Polyamines in human and animal milk

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Polyamines are highly regulated polycations which are essentially involved in cell growth and differentiation. Polyamines in food significantly contribute to the polyamine body pool. Dietary polyamines exert various direct and indirect trophic effects on the rat's immature intestine and play an important role during intestinal maturation. Human milk and that of other mammalians contain relatively high levels of polyamines which are essential luminal growth and maturation factors. The polyamines spermidine and spermine as well as their diamine precursor putrescine are ubiquitous normal constituents of all prokaryotic and eukaryotic cells and are essentially involved in various processes of cell growth and differentiation (Pegg & McCann, 1982; Tabor & Tabor, 1984; Seiler, 1990).

Polyamines: Milk: Gut maturation: Putrescine: Spermidine

Polyamine metabolism

Polyamines are aliphatic, low-molecular polycations with polybasic character. Polyamines are structurally flexible, positively loaded carbon chains with the possibility to form ion pairs with negatively loaded ions over a certain distance. Polyamines are ubiquitous, essential constituents of all mammalian cells. During evolution polyamine metabolism is highly preserved and several regulatory mechanisms contribute to the complex regulation of intracellular polyamine homeostasis. Intracellular polyamine concentrations are primarily regulated by intracellular polyamine *de novo* synthesis, conversion and degradation as well as uptake of extracellular polyamines (Pegg & McCann, 1982; Tabor & Tabor, 1984; Seiler, 1990; Löser & Fölsch, 1993). The intracellular polyamine metabolism is depicted in Fig. 1. Preliminary regulatory mechanisms are (1) intracellular polyamine *de novo* synthesis via ornithine decarboxylase (ODC) as the key regulatory enzyme of polyamine metabolism, (2) reconversion of polyamines via the interconversion pathway (spermine/spermidine N^1-acetyltransferase and polyamine oxidase) and (3) oxidative degradation of polyamines.

Uptake of dietary polyamines

Besides intracellular polyamine *de novo* synthesis, uptake of extracellular polyamines from the gut lumen was found to be a further important regulatory mechanism of polyamine metabolism (Pegg & McCann, 1982; Seiler, 1990; Seiler *et al.* 1996; Seidel & Scemama, 1997; Löser *et al.* 1997). It is well established that the alimentary tract is an important source of polyamines and that dietary as well as to some extent gut bacterial-derived polyamines significantly contribute to the total polyamine body pool. These findings stress the importance of a rapid uptake of dietary, luminal polyamines by the intestinal mucosa upon demand with a consecutive passage through the basolateral membrane to the body. Recently various specific intestinal polyamine transport and uptake systems have been characterized and these are found in virtually all mammalian cells (Grillo & Colombatto, 1994; Milovic *et al.* 1995; Seiler *et al.* 1996).

Importance and function of polyamines

Polyamines are essentially involved in various processes of cell growth and cell differentiation (Pegg & McCann, 1982; Tabor & Tabor, 1984; Seiler, 1990). The important role of intracellular polyamine *de novo* synthesis and uptake of extracellular primarily dietary polyamines for normal and adaptive growth in various organ systems is well established (Luk, 1992; Kobayashi *et al.* 1992; Bardócz *et al.* 1995). Furthermore, polyamines are essentially involved in carcinogenesis and tumour growth (Pegg & McCann, 1982; Tabor & Tabor, 1984; Pegg, 1988). During normal, adaptive and malignant growth polyamines are involved in a variety of different growth-related processes which reflects their multifunctional character during growth. Polyamines have various electrostatic interactions with macromolecules especially DNA, RNA and proteins. Polyamines are involved in the regulation and stimulation of DNA, RNA and protein synthesis, they interact with different components of the cell membrane thus modulating membrane functions, they stimulate cell differentiation and interact and modulate various intracellular messenger

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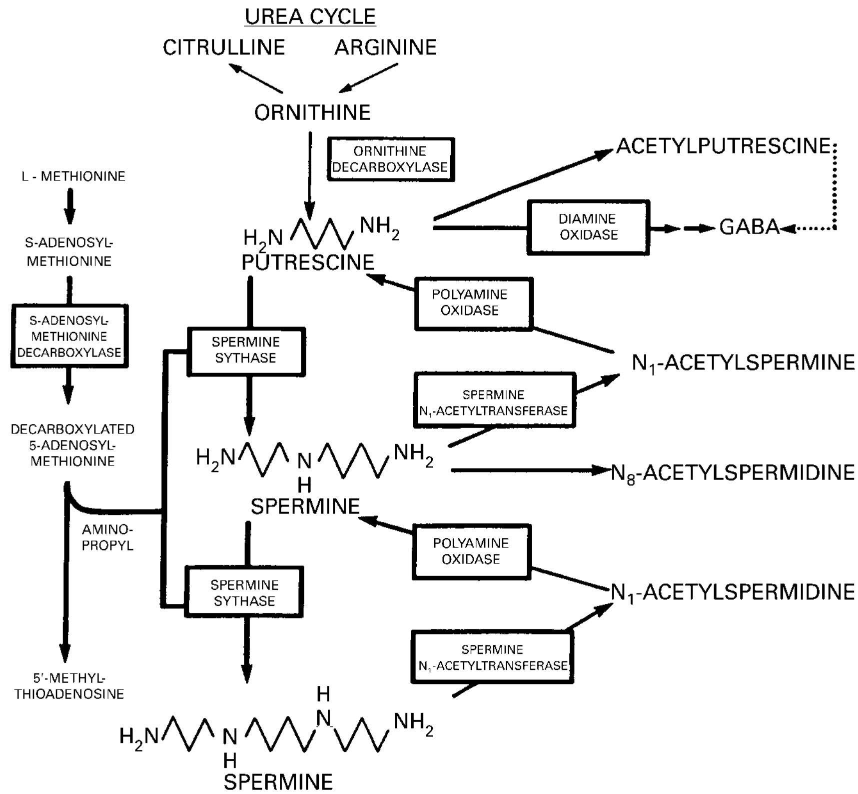

Fig. 1. Polyamine metabolism.

systems (Pegg & McCann, 1982; Tabor & Tabor, 1984; Pegg, 1988; Seiler, 1990; Persson *et al.* 1992).

Polyamines in growth and maturation of gut mucosa

Because of its high proliferation rate, intestinal mucosa has a high demand for polyamines. During the third postnatal week rapid intestinal maturation occurs in the rat and this period is characterized by an increase in epithelial cell proliferation and differentiation resulting in histological and enzymatic maturation of the small bowel epithelium. Prior to this process of epithelial maturation a ten- to twentyfold increase in mucosal ODC activity and a concomitant increase in SAM-DC activity as well as polyamine content were found; simultaneous administration of α-difluoromethylornithine (DFMO), a potent, specific ODC inhibitor, resulted in a marked attenuation of the increase in mucosal ODC and polyamine content which was followed by a significant delay in biochemical and histological maturation of the intestinal epithelium (Luk, 1992). The increase in ODC activity seems to be critical for the activation of intestinal maturation and polyamines in general are important intracellular messengers involved in the maturation of the small and large intestine.

Small intestinal maturation is a nutritive-sensitive process, and weaning, with consecutive transition of factors from the milk is necessary to complete epithelial maturation. Dietary polyamines exert various direct and indirect trophic effects on the rat's immature intestine and play an important role during intestinal maturation (Dufour *et al.* 1988; Buts *et al.* 1993; Wild *et al.* 1993; Kaouass *et al.* 1994; Wéry *et al.* 1996). Oral administration of exogenous spermine or spermidine resulted in a marked acceleration of mucosal maturation and proliferation (Dufour *et al.* 1988; Buts *et al.* 1993; Wild *et al.* 1993; Kaouass *et al.* 1994; Wéry *et al.* 1996). These experiments clearly prove the important role of dietary polyamines as essential luminal nutritional factors for gut growth and development (Löser *et al.* 1999). Although the important role of intracellular *de novo* polyamine synthesis, as well as cellular uptake of dietary polyamines for mucosal growth and maturation is well-established in different experiments, the complex intracellular mechanisms whereby polyamines effect mucosal proliferation and differentiation are not completely understood and need further evaluation (Luk, 1992; Seidel & Scemama, 1997; Löser *et al.* 1999).

Polyamine determination and analysis

Polyamine concentrations in food, organ tissues, blood, urine or fluids like milk can easily be detected by routine analytical methods such as, for example, HPLC or gas chromatography. Recently we established an improved, highly sensitive HPLC method for reversed-phase liquid chromatographic separation and fluorimetric detection of polyamines and their various acetylated derivatives in human and animal tissue, urine, serum and milk samples (Löser *et al.* 1988). This easy HPLC method for polyamine analysis is suitable for rapid routine application.

Table 1. Polyamine concentrations in human milk and cow's milk (nmol/dl)

	Putrescine	Spermidine	Spermine
Human milk			
Pollack *et al.* (1992) - day 7	33·8	224·4	276·2
Romain *et al.* (1992) - day 7	129±21	711±109	663±136
Buts (1996) - day 7	24±3·5	220±20	313±16
Darhout *et al.* (1996) - day 16	77	454	376
Sanguansermsri *et al.* (1974) - day 5		11·6±3	6·8±1·7
Cow's milk			
Motyl *et al.* (1995) - day 30		470±280	~400
Sanguansermsri *et al.* (1974) - day 28		19·8±0·7	8·4±3
Bardocz *et al.* (1993) - full cream milk	100	100–300	100–300
Okamoto *et al.* (1997)	n.d.	n.d.	n.d.

n.d.=not detected.

Nutritional polyamine support

Though the importance of polyamines for human growth and development is well-investigated, little is known with regard to the amount and role of polyamines in daily food. It has been calculated that the average daily polyamine consumption in adults amounts to 350–500 μmol/person/day (Bardócz *et al.* 1995). Data from Bardócz *et al.* (1993, 1995) reveal that the major sources of putrescine were fruit, cheese and non-green vegetables, whereas nearly all foods contribute similar amounts of spermidine to the diet and meat was the richest source of spermine. The well-established important role of dietary polyamines for intestinal mucosal maturation and growth (Dufour *et al.* 1988; Buts *et al.* 1993; Wild *et al.* 1993; Kaouass *et al.* 1994; Wéry *et al.* 1996) and the significant contribution of dietary polyamines to the polyamine body pool with an essential function in organ growth and development clearly stresses the importance of polyamines in milk for infant nutrition.

Polyamines in cow's and mother's milk

Breast-feeding is unequivocally the optimal form of nutrition in human infants and it is well known that mother's milk – besides essential nutrients such as proteins, fat, carbohydrates, minerals and vitamins – also contains a variety of biologically active substances which modulate the newborn's metabolism. Human milk and that of other mammalians contains relatively high levels of polyamines. The available data on polyamine concentrations in human and cow's milk are given in Table 1. Though the results of the various analyses are not uniform and show distinct variations (Table 1) it is evident that human milk contains substantial amounts of mainly spermine and spermidine with a lower quantity of putrescine. Polyamine concentrations in human milk increase during the first weeks postpartum, reach a maximum after one to two weeks and then decline (Romain *et al.* 1992; Buts, 1996; Dorhout *et al.* 1996).

Factors that influence polyamine milk concentrations

Various studies demonstrated considerable individual variabilities in the polyamine concentration in human and cow's milk. Besides interspecies and individual variabilities several significant regulatory influences on milk polyamine pattern during lactation have been observed in different species. There are several established factors that influence polyamine milk concentrations (Buts *et al.* 1995; Motyl *et al.* 1995; Buts, 1996): mother's age, genetic influence, ethnic origin, circadian rhythm of polyamine secretion, nutritional status, amount of dietary polyamine intake, duration of lactation, environmental influences, amount of milk in the breast, possible bacterial contamination etc.

Cow's milk samples and dairy products have lower polyamine contents compared to human milk (Table 1). The reason for this is the high rate of polyamine degradation in cow's milk as a consequence of the high activities of enzymes such as diamine oxidase (DOA) or polyamine oxidase (PAO) in cow's milk.

Polyamine concentrations in formula milk

Based on the results of published investigations and our present knowledge, an adequately high amount of polyamines in milk seems to be important for early infant nutrition. Nevertheless, polyamine concentrations in commercially available infant artificial formulas are very low (Romain *et al.* 1992; Buts *et al.* 1995; Buts, 1996). In artificial powdered formulas, the polyamine concentrations were about ten time lower than in human milk, with no differences in putrescine and spermine concentrations between first age and second age formulas (Buts *et al.* 1995). Measurement of polyamine concentrations in 18 powdered infant formulas revealed variable concentrations of polyamines with interestingly higher putrescine than spermidine and spermine concentrations and a general polyamine concentration which is more than ten times lower compared to natural human milk (Romain *et al.* 1992).

With regard to the evident importance for human organ growth and maturation it is important that further studies improve our knowledge of the necessary amount, general importance and, under certain circumstances, possible artificial enrichment of polyamine milk concentrations for human nutrition.

Conclusion

Human milk and that of other mammals contains substantial amounts of mainly spermine and spermidine with a lower quantity of putrescine. Polyamines in milk exert various direct and indirect trophic effects on the immature intestine and play an important role as luminal growth factors for intestinal maturation and growth.

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