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SHORT COMMUNICATIONS

5-Hydroxytryptamine (5-HT) transport in Down's Syndrome blood platelets: Effect of K^+ loading

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It has been postulated that blood platelets may act as an accessible model for certain aspects of amine metabolism in the CNS, particularly for the transport and storage of 5-hydroxytryptamine (5-HT) [1, 2]. In blood platelets, 5-HT transport is a complex process thought to involve multiple components including: (a) binding of 5-HT to the outer surface of the membrane [3], (b) transport across the platelet membrane, probably involving a mobile carrier [4, 5], (c) discharge of 5-HT at the inner surface of the membrane, and (d) storage in intracellular granules [6]. Some or all of these components are sensitive to the levels of Na^+, K^+ or Cl^- both inside and outside the cell.

Blood platelets from subjects with Down's Syndrome (D.S.) exhibit decreased accumulation and storage of 5-HT [7, 8], and previous work from this laboratory has shown that outward Na^+ transport, ouabain binding and Na^+/K^+-ATPase activities are decreased in D.S. platelets [9]. In addition, D.S. platelets have an increased content of Na^+ and a decreased content of K^+ suggesting that decreased 5-HT transport could be secondary to disturbances in the transmembrane ion gradients. The present experiments were designed to test the hypothesis that low intracellular K^+ contributes to the decreased uptake of 5-HT in D.S. platelets.

Methods

Subjects. Blood was obtained from Down's Syndrome subjects (10–18 years of age) living at home and attending the Winnifred Stewart School for Retarded Children, upon written consent of parent or guardian. Laboratory staff or students (18–35 years of age) served as controls. The project was approved by the Special Services and Research Committee of the University of Alberta Hospital.

Collection of venous blood and isolation of platelet rich plasma (PRP) were carried out as previously described [9].

$^{42}[K^+]$ *Efflux.* ^{42}KCl was prepared as previously described [10] and diluted to a concentration of 5.28 mM (sp. act. 195 Ci/mole K^+) Blood platelets were centrifuged from PRP and resuspended in a K^+-free buffer (150 mM NaCl, 20 mM imidazole, 11 mM glucose, 2.5 mM $MgCl_2$, 5 mM ATP and 1.5% albumin). KCl (0.1 ml) was added to 1 ml of platelet suspension to give a final conentration of either 5 mM (0.1 Ci $^{42}K^+$) or 30 mM (0.6 Ci $^{42}K^+$) and incubated for 40 min at 37° to obtain $^{42}K^+$ equilibrium across the platelet membrane. The platelets were centrifuged again and resuspended in the above buffer containing unlabeled KCl (5.28 mM). The efflux of $^{42}K^+$ was followed at 30-sec intervals for 2 min by removing 100 μl aliquots, diluting into 1.5 ml ice-cold buffer (NaCl 160 mM, EDTA 0.1 mM), and centrifuging for 60 sec at 800 *g* in a microcentrifuge. The supernatant fraction was discarded, and the inside of the tube was wiped dry. Potassium efflux was determined from the residual $^{42}K^+$ present in the platelet pellet, using a Searle Gamma Counter model 1185.

Serotonin uptake. Platelets suspended in imidazole buffer were preincubated at 37° for 10 min before transferring 100 μl samples to centrifuge tubes containing 0.8 μM 5-HT (+ 0.01 μCi[^{3}H]-5-HT, sp. act. 3.125 Ci/mmole) at 37°. Uptake was terminated after 2 min by the addition of 10 vol. of ice-cold NaCl/EDTA buffer, followed by rapid centrifugation. The supernatant fraction was aspirated and the platelet pellet was washed with a further 10 ml of NaCl/EDTA. After aspiration and drying the tube, the platelet pellet was dissolved in 0.5% Triton X-100 and sampled for scintillation counting. Extracellular contamination was determined by incubation at 0°. Initial rate of uptake was calculated in terms of nmoles·hr^{-1}·10^9 platelets from the accumulation at 2 min. Previous experiments had determined that the uptake of [^{3}H]-5-HT under these conditions was still linear at 2 min.

Measurement of intracellular Na^+ *and* K^+. To appropriate samples of the platelet suspension, 1 μCi of [^{14}C]inulin was added, and the platelets were centrifuged. The platelet pellet was washed twice with isotonic Tris–choline (148 mM Tris, 2 mM choline chloride, pH 7.2), the tubes were wiped dry, and the final pellet was digested overnight with 0.5 ml of conc. HNO_3. The acid digest was made up to 4 ml with deionized water, and the precipitate was removed by centrifugation. The ionic content of the supernatant fraction was determined by atomic absorption spectrophotometry, and the values were corrected for extracellular contamination.

Table 1. Intracellular K^+ content of normal and D.S. platelets before and after K^+ loading*

	Intracellular K^+ (μmoles/10^9 platelets)			
	Controls, 5 mM K^+	20 min at 30 mM K^+	% increase	P
Normal	0.42 ± 0.06 (11)	0.60 ± 0.06 (11)	38.9	<0.01
D.S.	0.28 ± 0.03 (11)	0.46 ± 0.05 (11)	64.0	<0.01
P	<0.005			

*Values in parentheses are the number of subjects. Statistical analysis was by a two-tail probability test. The external K^+ concentration was adjusted to 30 mM by the addition of small volumes of 1.3 M KCl. $^{42}K^+$ was omitted.

Table 2. Rate of K^+ efflux in normal and D.S. platelets before and after K^+ loading*

	K^+ efflux (μmoles · hr^{-1} · 10^9 platelets)			
	Controls	K^+ loaded	% increase	P
Normal	1.14 ± 0.13 (8)	1.46 ± 0.15 (8)	28	<0.2
D.S.	0.53 ± 0.06 (10)	1.04 ± 0.08 (10)	96	<0.001
P	<0.001	<0.025		

* Platelets were incubated in normal (5 mM K^+) or high (30 mM K^+) imidazole buffer containing $^{42}K^+$ for 40 min at 37°. After transferring to control incubation medium, the initial rate of $^{42}K^+$ efflux was determined over a 2-min interval.

Results

Intracellular K^+ levels. The intracellular K^+ levels in blood platelets from normal and D.S. subjects before and after incubation in high K^+ (30 mM) are shown in Table 1. The difference between a value of 0.28 ± 0.03 μmole/10^9 platelets for D.S. subjects and 0.42 ± 0.06 μmole/10^9 platelets for normal subjects in imidazole buffer containing 5 mM K^+ was statistically significant ($P < 0.005$, $N = 11$) and is comparable with values previously reported [11], demonstrating that D.S. platelets contain significantly less K^+ than normal platelets. Following 20 min of incubation at 36° in media where the K^+ concentration had been raised to 30 mM by the addition of small volumes of 1.3 M KCl, platelets from both groups gained approximately 0.17 μmole/10^9 platelets, increasing the intracellular concentration in D.S. platelets to 0.46 μmole/10^9 platelets, representing a 64% increase, and for normal platelets to 0.60 ± 0.06 μmole/10^9 platelets, a 39% increase over control values.

Intracellular Na^+ levels. Platelets from normal subjects had an intracellular Na^+ concentration of approximately 0.13 μmole/10^9 platelets, and this did not change significantly after K^+ loading; in D.S. platelets the intracellular Na^+ fell from 0.30 μmole/10^9 platelets to 0.22 μmole/10^9 platelets ($P = 0.06$, $N = 10$) after incubation in high K^+ medium.

K^+ efflux. The efflux of $^{42}K^+$ in D.S. platelets following labeling with $^{42}K^+$ at an external K^+ concentration of 5 mM was calculated to be 0.53 ± 0.06 μmol · hr^{-1} · 10^9 platelets and after K^+ loading following incubation with 30 mM K^+ the $^{42}K^+$ efflux increased to 1.04 ± 0.08 μmole · hr^{-1} · 10^9 platelets, representing a 96% increase (Table 2). Normal platelet K^+ efflux following incubation in 5 mM K^+ buffer was 1.14 ± 0.13 μmole · hr^{-1} · 10^9 platelets, significantly higher than that seen in D.S. platelets ($P < 0.001$), and following K^+ loading there was a 28% increase in efflux rate for $^{42}K^+$ in normal platelets.

[^{3}H]-5-HT uptake. In D.S. platelets, the initial rate of [^{3}H]-5-HT uptake was 4.70 ± 0.61 nmoles · hr^{-1} · 10^9 platelets, significantly lower than the values of 8.62 ± 0.77 calculated for normal platelets ($P < 0.005$) (Table 3). After K^+ loading, washing and transferring the platelets to standard incubation medium, D.S. platelets showed a 62% increase in 5-HT influx to 7.63 ± 0.62 nmoles · hr^{-1} · 10^9 platelets, whereas under the same conditions normal platelets showed an insignificant increase in 5-HT influx.

Discussion

Stahl [2] and Sneddon [12] demonstrated that 5-HT transport is dependent upon external Na^+ and suggested that the energy for transport derived from an inwardly directed Na^+ gradient. Rudnick [13], using isolated platelet vesicles as a model system which permits artificial control of the ionic environment on either side of the vesicular membrane, confirmed and extended these observations to suggest that Na^+ and Cl^- are involved in the binding of 5-HT to the carrier [14, 15] which translocates to the internal surface of the membrane where 5-HT, Na^+ and Cl^- dissociate from the carrier by the process facilitated by high K^+. The carrier then returns to the outside of the membrane assisted by the outwardly directed K^+ gradient [14]. According to these ideas, normal 5-HT transport in blood platelets is intimately linked to intracellular and extracellular concentrations of Na^+ and K^+. Our results confirm the observations of low internal potassium [9] and decreased 5-HT uptake [7, 8] in D.S. blood platelets and suggest that the two observations may be causally related. Blood platelets rapidly exchange internal and external K^+ [16, 17] and our results show that, if the external K^+ concentration is raised above physiological levels, it is possible to increase intracellular K^+ levels in both D.S. and normal platelets. However, D.S. platelets are less able to retain this accumulated K^+ as indicated by the significantly greater K^+ efflux seen when the K^+ loaded platelets are returned to incubation medium contain physiological levels of K^+.

Rudnick and Nelson [14], using membrane vesicles derived from porcine platelets, demonstrated that internal K^+ is necessary for the maximum influx of 5-HT, and they postulated that K^+ increases the rate of return of the 5-HT carrier to the outside of the membrane. Our results are compatible with this hypothesis, where increasing the internal concentration of K^+ in D.S. platelets would stimu-

Table 3. Initial rate of [^{3}H]-5-HT uptake in normal and D.S. platelets before and after K^+ loading*

	[^{3}H]-5-HT uptake (μmoles · hr^{-1} · 10^9 platelets)			
	Controls	K^+ loaded	% increase	P
Normal	8.62 ± 0.77 (13)	9.63 ± 0.85 (13)	6	NS
D.S.	4.70 ± 0.61 (10)	7.63 ± 0.62 (10)	62	<0.005
P	<0.005			

* Initial rates of [^{3}H]-5-HT were determined after a 2-min incubation at a final concentration of 0.8 μM. Significance was determined by a two-tailed probability test.

late 5-HT influx by permitting a faster return of the carrier to the outside of the membrane; K^+ may act by altering the conformation of the carrier and/or the outward directed K^+ efflux may provide part of the energy for the movement of the carrier. The failure to stimulate 5-HT influx in normal platelets following K^+ loading may indicate that in this case the K^+-induced return of the carrier is operating at maximum efficiency and, therefore, not influenced by increasing the internal K^+ concentration.

Our results suggest that a component of the functional impairment of 5-HT transport seen in D.S. platelets is due to disturbances in the Na^+ and K^+ gradients and that temporarily increasing the intracellular K^+ concentration restores functional integrity to the membrane carrier.

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Effect of prolonged alcohol administration on activities of various enzymes scavenging activated oxygen radicals and lipid peroxide level in the liver of rats

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Many studies have demonstrated that biochemical changes as found in humans with alcoholic liver injury occur in the liver of animals treated with alcohol for a long period [1, 2]. It has been suggested that lipid peroxidation among the biochemical changes is involved in liver damage due to chronic alcohol ingestion [3, 4]. MacDonald [5] showed that the activities of hepatic glutathione peroxidase (GSH peroxidase) and glutathione reductase (GSSG reductase) were enhanced with increases in hepatic mitochondrial and microsomal lipid peroxide contents in rats following chronic alcohol administration, implying the involvement of activated oxygen radicals formed in alcohol metabolism in lipid peroxidation. Hence, the level of lipid peroxides in the liver of alcohol ingesting animals can be considered to vary with the balance between the potential to form activated oxygen radicals and the capacity to remove the activated oxygen radicals and lipid peroxides.

The present study was undertaken to clarify the relationship between the generation of activated oxygen radicals and the accumulation of lipid peroxides in the liver of rats given 20% alcohol as drinking water for 4 weeks. We determined the contents of hepatic lipid peroxide and reduced glutathione (GSH), which participates in decomposing hydrogen peroxide (H_2O_2) and lipid peroxides [6] and scavenging superoxide anions (O_2^-) [7], and the changes in activities of various enzymes to produce or to eliminate activated oxygen radicals closely related to the formation of lipid peroxides.

Materials and methods

Male Wistar rats weighing about 250 g were separated in two groups. The diets were closely controlled and consisted of Oriental M powder diet (Oriental Yeast Co. Ltd.) (3.7 kcal/g) which contained 55% carbohydrate, 24% protein and 5% lipids. The contents of vitamin E, vitamin C, choline, cystine and methionine in this diet were 0.006, 0.005, 0.1, 0.3 and 0.3%, respectively. Both groups of rats were maintained on this powder diet for one week before used and during the period of the experiment, four weeks. One group of rats received 20% ethanol as the only source of drinking water; the second one received an isocaloric amount of glucose in its drinking water, throughout the period in which the experiment was performed. The ingestion of ethanol amounted to 9.4 ± 1.1 g/kg body weight per day ($n = 10$). In this manner, the experimental animals consumed 30% of their total daily calories in the form of ethanol. Totally they gained 203.1 ± 43.9 kcal/kg per day ($n = 10$), whereas the control rats gained 220.3 ± 42.5 kcal/kg per day ($n = 5$). There was no difference in body weight gain or liver weight between control and alcohol-treated rats. Both groups of rats were starved for 15 hr prior to sacrifice. Liver were removed, washed and homogenized in 4 vol. of ice-cold 0.15 M KCl. The homogenates were centrifuged at 10,000 *g* for 20 min, and then the postmitochondrial supernatants were recentrifuged at 105,000 *g* for 60 min to prepare microsomes and soluble