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Further characterization of adenosine transport in renal brush-border membranes

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Adenosine transport has been further characterized in rat renal brush-border membranes (BBM). The uptake shows two components, one sodium-independent and one sodium-dependent. Both components reflect, at least partly, translocation via a carrier mechanism, since the presence of adenosine inside the vesicles stimulates adenosine uptake in the presence as well as in the absence of sodium outside the vesicles. The sodium-dependent component is saturable (K_m adenosine = 2.9 μM, V_{max} = 142 pmol/min per mg protein) and is abolished at low temperatures. The sodium-independent uptake has apparently two components: one saturable (K_m = 4–10 μM, V_{max} = 174 pmol/min per mg protein) and one non-saturable (V_{max} = 3.4 pmol/min per mg protein, K_m > 2000 μM). Inosine, guanosine, 2-chloroadenosine and 2′-deoxyadenosine inhibit the sodium-dependent and -independent transport, as shown by trans-stimulation experiments, probably because of translocation via the respective transporter. Uridine and dipyridamole inhibited only the sodium-dependent uptake. Other analogs of adenosine showed no inhibition. The kinetic parameters of the inhibitors of the sodium-dependent component were further investigated. Inosine was the most potent inhibitor with a K_i (1.9 μM) less than the K_m of adenosine. This suggests a physiological role for the BBM ecto-adenosine deaminase (enzyme which extracellularly converts adenosine to inosine), balancing the amount of nucleoside taken up as adenosine or inosine by the renal proximal tubule cell.

Introduction

Adenosine has been shown to have a wide range of profound effects on renal function. Infusion of adenosine 'in vivo' produces renal vasal constriction [1–3], inhibits renal adenylate cyclase [4] and neurotransmitter release [5], reduces glomerular filtration [6] and sodium excretion [3] and inhibits renin release [7,8]. Many of these actions are mediated via specific receptors named P1 purinoceptors. From another point of view, the study of the physiology of adenosine in kidney is very important, since filtration and reabsorption of the nucleoside are important ways of conserving the purine ring. Also, excretion and metabolism of adenosine are vital in preventing an excessive intracellular accumulation of this potent regulatory and potentially cytotoxic nucleoside.

Extracellular adenosine is inactivated by two different mechanisms: degradation via ecto-adenosine deaminase and cellular uptake. Recently, a hypothesis concerning the role of ecto-adenosine deaminase has been proposed by Franco et al. [9]. In short, the enzyme could either directly participate in the transport process or convert adenosine into inosine, which then can also be transported by cells.

Trimble and Coulson [10] have demonstrated that adenosine undergoes carrier-mediated transport across both luminal and antiluminal membrane vesicles isolated from rat renal cortex. At physiological adenosine concentrations, the net transport of the nucleoside is in the direction of reabsorption. These authors reported, in the absence of sodium, a single transport system of low K_m (5 μM) in antiluminal vesicles and two systems, of low (4.4 μM) and high (43 μM) K_m in luminal vesicles. Although the high-K_m system was insensitive to sodium, the low-K_m system was stimulated by the presence of an inwardly directed sodium gradient. Moreover, Le Hir and Dubach [11] were able to demonstrate, in renal BBM vesicles, an overshoot in the presence of a sodium

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Abbreviation: BBM, brush-border membrane(s).

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gradient. The K_m corresponding to the sodium-dependent system was 1.1 μM.

From the point of view of specific inhibitors, adenosine uptake is extremely complex, and the transporter seems to transport other nucleosides as well [12,13]. Differences in the sensitivity of adenosine transport in erythrocytes and cultured cells to inhibition by nitrobenzylthioinosine and dipyridamole are largely species specific [14,15]. Detailed studies of dipyridamole-induced dissociation of nitrobenzylthioinosine from its sites lead to the conclusion that multiple forms of nitrobenzylthioinosine binding sites might correspond to interconvertible conformations of a single protein or macromolecular assembly [16]. On the other hand, Le Hir and Dubach [17] and Jarvis [12] have demonstrated, working with BBM from different sources, that the Na^+-dependent component of the transport is shared by other nucleosides such as inosine, guanosine and uridine. In this paper, both the sodium-dependent and the sodium-independent components of the adenosine transport system have been studied in renal cortical BBM vesicles; in particular using inosine, dipyridamole, nitrobenzylthioinosine, adenosine agonists and other adenosine analogs, with regard to their substrate and inhibitor specificity. A clear difference between the two systems could be demonstrated.

Materials and Methods

Membrane preparation, determination of enzyme activities and protein content

The preparation of the BBM vesicles was carried out at 4° C. Wistar rats (200–300 g) were killed by a blow on the neck and the kidneys were removed immediately. Thin slices of renal cortex were prepared with a razor blade. Brush-border vesicles were prepared by an $MgCl_2$ precipitation technique [18]. Protein was measured by the method of Lowry et al. [19], using bovine serum albumin as standard. Alkaline phosphatase was determined as described by Berner and Kinne [20]. The purity of the preparation was assessed by the enrichment of alkaline phosphatase (EC 3.1.3.1) in the vesicles as compared to the starting homogenate, which varied between 9.5- and 13-fold. The enrichment factor of Na^+/K^+-ATPase (EC 3.6.1.3), a basal-lateral enzyme marker, was less than 0.15. The purified vesicles were suspended in a medium containing 100 mM mannitol and 20 mM Hepes-Tris (pH 7.4). They were stored at −70° C and, prior to use, were rapidly thawed in a 37° C water bath and homogenized five times through a 23 gauge needle.

Uptake studies

The uptake of [2,5′,8′-^{3}H]adenosine was measured in triplicate by a rapid filtration technique (Millipore HAWPO2500, 0.45 μm pore size filters). The composition of the media is given for each experiment in the figure legends. When indicated, deoxycoformycin, an inhibitor of adenosine deaminase, was added to incubation mixtures to prevent deamination of adenosine. 100 μl of incubation medium and 10 μl of vesicles suspension (90–110 μg protein) both kept at 20° C were pipetted separately onto the bottom of plastic test-tubes. Transport was started by mixing vigorously the two droplets on a Vortex mixer. The transport was terminated with 1 ml of an ice-cold solution containing 150 mM NaCl, 100 mM mannitol and 20 mM Hepes-Tris (pH 7.4). The suspension was quickly filtered and the filter was washed once with 4 ml of ice-cold stop solution. To assess the performance of the stop procedure, filtrations were performed at different times, after the addition of 1 ml of cold stop solution. Radioactivity remaining in the filters did not vary for up to 60 s, thus indicating that, in these conditions, there is no release of radiolabelled adenosine from vesicles. The filters were transferred to scintillation vials and 7 ml of scintillation cocktail were added. The data were corrected for the radioactivity remaining on the filter in the absence of membrane vesicles in the incubation medium. All media and solutions used for isolation and transport of vesicles were filtered through Millipore filters (0.22 μm pore size) immediately before use, to avoid bacterial contamination. The time-course of the uptake in the absence or presence of sodium was linear up to 10 s. 8 s was chosen as the time for the zero-trans experiments. To differentiate uptake into vesicles from binding, osmotically active vesicular space was systematically reduced by addition of sucrose to the incubation medium. At the highest osmolarity tested (1100 mosM), the transport was 18% of that obtained in the usual assay conditions. This indicates, in fact, that most of the radioactivity measured in the vesicles represents a transport rather than a binding on the outside of the membranes.

Handling of data

Kinetic parameters (K_m, V_{max}, constant for non-specific binding and Hill coefficient) were calculated by non-linear regression using the appropriate equation. The adenosine and inhibitor concentrations used were previously determined using a program for continuous D-optimal experimental design [21]. A relative error was assumed in the design and a preliminary experiment was carried out to find an estimated value of all the kinetic constants. Data corresponding to inhibition studies were fitted assuming both competitive and non-competitive inhibition; results corresponding to the best fit (as indicated by the standard deviation of the fit) are presented. The program devised by Canela [22] was used. Goodness of fit was tested according to the reduced χ^2 or S.D. values given by the program. The modified F test was used to analyze whether the fit to

one kind of model significantly improves the fit to another model (for instance comparing competitive and non-competitive inhibition models). The equation applied in this test is the following:

$$F = \frac{(SS_1 - SS_2)/(df_1 - df_2)}{SS_2/df_2}$$

where SS_1 and SS_2 are residual sums of squares with the corresponding degrees of freedom df_1 and df_2. The F-values were calculated using (df_1-df_2) degrees of freedom in the numerator and df_2 degrees of freedom in the denominator [23]. Where indicated, data are presented as mean ± S.E. Statistical significance was determined by Student's two-sample t calculation with a $P < 0.05$ level considered as significant.

Thin-layer chromatography

Composition of the radioactive label in the incubation medium and within the vesicles after 8 s of [^{3}H]adenosine uptake was measured by thin-layer chromatography using silica-gel F_{254} plates (Merck). The eluent was *n*-butanol/ethyl acetate/methanol/ammonium hydroxide (7:4:3:4, v/v). It permitted a good resolution between adenosine, inosine, hypoxanthine and adenine nucleotides. The assays to study the distribution of radioactivity within the vesicles were performed as usual (see above). After filtration, vesicles were extracted with a 2 M solution of NH_4OH containing 10^{-7} M deoxycoformycin. The solvent was removed by lyophilization and the residue was dissolved with an aqueous mixture of standards (adenosine, inosine, hypoxanthine and AMP, 1 mM each) containing 10^{-7} M deoxycoformycin. The extent to which [^{3}H]adenosine was extravesicularly deaminated in 8 s was measured by including 2 μM unlabelled adenosine and deoxycoformycin (10^{-7} M) in the cold stop solution.

Vesicles were quickly pelleted (5 min 16000 × *g*) and an aliquot was taken for chromatographic analysis. After chromatography, standards were detected under an ultraviolet lamp and silica corresponding to each spot (4 per sample) was scraped, put into a scintillation vial together with 10 ml of cocktail and counted. After 8 s incubation, 90–95% of radioactivity was present in the interior of the vesicles or in the incubation medium, with the same R_F value as unlabeled adenosine.

Deoxycoformycin, an inhibitor of adenosine deaminase, prevents the metabolism of adenosine due to the enzyme of BBM. Vesicles were incubated for 90 min at 20 °C with respectively 50 μM of adenosine, inosine, guanosine, 2′-deoxyadenosine, 2-chloroadenosine or uridine with a medium containing 10^{-7} M deoxycoformycin. After extraction with a 2 M solution of NH_4OH containing 10^{-7} M deoxycoformycin, vesicles were pelleted (5 min 16000 × *g*) and an aliquot of the supernatant was chromatographed, as described above. The chromatography was developed by the fluorimetric method described by Guebitz et al. [24]. The vesicle extracts and the nucleoside blanks show the same patterns. This indicates that no significant metabolism occurs under these conditions. The limit of detection of the method is 5 ng of purine compound per chromatographic zone [24].

Results

Time-course of adenosine uptake

Adenosine uptake has a sodium-dependent component (see below). The time-course of the uptake in absence or in presence of sodium was linear up to 10 s (Fig. 1). 8 s was chosen as the time for the zero-trans experiments. As it is indicated in Materials and Methods, at this time there is no significant metabolization of either intra- or extra-vesicular adenosine.

Cation dependence of adenosine uptake

Compared to a mannitol medium, initial (8 s) uptake of adenosine increased in presence of sodium but not of other monovalent (potassium, rubidium, lithium or choline) or divalent (calcium) ions (Fig. 2).

Kinetic characterization of adenosine uptake

Previous authors have demonstrated the presence of sodium-dependent and sodium-independent adenosine transport systems in BBM. As shown in Fig. 3, in our experiments, the sodium-dependent component of the transport was saturable and displayed a K_m (adenosine) of 2.9 ± 0.6 μM and a V_{max} of 142 ± 18 pmol/min per mg protein (mean values ± S.E. of six experiments).

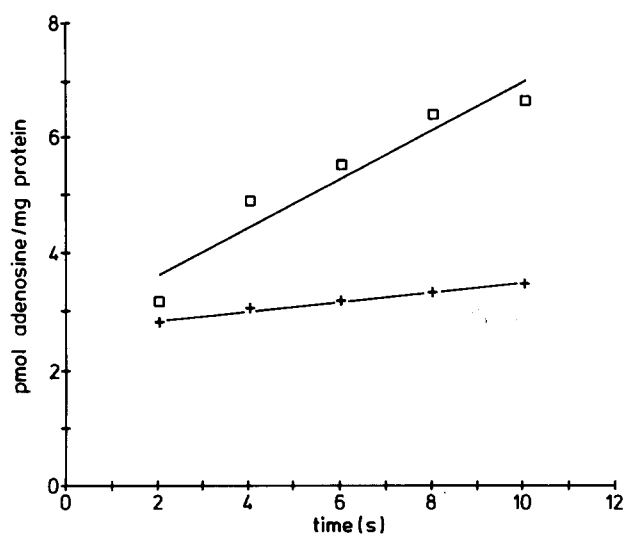

Fig. 1. Initial time-course of uptake of adenosine by rat kidney brush-border vesicles. The incubation media contained (final concentration) 100 mM mannitol, 5 μM deoxycoformycin, 20 mM Hepes-Tris (pH 7.4), 1.3 μM [^{3}H]adenosine and (□) 50 mM sodium nitrate or (+) 50 mM potassium nitrate. Each point is the mean of three experiments with triplicates. Temperature was 20 °C.

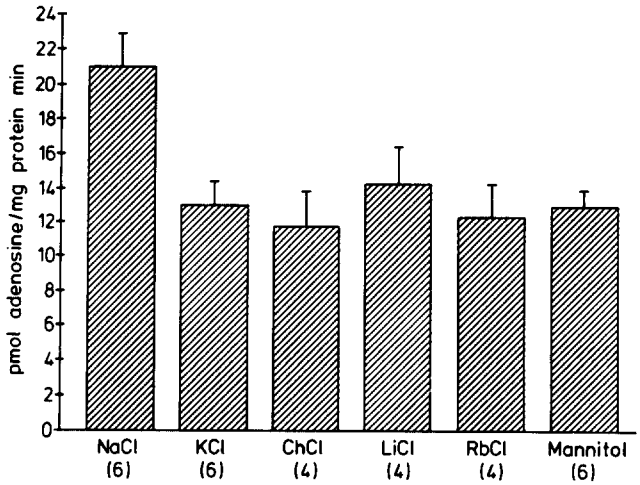

Fig. 2. Cation dependence of adenosine transport in brush-border vesicles. The incubation media contained (final concentration) 100 mM mannitol, 5 μM deoxycoformycin, 20 mM Hepes-Tris (pH 7.4), 1.3 μM [^{3}H]adenosine and 50 mM of the corresponding chloride salt. Incubation time was 8 s. All the differences with respect to mannitol (except for NaCl ($P < 0.05$)) are statistically non-significant. n is shown under the corresponding bar. ChCl = choline chloride.

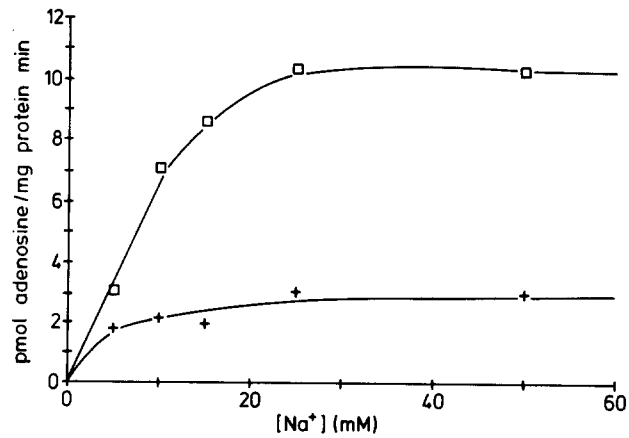

Fig. 4. Effect of sodium on the initial rate (8 s) of sodium-dependent adenosine uptake by kidney brush-border vesicles. The incubation media contained (final concentration) 100 mM mannitol, 5 μM deoxycoformycin, 20 mM Hepes-Tris (pH 7.4), 1.3 μM adenosine and different concentrations of (□) sodium nitrate or (+) sodium sulfate. Potassium nitrate or sulfate was, respectively, added in order to have a 50 mM final concentration. Each point is mean of two experiments with triplicates.

In addition, for the sodium-dependent uptake the stoichiometry and the affinity for sodium were determined, by varying the sodium concentration in the incubation medium. The data obtained with sodium nitrate or sodium sulfate are presented in Fig. 4. Fitting the data to the Hill equation (Fig. 5) an n ranging between 0.9 and 1.2 and a K_m (sodium) of 2.1 ± 0.4 mM were obtained (mean value $\pm$ S.E. of four separate experiments). The data depicted in Fig. 4 also show electrogenicity of the sodium-dependent adenosine transport, a result expected for sodium-adenosine cotransport.

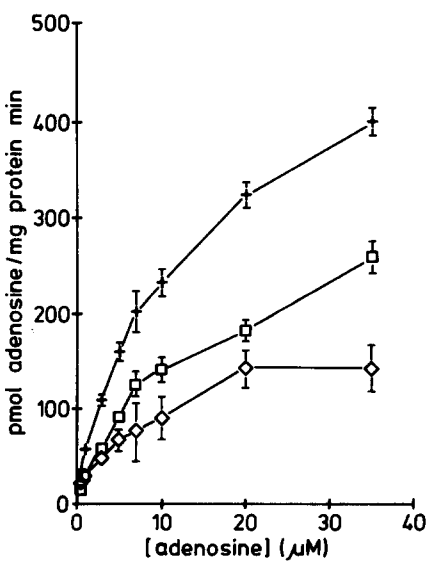

Fig. 3. Concentration dependence of the initial rate (8 s) of uptake of adenosine by kidney brush-border vesicles. The incubation media contained (final concentration) 50 mM sodium nitrate (+) or 50 mM potassium nitrate (□), 100 mM mannitol, 5 μM deoxycoformycin, 20 mM Hepes-Tris (pH 7.4) and adenosine between 0.5 μM and 70 μM. (◇) Difference between uptake in presence of Na^+ and K^+, i.e., sodium-dependent transport. The experiment shown is representative for six experiments with triplicates.

The saturation data obtained in the absence of sodium are, when fitted with the computer program, consistent with two processes, one saturable and another non-saturable (Fig. 3). The fit to two centers, one saturable and another nonsaturable, was significantly better than the fit to only one center (see Materials and Methods). The saturable system, which more likely corresponds to a transporter, displayed a K_m (adenosine) in the range 4–10 μM and a V_{max} of 174 ± 40 pmol/min per mg protein. The unsaturable system, which may correspond to simple diffusion, displayed a kinetic value of 3.4 ± 0.8 pmol/min per mg protein (mean values $\pm$ S.E. of six separate experiments).

In order to further characterize the sodium-dependent and -independent components of the transport,

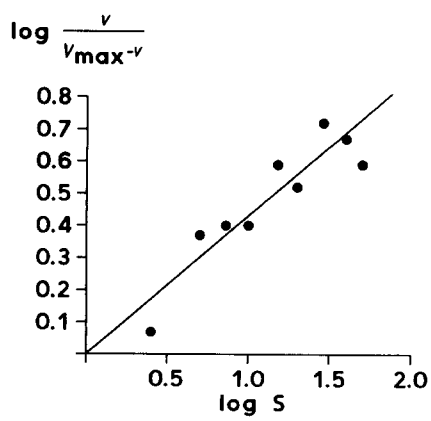

Fig. 5. Hill plot corresponding to data of Fig. 4.

uptake was studied at different temperatures. The results indicate that at 0 °C the sodium-dependent component is almost completely abolished. Identical results were obtained at two adenosine concentrations (1.3 and 15 μM) (Table I). At 0 °C, saturation experiments permit the fit of the data of the sodium-independent component to two systems, one saturable K_m (adenosine) 3.2–5.6 μM, V_{max} 26–32 pmol/min per mg protein (15–20% of the V_{max} at 19 °C; values for two separate experiments) and another non-saturable (K 0.6–0.8 pmol/min mg protein). Comparing these results with those obtained at 19 °C, K_m and K for both processes are only slightly changed, whereas the V_{max} is strongly decreased (see above). Due to the low sodium effect at 0 °C it was not possible to define the kinetic parameters (K_m and V_{max}) of the sodium-dependent component at 0 °C.

Substrate specificity and inhibitor sensitivity

In order to investigate the specificity of the adenosine transporter, inhibitors of adenosine deaminase, adenosine agonists, analogs of adenosine and known inhibitors of the adenosine uptake were tested in assays where adenosine translocation was measured at 19 °C in the presence of either potassium or sodium.

Hypoxanthine (100 μM), caffeine (50 μM), 3′-deoxyadenosine (50 μM), the adenosine agonists L-PIA (50 μM) and NECA (50 μM), and deoxycoformycin (100 μM), which is an inhibitor of adenosine deaminase, did not inhibit adenosine uptake. Nitrobenzylthioinosine, a potent inhibitor of the adenosine transport in other systems, did not inhibit (40 μM) the transport by BBM.

Inosine, guanosine, 2′-deoxyadenosine, 2-chloroadenosine and uridine, as well as dipyridamole, dis-

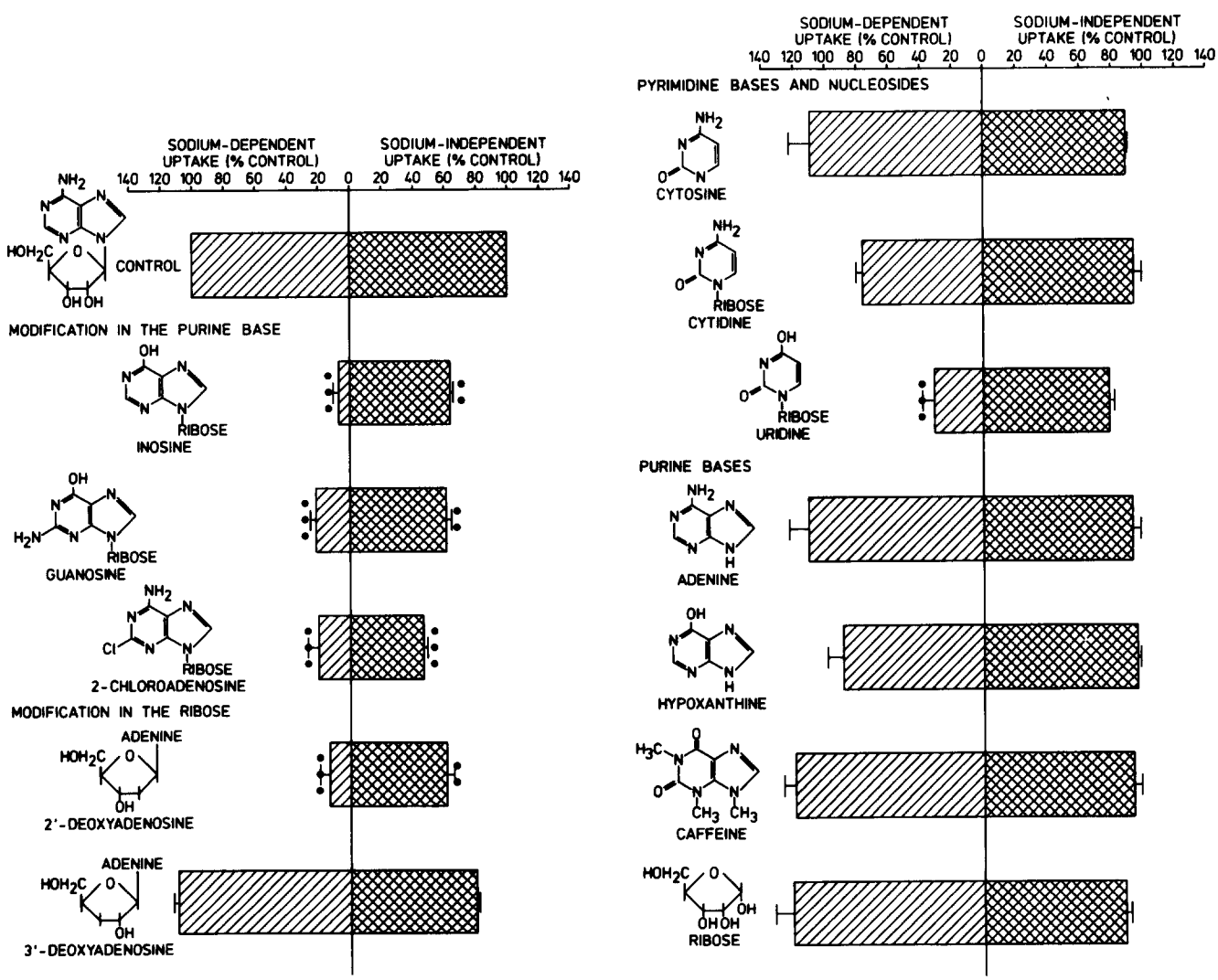

Fig. 6. Effect of some compounds on adenosine transport. Values are given in % with respect to the sodium-dependent (left side) or the sodium-independent (right side) adenosine transport control. The incubation media contained (final concentration) 100 mM mannitol, 20 mM Hepes-Tris (pH 7.4), 1.3 μM [^{3}H]adenosine, 50 mM sodium or potassium nitrate and 50 μM of the corresponding compound. (***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.1$ (vs. the corresponding control).

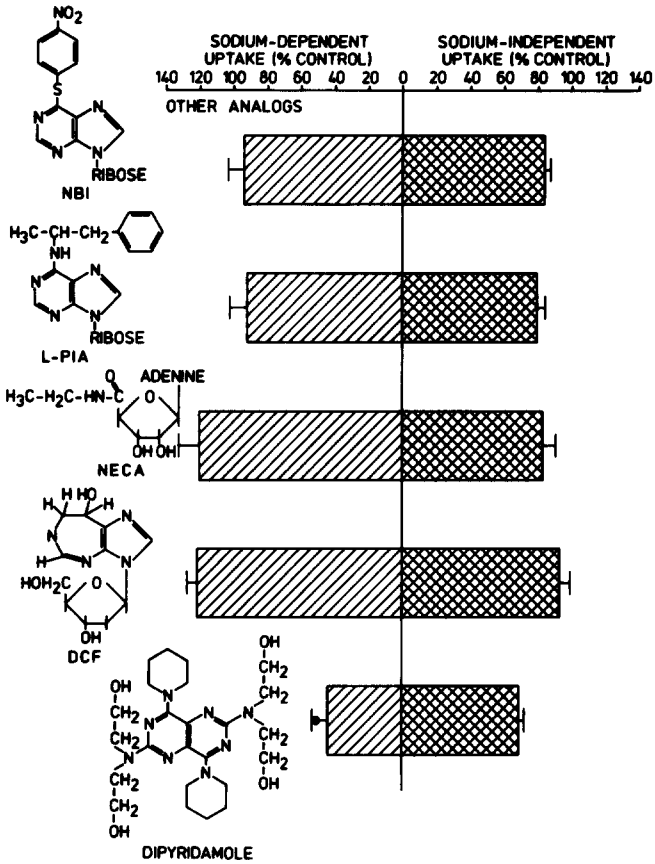

Fig. 6 (continued).

played a different pattern of inhibition, as indicated in Fig. 6. Dipyridamole and uridine depressed mainly the sodium-dependent component of the uptake, whereas the potassium-dependent component is not significantly affected. Inosine, guanosine, 2′-deoxyadenosine and 2-chloroadenosine (50 μM) abolished the sodium-dependent component almost completely, whereas the sodium-independent component was less affected.

TABLE I

Temperature dependence of adenosine uptake in brush-border membrane vesicles

The incubation media contained (final concentration) 100 mM mannitol, 5 μM deoxycoformycin, 20 mM Hepes-Tris (pH 7.4), adenosine at the concentration indicated and 50 mM sodium or potassium nitrate (see Materials and Methods). Sodium-dependent uptake represents the difference between uptake in the presence and absence of sodium. Values (in pmol adenosine/mg protein per min) are means ± S.D. of at least four experiments.

Adenosine:	1.3 μM			15 μM		
T (°C)	$+Na^+$	$-Na^+$	sodium dependent	$+Na^+$	$-Na^+$	sodium dependent
0	17±4	13±4	4	89±13	83±10	6
19	39±7	22±4	17	204±21	144±10	60
30	48±5	24±6	24	300±18	200±13	100
37	49±6	32±5	17	316±14	224±14	92

TABLE II

Inhibition of sodium-dependent adenosine transport system by various compounds

Inhibition constants were determined by non-linear regression of a plot v vs. S. Three concentrations of adenosine (1 μM, 2 μM and 40 μM) were used. Mean values of three experiments performed in triplicate were used for the calculations (values ± S.D.). All inhibition was of the competitive type.

Inhibitor	Apparent K_i (μM)
Inosine	1.9±0.3
Guanosine	7 ±1
Uridine	28 ±4
2-Chloroadenosine	7.8±0.7
2′-Deoxyadenosine	11.2±0.8

Inhibitors of the sodium-dependent uptake were variable in potency, as indicated by their apparent K_i for competitive inhibition of adenosine sodium-dependent uptake (Table II). Competitive inhibition showed in all cases the best fit (with $r > 0.994$).

TABLE III

Trans stimulation studies by inosine, guanosine, uridine, 2-chloroadenosine and 2′-deoxyadenosine

Vesicles were preincubated 90 min at 30 °C in the presence of 100 mM mannitol, 20 mM Hepes-Tris (pH 7.4), 5 μM deoxycoformycin and the corresponding nucleoside indicated. In the vesicles, which were not preincubated with adenosine and/or inhibitor, 0.909 μM adenosine and/or 4.545 μM inhibitor were included in the uptake media. The uptake media contained (final concentration) 100 mM mannitol, 5 μM deoxycoformycin, 20 mM Hepes-Tris (pH 7.4), 1.3 μM [^{3}H]adenosine, and 50 mM sodium ($+Na^+$) or potassium ($-Na^+$) nitrate. Sodium-dependent uptake represents the difference between uptake in the presence and absence of sodium. Mean values and standard deviations derived from three experiments are given (*) $P < 0.05$ in the paired Student's t-test.

Trans Side	% adenosine transported		
	$+Na^+$	$-Na^+$	sodium dependent
0	100	100	100
10 μM Adenosine	135± 7(*)	127± 8(*)	145
50 μM Inosine	137± 8(*)	115± 7(*)	165
0	100	100	100
10 μM Adenosine	126± 4(*)	114± 7(*)	141
50 μM Guanosine	133±15(*)	132± 6(*)	135
0	100	100	100
10 μM Adenosine	120±21(*)	127±20(*)	112
50 μM Uridine	136± 3(*)	92± 4	193
0	100	100	100
10 μM Adenosine	110± 3(*)	103± 6	119
50 μM 2-Chloroadenosine	129± 7(*)	133± 8(*)	123
0	100	100	100
10 μM Adenosine	127± 7(*)	123± 7(*)	132
50 μM 2′-Deoxyadenosine	145± 9(*)	132±10(*)	162

Competitive inhibition might be due to competition for binding at the transporter and/or competition for translocation. In order to test whether the inhibitory effect of inosine, guanosine and uridine is due to transport by the same transporter, trans-stimulation experiments were performed. The results (Table III) indicated that there exists a clear stimulation of adenosine influx when inosine, guanosine or uridine are inside the vesicles. Adenosine inside was used as a control and it was demonstrated that inosine as well as guanosine and adenosine enhance both, sodium-dependent and independent components. In agreement with the inhibition studies, uridine enhances solely the sodium-dependent component of the uptake. This is a clear indication that the nucleosides are, at least partially, translocated by the same transporter as adenosine.

Discussion

In this paper we have further characterized the sodium-dependent and sodium-independent component of adenosine uptake by BBM from rat kidney. It was shown that there exists a sodium-dependent component of the uptake which possesses a very low K_m for sodium (2.1 mM), if compared with the uptake of other substances such as glucose (14 mM, [25]) or glutamate (76.9 mM, [26]) by BBM. The Na^+-independent transport was insensitive to ions such as K^+, choline, Li^+ and Rb^+ (Fig. 2). These results are in contrast with those found by Lee et al. [27], since these authors demonstrate a K^+-dependent transport of uridine in BBM from rat kidney.

The experiments of adenosine uptake performed at different temperatures suggest that the sodium-dependent component as well as the saturable sodium-independent component are strongly dependent on the physical state of the membrane lipids. Studies with other sodium-dependent systems also show a dependence on temperature. For example, De Smedt and Kinne [28], working with hog renal BBM vesicles, observed a discontinuity between 12 and 15 °C in the Arrhenius plot of the sodium-dependent D-glucose and phosphate uptake.

The sodium-dependent component of the system displayed a K_m of 2.9 μM for adenosine, which is somewhat higher than that found by Le Hir and Dubach [11] working also with rat kidney BBM. These authors were able to find an overshoot in the presence of an inwardly directed sodium gradient. The overshoot was also found by Le Hir and Dubach [17] when measuring the uptake of other purine nucleosides such as 2′-deoxyadenosine, guanosine and inosine. Thus, it seems that the sodium-dependent component of the transport is shared by different nucleosides. Our trans-stimulation results shown in Table III also support this hypothesis.

Trimble and Coulson [10], working with rat kidney luminal vesicles, found, in the absence of sodium, two saturable systems of low (4.4 μM) and high (43 μM) affinity for adenosine. In the absence of sodium, we have found two systems, but one was saturable (K_m = 4–10 μM) and the other non-saturable (K_m > 300 μM). In all experiments performed the complete set of data could not be fitted to a two saturable centers system, but to one saturable and another non-saturable. It should also be noted that Trimble and Coulson [10] found a sodium stimulation only in the case of the low-affinity system, but not in the case of the high-affinity one. These differences can be due to a different way of preparation of BBM and of designing the experiment.

The different pattern of inhibition demonstrates that the sodium-dependent component and the saturable component of the sodium-independent uptake correspond to separate systems of adenosine transport. The structural requirement of the sodium-adenosine cotransport system is low, since in addition to nucleosides of purine, it also interacts with the pyrimidine nucleoside uridine. In contrast, the sodium-independent component deals only with purine nucleosides.

Plagemann and Woffendin [29] observed in mouse spleen cells also two systems for the nucleoside transport: a sodium-independent transporter with broad substrate specificity and a sodium-dependent transporter, specific for uridine and purine nucleoside. Other authors have also observed a relation between uridine transporter and purine nucleoside transport; so the sodium-uridine transporter presents a stoichiometry 1 : 1 [27], as does the sodium-adenosine transporter. Jarvis [12], working with rabbit intestinal BBM, observed an inhibition of sodium-dependent uridine transport by adenosine, inosine, 2′-deoxyinosine and 2-chloroadenosine. Le Hir and Dubach [17] also demonstrated in BBM from rat kidney a strong competitive inhibition of Na^+-dependent adenosine transport by inosine, guanosine, 2-chloroadenosine and 2′-deoxyadenosine. These and our results presented in Table II indicate that K_i of inosine and guanosine is low and comparable with K_m (adenosine).

The fact that inosine inhibits adenosine uptake by presumably being transported by the same system as adenosine is very important from a functional point of view. Since ecto-adenosine deaminase can transform extracellular adenosine to inosine, the enzyme could balance the quantity of nucleoside (either adenosine or inosine) which enters the cell. The same could apply for 2′-deoxyadenosine and deoxyinosine, although it is not likely to find these compounds extracellularly. For guanosine, which also strongly inhibits adenosine uptake, similar functional implications cannot be established due to the lack of metabolizing enzymes outside the cell.

Our BBM preparation was almost insensitive to dipyridamole, a strong inhibitor of adenosine transport in other systems. Even in the same kind of BBM, Trimble and Coulson [10] were able to find a relatively high inhibition by the compound assayed at a concentration of 200 μM. Although in some cells there exists dipyridamole-resistant nucleoside transporter, this varies from species to species and within a given species it depends on the type of cell [14]. This heterogeneity of results supports the view of Hammond and Clanachan [16] of the existence of different conformations for the transporter; we suggest that the equilibrium between these forms can be modified depending upon the way of obtaining the experimental preparations.

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