Acta Biologica Szeged. *21* (1—4), pp. 95—97 (1975)

THE LIPIDS OF THE BOVINE PINEAL GLAND
(Short communication)

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(Received March 8, 1975)

Abstract

The phospholipid composition and cholesterol content of 2-year-old bovine pineal gland extracts were determined. The total lipid content was 3.7—5.6% of the wet weight. In general 71% of the total lipids consists of various phospholipids, and 15% of free cholesterol.

The study of the lipids of the pineal gland was considered necessary becuase the hormone of this organ, melatonin, is a lipophilic substance (Index Merck, 1968), and because large changes take place in the mutual proportions of the lipids (mainly phospholipids) during various pathological processes (ZWEENS, 1963) and pathological changes (KAHÁN, 1971). In addition, various transmitters similarly affect the proportions of the phospholipids of the pineal gland and the uptake of $H_3{}^{32}PO_4$ ($^{32}P_i$)** (BASINSKA et al., 1973). The role of the neutral lipids and fatty acids is predominantly to act as a store of reserve nutriment, in the form of drops which can be detected histochemically in the cytoplasm of the pineal gland (QUAY, 1957; ROOZEMOND et al., 1970).

Materials and Methods

The bovine pineal glands used in the study were obtained from the Szeged abattoir. The study material was maintained at −4 °C. It was washed in physiological salt solution and the weight of the glands measured. The weight of one gland was 0.2—0.25 g. Samples taken for examination consisted of 5—10 glands. The samples were homogenized with a cold mixture of chloroform and methanol (2:1) (FOLCH et al., 1951), and the crude extract was washed with 0.88% potassium chloride (FOLCH et al., 1957). Other samples were treated in the following way: the material was homogenized in the above-mentioned mixture, and extracted under shaking for 30 min, and the water-soluble impurities were removed by dialyzation against distilled water. The extracts were evaporated to dryness and determined gravimetrically (KREMMER et al., 1969). The evaporated lipids were dissolved in chloroform — methanol (2:1); in general a 3% solution was prepared.

For purposes of TLC, Kiesegel G nach Stahl adsorbent was applied in a thickness of 0.25 mm to 10×20 cm and 20×20 cm glass plates with the aid of a Desaga apparatus. The plates were activated at 110 °C for 20 min.

Lecithin, stearic acid, palmitic acid, cholesterol and cholesterol acetate were used as comparative standards.

The solvent used to separate the individual components of the total lipid solution was n-hexane — diethyl ether — glacial acetic acid (73:25:2). The following fractions were obtained:

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** Abbreviations used: P_i inorganic orthophosphate; TLC thin layer-chromatography.

phospholipid, cholesterol, mono-, di- and triglycerides, fatty acids, and cholesterol esters. The plates were placed for a short time in iodine vapour, and the spots of the developed lipid fractions were marked. The lipid fractions on the chromatogram were next collected quantitatively into test-tubes (ALTHAUS et al., 1973). The individual components of the phospholipid fraction were separated further by ascending TLC on the above adsorbent.

The following solvents were employed to separate the individual members of the phospholipid fractions: chloroform — methanol — water — acetic acid (65:35:3.6:2), and chloroform — methanol — 25% ammonia (70:30:5). The separated components were collected individually and determined quantitatively, the phospholipids by the Fiske—Subbarow method (MÜHLRÁD, 1970), and cholesterol by the Lievermann—Burhard reaction (LIEBERMANN, 1885).

Results

It was found gravimetrically that the lipid content was 3.7—5.6% of the wet weight of the bovine pineal gland. The lipid content increased with the age of the pineal gland:

1 year	3.7%;
2 years	4.1%;
3 or more years	4.9—5.6%.

With the above methods five phospholipids could be identified: sphingomyelin, phosphatidly inositol, phosphatidyl choline, phosphatidyl serine and phosphatidyl ethanolamine.

The cholesterol content was found to be 15% of the total lipids.

It was also established that the phospholipids make up 71% of the total lipid content. The percentage distribution of these is shown in the following Table:

Table

Pineal gland phospholipid fraction	Proportion of the phospholipid component (in %)
unknown	1.03
sphingomyelin	12.20
phosphatidyl choline	48.30
phosphatidyl inositol	5.65
phosphatidyl serine	7.91
phosphatidyl ethanolamine	19.41
unknown II	5.5
total	99.99
(total phospholipid content of the extract)	97.33

With the aid of the phosphate calibration curve the phosphate contents of the indiviudal phospholipid fractions were obtained in mg, and from these the amounts of the individual components were calculated in mg and in %. Phosphate determination was also carried out on the original total phospholipid solution, for the sake of comparison. The values for the individual components were added together and the result was compared with the value calculated from the phospholipid solution; it was found that the error was $\pm 1.33\%$.

Discussion

86% of the lipids in the total-lipid extract of the pineal gland was identified. The residual 14% comprises cholesterol esters, mono- and diglycerides and waxes.

Of the components of the phospholipid fraction (which constitues 71% of the total lipids), only 6.53% could not be identified. The smaller part of this (unknown I) is situated near the TLC front, and can probably be identified as triglycerides accompanying the lipids. The larger part of it (unknown II) remains near the start point; this contains several components (cerebroside, ganglioside, etc.).

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