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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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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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TABLE 1. Effect of cis-$Pt(NH_3)_2Cl_2$ and trans-$Pt(NH_3)_2Cl_2$ on ATPase Activity of Isolated Rat Liver Mitochondria (M ± m)

Experimental conditions	Incubation time			
	5 min	2 h	24 h (with ATP)	24 h (without ATP)
Control	7,59±2,50	6,17±1,22	23,59±4,61	34,10±11,39
cis-$Pt(NH_3)_2Cl_2$	7,47±1,58	8,20±0,25	30,13±4,48	36,83±13,67
trans-$Pt(NH_3)_2Cl_2$	6,15±0,69	11,07±2,37*	59,81±1,82*	80,81±21,07*

Legend. Here and in Table 2: activity expressed in nmoles P_i/min/mg protein; an asterisk indicates significance of differences compared with control ($P < 0.01$).

EXPERIMENTAL METHOD

Mitochondria were isolated from rat liver and ATPase activity determined as described previously [1]. A suspension of mitochondria (1.5-2 mg protein/ml was incubated at 0°C in medium for the ATPase reaction (0.25 M sucrose, 5 mM $MgCl_2$, 12 mM Tris-HCl, pH 7.4) and with cis-$Pt(NH_3)_2Cl_2$ or trans-$Pt(NH_3)_2Cl_2$ in a concentration of 0.1 mM for 5 min or 2 or 24 h (in the presence or absence of 6.7 mM ATP). After addition of ATP, the samples were incubated for 20 min at 25°C. The volume of the samples was 2 ml. The reaction was stopped by the addition of 1 ml of cold TCA. Inorganic phosphate (P_i) formed in the course of the reaction was determined as described previously [1].

Membrane-bound Na^+,K^+-ATPase was obtained from the bovine cerebral cortex by the method of Boldyrev et al. [6] with certain modifications. After the last washing and centrifugation the enzyme preparation was frozen in small portions in liquid nitrogen and kept in the same medium. Before work the enzyme was thawed at room temperature, the fineness of the suspension was increased by forcing it through a pipet with a drawn out tip, and it was then diluted with 50 mM imidazole buffer, pH 7.2, containing 0.25 M sucrose and 1 mM EDTA to a protein concentration of 100 µg/ml. The resulting enzyme suspension, in a volume of 0.1 mM, was added to a reaction mixture, pH 7.4, containing 50 mM imidazole, 0.1 mM EDTA, 0.1 M NaCl, 20 mM KCl, and 10 mM $MgCl_2$, and the test concentration of cis-$Pt(NH_3)_2Cl_2$ and trans-$Pt(NH_3)_2Cl_2$. The solution was incubated at 37°C for 5 min or 2 or 24 h. Meanwhile the same solutions also were incubated but in the presence of 0.1 mM ouabain. The ATPase reaction, induced by addition of ATP-Na to a final concentration of 3 mM, was then carried out. The volume of the sample was 1 ml. The reaction was stopped after 10 min by the addition of 1 ml of cold 15% TCA. The P_i concentration was determined by Stanton's method [1]. The required activity was calculated by subtracting activity of the experimental sample in the presence of 0.1 mM ouabain from activity obtained in the absence of ouabain. The Na^+,K^+-ATPase activity of the preparation was about 99% of total ATPase activity. Each experiment was repeated at least three times.

Solutions for studying Na^+,K^+-ATPase activity were made up in thrice distilled water, the rest in bidistilled water. ATP was obtained from Reanal (Hungary), ouabain from Calbiochem (USA). Preparations of cis-$Pt(NH_3)_2Cl_2$ and trans-$Pt(NH_3)_2Cl_2$ were generously provided by V. B. Ivanov.

EXPERIMENTAL RESULTS

It was found that cis-$Pt(NH_3)_2Cl_2$ in a concentration of 0.1 mM did not induce activation of latent ATPase even on incubation for 24 h with the mitochondrial suspension (Table 1). This compound, in the concentration tested, is thus not toxic for mitochondria, which remain in a coupled state, i.e., capable of synthesizing ATP normally. In concentrations above 0.1 mM cis-$Pt(NH_3)_2Cl_2$ is known to increase mitochondrial respiration in the presence of succinate and ADP and also to increase the outflow at Ca^{2+} from mitochondria [4]. So far as the action of trans-$Pt(NH_3)_2Cl_2$ is concerned, in a concentration of 0.1 mM this compound activates latent mitochondrial ATPase during incubation with a suspension of mitochondria for 2 h by 79%, and for 24 h by 154%. Incubation for 5 min is clearly too short for the reaction of substitution of ligands in the platinum complex for protein or lipoprotein groupings. ATP does not protect the enzyme against the action of trans-$Pt(NH_3)_2Cl_2$. A low temperature (0°C) was chosen in

TABLE 2. Effect of cis-$Pt(NH_3)_2Cl_2$ and trans-$Pt(NH_3)_2Cl_2$ on Na^+,K^+-ATPase Activity Isolated from Bovine Cerebral Cortex (M ± m)

Experimental conditions	Incubation time		
	5 min	2 h	24 h
Control	8,81±0,34	8,74±0,47	5,92±0,15
cis-$Pt(NH_3)_2Cl_2$	8,05±0,04	8,09±0,25	3,77±0,33
trans-$Pt(NH_3)_2Cl_2$	7,78±0,04	6,83±0,21*	2,86±0,18*

experiments to keep the isolated mitochondria in the coupled state. At 25°C the ATPase activity of the mitochondrial suspension increased with the passage of time, evidence of thermal injury to the mitochondria. During incubation of the mitochondria at 25°C for 2 h, for instance, ATPase activity increased from 6.2 to 78.1 nmoles P_i/min/mg protein. However, in this case also trans-$Pt(NH_3)_2Cl_2$ activated mitochondrial ATPase by 76%. These results indicate that trans-$Pt(NH_3)_2Cl_2$ reacts more readily with the components of the inner mitochondrial membranes than cis-$Pt(NH_3)_2Cl_2$. The latter compound has low toxicity for isolated mitochondria.

So far as the effect of platinum complexes on membrane-bound Na^+,K^+-ATPase activity is concerned (Table 2), the inhibitory action of trans-$Pt(NH_3)_2Cl_2$ was found to be stronger in the case of this enzyme also. For instance, in a concentration of 0.1 mM, cis-$Pt(NH_3)_2Cl_2$ inhibited Na^+,K^+-ATPase by 36% but only after a long period of incubation (24 h) with the enzyme, whereas trans-$Pt(NH_3)_2Cl_2$ inhibited this enzyme by 22% in the case of incubation for 2 h and by 52% in the case of incubation for 24 h at 37°C.

Little is known of the effect of platinum compounds on other cell enzyme systems. It has been shown that cis-$Pt(NH_3)_2Cl_2$, in a concentration of 0.1 mM, inhibits Ca^{2+},Mg^{2+}-ATPase activity of the sarcoplasmic reticulum by 45% in the absence of ATP and by 70% in its presence [3]. Under the influence of a number of complex compounds of platinum, activity of certain dehydrogenases and of leucineaminopeptidase has been shown to be inhibited also [9]. Under these circumstances, just as with the enzymes studied in the present investigation, the trans-isomers proved to be more active inhibitors. The most reactive groups in proteins in reactions of ligand substitution in platinum coordination compounds are the thiol groups of methionine and cysteine, followed by histidine residues. Increased affinity of trans-$Pt(NH_3)_2Cl_2$ for sulfur-containing amino acids of the tested enzymes is evidently responsible for the stronger inhibitory effect of this compound than of cis-$Pt(NH_3)_2Cl_2$.

Despite differences in the effects *in vitro* and *in vivo*, it can be tentatively suggested that the antitumor action of cis-$Pt(NH_3)_2Cl_2$ depends only a little both on its effect on the mitochondrial cell fractions and on its action on the Na^+,K^+-ATPase of the plasma membranes. The trans-isomer gives rise to much stronger effects but is not an antitumor agent. Inhibition of Na^+,K^+-ATPase by cis-$Pt(NH_3)_2Cl_2$ may be responsible for the nephrotoxic side effects of this compound.

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