cholamine hormones in the regulation of metabolism and to study the detailed mechanism of action of reserpine.

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Formation of a Dead Code Triplet through Replacement of the Terminal Ribonucleoside in Guanylyl-Uridylyl-Uridine and Guanylyl-Uridylyl-Cytidine by 6-Azacytidine

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SUMMARY

The binding of valyl-tRNA to ribosomes *in vitro*, stimulated by guanylyl-uridylyl-uridine and guanylyl-uridylyl-cytidine is not effected by guanylyl-uridylyl-6-azacytidine. This finding indicates the high chemical specificity of the pyrimidine components of the codon.

Some 6-azapyrimidine derivatives belong among effective inhibitors of the metabolism of nucleic acid components (1). 6-Azauridine 5′-monophosphate (2) and 6-azacytidine 5′-monophosphate (3) are potent inhibitors of orotidylic acid decarboxylase, 6-azauridine 5′-diphosphate inhibits polynucleotide phosphorylase (4), and 6-azauridine 5′-triphosphate inhibits RNA polymerase (5). It was found in the course of the study of the biological effects of 6-azapyrimidine derivatives that 6-azacytidine 5′-diphosphate, in contrast with 6-azauridine 5′-diphosphate, can be incorporated *in vitro* into polyribonucleotides with the aid of polynucleotide phosphorylase (6).

It was considered to be important to establish the consequences for the genetic code of replacing a pyrimidine base by 6-azacytosine in the active code triplet. To this end we used a comparative study of the coding properties of trinucleotides in a cell-free system (7). Under the given conditions it was suitable from the experimental point of view to apply triplets of guanylyl-uridylyl-uridine and guanylyl-uridylyl-cytidine which code for valine (8, 9) and to compare their coding properties with those of a triplet containing the 6-aza analog of the pyrimidine base.

For preparing the trinucleotides we used the specific enzyme reaction of guanosine

2′,3′-cyclophosphate with synthetic dinucleoside phosphates, as catalyzed by T1 ribonuclease (10). The trinucleotides prepared here were isolated from the incubation mixture by paper chromatography (Whatman No. 3, isopropyl alcohol–ammonia–water, 7:1:2) and purified by paper electrophoresis (Whatman No. 3, 0.1 M triethylammonium carbonate buffer of pH 8.5, 500 volts). The electrophoretic purification of the trinucleotides is essential as the substances isolated by chromatography always contain a considerable amount of guanosine 3′-monophosphate. For the enzymic preparation of the trinucleotides we had at our disposal uridylyl(3′→5′)uridine (11), uridylyl(3′→5′)cytidine and uridylyl(3′→5′)6-azacytidine (A. Holý and J. Smrt, unpublished results). The identity of the isolated trinucleoside diphosphates was confirmed by reversed enzymic cleavage by T1 ribonuclease and subsequent analysis of the cleavage products by paper chromatography. The trinucleoside diphosphates obtained showed the expected spectral characteristics in the UV region.

^{14}C-Valyl-tRNA was prepared from *Escherichia coli* sRNA by a procedure described for the preparation of lysyl-tRNA (12), and it contained 65,000 cpm per milligram of total sRNA. Ribosomes were prepared from *E. coli* by the conventional procedure (13). The binding of ^{14}C-valyl-tRNA to the ribosomes was carried out as described earlier (8). All the experiments were carried out twice (including the preparation of the trinucleoside diphosphates) with completely identical results.

The results of the stimulation of binding of valyl-tRNA to ribosomes by the triplets studied are presented in Fig. 1. As may be seen from the values obtained the replacement by 6-azacytidine of the terminal uridine or cytidine in the functional triplets of guanylyl-uridylyl-uridine or guanylyl-uridylyl-cytidine results in a complete loss of coding properties for valine. These results point to a high specificity of the structure of the pyrimidine components of the triplets even with regard to the third nucleotide which can generally be represented by any natural nucleotide in the coding triplets. It has not been tested whether the anomalous triplet can stimulate the binding of ribosomes to other aminoacyl-tRNA's besides the valyl-tRNA.

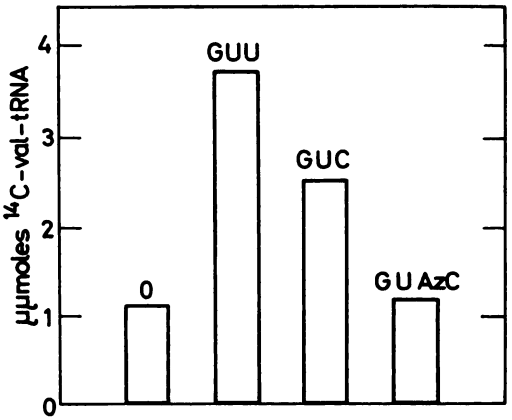

FIG. 1. *Stimulation of the binding of ^{14}C-valyl-tRNA to the ribosomes with the aid of trinucleoside diphosphates*

Incubation mixture composition: 45 μl mixture of equal volumes of 1 M Tris-HCl of pH 7.5, 0.2 M magnesium acetate, and 0.5 M KCl, 50 μl trinucleotide solution (always 0.5 A^{260} units), 25 μl ribosome suspension (2.5 A^{260} units) and 10 μl solution of ^{14}C-valyl-tRNA (95 μμmoles). Incubated for 20 min at 24° and filtered through a membrane filter with 0.3–0.5 μm pores; the filters were washed three times with a solution of 0.01 M Tris-acetate of pH 7.2, 0.02 M magnesium acetate, and 0.05 M KCl. Radioactivity was estimated in a 2π flow counter. Abbreviations used: GUU, guanylyl(3′→5′)uridylyl(3′→5′)uridine; GUC, guanylyl(3′→5′)uridylyl(3′→5′)cytidine; GUAz-C, guanylyl(3′→5′)uridylyl(3′→5′)6-azacytidine.

The specificity of the chemical structure of the pyrimidine components of the codon is suggested by findings (14, 15) showing the loss of coding properties for valine through replacement of uridylic acid by dihydrouridylic acid. It can be assumed that a dead triplet would also be formed on replacing the second nucleotide by 6-azacytidylic acid as follows from the investigation of alteration replacements of uridylic acid residues by a dihydrouridylic acid residue in guanylyl-uridylyl-uridine (15). On replacement of guanylic acid in guanylyl-uridylyl-uridine by 8-azaguanylic acid, a complete loss of the coding properties of the triplet was not observed (16).

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Increased Synthesis of Norepinephrine in the Rat Heart on Electrical Stimulation of the Stellate Ganglia

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SUMMARY

Electrical stimulation of the stellate ganglia results in increased formation of radioactive norepinephrine (NE) in the rat heart from L-tyrosine-^{14}C but not from L-dopa-^{3}H. These findings represent additional evidence that the activity of tyrosine hydroxylase, the rate-limiting step in NE synthesis, is increased by sympathetic nerve activity.

INTRODUCTION

The increased sympathetic stimulation associated with exercise or exposure to cold results in accelerated synthesis of norepinephrine (NE) and epinephrine in sympathetically innervated tissues of the intact

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