EFFECT OF CYTARABINE AND BLEOMYCIN ON THE PHOSPHORYLATION OF NUCLEAR ACIDIC PROTEIN

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1. Introduction

A single injection of isoproterenol into rodents causes a marked stimulation of DNA synthesis in the salivary glands after a lag period of 20 h, followed by a cell proliferation without hypertrophy [1,2]. Biochemical changes during the pre-replicative phase have been studied by using this model [3,4]. Recently attention has focused on nuclear non-histone proteins because of their possibility as regulatory molecules of gene activity in eukaryotic cells [5]. It is one of the most attractive properties of non-histone proteins that these fractions are phosphorylated actively and reversively. One of us (H.I.) reported that the phosphorylation of non-histone proteins which was catalyzed by chromatin associated protein kinase might be an important regulator in the control of DNA synthesis in the isoproterenol-stimulated rat submandibular glands [6,7]. Many investigators also have suggested this possibility by using several models of stimulated DNA synthesis [5]. The aim of the present investigation was to get insight on the role that cytarabine and bleomycin may have in the regulation of the phosphorylation of non-histone proteins with reference to DNA synthesis.

2. Material and methods

Male Sprague–Dawley rats, weighing 250–300 g, were used throughout this study. They were given standard laboratory diet (Oriental Yeast Company Ltd. Tokyo, Japan) and water ad libitum until sacrifice. Submandibular glands were pooled to isolate the nuclei according to the method described previously [6]. The purity of the preparation was checked and found to be the same as reported in other papers [6,8].

Nuclei (about 1 mg of protein) were incubated at 37 for 10 min in a reaction medium consisting of 6 mM $MgCl_2$, 115 mM KCl, 30 mM Tris–HCl buffer (pH 7.4), 1 mM Dithiothreitol and 3 mM ATP–Tris containing trace amount of [r-^{32}P]ATP, in a final volume of 2 ml. The specific activity of the ATP mixture in the reaction was generally 2000 dpm per nmol of ATP. The reaction was terminated by the addition of cold ethanol (final concentration 70% v/v). Histone and non-histone proteins were separated from the ethanol precipitated proteins and used to isolate phosphoproteins by the previous procedure [6]. The protein fractions were finally hydrolyzed overnight in 1 N-NaOH. Each sample was assayed for protein content by the method of Lowry et al. [9] and the radioactivity of the phosphate released was measured [6]. [r-^{32}P]ATP was prepared according to the method of Glynn and Chappell [10] and converted Tris salt by the method reported previously [11].

2.1. *Chemicals*

Isoproterenol–HCl was obtained from Aldrich Chemical Company Ltd. (Milwaukee, USA). Cytarabine and bleomycin were the gifts from Nippon Shinyaku Company Ltd. (Kyoto, Japan) and Nippon Kayaku Company Ltd. (Tokyo, Japan), respectively.

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3. Results and discussion

Rat submandibular glands nuclei incubated with [r-^{32}P]ATP incorporated rapidly ^{32}P into histone and non-histone proteins. A major portion of the radioactivity was present in the non-histone proteins. Following a single injection of isoproterenol, the incorporation of ^{32}P from [r-^{32}P]ATP into nuclear non-histone proteins decreased initially but begun to increase at 4 h, reaching a maximum at 24 h at a higher level compared with normal control, and returned to normal level at 40 h after the injection. On the other hand, the phosphorylation of histone proteins also declined after the injection of isoproterenol, but the rate of phosphorylation was not recovered until 16 h, reaching to the control level at 24 h after the injection. The increase of the phosphorylation of non-histone proteins caused by isoproterenol injection was found in both the initial and the total levels of phosphorylation. Further, it was also obvious that phenol-soluble acidic phosphoproteins associated with chromatin DNA were affected by the treatment of rat with isoproterenol (table 1).

Cytarabine [12] and bleomycin [13] given to rats causes the inhibition of the onset of DNA synthesis, but the metabolic events influenced by these drugs are not clearly known. And it is of interest to examine the effect of cytarabine and bleomycin on the phosphorylation of non-histone proteins stimulated at 24 h after the injection of isoproterenol. The result given in the table shows that both cytarabine and bleomycin at the doses 5 to 40 mg inhibit the incorporation of ^{32}P from [r-^{32}P]ATP into nuclear non-histone proteins. The inhibition of phosphorylation of histone proteins was not observed. The doses of these drugs used in this experiment were very low, thus the inhibitory effect of the phosphorylation of non-histone proteins can not be attributed to toxic effect of these drugs.

It is reported previously [6,7] that the treatment of rats with actinomycin D or cycloheximide, which block the stimulation of DNA synthesis caused by the injection of isoproterenol, inhibits the stimulation of phosphorylation of non-histone proteins at 24 h after isoproterenol. It is therefore, suggested that cytarabine and bleomycin inhibit the DNA synthesis by the block of the stimulation of phosphorylation of nuclear non-histone proteins.

Table 1
Effect of cytarabine and bleomycin on the incorporation of ^{32}P from [r-^{32}P]ATP into nuclear non-histone proteins of submandibular gland stimulated by isoproterenol

Treatment		^{32}P-incorporated (nmoles/mg of protein)	Per cent of control
None		1.38	
Ipr only		2.08	100
Ipr + ARA-C	(5)	1.70	82
	(10)	1.81	87
	(40)	1.61	77
Ipr + BLM	(5)	1.97	95
	(10)	1.91	92
	(20)	1.71	82
	(40)	1.59	76

Nuclei were prepared from rat submandibular glands after 24 h following the injection of Ipr (160 mg/kg body weight in 0.9% NaCl). ARA-C and BLM (in 0.9% NaCl, respectively) were given intraperitoneally 30 min before Ipr. The results were expressed as nmoles of ^{32}P per mg protein and were calculated from the specific radioactivity of ATP in the reaction. Each experiment was confirmed at least 3 times. All other experimental details were the same as described in the text. Abbreviations used in this table: Ipr, isoproterenol; ARA-C, cytarabine; BLM, bleomycin.

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