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Analytical Methods

Contribution to explanation of the effect of supplemented creatine in human metabolism

T. Navrátil [a,*], E. Kohlíková [b], M. Petr [b], M. Heyrovský [a], D. Pelclová [c], K. Přistoupilová [d], T.I. Přistoupil [d], Z. Šenholdová [c]

[a] J. Heyrovský Institute of Physical Chemistry of ASCR, v.v.i., Dolejškova 3, 182 23 Prague 8, Czech Republic
[b] Department of Physiology and Biochemistry, Faculty of Physical Education and Sport, Charles University, José Martího, 162 52 Prague 6, Czech Republic
[c] Department of Occupational Medicine, Toxicological Laboratory, First Faculty of Medicine, Charles University in Prague, Na Bojišti 1, 128 00 Prague 2, Czech Republic
[d] Na Hřebenkách 19, 150 00 Prague 5, Czech Republic

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ABSTRACT

Simple voltammetric determination of thiodiglycolic acid (TDGA) offers the possibility to follow individual deviations in metabolism of thiocompounds and one-carbon (1c) and two-carbon (2c) units, which take part in endogenous synthesis of creatine (CR). In three groups of young men the levels of TDGA in urine were followed after application of CR given as food supplement in 5 g daily doses. In the first group (7 men) it was found that the level of TDGA increased independently of the day time of application of CR. In the second group (9 men) the level of TDGA increased within an interval of 3–8.5 h after CR application and then dropped during 2 h to the normal level (20 mg L^{-1}). In the third group (11 men), in 4 days' study the effects of CR were compared in alternation to vitamin B_{12}. Vitamin B_{12} was given in the evening of the 1st and 3rd day and CR in the morning of the 3rd and 4th day. CR increased the excretion of TDGA in all men, while B_{12} only in four men independently of CR application.

1. Introduction

At present time the voltammetric method (among other methods, e.g., Chylkova & Fadrna, 2004; Samcova, Kvasnicova, Urban, Jelinek, & Coufal, 1999) is being used in toxicology (Dlaskova, Navratil, Heyrovsky, Pelclova, & Novotny, 2003) to measure levels of thiodiglycolic acid (TDGA) in urine. It helps to monitor the exposure of workers in chemical plants to some carcinogenic compounds, e.g., to vinylchloride monomer (VCM) or to ethylene dichloride (EDC) in factories producing polyvinyl chloride (Dlaskova et al., 2003; Senholdova-Dlaskova, 2002). Similarly as in the case of VCM exposure, the TDGA level in urine increases after intake of some remedies (Navratil et al., 2004), victuals (Navratil et al., 2004), thiolic compounds (Steventon, 1999) or of compounds, which affect oxidative metabolic pathways accompanied by release of two-carbon (2C) units (e.g., ethanol, VCM) (Navratil et al., 2004). In these oxidative pathways coenzyme P-450 may participate (Ambrosi, Soleo, Elia, & Attimotelli, 1989) as well.

The oxidative degradation of xenobiotics via TDGA decreases the cell pool of glutathione (GSH). In some critical cases all disposable GSH can get exhausted (Murray, Granner, Mayes, & Rodwell, 2003). This endangers all metabolic pathways dependent on the presence of GSH. Increased level of TDGA in urine indicates a sign of disbalanced cooperation among thiocompounds, 2C units and supply of oxygen radicals (Ermakova et al., 2002a, 2002b). The TDGA concentrations determined in samples of urine in healthy individuals (Dlaskova et al., 2003) do not normally exceed 20 mg L^{-1}.

According to our earlier papers, the appearance of TDGA in urine resulted from the disturbance of metabolic processes coordinated by betaine and vitamins B_{12} and folic acid (Navratil et al., 2007; Pristoupilova et al., 2005). They include transformation of homocysteine – the source of cysteine, and glycine (the source of 2C units), which both take part in endogenous formation of CR.

Creatine (CR) (methyl guanidine acetic acid) represents one of the most important nitrogen containing compounds playing role in energetic metabolism (Webber, 2006). CR is not an essential component of food, because it is formed naturally in human body. CR can be supplied by meaty food or by special food supplementation too. Two thirds of CR are present in the human body as creatine phosphate (PCR), the rest as free CR (Webber, 2006). In normal, healthy man, the turnover of CR is about 1–2 g daily, which is covered by its endogenous synthesis from amino acids (arginine,

* Corresponding author.
E-mail address: navratil@jh-inst.cas.cz (T. Navrátil).

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glycine, methionine) in liver and kidneys (Murray et al., 2003), and by food (from animal sources as meat). Under identical conditions the same amount (about 2 g daily) of CR is degraded by non-enzymatic dehydration to creatinine and excreted into urine (Murray et al., 2003).

It is recommended to apply CR as food supplement in amount corresponding to its natural level in meaty food. It is supposed that the human organism uses it for formation of CR phosphate, which is necessary as energetic source for muscular work.

More than 5 million kg of CR is sold yearly (Hespel, Op't Eijnde, Derave & Richter, 2001), mostly for purposes of food supplement for sportsmen. Exogenously applied CR is also used in treatment of neurodegenerative diseases – dystrophy, myalgia, rheumatoid arthritis, etc. (Felber et al., 2000; Petr, 2007; Tarnopolsky & Martin, 1999).

TDGA molecule is built up of similar subunits as CR. The changes in TDGA levels in urine after loading tests with different substances may reveal hitherto unknown relationships among metabolic pathways.

In this study we wanted to elucidate: (1) how rapidly does the organism respond to the administered CR by the release of TDGA into urine; (2) whether there exists a difference between the release of TDGA after separate administration of either CR or of vitamin B_{12}.

2. Experimental

2.1. Analytical methods

Urine samples were analyzed for TDGA by the voltammetric technique described in our previous papers (e.g., Dlaskova et al., 2003; Senholdova-Dlaskova, 2002). The preparation of the sample was realized in a column of powdered PVC, the urine sample was transferred to the top of the column and eluted by 0.2 M perchloric acid. The resulting eluate was introduced into the electrolytic cell, deaerated by a stream of nitrogen (purity 99.999%), and then subjected to direct current (D.C.) voltammetric analysis. The measurement was started by accumulation for 10 s under stirring at initial potential of −800 mV vs. Ag/AgCl/ 1 mol L^{-1} KCl, followed by rest period of 15 s, and then by potential scan at the rate of −10 mV·s^{-1} to the final potential of −1200 mV. The values of potentials given in this paper are referred to that Ag/AgCl/1 mol L^{-1} KCl reference electrode, which is at 25 °C by 9 mV more negative than the SCE. For quantitative evaluation the method of double standard addition appeared best suited. The determination of TDGA was carried out by the computer-controlled Eco-Tribo Polarograph using the software polar 5.1 version for Windows (Polaro-Sensors, spol. s r. o., Czech Republic), on pen type hanging mercury drop electrode (HMDE) (Polaro-Sensors, spol. s r. o., Czech Republic), on mercury meniscus modified silver solid amalgam electrode (e.g., Barek et al., 2003; Barek, Fischer, Navratil, Peckova, & Yosypchuk, 2006; Yosypchuk & Novotny, 2002, 2002b), or on solid composite electrodes (e.g., Barek et al., 2007; Navratil & Kopanica, 2002a, 2002b; Navratil, Kopanica, & Krista, 2003; Sebkova, Navratil, & Kopanica, 2004; Sebkova, Navratil, & Kopanica, 2005; Yosypchuk, Navratil, Lukina, Peckova, & Barek, 2007). The results, achieved using all three above mentioned working electrodes, were equivalent. More precisely, the calculated confidence intervals of results overlapped with probability higher than 95%. Nevertheless, the hanging mercury drop electrode was most "user friendly", its surface was the easiest and the fastest renewable, the repeatability of results achieved by it was the best. Smaller amount of mercury on the electrode surface (mercury meniscus, mercury film, polished amalgam surface or amalgam composite surface) causes smaller sensitivity to TDGA concentration. Responses of such electrodes are lower and worse reproducible. Therefore in most experiments presented in this manuscript, the HMDE electrode was used.

Under identical conditions of elution as in case of TDGA model samples, cysteine and carboxymethyl of the same and of 10 times higher concentrations, glutathione (incidentally, the presence of glutathione in urine cannot be expected under normal conditions) and phytochelatins PC2 and PC3 in micromolar range concentrations were determined. The parameters of voltammetric determinations were equal as well. None of all tested substances did yield any voltammetric signal in potential range in which TDGA did. Furthermore, TDGA was determined in presence of these substances and they did not affect TDGA determination (Senholdova-Dlaskova, 2002). From the experiments with thiols it is evident that these compounds are chemisorbed at about zero potential on the mercury electrode surface and desorbed at about −500 mV (in presence of Cu^{2+} ions even at more positive potentials). Because the accumulation potential of TDGA was −800 mV, and the scan was in negative direction, the thiols cannot affect TDGA determination, as it was proven experimentally. CR and creatinine were determined using Specord 200 (Bardodej, David, Sedivec, Skramovsky, & Teisinger, 1989). Compound levels in blood were determined in a commercial laboratory by usual methods. Urine sample was alkalified by NaOH and mixed with saturated solution of picric acid. The reaction lasted 10 min. The solution was analyzed spectrophotometrically at the wave length 530 nm against blank. pH was measured by digital laboratory pH-meter Inolab (Benella CZ, Praha).

2.2. Reagents and materials

The volunteers were supplemented with (a) folic acid with vitamin B_{12} and (b) CR.

Supplementation with folic acid and vitamin B_{12} per os (p.o.) was realized using the food supplement "Kyselina listová, Forte" (in Czech) - "Folic acid, Forte", produced by Agrochemie Ltd., Zlín, containing 0.2 mg of folic acid and 1 μg of vitamin B_{12} of natural origin in 1 tablet (1 dose = 1 tablet).

"Creatine-monohydrate – a special nutritional supplement for athletes" (Plutino, CR) was administered p.o. in 5 g doses, diluted in tepid water.

Other chemicals used were of analytical reagent purity grade. Doubly distilled water was applied throughout the work (conductivity < 1 μS cm^{-1}). All experiments were carried out at room temperature (25 ± 2 °C).

2.3. Proband group characterization, sampling and CR supplementation

There were three groups of volunteers involved in our study; the men were students of Faculty of Physical Education and Sport, Charles University in Prague of the age from 20 to 37. Women were not subjected to the present study due to complicated hormonal changes during month, which affect cell water content substantially. All probands were young, healthy, physically active persons, dealing with sportive activities (ice hockey, football, horsemanship, and athletics) on professional level. Sportive activities were very carefully observed, however, they were not consistent. All probands signed the informed consent. None of volunteers in the second and in the third experimental group applied any other food supplements, stimulants, drugs, vitamin preparations, they did not consume victuals containing onion, garlic etc., alcohol, remedies containing carboxymethyl cysteine (CMC) (ACC 100 etc.), and vitamin B_{12}, folic acid, during this study and one day before. They registered all administered remedies and important victuals. They drank about 2 L of fluids per day (Petr, 2007).

CR was administered p.o. in 5 g doses, diluted in tepid water.