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PRELIMINARY COMMUNICATIONS

A POSSIBLE ASSOCIATION BETWEEN THE NUCLEOSIDE TRANSPORT SYSTEM OF HUMAN ERYTHROCYTES AND ADENOSINE DEAMINASE

R. P. Agarwal and R. E. Parks, Jr.

Section of Biochemical Pharmacology

Division of Biological and Medical Sciences

Brown University, Providence, R. I. 02912, USA

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Recently this laboratory has examined the relative roles of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4; ADAase) and adenosine kinase (ATP:adenosine 5'-phosphotransferase, EC 2.7.1.20) in the control of human erythrocytic adenosine metabolism (1-3). These studies have been facilitated greatly by the technic of high pressure liquid chromatography and by the availability of several unique biochemical tools such as 2-fluoroadenosine, N^6-phenyladenosine and coformycin (1-3). Recently, in an attempt to assess the relative significance of simple diffusion and of the specific facilitated diffusion mechanism described in the elegant studies of Paterson and his colleagues (4,5), an experiment was performed with washed intact human erythrocytes in which the rate of ammonia liberation was measured from relatively high concentrations of adenosine (1.0 mM) in the presence and absence of a specific inhibitor of the erythrocytic facilitated transport mechanism, p-nitrobenzylthioguanosine (NBTGR) (5). As seen in Figure 1, in the presence of 10 μM NBTGR and 1.0 mM adenosine, ammonia liberation was almost completely inhibited in intact erythrocytes. However, no inhibition of ammonia liberation from adenosine occurred in hemolysates prepared from normal erythrocytes or from cells pretreated with NBTGR, even when the inhibitor (20 μM) was added subsequent to hemolysis. As

described elsewhere (3), the addition of the specific tight-binding ADAase inhibitor, coformycin (1 μg per ml of suspension), either to intact erythrocytes or to hemolysates, completely abolished ammonia liberation from adenosine, which indicates that essentially all of the ammonia formed under these conditions results from the ADAase reaction.

In order to assess the disposition of adenosine in erythrocytes treated with the nucleoside transport inhibitor, NBTGR, the experiment presented in Figure 2 was performed. Washed human erythrocytes were incubated for 30 minutes with varying concentrations of adenosine-8-^{14}C in the presence and absence of NBTGR (10 μM). Following incubation, the cells were extracted with perchloric acid and subjected to thin-layer chromatography on PEI-cellulose as described by Crabtree and Henderson (7). Following chromatography, the individual nucleotide spots were visualized with UV light, cut out and counted in a toluene-based scintillation fluid in a Packard scintillation counter. Consistent with earlier observations (3), when normal erythrocytes were incubated with adenosine-8-^{14}C, most of the radioactivity in the nucleotide pools was found in IMP with much smaller quantities in the adenine nucleotides (predominantly in ATP). In the presence of the nucleoside transport inhibitor, NBTGR, however, this pattern of erythrocytic nucleotide formation was drastically altered. Most strikingly, and consistent with the decreased ammonia liberation seen in Figure 1, the formation of IMP was markedly reduced (about 5 fold at 200 μM adenosine). On the other hand, the incorporation of adenosine-8-^{14}C into the adenine nucleotides (principally ATP) was markedly increased (greater than 4 fold at 200 μM adenosine). These observations are also consistent with the rapid formation of large amounts of nucleotides from 2-fluoroadenosine, an analog that is not a substrate for ADAase (1,2).

A reasonable hypothesis to explain these unexpected and unusual observations is that the enzyme, ADAase, although in the soluble fraction in hemolysates, is physically associated in the intact erythrocyte with the cell membrane at a site adjacent to the nucleoside transport system. One might specu-

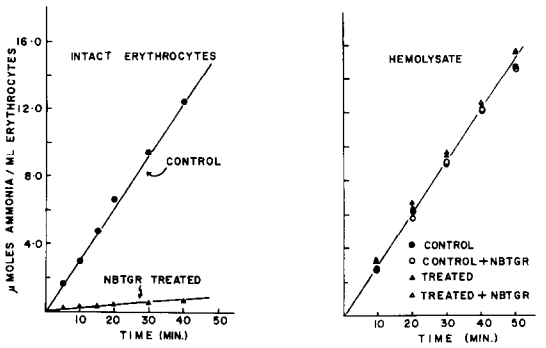

Fig. 1 Effect of NBTGR on deamination of adenosine by intact human erythrocytes or hemolysates. Erythrocytic suspensions (about 2.5%) in a medium containing phosphate buffer, 50 mM, pH 7.5; NaCl, 75 mM; $MgSO_4$, 2 mM and glucose 10 mM were preincubated at 30° with 10 μM NBTGR. Parallel experiments with no addition of NBTGR served as controls. After 20 min of preincubation adenosine (1 mM) was added and ammonia formation was determined at various time intervals by the method of Chaney and Marbach (6). For the hemolysate studies, cells were pretreated with NBTGR as above, collected by centrifugation and hemolyzed by adding 4 vol of cold distilled water. After hemolysis (30 min), an equal volume of double strength medium was added to achieve a final concentration of medium described above and both control and NBTGR-treated hemolysates were divided into two portions. One portion of each was treated with additional NBTGR (20 μM) for 20 min at 30°. Adenosine (1 mM) was added to all portions and ammonia was determined as above.

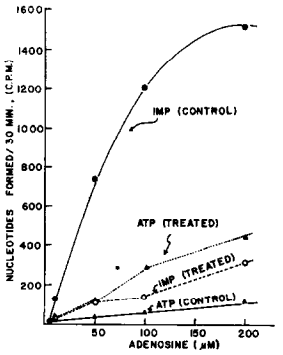

Fig. 2 Formation of nucleotides from adenosine-8-^{14}C in human erythrocytes. Erythrocytic suspensions (20%) were preincubated with or without 10 μM NBTGR as described in Figure 1. Then adenosine-8-^{14}C (0.98 mCi/mmole) was added at various concentrations. After incubation for 30 min at 30°, cells were extracted with perchloric acid, the acid-soluble supernatant fluids were neutralized with KOH, and the nucleotides were separated by thin-layer chromatography (Ref. 8) and the radioactivity determined.

late that ADAase is loosely bound to the membrane by hydrophobic bonds. If this were so, most of the adenosine that entered the erythrocyte via the nucleoside transport system would be preferentially deaminated forming inosine and would not become available for phosphorylation by adenosine kinase. On the other hand, when the transport system is blocked, it appears that much more of the adenosine that enters the cell by simple diffusion becomes available for phosphorylation by the adenosine kinase reaction. This hypothesis is also consistent with observations reported by De Bruyn and Oei (8) which suggest that ADAase is associated with the erythrocytic membrane. Further studies are planned to attempt to demonstrate directly an association between ADAase (and perhaps other enzymes) with the erythrocytic membrane.

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