Cation Transport in Duchenne Muscular Dystrophy Erythrocytes (36802)

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Recent reports (1–3) have indicated that a red blood cell (RBC) membrane ATPase obtained from patients with Duchenne muscular dystrophy (DMD) is abnormal in that it is stimulated by ouabain. This is in marked contrast to inhibition by ouabain of an RBC membrane ATPase obtained by the same method from normal patients. These observations raise the question of a generalized alteration in active cation transport in DMD patients since in at least two reports (1, 3) the enzyme stimulated was considered to be the "transport" or Na–K-dependent ATPase (1). Because of the importance of the latter possibility, the effect of ouabain on cation transport was studied in RBC from normal subjects and those with DMD.

Materials and Methods. ^{86}Rb uptake (4, 5) was utilized to measure active cation transport because this isotope has a longer half-life than ^{42}K (18.6 days as opposed to 12.4 hr) and ^{86}Rb has been shown to be actively transported by the RBC Na–K pump (6, 7). The RBC uptake of Rb is readily inhibited by cardiac glycosides (4, 5).

In each of the five experiments DMD and normal RBC preparations were studied simultaneously. The heparinized blood samples were centrifuged at 4°, and the plasma and buffy layers were removed. The RBC were then washed three times with cold (4°) K-free modified Krebs–Ringer phosphate buffer solution (5) and packed to a hematocrit of approximately 80%. RBC aliquots were pipetted into incubation tubes and either 0.4 ml (control tubes) or 0.3 ml (ouabain tubes) of the Krebs–Ringer solution was added. Ouabain solution (0.1 ml) was added (ultimate tube concn, 10^{-4} to 10^{-10} M) to noncontrol tubes. All tubes were incubated at 37° (in a shaker bath) for 45 min. RbCl solution (0.1 ml) containing enough ^{86}RbCl to give 3.3 μCi/sample was added to give final Rb concentration of 2.2×10^{-3} M and the incubation continued for an additional 2 hr. The RBC were then washed twice with cold normal saline and radioactivity counted in a gamma well counter (Nuclear Chicago, Model No. 1085).

Each experiment was carried out in duplicate and each experimental point is the average value obtained. ^{86}Rb uptake curves (over the range of ouabain concentrations) were constructed for the DMD and normal cells.

Results. As shown in Fig. 1a, there was no ouabain stimulation of ^{86}Rb uptake in either control or DMD RBC; rather, typical inhibitory effects were present. Although the ouabain inhibition in DMD RBC appeared greater than that of controls, the difference was not significant at any ouabain concentration (Student's t test).

Because of a report that DMD plasma contained a factor that modified the RBC ATPase and caused the ouabain stimulation (2), two DMD patients and controls were restudied with the addition of 0.1 ml of homologous plasma to each incubation tube. The results (Fig. 1b) show that ouabain inhibited ^{86}Rb uptake of both control and DMD RBC in the presence of homologous plasma. The differences between the two curves were not significant.

As an additional test 0.2 ml of homologous plasma was added to duplicate samples of normal and DMD RBC which were then incubated with or without 10^{-4} M ouabain in the fashion described above (data not shown), and ouabain also inhibited both normal and DMD RBC equally at this plasma concentration.

Discussion. These data demonstrate that ouabain is an effective inhibitor of ^{86}Rb uptake in DMD as well as normal RBC. It is also clear that DMD plasma, acting on the

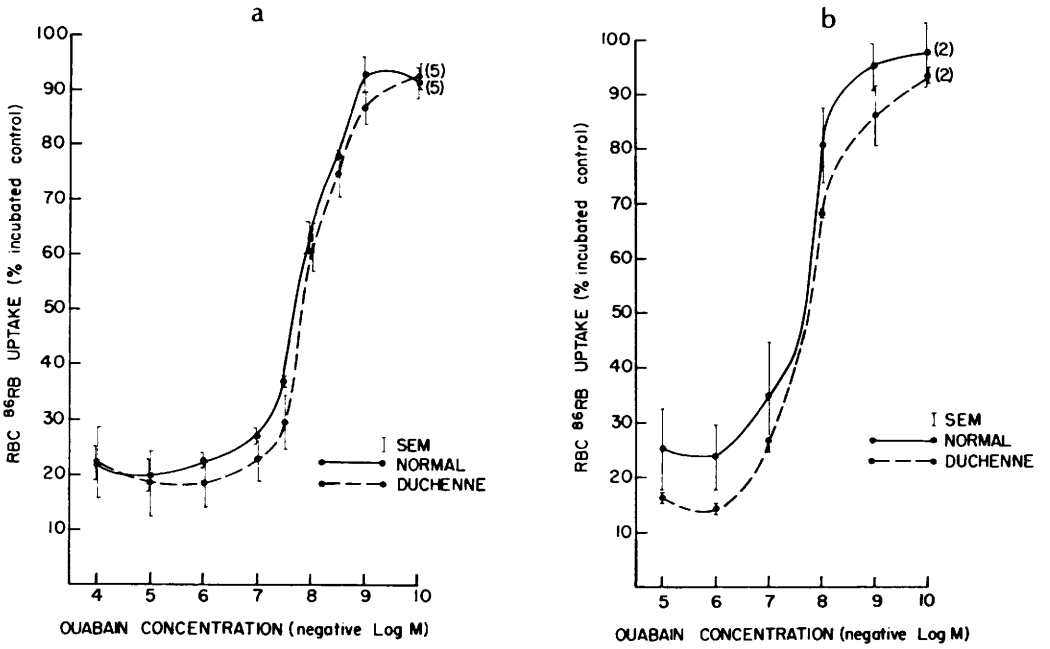

FIG. 1. Washed RBC were preincubated in K-free modified Krebs–Ringer solution (5) with varying concentrations of ouabain for 45 min at 37° and then 3.3 μCi of ^{86}RbCl were added and the incubation was continued for another 2 hr. The RBC were then washed and the ^{86}Rb activity was counted. (a) ^{86}Rb uptakes (expressed as percentage of control uptakes) in normal and DMD RBC were almost identical over the range of ouabain concentrations. There was no statistical difference in ouabain inhibition of uptake between the two groups at any point (Student's t test). (b) ^{86}Rb uptakes in normal and DMD RBC with 0.1 ml homologous plasma added to each incubation tube were comparable. There was no statistically significant difference between the two groups at any point (Student's t test).

outer surface of the RBC membrane, does not alter the effect of ouabain on ^{86}Rb transport.

In each experiment, the absolute uptakes of ^{86}Rb (cpm) in the normal and DMD RBC control tubes were also compared. The hematocrits of all of the stock washed packed RBC samples were approximately equal (ca. 80%) and all other incubation conditions were identical.

In spite of the possible small variations in the hematocrits between the normal and DMD samples, their control uptakes (actual counts) were not statistically different, suggesting similar base line transport activity.

The aforementioned conclusions are supported by a recent study of RBC ATPase activity in control and DMD RBC in which "classical" Na–K-dependent ATPase activity was studied (8). No differences were found in either total or ouabain-sensitive ATPase activity.

The ATPase assay conditions (low concentrations of Na + K) described by Brown, Chattopadhyay and Patel (1) and Peter, Worsfold and Pearson (2) and also Araki and Mawatari (3) were such as to make it unlikely that these authors were measuring Na–K-dependent ATPase activity. It would, therefore, not be expected that the observations would be relevant to active cation transport since that function is generally considered to be dependent upon the activity of the Na–K-dependent ATPase. Whether or not the described ouabain stimulation of a non-transport ATPase from DMD RBC has pathophysiological significance remains to be elucidated.

Summary. ^{86}Rb transport was studied in normal and DMD RBC. ^{86}Rb transport in

both groups was found to be inhibited equally by ouabain even in the presence of homologous plasma. These data fail to demonstrate an intrinsic or extrinsic (plasma-induced) modification of ouabain inhibition of ^{86}Rb transport in DMD RBC.

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1. Brown, H. D., Chattopadhyay, S. K., and Patel, A. B., Science **157**, 1577 (1967).

2. Peter, J. B., Worsfold, M., and Pearson, C. M., J. Lab. Clin. Med. **74**, 103 (1969).

3. Araki, S., and Mawatari, S., Arch. Neurol. **24**, 187 (1971).

4. Lowenstein, J. M., and Corrill, E. M., J. Lab. Clin. Med. **67**, 1048 (1966).

5. Grahame-Smith, D. B., and Everest, M. S., Brit. Med. J. **1**, 286 (1969).

6. Love, W. D., and Burch, G. E., J. Lab. Clin. Med. **41**, 351 (1953).

7. Kahn, J. B., Jr., J. Pharmacol. Exp. Ther. **136**, 197 (1962).

8. Klassen, G. A., and Blostein, R., Science **163**, 492 (1969).

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