

Study of adrenal polyunsaturated fatty acids by GC/MS with reference to RXR ligands

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Retinoid-X-receptor (RXR) is a nuclear receptor which mediates gene expression for regulating diverse metabolic reactions in the body. RXR is activated by 9-cis-retinoic acid (RA) and specific polyunsaturated fatty acids (PUFAs), namely, cervonic acid, docosapentaenoic acid, adrenic acid and arachidonic acid. However, 9-cis-RA is not found or found only in a trace amount in most tissues. To serve as a RXR-ligand, the PUFAs should present in unesterified form. Consequently, the tissue availability of unesterified RXR-specific PUFAs is important for study. We had observed that the rat adrenal expresses RXR α and RXR β , but their roles in steroidogenesis remain unknown. To shed light on the investigation, a method was developed for methylation of unesterified fatty acids for GC/MS analysis, with minimal cross-reactions with esterified fatty acids. We employed this method to study adrenal availability of these RXR-specific PUFAs in the rat. Experimental: Prior to extraction, to an 1 ml adrenal sample, 2.5 μ g pentadecanoic acid was added as internal standard (IS). Unesterified fatty acids were extracted by 5 ml acidified hexane (0.01% HCl, v/v). Fatty methyl esters were prepared using a BF₃-methanol reagent, by heating the mixtures at 60°C for 3 min precisely, and then subject to analysis by GC/MS.

Results and Discussion:

This "time-controlled" methylation permitted a primary detection of unesterified fatty acids; undesired cross-reactions with tripalmitin, heptadecanoyl-CoA, cholesterol heptadecanoate, and dipalmitoylphosphatidylcholine were minimized. Analysis of adrenal extracts revealed the presence of unesterified fatty acids including the four RXR-specific PUFAs, but only adrenic acid and arachidonic acid were detected in the nuclear fraction. We speculate that these two PUFAs may serve as ligands to activate RXR in the rat adrenal.

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