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Blood Polyamines in the Rat

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Abstract

The physiological concentrations of polyamines in plasma, serum and red blood cells were determined in male Wistar rats, using HPLC with fluorometric detection.

The analysis of the metabolic ratio between polyamines and the frontal cortex/plasma relationship for putrescine, spermidine and spermine, suggest the existence of common mechanisms in the regulation of spermidine in blood and brain.

Polyamines, putrescine, spermidine and spermine are biologically active compounds, widely distributed in living organisms. The regulation of the cellular polyamine content involves metabolic synthesis, interconversion between polyamines, their uptake from the circulation by tissues and their release. The key regulatory enzymes of polyamine metabolism are inducible and have a high turnover rate. In addition, polyamines have a complex pharmacological and toxicological profile. For reviews see Seiler (1990, 1991; Moulinoux et al 1991; Paschen 1992).

Modifications in polyamine concentrations in blood or tissues have been found in different pathological states, e.g. malignant proliferation (Moulinoux et al 1991) and brain injuries (Paschen 1992). The study of polyamines as biological markers in pathology is an active field of research.

Data concerning the concentrations of polyamines in the blood or in the brain of the rat, using chromatographic methods, are spread in the recent literature (Asai et al 1985; Desiderio et al 1987; Moulinoux et al 1987; Driessen et al 1989; Minuk et al 1990; Martínez et al 1991; Grossier et al 1992), but to our knowledge the concurrent determination of polyamines in blood and brain samples of the same animals has not been reported before. This paper attempts to provide reference values for polyamines in different blood samples in the rat in physiological conditions. For this purpose we determined simultaneously the polyamine content in plasma, serum and red blood cells (RBC) of adult rats. Data are compared with the corresponding frontal cortex values.

Materials and Methods

Nineteen male Wistar rats (IFFA-CREDO, Belgium), 290–320 g, were used. Rats were killed by decapitation and the brains rapidly removed from the skull. Frontal cortices were dissected, weighed and stored at −20°C until analysis. Samples of about 1 mL of whole trunk blood were collected into heparinized tubes and centrifuged immediately at 10 000 *g* for 2 min. After removing the plasma and the buffy coat layer, the pellet of RBC was washed three times with 2 vol 0·14 M NaCl. Washed cells were resuspended in 0·14 M NaCl and the erythrocyte number was determined using a Coulter counter Model ZM (Coulter Electronics Ltd). RBC count was also carried out in samples of whole blood. For serum collection, blood was drawn into glass tubes without heparin. All samples were stored at −20°C until analysis.

The brain polyamine analysis was performed using a previously described method (Martínez et al 1991) and the blood polyamine determination by an adaptation of this method as follows: 20 μL perchloric acid (70%) was added to 500 μL plasma or serum and the samples were vigorously shaken and centrifuged at 10 000 *g* for 15 min. To the supernatants was added 300 μL Na_2CO_3 (2·5 M) and 500 μL dansyl chloride (5 mg mL^{-1} in acetone) and the mixture was kept for 1 h at 50°C. The derivatized samples were then centrifuged for 3 min at 10 000 *g* and the supernatants carefully separated and extracted with benzene, according to previously described procedures (Desiderio et al 1987; Martínez et al 1991). RBC were homogenized ultrasonically for 30 s at 40 W, 20 kHz, centrifuged at 10 000 *g* for 20 min, and 500 μL of the supernatant processed as for plasma and serum samples. 1,6-Diaminohexane was used as internal standard. Benzene extract (200 μL) was evaporated under helium and redissolved in 100 μL methanol. Before the injection, the samples were filtered through 0·45 μm membrane filters (Versapor, Gelman Sciences). The HPLC equipment consisted of a gradient solvent-delivery pump Waters 600E, a WISP 712 automatic injector and a Waters 470 fluorescence detector (350 and 520 nm for excitation and emission wavelengths, respectively). A Spherisorb ODS1 column (150 × 4·6 mm, particle size 5 μm) was used. The elution was performed with a gradient consisting of solvent A (1·2 mM Na_2HPO_4 and 12 mM NaCl) and solvent B (methanol). Initial conditions (60% B) were maintained for 3·5 min, and then an 18-min linear gradient from 60 to 90% solvent B was run. Final conditions were maintained for 4·5 min. A short (2 min) reverse program was run to return to initial conditions. The flow rate was adjusted to 1·2 mL min^{-1}.

The retention time (min, mean ± s.e. of 27 determinations)

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Table 1. Polyamines in plasma, serum, red blood cells (RBC) and frontal cortex in rats.

	Plasma (nmol mL^{-1})	Serum (nmol mL^{-1})	RBC (nmol in 8×10^9 RBC)	Frontal cortex (nmol g^{-1})	Brain/plasma ratio (mL g^{-1})
Putrescine	1·03 ± 0·06	1·01 ± 0·05	0·84 ± 0·07	7·03 ± 0·81	6·7 ± 0·5
Spermidine	2·82 ± 0·21	5·30 ± 0·38	18·31 ± 1·19	343·10 ± 10·55	133·1 ± 10·7
Spermine	0·46 ± 0·05	0·25 ± 0·02	1·28 ± 0·09	270·60 ± 6·19	690·6 ± 56·6

Mean ± s.e., n = 19.

was 12·75 ± 0·04 for putrescine, 15·16 ± 0.03 for internal standard, 19·74 ± 0·02 for spermidine and 23·68 ± 0·02 for spermine. The regression coefficients for adjusted standards were higher than r = 0·999 for the three polyamines, in the range of concentrations 1 : 40. Intra-assay variability was estimated to be lower than 4·0% (n = 5) for putrescine. Inter-assay variability for putrescine was calculated to be 18·1% (n = 7).

Results and Discussion

The concentrations of polyamines in plasma and serum were of the same order (Table 1), and in agreement with data in the literature (Asai et al 1985; Driessen et al 1989; Minuk et al 1990). In RBC, spermidine was also the most concentrated polyamine (Table 1) but in this compartment putrescine was the minor constituent, also in agreement with other authors (Moulinoux et al 1987; Grossier et al 1992). Because there were $5.87 \pm 0{\cdot}17 \times 10^9$ RBC mL^{-1} in whole blood (n = 19) the concentration of total polyamines in RBC can be estimated as 15 nmol mL^{-1} blood. One millilitre of blood results approximately in 0·5 mL plasma and consequently in 2 nmol polyamines. Thus, both plasma and RBC pools amount to 17 nmol polyamines in 1 mL whole blood. Our results indicate that in 1 mL blood, the putrescine content is equally distributed in plasma (46%) and in RBC (54%), whereas spermidine and spermine are concentrated in RBC (90 and 80%, respectively) and only 10 and 20%, respectively, is found in plasma.

In agreement with previous work (Desiderio et al 1987; Martínez et al 1991) we found that in frontal cortex (Table 1) the concentrations of spermidine and spermine were of the same order of magnitude, whereas putrescine was a minor constituent. The comparison between the content of polyamines in blood and in brain shows that the most remarkable difference is the higher brain concentration of polyamines, specially spermidine and spermine (Table 1).

The mean ratio between spermine concentration in frontal cortex and in plasma, calculated for each animal, is 690·6. This ratio is 133·1 and 6·7 for spermidine and putrescine, respectively. These important physiological differences in the polyamine content between plasma and brain reflect the different regulation of both pools (Seiler 1990; Moulinoux et al 1991; Gilad & Gilad 1992). However, it is remarkable that the metabolic ratio spermidine/putrescine in brain is of the same order of magnitude as in RBC (59·9 ± 6·2 and 23·8 ± 1·7, respectively) and there is a positive correlation between the respective individual values (r = 0·6535, $P < 0{\cdot}01$, n = 19). This relationship suggests a common mechanism in the regulation of spermidine in brain and blood of rats in physiological conditions.

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