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EFFECT OF CORTISOL ON REPARATIVE SYNTHESIS AND METHYLATION OF RAT LIVER DNA

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KEY WORDS: cortisol; reparative synthesis; methylation of DNA.

Corticosteroid hormones are known to inhibit total DNA synthesis [9], to affect methylation of DNA [12], to activate transcription [6], and to increase the fraction of active chromatin [10] in rat liver cells. Against the background of general inhibition, some degree of activation of DNA synthesis is observed, coinciding in time with activation of transcription [6]. Amplification of the transcribed genes is possible under these circumstances [4], evidence for which is given by a change in the primary structure of DNA under the influence of the corticosteroid analog dexamethasone [3]. It has recently been suggested that demethylation of DNA by excision of 5-methylcytosine (MC), and also activation of nuclease, correlating with gene expression, can induce DNA injuries [1]. As a result of these injuries corresponding activation of reparative DNA synthesis may arise.

The object of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used and the experiments were done in April. Cortisol (Richter, Hungary) was injected intraperitoneally in a dose of 2 mg/100 g. To assess reparative DNA synthesis, hydroxyurea, which inhibits replicative DNA snythesis, was injected into the animals 1 h before sacrifice in a dose of 50 mg/100 g, and this was followed 10 min later by injection of [^{3}H]thymidine (55 Ci/mmole, USSR) in a dose of 100 μCi/100 g [5]. The animals were killed by decapitation. The freshly removed liver was quickly cooled on ice and homogenized in a Dounce homogenizer at 0°C in a solution containing 0.15 M NaCl, 0.1 M EDTA, pH 8.0, an equal volume of 1 N NaOH was added, and the mixture was allowed to stand for 12 h at 37°C, after which treatment of the material followed the usual lines [11]. The acid-insoluble residue was collected on membrane filters, washed with ethanol, and transferred to scintillation cuvettes. The filters were covered with 1 ml methylcellosolve and, a few hours later (after they had dissolved) 9 ml of toluene scintillator (4 g PPO + 400 mg POPOP to 1 liter toluene) was added and radioactivity was measured on a liquid scintillation counter.

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TABLE 1. Correlation between Methylation and Reparative Synthesis of DNA

Parameter	Control	Time after injection of hormone, h							
		0,25	0,5	1	2	3	4	6	8	10
Content of MC in DNA, moles %	1,24	1,20	1,23	1,24	1,29	1,23	1,08	0,89	0,82	0,90
Incorporation of ^{3}H-thymidine, cpm/mg DNA	201	288	270	269	198	299	303	320	388	361

Legend. The error in the MC content, expressed as standard deviation, did not exceed 0.07%. The error in counting radioactivity did not exceed 22 cpm.

TABLE 2. Content of Pyrimidine Sequences of Different Lengths in Rat Liver DNA

Experimental conditions	Pyrimidine isopliths, moles %						
	mono	di	tri	tetra	penta	hexa	7
Injection of hydroxyurea	11,7	9,7	8,7	6,1	4,8	2,9	6,1
Injection of hormone and hydroxyurea	11,9	9,5	8,9	5,6	5,0	2,6	6,5

Legend. Error did not exceed 0.4%.

The nucleotide and isoplith composition was determined by the use of DNA preparations [8] by thin-layer chromatography [2, 4]. To obtain each preparation a group of three animals was used. To obtain each value, three preparations were isolated.

EXPERIMENTAL RESULTS

As the data (Table 1) show, levels of reparative synthesis and methylation of DNA bore a reciprocal relationship to one another. Appreciable activation of reparative synthesis of DNA against the background of its demethylation is evidence in support of excision of MC followed by repair of the corresponding loci of the polynucleotide chain of DNA. The possibility cannot be ruled out that demethylation of DNA takes place through excision repair under normal conditions also.

Cortisol is a genic inducer. It can accordingly be concluded from the results that activation of gene expression is accompanied by activation of reparative DNA synthesis. Repression–derepression cycles of the genome of various cells, notably hepatocytes, in the body can periodically replace one another under the influence of a whole range of factors even, for example, depending on the time of day [7]. The frequency of repression–derepression cycles of the genome under these circumstances may correlate directly, because of the facts described above with the level of reparative DNA synthesis. We know that reparative DNA synthesis may be the source of genetic mistakes [13]. It can thus be concluded that different exogenous and endogenous factors acting on the body and capable of changing the level of gene expression in different types of cells can thereby accelerate the process of age accumulation of genetic mistakes in the body.

Changes in the nucleotide sequence of DNA similar to those arising under the influence of dexamethasone [3] were not found in the present investigation (Table 2). This is evidence that the amplification of the transcribed regions of DNA due to the hormone [4] takes place through replicative synthesis and can also be inhibited by hydroxyurea.

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EFFECT OF THE ANTITUMOR AGENT CIS-$Pt(NH_3)_2Cl_2$ AND ITS ISOMER TRANS-$Pt(NH_3)_2Cl_2$ ON LATENT ATPase ACTIVITY OF ISOLATED RAT LIVER MITOCHONDRIA AND ON MEMBRANE-BOUND Na^+,K^+-ATPase ACTIVITY OF BOVINE CEREBRAL CORTEX

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KEY WORDS: antitumor action; platinum compounds; mitochondrial ATPase; Na,K-ATPase.

One of the preparations currently used in the treatment of several human malignant tumors, in combination with bleomycin, adriamycin, and vinblastine, is the compound cis-$Pt(NH_3)_2Cl_2$ [9]. Initially Rosenberg et al. [11] showed that cis-$Pt(NH_3)_2Cl_2$ inhibits cell division of *Escherichia coli* without inhibiting growth of the bacterial cells. It has also been found that cis-$Pt(NH_3)_2Cl_2$ has a selective antimitotic action [2]. trans-$Pt(NH_3)_2Cl_2$ has no such effects. After the discovery of the antitumor action of cis-$Pt(NH_3)_2Cl_2$ the search for active complex compounds of platinum began. The molecular target for their action was considered to be cellular DNA [5]. In fact, active inhibitors of L1210 leukemia in mice induced lysogeny in lysogenic bacteria and were mutagenic in the system developed by Ames et al. cis-$Pt(NH_3)_2Cl_2$ inhibits DNA polymerase activity more effectively than the trans-isomer [10]. RNA-polymerase also is inhibited more strongly by cis-$Pt(NH_3)_2Cl_2$ than by the trans-isomer; low concentrations of the order of $5\cdot10^{-7}$-10^{-5} M, moreover, are active. Inhibition is connected with modification of the DNA substrate and not of the enzyme [12]. However, no direct correlation could be found *in vitro* between the antitumor activity of platinum compounds and their ability to form complexes with DNA. For instance, the inactive trans-$Pt(NH_3)_2Cl_2$ binds with DNA in amounts comparable with the cis-isomer and modifies the conformation of the double helix [8]. It is evident that only a certain special type of interaction between platinum compounds and DNA leads to the antitumor effect *in vitro*. Some workers suggest that this is covalent bonding of two neighboring guanine residues, located on the same DNA strand [13], by two valence bonds of cis-$Pt(NH_3)_2Cl_2$.

The presence of targets for platinum coordination compounds other than DNA in the cell likewise cannot be ruled out. Among the most important, besides reactions of nucleic acid synthesis, breakdown, and repair, we can distinguish cell processes connected with energy metabolism and also with the transport of ions and other physiologically important substances through the membrane. Meanwhile the effect of platinum compounds on mitochondria and on other organelles and also on transport processes and individual enzymes involved in these reactions has received very litte study. Information on this problem is interesting also in connection with the explanation of the mechanism of the toxic side effects of antitumor compounds of platinum, especially their nephrotoxicity [7].

The object of this investigation was to study the action of cis-$Pt(NH_3)_2Cl_2$ and its trans-isomer on latent ATPase activity of isolated rat liver mitochondria and on the activity of membrane-bound Na^+,K^+-ATPase from the gray matter of the bovine brain.

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