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EFFECTS OF CHLORPROMAZINE ON BEHAVIOUR OF SOME ENZYMES OF RAT LIVER IN DIFFERENT EXPERIMENTAL CONDITIONS

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Abstract—The behaviour of three lysosomal hydrolases (acid phosphatase, cathepsin and β-glucuronidase), of two mitochondrial enzymes (cytochrome oxidase and malic dehydrogenase) and of the glucose-6-phosphatase of the rat liver in three different experimental conditions (injection of dextran, fasting and ischaemia) has been studied. The common effect of all these treatments is a marked increase of the "non-sedimentable" activities of the lysosomal hydrolases. Many of the unfavourable effects of these treatments can be antagonized by chlorpromazine.

IT HAS been reported[1] that chlorpromazine and other phenothiazine derivatives exert *in vitro* a biphasic effect on subcellular particles: at concentration levels below 10^{-4} M, they have a stabilizing action on mitochondria and lysosomes; at a higher concentration they cause swelling of the mitochondria and rupture of lysosomes. This effect has been related to the action of phenothiazines on the membranes, especially on their permeability,[2] perhaps by inhibiting the formation of peroxides of lipoproteins.[3]

Similar results have been obtained in the only research which, to our knowledge, has been carried out *in vivo* on the action of chlorpromazine on liver lysosomes:[4] a dose of 2 mg/kg protects lysosomes against the labilizing action of vitamin A; a dose of 20 mg/kg exerts, with regard to the lysosomes, both a protective action against *Escherichia coli* endotoxin and an increased release of acid phosphatase, proving damage to these particles. On the other hand, Eger and Schulz[5] showed that two successive doses, each of 15–20 mg/kg, administered with an interval of 14 hr, induce a clear protective action against necrosis and oedema caused by dextran. The effects of dextran and other polysaccharides on the lysosomes have been studied and reviewed in detail by Wattiaux.[6]

The present study was designed to determine the behaviour of enzymes present in mitochondria, lysosomes and endoplasmic reticulum under various experimental conditions (injection of dextran, fasting, liver ischaemia) and their modifications following treatment with relatively high doses of chlorpromazine.

In particular, we have attempted to investigate the action of chlorpromazine on phenomena of endocytosis caused by i.v. injection of a foreign substance, such as dextran, and of intracellular autophagy induced by failure of nutritive supplies; the participation of lysosomes in these processes (see Weissmann[7] and Jacques[8]) is well known.

As a measure of lysosome integrity, we have determined the "total" and "non-sedimentable" activities of a number of acid hydrolasase (acid phosphates, cathepsin

and β-glucuronidase), which are associated with these granules; especially important are the "non-sedimentable" activities, which are found to increase in many cases of lysosomal injury.[9, 10] The activities of cytochrome oxidase and malic dehydrogenase have been taken as a criterion of mitochondrial integrity. A decrease of the activity of glucose-6-phosphatase, a cahracteristic enzyme of the endoplasmic reticulum, was considered an early and reliable indication of damage.[11]

EXPERIMENTAL AND RESULTS

Albino rats of the Wistar strain were used in all experiments. The number, sex and body weight of the animals is indicated in the Tables. The animals were maintained on an ordinary stock diet until the beginning of the experiments.

After the various treatments described below, the animals were weighed and killed by decapitation; the livers were immediately removed and weighed in ice-cold 0·25 M sucrose; they were cut in small pieces and homogenized by three up-and-down runs of the pestle of a Potter homogenizer, rotating at 780 rpm, in about 15 ml of 0·25 M sucrose. The homogenates were then brought to a volume which was equivalent to ten times the weight of the original organ, by adding 0·25 M sucrose. An aliquot of all homogenates was used to determine total nitrogen by the Kjeldahl method and of the "total" enzymic activities. Another aliquot was centrifuged in a M. Christ Omega type refrigerated ultracentrifuge at 40,000 rpm for 30 min (3,500,000 *g*-min).

The "non-sedimentable" activities of hydrolases were determined in the supernatant.

Glucose-6-phosphatase, β-glucuronidase, acid phosphatase, cathepsin and malic dehydrogenase were determined by the methods of de Duve *et al.*,[12, 13] cytochrome oxidase was determined by the method of Cooperstein and Lazarow,[14] as modified by Appelmans *et al.*[15] The activities are expressed in I.U./g of liver nitrogen, except for cytochrome oxidase, whose activity is defined according to Cooperstein and Lazarow.[14]

First series of experiments (dextran and chlorpromazine)[16]

The experiments were performed on five groups of rats. The first group served as control. The second group received, intravenously, dextran* at a dose of 3 g/kg body weight. The third group received, in addition, chlorpromazine i.p. (20 mg/kg 30 min before dextran and again 20 mg/kg 14 hr thereafter). The fourth group received only chlorpromazine, at the same dosages and times as the third group. Since animals injected with chlorpromazine fell asleep and were therefore unable to eat, the animals of the first and second group were also fasted during the experiment, which lasted 36 hr; all the animals received water *ad libitum*. In order to attenuate the effects of fasting, the animals received, 16 hr before killing, 1 g of glucose s.c. Finally, for better understanding of the effects of dextran independently from starvation, a fifth group received dextran, as did second and third ones, but was allowed to feed freely.

The mean values of our results are summarized in Table 1.

Second series of experiments (fasting and chlorpromazine)[17]

From the results of Table 1, the possibility arises that chlorpromazine exerts a

* "Macrodex" Baxter (6% solution w/v in 0·154 M NaCl) ; mean molecular weight about 75,000.

TABLE 1. EFFECTS OF DEXTRAN AND OF CHLORPROMAZINE ON THE LIVER OF MALE RATS (MEAN BODY WEIGHT 100 g). MEAN VALUES ± S.D.

Groups	I Controls (8)	II Dextran fasting (8)	III Dextran + chlorpromazine (9)	IV Chlorpromazine (4)	V Dextran no fasting (6)
Treatments at following times:					
hr 0	Beginning of fasting	Beginning of fasting	Beginning of fasting	Beginning of fasting	
hr 9½			Chlorpromazine	Chlorpromazine	
hr 10		Dextran	Dextran		Dextran
hr 20	Glucose	Glucose	Glucose	Glucose	
hr 24			Chlorpromazine	Chlorpromazine	
hr 36	Sacrifice	Sacrifice	Sacrifice	Sacrifice	Sacrifice
Liver weight (g/100 g of body weight)	3·46 ± 0·37	3·65 ± 0·37	4·17 ± 0·06‡	3·86 ± 0·04	3·74 ± 0·4
Liver N (mg/g of wet tissue)	33·4 ± 5·0	32·4 ± 2·5	31·8 ± 2·6	32·9 ± 2·9	35·3 ± 3·0
Liver N (mg/100 g of body weight)	115 ± 018	119 ± 11	133 ± 15	127 ± 14	132 ± 14
"Total" enzymatic activities (Units /g of N):					
Cytochrome oxidase	517 ± 118	480 ± 20	430 ± 162	410 ± 180	448 ± 128
Glucose-6-phosphatase	1075 ± 250	1082 ± 152	760 ± 163*	1046 ± 167	1034 ± 71
β-Glucuronidase	43·0 ± 8·2	40·0 ± 9·9	32·8 ± 6·0	27·5 ± 2·4‡	30·4 ± 0·5†
Acid phosphatase	236 ± 42	230 ± 29	231 ± 21	227 ± 20	249 ± 35
Cathepsin	49·9 ± 11·0	42·4 ± 9·5	52·4 ± 7·2	59·7 ± 4·5	54·2 ± 7·3
"Non-sedimentable" activities (% of total):					
β-Glucuronidase	6·85 ± 1·42	17·3 ± 3·5‡	13·6 ± 2·2‡§	4·46 ± 0·54†	13·5 ± 1·3‡‖
Acid phosphatase	7·92 ± 1·36	15·9 ± 2·4‡	13·1 ± 2·0‡§	5·05 ± 0·59†	12·2 ± 1·3‡¶
Cathepsin	8·33 ± 2·65	16·7 ± 5·5‡	11·4 ± 3·4‡§	4·10 ± 0·82*	13·4 ± 2·2†

( ) Number of animals.
* Difference from controls significant at $0·01 < P < 0·05$.
† Difference from controls significant at $0·001 < P < 0·01$.
‡ Difference from controls significant at $P < 0·001$.
§ Difference between groups II and III significant at $0·01 < P < 0·05$.
‖ Difference between groups II and V significant at $0·01 < P < 0·05$.
¶ Difference between groups II and V significant at $0·001 < P < 0·01$.

TABLE 2. EFFECTS OF FASTING AND OF ADMINISTRATION OF CHLORPROMAZINE ON THE LIVER OF FEMALE RATS (MEAN BODY WEIGHT 10 g). MEAN VALUES ± S.D.

Measured parameters	Controls (5)	Fasting (6)	Fasting + Chlorpromazine i.p. (5)	Fasting + Chlorpromazine s.c. (5)
Liver weight (g/100 g of body weight)	4·68 ±0·33	3·43 ± 0·33‡	4·43 ± 0·55	4·14 ± 0·39
Liver N (mg/g of wet tissue)	28·3 ± 1·0	35·8 ± 2·9‡	32·8 ± 3·1*	32·2 ± 3·4*
Liver N (mg/100 g of body weight)	133 ± 5	120 ± 8*	139 ± 17§	133 ± 10
"Total" enzymic activities (Units/g of N):				
Cytochrome oxidase	567 ± 58	555 ± 120	527 ± 149	633 ± 117
Malic dehydrogenase	129 ± 32	112 ± 16	110 ± 2	125 ± 30
Glucose-6-phosphatase	336 ± 107	700 ± 170†	722 ± 149‡	958 ± 129‡‖
β-Glucuronidase	45·7 ± 9·2	49·1 ± 2·5	49·8 ± 9·2	413 ± 5·9§
Acid phosphatase	189 ± 21	234 ± 25*	220 ± 10*	214 ± 39*
"Non-sedimentable" activities (% of total):				
β-Glucuronidase	3·46 ± 1·48	6·44 ± 1·76*	4·68 ± 1·34	5·71 ± 0·62
Acid phosphatase	5·45 ± 0·35	7·48 ± 1·02†	6·09 ± 0·32	6·53 ± 0·76
Cathepsin	2·37 ± 1·15	3·94 ± 0·77*	1·80 ± 0·56¶	1·84 ± 0·42¶

( ) Number of animals.
* Difference from controls significant at $0·01 < P < 0·05$.
† Difference from controls significant at $0·001 < P < 0·01$.
‡ Difference from controls significant at $P < 0·001$.
§ Difference from fasting significant at $0·01 < P < 0·05$.
‖ Difference from fasting significant at $0·001 < P < 0·01$.
¶ Difference from fasting significant at $P < 0·001$.

TABLE 3. EFFECTS OF CHLORPROMAZINE (15 mg/kg i.p.) ON THE ENZYMIC ALTERATIONS INDUCED BY ISCHAEMIA IN THE LIVER OF FEMALE RATS (MEAN BODY WEIGHT 200 g). MEAN VALUES ± S.D.

Measured parameters	Controls		Chlorpromazine		Ratios Ischaemic/normal	
	Normal	Ischaemic	Normal	Ischaemic	Controls	Chlorpromazine
Liver N (mg/g of wet tissue)	31·7 ± 1·1 (7)	24·3 ± 1·8 (7)	31·0 ± 1·8 (10)	25·2 ± 2·6 (10)	0·77 ± 0·07	0·79 ± 0·06
"Total" enzymic activities (Units/g of N):						
Cytochrome oxidase	416 ± 53 (5)	198 ± 51 (5)	404 ± 66 (5)	422 ± 90 (5)	0·48 ± 0·11	1·04 ± 0·09‡
Malic dehydrogenase	1112 ± 670 (5)	966 ± 136 (5)	879 ± 329 (8)	817 ± 253 (8)	0·88 ± 0·17	0·95 ± 0·12
Glucose-6-phosphatase	1031 ± 221 (7)	510 ± 83 (7)	1134 ± 184 (9)	674 ± 150 (9)	0·51 ± 0·01	0·60 ± 0·13
β-Glucuronidase	36·3 ± 6·6 (5)	41·7 ± 8·2 (5)	35·8 ± 5·5 (5)	41·1 ± 9·1 (5)	1·18 ± 0·29	1·15 ± 0·18
Acid phosphatase	182 ± 19 (5)	159 ± 20 (5)	194 ± 25 (5)	186 ± 22 (6)	0·89 ± 0·13	0·97 ± 0·10
Cathepsin	30·0 ± 5·7 (5)	19·0 ± 3·5 (5)	33·2 ± 7·5 (5)	29·8 ± 5·0 (5)	0·64 ± 0·12	0·92 ± 0·17*
"Non-sedimentable" activities (% of total):						
β-Glucuronidase	3·52 ± 0·52	14·5 ± 6·1	4·46 ± 1·06	7·32 ± 2·20	4·14 ± 1·15	1·67 ± 0·44†
Acid phosphatase	7·31 ± 1·43	29·8 ± 10·3	6·50 ± 1·12	13·4 ± 2·9	4·18 ± 1·45	2·11 ± 0·50†
Cathepsin	3·71 ± 1·04	28·9 ± 12·8	4·54 ± 2·15	11·7 ± 4·5	7·96 ± 2·64	2·89 ± 1·59‡

( ) Number of animals.
* Difference from controls significant at $0·01 < P < 0·05$.
† Difference from controls significant at $0·005 < P < 0·01$.
‡ Difference from controls significant at $P < 0·001$.

protective action not only against the effects of dextran, but also against those of starvation.

In order to substantiate this supposition and to further elucidate the action of chlorpromazine, we have carried out a second series of experiments. We worked with 4 groups of rats. The first group (controls) was allowed to feed freely; the second group remained fasting for 36 hr; the third and fourth ones (also fasting) received chlorpromazine (20 mg/kg), respectively intraperitoneally and subcutaneously, 12 hr after the beginning of starvation and, for a second time, 12 hr thereafter.

The results are summarized in Table 2.

Third series of experiments (ischaemia and chlorpromazine)[18]

These experiments were performed with 2 groups of rats. The animals were laparatomized under ether anaesthesia. The vascular pedicle of the left liver lobe was firmly, but carefully, ligated with fine thread; then the abdominal cavity was closed. This technique has been used by de Duve and Beaufay.[9] The first group served as control; the other received 30 min before the operation 15 mg/kg of chlorpromazine i.p.; 5 hr after ligature the animals were killed and the two liver parts were cooled, weighed and homogenized in the usual manner. The results are summarized in Table 3, where they are reported as such and as ratios between values for the ligated and unligated parts.

DISCUSSION

From Table 1 it is evident that dextran causes a great increase of the "non-sedimentable" activities of acid hydrolases; the increase is significantly less marked in animals given dextran plus chlorpromazine; on the other hand, chlorpromazine alone elicits a decrease of these activities. It should be pointed out that non fasting animals, receiving dextran, show lower levels (but still relatively high) than the fasting ones. It seems therefore that, in rats of the second group, the effects of dextran and starvation (that is of endocytosis and autophagy) are superimposed.

In order to confirm this hypothesis, we performed, as already said, the experiments of the second series, the results of which are shown in Table 2. From this table, it can be seen that starvation causes an increase of the "non-sedimentable" activities of the acid hydrolases, partially antagonized by chlorpromazine, except for β-glucuronidase. Subcutaneously administered chlorpromazine appears less active than intraperitoneally.

Since it could be argued from these experiments that there might be an inhibitory effect of chlorpromazine on autophagic phenomena, we tested this drug in similar, but heavier, phenomena caused by ischaemia (third series of experiments). Table 3 shows the great enhancement of "non-sedimentable" activities in the ischaemic lobe of the liver in comparison with the non ligated one. In this case, also chlorpromazine is able to relieve the tissue injury.

With regard to total activities of the lysosomal enzymes, β-glucuronidase activity is lowered by chlorpromazine in fasting rats (Tables 1 and 2); the activity of acid phosphatase is increased by starvation; the level of cathepsin seems to be slightly raised as a consequence of starvation (Table 2), but it is strongly decreased following

ischaemia (Table 3). This last effect is almost completely inhibited by chlorpromazine.

Of the mitochondrial enzymes, cytochrome oxidase is the most affected by ischaemia, which decreases its activity by 50 per cent. Its value returns to normal after chlorpromazine. However, the drug, despite its favourable effects on lysosomes and mitochondria, seems to be unable to normalize the level of glucose-6-phosphatase: its activity, in agreement with de Duve and Beaufay[6] and Beaufay *et al.*,[13] is about halved by ischaemia and doubled by fasting. It should be considered, on the other hand, that, while the first effect is a sign of damage of endoplasmic reticulum, the second could be correlated with an increase of glycogenolysis in starving rats.

Moreover, it should be pointed out that a simultaneous treatment with dextran and chlorpromazine results, in fasting animals, in a significant lowering of this enzyme; from this observation, we believe that in this case the liver, impaired by the polysaccharide, becomes an easier target for the well-known, unfavourable side effects of chlorpromazine.

Our results indicate that the three series of treatments cause a more or less marked injury in the liver tissue, which is partially mitigated by chlorpromazine. Although this aspect of our results is in agreement with the histological observations of Eger and Schulz [5] concerning the effects of dextran and their attenuation by means of chlorpromazine, we were unable to confirm the oedematigenous action of dextran that was found by the German authors: in fact, the weight and the nitrogen content of the liver, in the rats injected with dextran were not significantly affected in comparison with those of control animals. On the contrary, in agreement with de Duve and Beaufay,[9] we found the oedema in ligated liver lobes.

The positive effect usually exerted in our experiments by chlorpromazine could be ascribed to the fact that, with the dosage levels we have used (about 1/4 of LD_{50} for the rat), the drug concentration reached in the liver is lower than the one which, as mentioned before, is critical for the biphasic action of phenothiazines. Probably by employing higher dose the injurious effects would prevail over the protective ones.

On the other hand, Guth *et al.*[4] were able to obtain, with doses similar to the ones we used, in addition to a clear damage, a protective action against *Escherichia coli* endotoxin.

It may be that the critical dose depends upon both animal sensitivity and different kinds of injuring agents. Another factor that should be considered is the time delay between the injection of the drug and the sacrifice of animals.

On the whole, our results indicate that chlorpromazine has a clear inhibitory effect on cellular autophagic processes, at least on those caused in the liver by starvation and ischaemia. Perhaps the drug, whatever its mechanism of action,[19] is capable of stabilizing the membranes of autophagic vacuoles, or eventually of avoiding their formation, preventing in such a way any dangerous cellular alteration.

The question remains open whether the apparent protection of the mitochondria is a primary effect or a secondary one, caused by the inhibition of autophagic processes, or elicited by a probably decreased turnover of cytochrome oxidase.

Preliminary experiments[20] with various phenothiazinic and/or antihistaminic drugs have shown that the best results in inhibiting ischaemia necrosis have been obtained by means of chlorpromazine, whose action seems therefore to be independent from either neuroleptic or antihistiminic activity.

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