.8 $\mu\text{mol}/\text{time unit}$.

Internal inosine withdrawal

The rate equation for the inosine degradation to hypoxanthine, by purine nucleoside phosphorylase (EC 2.4.2.1), is considered Michaelian. $K_{\text{m(inosine)}} = 97 \mu\text{M}$ [7]. V_9 for the conditions of Fig. 1(b) (case B₁) is 9.7 $\mu\text{mol}/\text{time unit}$.

Internal adenosine kinase (EC 2.7.2.1)

Adenosine kinase catalyses the phosphorylation of adenosine to AMP by ATP. The mechanism of the reaction is described by Hawkins & Bagnara [8], AMP being a competitive inhibitor, and adenosine inhibiting by substrate excess. [ATP] was fixed and included in V_{10} . For this enzyme $K_{\text{m(adenosine)}} = 2.8 \mu\text{M}$ [9], $K_{\text{i(adenosine)}} = 10 \mu\text{M}$ [10], $K_{\text{m(AMP)}} = 0.2 \text{ mM}$ [1] and V_{10} for the conditions of Fig. 1(b) (case B₁) is 21.7 $\mu\text{mol}/\text{time unit}$.

Internal 5'-nucleotidase (EC 3.1.3.5)

Inside the vesicles, where AMP and IMP are present, the enzyme can use both substrates (see Fig. 1). Rate equations: v_{11} for AMP_i dephosphorylation and v_{12} for IMP_i dephosphorylation were given according to Franco & Canela [1]. Adenosine is a competitive inhibitor of the enzyme in both equations, and IMP and AMP competitive inhibitors for v_{11} and v_{12} respectively. Constants are: $K_{\text{m(AMP)}} = 24 \mu\text{M}$, $K_{\text{m(IMP)}} = 0.11 \text{ mM}$, $K_{\text{i(adenosine)}} = 0.24 \text{ mM}$ [7] and V_{11} and V_{12} for the conditions of Fig. 1(b) (case B₁) are respectively 30 and 19 $\text{nmol}/\text{time unit}$.

Internal AMP deaminase (EC 3.5.4.6)

The reaction catalysed by AMP deaminase was considered to follow Michaelian behaviour, where IMP_i competitively inhibits the enzyme. For this enzyme $K_{\text{m(AMP)}} = 3.4 \text{ mM}$, $K_{\text{i(IMP)}} = 3.4 \text{ mM}$ [11]. V_{13} for the conditions of Fig. 1(b) (case B₁) is 28.6 $\mu\text{mol}/\text{time unit}$.

Internal adenylosuccinate synthase (EC 6.3.4.3)

This enzyme catalyses the following irreversible reaction:

Previous studies suggest that the reaction mechanism is of the ter-ter ordered type [12,13], but the rate equation is Michaelian

when [GTP] and [aspartate] are constant and included in the maximal velocity value. Adenylosuccinate was considered a competitive inhibitor. Constants were: $K_{m(\text{IMP})} = 54 \mu\text{M}$, $K_{i(\text{adenylosuccinate})} = 0.27 \text{ mM}$ [12]. V_{14} for the conditions of Fig. 1(b) (case B₁) is 9.9 nmol/time unit.

Internal adenylosuccinate lyase (EC 4.3.2.2)

The enzyme catalyses the formation of fumarate_i and AMP_i from adenylosuccinate_i. The equation is Michaelian and AMP_i inhibits competitively. Constants are: $K_{m(\text{adenylosuccinate})} = 12 \mu\text{M}$, $K_{i(\text{AMP})} = 48 \mu\text{M}$ [14]. V_{15} for the conditions of Fig. 1(b) (case B₁) is 0.2 $\mu\text{mol/time unit}$.

Intracellular anabolism towards ADP and ATP

The rate equation for the process $\text{AMP}_i \rightarrow \text{ADP}_i \rightarrow \text{ATP}_i$ which is the anabolic net flux towards ATP (i.e. anabolic flux from AMP_i minus catabolic flux from ADP and ATP towards AMP_i) is assumed to be Michaelian. Constants are $K_{m(\text{AMP})} = 3.4 \text{ mM}$ (assumed) and V_{16} for the conditions of Fig. 1(b) (case B₁) is 0.5 $\mu\text{mol/time unit}$.

IMP withdrawal

The rate equation which corresponds to the pathway hypoxanthine $\rightarrow$ IMP_i $\rightarrow$ GMP_i is assumed to be Michaelian.

Constants are $K_{m(\text{IMP})} = 11.6 \mu\text{M}$ (assumed) and V_{17} for the conditions of Fig. 1(b) (case B₁) 10.5 nmol/time unit.

External inosine withdrawal

This step is identical with step 5. V_{18} for the conditions of Fig. 1(b) (case B₁) is 21.3 $\mu\text{mol/time unit}$.

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