

Quebec Cooperative Study of  
Friedreich's Ataxia

## Platelet Taurine Uptake in Spinocerebellar Degeneration

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**SUMMARY:** *The uptake of  $^{14}\text{C}$ -taurine was studied in the platelets of 20 ataxic patients and 20 age-matched normal control subjects. No significant differences were found in uptake or kinetics of taurine between the two groups of subjects. If a transport defect in taurine exists in Friedreich's ataxia, it is not present in all tissues. Preliminary indication was obtained in favor of heterogeneity of the uptake pattern between ataxic individuals.*

**RÉSUMÉ:** *La captation de la taurine  $\text{C}^{14}$  fut étudiée dans les plaquettes de 20 patients ataxiques et 20 sujets normaux contrôlés appariés pour l'âge. Aucune différence significative ne fut trouvée dans la captation ou dans les caractéristiques cinétiques de la taurine entre les deux groupes de sujets. Ceci indique que s'il existe un défaut dans le transport de la taurine dans l'ataxie de Friedreich, il n'est certainement pas présent dans tous les tissus. Nous avons obtenu quelques indices préliminaires d'une hétérogénéité de la captation entre les individus ataxiques étudiés.*

### INTRODUCTION

Platelets appear to be a model for uptake and binding systems in serotonergic (Stacey, 1961; Pletscher, 1968; Pletscher et al., 1971; Meynert et al., 1968) and dopaminergic (Boullin, 1970) nerve endings in the brain and sympathetic nerve endings in the periphery (Abrams, 1969; Paasonen, 1973). This evidence has prompted studies of the monoamine and enzymatic content of platelets in several neurological disorders known to have alterations in biogenic amines in the brain, like endogenous depression (Baldessarini, 1972), phenylketonuria (Pare, 1959), Down's syndrome (Lott, 1972), schizophrenia (Murphy et al., 1972; Wyatt et al., 1973; Buchsbaum et al., 1976), Huntington's chorea (Butterworth et al., 1977) and Parkinson's disease (Boullin et al., 1970; Yamaguchi et al., 1972; Barbeau et al., 1975).

Other studies showed that platelets are rich in taurine (Fredo et al., 1959), that the transport of taurine into human platelets is a metabolically dependent process (Ahtee et al., 1974), that the uptake can be suppressed by metabolic inhibitors, structural analogs, putative neurotransmitters and convulsants, and that there is a similarity of taurine uptake by human platelets and rat brain slices (Gaut et al., 1976). These data, according to Gaut and Nauss (1976), suggested that platelets may also serve as model for taurine uptake in the brain. A decreased  $V_{\max}$ , without change of  $K_m$  and endogenous taurine, has been reported in mongoloid trisomy 21 and mentally retarded patients (Boullin et al., 1975).

Moreover, taurine seems to be

implicated in many of the metabolic abnormalities observed in Friedreich's ataxia (Barbeau, 1976). In patients with this disease and other types of ataxia we found an increase in the urinary excretion of taurine,  $\beta$ -alanine, and BAIB ( $\beta$ -amino-isobutyric acid) with normal plasma levels and with marked increases in the renal clearance rate of taurine (Lemieux et al., 1976). Increased renal clearance rates in the absence of overflow may indicate impaired tubular reabsorption, a membrane transport defect. It has been demonstrated (Scriver and Rosenberg, 1973) that taurine,  $\beta$ -alanine and BAIB share the same transport system. A specific defect in this system has been described in the mouse (Goldman and Scriver, 1967; Chesney et al., 1975).

It seemed worthwhile to explore the possibility that the renal defect found in the ataxic patients might also be reflected in platelets. If present, it would suggest a generalized defect of uptake and storage of taurine in this disease.

### SUBJECTS, MATERIALS AND METHODS

#### 1) Subjects

##### a) Controls

Twenty normal volunteer subjects of either sex ranging in age from 20 to 35 years were tested.

##### b) Patients

Twenty patients of either sex, ranging from 16 to 49 years, were tested. All were affected with Friedreich's ataxia or with other kinds of ataxia. They were divided into sub-groups according to the classification of Barbeau (1976) (Table 1). Two patients were receiv-

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TABLE 1

ATAXIC PATIENTS

GROUP	NO.	DESCRIPTION
GROUP Ia	10	"Typical" Friedreich's ataxia - Complete picture
GROUP Ib	1	"Typical" Friedreich's ataxia - Incomplete picture (without pes cavus)
GROUP IIa	7	Atypical Friedreich's ataxia - Possible autosomal recessive Roussy-Levy
GROUP IIb	2	Not Friedreich's ataxia - Other diagnosis proposed

TABLE 2

PLATELET COUNTS

	N	Mean X 10 <sup>3</sup> (mm <sup>2</sup> )	± S.E.
CONTROLS	20	304.86	10.46
PATIENTS	20	311.08	13.23

Values are the mean ± S.E. of the mean (± S.E.)

obtained in the number of subjects indicated (n).

ing treatment with insulin because of diabetes.

2) Materials

(<sup>14</sup>C-1,2) Taurine, specific activity 56.08 mCi/mmol, was obtained from New England Nuclear Corp., Boston, Mass., U.S.A.

3) Methods

a) Preparation of platelets rich plasma

Blood was collected by venipuncture at 9 a.m. from fasting subjects in 20 ml plastic syringes, each contain-

ing 1.5 ml sodium ethylenediamine-tetracetic acid (EDTA) solution; 13.5 ml of blood was taken per syringe (final EDTA concentration 0.1%). The total blood volume withdrawn was 60 ml. Blood was transferred immediately to centrifuge tubes and cooled in ice. Each patient was matched with a control subject done the same day. Tubes were centrifuged (120 g, 15 minutes, 4°C) to obtain platelet rich plasma. Platelet count was done microscopically in platelet rich plasma. Four counts were performed on each platelet rich

sample according to the Rees-Ecker method and the mean value per mm<sup>3</sup> used for calculation.

b) Uptake of (<sup>14</sup>C-1,2)

Taurine by platelets in vitro

One ml samples of platelet-rich plasma were incubated in an atmosphere of 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 37°C, using a Dubnoff metabolic incubator (Precision Scientific Co., Chicago). After 10 minutes of preincubation, 1 n mol of (<sup>14</sup>C-1,2) taurine in 10 μl of a solution of 0.01 N HCl was added to each sample and rapidly mixed

TABLE 3

UPTAKE OF TAURINE BY PLATELETS

TIME OF INCUBATION (minutes)	UPTAKE EXPRESSED AS nmol OF ( <sup>14</sup> C-1,2) TAURINE PER 10 <sup>11</sup> PLATELETS		
	CONTROLS	PATIENTS	P
	(mean ± S.E.)	(mean ± S.E.)	
10	4.63 ± 0.39	5.51 ± 0.42	p < 0.2 N.S.
30	14.77 ± 0.81	14.64 ± 0.85	p > 0.5 N.S.
60	26.94 ± 1.49	26.00 ± 1.48	p > 0.5 N.S.
90	36.81 ± 1.67	36.05 ± 1.65	p > 0.5 N.S.
120	47.51 ± 2.06	47.65 ± 2.06	p > 0.5 N.S.

The above results were obtained in experiments with platelets from 20 ataxic and 20 controls incubated with 1 μM of (<sup>14</sup>C-1,2) taurine for different times.

TABLE 4

UPTAKE OF TAURINE IN PATIENTS DIVIDED INTO SUB-GROUPS

SUB-GROUPS	UPTAKE AFTER 120 MIN	P
Ia + Ib	47.20 ± 3.20	p > 0.5
Ia	47.34 ± 3.54	p > 0.5
IIa + IIb	48.19 ± 2.56	p > 0.5
IIa	46.07 ± 2.68	p > 0.5
IIb	55.62 ± 3.90	p > 0.4

The value are mean ± S.E. and are expressed as nmol/10<sup>11</sup> platelets after 120 min of incubation - Student's t-test was used.

(Vortex Mixer) to give a final ( $^{14}\text{C}$ -1,2) taurine concentration of 1  $\mu\text{M}$ . Incubation then was continued for samples of each subject for periods of 10, 30, 60, 90, 120 minutes.

c) *Kinetics of uptake of ( $^{14}\text{C}$ -1,2) taurine by platelets*

1 n mol of labelled taurine and different concentrations of cold taurine were added to the samples to give four different final concentrations of taurine: 48  $\mu\text{M}$ , 24  $\mu\text{M}$ , 4.8  $\mu\text{M}$ , 2.4  $\mu\text{M}$ . The incubation was continued for 15 minutes and was then stopped by cooling in ice: Platelets were isolated by centrifugation (20,000 G, 5 minutes, 4°C) and platelet poor plasma was decanted directly into liquid scintillation vials, containing 12 ml of a mixture of Triton X-100 and toluene (1:2 v/v) containing PPO 8.25 g; dimethyl POPOP 0.25 g, in "scintanalyzed grade" toluene 1,000 ml (Fisher Scientific Co., Ltd). To each platelet pellet was added 1 ml bidistilled water and the platelets were disrupted by sonication (15 seconds, Sonic 300 dismembrator, setting power 30% of the maximum). Platelet lysates were transferred to liquid scintillation vials. Platelets and platelet poor plasma radioactivity was determined by liquid scintillation spectrometry using a Packard model 3375 Tricarb liquid scintillation counter. Each sample was counted 5 minutes and corrected for machine efficiency.

d) *Statistical analysis*

Student's t-test was carried out according to Goldstein (1964).

## RESULTS

### *Platelet counts*

There is no significant difference between controls and ataxic patients (Table 2).

### *Uptake of taurine by platelets*

When platelets were incubated in plasma containing ( $^{14}\text{C}$ -1,2) taurine, substantial accumulation took place, which did not reach saturation after 120 minutes of incubation. We could not find any statistical difference between controls and ataxic patients,

TABLE 5

### KINETICS OF TAURINE UPTAKE BY PLATELETS

	$V_{\max}$ (nmol $\times 10^{-6}$ /15 min/ $10^{11}$ platelets)	$K_m$ (M $\times 10^{-5}$ )
CONTROLS	0.44 $\pm$ 0.06	5.33 $\pm$ 0.74
PATIENTS	0.48 $\pm$ 0.09	5.12 $\pm$ 0.79
P	p > 0.5 N.S.	p > 0.5 N.S.

The results (mean  $\pm$  S.E.) were obtained using platelets from 20 ataxics and 20 controls. Platelets were incubated with 2.4, 4.8, 24, 48  $\mu\text{M}$  ( $^{14}\text{C}$ -1,2) taurine for 15 minutes.  $K_m$  and  $V_{\max}$  were calculated according to the Michaelis-Menten equation used to describe saturable enzyme/substrate interactions.

even when we divided them into sub-groups (Tables 3 and 4).

Moreover, in the two tested families affected by typical Friedreich's ataxia, we found that all the members from the same family had similar uptakes. All three patients from family 1 were low uptakers (mean = 36,52 n mol/ $10^{11}$

platelets; S.E.  $\pm$  1.4) and all the three members from family 2 were high uptakers (mean  $\pm$  57.73 n mol/ $10^{11}$  platelets; S.E.  $\pm$  5.8).

### *Kinetics of uptake of taurine by platelets*

Analyses of the data according to the Lineweaver-Burk method (1934)

TABLE 6

### KINETICS OF TAURINE UPTAKE IN SUB-GROUPS OF PATIENTS

SUB-GROUPS	$V_{\max}$ (nmol $\times 10^{-6}$ /15 min/ $10^{11}$ platelets) (mean $\pm$ S.E.)	P	$K_m$ (M $\times 10^{-5}$ ) (mean $\pm$ S.E.)	P
Ia + Ib	0.37 $\pm$ 0.09	p > 0.5	3.68 $\pm$ 0.80	p < 0.2
Ia	0.39 $\pm$ 0.10	p > 0.5	3.84 $\pm$ 0.87	p < 0.4
IIa + IIb	0.59 $\pm$ 0.15	p < 0.4	6.87 $\pm$ 1.29	p < 0.4
IIa	0.48 $\pm$ 0.01	p > 0.5	6.07 $\pm$ 0.95	p > 0.5
IIb	0.97 $\pm$ 0.67	p < 0.1	9.65 $\pm$ 5.64	p < 0.2

$V_{\max}$  and  $K_m$  of each sub-group was compared with the values of the controls and student's t-test calculated.

indicated that there is no significant difference between controls and ataxic patients, even when we divided them into their clinical subgroups (Tables 5, 6).

#### DISCUSSION

Previous studies (Lemieux et al., 1976) have indicated that the urinary excretion and the renal clearance rate of taurine and  $\beta$ -alanine are markedly elevated in most patients with Friedreich's ataxia. This could be the result of a genetic transport defect in the reuptake of taurine in the proximal tubules of the kidney. Alternatively, the taurine loss could be the secondary manifestation of competition for the same transport system by excessive  $\beta$ -alanine excretion. Finally, abnormally elevated secretion of taurine or of  $\beta$ -alanine from the distal tubule could explain the initial observation. In the latter case, one would expect elevated tissue concentrations of taurine, at least in the kidney.

In order to investigate the first possibility, a genetic transport defect in taurine, we looked at the transport and kinetics of taurine in the platelet, considered a model of brain transport. We found no significant change in platelet taurine uptake or kinetics. This indicates that there is no generalized transport defect for taurine in Friedreich's ataxia. This, of course, does not eliminate the possibility that the defect is localized in the kidney, or in other tissues such as the intestine.

Our studies also offer preliminary evidence for a familial characteristic in uptake of taurine in platelets. High uptake families could be distinguished from low uptake families, with relatively constant patterns between individuals of the same family group. Moreover, repeat determinations of taurine uptake in the same individual over a period of time were remarkably constant, giving more importance to our finding of interfamily heterogeneity in taurine uptake. This observation may be of importance in the interpretation of the overall role of taurine in the

pathogenesis of Friedreich's ataxia (Barbeau, 1976).

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