Fatty Acid Composition of the Phosphatidylcholine Fractions of Denervated and Tendonectomized Rat Skeletal Muscle

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The differences in the phosphatidylcholine (lecithin) content of red muscle and white muscle (Masoro, Rowell & McDonald, 1964) and also normal and denervated muscle (Graff, Hudson & Strickland, 1965) have been discussed in terms of the electrical properties of these muscles (Albuquerque & Thesleff, 1967; Thesleff & Albuquerque, 1967). However, little is known of the changes in fatty acid composition of muscle phosphatidylcholine under these different physiological states, although the role of the fatty acid composition of phospholipids in determining the behaviour of membranes is of current interest (Van Deenen, 1968). Preliminary studies have suggested differences in the degree of unsaturation of the total lipid fraction derived from normal and denervated muscles (Zatti, Patriarca, Terribile & Rossi, 1969). In this paper the effects of denervation and tendonectomy on the fatty acid composition of the phosphatidylcholine fraction from rat gastrocnemius muscle are described.

Methods. Male rats were unilaterally denervated or tendonectomized under Avertin anaesthesia by excising 1 cm. of sciatic nerve or gastrocnemius tendon. The animals were killed 20 days after the operation, the normal and affected gastrocnemius muscles were removed and freed of superficial fat as completely as possible, and the lipids were extracted by the method of Folch, Lees & Sloane-Stanley (1957). The phosphatidylcholine fraction was isolated by t.l.c. on silica gel G with chloroform-methanol-water (60:25:4, by vol.) as solvent.

The methyl esters of the phosphatidylcholine fatty acids were formed by transesterification on the silica gel in 5% (v/v) H₂SO₄ in methanol, and were then extracted into light petroleum (b.p. 40–60°). G.l.c. was performed on a Pye 104 Gas Chromatograph with a dual hydrogen flame detector and 10% diethylene glycol succinate columns. Fatty acids and methyl esters were identified by comparison with standard mixtures (Applied Science Laboratories, State College, Pa., U.S.A.) and cod liver oil methyl esters both on diethylene glycol succinate and Apiezon L stationary phases before and after t.l.c. on AgNO₃-impregnated silica gel.

Results and discussion. The effects of denervation on the fatty acid composition of muscle phosphatidylcholine are shown in Table 1 (Expt. 1). These results show the appreciable alteration in fatty acid composition after denervation and that the changes mainly affect the polyunsaturated fatty acids. There is a 50% increase in the arachidonate fraction, a smaller increase in the octadecenoate fraction and equivalent falls in the octadecadienoate and docosahexenoate fractions. To demonstrate that these changes were in fact due to lack of nervous stimulus and not due to immobilization alone, the effect of tendonectomy atrophy on phosphatidylcholine fatty acid composition was then

shown in Table 1 (Expt. 2). These results show the appreciable alteration in fatty acid composition after denervation and that the changes mainly affect the polyunsaturated fatty acids. There is a 50% increase in the arachidonate fraction, a smaller increase in the octadecenoate fraction and equivalent falls in the octadecadienoate and docosahexenoate fractions. To demonstrate that these changes were in fact due to lack of nervous stimulus and not due to immobilization alone, the effect of tendonectomy atrophy on phosphatidylcholine fatty acid composition was then

Table 1. *Fatty acid composition of the phosphatidylcholine fractions of denervated rat gastrocnemius and of tendonectomized rat gastrocnemius*

The results are given as means $\pm$ S.D. of six rats (denervated muscle) or three rats (tendonectomized muscle), and represent the analyses of the denervated or tendonectomized muscle and its contralateral control from each rat. The *P* values were derived from paired *t* tests: N.S., not significant.

Expt. 1	Fatty acid	Fatty acid composition of muscle phosphatidylcholine (%)		<i>P</i>
		Normal	Denervated	
	C _{16:0}	39.0 $\pm$ 4.3	42.5 $\pm$ 3.9	N.S.
	C _{18:0}	7.9 $\pm$ 1.4	8.1 $\pm$ 1.2	N.S.
	C _{18:1}	10.5 $\pm$ 0.6	12.9 $\pm$ 0.9	< 0.001
	C _{18:2}	19.2 $\pm$ 2.3	14.0 $\pm$ 1.6	< 0.001
	C _{20:4}	10.0 $\pm$ 1.3	15.0 $\pm$ 2.4	< 0.001
	C _{22:5}	1.7 $\pm$ 0.3	0.9 $\pm$ 0.5	0.01
	C _{22:6}	8.4 $\pm$ 1.4	3.0 $\pm$ 0.6	< 0.001
	Minor components	3.6 $\pm$ 1.9	3.6 $\pm$ 1.4	N.S.
Expt. 2	Fatty acid	Fatty acid composition of muscle phosphatidylcholine (%)		<i>P</i>
		Normal	Tendonectomized	
	C _{16:0}	33.1 $\pm$ 3.0	34.7 $\pm$ 2.3	N.S.
	C _{18:0}	10.0 $\pm$ 0.2	8.0 $\pm$ 0.4	N.S.
	C _{18:1}	9.9 $\pm$ 0.3	9.2 $\pm$ 0.2	N.S.
	C _{18:2}	19.8 $\pm$ 1.9	21.0 $\pm$ 2.5	N.S.
	C _{20:4}	12.8 $\pm$ 1.2	12.7 $\pm$ 1.5	N.S.
	C _{22:5}	1.8 $\pm$ 0.2	1.5 $\pm$ 0.2	N.S.
	C _{22:6}	9.5 $\pm$ 0.5	9.3 $\pm$ 0.8	N.S.
	Minor components	3.7 $\pm$ 1.0	3.3 $\pm$ 1.2	N.S.

studied (Table 1, Expt. 2). The results of this experiment suggest that the changes seen after denervation are specific and do not relate to the atrophy associated with immobilization.

The denervated muscle has been shown to have an increased turnover of phospholipids (Artom, 1941; Zatti & Rossi, 1962; Graff *et al.* 1965; Patriarca, Zatti, Rossi & Rossi, 1968), and this may be associated with an instability of its membranes as shown by changes both in structural (Schrodt, 1966) and functional properties of several of the membranous systems of the muscle cell (Muscatello & Patriarca, 1968). The changes in the fatty acid composition of the phosphatidylcholine fraction demonstrated in the present study are further evidence for appreciable phospholipid turnover.

The phosphatidylcholine fraction of muscle, as is the case with other tissues, probably shows a great metabolic heterogeneity of its molecular species with regard to both synthetic and degradative reactions. Although the mechanism by which the fatty acid composition is changed after denervation is not yet understood, the increased percentage of arachidonate present under these conditions may suggest a tentative explanation of the findings described by Albuquerque & Thesleff (1967) and Thesleff & Albuquerque (1967). These authors showed that the input resistance and the resting membrane potential are decreased on incubation of the muscle with phospholipase C, the decrease occurring at a lower rate in denervated than in normal muscle. The increase in the arachidonoyl content of the denervated-muscle phosphatidylcholine might well be associated with an increased resistance of the muscle phospholipids to phospholipase C, (arachidonoyl) phosphatidylcholine being more resistant to this enzyme than other phosphatidylcholine species (Graf & Stein, 1966).

The degree of unsaturation of muscle fatty acids seems to be of importance in the maintenance of

some of the properties of muscle fragments; Balzer, Makinose, Fiehn & Hasselbach (1968) demonstrated that hydrogenation of the lipids of the fragmented sarcoplasmic reticulum abolishes the microsomal adenosine triphosphatase activity. It may be possible to correlate the changes in phosphatidylcholine fatty acid composition with the alterations in microsomal adenosine triphosphatase activity that have been shown to occur in the denervated muscle (P. Patriarca, M. Zatti, U. Carraro & F. Rossi, unpublished work).

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