REVIEW ARTICLE

NATURAL POLYAMINES AND THEIR BIOLOGICAL CONSEQUENCE IN MAMMALS

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Summary: The polyamines (putrescine, cadaverine, agmatine, spermidine and spermine), wide-spread in all organisms, have been shown to play a role in regulation of growth and differentiation of virtually all types of cells. Their role in many physiological and pathophysiological processes have been studied very intensively during the last two decades. Inhibitors of polyamine biosynthesis have potential clinical uses as antitumor and antiparasitic agents. The brief summary with regard to their biological consequences in mammals is discussed in this paper.

Key words: *Polyamine; Agmatine; Putrescine; Spermidine; Spermine; Ornithine dacarboxylase*

Introduction

The polyamines, putrescine, cadaverine, agmatine, spermidine, and spermine, are low-molecular weight substances, synthesized in eukaryotic cells from their immediate precursors, ornithine, lysine, or arginine. Their chemical structures are given in Fig. 1.

These nitrogenous compounds are essential for growth. An animal's endogenous supply of these metabolites derives from biosynthesis, the diet, or by synthesis in the intestinal flora. The polyamines are found in fruits and vegetables, many foods of animal origin (milk, eggs, fish, and meat), and fermented food products (cheese, beer, and sauerkraut). Being nitrogenated compounds, they are considered as "minor" components of the diet. Ornithine decarboxylase (ODC), an enzyme of short half-life, is the rate-limiting catalyst for biosynthesis of putrescine, spermidine, and spermine. The relevant biochemical pathway is schematically displayed in Fig. 2. In some cells, this enzyme is phosphorylated by a protein kinase reaction that is dependent on spermidine and spermine. Putrescine antagonizes the phosphorylation (7).

Cellular polyamines are found in free or complexed forms, the latter made possible, above all, by the presence of positive charges at their protonated nitrogen atoms. Their particular structure facilitates interaction with anions and binding to nuclear and membrane structures, particularly phospholipids, proteins, and DNA. The natural polyamines, spermine and spermidine, their biosynthetic precursor putrescine, and their analogue cadaverine derived from lysine, stimulate GTPase activity (1). Mammalian requirements for these substances are elevated during pha-

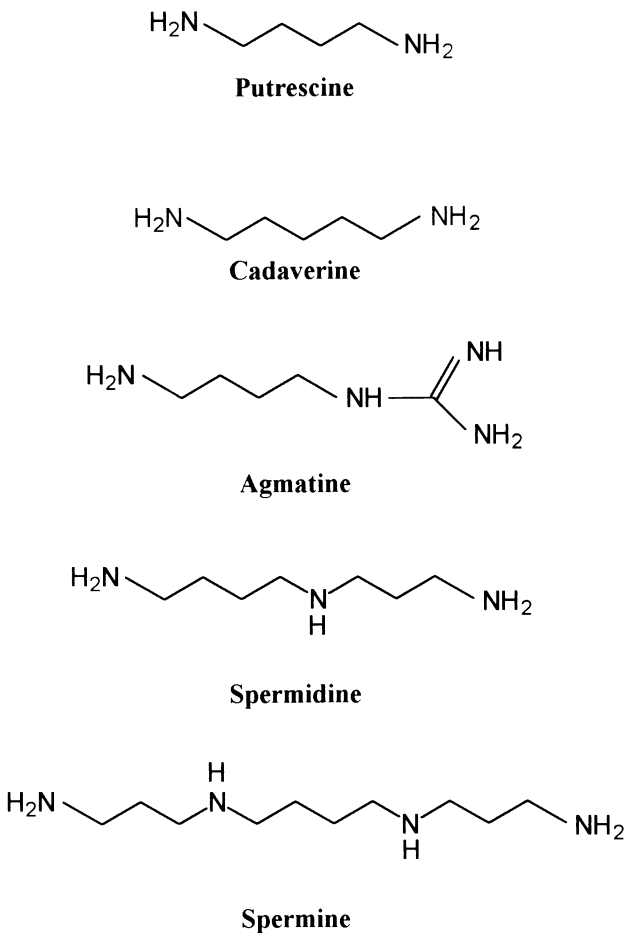


Fig. 1: Chemical structures of common polyamines.

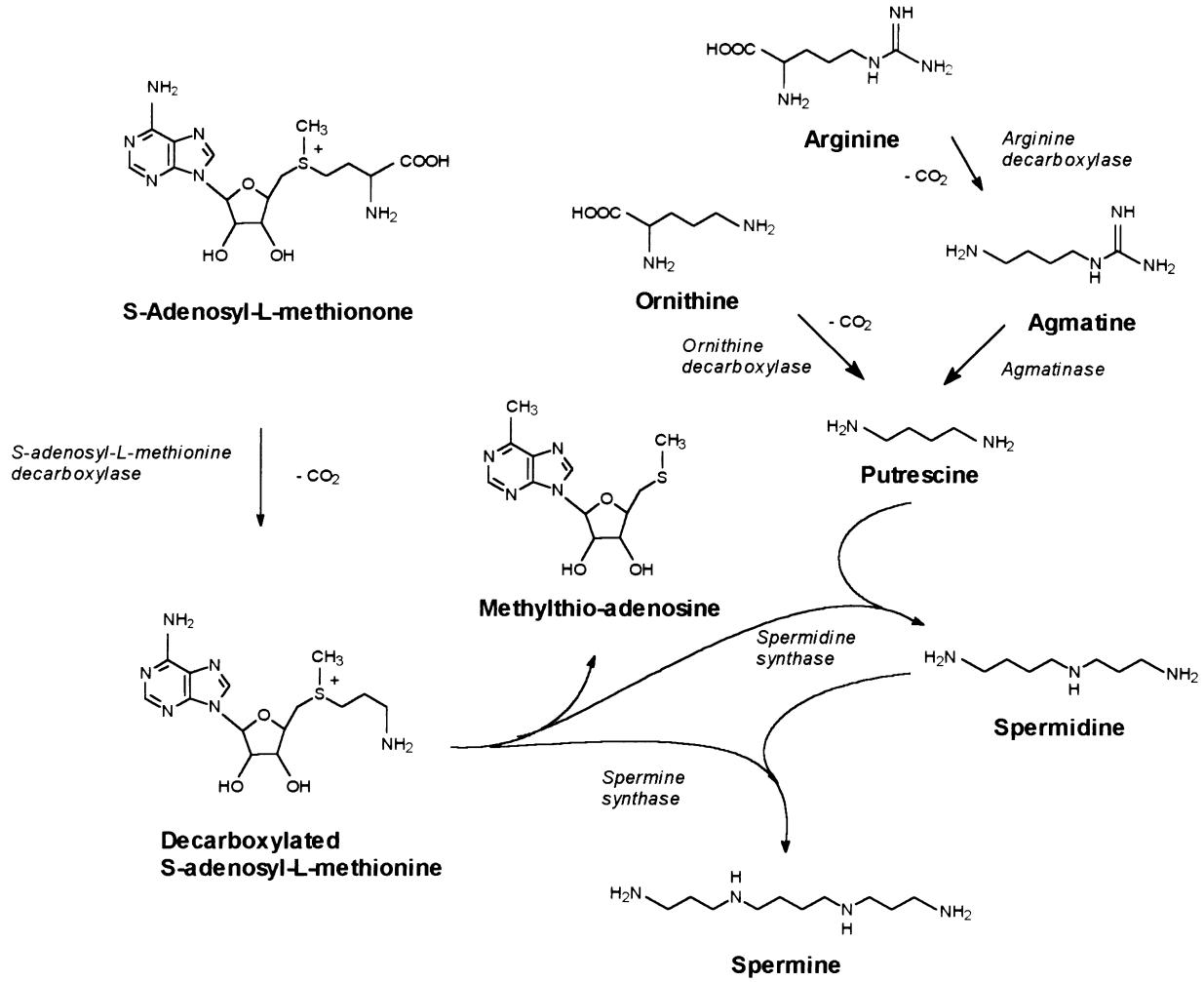


Fig. 2: Polyamine biosynthetic pathway.

ses of intense growth rate or increased biosynthetic demand. Thus, the nutritional supply can be crucial during developmental processes that involve a high degree of loss combined with deficits in endogenous biosynthesis. Increased tissue and organ polyamine concentrations correlate with diseases of neoplastic origin. It has been hypothesized that inhibition of polyamine biosynthesis could be a therapeutic mechanism for such conditions (11).

Polyamines are required for initiation and progression of the cell cycle. Polyamines regulate DNA, RNA, and protein syntheses; they stabilize ribosomes, membranes, and nucleic acids; and, they protect the cell from lipid peroxidation. Therefore, augmentation of polyamine levels is essential for cellular transformation. Polyamines are autoregulated through induction of a protein factor, antizyme, which represses both the rate-limiting polyamine biosynthetic enzyme, ODC, and cellular polyamine transport. Polyamines also serve the dual regulatory functions of suppressing polyamine biosynthesis and cellular polyamine uptake through induction of antizyme.

Extensive evidence indicates that the excessive accumulation of putrescine and spermidine favors malignant transformation of cells. Selective depletion of putrescine has been shown to restore the normal phenotype in some transformed cells. Inhibition of polyamine formation appears, therefore, to be a rational target in chemoprevention. Clinical trials with 2-(difluoromethyl)ornithine, a selective suicide-substrate inhibitor of ODC, the initial enzyme of polyamine biosynthesis, are promising. Structural analogs of the polyamines with polyamine-mimetic or antagonist properties, and calmodulin antagonists are other types of drugs which affect several key reactions of polyamine metabolism, and appear to be candidates for the prevention of carcinogenesis, especially of the gastroinstestinal tract (25).

Putrescine

Putrescine (1,4-diaminobutane) is a product of conversion of agmatine by agmatinase or by decarboxylation of ornithine by ODC. In experiments with a variety of tissues

and cultured cells, it was found that an early rise in the level of putrescine is important for hormonal and/or agonist stimulation of DNA synthesis (16). Putrescine binding to nuclear macromolecules has been proposed to modulate DNA synthesis and transcription. In many experimental cell systems, the polyamines sometime act synergistically or antagonistically, making it difficult to distinguish their individual effects. Polyamines, spermine, spermidine, and putrescine, all exhibit antimutagenic potential against ethylmethane sulfonate (EMS)-induced reversions. In addition, spermidine and putrescine demonstrate potential to reduce the number of spontaneous revertants in modified Ames tests (17). The loss of feedback regulation of the polyamine transport system is sufficient to induce apoptosis (29).

Cadaverine

Cadaverine (1,5-diaminopentane) is formed by the decarboxylation of lysine. This catabolism of lysine is characteristic of postmortem changes in animals. Therefore the estimation of cadaverine, as well as putrescine, levels in fish products is used for the estimation of food quality and safety (2,27). The ingestion of fish, which have been improperly handled or stored, is very often connected with so called scombroid toxicity. The toxin is believed to consist of histamine, and possibly putrescine and cadaverine, which potentiate the toxicity of histamine. Putrescine and cadaverine inhibit the histamine-metabolizing enzymes, diamine oxidase and histamine N-methyl transferase (27). All physiological functions of cadaverine, if any, are yet unknown.

Agmatine

Agmatine (1-amino-4-guanidobutane) is an amine derived from the decarboxylation of L-arginine ([1-carboxy,1-amino]-4-guanidobutane) catalyzed by arginine decarboxylase (ADC). Agmatine is metabolized to putrescine by agmatinase. While prevalent in bacteria and plants, agmatine and its metabolic enzymes have only recently been identified in mammalian tissues. Agmatine has been proposed as the physiological ligand for the imidazoline receptors (6) and may be a novel neurotransmitter (20). It is not known whether agmatine is also involved in the homeostasis of intracellular polyamine content, but its physiological significance is probably much more important than previously surmised just a few years ago.

Agmatine, which in other life forms serves as a metabolic intermediate for polyamine biosynthesis, appears to have properties in mammals that are consistent with its action as a neurotransmitter/neuromodulator. Thus, agmatine is synthesized unequally in brain by ADC; is stored in neurons and axon terminals with a heterogeneous distribution; is released from synaptosomes by depolarizations; is enzymatically converted by agmatinase to putrescine; interacts not only with alpha2-adrenergic and I-receptors in the CNS, but also may selectively block NMDA receptor channels; and, when administered centrally, has several potent biological actions (20,21). Clarification of its role in normal brain function, however, has not yet been fully established, in part because of the absence of agents that selectively affect its biosynthesis or degradation. Agmatine is also a putative endogenous ligand for imidazoline binding sites of monoamino oxidase (MAO). It decreases MAO enzymatic activity (IC_{50} = 168 μM), whereas its precursor, L-arginine, and its metabolic conversion product, putrescine, have no effects on MAO (18).

Endogenous levels of agmatine are very low in the CNS and retinas of vertebrates. However, both agmatine and arginine display the capacity to permeate ionotropic glutamate gated AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionoc acid), KA (kainic acid), and NMDA (N-methyl-D-aspartate) receptor/channels with differential effectiveness. Agmatine selectively blocks the NMDA subclass of glutamate receptor channels (30), but permeates all three (except for certain low conductance AMPA receptor/channel complexes). In addition, it seems to permeate some additional nonselective channels in some receptor cells such as in retina, olfactory epithelium, gustatory epithelium and lateral line. Arginine permeates NMDA receptor/channel complexes and a subset of AMPA receptor/channel complexes but does not measurably permeate cyclic nucleotide gated channels (9). Furthermore, arginine permeation seems to lead to substantial cytotoxicity in certain cells, possibly those containing high levels of NO synthase (nitric oxide synthase).

Agmatine is a competitive NO synthase inhibitor but is not an NO precursor. In vitro K_I values are approximately 660 mM for NO synthase (NOS-1), 220 mM for NO synthase-2, and 7.5 mM for NO synthase-3. Structurally related polyamines do no inhibit NOS activity. Agmatine, therefore may be an endogenous regulator of NO production in mammals, although the requisite concentrations for inhibition of NOS-1 and NOS-2 are prohibitively high for effects requiring direct interactions. Regunathan et al. (19) observed that agmatine decreased the activity of NOS-2 by reducing the levels of enzyme protein as measured by immunoblot and immunocytochemistry. It was observed that agmatine, as well as some other imidazoline agents inhibit the expression of NOS-2 and proliferation in primary glial cells and vascular smooth muscle cells (VSMC). Agmatine was also found in axons and axon terminals associated with small synaptic vesicles in rat hippocampus (22). These findings further implicate agmatine as an endogenous neurotrasmitter which may be co-stored with L-glutamate and may act as a blocker of NOS and the NMDA receptor.

The distribution of agmatine was mapped in the CNS of the rat. Agmatine-containing neurons were present in the cerebral cortex, predominantly within laminae VI and V, and to a lesser extent, III, and mainly in retrosplenial, cingulate, primary somatosensory and auditory cortices, and the subiculum. In the lower brainstem, these neurons were selectively localized to visceral relay nuclei; the nucleus tractus solitarii and pontine parabrachial complex, and pe-

riventricular areas including the laterodorsal nucleus, locus coeruleus and dorsal raphe. In the midbrain, these cells were concentrated in the ventral tegmental area and periaqueductal gray. In the forebrain, subcortical agmatine-containing neurons were obtained predominantly in the preoptic area, amygdala, septum, bed nucleus of the stria terminalis, midline thalamus, and the hypothalamus. Agmatine immunoreactivity was also affiliated with endoplasmic reticulum and the plasmalemma (14). The central distribution of agmatine is consistent with the hypothesis that the amine may be a novel neurotransmitter of neurons involved in behavioral and visceral control.

Agmatine uptake into rat synaptosomes was investigated by Sastre et al. (23). They found that transport was not inhibited by amino acids, polyamines, or monoamines, indicating that the uptake is not mediated by any primary amine-bearing compounds of these types. When they examined the effects of some ion-channel agents on agmatine uptake, Ca^{2+} ion was observed to increase it. In addition, some imidazoline drugs, such as idazoxan and phentolamine, were strong noncompetitive inhibitors of agmatine uptake. Thus, a selective, Na^{+} ion-independent uptake system for agmatine exists in brain and may be important in regulating the extracellular concentration of agmatine.

Plasma agmatine concentrations are very low in humans. However, they are significantly elevated in depressed patients compared to healthy controls. Treatment with the antidepressant bupropion normalized plasma agmatine levels. Correlative evidence has been published that reports a change in plasma agmatine levels may lead to similar changes in platelet I1 imidazoline receptors (4). Systemic infusion of agmatine into rats causes hypotension (12).

Paradoxically, agmatine appears to act directly on endothelial cells to increase the synthesis of NO as opposed to examples of NO inhibition mentioned earlier in this review. As was observed by Schwartz et al. (26), agmatine also exerts stimulatory effects on glomerular ultrafiltration via a constitutive NOS-dependent mechanism and this does not require the participation of alpha 2-adrenoreceptors.

It has been demonstrated that the gastric pathogen, Helicobacter pylori, is able to form and release the endogenous imidazoline receptor ligand, agmatine, and that considerable amounts of agmatine are present in human gastric juice. The quantities of agmatine were higher in gastric juice from H. pylori-positive patients than patients who are H. pylori-negative (10).

Satriano et al. (24) proposed a novel regulatory pathway in which agmatine acts as an antiproliferative molecule and potential tumor suppressor by restricting the cellular polyamine supply required to support growth.

Spermidine and spermine

Spermidine (1,8-diamino-5-azaoctane) and spermine (1,12-diamino-5,9-diazadodecane) are very ubiquitous tri- and tetra-amines, respectively, which frequently occur simultaneously in animal cells. Their physiological functions are generally similar. Their biosyntheses originate through initial aminopropylation of one primary amine group of putrescine to form spermidine. This is followed by a second aminopropylation addition to the primary amine group of spermidine, which initially derived from putrescine, thus forming spermine. Both reactions require decarboxylated S-adenosylmethionine as the propylamine donor. Spermidine synthesis from putrescine is catalyzed by putrescine aminopropyltransferase (PAPT) and spermine synthesis from spermidine is catalyzed by spermidine aminopropyltransferase (SAPT). Spermidine and spermine are retroconverted to putrescine and spermidine, respectively, by initial N-acetylation and subsequent polyamine oxidation. The intermediate N-acetylputrescine, N^{1}-acetylspermidine and N^{8}-acetylspermidine are the major urinary N-acetylpolyamines. Polyamines and N-acetylpolyamines are terminally degraded to non-amino acid metabolites by oxidative deamination and aldehyde dehydrogenation. Polyamine oxidation, catalyzed by polyamine oxidase, has recently been hypothesized to be a major contributor of cellular hydrogen peroxide, which commits many types of eukaryotic cells into an apoptotic pathway of cell death (3,15).

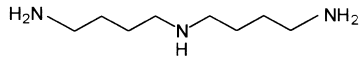
Spermine has been identified as a potent antioxidant and an anti-inflammatory agent. The compound is present in all animal cells and all organs. The concentration is exceptionally high in skin, and spermine constitutes a prime defense against radiation damage. This hypothesis is substantiated by the fact that ODC, the rate-limiting enzyme of spermine biosynthesis, is induced by UVB-irradiation and oxidative stress. Moreover, inhibition of ODC makes cells more sensitive to radiation damage. The antioxidative effect of spermine may be due to metal chelation and/or to prevention of superoxide generation from stimulated neutrophils (8).

Unusual polyamines

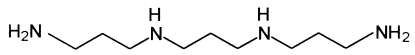
Unusual polyamines are found in some plants and thermophilic microorganisms. Very often these unusual polyamines occur simultaneously with the usual polyamines such as diaminopropane, putrescine, cadaverine, spermidine, spermine, and agmatine. Aminopropylhomospermidine has been reported in the aquatic plants *Brasenia schreberi* and *Nuphar japonicum* belonging to the family *Nymphaeaceae*. Norspermidine and norspermine were detected in the blackweed *Hydrilla verticillata* belonging to *Hydrocharitaceae*. The same unusual polyamines have been identified in water-deficit stressed *Medicago sativa* L. (alfalfa) and the photosynthetic acidothermophilic alga, *Cyanidium caldarium*. Thermospermine was detected in *Brasenia schreberi* and more recently in *Medicago sativa* L. N,N(-Bis(3-aminopropyl)-1,2-ethanediamine [$H_2N(CH_2)_3NH(CH_2)_2NH(CH_2)_3NH_2$], was discovered in the aquatic plant *Nuphar japonicum*. N^{4}-Methylspermidine [$H_2N(CH_2)_3N(CH_3)(CH_2)_4NH_2$] was discovered in the water chestnut

Trapa natas belonging to the family *Hydrocaryaceae.* γ-Guanidinooxypropylamine [$H_2N(NH=)CNHO(CH_2)_3NH_2$], a new guanidinooxyamine was found in *Wistaria floribunda* seeds and seedlings of the sword bean, *Canavalia gladiata* (5).

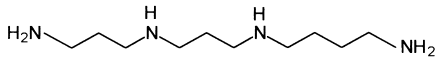
Many unusual polyamines have been identified in extreme thermophiles (13), such as thermine (norspermine), canavalmine, caldopentamine, caldohexamine, tris(3-aminopropyl)amine, thermospermidine, caldine (norspermidine), or tetrakis(3-aminopropyl) ammonium and others. Chemical structures for some unusual polyamines are given in Fig. 3.



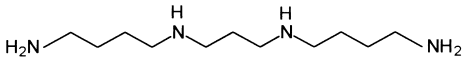
Caldine



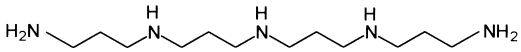
Thermine (Norspermine)



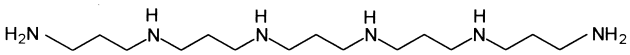
Thermospermine



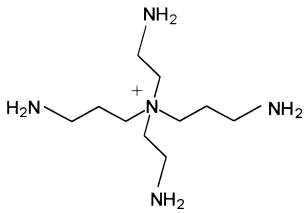
Canavalmine



Caldopentamine



Caldohexamine



Tetrakis(3-aminopropyl)-ammonium

Fig. 3: Chemical structures of some uncommon (unusual) polyamines.

Conclusions

Natural polyamines represent a group of compounds having major physiological significances. Since the biosyntheses of the polyamines is tightly regulated and involved in the control of many biological processes such as carcinogenesis, cell growth, cell differentiation, gene transcription and translation, their continued study is very important for understanding critical processes in biological systems. Inhibitors of polyamine biosynthesis have potential clinical uses as antitumor and antiparasitic agents (28).

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