Br. J. Pharmac. (1980), **71**, 553–556

A STUDY OF γ-AMINOBUTYRIC ACID UPTAKE IN NORMAL AND DOWN'S SYNDROME PLATELETS

LOUISE ENNS & E.E. McCOY

Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada

1 Initial uptake rates of γ-aminobutyric acid (GABA) were compared in Down's syndrome (D.S.) and normal platelets. GABA uptake was decreased in D.S. platelets (1.10 ± 0.10 nmol h^{-1} 10^{-9}) compared to uptake by normal platelets (1.89 ± 0.21 nmol h^{-1} 10^{-9}), $P < 0.005$.

2 The effect of varying the Na^+ concentration was similar on D.S. and normal platelets. Increasing the media Na^+ concentration resulted in increased rates of GABA uptake in both D.S. and normal platelets.

3 GABA uptake in the presence of 2,4-dinitrophenol or at 2°C is approximately 56% of the uptake at 37°C for both D.S. and normal platelets.

4 Extrapolation of a reciprocal plot indicates a two affinity uptake system: a high affinity and a low affinity mechanism.

5 A significant defect in GABA uptake exists in D.S. platelets.

Introduction

The uptake and storage of monoamines by platelets have been proposed as a model for similar processes in synaptosomes (Pletscher, 1968; Sneddon, 1973; Stahl & Meltzer, 1978). Platelets from subjects with Down's syndrome (D.S.) have been shown to have both a decreased rate of uptake and a decreased content of 5-hydroxytryptamine (5-HT) (Boullin & O'Brien, 1971; Bayer & McCoy, 1974; McCoy & Enns, 1978).

γ-Aminobutyric acid (GABA) is an important inhibitor of central nervous system neurones (Krnjević & Phillis, 1963). Intracellular recording of membrane resistance has shown marked similarity of effects produced by synaptic inhibition and GABA application (Krnjević & Schwartz, 1967; Dreifuss, Kelly & Krnjević, 1971). As both GABA (Weinstein, Varon, Muhleman & Roberts, 1965; Hutchison, Werrbach, Vance & Haber, 1974) and 5-HT (Sneddon, 1971) are transported by Na^+-dependent mechanisms, a study was undertaken to determine whether the decreased uptake by D.S. platelets was confined to 5-HT or was more general, affecting other amines such as GABA.

Methods

Blood was obtained, after written permission was given by parents or guardians, from D.S. students aged 15 to 18 years attending the Winnifred Stewart School for Retarded Children or D.S. adults aged 18 to 40 years working at the Western Industrial Research and Training Centre in Edmonton, Alberta. Control blood samples were obtained from laboratory staff or medical students aged 20 to 30 years.

The blood was taken in a polyethylene syringe with 0.1 M disodium edetate (EDTA) in 150 mM NaCl as an anticoagulant. Polyethylene tubes and polyethylene pipettes were used throughout. Platelets were isolated by previously published methods (McCoy & Enns, 1978).

GABA uptake

The platelet pellet was resuspended in 0.9 ml of the incubation buffer (Henn & Hamberger, 1971) (composition (mM) NaCl 120, KCl 5, $MgCl_2$ 2.5, glucose 20, $CaCl_2$ 2, Tris-HCl 35, plus 5 mg/ml albumin (BSA), pH 7.4) and preincubated for 5 min at 37°C. To the platelet suspension 0.1 ml [1-^{14}C]-γ-aminobutyric acid (1 μCi, 49.4 μCi/mmol, 2 μM final concentration) was added and mixed. The total [^{14}C]-GABA uptake was determined by removal of duplicate 0.1 ml aliquots at 0, 1, 2, 5 and 10 min. Termination was by dilution in 20 volumes of ice cold saline and centrifugation at 1500 *g* for 12 min at 4°C. The initial rate of total uptake of GABA was expressed as nmol h^{-1} 10^{-9} platelets. As determined, total uptake includes both energy-independent and energy-dependent GABA uptake.

0007-1188/80/140553-04 $01.00

GABA uptake and Na^+ dependence

Platelets were resuspended in buffer containing either 0, 12, 24, 60, 120, 150, 180 mM NaCl and preincubated for 5 min. Choline chloride was added in appropriate amounts to maintain equal osmotic pressure. [^{14}C]-GABA uptake was determined at intervals for 5 min at 37°C. The reaction was terminated and radioactivity determined as described in the GABA uptake section.

GABA uptake in presence of dinitrophenol and sodium fluoride

GABA uptake was determined in platelets poisoned by inhibitors of both major energy pathways. Platelets were preincubated with or without 1.5 mM 2,4-dinitrophenol (2,4 DNP) and 40 mM NaF for 5 min. GABA was added and uptake proceeded for 1 to 5 min. The uptake was terminated and radioactivity determined as described for GABA uptake.

GABA uptake at 2°C

GABA uptake was studied at 2°C to determine the amount of non-energy requiring GABA uptake by a second method. The method followed was as for the original GABA uptake except that from one donor two series were run, at 37°C and at 2°C. The assay was carried out for 1 to 5 min in duplicate.

Platelet uptake of GABA at various concentrations

GABA uptake was determined in a reaction buffer at 120 mM Na^+ and concentrations of GABA at 0.2, 1, 2, 5, 10 and 20 μM. Incubation was terminated after 5 min and accumulated radioactivity determined as described for GABA uptake. From this data Michaelis-Menton plots were obtained for K_m and V_{max}.

Table 1 Total γ-aminobutyric acid (GABA) uptake by platelets (nmol h^{-1} 10^{-9} platelets) from control subjects and subjects with Down's syndrome

	Control	*Down's syndrome*
	1.49	0.89
	1.29	0.93
	1.57	1.08
	1.15	1.20
	2.60	1.01
	1.60	0.67
	1.95	1.79
	2.88	1.27
	2.49	1.05
Average	1.89	1.10
± s.e. mean	±0.21	±0.10
		$p < 0.005$

Initial rates of [^{14}C]-GABA uptake were followed in D.S. and control platelets. Details as in methods; s.e. mean determined by unpaired *t* test.

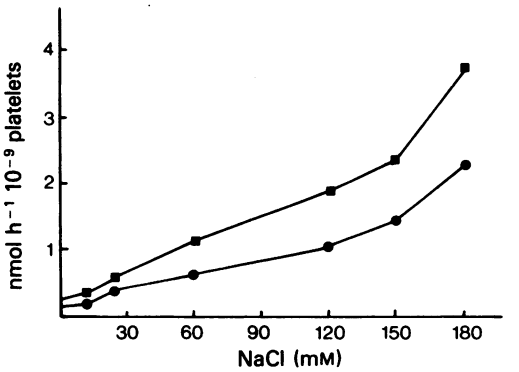

Figure 1 Effect of various external Na^+ concentrations on [^{14}C]-γ-aminobutyric acid [^{14}C]-GABA uptake by platelets: expressed as rate of uptake of GABA at different Na^+ concentrations. Each point is the average of four individual determinations, each done in duplicate: Down's syndrome platelets (●); control platelets (■).

Results

GABA uptake at various Na^+ concentrations

Under standard incubation conditions as described in the methods, GABA uptake was compared in D.S. and control subjects (Table 1). GABA uptake into D.S. platelets was significantly slower (1.10 ± 0.10 nmol h^{-1} 10^{-9} platelets) than into normal platelets (1.89 ± 0.21 nmol h^{-1} 10^{-9} platelets) ($P < 0.005$).

The effect of different external Na^+ concentrations on GABA uptake was studied. As shown in Figure 1, at low Na^+ concentrations a low uptake of GABA occurred. As Na^+ concentrations increased, a parallel increase in GABA uptake was observed in D.S. and normal platelets. However, at each concentration of Na^+ the rate of uptake of GABA was less in D.S. than in normal platelets. Increasing the Na^+ concentration above 120 mM (normally used in uptake studies), to 150 mM and 180 mM resulted in further increased rates of GABA uptake. It is presumed that GABA, like 5-HT, is co-transported with Na^+ when it enters platelets via an electrochemical gradient (Weinstein *et al.*, 1965). With increased external Na^+ concentration, a larger electrochemical gradient for Na^+ should occur (Sneddon, 1971) with a resulting increased rate of inward movement of Na^+ and also of GABA.

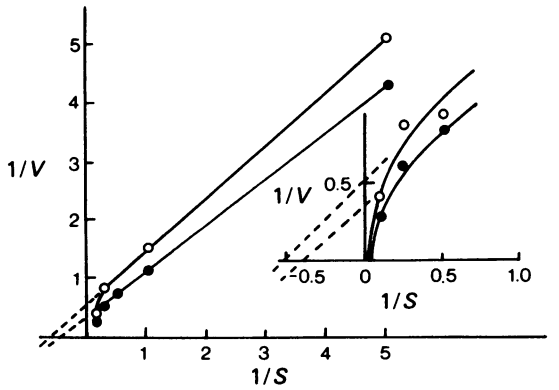

Figure 2 Lineweaver-Burk plot showing extrapolated K_m and V_{max} values for high affinity γ-aminobutyric acid uptake by Down's syndrome (○) and normal (●) platelets. The inset shows the origin, in greater detail.

When the uptake rates of GABA into D.S. and normal platelets were compared at various Na^+ concentrations it was observed that the D.S./normal ratio of GABA uptake did not change significantly from one Na^+ concentration to another. Even at higher external Na^+ concentrations than normally used, the ratio remained constant, indicating that the Na^+ dependency of GABA uptake is similar in D.S. and normal platelets.

GABA uptake into platelets poisoned by 2,4-dinitrophenol and sodium fluoride

GABA uptake was determined in platelets in which oxidative phosphorylation was blocked by 2,4-DNP and glycolysis by NaF. The fact that some GABA uptake occurred even with the energy pathways blocked (Table 2), may be due to the high external to internal Na^+ ratio which would allow Na^+ to enter the platelet and co-transport GABA even when the platelet metabolic activity was decreased.

As is evident from Table 2, inhibition by 2,4-DNP/NaF of GABA uptake in both D.S. and normal platelets was similar to that seen at 2°C. Uptake at 2°C is though to represent non-energy requiring transport. The uptake by D.S. and normal platelets appeared to be decreased proportionally.

The ratio of uptake at 2°C to uptake at 37°C remained constant at all time points assayed for both D.S. and normal platelets. This does not affect the relative decrease in GABA uptake observed in D.S. platelets compared to uptake in normal platelets.

Uptake at various GABA concentrations

GABA concentrations were varied between 0.2 and 20 μM. Two distinct slopes were obtained (Figure 2), one for low concentrations from 0.2 to 2.0 μM and another for concentrations from 5 to 20 μM.

If the results are plotted as a conventional double reciprocal plot, an average high affinity V_{max} of 3.30 nmol h^{-1} 10^{-9} platelets is obtained for normal platelets and 2.00 nmol h^{-1} 10^{-9} platelets for D.S. subjects ($n = 5$) ($P < 0.01$). By extrapolation, the K_m for normal platelets was found to be 2.5 μM and for D.S. platelets 2.0 μM ($n = 5$) ($P < 0.5$); not significantly different. V_{max} and K_m for the low affinity mechanism are difficult to determine since upon extrapolation of the lines, they appear to pass through the origin, indicating infinitely large figures for V_{max} and K_m (usually considered to be diffusion).

Discussion

The objective of the present study was to determine whether decreased aimine uptake into D.S. platelets was restricted to 5-HT or whether other monoamines also had decreased rate of uptake. These studies show that GABA uptake is decreased 42% in D.S. compared to normal platelets. We have recently shown that the uptake of choline, the precursor of acetylcholine, is similar in D.S. and normal platelets (McCoy, Strynadka & Masserelli, unpublished observations). These data indicate that decreases in uptake are selective and not a generalized property of D.S. platelets.

Table 2 The effect of 2,4-dinitrophenol (DNP) sodium fluoride (NaF) and low temperature on [^{14}C]-γ-aminobutyric acid uptake by D.S. and normal platelets

Conditions	*Normal*	*D.S.*	*Significance (P)*
37°C	1.89 ± 0.21 ($n = 9$)	1.10 ± 0.10 ($n = 9$)	<0.005
2°C	1.08 ± 0.12 ($n = 4$)	0.62 ± 0.05 (n = 5)	<0.005
DNP/NaF	1.00 ± 0.11 ($n = 4$)	0.58 ± 0.05 (n = 5)	<0.005

Initial uptake rates were determined under various conditions as described in the text; n = number of experiments (done in duplicate).

Some transmitter amines, such as dopamine and 5-HT are transported into the cell by a common uptake system (Omenn & Smith, 1978) but preliminary data indicate that GABA and 5-HT are transported by separate systems. Chlorimipramine and (±)-*p*-chloro-amphetamine have no apparent effect on the uptake of GABA while inhibiting >90% the uptake of 5-HT (unpublished observation).

GABA uptake into platelets is a Na^+-dependent process (Hutchison *et al.*, 1974). At low Na^+ concentrations the uptake of GABA was decreased and as Na^+ concentration increased, there was a parallel increase in the rate of GABA uptake in both D.S. and normal platelets. At low temperatures and when both oxidative phosphorylation and glycolysis were blocked, GABA uptake was decreased by approximately 50%. Sneddon (1971) has shown that when energy pathways were blocked in a similar manner in rat platelets, Na^+ and 5-HT uptake occurred when an external to internal Na^+ gradient was present. It is proposed that the present results for GABA uptake in poisoned platelets can be explained on a similar basis, that is, the non-energy requiring uptake of GABA is along an electrochemical gradient.

High and low affinity uptake has been demonstrated for several amines and in different species (Hutchison *et al.*, 1974; Stahl & Meltzer, 1978). Two distinct slopes are present in Lineweaver-Burk plots of GABA uptake for both D.S. and normal platelets, suggesting that a high and low affinity uptake for GABA is present in human platelets.

It has been proposed that the uptake of 5-HT into platelets is a model for the uptake of amines into synaptosomes (Pletscher, 1968; Sneddon, 1973; Stahl & Meltzer, 1978). Recent studies have demonstrated the similarity of 5-HT uptake into synaptosomes and platelets from the same animal (Stahl & Meltzer, 1978). The demonstration that the uptake of another transmitter is defective in D.S. platelets increases the possibility that decreased amine uptake and altered neurotransmission may be present in D.S. synaptosomes.

This work was supported in part by a Medical Research Council of Canada Grant.

References

BAYER, S.M. & McCOY E.E. (1974). A comparison of the serotonin and ATP content in platelets from subjects with Down's syndrome. *Biochem. Med.*, **9**, 225–232.

BOULLIN, D.J. & O'BRIEN R.A. (1971). Abnormalities of 5-hydroxytryptamine uptake and binding by blood platelets from children with Down's syndrome. *J. Physiol.*, **212**, 287–291.

DREIFUSS, J.J., KELLY, J.S. & KRNJEVIĆ K. (1969). Cortical inhibition and γ-aminobutyric acid. *Exp. Brain Res.*, **9**, 137–142.

HENN, F.A. & HAMBERGER, A.A. (1971). Glial cell function: uptake of transmitter substances. *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2686–2690.

HUTCHISON, H.T., WERRBACH K., VANCE, C. & HABER, B. (1974). Uptake of neurotransmitters by clonal lines of astrocytoma and neuroblastoma in culture. I. Transport of α-aminobutyric acid. *Brain Res.*, **66**, 265–274.

KRNJEVIĆ, K. & PHILLIS, J.W. (1963). Ionophoretic studies of neurones in the mammalian cerebral cortex. *J. Physiol.*, **165**, 274–285.

KRNJEVIĆ, K. & SCHWARTZ, S. (1967). The action of γ-aminobutyric acid on cortical neurons. *Exp. Brain Res.*, **2**, 320–325.

McCOY E. E. & ENNS L. (1978). Sodium transport, ouabain binding, and (Na^+/K^+)-ATP activity in Down's syndrome platelets. *Ped. Res.*, **12**, 685–689.

OMENN, G.S. & SMITH, L.T. (1978). A common uptake system for serotonin and dopamine in human platelets. *J. clin. Invest.*, **62**, 235–240.

PLETSCHER, A. (1968). Metabolism, transfer and storage of 5-hydroxytryptamine in blood platelets. *Br. J. Pharmac.*, **32**, 1–16.

SNEDDON, J.M. (1971). Relationship between internal Na^+/K^+ and the accumulation of [^{14}C]-5-hydroxytryptamine by rat platelets. *Br. J. Pharmac.*, **43**, 834–844.

SNEDDON, J.M. (1973). Blood platelets as a model for monoamine-containing neurons. *Progr. Neurobiol.*, **1**, 151–198.

STAHL, S.M. & MELTZER, H.Y. (1978). A kinetic and pharmacologic analysis of 5-hydroxytryptamine transport by human platelets and platelet storage granules: comparison with serotonergic neurons. *J. Pharmac. exp. Ther.*, **205**, 118–132.

WEINSTEIN, H., VARON, S., MUHLEMAN, D.R. & ROBERTS, E. (1965). A carrier-mediated transfer model for the accumulation of ^{14}C-γ-aminobutyric acid by subcellular brain particles. *Biochem. Pharmac.*, **14**, 273–288.

(Received March 3, 1980. Revised June 30, 1980.)