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Stimulation of the Release of Unesterified Fatty Acids in Synaptosomes by Noradrenaline and 5-Hydroxytryptamine

By C. J. PRICE and C. E. ROWE (*Department of Biochemistry, P.O. Box 363, Birmingham B15 2TT, U.K.*)

Intraventricular injection of eserine or carbamoylcholine into rat brain caused up to 40-fold increases in cerebral unesterified fatty acid (Lunt & Rowe, 1971), suggesting that unbound acetylcholine stimulates fatty acid release *in vivo*. Noradrenaline and 5-hydroxytryptamine are concentrated in nerve endings in brain (see Michaelson & Whittaker, 1963) and are putative transmitter substances. The question arises as to whether these compounds also stimulate the release of unesterified fatty acid. The effects were investigated of noradrenaline and 5-hydroxytryptamine on the concentration of unesterified fatty acid in isolated nerve endings (synaptosomes).

Synaptosomes isolated from guinea-pig cortex and cerebellum (Eichberg, Whittaker & Dawson, 1964) were incubated in Krebs-Ringer bicarbonate medium, containing glucose (11 mM), at 37°C for 90 min with shaking. Unesterified fatty acids were extracted (Dole, 1956) and determined as their methyl esters by g.l.c. (Rowe, 1964). Noradrenaline (1.0 mM) caused up to threefold increases in the yields of unesterified palmitate, stearate, oleate and arachidonate. Similar increases were obtained with 5-hydroxytryptamine and adrenaline, but not with acetylcholine in the presence of eserine, or with methylamine. Fluorimetric determinations of noradrenaline (Miller, Cox, Snodgrass & Maickel, 1970) and 5-hydroxytryptamine (Curzon & Green, 1970) indicated that concentrations of these substances in the synaptosomes were considerably less than in the incubation media.

Synaptosomes were ruptured by osmotic shock (Whittaker, Michaelson & Kirkland, 1964) and the suspension was incubated in 50 mM-tris buffer, pH 7.4. Iproniazid phosphate (100 µg/ml) increased the yield of acid (from 17%, w/w, to 101%, w/w). This increase was maximal after 15–20 min. In the presence of iproniazid phosphate relatively low

concentrations of noradrenaline (0.01 mM) could stimulate further release of fatty acids. Higher concentrations (1.0 mM), however, depressed fatty acid yields. An analogous depression was also obtained with 5-hydroxytryptamine.

Synaptosomal membrane fractions and vesicle fractions were isolated from an osmotically shocked crude mitochondrial pellet (Lapetina, Soto & De Robertis, 1967). After incubation in tris buffer for 30 min unesterified fatty acids were determined as their [³H]methyl esters. Noradrenaline (0.01 mM) and 5-hydroxytryptamine (0.01 mM) stimulated the release of fatty acids in both these fractions.

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Synthesis of Fatty Acids from [1-¹⁴C]Acetyl-Coenzyme A in Subcellular Particles of Rat Epididymal Adipose Tissue

By H. KANOH and D. B. LINDSAY. (*Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AG, U.K.*)

Fatty acid synthesis from [1-¹⁴C]acetyl-CoA was studied in mitochondria and microsomes of adipose tissue. We also studied the relationship between particulate fatty acid synthesis and triglyceride formation, which has been reported to be a major function of subcellular particles of this tissue (Roncari & Hollenberg, 1967; Angel & Roncari, 1967).

Yield of particles (means ± S.E.M.) was small (mitochondria: 1.33 ± 0.07 mg of protein/g wet wt. of tissue; microsomal fraction: 0.75 ± 0.05 mg of protein/g wet wt. of tissue), but represented about 20% of protein obtained in a defatted homogenate.

The two types of particle were clearly distinguished (1) by fatty acid composition of total lipids and (2) by the distribution of various enzyme