EFFECT OF CARBON TETRACHLORIDE AND ALDACTONE ON ACTIVITY OF ACID HYDROLASES AND SOME DEAMINATION ENZYMES IN THE RAT LIVER

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The effect of aldactone and carbon tetrachloride, separately and combined, on β-glucuronidase, acid phosphatase, guanine deaminase, and histidine-ammonia liase activity in the liver tissue and blood serum of male rats was investigated. Combined administration of aldactone and CCl_4 was found to potentiate the toxic action of CCl_4 and was accompanied by a decrease in the total activity of all the enzymes in the liver tissue and an increase in free β-glucuronidase and acid phosphatase activity in the liver tissue and blood serum. Preliminary administration of aldactone prevented the decrease in activity of the enzymes but did not affect the stability of the lysosomes during CCl_4 poisoning.

Aldactone, a virtually nontoxic substance, is known to have a protective action against poisoning by various substances producing necrosis of parenchymatous organs [7-9]. The preventive action of aldactone against liver damage by CCl_4 has never been studied and, in particular, its effect is not known on the stability of the lysosomal membranes, a disturbance of the integrity of which is followed by release of acid hydrolases into the cytoplasm with the development of further destructive changes in the hepatocytes [2].

It was accordingly decided to investigate the activity of β-D-glucuronide-glucurono-hydrolase (β-glucuronidase, β-GL), acid phosphatase (β-glycerophosphatase, AP), histidine-ammonia liase (HAL), and guanine deaminase (GDA) in the liver of intact rats and rats poisoned with CCl_4, given alone or in conjunction with aldactone.

EXPERIMENTAL METHOD

Noninbred male albino rats aged 7-8 weeks and weighing 100-130 g were divided into the following groups: 1) intact rats; 2) rats receiving aldactone for 3 days in a dose of 5 mg/100 g body weight twice a day; 3) animals receiving CCl_4 for 3 days in a dose of 0.2 ml of a 25% oily solution/100 g body weight daily; 4) the same as group 3 but the rats were sacrificed 72 h, not 24 h, after stopping the poison; 5) rats receiving CCl_4 for the first 3 days and aldactone for the next 3 days; 6) rats receiving aldactone for the first 3 days and CCl_4 for the next 3 days and sacrificed 24 h after stopping the CCl_4. Aldactone and CCl_4 were administered to the rats by means of a metallic intragastric tube.

β-GL activity in the blood serum and liver tissue was determined by hydrolysis of 1 mmole phenolphthalein glucuronide in 0.1 M acetate buffer, pH 5.0 [5]. The free β-GL activity was determined in the supernatant of a homogenate (the $2 \cdot 10^6$ g, 1 min fraction) made up in 0.25 M sucrose (1:10) with 3 mM EDTA. Total enzyme activity was determined in the total homogenate in 0.85% NaCl solution. The incubation time was 5 h and 10 min respectively for free and total enzyme activity. AP activity was determined by hydrolysis of 16.6 mmoles β-glycerophosphate. Activity of the enzymes was expressed for tissues in micromoles/g per minute and for blood serum in micromoles/ml per minute. HAL activity was deter-

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TABLE 1. Activity of Enzymes in Liver Tissue and Blood Serum of Experimental Groups of Rats (M ± m)

Group of animals	No. of animals	Liver tissue: total activity (in μmoles/g/min)				Liver tissue: free activity (in % of total)		Blood serum: μmoles · 10^{-3}/min/liter^{-1}	
		GDA μmoles·10^{-2}	HAL, units	β-GL μmoles·10^{-3}	AP, μmoles·10^{-2}	β-GL	AP	β-GL	AP
1	16	119±8	280±25	864±66	160±10	1,8±0,25	4,7±0,31	1,72±0,07	4,46±0,45
	14	47±6	142±18	471±72	155±12	—	—	1,84±0,04	5,64±0,54
	P	<0,001	<0,001	<0,001				>0,5	0,5
2	12	75±9	133±8	764±70	126±8	2,8±0,60	5,1±0,82	2,10±0,15	4,62±0,60
	P	<0,001	<0,001	>0,5	<0,02	<0,2	<0,2	<0,05	>0,5
3	12	34±8	57±8	670±84	127±7	3,2±0,39	7,1±0,36	2,16±0,17	3,76±0,60
	P	<0,001	<0,001	<0,2	<0,02	<0,01	<0,01	<0,05	<0,2
4	12	41±7	49±3	468±63	103±8	4,6±0,76	8,0±0,95	2,17±0,13	5,70±0,55
	P	<0,001	<0,001	<0,001	<0,001	<0,01	<0,01	<0,02	<0,1
5	12	77±9	132±6	644±72	118±11	3,4±0,85	7,5±1,01	2,90±0,35	5,0±1,20
	P	<0,001	0,001	0,05	0,01	0,05	0,02	0,01	>0,5
6	12	113±7	300±22	1006±72	157±14	2,4±0,40	6,9±0,66	2,16±0,12	6,23±0,73
	P		>0,5	>0,5		<0,5	<0,01	<0,02	<0,1
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mined by the method of Mardashev and Burobin [1] and expressed in units of urocainic acid, while GDA activity was tested by deamination of guanine [4] and expressed in micromoles NH_3/g per minute.

EXPERIMENTAL RESULTS

It is clear from Table 1 that administration of aldactone for 3 days led to a marked decrease in the total activity of β-GL, HAL, and GDA. Poisoning of the rats with CCl_4 caused a marked decrease in the total activity of all the enzymes tested, which was most marked on the third day after stopping the poison (group 4), especially for HAL and GDA. A disturbance of the permeability of the lysosomes and cell membrane of the hepatocytes was accompanied by a definite increase in free activity of β-GL and AP in the liver tissue and an increase in β-GL activity in the blood serum. Consequently, after administration of small doses of CCl_4 for 3 days definite disturbances were found in the hepatocytes. Simultaneous administration of aldactone and CCl_4 caused even greater changes in the levels of activity of these enzymes (group 5). It was also shown that preliminary administration of aldactone alone to the animals (group 7) prevented the decrease in activity of all the enzymes studied observed in CCl_4 poisoning. At the same time, aldactone had no significant effect on stabilization of the lysosomes or a return to normal β-GL and AP activity of the blood serum.

It can be concluded that the effect of aldactone on enzyme activity depends on the experimental conditions, such as whether the aldactone and CCl_4 were given together, separately, or one after the other. CCl_4 is known to inhibit enzyme activity of the endoplasmic reticulum [3]. Hydroxylation and dimethylation are sharply inhibited [6] while the activity of enzymes accelerating the formation of toxic products from CCl_4 [10] is intensified. Under these conditions aldactone evidently not only has no protective action but it may actually facilitate the toxic effect of CCl_4 and thereby throw an additional load on the hepatocytes if given simultaneously with the hepatotropic poison. This is shown by the greater increase in free β-GL and AP activity of the hepatocytes and the sharp decrease in the total activity of all enzymes tested. The possibility cannot be ruled out that CCl_4 inhibits the activity of enzymes directly concerned with the conversion of aldactone itself.

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