Quantitative Determination of Hydrolysis Products of Phospholipids in the Ischaemic Rat Brain *

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Summary. 1. Glyceryl-phosphoryl- and phosphoryl derivatives of choline, ethanolamine, serine and inositol, as well as glycerol were determined in rat brain following various periods of ischaemia. The phosphate esters were separated by two-dimensional thin-layer chromatography and quantitated by phosphate determination. Free glycerol was analysed enzymatically.

2. The concentrations of phosphate esters ranging between 0.016 and 0.087 μmoles/g wet wt. in normal brain tissue, increased to levels of 0.189 to 0.327 μmoles/g wet wt. after 1 h of ischaemia.

3. Glycerol, undetectable in normal brain, reached a concentration of 1.3 μmoles/g wet wt. after 1 h; glycerol formation slowed down within the second hour, and from 2—8 h steadily rose to 5.4 μmoles/g wet wt.

4. Based on the amount of intermediates and glycerol formed the rate of phospholipid catabolism during ischaemia at normothermia was calculated. The metabolites indicated hydrolysis of phospholipids via two pathways catalysed either by phospholipases A₁ and A₂ (EC 3.1.1.4), lysophospholipase (EC 3.1.1.5), and glycerophosphoryl choline diesterase (EC 3.1.4.2), or by phospholipase C (EC 3.1.4.3) and tissue lipases (EC 3.1.1.3).

Key words: Brain — Ischemia — Phospholipid Catabolism — Glycerol.

The hydrolytic breakdown of phospholipids in the ischaemic brain was estimated either by decrease in phospholipid content (Hinzen et al., 1970) or by rise in free fatty acids (Bazán, 1970). Decrease in phospholipid content did not give information about metabolites and endproducts of catabolism. Concerning the increase of free fatty acids it was uncertain which type of fatty acid ester was hydrolysed and how the non-fatty acid moiety was further metabolized.

In order to study the stepwise hydrolysis of the complex lipids, the assumed intermediates such as the glycerylphosphoryl derivatives and the phosphate esters of choline, ethanolamine, serine, and inositol as well as the final product glycerol were analysed. The changes in concen-

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Abbreviations used. CP, choline phosphate; EP, ethanolamine phosphate; FA, fatty acid(s); GPC, glycerylphosphoryl choline; GPE, glycerylphosphoryl ethanolamine; GPI, glycerylphosphoryl inositol; GPS, glycerylphosphoryl serine; IP, inositol phosphate; SP, serine phosphate; TLC, thin layer chromatography.
trations of these compounds provided information not only about the rate of phospholipid loss but also about the preferred catabolic pathway and turnover in the individual reactions.

Within a short period of ischaemia all biosynthetic reactions were terminated due to a lack of substrates and loss of energy requiring substrate activation. Since the constant lipid content in the mature brain may be assumed to result from an equilibrium between anabolic and catabolic reactions, the catabolic rate during the first few minutes of ischaemia should equal the turnover rate of the phospholipids in the normal brain, provided that no activation and liberation of lysosomal hydrolytic enzymes occurs.

The functional importance of catabolic reactions is obvious in the exchanges and alterations of membrane lipids (e.g. deacylation-acylation cycle, Lands, 1960). It is, however, still questionable in which way the membrane characteristics are changed by the lipid breakdown in ischaemia, and whether the damage of membranes is causally related to the revival time of the brain.

Methods and Materials

Albino-rats (weighing 250—350 g) were anaesthetized by intraperitoneal administration of pentobarbital sodium (5 mg/100 g). The duration of anaesthesia was 5 min.

Control Brains. The heads were deeply frozen in liquid air without prior interruption of the circulation.

Ischaemic Brains. After decapitating the rats, the heads were incubated in a moist chamber in an incubator at 38°C for 5, 15, 30, 60 min and 2, 3, 4, 6 and 8 h.

Cerebrum and cerebellum were dissected from the frozen heads, weighed and pulverized in a mortar with dry-ice. Referring to brain weight, an eight-fold amount of 0.33 M perchloric acid was added to the mixture of dry-ice and pulverized brain. Perchloric acid freezing in the mortar was broken up, mixed with the tissue powder and thawed. The homogenate was centrifuged and the supernatant neutralized with 2 N KOH. After sedimentation of potassium perchlorate, the extract was evaporated to dryness in vacuo at 26°C in a rotary vacuum evaporator. The residue was dissolved in 0.5 ml of bidistilled water and stored at —20°C.

Thin Layer Chromatography. The phosphate esters were separated by two-dimensional TLC. A mixture of 15 g cellulose MN 300 and 120 ml of water was stirred in an electric blender for one min and the slurry was immediately applied as a 0.5 mm thin layer onto 20×20 cm glass plates. Plates were air-dried for 6—8 h, activated overnight at 120°C and stored in a desiccator. 40 μl of sample were applied as a small spot about 2 cm from the corner of the plate with a Hamilton microsyringe. The plate was developed in the first direction in propanol/25% ammonia/water (5:3:2, v/v) to a height of 16 cm and in the second direction with acetic acid/methanol/water (1:6:1, v/v) to a height of 18 cm.

Glycerylphosphoryl derivatives could not be separated from the corresponding phosphoryl compounds by TLC on silica gel HR with the solvent system for paper chromatography of phosphoglycerolipid hydrolysis products as developed by Dawson (1960). Similar disadvantages were observed in TLC on cellulose MN 300, using the