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Biogenic Amines and Polyamines: Similar Biochemistry for Different Physiological Missions and Biomedical Applications

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ABSTRACT: Biogenic amines are organic polycations derived from aromatic or cationic amino acids. All of them have one or more positive charges and a hydrophobic skeleton. Nature has evolved these molecules to play different physiological roles in mammals, but maintains similar patterns for their metabolic and intracellular handling. As deduced from this review, many questions still remain to be solved around their biochemistry and molecular biology, blocking our aims to control the relevant pathologies in which they are involved (cancer and immunological, neurological, and gastrointestinal diseases). Advances in this knowledge are dispersed among groups working on different biomedical areas. In these pages, we put together the most relevant information to remark how fruitful it can be to learn from Nature and to take advantage of the biochemical similarities (key protein structures and their regulation data on metabolic interplays and binding properties) to generate new hypothesis and develop different biomedical strategies based on biochemistry and molecular biology of these compounds.

KEY WORDS: arginine/ornithine-derived amines, histamine, neurotransmitter amines, decarboxylases, amino oxidases, amine transport systems.

I. INTRODUCTION

Biogenic amines are natural products related to intercellular communication and, as such, present in specific cell types, globally called amine-handling cells. The most relevant ones in mammalian physiology are serotonin, histamine, dopamine, and noradrenaline. On the other hand, arginine/ornithine-derived polyamines, although they might also be considered biogenic amines, are not only involved in biosignaling and are present not only in amine-handling cells. They are ubiquitous in localization (in fact, they are present in almost every kind of cell) and have pleiotropic effects, with a recognized major role as essential compounds in maintaining macromolecular synthesis and cell proliferation rates (Cohen, 1998). Figure 1 shows the main biogenic amines and arginine/ornithine-derived polyamines. The relationship between these amine compounds and human pathologies

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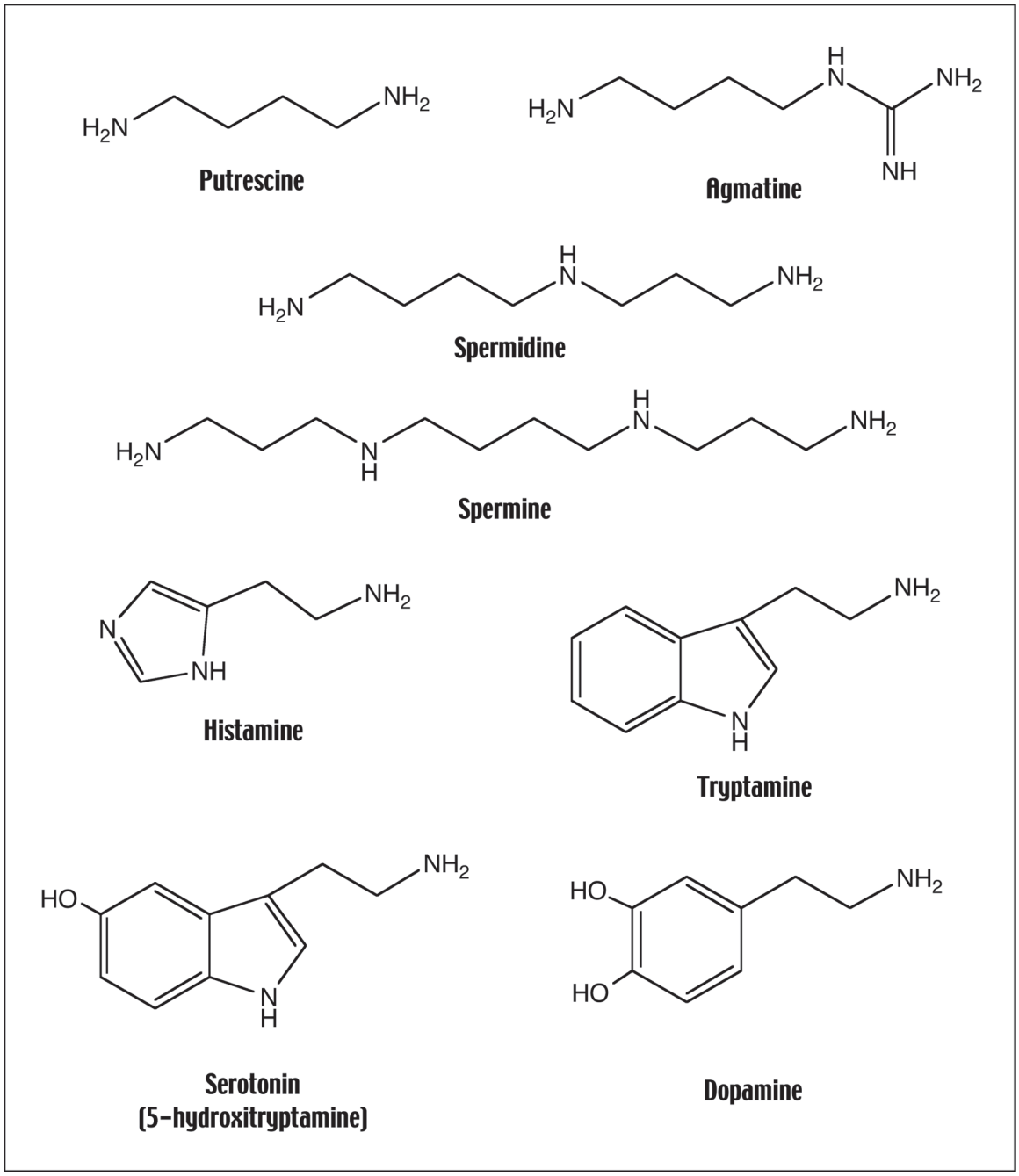

FIGURE 1. The main biogenic amines and polyamines.

has been known for more than 150 years. Currently, in fact, it is known that they are involved in some of the most prevalent human pathologies, including carcinogenesis and tumor invasion (ornithine-derived polyamines and histamine), allergy and immune response in general (histamine), and neurological disorders such as Parkinson, Alzheimers, depression, and anorexia (serotonin, dopamine, histamine). Table 1 cites review articles devoted to these connections.

Despite the relevance of these compounds and their related diseases, there are still many obscure points in their biochemistry and molecular biology. The major aim of this review is not to make update the present knowledge on biogenic amine and polyamine biochemistry and physiology, but to present the major questions on this topic and present a comprehensive view of what and how we should try to know more about certain areas and to apply this knowledge to the specific biomedical problems related to them.

II. AMINE-HANDLING CELLS

With the exception of arginine/ornithine-derived polyamines that are produced by all proliferating cell types, the other biogenic amines are only synthetized by a very small set of cells (amine-handling cells), which are sometimes dispersed in the organisms, as is the case for histamine and mast cells. Amine-handling cells are cells that fulfill those requirements: (1) They contain the enzymes involved in the biosynthesis of at least one biogenic amine. (2) They contain intracellular transporters for storage of biogenic amines in secretory vesicles. (3) They present plasma membrane transporters for scavenging and recycling biogenic amines from extracellular space once they have played their biosignaling role. Table 2 shows a classification of amine-handling cells.

A major problem for the molecular characterization of the metabolic regulation of biogenic amines comes from difficulties in having good cultured amine-handling cell models. Most of the amine-handling cell types are highly differentiated cells that are difficult to be cultured *in vitro*, and transformed counterparts could lack or have distorted any of the relevant regulatory mechanisms acting in the original cell type *in vivo*. For instance, human mast HMC-1 cell line expresses a mutant c-*kit* receptor, disturbing their biosignaling pathways (Welker et al., 1998). However, these amine-handling cells are the only cellular systems in which the metabolic interplays between biogenic amines involved in biosignaling and arginine/ornithine-derived polyamines can be properly studied. Thus, new efforts to get better and more "physiological" cell culture models are required.

III. METABOLIC PATHWAYS FOR BIOSYNTHESIS AND CATABOLISM OF BIOGENIC AMINES AND POLYAMINES

Figures 2 and 3 depict the metabolic pathways for biosynthesis and catabolism of biogenic amines and polyamines, respectively. Figure 4 summarizes the common features of these metabolic pathways. In the biosynthesis of all these amines there is a key enzyme with amino acid decarboxylase activity: aromatic amino acid decarboxylase (DDC) for serotonin, tryptamin and dopamine, histidine decarboxylase (HDC) for histamine, arginine decarboxylase (ADC) for agmatine, and ornithine decarboxylase (ODC) for putrescine. In the degradation of these amines, there are always some oxido-reduction reactions catalized by amine oxidase and aldehyde dehydrogenases. In the degradation of biogenic amines, but not in

TABLE 1
Amines and Related Pathological Disorders

Amine	Disorder	Reference
Histamine	Cancer (skin cancer), oxidative stress, schizophrenia, allergy, inmunotherapy	(Heleniak & O'Desky, 1999; Medina et al., 1999 Hellstrand et al., 2000; Hart et al., 2001; Hellstrand, 2002)
Serotonin	Depression, schizophrenia, Alzheimer, Parkinson, anxiety, panic, migraine, obesity, encephalopathy, platelet inhibition	(Baldwin & Rudge, 1995; Iqbal & van Praag, 1995; Wurtman & Wurtman, 1996; Kapur & Remington, 1996; Miyawaki et al., 1997; Mathew, 1997; Deakin, 1998; Nair et al., 1999; Aghajanian & Marek, 2000; Bengtsson, 1999; Berk, 2000; Mossner et al., 2000; Solai et al., 2001)
Dopamine	Schizophrenia, Alzheimer, Parkinson	(Kapur & Remington, 1996 Miyawaki et al., 1997; van Veelen & Kahn, 1999)
Tyramine	Migraine, hypertension, schizophrenia, Parkinson, depression	(Premont et al., 2001)
Tryptamine	Depression, schizophrenia, hepatic encephalopathy	(Premont et al., 2001)
Polyamines	Ischemia (neuronal injury), muscular dystrophy, epilepsy, Alzheimer, psoriasis, cystic fibrosis, cancer	(Russell, 1983; Seiler et al., 1998; Bernstein & Muller, 1999; Davidson et al., 1999)
Adrenaline	Hyperactivity	(Mefford & Potter, 1989)
Noradrenaline	Hyperactivity disorder, Post-traumatic stress disorder, schizophrenia, Alzheimer	(Friedman et al., 1999; Southwick et al., 1999)

TABLE 2
Types of Amine-Handling Cells, According to Weihe and Eiden (2000)

Cell group	Cell type	Amines
Nervous system cell	Neuron of CNS	Noradrenaline, adrenaline, dopamine, serotonin, histamine
	Neurons of PNS	Noradrenaline, adrenaline, histamine
Neuroendocrine cells	SIF cell	Dopamine
	Enterochromaffin-like cell	Histamine
	Enterochromaffin cell	Dopamine
	Chromaffin-like cell	Dopamine
	Gastrin containing cell (stomach and duodenum)	Histamine
Cells from the immune/inflammatory axis	Mast cell (brain, tonsils and skin)	Histamine
	Merkel cell (skin)	Histamine
	Megakaryocyte	Serotonin
	Platelets	Serotonin

that of polyamines, there are methylation reactions using *S*-adenosylmethionine as the high-energy methyl donor. A very specific feature of polyamine metabolism is their interconversion pathway. It allows increases in the size of the molecules from putrescine to spermidine and from spermidine to spermine by sequential transfer of aminopropyl groups, in reactions catalized by spermidine and spermine synthase, respectively. It is noteworthy that *S*-adenosylmethinine decarboxylase (SAMDC) has to decarboxylate *S*-adenosylmethione to render it as a suitable aminopropyl group donor. In the return direction of the interconversion pathway, spermine and spermidine can be N^1-acetylated by spermidine/spermine N^1-acetyltransferase (SSAT) to produce compounds suitable for oxidation by polyamine oxidase (PAO), finally yielding spermidine from spermine and putrescine from spermidine. Thus, *S*-adenosylmethionine availability could play an important role in biogenic amine metabolism regulation in amine-handling cells.

To have a complete view of the metabolism of biogenic amines and polyamines,

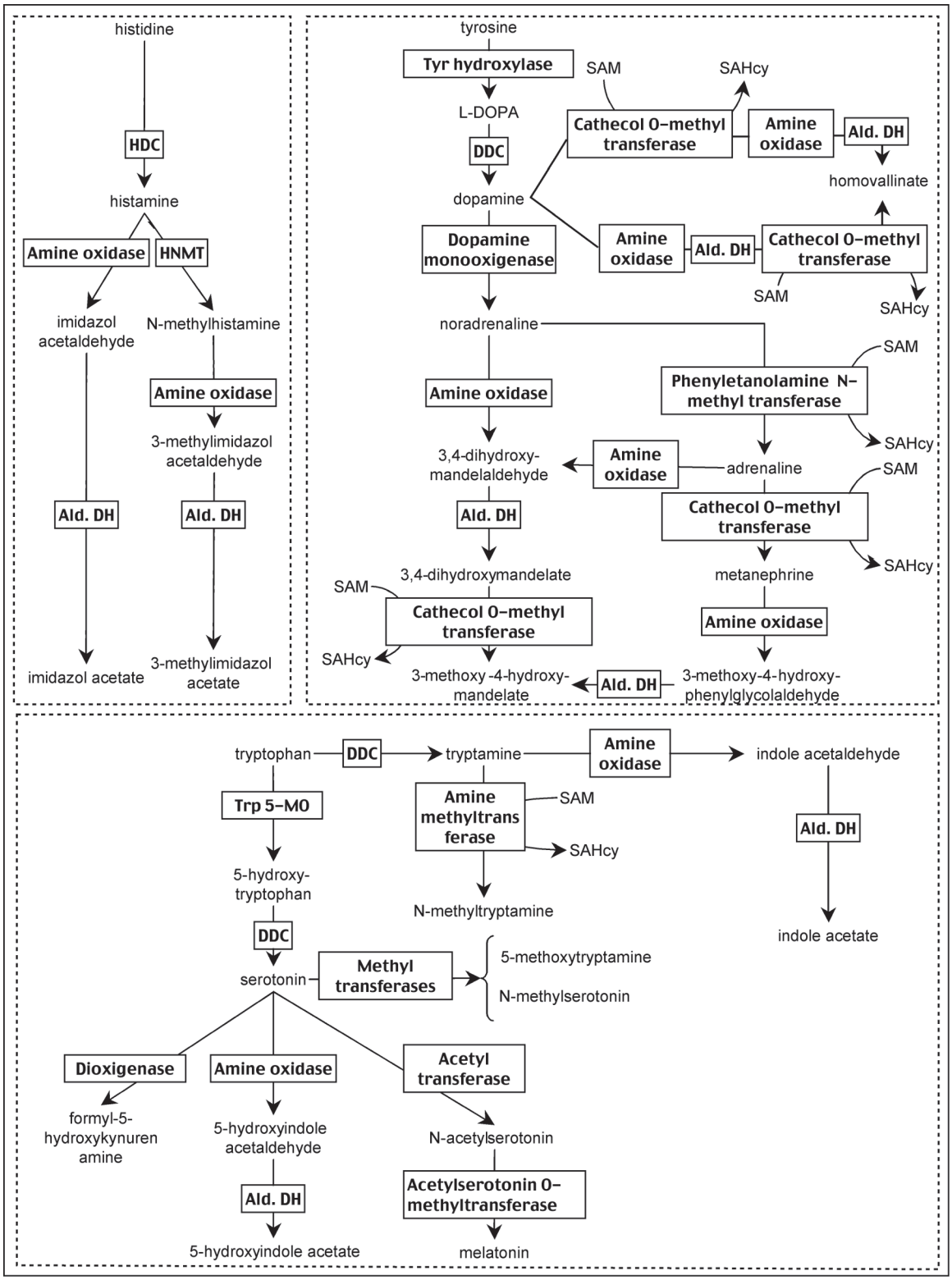

FIGURE 2. Metabolism of biogenic amines in mammals. Abbreviations: aldehyde dehydrogenase (Ald. DH), aromatic L-amino acid decarboxylase (DDC), histamine *N*-methyltransferase (HNMT), histidine decarboxylase (HDC), *S*-adenosylhomocysteine (SAHcy), *S*-adenosylmethionine (SAM), tryptophan 5-monooxigenase (Trp 5-MO).

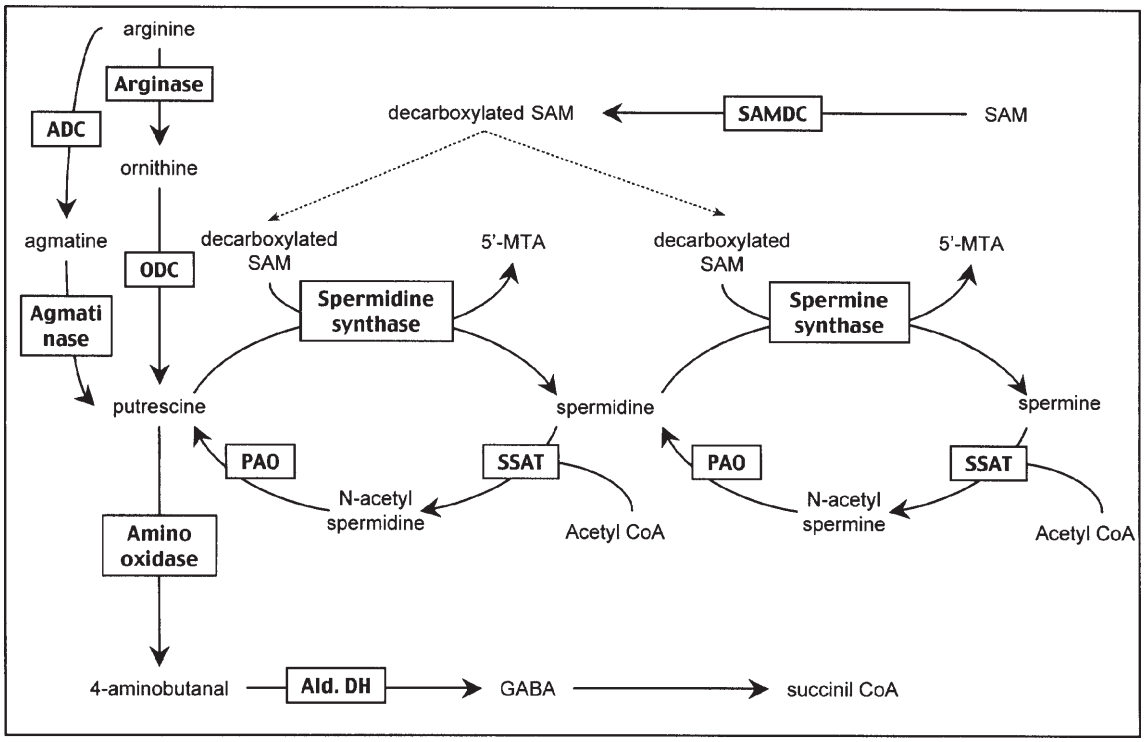

FIGURE 3. Metabolism of polyamines in mammals. Abbreviations: aldehyde dehydrogenase (Ald. DH), arginine decarboxylase (ADC), 5'-methylthioadenosine (5'-MTA), ornithine decarboxylase (ODC), polyamine oxidase (PAO), *S*-adenosylmethionine (SAM), *S*-adenosylmethionine decarboxylase (SAMDC), spermidine/spermine N^1-acetyltransferase (SSAT).

mechanisms of transport, compartmentation, and conjugation should also be taken into account, as discussed below.

There still is an important pending question in the metabolic picture of arginine-derived amines in mammals. This is agmatine biosynthesis. It is well established that arginine is a substrate for arginine decarboxylase (ADC) in bacteria and plants (Wu and Morris, 1973), yielding the amine agmatine, but it was believed that this enzyme was not expressed in mammals. In 1994, agmatine was identified as an endogenous clonidine-displacing substance in the brain (Li et al., 1994). Afterward, agmatine has been detected in many different tissues and organs, including aorta, spleen, adrenals, small intestine, skeletal muscle, stomach, brain, liver, and kidney (Raasch et al., 1995; Morrissey et al., 1996; Stickle et al., 1996; Molderings et al., 1999). Although agmatine is also present in food and intestinal flora, the distribution of tissue agmatine does not correlate to tissue blood flow, suggesting local synthesis by ADC. In fact, ADC has been partially cloned in rat kidney (Morrissey et al., 1996), and its activity has been demonstrated in a number of cells, tissues, and organs (Li et al., 1995; Regunathan et al., 1995, 1996; Lortie et al., 1996; Regunathan and Reis, 2000). However, neither a mammalian ADC gene nor a full-length ADC cDNA have been reported so far.

Nevertheless, agmatine is another bioactive metabolite of arginine in mammals. In plants and bacteria, agmatine is a metabolic intermediate in a pathway for polyamine biosynthesis; in fact, hydrolysis of agmatine by agmatinase yields putrescine, a precursor for spermidine and spermine biosynthesis. Recently, agmatinase activity

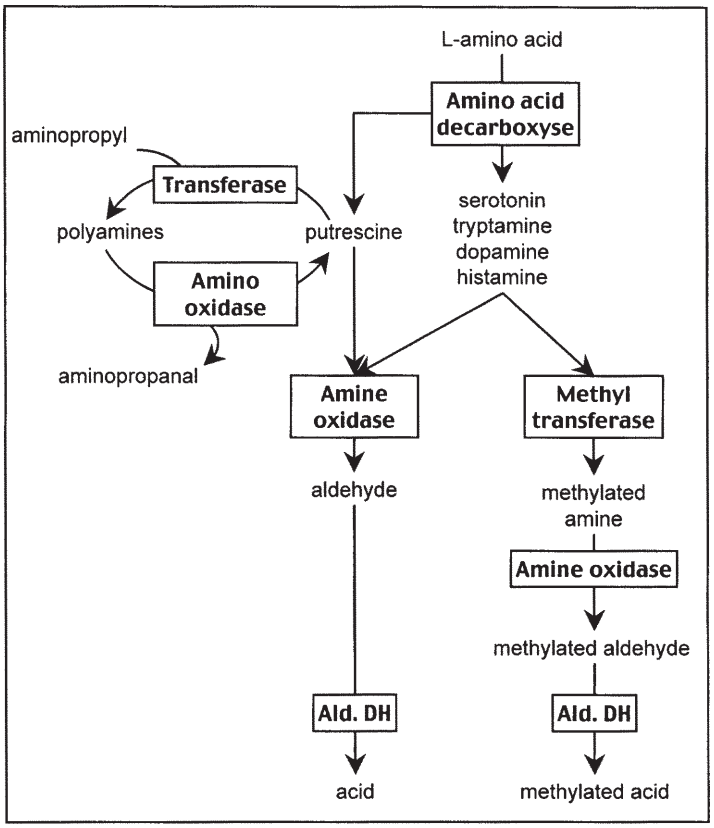

FIGURE 4. Common motifs of biogenic amine and polyamine metabolism in mammals. Abbreviation: aldehyde dehydrogenase (Ald. DH).

has been found and cloned in mammals (Sastre et al., 1996; Wu and Morris, 1998; Mistry et al., 2002). Thus, agmatine may also be a precursor for polyamine biosynthesis in mammals.

Several effects are induced by agmatine, including the promotion of catecholamine release from adrenal chromaffin cells (Li et al., 1994), stimulation of insulin release (Sener et al., 1989), and the inhibition of human coronary artery vascular smooth muscle cell growth (Regunathan and Reis, 1997). The multiple modulator effects of agmatine on arginine and polyamine metabolism seem especially relevant: agmatine has been shown to be a competititve inhibitor of nitric oxide synthases (Galea et al., 1996; Regunathan et al., 1999), an activator of *S*-adenosylmethionine decarboxylase (Vargiu et al., 1999), an inducer of translational frameshift of antizyme mRNA (Satriano et al., 1998), an inhibitor of ornithine decarboxylase and polyamine uptake (Vargiu et al., 1999; Satriano et al., 1998), and a potent inducer of spermidine/spermine acetyltransferase activity (Vargiu et al., 1999).

In kidney, agmatine is degraded to guanidino-butylaldehyde by diamine oxidase. The production and degradation of agmatine has been described as a novel endogenous regulatory system in the kidney (Regunathan et al., 1995). In fact, agmatine seems to control natriuresis by functioning as a physiological agonist of I1 imidazoline receptors (Penner and Smyth, 1996), and it can increase absolute proximal reabsorption and single nephron glomerular filtration rate through I2 imidazoline receptors (Regunathan et al., 1995). Thus, the full

molecular characterization of the agmatine metabolism and physiological roles appear to be an interesting open topic in the field.

IV. METABOLIC INTERPLAYS BETWEEN BIOGENIC AMINES AND POLYAMINES

The structural similarities among biogenic amines and polyamines and the common features in their respective metabolic pathways lead to the suspicion that there should be mutual interferences. To our knowledge, these metabolic interplays have been more deeply studied for histamine and polyamines than for other biogenic amines and polyamines, up to now.

A. Histamine and Polyamine Metabolic Interplays in Mammalian Cells

Histamine-handling cells include histamine-producing neurones, enterochromaffin-like and gastrin-containing cells of the gastric mucosa, certain types of tumors and basophilic cells. As mentioned above, most of these cell types are very specialized and cannot be manipulated easily in cultures. This is a major problem for such metabolic studies.

It is well known that the NMDA receptor has a binding site for polyamines (Purcell et al., 1996; Williams, 1997; Yamakura and Shimoji, 1999). On the other hand, agmatine has been shown to selectively block this receptor in rat hippocampal neurons (Yang and Reis, 1999). Through this molecular interaction polyamine can regulate neuronal viability and function. It has also been reported that histamine can modulate some of the NMDA-induced effects (Meyer et al., 1998; Brown et al., 2001).

In gastric mucosa, the roles of histamine and polyamines seem to be clearly differentiated and somehow opposite. Polyamines have been related to stomach epitelium protection and turnover, and histamine is related to acid secretion and consequently is related to peptic ulcer formation. Polyamines are able to inhibit acid production as reported by Ostrowski et al. (1993). It has been reported that aspirin and other promoting factors of peptic ulcer lead to a decrease in stomach polyamine content (Al-shabanah et al., 1999). On the contrary, cimetidine and other H2-receptor antagonists, with an strong inhibitory effect on gastric lesions, protect against polyamine depletion. Moreover, the long-term administration of cimetidine causes precancerous changes with increases in polyamine levels (Tsutsumi et al., 1998). The molecular causes for this increase in the polyamine content by the histamine receptor blocker is not yet fully known. On the other hand, Araki et al. (1991) and Ding et al. (1996) have observed simultaneous ODC and HDC inductions in gastric mucosa in response to different hormones and other treatments. In any case, histamine and polyamine metabolisms seem to be interregulated, and the equilibrium between both biogenic amine groups seems to be important for maintaining gastric integrity and function.

Lung and hepatic tumors have been reported in which growth stimuli induce both ODC and HDC simultaneously *in vivo* (Medina et al., 1999). In these cases, histamine production seems to contribute to maintain tumor growth, as do polyamines, because HDC inhibition by the substrate analog monofluoromethylhistidine reduces tumor growth. In humans, ODC inhibiton by difluormethylornithine induces cytostatic effects on small cell lung carcinoma (Porter and Bergeron, 1988), which very recently have been shown to be histaminergic (Graff et al., 2002). In our lab, we have character-

ized polyamine uptake by Ehrlich carcinoma, originally a mouse mammary carcinoma widely used for *in vivo* polyamine metabolism studies, with nondetectable HDC activity and histamine contents. Ehrlich cell polyamine uptake is energy dependent and highly specific for spermidine and spermine, and not affected by the presence of histamine and other natural and non-natural diamines (Paz et al., 2001). However, in these cells, putrescine and histamine produced equally effective inhibition of ornithine by the cationic amino acid y^+ transport system (Medina et al., 1991). This transport system is widely extended in tumor and nontransformed mammalian cells, so that this regulation point of polyamine metabolism by histamine should function in many other mammalian cell types.

Basophils and mast cells are specialized in immune cell communication, histamine being one of the most important cell mediators produced and secreted by these cells. Because basophilic cells can also proliferate, they also express both ODC and HDC, so that they could provide a good model for the study of polyamine and histamine metabolic interplay. Recently, working with different basophilic cell lines and treatments, all results indicated some kind of antagonisms between polyamine and histamine content. An increase in the intracellular histamine concentrations led to a reduction in total polyamine content (Fajardo et al., 2001a). Spermine uptake was reduced in both C57.1 mouse mast cells treated with exogenous histamine and histamine-preloaded cells (Fajardo et al., 2001b). This antagonism between polyamine and histamine content in basophilic cells is also suspected from results of other authors using different experimental approaches. Treatments of basophilic cells with extracellular polyamines at millimolar range elicit a release of histamine. Some authors claim that the NMDA receptor is involved in this response (Purcell et al., 1996). Others explain this effect by the activation of G proteins that would induce basophilic degranulation (Daeffler et al., 1999).

The above-mentioned results from different groups clearly indicate a polyamine/histamine interplay in mammalian cells for which both histamine and polyamines have physiological importance. However, many questions still remain to be answered about the molecular mechanisms underlying these observations and their physiological consequences.

B. Other Biogenic Amines and Polyamine Metabolic Interplays in Mammalian Cells

The studies on metabolic interplays between polyamines and biogenic amines other than histamine are very scarce. Furthermore, most of the little data currently available concerns the roles of these biogenic amines as extracellular biosignaling molecules. Thus, dopamine and serotonin have been shown to induce ODC activity in several target cell types (D'Amore et al., 1978; Persson and Rosengren, 1987; Townsend et al., 1993). On the contrary, dopamine agonist administration decreased *S*-adensylmethionine decarboxylase activity in rat adrenal gland (Ekker and Sourkes, 1987), and serotinin and tryptamine suppressed the induction of ODC by ornithine in Ehrlich tumor cells (Matés et al., 1991).

Some physicopharmacological results seem to indicate a selective action of polyamines on mesolimbic dopamine behavior (Hirsch et al., 1987). It has also been shown that spermine interacts with cocaine binding sites on dopamine transporters (Ritz et al., 1994).

More comprehensive and systematic studies in this area are required.

V. KEY ENZYMES INVOLVED IN THE BIOSYNTHESIS AND CATABOLISM OF BIOGENIC AMINES AND POLYAMINES

At least theoretically, there should be two ways to effectively control amine production: through managing either its synthesis or its degradation. An amine-handling cell cannot store or secrete amines if they are not synthetized or are destroyed immediately after synthesis. These approaches have found several major problems, so the final goal is still a long way away.

As mentioned above, in the biosynthetic pathways of all the biogenic amines and polyamines there is a key enzyme with L-amino acid decarboxylase activity. These enzymes are good targets to control amine production. Unfortunately, they are very unstable and minority proteins. This fact has retarded their full characterization up to the point that only two of these mammalian structures have been X-ray solved very recently: ODC (Grishin et al., 1995; Kern et al., 1999) and DDC (Burkhard et al., 2001). Many efforts have been done to develop specific ODC inhibitors able to reduce polyamine synthesis in mammalian cells as an antiproliferative strategy (Seiler et al., 1998). The development of new DDC inhibitors is also one of the major aims from the structural characterization of this enzyme (Burkhard et al., 2001).

On the other hand, amine degradation involves cleavage of amino groups and the oxidation of the carbon skeleton by oxidases and, in many cases, transferease reactions. Many mono, di-, and polyamine oxidases have been described in mammalian cells (Jalkanen and Salmi, 2001; Binda et al., 2002); however, as deduced from the most recent bibliography, different proteins with different specificities, mechanisms of action, and intra- or extracellular location are included under this denomination. Up to now, it is not clear either which kind of amine oxidases are expressed by each cell type or even how many different proteins there are for these activities. Despite these questions, some authors are starting to claim for a biotechnological application of these enzymes in therapy. Among the enzymes involved in transferase reactions, the best characterized in the last few years is SSAT, a key enzyme in polyamine interconversion with a very rapid turnover in mammalian cells (Casero and Pegg, 1993).

A. L-Amino Acid Decarboxylases

Biogenic amines and polyamines are synthetized by the decarboxylation of amino acids. In mammals, these L-amino acid decarboxylases are pyridoxal-5'-phosphate (PLP)-dependent enzymes. Different PLP-dependent enzymes catalize reactions involved in amino acid metabolism (transamination, decarboxylation, racemization, aldol cleavage, β- and γ-substitution and β- and γ-elimination). On the basis of sequence comparison, PLP enzymes can be grouped in four independent families of paralogous proteins (Sandmeier et al., 1994; Grishin et al., 1995; Momany et al., 1995; Mehta and Christen, 2000). The eukaryotic decarboxylases involved in the biosynthesis of biogenic amines and polyamines are included in two different groups. Mammalian ODC belongs to the alanine racemase family (β/α–barrel proteins) or group IV, while HDC and DDC belong to the α family or group II, with aspartate aminotransferase as a prototype enzyme (Grishin et al., 1995; Mehta and Christen, 2000). The PLP-binding domain of ODC folds in an alpha/beta barrel model (Kern et al., 1999; Almrud et al., 2000). On the other hand, the tertiary structure of mammalian HDC is still

unknown. Recently, the crystalographic structure of pig DDC has been solved (Burkhard et al., 2001). The PLP-binding domain of DDC consists of different α/β folds with seven stranded mixed β-sheet surrounded by eight α-helices (Burkhard et al., 2001).

Despite their different evolutionary origins, these decarboxylases seem to have similar protein motifs in common, so that they could share some mechanistic and regulatory characteristics in mammalian organisms. In 1994, we detected some common motifs between these enzymes for the first time (Viguera et al., 1994). During the last few years, experimental data have been obtained by our group and others reinforcing some of our initial hypothesis (Fleming and Wang, 2000; Olmo et al., 2000).

In the last decade, a great effort has been made to elucidate the structure/function relationships of ODC. Residues involved in catalysis have been located by directed mutagenesis (Jackson et al., 2000). A catalytic mechanism has been proposed (Brooks and Phillips, 1997). As far as the catalytic mechanism of HDC homologous proteins is known (Hayashi, 1995), it seems to be similar to the mechanism described for ODC. Figure 5 summarizes the mechanism of action of these PLP-dependent decarboxylases. Recent studies of our group, working with recombinant rat HDC, have characterized enzyme/substrate complexes during histidine decarboxylation (Olmo et al., 2002); this has allowed us to compare the properties of the enzyme to those of other homologous decarboxylases.

These enzymes are active as dimers. In ODC, the catalytic centre involves the N-terminal part of a monomer and the C-terminal part of the other one (Coleman et al., 1994). In DDC, the active site is located near the monomer-monomer interface but is composed mainly of residues from one of the monomers (Burkhard et al., 2001). The catalytic center of HDC is still unresolved. In rodents, native HDC also seems to be a homodimer (around 110 kDa), the primary translation product (74 kDa) being inactive (Yamamoto et al., 1990, 1993). However, in human HDC, activity has been detected from the 74-kDa polypeptide, which seems to function as a monomeric enzyme (Yatsunami et al., 1995).

Both mammalian ODC and HDC are described as homodimeric and unstable proteins (Yamamoto et al., 1990, 1993; Coleman et al., 1994). Mammalian ODC has one of the shortest half-lives described for mammalian proteins (Rogers et al., 1986; Heby and Persson, 1990). This is explained by the presence of PEST regions (clusters rich in proline, aspartate, glutamate, serine, and threonine), and more specifically the PEST region located in the carboxyl-terminus of the protein (Figure 6). ODC degradation is regulated by polyamines; different domains in the protein confer constitutive degradation and polyamine responsiveness to ODC (Ghoda et al., 1992). Polyamines induce the expression of a proteic ODC inhibitor, called antizyme (Hayashi et al., 1996). Antizyme binds to ODC momomer blocking the formation of the active dimer. On the other hand, antizyme binding provokes a conformational change in ODC monomer, exposing the carboxy-terminal region to proteasoma 26S, which degraded ODC to peptides of 5–11 amino acid, whereas antizyme is released and recycled to destabilize more ODC monomers. Antizyme also supresses cellular uptake of polyamines and can bind an antizyme inhibitor with higher affinity than ODC momomer. This antizyme inhibitor stabilizes ODC by reducing the amount of antizyme available for ODC destabilization (Hayashi et al., 1996). Thus, antizyme plays a central role in the regulation of polyamine levels by both promoting ODC degradation and suppressing polyamine uptake.

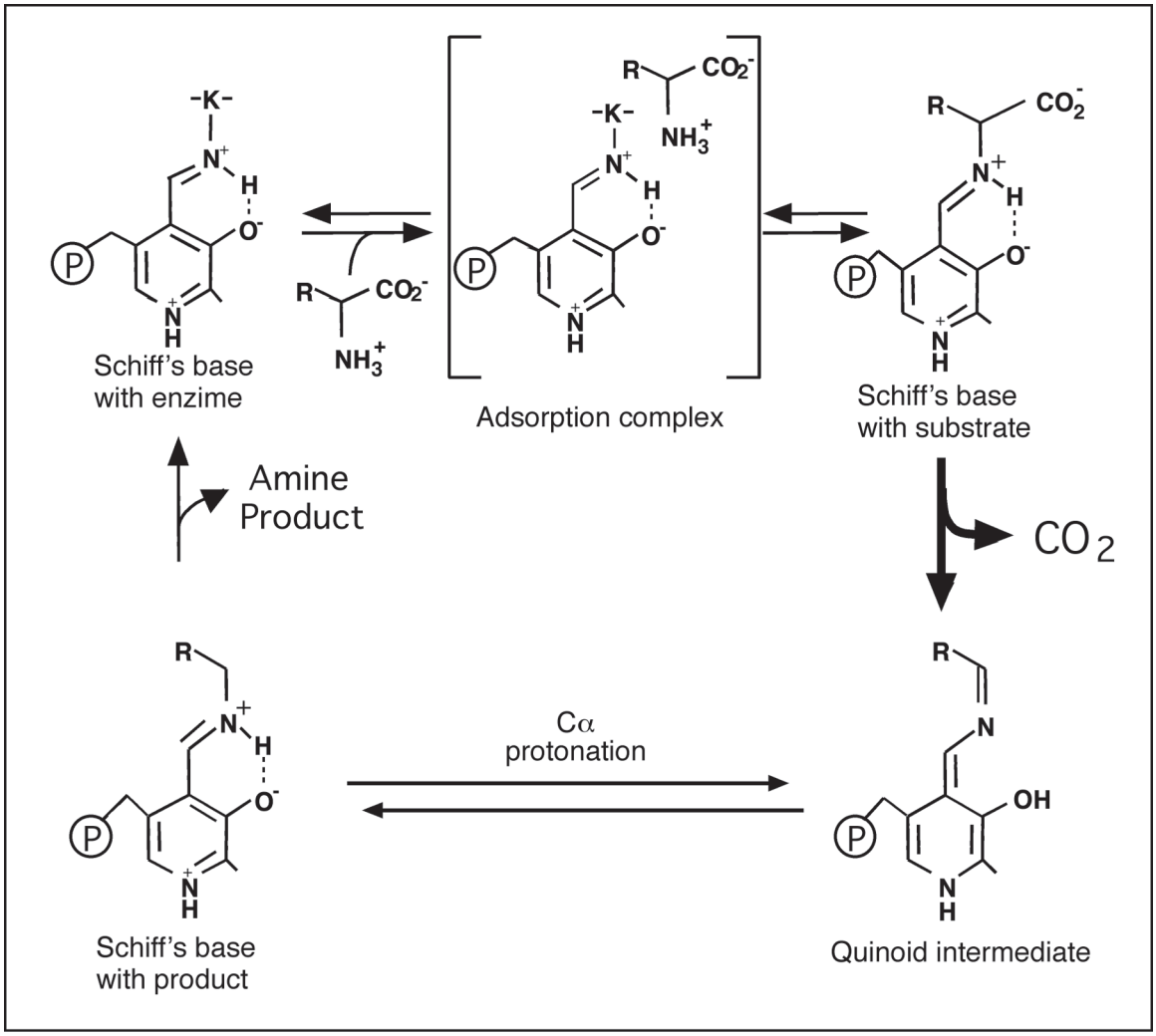

FIGURE 5. Mechanism of action of pyridoxal 5'-phosphate-dependent decarboxylases, based on models proposed by Hayashi (1995) and Brooks and Phillips (1997). Different tautomeric forms could be found in some stages among different families.

In vivo, mammalian HDC seems to be as unstable as mammalian ODC (Bartholeyns and Bouclier, 1984; Endo, 1989; Tanaka et al., 1997). In addition, the primary translation product of HDC mRNA (74 kDa) must be processed to a shorter polypeptide (Yamamoto et al., 1990, 1993). Controversy exists on the final size, in the 53 to 69 kDa range of the mature polypeptide in mammalian tissues (Yamamoto et al., 1990, 1993; Dartsch et al., 1998, 1999; Fajardo et al., 2001a). Experimental data show that essential residues for enzymic activity must be located in the primary structure betweeen residues 69 (Engel et al., 1996) to 480 (Yamamoto et al., 1990, 1993). In any case, proteolytic mechanisms must operate for both processing and rapid degradation of the enzyme. The mechanisms operating for both processes and the exact points for HDC cleavage during processing are not elucidated yet.

We have detected at least two PEST regions in every mammalian HDC (Figure 6) described so far (Viguera et al., 1994). The first one is located between residues 40 to 70. At least another PEST region is found after residue 500 in mammalian HDCs. From

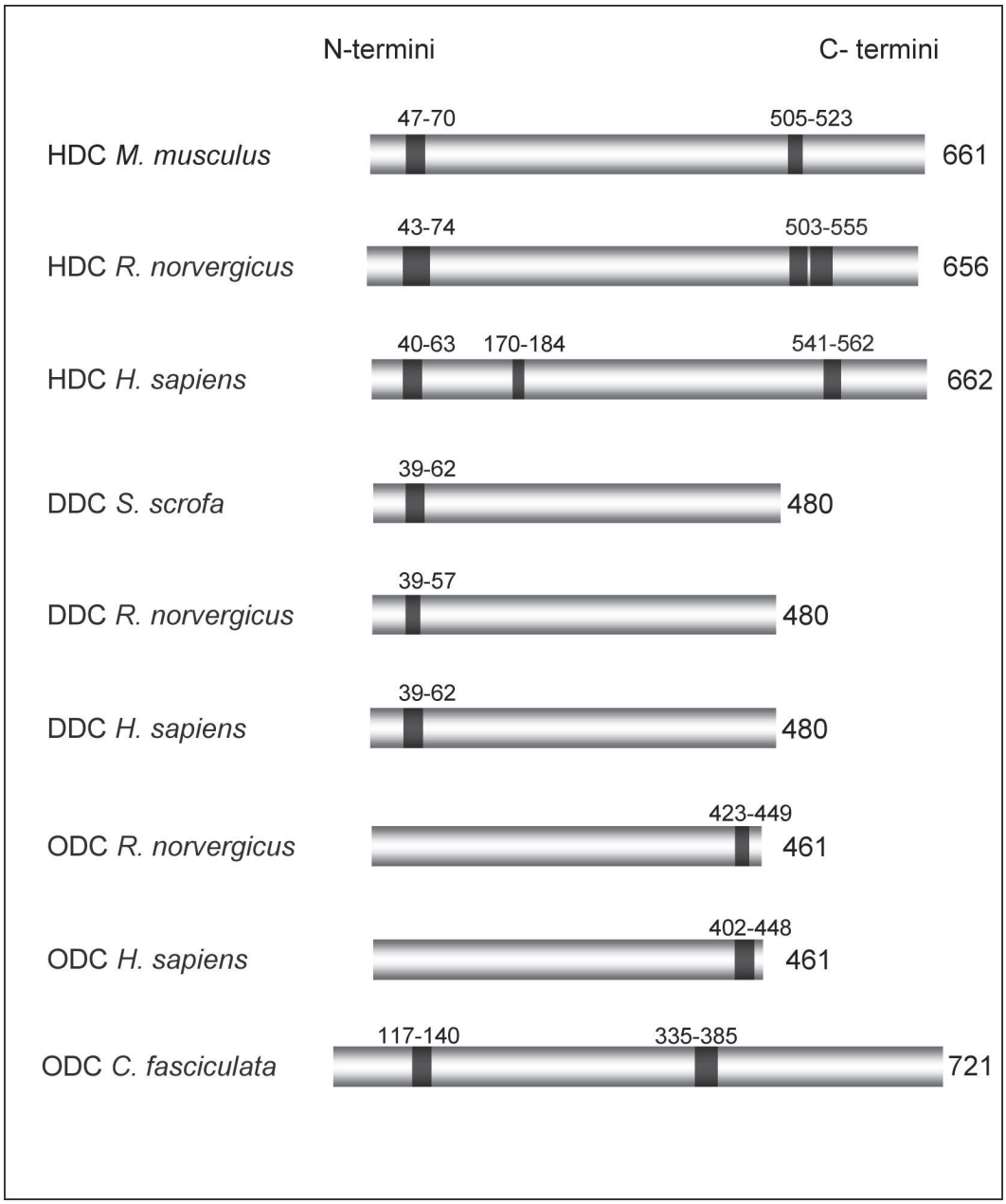

FIGURE 6. The position of the PEST regions in eukaryotic DDC, HDC, and ODC.

in vitro proteolysis experiments with commercial proteases, Ichikawa's group postulated that HDC should be processed *in vivo* by cleavage at some point around residue 480, but this fact is not fully proven yet (Yamamoto et al., 1990, 1993; Tanaka et al., 1997; Suzuki et al., 1998). Working with deletion mutants of rat HDC expressed *in vitro* (Olmo et al., 2000), we have observed that the N-terminal PEST region of HDC, but not the C-terminal ones, is involved in ATP-dependent degradation of both 1–656 and 1–512 HDCs (Olmo et al., 2000). The C-terminal PEST regions of rat HDC cannot replace that of ODC in its central role on the turnover of this protein (Olmo et al., 1999). The importance of C-terminal PEST regions in mammalian HDC has been studied by Fleming and Wang (2000). At the moment, the available data seem to indicate that PEST region-induced degradation of mammalian HDC is an ubiquitin-dependent process and no "HDC antyzme" has been detected. Recently, an unstable ODC with an N-terminal PEST-region has been described in *Critidia fasciculata* (Svensson et al., 1997), and its degradation via proteasome 26S is not antizyme dependent, but ubiquitin dependent. Thus, both eukaryotic ODC and HDC present N- and/or C-terminal PEST regions involved in their turnover rates (Figure 6).

B. Transferases

Spermidine/spermine N^1-acetyltransferase (SSAT), the key enzyme in the polyamine interconversion, is also inducible and has a rapid turnover in mammalian cells (Casero and Pegg, 1993). SSAT activity controls the catabolism and excretion of polyamines. This enzyme catalizes the acetylation of the primary amino group using acetyl CoA as the acetyl donor. Acetylation marks polyamines for their reutilization in the synthesis of other polyamines or their oxidation for excretion.

In normal tissues, SSAT activity is very low, and therefore the regulation of this enzyme under cellular conditions where it is not induced has been very difficult to study (Pegg, 1986). SSAT protein has a very short half-life, and it is degraded through the ubiquitin/proteasome pathway (Coleman and Pegg, 1997). Studies of SSAT induction have provided evidence of regulation at different levels: gene transcription, mRNA translation and stabilization, and protein degradation. Spermidine and spermine induce SSAT activity (Erwin and Pegg, 1986). The depletion of putrescine or treatment with difluormethylornithine, an inhibitor of ODC, provokes a decrease of enzyme activity and expression of its mRNA (Pegg and Erwin, 1985; Shappell et al., 1993). The induction of SSAT gene transcription appears to be modulated through the association between the nuclear protein factor Nrf-2 and a *cis*-element described as the polyamine-responsive element (PRE) (Wang et al., 1998, 1999). We have shown that histamine can enhance the effect of "superinducers" on SSAT activity (Fajardo et al., 2001b). Recent data suggest that SSAT may play a role in activity-dependent neuronal plasticity and development (Ingi et al., 2001).

Catechol-*O*-methyltransferase (COMT) catalyzes the transfer of the methyl group of *S*-adensylmethionine to one of the hydroxyl groups of a catechol subtrate. The general function of COMT is the elimination of biologically active or toxic catechols and some other hydroxylated metabolites. COMT activity also regulates the amount of active dopamine and noradrenaline in the brain, intestine tract, and kidney (Mannisto and Kaakkola, 1999). There is one single gene for COMT, which codes for both soluble (S-COMT) and membrane-bound COMT (MB-COMT) (Lundstrom et al., 1995). COMT is not easily induced or

supressed. Some special treatments or situations may increase COMT activity, but most of them cause at the best only a doubling of activity (Mannisto and Kaakkola, 1999).

Histamine-*N*-methyltransferase (HNMT) catalizes the Ntau-methylation of histamine. Metoprine is the most commonly used specific inhibitor of HNMT (Lecklin et al., 1999). It is known that HNMT is inhibited by biogenic amines, the most potent being tyramine and tryptamine (Fuhr and Kownatzki, 1986). Several mammalinan HNMTs have been cloned, including those from rat (Takemura et al., 1992), guinea pig (Kitanaka et al., 2001), mouse (Wang et al., 2001), and human (Girard et al., 1994). In humans, HNMT is highly expressed in the kidney and shows several common genetic polymorphisms that alter its activity (Preuss et al., 1998). One of these polymorphisms is Thr105Ile, which decreases enzymatic activity and is a risk factor for asthma (Pang et al., 2001). Very recently, two ternary structures of human HNMT have been determined: the Thr105 variant complexed with its substrate histamine and reaction product *S*-adensylhomocysteine, and the Ile105 variant complexed with an inhibitor (quinacrine) and *S*-adenosylhomocysteine (Horton et al., 2001). This structural study concludes that human HNMT has a 2 domain structure, including a consensus *S*-adenosylmethionine binding domain, where the residue 105 is located on the surface, consistent with the kinetic data that the polymorphism does not affect overall protein stability at physiological temperatures but changes the affinity constant values. Using the threading method and molecular dynamics simulations in water, a theoretical 3-dimensional model of human HNMT has also been reported that reveals that the polymorphic 105 residue is located in the turn between a beta strand and an alpha helix on the protein surface away from the active site of the enzyme and that Ile105 energetically destabilizes folded HNMT, promoting the formation of misfolded proteins that are prone to clearance by proteasome (Pang et al., 2001). These data support the hypothesis that the decreased HNMT activity for allozyme Ile105 is due to a decreased concentration of allozyme Ile105 and not to changes in the active-site topology. This is a good example of how bioinformatics tools can contribute significantly to a deeper knowledge of the structure/function relationships of biomolecules. These results also indicate that proteasomal activity is important for both histamine synthesis and degradation.

C. Amine Oxidase

Oxidation of biogenic amines and polyamines can be catalyzed by different types of amine oxidases. The Enzyme Nomenclature Committiee of the International Union of Biochemistry and Molecular Biology lists four EC numbers: EC 1.4.3.4 amine oxidase (flavin-containing) (trivial names: monoamine oxidase, amine oxidase, and tyramine oxidase), EC 1.4.3.6 amine oxidase (copper-containing) (trivial names: diamine oxidase, amine oxidase, and histaminase), EC 1.4.3.10 (putrescine oxidase) and EC1.5.3.11 polyamine oxidase. Traditionally, amine oxidases are divided into two main groups based on the cofactor attached. One group has flavin-adenin dinucluotide (FAD) and includes both monoamine oxidases (MAO) and polyamine oxidases (PAO). The second group has one or more carbonyl groups, which appears to be topaquinone (TPQ) in most cases; they are usually called copper-containing semicarbazide-sensitive amine oxidases (SSAO) and include diamine oxidases (DAO), cell-surface and soluble SSAOs, and extracellu-

lar lysyloxidase (Jalkanen and Salmi, 2001). Several amine oxidases have been cloned and structurally characterized. FAD- and the TPQ-containing amine oxidases do not only differ in their cofactors, but they present differences in subcellular distribution, inhibitors, and biological function (for recent reviews see Jalkanen and Salmi, 2001; Binda et al., 2002).

Diamino oxidase and related copper-containing amine oxidases are also able to eliminate putrescine and other polyamines from many tissues by oxidative deamination (Seiler and Douaund, 1998). Some of them, like serum amino oxidase, are able to accept both as substrates. Polyamine oxidation products of this activity (acrolen and ROS) are strong cytotoxic agents inducing apoptosis (Fajardo et al., 2000; Sharmin et al., 2001).

VI. TRANSPORTERS AND COMPARTMENTATION

As for every other metabolic pathway, the steady state intracellular concentrations of biogenic amines and polyamines are influenced by the regulation of the key activities of their endogenous biosynthetic and catabolic pathways (as mentioned above), but also by the regulation of transporters for their uptake and release. On the other hand, the storage of very important quantities of amines into vesicles of their producing cells is a well-known fact for di- and monoamines, and recently it has also been proposed for arginine/ornithine-derived polyamines (Aziz et al., 1998; Cullis et al., 1999). Since most of the mono- and di-amines are involved in intercellular communication, the stored amines are selectively secreted by each cell type in response to very diverse stimuli. In a few minutes, an amine-handling cell could change its intracellular pool of amine by several orders of magnitude. This fact and the lack of good *in vitro* models for cellular and molecular characterization of these processes (mentioned above) have made it very difficult to control the metabolism of these compounds in their respective producing cells. Thus, most of the pharmacology developed around biogenic amines is devoted to block or mimick the effects of these amines on the target cell receptors, as several receptors have been described, cloned, and characterized and specific agonists and antagonists are available (Del Valle and Gantz, 1997; Leurs et al., 1998; Van der Goot and Timmerman, 2000; Brown et al., 2001; Stark et al., 2001). In general, important questions still remain to be clarified on the compartimentalization of biogenic amines and the involved transport system, especially in the case of arginine/ornithine-derived polyamines. A better characterization of the transporters could make a different way of pharmacological intervention possible in pathophysiological processes related to biogenic amines and/or polyamines.

Two different vesicular monoamine transporters have been structurally characterized (Eiden, 2000). Organic inhibitors highly specific for these transporters could provide a promising way to image *in vivo* nerve terminals and to study synaptic patency in human brain in neurodegenerative and other diseases (Kuhl et al., 1996; Efange, 2000). Different reuptake systems for serotonin, dopamine, and noradrenaline expressed in the plasma membrane of amine-handling cells have also been cloned and characterized (Iversen, 2000; Miller et al., 2001; Oh et al., 2001).

The state of the art in the research on polyamine transporters is very different. In animals, different plasma membrane transport systems for arginine/ornithine-derived

polyamines have been detected and their kinetics, substrate specificities, and ionic and osmotic dependences have been described (for a review see Seiler et al., 1996). In the same way, some kinetics and bioenergetic aspects of intracellular organelle polyamine transporters have been studied (Toninello et al., 1992). However, multiple efforts to clone these transporters in animals based on homology with those previously cloned in prokaryotes and yeasts (Igarashi and Kashiwagi, 1999; Tomitori et al., 1999, 2001) have not been successful yet. Several authors have claimed for a particulated internalization of exogenous polyamines (Aziz et al., 1998; Cullis et al., 1999), so that the transport system could share characteristics of membrane receptors. Whether spermidine/spermine uptake is mediated by a membrane receptor-like protein is a very suggestive hypothesis that needs further work.

In any case, a full characterization of plasma membrane and intracellular polyamine transporters should be a priority in polyamine research for at least two reasons: (1) antiproliferative strategies based on the inhibition of polyamine synthesis have frequently been unsuccessful, due to the upregulation of the polyamine uptake in polyamine-deprived cells (Babal et al., 2001). A simultaneous intervention in both processes would be a more promising strategy. The characterization of the transporters and their evolutionary lineage will help the development of specific inhibitors. (2) Questions still pending around polyamine transport systems block a comprehensive view of polyamine compartmentalization, which is a major problem to achieve clear conclusions on the physiological/metabolic meaning of intracellular polyamine content determinations.

Most probably, we should change our mind and strategies. Perhaps the full elucidation of these transport systems and their cellular compartmentalization needs the cooperation of different approaches: location of putative sequences in genome data banks, protein modeling analysis of these primary sequences, overexpression of these proteins in homologous or heterologous cell models, standard kinetic analysis, and biophysical/cell biology methods for *in situ* detection of biomolecules.

VII. INTERACTIONS WITH BIOMACROMOLECULES

From a chemical point of view, all of these amines have at least a positive charge bound to a carbon skeleton (or heterocycle) able to establish van der Waals interactions with other molecules. Due to their cationic nature, they could interact with biopolyanions (phospholipids, proteins, and nucleic acids, among others). Most of the biological roles of these compounds are exerted through these interactions. Due to the advances in molecular modeling and macromolecular structure data bases, bioinformatic approaches also seem to be a promising complement to this area of research. Due to the small size of amines, possibilities of interactions with different biomolecules must be extremely high, most of them being unspecific for any other inorganic cation present in the cell at higher concentration. Consequently, these bioinformatic approaches should be bound to biophysical experimental studies, to distinguish specific from nonspecific amine interaction modes and conformational changes caused by these compounds on macromolecules.

A. Interactions with Nucleic Acids

The discovery of high levels of ornithine decarboxylase and polyamines in rapidly growing cells suggested to investiga-

tors the idea that polyamines could directly interact with nucleic acids (Tabor and Tabor, 1984; Heby and Persson, 1990). Further studies even indicated that the interaction with nucleic acids could explain some of the important biological functions of polyamines (Cohen, 1998). This fact encouraged investigators to deeply analyze the binding possibilities between DNA and the biogenic polyamines, using the available techniques to study interacting systems (summarized in Table 3).

Up to now, the studies aimed to describe details of the DNA-polyamines interaction have been largely performed *in vitro*. This fact has given rise to a disagreement among the proposed binding models based on the aforementioned tools. Early experimental works were related to the influence of spermidine and spermine on DNA pre-

TABLE 3
Techniques Used for the Study of Polyamine/Nucleic Acid Interactions

Technique	Application	Commentary
Vibrational spectroscopy	• Structural details of molecular interactions, specially Raman.	• Great amount of spectral information available • Technique to study molecules in aqueous solution
Electronic spectroscopy	• Structural information at macromolecular level (secondary and tertiary structures), especially electronic circular dichroism..	• It requires concentrations lower than Raman spectroscopy.
Magnetic resonance spectroscopy	• To investigate the nature of the DNA-drug complexes and the drug mobility in DNA.	• More applied for proteins than nucleic acids. • To perform studies in solution.
X-ray diffraction	• The best method to know how two molecules are bound.	• It was the first technique used in this topic. • Necessity of growing suitable crystals. • Not all interactions are valid in solution.
Theoretical studies	• To check the energetic availability of the models proposed on the basis of the experimental data.	• They use molecular and quantum calculation mechanics.
Thermodynamical studies	• To study the stability of biological macromolecules.	• For a quantitative description of chemical interaction by thermodynamical parameters. • Problems for the thermodynamical interpretation of data.

cipitation and thermal denaturation (Tabor, 1962; Tabor and Tabor, 1984). They were found to precipitate and stabilize DNA. These effects were immediately attributed to the electric charge neutralization, giving rise to binding models based on the phosphate-amino electrostatic interaction (Tsuboi, 1964; Liquori et al., 1967; Suwalsky et al., 1969).

In order to determine the exact position of spermine when interacting with DNA, several laboratories focused on the crystallization of the complexes to be analyzed by X-ray diffraction. Drew and Dickerson obtained crystals of the dodecamer d(CGCGAATTCGCG) in the presence of spermine (Drew and Dickerson, 1981). The X-ray spectra found spermine molecules attached to phosphates across the oligonucleotide major groove, which supported the electrostatic models. However, the inner protonated secondary amine groups were found to interact with the base residues, which supported the existence of structural specifities. An outstanding fact is that the dodecamer was crystallized in the B form, which is the natural conformation of DNA in solution. Other X-ray studies involved the A and Z conformations of different oligonucleotides (Jain et al., 1989; Egli et al., 1991). Spermine molecules were bound to bases in the minor groove of the A-form octamer d(GTGTACAC). The formation of left-handed Z-DNA structures usually has been related to specific DNA sequences, in which the alternating GC sequence was predominant (Drew et al., 1980; Wang et al., 1981). In these cases, spermine was able to change the secondary structure of the oligonucleotide from B to Z. X-ray studies on the complex d(CGCGCG)-spermine demonstrated the binding of this polyamine to guanine bases (Egli et al., 1991). Spermidine and putrescine were found to be less effective in inducing structural changes in DNA sequences (Schellman and Parthasarathy, 1984).

X-ray diffraction studies allowed the confirmation of the existence of specific binding sites of the polyamines in DNA. However, the results were not directly extended to the interaction in physiological conditions. Nuclear magnetic resonance (NMR) studies in solution showed that the spermine molecules still had high mobility in their complexes with DNA (Wemmer et al., 1985; Andreasson et al., 1993, 1996). Nevertheless, they did not exclude the possibility of specific binding sites. An interesting result obtained from electron paramagnetic resonance (EPR) indicated the ability of spermine to protect DNA from reactive oxygen species-induced damage (Ha et al., 1998).

A great amount of work has been carried out in recent years concerning DNA-polyamines studies using both electronic absorption (UV) and circular dichroism (CD) spectroscopies. Many of them were performed on selected oligonucleotide chains. Thus, studies on alternating purine-pirimidine sequences have demonstrated that polyamines interact differently with GC and AT sequences (Marquet and Houssier, 1988). Spermine was found to induce a B to Z conformational transition on GC-rich oligonucleotides, while it exhibited a lower affinity for AT-rich regions (Thomas and Messner, 1986; Hasan et al., 1995). On the other hand, an anormal increase of CD signal has been observed for moderate-sized DNA chains in the presence of spermine and spermidine (Thomas et al., 1996). This feature is evidence of highly ordered structures in solution, which has suggested the formation of a new tertiary structure named as Ψ-DNA, formed by the aggregation of alternating DNA and polyamine chains, similary to a liquid crystal or cholesteric phase (Grosberg et al., 1986; Reich et al., 1994). An interesting result was obtained from CD and UV studies of the binding between the estrogen receptor (ER) and the

estrogen response element (ERE) in the presence of biogenic polyamines (Thomas et al., 1997, 1999). These studies have shown that polyamines modulate the binding ER-ERE. It is possible that polyamines can modulate other *cis*/*trans* element bindings, and this would explain their role in gene regulation.

DNA-polyamine interactions have received relatively little attention in the past from the vibrational point of view. Nowadays, the combination of Raman and Fourier transform (FT) spectroscopies has allowed studying biological systems in physiological conditions. Recent FT-Raman studies have supported the role of the structural specificities on the DNA-polyamine binding (Ruiz-Chica et al., 2001a). These results have confirmed the preference of spermine to interact with DNA by the major groove, while spermidine and putrescine would interact by the minor groove. Predominant involved reactive sites were purine-N7, thymine-O2, and thymine-CH_3 positions (hydrophobic contacts) at the major groove, and purine-N3 and pyrimidine-O2 positions at the minor groove. Electrostatic attractions between phosphates and protonated amino groups were also supported. Other authors have suggested, on the basis of Raman measurements, that phosphates could be primary targets of interaction of DNA with spermine and spermidine, although they did not exclude interaction by the bases (Deng et al., 2000). Concerning GC sequences, Raman evidence of the formation of polyamine-induced aggregates (Ψ-DNA) has been achieved (Ruiz-Chica et al., 2001b, 2001c). The different affinity of the polyamines for GC and AT sequences has also been demonstrated. Therefore, no cholesteric phases were observed by Raman spectroscopy for a 15-mer alternating AT oligonucleotide (Ruiz-Chica et al., unpublished results). These differential effects on conformation of GC- and AT-enriched sequences could be related *in vivo* to the observed role of polyamines on the regulation of gene expression and cell survival, because the role of these sequences on nucleic acid dynamics is somehow the opposite. GC-enriched sequences are frequently involved in macromolecular aggregation, while AT-enriched sequences are related to open complex formations.

Some laboratories have applied theoretical methods to the DNA-polyamine interaction. Feuerstein et al. (1986, 1991) used molecular modeling calculations to study DNA-spermine models, which predicted preferential interaction by the major groove. They also studied the binding of spermine with several oligonucleotide chains (Feuerstein et al., 1990). The results indicated different affinities for different base sequences. Recent works based on molecular dynamic calculations have suggested that the binding of spermine by the DNA major groove is limited to GC-rich regions, thus proposing preferential interaction by the minor groove in alternating AT sequences (Korolev et al., 2001). Theoretical models have supported the experimental evidence that spermine can act as a DNA protector against radiative breaking (Sy et al., 1999).

Despite the great amount of experimental and theoretical works carried out up to now, we cannot give an undoubted explanation about how the polyamines bind to DNA. Probably, there is no single way of interaction, and it would be better to talk of preferential binding sites, which will not be identical for the three biogenic polyamines. However, it seems to be widely accepted that the models exclusively based on electrostatic attraction cannot give response to all the proposed questions.

It seems that most of intracellular polyamines are mainly associated RNA molecules (Igarashi and Kashiwagi, 2000). Thus, more specific theoretical and experimental studies of polyamine-RNA binding

are required. On the other hand, it has been suggested that other biogenic and synthetic amines could also bind to nucleic acid because it became evident that nucleic acid and the biogenic polyamines interact. This is also a research area deserving further efforts. DNA-histamine interactions are currently being studied by both experimental (Raman, CD) and theoretical (QM-MM) techniques. These possibilities are not constrained to groove and phosphate interactions, but the presence of an imidazole ring could allow histamine to intercalate between adjacent base pairs (Ruiz-Chica et al., 1999). This has been demonstrated for some histamine analogs with aromatic moieties (Medina et al., 1998). The antihistaminic chlorpheniramine can bind to both DNA and RNA, affecting protein synthesis, in general, and ODC translation, in particular (Medina et al., 1995, 1998). Much work still remains to be done to characterize the effects of biogenic amines and their analogs as gene expression modulators.

B. Interactions with Proteins

As mentioned above, intracellular polyamines seem to be mainly associated to nucleic acids. Nevertheless, the quantitatively minor binding of polyamines to proteins does not mean that studies on polyamine-protein binding cannot be relevant. In fact, the most important proteins in the regulation of cell proliferation/differentiation are not abundant. Due to the chemical nature of polyamines and other biogenic amines — a hydrophobic backbone with 1–4 positive charges separated by 3 to 5 Å — it is hard to think that they are only able to bind to a few set of proteins reviewed recently, in the case of polyamines (Igarashi and Kashiwagi, 2000). As mentioned before, many different membrane receptors, and some intracellular binding proteins, have been described for other biogenic amines. It is noteworthy that polyamines are also ligands of some of them.

It should be taken into account that biogenic amines (including polyamines) can also covalently bind to other molecules. It can also be mentioned that some transglutaminase activities are able to use both polyamines and histamine as substrates for posttranslational protein modification (Piacentini et al., 2000) These protein modifications seem to be related to the regulation of cell death, cell mobility, and tumor invasion, among others (Melino and Piacentini, 1998; Haroon et al., 1999; Lesort et al., 2000; Facchiano et al., 2001).

VIII. FUTURE PROSPECTS

Current knowledge about natural amines has risen from different biomedical areas (mainly neurobiology, immunology, food sciences, and oncology). As molecular and biochemical bases are being characterized, some common features are being revealed concerning their properties of binding to nucleic acids and proteins, the structures and the reaction mechanisms of the metabolism-related protein, and the regulation of their metabolic pathways. It is clear that all of these natural amino acid-derived polycations have evolved to play distinct physiological roles; however, from a biochemical point of view, Nature uses some similar patterns to manage the intracellular concentrations of these compounds. Humans also want to control amine levels, since these compounds are related to the very important pathologies; so, useful strategies for controlling metabolism or biological effects of any of these amines could also be beneficial for the intervention in biochemistry of the others.

In the polyamine field, two activities have been chosen as the most promising targets for polyamine depletion as an antitumoural strategy: inhibition of the key biosynthetic enzyme ODC (Batholeyns, 1983), and induction of the key degradative enzyme of higher polyamines SSAT (Chen et al., 2001). However, for pathologies related to products of group II decarboxylases (histamine, serotonin, dopamine, etc.), the major efforts have been devoted to interfere with (antagonists) or mimick (agonists) the reception of the amines by the target cells. They are two different strategies that should be considered complementary in both fields. In fact, one of the major problems for success in antitumoral strategies based on polaymine depletion comes from the fact that low intracellular levels of polyamines lead to an increased polyamine uptake from the extracellular medium (coming, for instance, from diet). Some polyamine analogs have been synthetized acting as blockers of polyamine uptake (Tomasi et al., 1998) that could be a perfect complement in anticancer therapies based on polyamine depletion.

In addition, for years enough knowledge about group II decarboxylases has been accumulated to start thinking about a selective inhibition of these homologous enzymes (Fleming and Wang, 2000; Bertoldi et al., 2001; Burkhard et al., 2001; Olmo et al., 2002). The development of specific inhibitors against group II decarboxylases could take into account one or more of the following facts: (1) dimerization seems to be necessary for these activities, so that blocking/promoting dimerization by either low molecular inhibitors or peptides should avoid/increase biogenic amine synthesis. In fact, in the case of ODC, a similar regulatory mechanism is carried out by the natural inhibitor antizyme. (2) At least for ODC and HDC, proteolysis plays an important role in short-term regulation of the intracellular levels of these enzymic activities (Hayashi et al., 1996; Tanaka et al., 1997; Fleming and Wang 2000; Olmo et al., 2001). In the case of HDC, in addition to proteasomal degradation, calcium-dependent proteolysis also seems to control the intracellular levels of this protein (Rodríguez-Agudo et al., 2000). This is an important point that deserves a deeper characterization, because calcium movements also regulate histamine secretion. (3) Slight differences have been detected among human group II decarboxylases at mechanistic level, which could provide the bases for selective inhibition. HDC seems to have a very specific catalytic site being the less efficient of all of the mentioned human L-amino acid decarboxylases (Olmo et al., 2002). Among these mammalian enzymes, only the structure of pig DDC has been solved (Burkhard et al., 2001). However, bioinformatics (protein modeling and molecular dynamics programs) could provide very useful tools for structure/function relationship studies of the others and for a rational design for inhibitors. (4) The actual levels of any enzymic activity in a given cell can be manipulated at the level of the catalytic mechanism, controling degradation or processing of the catalytic polypeptide or, alternatively, by interfering with its gene expression. However, the last strategy appears to be still distant, since the tissue-specific mechanisms acting for regulation of L-amino acid decarboxylases are still very badly characterized. Most probably functional genomic approaches will enormously facilitate this task.

We mentioned above that amine degradation pathways involves the action of different amino oxidases. We can suspect that they will become very promising biotech tools when their intracellular location, tissue-dependent regulation of expression, and turnover have been characterized. The best strategy to avoid the negative effect of any compound is to promote its metabolic degradation. However, in this case, we have to

take into account that all of these activities produce reactive oxygen species (ROS), which are toxic compounds; in addition, some of them also produce very toxic aldehydes, as is the case of acrolein produced from higher polyamines by serum amino oxidases. Thus, strategies leading to the overexpression of such an amino oxidase could not only lead to depletion of these compounds, but also to a selective death of polyamine-producing cells. This could be a promising approach against undesirable proliferating cells. This biotech strategy still needs further cell and molecular biology knowledge to make coincident substrates and enzymes in the same intracellular compartments. It is well known that polyamines bind to nucleic acids. At least *in vitro*, it has been observed that polyamines facilitate diamine oxidase binding to DNA (Bruun et al., 1998). If this fact could be mimicked *in vivo*, polyamines, which are essential compounds for cell proliferation, will turn to be enzymic-activated antiproliferative agents (ROS and toxic aldehydes) in the nucleus of the transfected cells.

The full characterization of intracellular amine compartmentation is not a solved topic either. There are two major noncovalent mechanisms used by cells to counteract the intracellular accumulation of these polycations: storage into vesicles (associated to polyanions like heparine) and binding to nucleic acids. The occurrence of the former mechanism in the case of biogenic amines is well known, and the occurrence of the latter for polyamines. However, recent reports indicate that polyamines could also be accumulated into vesicles (Cullis et al., 1999) and biogenic amines and their analogs could also bind to nucleic acids (Ruiz-Chica et al., 2001a). Both kinds of insights are interesting, especially in cell types in which both kinds of amines can be handled simultaneously. Polyamines, histamine, and serotonin have a 1,4-diamine structure and a hydrophobic carbon skeleton. At least one of these amino groups are charged, being able to interact with polyanions. There are several reports indicating that changes in the intracellular concentration of one of these amines influences the intracellular content of the others, suggesting some replacement of binding sites among them. Because the physiological roles of these amines are different, this kind of replacement in intracellular storages could also have important biological consequences. This fact can have a major physiological sense *in vivo* during immunological response to cancer cells or proliferating parasites, since both histamine and polyamines can be simultanously present in cells and/or in the extracellular medium. Due to the relative simple chemical structures of these compounds, there are important problems to distinguish specific from unspecific binding to other biomolecules. Modern biophysical techniques, such as vibrational spectroscopy, circular dichroism, and X-ray crystalography could help us to elucidate *in vitro* these binding modes and the affinity of different polycations by the same binding sites. Protein arrays could also be very useful for the characterization of the highest affinity binding sites and replacement from these binding sites by the others. Fluorescence correlation spectroscopy (for a review on the applications of this technique to biological problems see Medina and Schwille, 2002) could also provide a very valuable tool for the visualization of intracellular compartmentation of these compounds *in vivo*, since some fluorescent analogs of amines have been synthetized, apparently keeping the binding properties of the natural compounds (Cullis et al., 1999).

Among the natural polyanions that are able to interact with natural amines, we remark the specific binding modes of higher polyamines to nucleic acids and the confor-

mational changes induced by them in genetic material. It is demonstrated that spermine, and spermidine to a lesser extent, are able to induce dramatic changes in nucleic acid structure (Ruiz-Chica et al., 2001a, b, c). They are able to condensate DNA as cholesteric crystal in which information can be stored and protected. This fact is being valued by bioinformatics looking for new possibilities of information storage modes that take up as little space as possible. On the other hand, in the condensated state, DNA is protected from natural degradation mechanism, so that some molecular biologists claim for the usefulness of polyamine-condensated DNA for the development of non-viral-based gene therapy strategies (Byk et al., 1998; Rudloph et al., 2000).

We have to take into account that biogenic amines (including polyamines) can also covalently bind to other molecules. The regulatory roles of these covalent modifications deserve to be studied more deeply.

Finally, we have to remark that a comprehensive understanding of biogenic amine and polyamine metabolism and physiology goes far beyond a detailed description of metabolic pathways and a reductionistic study of purified enzymes. In fact, enzymes, metabolites, and modulators are interconnected in the metabolic network in a very complex fashion. Theories such as Control Flux Analysis try to understand metabolic regulation in a comprehensive way, allowing us to know the actual contribution of each change in the concentrations of any enzyme or metabolite in the flux through the whole metabolic pathway (Fell, 1997). There is a lot of disperse information in the scientific literature that could be useful for modeling and quantitative studies of metabolic regulation with the help of informatic tools.

CONCLUDING REMARKS

Despite the important efforts to study and characterize the biochemistry and physiology of biogenic amines (including polyamines), many questions still remain to be answered before being able to control these processes completely in amine-handling cells and amine-target cells. The lacking information can provide multiple possibilities to control the undesirable effects of these amines for human and animal health and to exploit benefits of these molecules for biotechnological applications. We encourage researchers to look at the biogenic amine field in a more comprehensive way, since these compounds have different biological missions but similar chemical structure and metabolic pathways. It is time to include polyamines under the general name of biogenic amines, and most probably this open view could facilitate the answer to some of the pending questions.

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