Article

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Mice, Rats and Guinea Pigs Exhibit Significant Variations in the Plasma, Urine and Tissue Levels of Taurine, Betaine, Sarcosine and Other Osmolyte-Active Amino Acids

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Background: Osmolytes are naturally occurring compounds that protect cells from osmotic stress in high-osmolarity tissues, such as the kidney medulla. Some amino acids, including taurine, betaine, glycine, alanine, and sarcosine, are known to act as osmolytes. This study aimed to establish the levels of these amino acids in body fluids and tissues of laboratory animals used as models for human diseases in biomedical research.

Methods: Liquid chromatography coupled with mass spectrometry was used to quantify taurine, glycine, betaine, alanine, beta-alanine, and sarcosine in plasma, urine, and tissues of adult, male mice, rats and guinea pigs.

Results: Among the species analyzed, taurine was found to have the highest tissue concentrations across all compounds, with the heart containing the greatest amount. In guinea pigs, betaine levels were higher in the renal medulla than in the renal cortex ($p < 0.01$), while in rats and mice, there were no significant differences in betaine levels between the kidney cortex and medulla. The urine of guinea pigs had lower levels of sarcosine compared to rats ($p < 0.001$), while the plasma ($p < 0.05$; > 0.05), heart ($p < 0.05$; < 0.05), lungs ($p < 0.01$; < 0.01), liver ($p < 0.001$; < 0.05), and kidneys ($p < 0.01$; < 0.01) of rats exhibited notably higher concentrations of sarcosine compared to both mice and guinea pigs, respectively.

Conclusions: There are pronounced differences in the concentrations of taurine, betaine, and other amino acids across the investigated species. It is important to acknowledge these differences when selecting animal models for preclinical studies and to account for variations in amino acid concentrations when selecting amino acids doses for interventional studies.

Keywords: osmolytes; piezolytes; amino acids; taurine; laboratory animals

Introduction

Amino acids serve numerous functions in animals. Some serve as building blocks for synthesizing proteins, substrates for producing hormones, or numerous compounds like glucose, long- and short-chain fatty acids, nitric oxide, glutathione, ammonia, and urea. Other amino acids are involved in the energy metabolism of a cell, mediate the communication between cells or serve as osmolytes, i.e., regulate cellular volume by protecting the structural and functional integrity of cells against osmotic and/or hydrostatic stress [1,2]. Organisms accumulate small organic solutes that balance the osmolalities of the cellular compartments to maintain the cellular volume. The osmotic activity of amino acids has been well-documented in marine fish and mammals, in which changes in osmolality correlate with the levels of taurine, glycine, betaine, and alanine in various organs [3,4]. The levels of osmolytes exhibit significant variability across various species, as well as within individuals and organs of an individual [1]. This variability can be attributed to the differential osmotic and hydrostatic pressures experienced by tissues. For example, renal medulla cells accumulate osmolytes due to their adaptation to the high osmotic environment present within extracellular fluids of the kidneys. Similarly, osmotic and hydrostatic forces within the cardiovascular system exhibit significant variation and fluctuation. Furthermore, the pulmonary microcirculation exhibits a unique osmotic characteristic, developing a pressure gradient that draws fluid from the interstitial space. Although osmolytes play a critical role in maintaining balance between hydrostatic and osmotic forces, their distribution across tissues and interspecies variations across different terrestrial mammalian species remains poorly understood.

Physiological levels of amino acids in the organism are crucial for maintaining homeostasis. Notably, alterations in the concentrations of amino acids are associated with impaired immune function and a higher risk of various cardiovascular, renal, and metabolic disorders [5–7]. In humans, supplementing some amino acids is practiced for multiple indications; however, convincing clinical evidence is lacking. For instance, taurine is commonly used in beverages that enhance alertness. It is distributed to the tissues and organs of the whole organism [8]. High taurine levels have been measured in the heart [8]. In addition, glycine, betaine, and beta-alanine are popular food supplements that potentially may promote athletic performance. Glycine is the simplest amino acid formed from serine, threonine, or choline, mainly in the liver and kidneys. It is one of the amino acids that are essential for the synthesis of proteins, but it also plays an essential role in regulating antioxidative defenses [9]. Interestingly, sarcosine, a precursor of glycine, has been suggested to act as an antidepressant agent [10]. Betaine is endogenously produced from choline in the liver and kidneys, and its primary functions are regulating the cellular volume and donating methyl groups to other biomolecules [11]. Alanine, a constituent of proteins and a crucial regulator of gluconeogenesis, comes from the conversion of pyruvate [12].

Mice, rats, and guinea pigs are the most commonly used animal models in biomedical research on human health and disease. It is well established that preclinical studies employing various animal models should consider the interspecies differences during data interpretation of interventional studies. For instance, several osmolyte pathways have been identified that regulate the response of the immune system in the organism [13,14]. In addition, imbalances in osmolyte regulation have been associated with higher cardiovascular risk and severity of chronic kidney disease [15]. However, tissue concentrations of osmotic active amino acids such as taurine, glycine, betaine, alanine, beta-alanine, and sarcosine and possible interspecies variations have not been elucidated. Thus, we aimed to determine and compare the levels of specific amino acids in the plasma, urine, and tissues of mice, rats, and guinea pigs.

Materials and Methods

Animals

The study was performed on 15–17-week-old male Sprague-Dawley rats (346 ± 27 g, n = 8), 10–12-week-old male BALB/c mice (22.4 ± 1.3 g, n = 8), and 7–8-month male American guinea pigs (679 ± 118 g, n = 8). Rats and mice were obtained from the Central Laboratory for Experimental Animals (Medical University of Warsaw, Poland). Guinea pigs were obtained from the Animal Breeding Laboratory in Ilkowice, Poland. Experiments were carried out in the Laboratory of the Centre for Preclinical Research (Medical University of Warsaw, Poland). The animals were quarantined for 2 weeks after they were brought to the laboratory. During this period, the animals were fed a standard laboratory diet for mice rats and guinea pigs, housed in groups of 2–4 in propylene cages, under a 12 hrs light/12 hrs dark cycle, at a constant temperature of 22–23 °C and humidity of 45–55%. After this period, the animals were anesthetized with urethane (1.5 g/kg body weight (BW)). Urine was collected from fresh urine samples produced during spontaneous voids before anesthesia. Blood had been withdrawn and collected by cardiac puncture. Rats and mice were euthanized by cervical vertebrae dislocation, and guinea pigs by decapitation. Heart, lung, liver, renal cortex and renal medulla were explanted and immediately frozen at –80 °C. Experiments were performed in the middle of light phase and animals were not fasted before sacrifice.

Chemicals and Solutions

Taurine, glycine, betaine hydrochloride alanine, beta-alanine, sarcosine, alanine-$^{13}C_3$,^{15}N, taurine-D_4, glycine-$^{13}C_2$, sodium bicarbonate, pentafluoropropionic acid dansyl chloride, *tert*-butyl bromoacetate (TBBA), ammonia solution and ammonium formate were purchased from Sigma Aldrich (Merck Life Science, Darmstadt, Germany). Sarcosine-$^{13}C_3$ and betaine-D_3 hydrochloride was purchased from Toronto Chemicals Research (Toronto Chemicals Research, North York, Canada). Amino acids stock solutions were prepared in water freshly, and betaine solution was prepared in methanol. Liquid chromatography and mass spectrometry (LC-MS) grade acetonitrile, high performance liquid chromatography (HPLC) grade acetone, HPLC grade acetonitrile, HPLC grade methanol, and formic acid were obtained from J.T. Baker (Phillipsburg, NJ, USA). Ultra-pure water (Mili-Q water) was produced by a water purification system (Mili-Q, Millipore, Milford, MA, USA).

To differentiate alanine, beta-alanine, and sarcosine a derivatization technique was applicated. The protocol was as follows: 20 µL of a sample (biological and calibration samples) was mixed with 50 µL of acetone containing internal standards and 100 µL of 0.5 M bicarbonate sodium. Samples were incubated with 100 µL of 2 mg/mL dansyl chloride for 30 min at 60 °C to form the dansyl derivatives. Next, the solution was centrifuged, and an aliquot was injected into the apparatus. For the determination of betaine, 50 µL of *tert*-butyl bromoacetate in acetonitrile, 10 µL of 2.5% ammonia solution, and 50 µL of acetone (containing internal standards) were added to 20 µL of a sample (biological and calibration samples). The mixture was mixed and incubated at room temperature. After 30 min, 25 µL of 0.5% formic acid in 50% acetonitrile was added. Next, the solution was centrifuged, and an aliquot was injected into the apparatus.

Liquid Chromatography and Mass Spectrometry

The levels of taurine, glycine, betaine, alanine, beta-alanine, and sarcosine were detected in plasma, urine, and tissue homogenates by liquid chromatography-mass spectrometry technique. All urine samples were diluted ten times using distilled water. Hearts, lungs, livers, renal cortex, and medulla were weighed, placed in 10% ethanol (90 µL per 10 mg of tissue), and homogenized using the Precellys Cryolys Evolution tissue homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France). Plasma, urine, and homogenates were stored at –80 °C until analysis. The concentrations of amino acids were evaluated using a Waters Acquity Ultra Performance Liquid Chromatograph (Waters, Milford, MA, USA) coupled to a Waters TQ-S triple-quadrupole mass spectrometer through an electrospray ionization source (Waters, Manchester, UK). For the instrument control and data acquisition Waters MassLynx v4.2 SCN1035 software was used (Waters, Manchester, UK). Waters TargetLynx was used to process data (Waters, Manchester, UK).

Analytes separation was accomplished using a Waters HSS T3 column (1.7 µm, 2.1 mm × 100 mm) (Waters, Milford, MA, USA) thermostatted at 55 °C. The mobile phase composition used during separation was 0.1% formic acid in Mili-Q water (mobile phase A) and 0.1% formic acid with 0.00005% pentafluoropropionic acid in acetonitrile (mobile phase B). The flow rate of the mobile phase was set at 0.5 mL/min, and the injection volume was 5 µL. Initially, the gradient scheme was 35% B, increasing to 70% B at 2 min. At 2.5 min, the mobile phase reverted to the initial condition (35% B). The total analysis time was 3 min, including re-equilibration time. To determine betaine, the chromatographic separation was performed using a Waters HILIC column (1.7 µm, 2.1 mm × 50 mm) (Waters, Milford, MA, USA) thermostatted at 60 °C. Mobile phase A was 15 mM ammonium formate in Mili-Q water, and mobile phase B was acetonitrile. The flow rate of the mobile phase was set at 0.5 mL/min. The total time of separation was 2.2 mins.

The mass spectrometer was operated in positive electrospray ionization (ESI+) mode with the following general conditions: capillary voltage = 2.0 kV, desolvation temperature = 450 °C, desolvation gas flow = 500 L/h, cone gas flow = 150 L/h, nebulizer gas pressure = 7.0 bar, source temperature = 150 °C. For betaine, the general conditions were set to capillary voltage = 2.5 kV, desolvation temperature = 350 °C, desolvation gas flow = 550 L/h, cone gas flow = 150 L/h, nebulizer gas pressure = 7.0 bar, source temperature = 150 °C. The mass spectrometer was operated in multiple-reaction monitoring (MRM) mode, and two MRM transitions were defined for each analyte. The MRM transitions, cone voltages, collision energies, and retention times used in the described method are presented in Table 1. Calibration standards were prepared in water; no amino acids-free serum or urine was available. Calibration curves were created by comparing a ratio of the analyte's peak area to the corresponding internal standard's peak area against known analyte concentrations. Biological samples (plasma, urine, stool extract) were compared with an obtained calibration curve. The mean R2 coefficients of calibration curves were not lower than 0.98. The lower limits of quantification were: 20 ng/mL for betaine, 0.1 µM for sarcosine, 1 µM for beta-alanine, and 10 µM for alanine, taurine, and glycine.

Statistics

Outliers were defined (results above Q3 + 1.5 × interquartile range and below Q1 – 1.5 × interquartile range) and removed from the statistical analysis. The Shapiro–Wilk test was used to test the normality of the distribution. Differences in the concentrations of taurine, glycine, betaine, alanine, beta-alanine, and sarcosine in the plasma and urine within one species were evaluated by the Mann-Whitney U Test. Differences in the taurine, glycine, betaine, alanine, beta-alanine and sarcosine concentrations in the tissue homogenates were evaluated by Kruskal-Walli's test followed by post-hoc Dunn's test. Differences in the concentrations of taurine, glycine, betaine, alanine, beta-alanine, and sarcosine in the plasma, urine, and tissue homogenates between the three species were evaluated by Kruskal-Walli's test followed by post-hoc Dunn's test. A value of two-sided $p < 0.05$ was considered significant. Statistical analysis was conducted using STATISTICA 13.3 (Stat Soft, Krakow, Poland).

Results

Comparison of Amino Acids in the Body Fluids and Tissues of Animals

Mice

The levels of all measured amino acids were significantly higher in the urine than in the plasma of mice (Table 2). The comparison of tissue homogenates (Table 3) showed that the taurine concentration was higher in the heart (4-fold, $p < 0.001$ and 2-fold, $p < 0.05$) compared to the lung and renal medulla, respectively. In contrast, the cardiac concentration of betaine (33-fold, $p < 0.001$; 23-fold, $p < 0.05$ and 28-fold, $p < 0.01$) and beta-alanine (28-fold, $p < 0.001$; 7-fold, $p < 0.01$ and 6-fold, $p < 0.05$) was lower than the concentration in the liver, renal cortex, and medulla, respectively. Significantly higher levels of taurine, betaine, and beta-alanine were found in the liver compared to the lungs (2-fold, $p < 0.01$; 11-fold, $p < 0.01$ and 12-fold, $p < 0.001$). Glycine and alanine were abundant in the kidneys of mice. Higher levels of glycine (10-fold, $p < 0.001$ and 3-fold, $p < 0.01$) and alanine (3-fold, $p < 0.001$ and 6-fold, $p < 0.001$) were determined in the renal cortex compared to the heart and lungs, respectively. The concentration of glycine and alanine in the renal medulla was significantly higher than the concentration in the heart and lung, respectively (9-fold, $p < 0.001$ and 4-fold, $p < 0.01$).

Table 1. Monitored transitions for dansyl derivative of analytes, cone voltages, collision energies, and retention times.

Analyte	MRM transition	Cone voltage [kV]	Collision energy	Retention time [min]
Taurine	359.07 > 157.1 (qt)	20	30	0.63
	359.07 > 170.1	20	20	
Taurine-D_4	363.09 > 170.1	20	20	0.63
	363.09 > 234.1	20	15	
Glycine	309.09 > 157.1 (qt)	20	50	1.36
	309.09 > 170.1	20	45	
Glycine-$^{13}C_2$	311.09 > 157.1	20	35	1.36
	311.09 > 170.1	20	35	
Betaine	118.086 > 58.09 (qt)	20	40	1.33
	118.086 > 59.09	20	40	
Betaine-d3	121.105 > 61.06	20	15	1.33
	121.105 > 62 (qt)	20	15	
Beta alanine	323.11 > 157.1 (qt)	20	25	1.47
	323.11 > 170.1	20	15	
Alanine	323.11 > 157.1 (qt)	20	50	1.57
	323.11 > 170.1	20	50	
Alanine-$^{13}C_3$,^{15}N	327.11 > 157.1	20	25	1.57
	327.11 > 170.1	20	20	
Sarcosine	323.11 > 157.1 (qt)	20	25	1.85
	323.11 > 170.1	20	15	
Sarcosine-$^{13}C_3$	326.12 > 157.1	20	30	1.85
	326.12 > 170.1	20	20	

MRM, multiple-reaction monitoring.

Table 2. The comparison of amino acid levels between plasma and urine in mice, rats and guinea pigs.

Parameter [µmol/L]	Plasma	Urine
Mice		
Taurine	1547.60 (1510.92; 2446.68)	46984.15 (38735.65; 94476.11)**
Glycine	289.11 (213.02; 335.91)	854.45 (421.26; 1648.62)**
Betaine	70.83 (52.66; 77.96)	649.35 (274.82; 1059.69)**
Alanine	138.97 (130.15; 144.52)	206.15 (167.84; 236.34)*
Beta alanine	5.53 (4.60; 8.51)	253.41 (171.74; 354.19)**
Sarcosine	0.48 (0.41; 0.55)	32.58 (11.33; 37.89)*
Rat		
Taurine	836.44 (806.69; 1060.56)	6468.90 (4832.21; 7750.36)**
Glycine	146.30 (139.69; 151.97)	3571.83 (3267.70; 3844.64)**
Betaine	184.62 (112.99; 312.69)	926.55 (669.41; 1285.18)**
Alanine	243.66 (226.07; 286.74)	249.9 (190.28; 350.57)
Beta alanine	2.83 (2.50; 3.76)	70.12 (52.98; 85.50)**
Sarcosine	3.55 (2.13; 4.95)	48.40 (39.65; 70.79)**
Guinea pig		
Taurine	90.10 (77.28; 119.77)	238.12 (96.23; 389.81)
Glycine	531.50 (471.15; 963.98)	445.95 (390.65; 818.58)
Betaine	158.36 (129.88; 174.84)	92.84 (73.44; 154.50)
Alanine	147.29 (124.98; 223.22)	80.72 (69.64; 107.35)**
Beta alanine	47.42 (42.56; 58.87)	48.22 (25.56; 63.69)
Sarcosine	0.58 (0.45; 2.14)	0.78 (0.57; 1.02)

All data are expressed as the median, Q1, Q3; Mann-Whitney U test. *$p < 0.05$; **$p < 0.01$.

We observed an accumulation of sarcosine in the liver of mice. Sarcosine levels were higher in the liver (5-fold, $p < 0.001$ and 7-fold, $p < 0.001$) than in the renal cortex and medulla, respectively. Significant differences in sarcosine levels were found between the heart and renal medulla (3-fold, $p < 0.05$).

Table 3. The comparison of amino acid levels in tissue homogenates in mice, rats and guinea pigs.

Parameter [µmol/L]	Heart	Lungs	Liver	Renal cortex	Renal medulla	Kruskal-Wallis test
Mice						
Taurine	40266.73 (37079.98; 41684.36)	10795.02 (10387.74; 11349.54)***	21299.09 (16992.27; 22131.22)##	17274.63 (16321.86; 19316.30)	16980.66 (15605.16; 17389.11)*	$p < 0.001$
Glycine	1388.96 (1200.51; 1594.40)	4962.88 (4538.49; 5503.22)	6064.25 (5470.84; 7000.17)	13807.21 (13152.49; 14898.13) ***##	11889.04 (11573.92; 12855.58)***	$p < 0.001$
Betaine	93.46 (74.75; 100.45)	274.30 (245.99; 298.65)	3116.67 (2500.72; 4067.46) ***##	2180.00 (1737.89; 2383.50) *	2570.43 (1892.51; 2664.78)**	$p < 0.001$
Alanine	3069.74 (2838.62; 3398.64)	1876.01 (1800.37; 2086.67)	6239.85 (5241.83; 7140.52)	10508.91 (10053.85; 11421.81) ***###	7707.77 (7156.15; 8420.56)##	$p < 0.001$
Beta alanine	16.60 (14.77; 17.66)	37.79 (34.93; 42.08)	467.66 (452.96; 708.94)***###	111.59 (108.03; 117.50)**	96.99 (95.49; 111.20)*	$p < 0.001$
Sarcosine	4.04 (3.02; 4.31)	3.15 (2.50; 4.11)	10.82 (8.55; 13.04)	2.01 (1.50; 2.17)^^^	1.60 (1.21; 1.77)*^^^	$p < 0.001$
Rat						
Taurine	36440.90 (35353.26; 38062.00)	10015.77 (9525.08; 10347.00)***	6324.36 (38062.00; 7789.85)***	13438.09 (12947.04; 15244.56)^^	12413.71 (11155.18; 13161.59)^	$p < 0.001$
Glycine	1130.91 (1087.98; 1147.01)	8005.07 (7461.08; 8275.74)	4191.65 (3961.89; 4317.71)	13739.10 (13398.82; 14205.12)***^^	13073.32 (12242.38; 13697.86)***^	$p < 0.001$
Betaine	267.63 (204.78; 309.47)	287.46 (273.73; 377.49)	2193.30 (1861.14; 2678.47)***##	1616.12 (1254.22; 2290.73)*	1632.82 (1496.47; 2321.91)**#	$p < 0.001$
Alanine	3031.34 (2983.71; 3173.51)	4618.17 (4163.61; 4757.13)	16058.49 (15434.67; 17780.62)***##	13355.17 (12426.25; 13933.06)**	13855.12 (12829.59; 14464.26)**	$p < 0.001$
Beta alanine	57.06 (54.65; 62.24)	54.96 (44.92; 65.19)	399.09 (382.55; 442.05)***###	56.02 (54.02; 70.17)^^	76.99 (69.85; 88.86)	$p < 0.001$
Sarcosine	6.74 (5.82; 8.48)	25.72 (17.58; 31.16)**	121.83 (90.15; 138.74)***	7.57 (6.30; 10.74)^^^	10.19 (8.23; 11.43)^^	$p < 0.001$
Guinea pig						
Taurine	15836.78 (12134.31; 17288.21)	2443.74 (2368.16; 2706.91)	214.07 (170.93; 239.13)***	2418.93 (2000.21; 3116.20)*	2710.60 (2300.49; 3244.02)	$p < 0.001$
Glycine	1653.54 (1531.23; 1706.09)	6983.79 (5944.10; 7822.00)	11816.89 (10000.83; 12452.86)*	14380.96 (12263.32; 14791.00)***##	11650.27 (11229.49; 13611.74)**	$p < 0.001$
Betaine	1104.62 (690.36; 1500.21)	988.44 (751.36; 1152.98)	1309.63 (427.99; 2257.33)	794.63 (778.84; 1000.99)	5388.27 (3237.94; 6920.75)*##§§	$p = 0.007$
Alanine	4243.48 (3204.17; 4946.69)	1652.57 (1533.21; 1765.42)**	5664.21 (4664.01; 6317.02)###	3200.34 (2700.89; 3614.47)	2745.46 (2435.07; 2989.11)^	$p < 0.001$
Beta alanine	24.22 (17.13; 32.27)	147.58 (131.43; 163.44)	362.91 (317.44; 411.26)***###	262.98 (237.02; 296.98)**	245.07 (217.06; 276.30)*	$p < 0.001$
Sarcosine	3.36 (2.38; 5.78)	3.64 (2.79; 3.93)	14.87 (11.09; 32.28)	1.52 (0.89; 2.51)^^^	1.51 (1.02; 2.04)^^^	$p < 0.001$

All data are expressed as the median, Q1, Q3; Kruskal-Wallis test followed by post-hoc Dunn's test. *$p < 0.05$ vs. heart; **$p < 0.01$ vs. heart; ***$p < 0.001$ vs. heart; #$p < 0.05$ vs. lung; ##$p < 0.01$ vs. lung; ###$p < 0.001$ vs. lung; ^$p < 0.05$ vs. liver; ^^$p < 0.01$ vs. liver; ^^^$p < 0.001$ vs. liver; §§$p < 0.01$ vs. renal cortex.

Rats

The alanine levels in rats' urine and plasma were not significantly different. All other measured amino acids were significantly higher in the urine than in the plasma (Table 2). The comparison of tissue homogenates (Table 3) showed that the taurine concentration was higher in the heart (4-fold, $p < 0.001$ and 6-fold, $p < 0.001$) in contrast to the lungs and liver of rats, respectively. Similarly, higher levels of taurine were in the renal cortex (2-fold, $p < 0.01$) and medulla (2-fold, $p < 0.05$) than in the liver. Glycine was abundant in the kidneys of rats. The glycine levels were significantly higher in the renal cortex (12-fold, $p < 0.001$ and 3-fold, $p < 0.01$) and medulla (12-fold, $p < 0.001$ and 3-fold, $p < 0.05$) compared to the heart and liver, respectively. We observed lower cardiac concentration of betaine (8-fold, $p < 0.001$; 6-fold, $p < 0.05$ and 6-fold, $p < 0.01$) and alanine (5-fold, $p < 0.001$; 4-fold, $p < 0.01$ and 5-fold, $p < 0.01$) than the concentration in the liver, renal cortex, and medulla, respectively. Beta-alanine and sarcosine were abundant in the liver of rats. Significantly higher levels of beta-alanine were in the liver (7-fold, $p < 0.001$; 7-fold, $p < 0.001$ and 7-fold, $p < 0.01$) than in the heart, lung, and renal cortex, respectively. Sarcosine levels in the liver were higher (18-fold, $p < 0.001$; 16-fold, $p < 0.001$ and 12-fold, $p < 0.01$) compared to the heart, renal cortex, and medulla, respectively.

Guinea Pigs

The levels of alanine were significantly higher in guinea pigs' plasma than in urine ($p < 0.01$). All other measured amino acids did not differ substantially in the body fluids (Table 2). The comparison of tissue homogenates (Table 3) showed that the taurine concentration was higher in the heart (74-fold, $p < 0.001$ and 7-fold, $p < 0.05$) compared to the liver and renal cortex, respectively. In contrast, the cardiac concentration of glycine (7-fold, $p < 0.05$; 9-fold, $p < 0.001$ and 7-fold, $p < 0.01$) and beta-alanine (15-fold, $p < 0.001$; 11-fold, $p < 0.01$ and 10-fold, $p < 0.05$) was lower than the concentration in the liver, renal cortex, and medulla, respectively. Higher levels of glycine (2-fold, $p < 0.01$) and beta-alanine (3-fold, $p < 0.001$) were found in the renal cortex and liver than in the lungs. Alanine concentration was significantly lower in the lungs (3-fold, $p < 0.01$ and 3-fold, $p < 0.001$) compared to the heart or liver. Betaine accumulated in guinea pigs' renal medulla. The levels of betaine were significantly higher in the renal medulla (5-fold, $p < 0.05$; 6-fold, $p < 0.01$ and 7-fold, $p < 0.01$) compared to the heart, lung, and renal cortex, respectively. Sarcosine was abundant in the liver of guinea pigs. The levels of sarcosine were significantly higher in the liver (10-fold, $p < 0.001$) in comparison to the levels in the kidneys.

Interspecies Comparison

The concentrations of taurine in the plasma (17-fold, $p < 0.001$ and 9-fold, $p > 0.05$), urine (200-fold, $p < 0.001$ and 27-fold, $p > 0.05$), heart (3-fold, $p < 0.01$ and 2-fold, $p < 0.01$), lungs (5-fold, $p < 0.001$ and 4-fold, $p < 0.05$), liver (100-fold, $p < 0.001$ and 29-fold, $p > 0.05$) and kidneys (7-fold, $p < 0.001$ and 6-fold, $p > 0.05$) of guinea pigs were lower than the concentrations in mice or rats, respectively. Glycine levels were higher in the plasma (4-fold, $p < 0.001$), heart (2-fold, $p < 0.01$), and liver (3-fold, $p < 0.001$) of guinea pigs compared to rats. Rats showed higher glycine levels in the urine (4-fold, $p < 0.05$ and 8-fold, $p < 0.01$) compared to mice or guinea pigs, respectively. Mouse glycine levels in the lungs were lower (2-fold, $p < 0.001$ and 1.4-fold, $p < 0.05$) in contrast to rats and guinea pigs. Betaine levels were lower in the urine (7-fold, $p < 0.05$ and 10-fold, $p < 0.001$), liver (2-fold, $p < 0.01$ and 2-fold, $p > 0.05$), and renal cortex (2-fold, $p < 0.01$ and 3-fold, $p < 0.05$) but higher in the heart (12-fold, $p < 0.001$ and 4-fold, $p > 0.05$), lungs (4-fold, $p < 0.001$ and 3-fold, $p < 0.01$) and renal medulla (2-fold, $p < 0.05$ and 3-fold, $p < 0.01$) of guinea pigs in comparison to mice or rats, respectively. The alanine concentration in guinea pigs' urine was lower than in mice or rats (1.2-fold, $p < 0.05$ and 3-fold, $p < 0.01$ respectively). Higher levels of alanine were detected in the lungs (2-fold, $p > 0.05$ and 3-fold, $p < 0.001$), liver (3-fold, $p < 0.01$ and 3-fold, $p < 0.001$), and kidneys (2-fold, $p > 0.05$ and 4-fold, $p < 0.001$) of rats in comparison to mice or guinea pigs. Beta-alanine levels were higher in the plasma (9-fold, $p > 0.05$ and 17-fold, $p < 0.001$), lungs (4-fold, $p < 0.001$ and 3-fold, $p < 0.05$), and kidneys (2-fold, $p > 0.05$ and 8-fold, $p < 0.001$) of guinea pigs in comparison to mice or rats. Mice showed a lower concentration of beta-alanine in the heart compared to rats (3-fold, $p < 0.001$) and a higher concentration of beta-alanine in the urine (4-fold, $p < 0.05$ and 5-fold, $p < 0.001$) compared to rats and guinea pigs and in the liver compared to guinea pigs (1.3-fold, $p < 0.01$). Sarcosine levels were lower in guinea pigs' urine (42-fold, $p > 0.05$ and 62-fold, $p < 0.001$) compared to mice or rats, respectively. Rats showed higher concentrations of sarcosine in the plasma (7-fold, $p < 0.05$ and 6-fold, $p > 0.05$), heart (2-fold, $p < 0.05$ and 2-fold, $p < 0.05$), lungs (8-fold, $p < 0.01$ and 7-fold, $p < 0.01$), liver (11-fold, $p < 0.001$ and 8-fold, $p < 0.05$), and kidneys (5-fold, $p < 0.01$ and 6-fold, $p < 0.01$) in comparison to mice or guinea pigs, respectively (Figs. 1, 2).

Discussion

Several amino acids, including taurine, glycine, betaine, alanine, beta-alanine, and sarcosine, serve as biological mediators, osmolytes and piezolytes protecting against changes in osmotic and hydrostatic pressure, respectively [1,11,16,17]. The role of those amino acids in various pathologies is increasingly studied in animal models of human diseases [11,18,19]. Mice, rats, and guinea pigs are

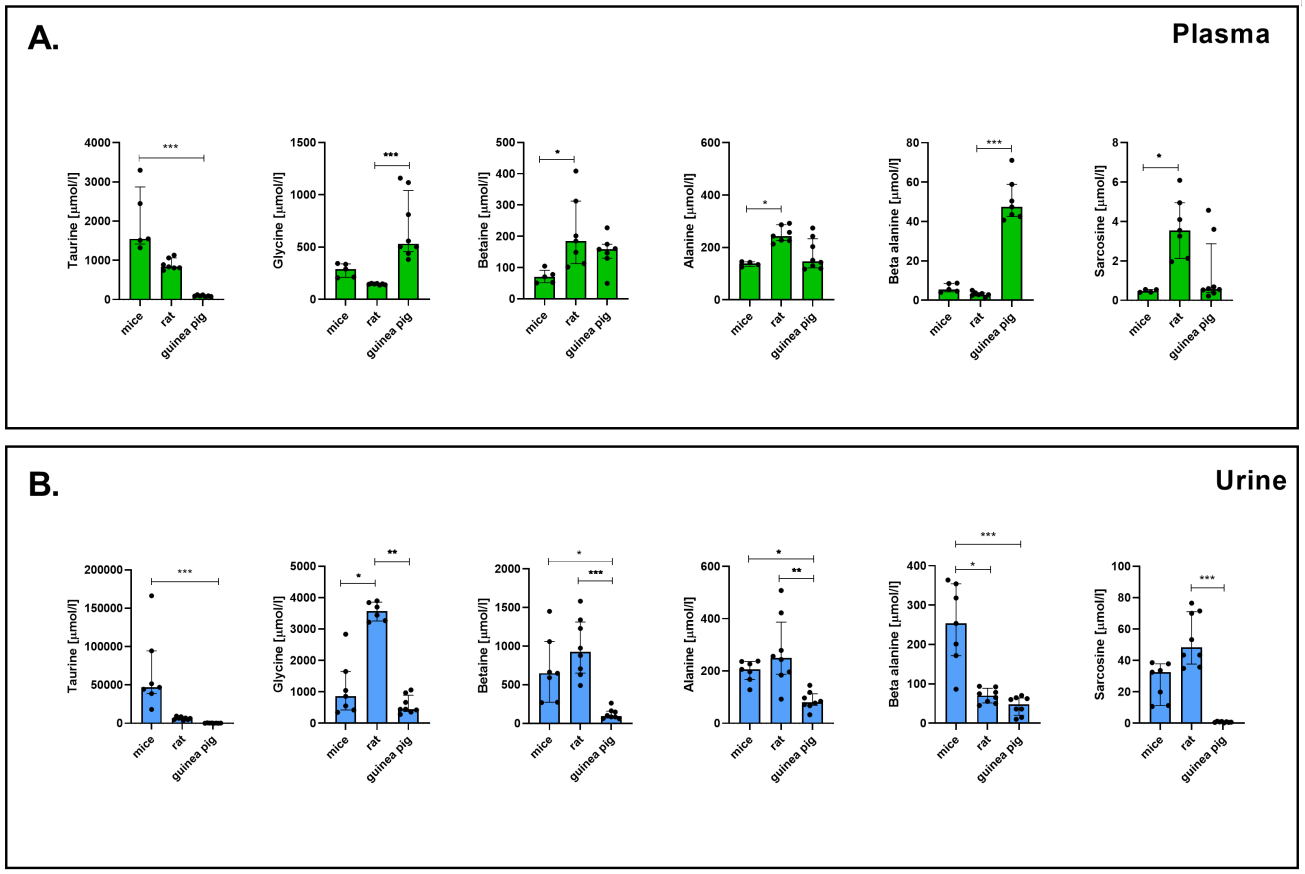

Fig. 1. Species-specific comparison of amino acid levels in body fluids. The levels of taurine, glycine betaine, alanine, beta-alanine, and sarcosine in the plasma (A) and urine (B) of mice, rats, and guinea pigs (n = 4–8). All data are expressed as the median, Q1, Q3; Kruskal-Wallis test followed by post-hoc Dunn's test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

the most commonly used animal models in biomedical research. However, tissue concentrations of these amino acids have not been elucidated. This study found significant inter-tissues and interspecies differences in the concentration of the amino acids. These findings imply that there are differences in the physiological importance of these amino acids in mice, rats, and guinea pigs. Those interspecies differences should be considered during dose selection and data interpretation of interventional studies.

Taurine

The present study found millimolar taurine concentrations in the tissues, whereas other amino acid levels were in the micromolar range. Furthermore, our study showed that cardiac levels of taurine were 41, 36, and 16 µmol/g in mice, rats, and guinea pigs, respectively, and were significantly higher than in other tissues (below 21, 13, and 3 µmol/g in lungs, liver, and kidney). These findings align with other studies that reported the cardiac accumulation of taurine [8]. Several studies showed the involvement of taurine in regulating osmotic pressure, oxidative stress, and the stabilization of cell membranes [17]. Other data shows that taurine contributes to the modulation of ion transport and electrophysiological activity of the heart [20]. Interestingly, low plasma levels of taurine were associated with the development of dilated cardiomyopathy in cats [21]. On the other hand, taurine transporter knockout mice showed disturbances in renal osmoregulation and the development of liver diseases, but the cardiac function was preserved [22]. Based on our experiments showing the accumulation of taurine in the hearts of the studied animals and the highest cardiac concentrations of taurine in mice, and the lowest in guinea pigs, a relationship may exist between the levels of taurine and the size of rodent and/or the heart rate. Further studies are needed to elucidate whether taurine plays a protective role in the heart, particularly in smaller animals with higher heart rates associated with higher metabolic rates and more frequent changes in hydrostatic pressure in the heart.

The analysis of body fluids showed that the levels of taurine in the plasma and urine were significantly higher in mice in comparison to guinea pigs. Interestingly, the reported concentration of taurine in human plasma [23] are similar to the plasmatic levels that we detected in guinea pigs.

Glycine

Glycine was the second most abundant amino acid among the evaluated compounds. The highest levels were

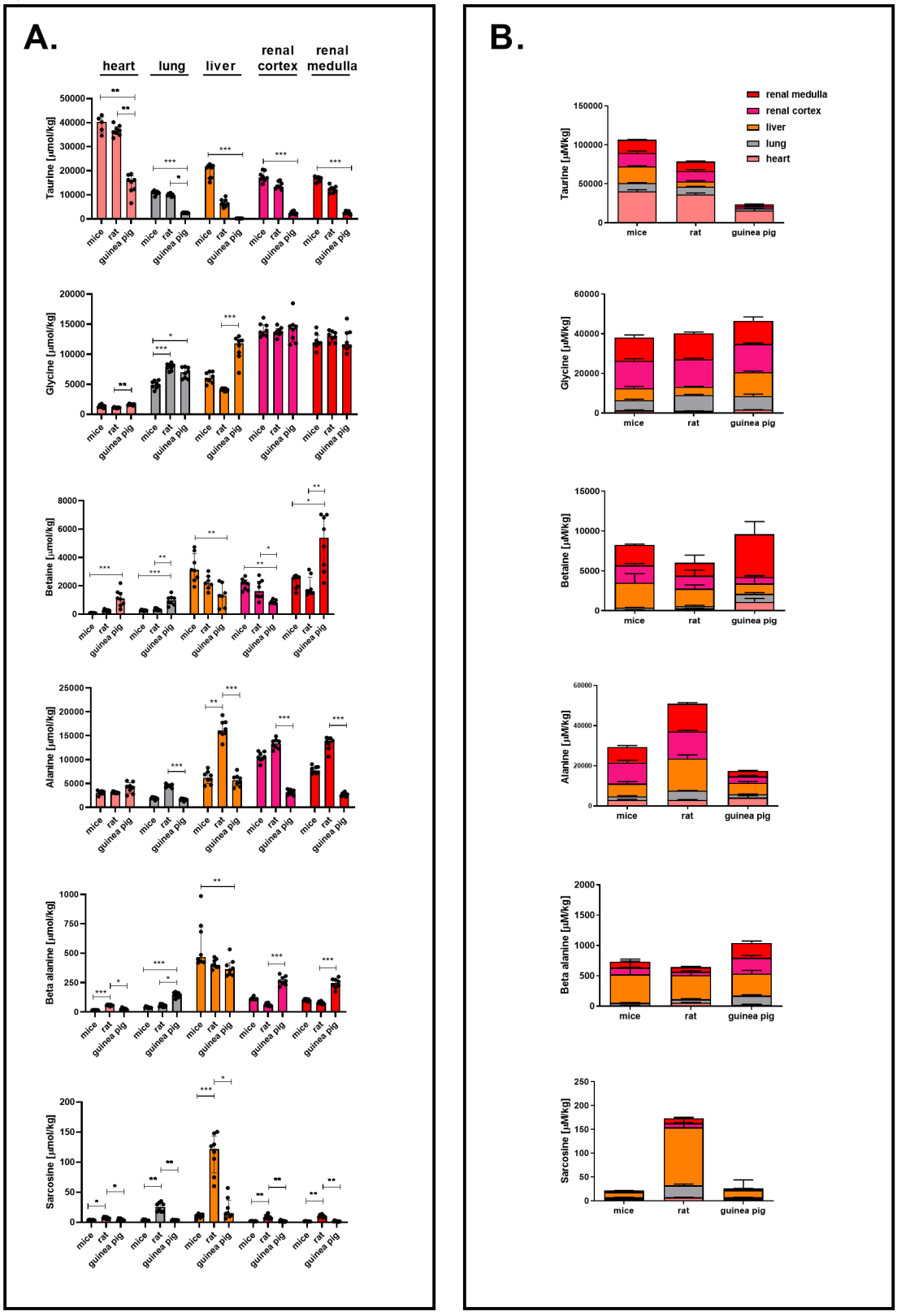

Fig. 2. Species-specific comparison of amino acid levels in tissue homogenates. The taurine, glycine betaine, alanine, beta-alanine, and sarcosine levels in the heart, lung, liver, renal cortex, and renal medulla of mice, rats, and guinea pigs (n = 5–8) (A). The distribution of taurine, glycine, betaine, alanine, beta-alanine, and sarcosine in the tissues of mice, rats, and guinea pigs (B). All data are expressed as the median, Q1, Q3; Kruskal-Wallis test followed by post-hoc Dunn's test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

detected in the kidneys of rodents. Several studies suggest that glycine stabilizes proteins and protects them against osmotic stress-induced inactivation [16]. Data shows that a decrease in urine osmolarity is associated with a significant reduction of glycine in the renal medulla [24]. Our study found no significant interspecies variations in mice, rats, or guinea pigs' glycine levels in the renal cortex or renal medulla. However, we found higher glycine levels in guinea pigs' plasma, liver, and heart compared to those same tissues in mice or rats. Interestingly, the plasmatic levels of glycine in mice and rats were in the reported range in humans, but higher in guinea pigs [25]. Importantly, glycine serves as a substrate in the production of other organic osmolytes [26] such as glycine betaine which is considered a major osmolyte in the mammalian kidney [27].

Betaine

Accumulating evidence confirms that betaine protects various cells and proteins against osmotic stress [11]. In addition, hyperosmolarity leads to an increased expression of betaine transporter in rat liver [28]. Previous reports showed that the osmotic activity of betaine is mainly used by the renal medulla [15]. In this study, guinea pigs showed higher levels of betaine in the kidney medulla than in the kidney cortex, which confirms the concentration of betaine in tissues exposed to high osmotic stress produced by accumulating salt and urea. However, we did not find this difference in the kidneys of mice or rats. This may suggest interspecies differences in the role of betaine as an osmolyte in the kidney medulla. Furthermore, we found that betaine levels were significantly lower in the urine but higher in the lungs and hearts of guinea pigs than in mice and rats, further confirming the preservation of betaine by guinea pigs. In comparison to the plasmatic levels of betaine in humans [29], mice in our study showed similar levels, but levels in rats and guinea pigs were higher.

Alanine

Alanine also plays a role in regulating osmolality in the kidneys [30]. We found lower levels of alanine in the urine and kidney of guinea pigs in comparison to mice and rats. In addition, higher levels of alanine were found in rats' lungs, liver, and kidneys in contrast to mice and guinea pigs. On the other hand, higher levels of beta-alanine were detected in the urine and kidney of guinea pigs in comparison to mice or rats. Guinea pigs showed lower levels of alanine and higher levels of beta alanine than the reported plasmatic values in humans [25].

Sarcosine

From the studied organic molecules, sarcosine was the least abundant solute in small rodents' plasma, urine, and tissues. The highest levels of sarcosine were found in the liver of these animals. Interestingly, the concentrations of sarcosine in body fluids and tissues were significantly higher in rats in comparison to mice or guinea pigs. The plasmatic levels of sarcosine in all investigated species were in the range reported for human plasma [31].

The current study has certain limitations that should be acknowledged. Further investigations are warranted to explore the potential origins of differences between the species, including factors such as dietary variation or divergent metabolic phenotypes. Subsequent research is necessary to assess the tissue concentrations of other osmolytes, such as sorbitol and inositol, in laboratory animals. Additionally, it would be valuable to investigate organs that accumulate osmolytes, such as the brain, as well as organs exposed to high osmotic pressure, like the spleen and thymus. Finally, comparing the levels of osmolytes among diverse strains and pathological models of laboratory animals would provide valuable insights.

Conclusions

To our knowledge, this is the first study evaluating interspecies and inter-tissue differences in the concentration of amino acids, including taurine, glycine, betaine, alanine, beta-alanine, and sarcosine in laboratory mice, rats, and guinea pigs. We found significant variations between species in tissue concentrations of the investigated amino acids, with guinea pigs showing the most distinct profile of the amino acid tissue levels. Notably, research suggests that the guinea pig may be the preferred model for preclinical biomedical research due to its lipoprotein profile, cholesterol metabolism, and heart electrophysiology being more like that in humans [32]. Based on our findings, it appears that guinea pigs share a closer metabolic resemblance to humans in terms of taurine metabolism. However, the concentrations of glycine, betaine, alanine, and beta alanine were significantly different in guinea pigs, indicating that mice and rats may be better suited as models for studying the biological effects of these amino acids in the organism and for translating data from animal studies to human research.

Abbreviations

LC-MS, liquid chromatography and mass spectrometry; MRM, multiple-reaction monitoring.

Availability of Data and Materials

Upon request, the corresponding author may provide the datasets generated during the current study.

Author Contributions

MU concepted, designed, supervised the study and revised the manuscript. KM and ES collected, analysed and interpreted the data and drafted the manuscript. DC interpreted the data, drafted and revised the manuscript. LT prepared the figures, interpreted the data and wrote the manuscript. All authors read and approved the final

manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

The study was performed according to Directive 1020/63 EU on the protection of animals used for scientific purposes and the local ethical committee (permission no. WAW2/046/2022).

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Conflict of Interest

The authors declare no conflict of interest.

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