COMBINED ACTION OF SALTS OF HEAVY METALS AND PHENOL ON ENERGY METABOLISM OF ISOLATED RAT LIVER MITOCHONDRIA

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An essential role in the mechanism of the toxic action of salts of heavy metals and phenol is played by disturbance of mitochondrial energy metabolism. There are data in the literature on a close connection between the action of various substances on mitochondrial respiration and their toxicometric characteristics [7]. Similar relationships also have been observed when other physiological parameters of various biological objects have been recorded [1, 2, 11, 12]. However, the advantage of data obtained on mitochondria is that it is possible to predict acute nonspecific toxicity, due to a wide range of membrane effects [5, 9, 14].

Under real conditions, the body is exposed not to individual compounds, but to their combinations, and without taking into account their synergistic or antagonistic effects, the prediction of toxicity is not always correct [4, 6].

The aim of the investigation described below was to study the combined action of salts of metals (lead, copper, nickel, zinc) and of phenol on the rate of electron transport in the respiratory chain and the degree of coupling of oxidation and phosphorylation in rat liver mitochondria.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 160-180 g. Mitochondria were isolated from the liver by the method described previously [3]. The incubation medium of the mitochondria contained 70 mM KCl, 5 mM KH_2PO_4, 4 mM glutamate, and 1 mM lactate ("Serva"), pH 7.5. All solutions were made up in triple distilled water. The intensity of mitochondrial respiration was determined with the aid of a closed Clark's oxygen electrode on an LP-7E polarograph (Czechoslovakia). Into a polarographic cell with a volume of 1 ml was introduced 2.5-3.0 mg mitochondrial protein at 28°C. The respiratory control (RC) was recorded after addition of 250 mM ADP ("Sigma") to the cell, and the rate of oxygen consumption was recorded after addition of 20 μM dinitrophenol. The results were subjected to statistical analysis by Student's t test for paired comparisons.

EXPERIMENTAL RESULTS

In the experiments of series 1 changes in the rate of oxygen consumption by the liver mitochondria under the influence of the test substances in different concentrations were studied. The experiments showed that the velocity of electron transport in the respiratory chain was reduced by 50% by phenol (PhOH) in a concentration of 0.12 mM, $ZnCl_2$ in a concentration of 0.4 mM, and $CuSO_4$ in a concentration of 2 mM; the same result was obtained by adding 6 mM Pb acetate ($PbAc_4$) or $NiCl_2$ to the incubation medium of the mitochondria. Thus according to the degree of inhibition of respiration, these chemical compounds could be arranged in the following order: PhOH > $ZnCl_2$ > $CuSO_4$ > $PbAc_4$ ≈ $NiCl_2$.

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TABLE 1. Effect of Salts of Heavy Metals and Phenol on Oxygen Consumption by Rat Liver Mitochondria [M ± m(n)]

Substances	Concentrations of components, mM		Percentage inhibition of O_2 consumption
$CuSO_4$:$PbAc_4$	0,25	3	10±1 (4)
	0,25	4	50±2 (5)
	0,5	2	50±1 (6)
$CuSO_4$:$NiCl_2$	0,5	2	76±2 (4)
	0,5	1	57±3 (5)
	0,5	0,5	50±2 (5)
$CuSO_4$:$ZnCl_2$	0,8	0,005	75±3 (6)
	0,8	0,0012	70±2 (6)
	0,8	0,0007	60±9 (5)
	0,8	0,00035	50±2 (5)
$CuSO_4$:$ZnCl_2$	0,5	0,25	90±2 (5)
	0,5	0,12	84±1 (5)
	0,5	0,035	76±3 (7)
	0,5	0,0025	70±2 (6)
	0,5	0,0005	50±1 (6)
PhOH:$CuSO_4$	0,06	0,5	80±1 (4)
	0,06	0,25	75±1 (4)
	0,06	0,12	67±2 (5)
	0,06	0,07	50±3 (5)

It is interesting to note that the order of toxicity of the metals, estimated on the basis of inhibition of mitochondrial respiration, is comparable with the results obtained by the neutral red test on a cell culture [8].

As the results in Table 1 show, in experiments with mixtures of substances a 50% decrease in the maximal velocity of electron transport in the mitochondrial respiratory chain was observed when lower concentrations of test substances were used in a mixture compared with the individual substances.

It can be concluded from these results that the total concentration of substances in a mixture, determining its toxic effect, is comparable with or lower than the acting concentration of that component of the mixture which possesses the highest toxicity.

The type of combined action can be determined as the sum of the ratios of Ci_{50} of the substance in the mixture to Ci_{50} of the individual substance for each component [6]. According to Table 1 this sum was 0.57, 0.33, 0.25, and 0.54 for $CuSO_4$ – $PbAc_4$, $CuSO_4$ – $NiCl_2$, $CuSO_4$ – $ZnCl_2$, and $CuSO_4$ – PhOH pairs respectively, indicating a potentiating effect during the combined action of the compounds tested.

In our experiments the test substances in a concentration giving 50% inhibition of mitochondrial respiration reduced the value of Chance's RC from 2.9 ± 0.1 or 2.6 ± 0.2 to 1.0. Addition of EDTA to the incubation medium of the mitochondria prevented the changes described above in mitochondrial energy metabolism. EDTA concentrations necessary in this case to equalize the toxic effects were 1.0, 0.5, 4.0, 4.0, and 4.0 mM respectively. However, the ratio between concentrations of EDTA and of the substances differed significantly, and amounted to 8.33, 1.25, 2.0, 0.67, and 0.67 for the same series. This result indicates that the effect of EDTA is due not only to binding with the test substances in equimolar proportions. We postulated that the EDTA concentration necessary to restore normal mitochondrial energy metabolism can be used as a criterion of toxicity of the medium. In fact, on the basis of polarograms for substances taken in 50% inhibition concentrations of mitochondrial respiration, and data on toxicometry revealed correlation between the EDTA concentration restoring the value of RC and LD_{50}. The sensitivity of this method, moreover, is 2-3 orders of magnitude higher than that of recording inhibition of respiration [5].

Since addition of EDTA to the incubation medium prevented damage to mitochondria by individual substances, the presence of a protective action of this agent may be suggested also for a mixture of toxic substances, and in addition it may be expected that the EDTA concentration required to bind the toxic agents will determine the toxicity of the mixture.

To test this hypothesis we studied the action of mixtures of substances in ratios causing 50% inhibition of mitochondrial respiration, in the presence of EDTA within the concentration range of 0-8 mM. Table 2 gives EDTA concentrations leading to maximal restoration of RC, with values of V_3 and V_4 for states 3 and 4 after Chance, in relative units. The data given in Table 2 indicate that in this case also potentiation of the effect is observed during the combined action of the substances. As the data in Table 2 and the relationships given above between concentrations of EDTA and substances determining leveling of the fall of RC given above, in order to restore its value in a PhOH/$CuSO_4$ mixture 3.1 times more, a $ZnCl_2$/$CuSO_4$ mixture 1.3-4.0 times, and $PbAc_4$/$CuSO_4$ and $NiCl_2$/$CuSO_4$ 3 times more EDTA are required.

TABLE 2. Restoration of Respiratory Control of Rat Liver Mitochondria by Different EDTA Concentrations [M ± m(n)]

Substances	Concentrations of substances and EDTA, mM		V_3, relative units	V , relative units	RC
$CuSO_4$	0,25		7,0±0,2	7,0±0,2	1,0±0,0
$PbAc_4$	4	0	(5)	(5)	(5)
«—»		4	27,6±0,6	17,0±1,1	1,6±0,2*
			(5)	(5)	(5)
$CuSO_4$	0,5		12,4±0,8	12,4±0,8	1,0±0,0
$PbAc_4$	2	0	(4)	(4)	(4)
«—»		4	21,2±0,8	13,6±0,3	1,6±0,1***
			(5)	(5)	(5)
$CuSO_4$	0,5		5,1±0,5	5,1±0,5	1,0±0,0
$ZnCl_2$	0,0005	0	(4)	(4)	(4)
«—»		4	24,1±1,4	12,2±0,6	2,0±0,1***
			(5)	(5)	(5)
$CuSO_4$	0,8		10,3±1,3	10,3±1,3	1,0±0,0
$ZnCl_2$	0,00035	0	(3)	(3)	(3)
«—»		2	31,9±1,1	8,6±0,5	3,8±0,7**
			(5)	(5)	(5)
$CuSO_4$	0,5		8,8±1,4	8,8±1,4	1,0±0,0
$NiCl_2$	0,5	0	(3)	(3)	(3)
«—»		2	26,4±1,2	13,5±1,5	2,0±0,1***
			(5)	(5)	(5)
$CuSO_4$	0,07		11,4±0,8	11,4±0,8	1,0±0,0
PhOH	0,06	0	(4)	(4)	(4)
«—»	0,06	2	20,1±2,2	10,9±0,5	1,8±0,1***
			(5)	(5)	(5)

It can be tentatively suggested that in this case EDTA prevents activation of phospholipase A_2 [13] and initiation of LPO in the mitochondrial membranes as toxic substances accumulate in them [5, 10].

Injury to mitochondria by pairs of salts of metals and phenol is thus characterized by synergism of its action on respiration and respiratory control. Taking account of the key role of energy metabolism in the vital activity of the cell, the results are evidence that toxicity in a mixture of substances may be increased, and that in some cases assessment of the integral toxicity of media on the basis of concentrations and toxicometric characteristics of individual compounds present in them may be incorrect in some cases. Moreover, the investigation indicates that both methods of biological testing on a mitochondrial test system used in the work may provide the basis for rapid prediction of the toxicity of multicomponent mixtures of substances.

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