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PRELIMINARY COMMUNICATIONS

PREVENTION BY CHLORPROMAZINE OF LYSOSOMAL ENZYME RELEASE CAUSED BY A TRANSITORY ISCHAEMIA. EFFECT OF HYPOTHERMIA

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In homogenates of rat-liver lobes made ischaemic for 2h. and recirculated with blood for 20 h., a large proportion of acid phosphatase and β-galactosidase exists in a free form. Animal treatment with chlorpromazine prevents the release of both lysosomal enzymes (Wattiaux and Wattiaux-De Coninck, 1980, 1981). In the present paper, we report that this apparently protective effect of chlorpromazine on lysosomes results from hypothermia induced by the drug.

Materials and Methods:

Transitory ischaemia was obtained following the method of Chien and Farber (1977). Male Wistar rats were laparotomized under ether anaesthesia and the vascular pedicle of the left lobe was clamped with a small forceps. 2h. later, the animals were again anaesthetized, the abdomen was re-opened, the forceps removed and the wound closed. The animals were killed 20 h. after restoration of the blood flow, the left lobe was removed, cut into several slices, examined for macroscopic signs of necrosis, weighed and homogenized in ice cold 0,25M sucrose by three strokes of a motor driven Teflon pestle in a Potter homogenizer (A.H. Thomas, Philadelphia, PA U.S.A.). The homogenized suspension was adjusted to a volume corresponding to ten times the weight of the tissue. Part of the homogenate was centrifuged for 40 min. at 40.000 rev/min. in the n° 40 rotor of the Spinco preparative ultracentrifuge. Free and total acid phosphatase was measured in homogenates as described by Wattiaux and de Duve (1956); total β-galactosidase activity was determined in homogenates and supernatants by the Vaes method (1966).

Results and discussion

When a rat, previously injected with chlorpromazine (2 mg/100 g body weight) after inducing ischaemia, is maintained at room temperature (20-22°C) its temperature falls progressively until it reaches 30°C - 32°C. If the treated animal is kept in a well ventilated room at 30° C. its temperature remains within normal limits (36° - 37°C). The effect of chlorpromazine treatment on the acid hydrolase latency modifications caused by ischaemia differs according to whether the rats are hypothermic or not. As shown by Fig.1 and in agreement with our previous results, the free activity of acid phosphatase and unsedimentable activity of β-galactosidase are markedly increased in the homogenates of rat liver lobes made ischaemic for 2 h. and re-circulated with blood for 20 h. Results are similar whether the rats are maintained at 20°C or 30°C. The injection of chlorpromazine largely prevents the release of both acid hydrolases when animals are kept at room temperature; the drug is almost ineffective when the rats are maintained at 30°C.

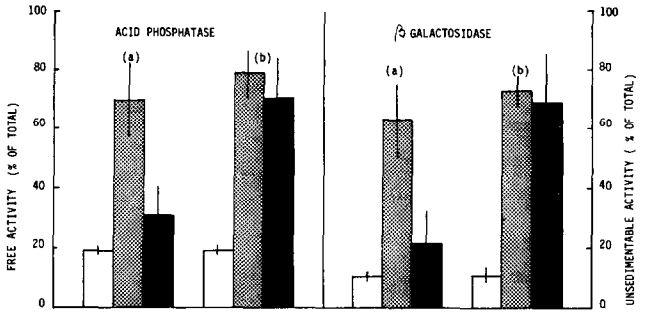

Fig.1. Influence of 2 h. ischaemia on the free activity of acid phosphatase and the unsedimentable activity of β-galactosidase. These activities are given as a percentage of the total activity. ▒▒▒ : Free or unsedimentable activities found in homogenates of liver lobes subjected to 2 h. ischaemia and re-circulated with blood for 20 h. ■■■ : id. but rats were injected with chlorpromazine (2 mg/100 g body weight), subcutaneously just after induction of ischaemia; □ : Free or unsedimentable activities found in homogenates of unligated lobes of operated animals treated or not with chlorpromazine. (a) Rats maintained at room temperature (20-22°C); (b) Rats maintained in a well ventilated room at 30°C. Means ± S.D. of five animals are presented.

The relationship between the duration of hypothermia and the prevention of hydrolase release was investigated. Rats were injected with chlorpromazine after ischaemia induction and maintained in different temperature conditions. One group of animals (a) was kept in cages immersed in a thermostatic bath at 30°C. Other groups (b,c,d,e) were kept at room temperature (20-22°C) for increasing times, then quickly warmed by transfering them in cages immersed in a thermostatic bath at 37°C during 30 min. After that they were put in other cages maintained in a bath at 30°C. The animals were killed 20 h. after re-establishment of the circulation. The evolution of the rectal temperature of the different groups of rats is reported in the upper part of Fig.2. In the lower part of Fig.2 the changes of free acid phosphatase and unsedimentable β-galactosidase are illustrated. Obviously hypothermia must be maintained during most of the ischaemic period to observe an effect of chlorpromazine on acid phosphatase and β-galactosidase latency but is no longer necessary after restoration of the blood flow.

These results indicate that it is not chlorpromazine "per se" but hypothermia induced by the drug that prevents the release of lysosomal hydrolases taking place after restoration of the blood flow in ischaemic liver lobes. They show that the cellular dysfunction, responsible for this release, occurs during the ischaemic period and is temperature sensitive. It is to note that in our experiments, each time the acid hydrolase release was high in a liver lobe homogenate, this lobe exhibited macroscopic signs of extensive necrosis and each time this release was low, the lobe was apparently normal. It is probable therefore that the protective effect of hypothermia on lysosomes we have described is parallel with a general protection of the liver cells against the deleterious effects of transitory ischaemia.

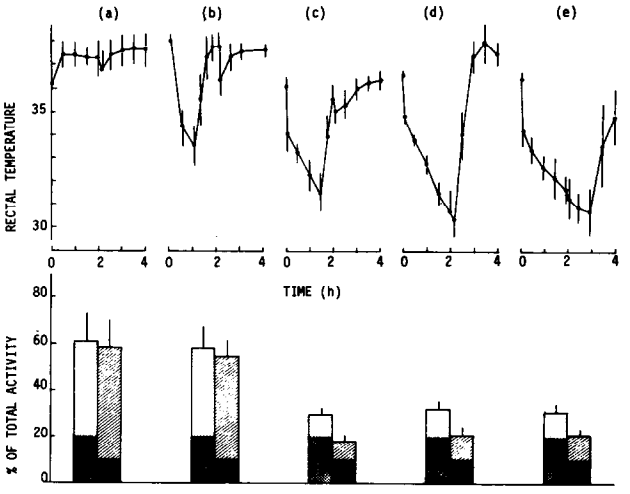

Fig.2. Influence of the duration of hypothermia on the chlorpromazine effect. Rats were injected with chlorpromazine (2mg/100 g body weight) just after ischaemia induction and maintained in different temperature conditions. Rats were kept in cages immersed in a thermostatic bath at 30°C (a) or at room temperature (20-22°C) for 60 min (b), 90 min (c), 120 min (d), 150 min (e), then quickly warmed as explained in the text. The animals were killed 20 h. after re-establishment of the circulation. The upper part of the Fig. illustrates the evolution of the rectal temperature during the four hours following ischaemia induction. In the lower part, the changes of free acid phosphatase and unsedimentable β-galactosidase are shown. ▒ : Free acid phosphatase, unligated lobes; □ :Free acid phosphatase, increment found in ischaemic lobes; ■ : Unsedimentable β-galactosidase, unligated lobe; ▨ : Unsedimentable β-galactosidase, increment found in ischaemic lobes. Means ± S.D. of five animals are presented.

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