Acta Medica Okayama

Volume 46, *Issue* 3 1992 *Article* 4

JUNE 1992

Concentration and uptake of taurine in umbilical blood platelets.

Hirofumi Kanemori* Kohei Ejiri† Shuichiro Akahori‡
Takafumi Kubo** Kaoru Sekiba††

*Okayama University,
†Okayama University,
‡Okayama Univerisity,
**Okayama University,
††Okayama University,

Concentration and uptake of taurine in umbilical blood platelets.*

Hirofumi Kanemori, Kohei Ejiri, Shuichiro Akahori, Takafumi Kubo, and Kaoru Sekiba

Abstract

The concentration and uptake of taurine in the umbilical and adult blood platelets were studied. Taurine was the most abundant free amino acid in both umbilical and adult blood platelets. The taurine concentration in umbilical blood platelets (2.30 pmoles/10(4) cells) was significantly lower than that of adult blood platelets (3.27 pmoles/10(4) cells) in contrast to the reverse relationship in taurine concentrations in umbilical and adult blood plasma. No other amino acid showed such significant difference in the concentrations between umbilical and adult blood platelets. Taurine uptake into umbilical blood platelets was temperature sensitive and sodium-dependent in a manner similar to that of adult blood platelets. The uptake conformed well to Hanes-plot. The Vmax of the uptake into adult blood platelets was about 3.6 times higher than that of umbilical blood platelets, but no significant difference was seen in the Km value between the two groups.

KEYWORDS: umbilical blood, platelet, taurine concentration, faurine uptake

*PMID: 1502921 [PubMed - indexed for MEDLINE]

Acta Med Okayama **46** (3) 169-174 (1992)

Concentration and Uptake of Taurine in Umbilical Blood Platelets

Hirofumi Kanemori, Kohei Ejiri*, Shuichiro Akahori, Takafumi Kudo and Kaoru Sekiba

Department of Obsterics and Gynecology, Okayama University Medical School, Okayama 700, Japan

The concentration and uptake of taurine in the umbilical and adult blood platelets were studied. Taurine was the most abundant free amino acid in both umbilical and adult blood platelets. The taurine concentration in umbilical blood platelets (2.30 pmoles/10^4 cells) was significantly lower than that of adult blood platelets (3.27 pmoles/10^4 cells) in contrast to the reverse relationship in taurine concentrations in umbilical and adult blood plasma. No other amino acid showed such significant difference in the concentrations between umbilical and adult blood platelets. Taurine uptake into umbilical blood platelets was temperature sensitive and sodium-dependent in a manner similar to that of adult blood platelets. The uptake conformed well to Hanes-plot. The V_{max} of the uptake into adult blood platelets was about 3.6 times higher than that of umbilical blood platelets, but no significant difference was seen in the *Km* value between the two groups.

Key words : **umbilical blood, platelet, taurine concentration, taurine uptake**

Taurine is one of the sulfur-containing amino acids which is widely distributed in the human body (1,2). Taurine is abundant in adult blood platelets (3,4). The concentration was 2-500 times higher than in the plasma (2). High concentrations appear in the tissues of fetuses and neonates (5,6). However, the taurine-forming activity in these periods of life is very low due to the delayed development of cystathionase (E.C. 1. 13.11.20) and to the low activity of cysteine sulfinic acid decarboxylase (E.C. 4.1.1.29) (7). Therefore, taurine must be supplied from the mother to the fetus across the placenta, and is incorporated into the fetal organs (1,8). Taurine is considered to be an essential amino acid in fetus and neonates (3,4), and is added to powdered milk. Very little data about the concentration and uptake of taurine in platelets during fetal and neonatal periods has been published. The present paper reports the investigation of these problems in umbilical blood platelets.

Materials and Methods

Materials

Fifteen appropriate-for-date fetuses at full term delivery without maternal complication and 15 normal non-pregnant adult women were used in this study.

Methods

Amino acid analysis. Blood samples from adult antecubital vein and newborn umbilical cord blood were

* To whom correspondence should be addressed.

withdrawn with a clean plastic syringe containing 0.1 volume of acid-citrate-dextrose solution (ACD, Terumo Co., Tokyo, Japan) containing 2.20% sodium citrate, 0.80% citric acid and, 2.20% glucose (all in W/V). Platelet rich plasma (PRP) was prepared by centrifugation of blood at 70 × g for 15 min at room temperature. Then, PRP was centrifuged after addition of 0.1 volume of 0.1 M EDTA (pH 7.4) at 750 × g for 15 min at room temperature to separate plasma and platelet pellet. A portion of the pellet resuspended in physiological saline of the original volume of PRP was subjected to freezing and thawing 3 times and centrifuged at 750 × g for 10 min. Free amino acids in the supernatant and the plasma obtained above were determined with an amino acid analyzer (model A-5500E, IRICA, Kyoto, Japan). Taurine concentrations in platelets and in plasma were expressed as pmoles/10^4 cells and μmoles/ml, respectively, and the values were expressed as the mean ± SD of separate determinations. The significance of the differences was examined by the Student's t-test.

Taurine uptake by platelet. The remaining portion of the platelet pellet was resuspended in the Krebs-Ringer-phosphate buffer, or the buffer substituted with LiCl for NaCl, of half the volume of the original supernatant. [^{14}C]-Taurine (5 mCi/mM, 100 μl) was added at the final concentration of 6.25 to 100 μM to 100 μl of the platelet suspension in a Eppendorf microtest tube No. 3810 preincubated in an atomosphere of 95% O_2 - 5% CO_2 at 37°C or 0°C for 5 min. Then the mixture was incubated for 5 min under the same condition. The reaction was stopped by the addition of 1 ml of icecold physiological saline, and the mixture was immediately centrifuged at 5,600 × g at 0°C for 30 sec. The supernatants were decanted, and tubes were swabbed with cotton-tipped applicators to remove excess fluid adhering the walls. The resulting platelet was lysed in 0.1 ml Soluene-350 (Packard Instrument Company, Inc., U.S.A.). The lysate was placed in a 20 ml glass scintillation counting vial with 0.5 ml of Clear-sol I scintillation fluid (Nacalai Tesque Company, Kyoto, Japan). The radioactivity was counted with a liquid scintillation counter (LSC-703, ALOKA, Tokyo, Japan).

Results were expressed as fmoles/10^4cells·min^{-1}. The values were expressed as the mean ± SD, of several separate determinations as shown below. The significance of the difference was examined using the Student's t-test.

Results

Free amino acid concentrations in plasma and platelet. As shown in Table 1, the concentrations of Tau, Ala, Glu, Ser and Lys in the umbilical blood plasma were significantly higher

Table 1 Free amino acid concentrations in the plasma and platelets of umbilical and adult blood [a]

Amino acid	Concentrations			
	Plasma (μmoles/ml)		Platelet (pmoles/10^4 cells)	
	Adult	Umbilical	Adult	Umbilical
Tau	0.04±0.01	0.11±0.02**	3.27±0.70	2.30±0.84**
Gln	1.00±0.17	0.70±0.09*	0.42±0.12	0.37±0.21
Ala	0.23±0.06	0.42±0.08**	0.19±0.07	0.39±0.29
Glu	0.03±0.01	0.12±0.01**	0.69±0.17	0.69±0.26
Gly	0.15±0.05	0.16±0.03	0.14±0.05	0.30±0.21
Ser	0.09±0.02	0.13±0.01*	0.15±0.05	0.26±0.13
Leu	0.07±0.01	0.08±0.01	0.12±0.05	0.19±0.13
Thr	0.04±0.01	0.05±0.01	0.09±0.03	0.19±0.11
Val	0.15±0.02	0.16±0.03	0.02±0.06	0.19±0.19
Lys	0.11±0.02	0.22±0.04*	0.07±0.03	0.16±0.11
Arg	0.04±0.01	0.02±0.01	0.03±0.05	0.08±0.01
Asn	0.06±0.01	0.06±0.01	0.09±0.06	0.04±0.03
Met	0.02±0.01	0.02±0.00	0.04±0.02	0.07±0.05

a: Results are expressed as the mean + SD of 15 determinations. Free amino acid concentrations of umbilical blood platelets which differ significantly from adult blood platelets are shown by * (p±0.05), and ** (p<0.01).

($p < 0.05$ or $p < 0.01$) than those in the adult blood, but the concentration of Gln in the umbilical blood plasma was significantly lower ($p < 0.05$) than that in the adult blood. The concentrations of other amino acids in plasma exhibited no significant difference between adult and umbilical blood. Taurine was the most abundant free amino acid both in the adult and the umbilical blood platelets as shown in Table 1. The concentration of taurine in the umbilical blood platelets was 2.30 pmoles/10^4 platelets, which was significantly lower ($p < 0.01$) than that of adult blood platelets (3.27 pmoles/10^4 platelets). No other amino acid showed significant difference between umbilical and adult blood platelets.

Effect of temperature on the taurine uptake into umbilical blood platelet. Taurine uptake into platelets of umbilical blood at 37°C or at 0°C was studied. As shown in Fig. 1, the uptake was less at 0°C than at 37°C.

Effect of sodium ion concentration on taurine uptake into umbilical blood platelet. As shown in Fig. 2, the taurine uptake into the umbilical blood platelets increased with a rise in the sodium ion concentration.

Taurine uptake into platelets at various concentrations of taurine in adult and umbilical blood. As shown in Fig. 3a, taurine uptake into platelets showed a saturating tendency in adult and the umbilical blood platelets (Fig. 3a). Taurine uptake was markedly decreased in umbilical blood platelets. The result was analyzed with the Hanes-plot (Fig. 3b). As shown in Table 2, the V_{max} values in adult and umbilical blood platelets were 0.98 and 0.27 fmoles/10^4 cells·min^{-1}, respectively. The V_{max} in the umbilical blood platelets was sigificantly lower than that in the adult blood platelets ($p < 0.01$). No sigificant difference was seen in *Km* between these two groups.

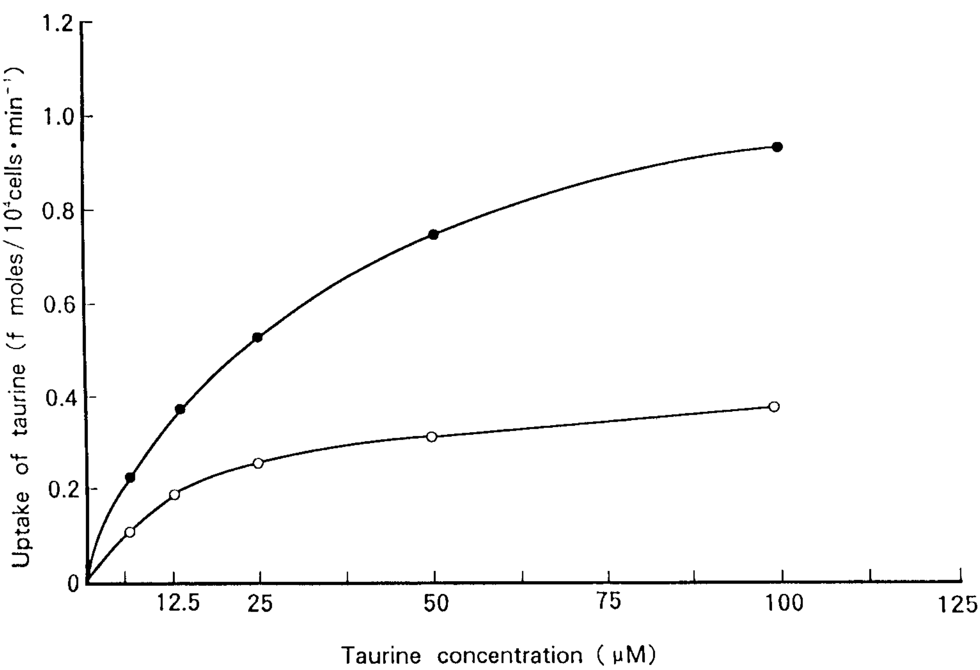

Fig. 1 Effect of temperature on the taurine uptake into umbilical blood platelets. The uptake of taurine at various concentrations at 37°C (●) and 0°C (○) is shown. Each point represents the mean value of 5 determinations.

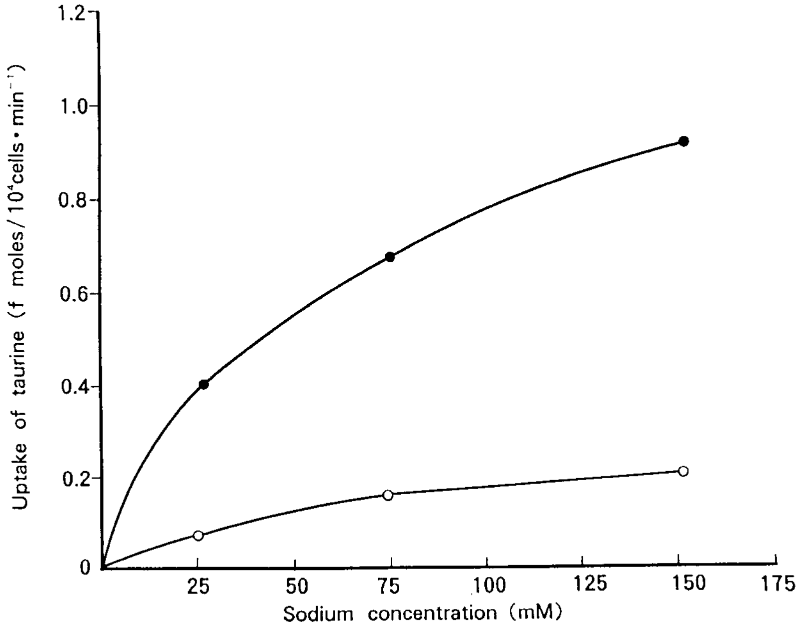

Fig. 2 Effect of the sodium ion concentration on the taurine uptake into umbilical blood platelets. The uptake of taurine at various sodium ion concentrations at 37°C (●) and 0°C (○) is shown. Each point represents the mean value of 5 determinations.

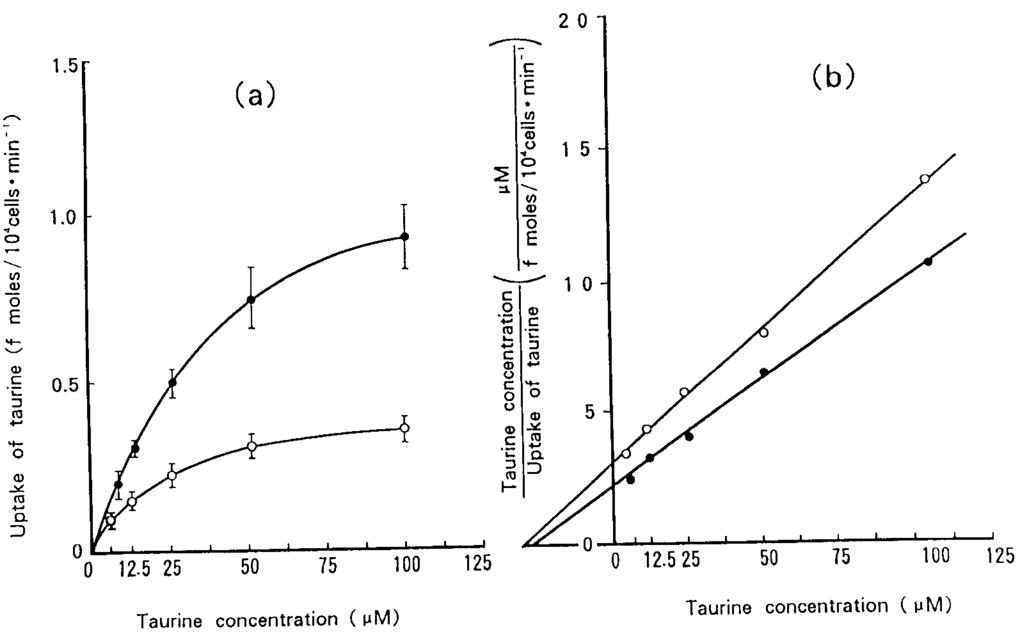

Fig. 3 Taurine uptake by platelets at various concentrations of taurine (3a) and Hanes-plot (3b) in adult and umbilical blood. The taurine uptake (the value at 37°C minus that at 0°C) is shown. Each point represents the mean value of 9 determinations in umbilical blood platelets (○) and 5 determinations in adult blood platelets (●). Vertical lines indicate standard deviation. In Hanes-plot (3b), taurine concentration/taurine uptake was plotted against taurine concentration.

Table 2 Kinetic constants of taurine uptake by platelet in adult and umbilical blood.[a]

Platelets	V_{max} (Fmoles/10^4 cells·min^{-1})	Km (μM)
Adult ($n=5$)	0.98 ± 0.35	52.0 ± 21.8
Umbilical ($n=5$)	0.27 ± 0.21*	54.0 ± 24.3

a: Results are expressed as the mean ± SD. Numbers of cases are shown in the parentheses. The V_{max} of umbilical blood platelet which differs significantly from adult blood platelet is shown by * ($p < 0.01$).

Discussion

Taurine is the most abundant free amino acid widely distributed in the human body (9,10,11), especially in the important organs such as brain, liver, myocardium and skeletal muscles (1,8), and in human body fluids (9,10). The concentrations of taurine in fetal organs and plasma are higher than those in the adult (1,2), and the plasma taurine concentration in the umbilical blood was 2.6 times higher than that in the adult blood (12) (Table 1). The elevated taurine levels in organs and plasma just after the birth decrease to the adult levels within a few days or during the first 2 weeks of life (13,14). The elevated taurine concentration in umbilical blood has been interpreted as the result of active transport of taurine across the placenta (15,16). Human milk contains a considerable amount of taurine (17–19). Taurine depletion in milk formula was associated with a sigificant growth depression (17,18). Therefore, the relationship between taurine and fetal and neonatal development has been suggested.

Adult blood platelets are known to contain higher amounts of taurine than other amino acids (4). The taurine concentration was reported to be as much as 6 times greater than that of any other amino acid (4). The concentration was in the range of 16–24 mmoles/kg of platelet water (20), which was 2–500 times higher than that in the plasma (2). However, there have been no reports concerning taurine synthesis in the platelets. Therefore, taurine must be actively transferred into the platelets. Our results confirm these findings in adult blood platelets. However, the taurine concentration in the fetal and neonatal platelets had not been investigated. Thus we studied the taurine concentration and taurine uptake in umbilical blood platelets.

Taurine was the most abundant free amino acid in the umbilical blood platelets. However, the concentration in umbilical platelets was sigficantly lower than that in the adult (Table 1). Moreover, taurine is the only amino acid, of which the concentration in the umbilical platelets was sigficantly lower than that in the adult blood platelets. The present study shows that the trurine concentration in the umbilical blood platelets (1.84 μmoles/ml) was about 20 times higher than in the umbilical plasma (0.11 μmoles/ml), while the taurine concentration in adult blood platelets (2.62 μmoles/ml) was about 70 times higher than that in the adult plasma (0.04 μmoles/ml) (Table 1). Thus, the ratio of platelet/plasma taurine concentration was decreased in umbilical blood. This result might suggest that the taurine uptake into umbilical blood platelets is low compared with that of the adult.

The taurine uptake into the umbilical blood platelets exhibited a saturating tendency and was dependent on the incubation temperature and sodium ion concentration as shown in Figs 1 and 2. The curve for taurine uptake approaches a plateau value, which is typical for the Michelis-Menten type of saturation phenomenon. Therefore, the Km and V_{max} values for taurine uptake were obtained by the Hanes-plot. The Km values for adult and umbilical blood platelets were essentially the same as shown in Table 2. However, the V_{max} of taurine uptake by the umbilical blood platelets was lower than that of the adult blood platelets. The V_{max} of taurine uptake reflects the capacity of taurine incorporation into platelets. Therefore, when the V_{max} is low, the intra-platelet taurine concentration is expected to be low. On the other hand, there was no sigficant difference in Km value between the two groups. The Km value reflects the affinity of the uptake system. Thus, the low concentration of taurine in the umbilical blood platelets reflects a lower capacity rather than the difference in the characteristics of taurine uptake system on the platelet membrane.

The present findings might suggest that a mechanism which allows a favorable uptake of taurine into the important organs such as the brain and liver is operating in the fetal and neonatal period, and that the platelets play a role in supplying taurine or regulating the taurine level. However, further studies are needed to elucidate the possible mechanism between platelet taurine and taurine supply for organs.

Acknowledgment. The author is deeply indebted to professor Toshihiko Ubuka, Department of Biochemistry, Okayama University Medical School, for his suggestions and to Dr. Hidetoshi Okada, Okayama Red Cross Blood Center, for the counting of platelets in this study.

References

1. Jacobasen JG and Smith LH: Biochemistry and physiology of taurine and taurine derivatives. Physiol Rev (1968) **48**, 424–511.
2. Soupart P: Free amino acid of blood and urine in the human; in Amino Acid Pools, Holden ed., Elsevier, Amsterdam (1962) pp 220–262.
3. Lisa A, Boullin DJ and Paasonen: Transport of taurine by nomal human blood platelets. Br J Phalmacol (1974) **52**, 245–251.
4. Maupin B: Blood Platelets in Man and Animals. Oxford Pergamon City (1969) pp 116–117.
5. Sturman JA and Hayes KC: The biology of taurine in nutrition and development. Adv Nutr Res (1980) **3**, 231.
6. Reigo J and Senterre J: Is taurine essential for the neonate. Biol Neonate (1977) **32**, 73–76.
7. Pasantes MH, Loriette C and Chatagner F: Regional and subcellular distribution of taurine synthesizing enzymes in the central nervous tissue. Neurochem Res (1977) **2**, 671–680.
8. Strumann JA, Rassin DK and Gaull GE: Taurine in the development of the central nervous system; in Taurine and Neurological Disorders, Barbeau and Huxtable eds, Raven Press, New York (1978) pp 49–71.
9. Meister A: Biochemistry of the Amino Acids, 2nd Ed, Academic Pless Inc, New York (1967) pp 757–818.
10. Macaione S, Tucci G and Di Giorgio RM: Taurine distribution in rat tissues during development. Ital J Biochem (Engl Ed) (1975) **24**, 162–174.
11. Ackermann PG and Kheim T: Plasma amino acids in young and older adult human subjects. Clin Chem (1964) **10**, 32–40.
12. Philipps AF, Horzman IR and Battagliia FC: Tissue concentration of free amino acids in term human placentas. Am J Obstet Gynecol (1978) **131**, 881–887.
13. Dickinson JC, Rosenblum H and Hamilton PB: Ion exchange chromatography of plasma acids of newborn. Pediatrics (1965) **36**, 2–13.
14. Ghadimi H and Pecora P: Plasma amino acids after birth. Pediatrics (1964) **34**, 182–191.
15. Ghadimi H and Pecore P: Free amino acids of cord plasma as compared with maternal plasma during pregnancy. Pediatrics (1964) **33**, 500–506.
16. Glendening MB, Margolis AJ and Page EW: Amino acid concentrations in fetal and maternal plasma. Am J Obstet Gynecol (1961) **81**, 591–593.
17. Gaull GE, Rassin DK and Heinonen K: Milk protein quantity in low birth weight infants. J Pediatr (1977) **90**, 348–355.
18. Gaull GE: Taurine, milk and maturation; in Human Milk, Freier and Editman eds., Excerpta Medica, Amsterdam-Oxford-Prinston (1980) pp 23–27.
19. Zaima K, Nisihara K and Kobayasi N: Taurine concentration in maternal serum, cord blood and breast milk. Sulfur Amino Acid (1986) **1**, 241–250.
20. Hino A: Amino acids in normal human blood functons. Nippon Naika Gakkai Zasshi (1956) **45**, 609-619 (in Japanese).

Received January 13, 1992; accepted February 12, 1992.