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Note

Liquid chromatographic determination of taurine in whole blood, plasma and platelets

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Taurine (2-aminoethanesulfonic acid) is ubiquitous in mammals; yet its function still is not well understood [1,2]. In the blood, there is an active uptake of taurine into the platelets, with a platelet/plasma gradient of over 400/1 [3,4]. This fact has prompted investigation in this area [4–12] and has raised questions about the role of taurine in platelet physiology.

Taurine is a stable amino acid amenable to analysis by ion-exchange chromatography followed by post-column derivatization with ninhydrin [4,7] or *o*-phthalaldehyde (OPA) [8]. Recently, reversed-phase separations with pre-column derivatization have been used [11,12]. Generally, these methods require long analysis times in order to resolve taurine from other amines and amino acids.

We report a method for taurine in plasma and whole blood that utilizes a previously reported [9–13] mixed-bed ion-exchange clean-up, followed by OPA derivatization and high-performance liquid chromatographic (HPLC) separation on a ion-pair reversed-phase C_{18} column. Platelet taurine was similarly analyzed without the necessity of a preliminary purification. The methods utilize an internal standard, 3-aminopropanesulfonic acid (APS), and permit rapid (< 10 min) HPLC analysis.

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EXPERIMENTAL

Reagent and chemicals

Taurine, APS, OPA, 3-mercaptopropionic acid (3MPA) and other amino acids were obtained from Sigma (St. Louis, MO, U.S.A.). Acetronitrile and methanol were glass-distilled and purchased from American Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Cation-exchange (50W-X8) and anion-exchange (AG1-X8) resins (200–400 mesh) were from Bio-Rad Labs. (Richmond, CA, U.S.A.). All other chemicals were reagent grade from local suppliers. All water was distilled and deionized.

Amino acid solutions were made up in distilled water and stored refrigerated. Stock solutions of 1 μg/μl were made up monthly and diluted to working concentration of 1 ng/μl each day. The OPA solution was made by dissolving 50 mg OPA in 4.5 ml methanol and 0.5 ml borate buffer. 3MPA (50 μl) was added and the solution stored refrigerated in the dark. Borate buffer was made by adjusting 0.5 *M* boric acid to pH 9.5 with 5 *M* sodium hydroxide. An OPA reaction mixture was prepared daily, consisting of 100 μl of OPA solution, 400 μl of borate buffer and 400 μl of methanol.

High-performance liquid chromatography

The HPLC system consisted of an Altex 110A pump, a Rheodyne 7125 injector with a 100-μl sample loop, an Altex Ultrasphere 250 mm×4.6 mm, 5-μm ODS column (all Rainin Instruments, Woburn, MA, U.S.A.) and a Shimadzu Model RF-530 fluorimeter (Columbia, MD, U.S.A.). Excitation and emission wavelengths were 330 and 440 nm, respectively, with 20-nm bandpasses. Output was recorded using a Kipp & Zonen BD-41 strip chart recorder. The mobile phase consisted of 0.1 *M* sodium dihydrogenphosphate containing 5 g/l tetrabutylammonium hydrogen sulfate and 100 mg/l Na_2EDTA–acetonitrile (60:40). The mobile phase was delivered at a flow-rate of 0.8–1.0 ml/min.

Ion-exchange columns

Columns were prepared as previously described [2]. Briefly, 50W-X8 and AG1-X8 resins were washed in 10 volumes of 4 *M* hydrochloric acid and rinsed with distilled water until neutral. Approximately 0.1 g of each resin was packed into a Pasteur pipette (0.5 cm I.D., packing 1.0 cm in height) and columns were rinsed with 10 ml distilled water. Columns were regenerated by washing with 10 ml of 4 *M* hydrochloric acid, rinsing with distilled water until neutral and then rinsing with an additional 10 ml distilled water.

Sample preparation

Blood samples were collected into tubes containing EDTA as anticoagulant. Platelet-rich plasma (PRP), platelet-poor plasma (PPP) and platelet pellets were prepared as follows. Whole blood (5–6 ml) was mixed by inversion and centrifuged at 300 *g* for 10 min at 10°C. Resulting PRP was transferred to a second tube, 200 μl removed for platelet count and 250 μl removed for PRP taurine analysis. A 1-ml volume of PRP was spun for 15 min at 800 *g* at 10°C. PPP was

removed and saved for analysis, the tube blotted dry and 1.0 ml of 0.9% sodium chloride was added without disruption of the pellet. After centrifugation at 2500 *g* for 15 min the supernatant was removed and the pellet saved for analysis.

Samples for analysis were prepared from 200 μl PRP, PPP or whole blood by deproteinization with 20 μl (PRP and PPP) or 40 μl (whole blood) of 3.2 *M* perchloric acid after 2 μg of APS had been added as internal standard. After being kept on ice for 5–10 min, samples were spun at 13 000 *g* for 5 min and the supernatant (approximately 200 μl) placed over the ion-exchange column. The column was washed with 800 μl distilled water; all eluent was collected. A 50-μl volume of the eluent was added to 450 ml of the OPA reaction mixture and vortex-mixed. After an incubation of 2.5 min, 20 μl of the sample reaction mixture were mixed with 180 μl of 0.1 *M* phosphoric acid and 50 μl loaded on the sample loop with a syringe and injected onto the column.

An extract of the platelet pellet was prepared by sonication (Branson Model 185 sonifier; Branson Sonic Power, Danbury, CT, U.S.A.) of the pellet in 1.0 ml distilled water to which 10 μg APS had been added as internal standard. Following sonication (1 min, setting 4) and the addition of 100 μl of 3.2 *M* perchloric acid, the sample was kept on ice for 5–10 min, then spun at 13 000 *g* for 5 min. Derivatization of 50 μl of the supernatant was carried out as described above.

RESULTS AND DISCUSSION

The chromatograms of standards and PRP are shown in Fig. 1. Analysis is rapid (approximately 10 min), and samples can be quantified easily without interference. Standard addition to a PRP sample demonstrated linear recovery between 1 and 10 μg/ml PRP. Average absolute recoveries of taurine and APS through the ion-exchange column were $104 \pm 4.1\%$ ($n=4$) and $98.0 \pm 5.6\%$ ($n=7$), respectively. Within-day ($n=4$) coefficients of variation observed for selected specimens varied from 0.7 to 5.1% and a day-to-day ($n=4$) coefficient of variation of 5.9% was observed for a pooled specimen (mean 10.9 μg/ml). Similar chromatograms and standard addition data were obtained with whole blood and PPP samples. Identification of taurine and APS peaks were confirmed by HPLC using a mobile phase consisting of 0.1 *M* sodium dihydrogenphosphate (pH 3.8) with 2 g/l tetrabutylammonium hydrogen sulfate–acetonitrile (60:40). No significant differences in values were observed.

A chromatogram of platelet pellet homogenate is shown in Fig. 2. Although no preliminary sample purification was employed, the sample was remarkably free of other peaks. The peak identities were confirmed using the mobile phase described above.

Standard addition to pellet homogenate demonstrated a linear recovery over the range 0–10 μg/ml homogenate. The average recoveries of taurine and APS were 105 ± 4.9 and $110 \pm 2.6\%$, respectively. Within-day ($n=4$) and day-to-day ($n=4$) coefficients of variation for a pooled specimen (mean 8.87 μg/ml) were 1.1 and 3.4%, respectively.

Sample preparation for plasma and blood analyses is not difficult, and the mixed-bed ion-exchange column provides excellent clean-up. Furthermore, we

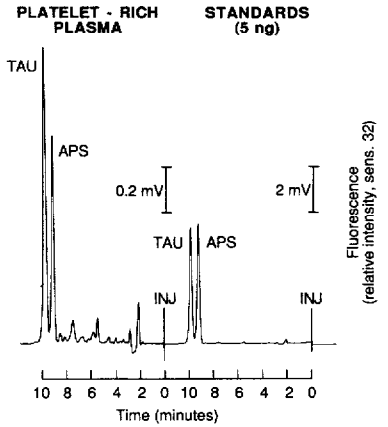

Fig. 1. Taurine (TAU) and APS standards (OPA derivatives) injected in 50 μl of acidified reaction mixture. Standard concentration in original standard solution was 10 μg/ml (5 ng injected). The sample injection of 50 μl acidified sample reaction mixture was equivalent to 0.1 μl of PRP. PRP contained 10 μg/ml APS as internal standard. A taurine concentration of 14.9 μg/ml was calculated for the sample shown. Note the difference in sensitivity between injections. See Experimental section for details of derivatization and chromatography.

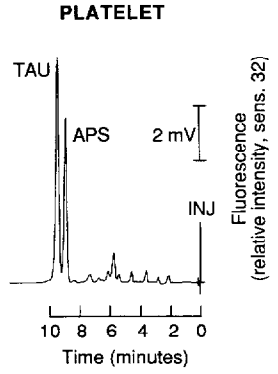

Fig. 2. Taurine (TAU) and APS determined in platelet homogenate. Platelet pellet from 1 ml of PRP ($0.40 \cdot 10^9$ platelets per ml) was homogenized in 1.0 ml of water with 10 μg/ml APS added. A 50-μl volume of the homogenate was added to 450 μl of OPA reaction mixture; 20 μl of this sample reaction mixture were added to 180 μl of 0.1 *M* phosphoric acid and 50 μl of the diluted, acidified solution were injected (injection equivalent to 0.5 μl of platelet homogenate). A taurine concentration of 14.2 μg/ml homogenate or 35.5 μg per 10^9 platelets was calculated for the sample shown.

have regenerated columns at least ten times with no decrease in recovery or selectivity. A 1.0-ml total eluent was found to be sufficient for complete recovery of both taurine and APS. No special precautions in protein precipitation were required, and perchloric and 5-sulfosalicylic acid yielded the same results (data not shown).

The separation of taurine and APS is accomplished by reversed-phase ion-pair chromatography with tetrabutylammonium as ion-pair reagent. The high concentration (5 g/l) of reagent was necessary to completely separate taurine and APS. Although a lower concentration of ion-pair reagent also effected a usable separation of the compounds, attempts to separate taurine and APS without ion-

pairing were unsuccessful. Variations of the mobile phae including pH, buffer composition and organic modifier did not resolve taurine and APS.

The OPA derivatives of taurine and APS exhibit marked stability with 3MPA compared to β-mercaptoethanol. This stability has been investigated for a number of thiols [14,15], and, while fluorescence yield decreases with increasing thiol size, 3MPA derivatives exhibit good fluorescence with enhanced stability. The half-lives of taurine and APS in 0.1 *M* phosphoric acid are 17 and 22 min, respectively, making APS an excellent internal standard for this application. The detection limits of the system were found to be 5–10 pg for taurine and APS. Due to the high concentration of taurine in the blood, analyses can be performed on as little as 25 μl of PPP, and with even less PRP and whole blood.

The values obtained for PPP, PRP, whole blood, packed cells and platelets are listed in Table I with previous literature values, where available. Our values generally are in agreement with the literature, although several values bear comment.

Reported concentrations for platelet taurine range from a low of 9.6 to a high of 35.8 μg per 10^9 platelets, with our value, 16.3 μg per 10^9 platelets, approaching the mean. When the platelet taurine concentration was converted to units of μg/ml whole blood (based on whole blood platelet counts) a mean value of 4.8 ± 2.2 μg/ml whole blood was obtained.

Our value for PPP taurine (4.90 ± 1.50 μg/ml PPP) was in good agreement with, though slightly lower than, previous reports. When converted to units of μg/ml whole blood (based on hematocrits) a mean value of 2.9 ± 0.9 μg/ml whole blood was calculated.

The mean PRP value of 11.3 ± 3.2 μg/ml was in good agreement with the mean of the sums (10.1 ± 2.6 μg/ml) of the PPP value and the platelet concentration in PRP (determined from the taurine concentration in the platelet pellet derived from 1.0 ml of PRP).

Conversely, platelet taurine concentrations could be determined by subtracting the PPP concentration from the PRP value. Mean values of platelet taurine concentrations determined directly (by measuring platelet pellet concentration,

TABLE I

TAURINE CONCENTRATIONS IN BLOOD

Reference	PPP (μg/ml)	Whole blood (cellular) (μg/ml)	Whole blood (μg/ml)	PRP (μg/ml)	Platelet (μg/10^9 platelets)
Frendo et al. (1959) [5]					9.6
Maupin (1969) [3]	6.00				16.9
Aoki et al. (1973) [7]	6.48	12.4	16.3		
Ahtee et al. (1974) [4]					35.8 ± 12.9
Paasonen et al. (1980) [16]					32.6
Fukuda and Usui (1983) [8]	8.15 ± 1.21	12.2 ± 1.1			
Vinton et al. (1986) [10]	9.25 ± 3.4	18.2 ± 5.2			32.1 ± 12.9
This work (1988) ($n=10$)	4.90 ± 1.50	17.0 ± 14.4	25.1 ± 15.4	11.3 ± 3.6	16.3 ± 3.0

16.7 ± 3.0 μg per 10^9 platelets) were in good agreement with those determined by difference (19.8 ± 4.7 μg per 10^9 platelets).

When the mean values for PPP (2.9 ± 0.9 μg/ml whole blood) and platelet (4.8 ± 2.2 μg/ml whole blood) taurine are subtracted from the whole blood value (25.1 ± 15.4 μg/ml) a cellular concentration of 17.4 ± 14.4 μg/ml whole blood can be estimated. This was in excellent agreement with the value of 18.2 ± 5.2 μg/ml estimated from the Vinton et al. [10] report of lymphocyte, granulocyte and erythrocyte concentrations.

Linear regression analysis was performed on a group of bloods (PPP, PRP, platelets, $n=10$; whole blood, $n=8$). Whole blood values were highly correlated with platelet ($r=0.894$, $p=0.003$) and PRP ($r=0.802$, $p=0.017$) values, while no correlation existed for whole blood and PPP ($r=0.174$, $p=0.681$). Platelet taurine was correlated with PRP taurine ($r=0.890$, $p<0.001$) but not with PPP taurine ($r=0.030$, $p=0.933$).

It is somewhat surprising that neither whole blood nor platelet levels are correlated with plasma free taurine levels. This suggests that platelet (and cellular) stores of taurine are not greatly influenced by plasma free taurine levels despite evidence that plasma concentrations are below the observed Michaelis constant (K_M) for platelet uptake of taurine.

In summary, a method for taurine analysis in blood is described. Whole blood, PRP and PPP samples are partially purified by use of a mixed-bed ion-exchange column, while platelet pellet analysis does not require this step. An internal standard, APS, provides accurate and precise quantitation. The samples were derivatized with OPA–3MPA and separated by reversed-phase ion-pair HPLC with fluorimetric detection. Analysis of human blood fractions gives results which generally are in good agreement with the literature.

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