

Biochemical Markers for Huntington's Chorea

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SUMMARY: *The uptake of dopamine (DA) by platelet rich plasma was assayed in 11 patients with Huntington's chorea (H.C.). The results confirmed the previous observation that platelets from H.C. patients take up, at equilibrium, more dopamine than do platelets from normal control subjects. The mean difference was 50% higher at DA substrate concentrations of 0.11 mM.*

However, attempts to confirm the higher $\text{Na}^+ - \text{K}^+$ ATPase activity of erythrocyte ghosts from Huntington's chorea patients were unsuccessful.

RÉSUMÉ: *L'uptake de dopamine par le plasma riche en plaquettes fut mesuré chez onze patients avec chorée de Huntington. Les résultats confirment l'observation préalable qui indiquait que les plaquettes des patients choréiques captent, en équilibre, plus de dopamine que les plaquettes des sujets contrôles. A une concentration de substrat dopamine de 0.11 mM la différence moyenne fut de 50% plus élevée.*

Cependant, il ne fut pas possible de confirmer l'activité supérieure en ATPase $\text{Na}^+ - \text{K}^+$ des érythrocytes de patients choréiques.

INTRODUCTION

It was reported that blood platelets from patients with Huntington's chorea (H.C.) take up more dopamine (DA) than do platelets from normal controls (Aminoff et al, 1974; McLean & Nihei, 1977). Subsequently, Butterworth et al (1977) and Omenn and Smith (1978) studied the *rate* of DA uptake by platelets finding no difference in the platelets of H.C. patients. These conflicting conclusions appear to be due mainly to different experimental design. The former studies measured the DA *capacity* of platelets (amount of DA taken up at equilibrium) whereas the latter investigators determined the *rate* of DA uptake.

This paper reports the results of the DA uptake assay in 11 H.C. patients and confirms our previous observation of high uptake capacity.

Butterfield et al (1978) reported a 30% higher $\text{Na}^+ - \text{K}^+$ ATPase activity in erythrocyte ghosts from H.C. patients. We assayed the $\text{Na}^+ - \text{K}^+$ ATPase activity in erythrocyte ghosts from 10 H.C. patients, but found the activity to be normal.

METHOD

Dopamine Uptake Assay:

The H.C. patients consisted of 7 males and 4 females ranging in age from 35 to 73. The diagnosis was made on the basis of family history and clinical examination. All medication was stopped two weeks before blood was drawn. Blood samples collected in Vacutainer tubes (B-D Co. Canada) containing EDTA and sorbitol were centrifuged at 200 g for 5 min at room temperature. Platelet rich plasma (PRP) was recovered as the supernatant. Patient and control samples were assayed concurrently.

In the DA uptake assay, 10 μl of the stock ^{14}C -dopamine solution was

added to each ml of PRP and incubated for 1 hour at 37°. The concentration of DA in PRP was adjusted by diluting the stock solution (25 $\mu\text{Ci}/55 \mu\text{mol}$) to 0.55, 0.275 or 0.11 mM. After incubation, 2 ml of cold 0.9% NaCl containing 0.15 $\mu\text{Ci}/\text{ml}$ of ^3H -inulin (Amersham Corp. Toronto/Chicago) was added to 1 ml of PRP, mixed by a vortex mixer, and the mixture was centrifuged at 8000 g for 10 min at 4°. 20 μl of the supernatant and the pellet were dissolved respectively in 1 ml of 1% sodium dodecyl-sulfate and the radioactivity was counted with 10 ml of Aquasol II (NEN Canada, Que.) in a Bechman SL-200 Scintillation counter. The levels of tritium and carbon-14 were calculated using two variable discriminators.

The purity of the ^{14}C -dopamine (Amersham Corp. Toronto/Chicago) and ^{12}C -dopamine (Calbiochem-Behring Corp. Calif.) was examined using a thin layer chromatography in n-butanol-acetic acid-water.

$\text{Na}^+ - \text{K}^+$ ATPase Assay:

Erythrocyte ghosts were obtained by the procedure of Philipson and Baumgartner (1979). The ATPase activity was determined as the ouabain sensitive portion of ATPase. Two types of incubation mixtures were used to compare the $\text{Na}^+ - \text{K}^+$ ATPase of erythrocyte ghosts from H.C. patients and healthy volunteers; one described by Wins and Schoffniels (1966) and Butterfield et al (1978), and the other by Jarrett and Penniston (1978). The amount of inorganic phosphate released from ATP was measured by the method of Jarrett and Penniston (1978).

The protein content of erythrocyte ghosts was assayed using the biuret method.

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TABLE 1

Summary of Dopamine Uptake Assay

Concentration of dopamine added to PRP (mM)	n moles of dopamine in 10 ⁸ platelets		difference of means	p-value
	control (n = 13)	H.C. (n = 11)		
	mean S.D.	mean S.D.		
0.550	2.58 ± 0.65	3.11 ± 0.87	0.53	< 0.05
0.275	1.65 ± 0.45	2.21 ± 0.77	0.56	< 0.025
0.110	1.00 ± 0.26	1.49 ± 0.29	0.49	< 0.005

TABLE 2

Na⁺ - K⁺ ATPase Activity of Erythrocyte Ghosts

	ATPase Activity (n moles/h.mg.) at 37°			
	Assay Method of Butterfield et al (1973)		Assay Method of Jarret and Penniston (1978)	
	mean	S.D.	mean	S.D.
H.C.	109 ± 86 (n = 10)		120 ± 78 (n = 6)	
CONTROL	134 ± 61 (n = 10)		128 ± 57 (n = 6)	

RESULTS

Table 1 shows the average uptake capacity of DA by platelet rich plasma from controls at DA substrate concentrations of 0.11, 0.275 and 0.55 mM. Depending upon the DA concentration, uptake capacity was 20 - 50% higher in H.C. patients. The actual uptake amounts are depicted in Fig. 1 and indicate that 8 of 11 patients were outside 1 standard deviation of the mean at a substrate concentration of 0.11 mM. The results were less impressive at concentrations of 0.275 and 0.55 mM.

Table 2 summarizes the results of the erythrocyte ghost Na⁺ - K⁺ ATPase assay for both the Butterfield et al (1978) and Jarrett and Penniston (1979) methods. The erythrocyte ghost ATPase activity was the same in Huntington's chorea patients and normal controls.

DISCUSSION

The results of this study on the DA uptake capacity by platelets from H.C. patients were consistent with those reported by Aminoff et al (1974) and ourselves (1977). Butterworth et al (1977) reported that the rate of DA uptake by platelets from 3 unmedicated Huntington's chorea patients was not abnormal. Omenn and Smith (1978) also measured the rate of DA uptake in platelets from 3 patients finding no abnormality. The distinction between the uptake capacity and the rate of uptake has not been clearly recognized by these authors. It appears that the DA uptake capacity is elevated in the patient's platelets, but not the rate of uptake.

There has been much work done on the mechanism of serotonin (5HT)

uptake and transport by platelets, but only a few studies on DA. The mechanism for 5HT and DA uptake is probably very similar, if not identical. DA is the substrate for a non-specific pump in the membrane of platelets which accumulates organic bases such as 5HT and nor-epinephrine (Solomon et al, 1970). Indeed, it has been shown that 5HT and DA are stored in the same intracellular vesicles of rabbit platelets (DaPrada & Pletscher, 1969). Although DA uptake is inhibited by

5HT, cold and various metabolic inhibitors (Solomon et al, 1970), it has been suggested (Stohl and Meltzer, 1978) that DA diffuses passively into human platelets and is accumulated in storage granules. However, haloperidol, which binds to DA receptor (Creese et al, 1977) has been shown to inhibit DA uptake (Solomon et al, 1970). In some preliminary experiments, we have found that 0.1 mM haloperidol inhibited 90% of the DA uptake in the presence of 0.25 mM DA. Also, as is

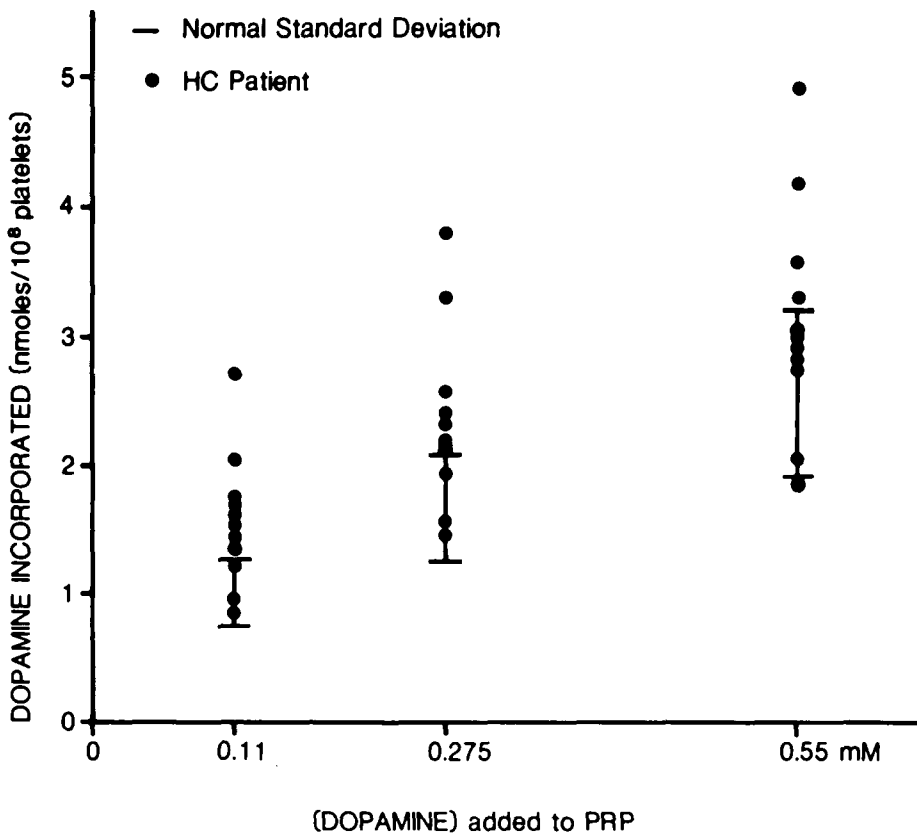


Figure 1 — Dopamine uptake by blood platelets from Huntington's chorea patients. The uptake values are determined after incubating PRP with ¹⁴C-dopamine for 60 minutes at 37°. The horizontal bars indicate the standard deviations of control uptake values.

the case with chromaffin granule ghosts from adrenal medulla (Ingebretsen and Flatmark, 1979), $(\text{NH}_4)\text{Cl}$ suppresses the DA uptake by platelets by collapsing the pH-gradient across plasma membranes. These observations suggest that platelets take up DA through an energy-dependent and receptor-mediated mechanism, as well as through the passive diffusion. We have not determined whether altered active uptake or passive diffusion causes the increased DA uptake in platelets from H.C. patients.

The DA concentration (.55 mM) in the substrate added to the platelet rich plasma has been criticized (Omenn and Smith, 1978) as being too high to reveal any characteristics of physiological importance. It is, however, at 10 mM that DA saturates the $(\text{NH}_4)\text{Cl}$ -sensitive and energy dependent DA transport in chromaffin granule system (Ingebretsen and Flatmark, 1979). Thus, the DA concentration range used in our studies does not seem unreasonably high.

It is difficult to correlate the abnormal uptake of DA by platelets with the elevated levels of brain DA found in patient with H.C. (Bird & Livesen, 1974). A common defect involving both platelets and certain central neurones in H.C. may explain these findings. It has been shown in rats that brain DA receptors increase after lesions of the nigrostriatal pathway but platelets have not been examined after central nervous system lesions. Changes in platelets would not be expected.

In this study, we could not confirm the results of Butterfield et al (1978) concerning the elevated $\text{Na}^+ - \text{K}^+$

ATPase activity of erythrocyte ghosts from H.C. patients. The control $\text{Na}^+ - \text{K}^+$ ATPase activity found by Butterfield et al (1978) was 66.1 ± 6.4 whereas our level was 134 ± 61 nmoles/h mg. The reason for this discrepancy is not clear. The $\text{Na}^+ - \text{K}^+$ ATPase activity of human erythrocyte ghosts ranges from 36 to 400 n moles per mg hr at 37° (Albers, 1967; Zewall et al, 1973). This wide variation in normal may reflect the capricious nature of the ghost preparation.

Based on the results of this study, the DA uptake assay should yield a bimodal distribution when applied to patients at risk if the same uptake abnormality exists in the pre-symptomatic patient.

ACKNOWLEDGEMENTS

This work was supported by the Alberta Mental Health Advisory Council.

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