Acta Biochimica Polonica

Vol. 47 No. 3/2000

877–879

QUARTERLY

Communication

Determination of adenine nucleotides and their metabolites in human saliva★

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Received: 03 November, 1999; revised: 17 February, 2000; accepted: 09 June, 2000

Key words: saliva, adenine nucleotides, HPLC, uric acid, inosine, hypoxanthine, xanthine

The profile and normal concentrations of nucleotide metabolites in human saliva and reproducibility of these determinations were analyzed. Samples of human saliva collected from healthy individuals at weekly intervals, were deproteinized and analysed for the content of adenine nucleotides and their metabolites by reversed-phase HPLC. Initial ATP, hypoxanthine and uric acid concentrations were 0.52 ± 0.15 μM, 1.91 ± 0.37 μM and 184 ± 22 μM respectively. A substantial individual variation persisted within 3 weeks of sampling excepted hypoxanthine which showed some unrelated variations. Determination of nucleotides and their catabolites in saliva due to its simplicity and reproducibility, may be of clinical value in diagnosis of local or systemic disorders.

Human saliva has been for a long time studied and used for diagnosis of local and systemic diseases [1–9]. Measurements of metabolites in saliva, could be a superior and non invasive alternative to blood or urine testing for the assessment of some physiological, biochemical and pharmacological parameters [1, 4, 7, 8, 10, 11]. Simplicity of saliva collection makes easy obtaining of multiple specimens, which could help clinical monitoring [1, 4–7, 10]. Highly specific and sensitive analytical methods are currently available allowing measurement of microconcentrations of various salivary components [6–8, 10, 12, 13]. The content of adenine nucleotides and their metabolites in human saliva could be a useful marker not only of teeth and oral diseases but also of general disease processes [1, 3, 5, 8,

★The paper was presended at the 7th Symposium of the European Society for the Study of Purine & Pyrimidine Metabolism in Man (Gdańsk, Poland, 15–19.09.1999)

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11–15]. However, it is important to establish variability of, and reference values for these compounds in human saliva under normal conditions. The aim of our studies was to determine the profile and normal concentration of adenine nucleotide metabolites in human saliva in healthy subjects and reproducibility of these determinations in the samples collected at successive time intervals.

MATERIALS AND METHODS

Healthy volunteers (n = 23, 13 females, 10 males, aged 21–44 years) without oral and teeth pathology were included in the study. Samples of whole saliva (1 ml) were collected from each subject under standardised conditions (without stimulation) between 9.00–9.30 a.m., 2 h after breakfast. The samples taken from each subject at weekly intervals were indexed as I, II and III, respectively. Immediately after collection saliva was extracted with perchloric acid and stored at –20°C until further analysis. The samples were analysed using reversed-phase high performance liquid chromatography (HPLC) according to Smolenski *et al.* [16, 17]. Results are presented as means +/– standard error of the mean (S.E.M.). Statistical analysis was performed using Friedman's test based on χ^2 statistics. $P < 0.05$ was considered a significant difference.

RESULTS AND DISCUSSION

As may be seen in Table 1, several nucleotides and their metabolites could be detected in saliva of healthy subjects with no oral pathology. According to our results obtained from 23 individuals, concentrations obtained for each subject were consistent in the samples collected within 15 days for all metabolites with the exception of hypoxanthine. However, quite substantial individual variation was found. Distribution of individual values was asymmetric for all metabolites measured except for uric acid, which showed normal distribution. In comparison with normal blood levels of these metabolites, uric acid concentration was found to be lower while concentrations of all other metabolites were higher in saliva than in whole blood or blood plasma measured by the same method [17]. Moore *et al.* [11] found that the total antioxidant activity of whole saliva, and salivary uric acid content was lower than its plasma level. Moreover, they have observed that stimulation of salivary flow is associated with increased ex-

Table 1. Concentration of adenine nucleotides and their metabolites of human saliva in healthy subjects.

The results expressed as μmol/l

	Time of sampling (weeks)		
	I	II	III
ATP	0.52 ± 0.15	0.47 ± 0.15	0.46 ± 0.15
ADP	1.23 ± 0.13	1.39 ± 0.16	1.26 ± 0.19
AMP	0.99 ± 0.16	1.24 ± 0.33	1.10 ± 0.29
Inosine	0.68 ± 0.20	0.96 ± 0.23	0.73 ± 0.16
Hypoxanthine*	1.91 ± 0.37	2.15 ± 0.34	1.11 ± 0.20
Xanthine	0.88 ± 0.31	1.81 ± 0.58	1.58 ± 0.39
Uric Acid	184 ± 22	178 ± 19	162 ± 17

Values are mean ± S.E.M., n = 23, *$P \leq 0.05$ according to Friedman's test indicating significant variation over time.

cretion of uric acid. Thus it seems absolutely essential to sample saliva under standardised conditions in order to obtain reproducible determinations of nucleotide metabolites. According to Kondakova *et al.* [3], uric acid content in saliva correlates with plasma uric acid, suggesting that the former is imported from plasma. This may be not true regarding other nucleotide metabolites because composition of whole saliva is also determined by exogenous factors like bacteria present in oral cavities [12, 13, 15]. Due to substantial individual variation and uncertain origin of metabolites measured in the present study, these results represent only a preliminary assessment of nucleotide metabolite concentrations in saliva of human population. Further studies on a much larger population are thus necessary to establish normal values of nucleotide metabolites in human saliva and their variation in pathology.

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