Effect of Chemically Modified Polyamine Analogs on the Biosynthesis of Polyamines and Putrescine in a Cell-Free System of Rat Hepatoma

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Increasing attention is being focused on the metabolism of polyamines, because it may serve as a new systematic approach in experiments to devise antitumor agents. Potential and specific inhibitors have already been described for most of the enzymes of polyamine biosynthesis [12,13]. A search for prospective antitumor agents is also being actively pursued among the synthetic substrate- and product-like polyamines [3,8,14].

Considerable attention has recently been paid to nucleotide derivatives as possible pharmacological drugs for the treatment of cancer in human beings [1,3]. Structural analogs of polyamines modified by nitrogenous bases have been synthesized at the Riga Institute of Organic Synthesis to produce compounds which are able to form not only ionic but also hydrogen bonds with certain nitrogenous bases, attention being paid mainly to structural variation due to the length of polyamine aliphatic chain. Some of these compounds, for example, (uracil-1)-alkyldiamines, have been found capable of forming complexes with DNA and exhibit a certain antitumor activity, depending mainly on the length of the polyamine hydrocarbon chain [4,5]. Similar analogs of polyamines (for example, thyminylputrescine) have been isolated from bacteriophage nuclease-resistant DNA [16].

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However, there are no data in the literature available on the effect of similar nucleotide derivatives of polyamines on the biosynthesis of putrescine, spermidine, and spermine. In the present study two groups of polyamine analogs modified by nitroge ous bases (Table 1) were investigated for a quantitative evaluation of their effect on polyamine biosynthesis in a cell-free system of rat hepatoma G-27.

MATERIALS AND METHODS

The polyamine analogs (I-IX) (Table 1) were synthesized at the Institute of Organic Synthesis, Latvian Academy of Sciences (Riga). A well-known inhibitor of ornithine decarboxylase (ODC), α-difluorodimethylornithine (DFMO) (Sigma, USA), was used as a reference compound. The substances were weighed and dissolved in warm distilled water. The final concentration of analogs and DFMO in the reaction medium was 10^{-4} M.

Outbred male albino rats weighing 100-120 g were injected subcutaneously with 1 ml of a cell suspension from transplanted solid hepatoma G-27. The rats were killed by decapitation on the 21st day after inoculation. The tumor tissue was isolated, weighed, and used immediately for further investigation.

The tumor tissue specimens, pounded by a metal press, were homogenized (m/v = 1/2) in 50 mM K,Na phosphate buffer saline (pH 6.6) containing 0.1 mM dithiothreitol (DTT) (Serva, Ger-

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TABLE 1. Structure of Polyamine Analogs Modified by Nitrogenous Bases.

Group 1: β-D-Xyl_F-(1-9)-Ade-8-NH_2 -$(CH_2)_n$ -R

Symbol	Systematic Name (IUPAC)	n	R
I	8-(2-oxyethyl)amino-9-β-D-xylofuranosyladenine	2	-OH
II	8-(3-oxypropyl)amino-9-β-D-xylofuranosyladenine	3	-OH
III	8-(2-aminoethyl)amino-9-β-D-xylofuranosyladenine	2	$-NH_2$
IV	8-(4-aminobutyl)amino-9-β-D-xylofuranosyladenine	4	$-NH_2$
V	8-(5-aminopentyl)amino-9-β-D-xylofuranosyladenine	5	$-NH_2$
VI	8-(6-aminohexyl)amino-9-β-D-xylofuranosyl-adenine	6	$-NH_2$

Group 2: Ura-5CH_2-NH_2 -$(CH_2)_n$ -NH_2 -CH_2 -(Ura-5)

Symbol	Systematic Name (IUPAC)	n
VII	1,3-bis(Uracilyl-5-methylene)-trimethylenediamine	3
VIII	1,4-bis(Uracilyl-5-methylene)-tetramethylenediamine	4
IX	1,6-bis(Uracilyl-5-methylene)-hexamethylenediamine	6

many) and 0.4 mM pyridoxal phosphate (PDP) (Reanal, Hungary) and centrifuged at 20,000 g for 20 min. The supernatant was used for the analysis of the enzymes of polyamine synthesis.

The test system contained 5 mM K,Na phosphate buffer saline, 1 mM DTT, 0.2 mM PDP, 4 mM L-ornithine, 300 μl supernatant, and the test substances in a concentration of 10^{-4} M in a final volume of 0.5 ml. The mixture was incubated in the presence of the substances at 37°C for 1 h. The reaction was stopped by adding 0.1 ml of 0.2 M $HClO_4$.

The levels of polyamines and putrescine were determined by the microfluorometric method on the basis of thin-layer chromatography on Silufol-UV 254 plates (Kavalier, Czechoslovakia) [7]. ODC activity was measured as described previously [2]. The protein concentration was determined after Lowry [11] with our modifications [6].

RESULTS

The polyamine analogs investigated here (Table 1) were divided into two groups. The first group contained hydroxy- (I, II) and amine (III-VI) analogs modified by adenosine with various hydrocarbon chain lengths (*n* is the number of methylene groups). The second group consisted of substances VII-IX modified by two uracil residues with various aliphatic chain lengths.

The results on the effect of the test substances and DFMO on polyamine synthesis in the cell-free system from hepatoma G-27 are summarized in Tables 2 and 3.

It should be noted that the cell-free test system contained all the necessary components for polyamine synthesis. Substrate and cofactor of ODC (L-ornithine and PDP) were added to the postmitochondrial supernatant of tumor tissue homogenate (the source of the enzymes of polyamine biosynthesis and polyamines themselves) to trigger the chain of reactions of polyamine biosynthesis. Thus, polyamine synthesis in the cell-free system was limited only by the amount of adenosylmethionine (AM), and in the supernatant by the amount of adenosyl-methionine decarboxylase substrate, AMD.

TABLE 2. Effect of Analogs on Polyamine Levels in Cell-Free Test System from Hepatoma G-27 ($M \pm m$, $n=6$).

Substance	Polyamines, nmol/mg protein			
	putrescine	spermidine	spermine	Σ polyamines
Without incubation	3.2±0.1	4.2±0.2	4.1±0.2	11.5±0.5
With incubation	5.3±0.2	8.2±0.2	9.3±0.4	22.8±0.9
I	3.2±0.2	4.7±0.1	6.6±0.002	14.5±0.3
II	9.1±0.3	11.9±0.4	15.3±0.7	36.3±1.4
III	9.7±0.9	12.9±0.7	10.1±0.6	32.7±2.2
IV	7.6±0.3	8.4	12.9	28.9±0.3
V	9.4±0.2	4.0±0.2	9.4±0.1	28.8±0.4
VI	4.2±0.2	7.1±0.5	8.3±0.7	19.6±1.4
VII	3.7±0.2	4.8±0.2	3.5±0.02	12.0±0.4
VIII	3.4±0.3	1.5±0.1	7.3±0.1	12.2±0.6
IX	3.7±0.02	2.0±0.2	5.8±0.8	11.5±0.9
DFMO	3.7±0.3	8.4±0.4	8.1±0.2	20.2±0.9

The incubation of the test system for 1 h at 37°C without test substances resulted in a 2-fold rise of the levels of putrescine and polyamines (Table 2). The system thus contained a sufficient amount of AM, which seems to be associated with a disturbance of methionine metabolism in the tumor tissue, since in normal tissue the content of AM is very low [1].

The incubation of the test substances under the same conditions in the test system showed the following results.

Substances I, VI, VII-IX and DPMO inhibited the activity of ODC, the analogs of the second group VII, VIII, and IX being more powerful than DPMO, more or less completely suppressing the synthesis of putrescine and polyamines (Tables 2 and 3). The drop of the levels of spermidine and spermine below the initial levels in tumor tissue against the background of the effect of substances VIII and IX, and VII, respectively, suggests a possible activation of polyamine oxidase by these substances.

The substances of the first group except for I and VI stimulated the polyamine synthesis system. They increased the contents of polyamines and putrescine in the test system in comparison with the control by 1.5 times. Compound I from this group was capable of inhibiting ODC, but seemed not to affect AMD. Substance V was inert with respect to ODC, whereas it considerably suppressed spermidine synthesis. It can be assumed that substance V has the property of inhibiting spermidine synthase because of some resemblance between the structure of substance V and 9-adenosyl-1,8-diamino-3-thiooctane, an inhibitor of spermidine synthase [10]. Compound VI acted like substance I, though to a considerably lesser degree. A drop of the total level of biogenic amines and a decrease of ODC activity were observed under the influence of substances I, VII, VIII, and IX. Thus, these substances may be considered as potential inhibitors of polyamine biosynthesis that are even more active than DPMO, a well-known ODC inhibitor, with respect to rat hepatoma G-27.

On the other hand, it should be pointed out that the substances from the first group are modified by adenosine; the structure of the latter is analogous to the structure of AM derivatives, among which inhibitors of polyamine biosynthesis enzymes have been found [12,13]. However, among the substances tested just compound I had marked properties of an inhibitor of the key enzyme. When comparing the structure of the substances of the first group, we may conclude that a substance's ability to suppress ODC activity is likely to be related to the presence of a terminal OH group and two methylene groups in the chain. Substitution of an NH_2 group for OH resulted in the loss of these properties, whereas elongation of the aliphatic chain (from 2 to 6 methylene groups) decreased the degree of activation of polyamine synthesis and probably increased the ability to inhibit this process.

TABLE 3. Effect of Polyamine Analogs on ODC Activity in Cell-Free Test System from Hepatoma G-27 ($M \pm m$, $n=6$)

Substance	ODC, nmol/h/mg protein	Inhibition, %
Control	11.3±0.2	0
I	3.0±0.1	73.5
II	24.8±0.3	+120
III	21.2±0.4	+87.6
IV	17.0±0.1	+50.4
V	11.3±0.2	0
VI	8.1±0.3	28.3
VII	0.5±0.1	95.6
VIII	0.7±0.2	93.8
IX	<0.001	100
DFMO	8.7±0.2	23.0

Note. +: activation of enzyme, %.

Among the analogs of the second group no definite dependence of the inhibitory properties on the length of the aliphatic chain was found. However, from the structural resemblance of the compounds VII-IX to bis-(benzyl)polyamine analogs [9] it may be assumed that uracil residues are responsible for the properties of polyamine biosynthesis inhibition. The inhibitory properties of adenosine-containing substance I are likely to be less pronounced in the cell system due to hindered penetration into the cell [15]. Thus, it may be concluded that among all the new synthetic analogs tested, the compounds VII-IX, modified by two uracil moieties, will prove to be more promising candidates as potential antitumor drugs.

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Role of Extracellular Calcium in Endothelin-1-Induced Vasoconstriction

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In early studies of the mechanism of action of endothelin-1 (ET-1), a vascular endothelium-derived vasoconstrictor peptide, it was suggested that it produces an effect typical of an endogenous antagonist of voltage-dependent channels and that its activity as a vasoconstrictor depends on the concentration of extracellular Ca^{2+} and is suppressed in the presence of antagonists of these channels [10,11]. However, later this hypothesis was not accepted by all authorities. Discrepancies in the data obtained were due to determinations of the pathways of Ca^{2+} entry into the cell and of the intracellular pathways of signal transfer from receptor to effector (cell contractile proteins). In particular, it was thought [7] that the effect of ET-1 upon Ca^{2+} channels is not a direct one, but is mediated by the penetration of cations through nonselective ion channels and by membrane depolarization to the level critical for Ca^{2+} entry. The present paper is a continuation of our studies [1] of the mechanism of the ET-1 effect on vascular smooth muscle in rats.

In this study the role of extracellular Ca^{2+} ions in the contractile response was investigated, along with the pathways whereby they enter smooth muscle cells.

MATERIALS AND METHODS

The experiments were carried out on deendothelized smooth muscle preparations: helical strips of the thoracic aorta of Wistar rats. The contractility of the aorta strips was recorded with the aid of a DU-1 isometric force transducer on a Gemini recorder (Italy). The initial load was 1.5 g. The strips were placed in a thermostatically controlled chamber filled with normal oxygen-saturated Krebs solution of the following composition (mM): NaCl 130, KCl 4.6, $NaHCO_3$ 3.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, HEPES 10, and glucose 11, pH 7.4. Na-free Krebs solution was prepared by replacing NaCl with an equimolar amount of N-methyl-D-glucosamine (NMDG). Ca-free solution was prepared

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