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Effects of phenobarbitone and leptazol on rat brain lysosomes

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Phenobarbitone influences the membrane function of erythrocytes (Coldman & Good, 1969), cerebral cortex slices (Gilbert, Ortiz & Millichap, 1966) and synaptosomes (Balfour & Gilbert, 1971), but effects on cerebral lysosomal membranes have received little attention. Many drugs stabilize or labilize lysosomes (Weissman, 1968) and it is possible that the effects of phenobarbitone on lysosomes of the central nervous system might be involved in the mechanism of its anticonvulsant effect. The effects of phenobarbitone on release of acid phosphatase from lysosomes of the rat cerebral cortex have been determined: (i) after injecting phenobarbitone into rats and subsequently incubating the mitochondrial fraction in 0·25 M sucrose solution and (ii) after incubating the mitochondrial fraction from untreated animals in media containing phenobarbitone.

Male Sprague-Dawley rats were injected intraperitoneally with phenobarbitone sodium, thiopentone sodium or with 0·9% NaCl solution, 1 h before decapitation. The mitochondrial fraction containing lysosomes, was prepared as described by Koenig, Gaines, McDonald, Gray & Scott (1964). It was incubated for 30 min in 0·25 M sucrose and released acid phosphatase activity was determined by the method of Gianetto & de Duve (1955). The activity of the enzyme released was calculated as a percentage of the total activity released by 0·01% Triton X-100.

In preliminary experiments, when an anaesthetic dose (150 mg/kg) of phenobarbitone sodium was used, the free acid phosphatase activity was reduced by 14% compared to controls ($P<0·01$), whereas in rats anaesthetized with thiopentone sodium (40 mg/kg), the free activity was reduced by 8% and this was not significant at the 5% level. When rats were injected with a smaller dose of phenobarbitone sodium (50 mg/kg) daily for 4 days, they did not appear to be sedated at the time of killing (18 h after the last injection) and the free acid phosphatase activity was reduced by 9% ($P<0·05$). When rats were injected with leptazol (64 mg/kg) and decapitated during a convulsion, the free acid phosphatase activity was increased by 18% ($P<0·01$).

In other experiments, the mitochondrial fraction obtained from cerebral cortex was incubated for 30 min in 0·17 M sucrose containing 83 mM tris-maleate buffer pH 7·4, with or without phenobarbitone (0·01, 0·1, 2·0 or 5·0 mM) and the medium was then made hypotonic by the addition of water (2 vol. to 3 vol. medium) and the incubation was continued for 30 minutes. The release of acid phosphatase activity by osmotic shock was decreased by 2·0 mM (by 61%) and 5·0 mM (by 66%) phenobarbitone sodium.

These results suggest that phenobarbitone stabilizes brain lysosomes to a significant extent.

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Effects of phenobarbitone on glucose transport and membrane ATP-ase activities of cerebral cortex

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Phenobarbitone, at anticonvulsant doses, elevates the concentration of glucose in brain (Gilbert, Gray & Heaton, 1971). Stimulation of glucose transport could be a contributing factor and this would be compatible with the observation that phenobarbitone stimulates xylose transport in cerebral cortex slices (Gilbert, Ortiz & Millichap, 1966). Direct evidence that the drug influences glucose transport has been lacking and the object of the present work was to test this possibility.

Guinea-pig cerebral cortex slices were incubated in oxygenated Krebs-Ringer bicarbonate or phosphate media containing pyruvate (4 mM), raffinose (10 mM) and half (1·2 mM) the recommended calcium concentration. Glucose transport was studied under the following conditions in the presence and absence of phenobarbitone sodium.

1. Glucose uptake was determined at 37° C in the bicarbonate medium containing iodoacetamide (1 mM) and glucose (10 mM) after preincubation for 30 min at 37° C in a similar medium lacking glucose.

2. Glucose efflux into the glucose-free bicarbonate medium containing iodoacetamide (1 mM) was determined at 37° C after preincubation for 30 min at 37° C in a similar medium containing glucose (17 mM).

3. Glucose uptake was determined at 1° C in the phosphate medium containing glucose (10 mM) after preincubation for 30 min at 37° C in a similar medium lacking glucose.

Glucose metabolism was negligible in all experiments. At 37° C, glucose uptake by the slices was rapid and its volume of distribution soon exceeded that of raffinose. The kinetics of permeation of glucose into the non-raffinose compartment conformed to carrier transport kinetics and phenobarbitone (2 mM) did not influence the process. At 37° C, glucose efflux from slices was rapid and was not significantly influenced by phenobarbitone. These results are compatible with previous work suggesting that iodoacetamide can prevent stimulation of sugar transport by some drugs. Glucose uptake at 1° C, in the absence of iodoacetamide, was slow and was significantly stimulated by phenobarbitone; the volume of distribution of glucose did not exceed that of raffinose and the kinetics of uptake were compatible with a simple diffusion process.