Lipid and Muscle Function

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Lipid in its various forms provides many functions in skeletal muscle. These functions include its role as a form of energy storage, as a source of energy, as an integral component of membranes and as a storage form of second messenger molecules. Each of these functions is critical to muscle growth, and critical to its very survival. Of the virtually innumerable topics/approaches in which the association between muscle function and lipids could be addressed, I will dwell upon two general areas, fatty acid and phosphoinositide metabolism. Several topics within these areas are shrouded in controversy and are, therefore, under intense investigation.

Developmental Aspects of Lipid Metabolism in Fetal Pig Muscle

Between 35 and 52 days of gestation, primary fibers are first formed in the skeletal muscle of fetal pigs (Beermann et al., 1978; Campion et al., 1981). Cytoplasmic lipid droplets are present in these first formed fibers and in the subsequently formed secondary fetal fibers which begin to appear after 52 days of gestation. It is not entirely clear to what extent these cytoplasmic stores can be mobilized in the fetus. At birth, all the fibers of the *semitendinosus* muscle stain positive for esterase activity (Hausman et al., 1983) which suggests the presence of hormone sensitive lipase activity. Working with 110 day fetal pig muscle in vitro, we have not been able to detect a quantitative loss of triacylglycerol over a two-hour incubation period (Campion et al., 1984). However, using radio-labeling techniques, we were able to verify the movement of ^{14}C atoms from triacylglycerol to CO_2, albeit at a very slow rate.

The ability of pig skeletal muscle to metabolize fatty acids develops fetally. At least by 70 days of gestation, the *biceps femoris* muscle was capable of oxidation of both acetate and palmitate to CO_2 in vitro (Campion and Wilson, 1986). While the rates of oxidation of these substrates were concentration dependent, the rates were not influenced by age (70, 90 and 110 days of gestation). The incorporation of palmitate into triacylglycerol and into phospholipid was also shown to be concentration dependent. The rate of incorporation into triacylglycerol was constant across the three fetal ages while the rate of incorporation into phospholipid was higher at 70 than at 90 or 110 days of gestation. The decrease in the rate of incorporation of palmitate into phospholipid between 70 and 90 days of gestation coincides with the time frame in which the mature number of fibers is established (Swatland, 1973; Campion et al., 1981). Thus, the relative demand for phospholipid synthesis, on a per-unit wet-weight basis, may have decreased after 70 days of gestation as fiber hypertrophy occurred.

De novo fatty acid synthesis was not detected in fetal pig muscle (Campion and Wilson, 1986). This inability is presumably associated with the inability of skeletal muscle to express the gene for fatty acid synthase (Goodridge, 1986).

The presence of lipoprotein lipase (LPL) activity was demonstrated in fetal pig muscle (McNamara and Martin, 1982; Campion et al., 1987). There is evidence to suggest that higher LPL activity is associated with greater muscle triacylglycerol content (Campion et al., 1987). Presence of this enzyme implies that the fetal muscle is capable of uptake of circulating lipids.

Factors that influence lipid metabolism (albeit in general terms) by fetal pig skeletal muscle at 110 days of gestation have been investigated, using several different pig models. The results of these studies are summarized in Table 1. The small, but significantly higher, dry matter content of the muscle from obese fetuses compared to lean fetuses may reflect a more advanced state of physiological maturity (Campion et al., 1987). While LPL activity was higher in the muscle of obese fetuses when compared to the lean fetuses, the two genetic types responded similarly to a lipid tolerance test (Campion et al., 1987). In addition, the oxidation and esterification rates of palmitate were not significantly different between the two types even when the differences in muscle protein or dry matter content were taken into account (Darnton et al., 1983). Thus, the mechanism by which the obese fetus deposited more lipid, other than through greater LPL activity, is not clear. But, differences in triacylglycerol turnover rates between the two types of fetuses cannot be ruled out.

Although expression of fatty acid oxidation and esterification rates on a per-unit protein basis revealed no differences in muscles from control or decapitated fetuses (MacLarty et al., 1984), the rates for decapitated fetuses were lower when expressed on the basis of dry matter or on total muscle weight. These rates are consistent with decreased lipid deposition in the muscle of the decapitated fetuses when compared to control fetuses. However, higher serum levels of total triglycerides and free fatty acids were observed in decapitated fetuses when compared to control fetuses (Martin et al., 1984). Thus, differences in LPL activity and in fatty acid uptake may also be involved. These mechanisms have not been investigated.

While the regulatory mechanisms for lipid metabolism in fetal pig muscle remain to be elucidated, the above studies

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Table 1. Metabolic Characteristics of Biceps Femoris Muscle from Several Fetal Pig Models[a].

Model	Dry matter %	Triacylgerol mg/g wet wt.	Palmitate oxidation to Co_2, nmole $\cdot 2h^{-1}$ mg $prot^{-1}$	Palmitate esterified[b], nmol$\cdot 2h^{-1}\cdot$ mg prot	LPL activity per mg prot	References
Lean vs	18.4[c]	1.2[c]	.17	2.6	3.4[c]	Darnton et al., 1983;
Obese	20.2	5.8	.12	1.8	4.4	Campion et al., 1987
Control vs	20.3	9	.42	6.1		MacLarty et al., 1984
Decapitated	12.9	5	.32	3.5		
Control vs	20.0	1.3	.45	.47		Campion et al., 1984
High Fat Fed	18.4-20.2	1.2-1.3	.39-.46	.37-.59		

[a]All fetuses were sampled at 110 days of gestation.
[b]For first and second model listed esterification to triacylglycerol and to phospholipid was not distinguised. In the high fat feeding study, esterification for triacylglycerol was measured separately from phospholipid. The rates of esterification to phospholipid were .08 for control fetuses and .06-.07 nmol$\cdot 2h^{-1}\cdot$mg $prot^{-1}$ for fetuses from fat fed dams.
[c]Comparison within model was significantly different ($P<.05$).

have amply demonstrated the activity of several metabolic pathways by which lipid is utilized as an energy store and as an energy substrate.

Fatty Acids, Prostaglandins and Myogenesis

In addition to fatty acid incorporation into energy storage forms (triacylglycerol) and to their direct utilization as an energy source, certain fatty acids were shown to have an integral role in myogenesis and in protein metabolism (Table 2). For example, both oleic and linoleic acid enhanced the fusion of cultured myoblasts when added to defined growth medium (Horowitz et al., 1978; Allen et al., 1985). The mechanism or mechanisms by which this occurs have not been defined. Furthermore, there is no evidence that this effect represents a physiological response.

However, at least one potential mechanism may be postulated as linoleic acid and may stimulate myoblast fusion through its conversion of prostaglandins (PG). Prostaglandin E_1 (PGE_1), a metabolite of linoleic acid metabolism, induced myoblasts in culture to precociously fuse (David and Higginbotham, 1981). PG-binding to myoblasts was recently shown to precede cell-cell aggregation (Hausman et al., 1987). Binding was also correlated with changes in membrane order (Santini et al., 1987). And, addition of inhibitors of PG synthesis to the culture medium delayed the onset of myoblast fusion (Zalin, 1977, 1979). McLennan (1987) tested the analogous situation in vivo. Chick embryos were injected with either inhibitors of PGE_1 synthesis (aspirin or indomethacin) or PGE_1 from the 4th to the 19th day in ovo. In both situations, the number of nuclei incorporated into the muscles of treated embryos were fewer when compared to the number incorporated into the muscles of control embryos. Why the inhibitors elicited the same response as PGE_1 is not known. Solution will require further experimentation. Since the PGs can be antagonistic among themselves, and bearing in mind that the inhibitors block synthesis of the various PGs, it could prove fruitful to examine the effects of other PGs on myoblast proliferation and fusion.

Fatty Acids, Prostaglandins and Protein Turnover

Dietary linoleic acid can be enzymatically degraded to arachidonic acid which, in turn, can be converted to leukotrienes by the action of lipooxygenase or to prostacyclin, prostaglandins, or thromboxanes by cyclooxygenase. Reeds and Palmer (1986) give an excellent review of the interrelations among arachidonic acid, the prostaglandins and protein turnover in skeletal muscle. The physiologically significant pool of arachidonic acid is that esterified at position 2 of the membrane phospholipids. Control of arachidonic acid catabolism is exercised by 1,2-diglyceride lipase with little evidence of regulation at the steps involving either lipooxygenase or cyclooxygenase activity. Generation of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

Table 2. Effects of Fatty Acids on Myoblast Fusion and on Protein Turnover.

Fatty acid	Function	Species	References
Linoleic	Enhance myoblast fusion	Rat	Allen et al., 1985
		Chick	Horowitz et al., 1978
Oleic	Enhance myoblast fusion	Chick	Horowitz et al., 1978
Arachidonic	Stimulate protein synthesis	Rat	Rodemann and Goldberg, 1982
		Rabbit	Smith et al., 1983;
		Rabbit	Palmer and Wahle, 1987
	Stimulate protein degradation	Rat	Rodemann and Goldberg, 1982
Dihomo-γ-linoleic	Stimulate protein synthesis	Rabbit	Palmer and Wahle, 1987

from arachidonic acid is of particular significance to protein turnover in skeletal muscle. In vitro, $PGF_{2\alpha}$ stimulated protein synthesis (Rodemann and Goldberg, 1982; Smith et al., 1983), while PGE_2 increased protein degradation at least in rat soleus muscle (Rodemann and Goldberg, 1982). Palmer and Wahle (1987) recently reported that the omega 6 fatty acids, arachidonic and dihomo-γ-linoleic acids, stimulated protein synthesis and release of $PGF_{2\alpha}$ in rabbit digit extensor muscle; no effect on protein degradation was detected.

On the other hand, the omega 3 fatty acids, eicosapentaenoic acid ($C_{20:5}$) and docosahexaenoic acid ($C_{22:6}$), were without effect on the basal rate of protein synthesis but did decrease the rate of PGF_2 release by rabbit muscle when measured in vitro (Palmer and Wahle, 1987). Smith et al. (1983) reported that inhibitors of PG synthesis did not reduce the basal rate of protein synthesis in skeletal muscle incubated in vitro. Thus, it appears that maintenance of basal rates of protein synthesis are sustained at extremely low levels of PGF_2 or are independent of PG-mediated control.

Thirty minutes after a single intravenous injection of indomethacin, an inhibitor of PG synthesis, the basal rate of muscle protein synthesis was not affected but a marked inhibitory effect on hormonally (insulin) elevated rates of protein synthesis occurred in the rat. In another in vivo experiment, feeding fenbufen (which releases an inhibitor of PG synthesis when metabolized in the liver) to rats for 7 days resulted in a significant reduction in protein synthesis and a 20% reduction in protein degradation (see Reeds and Palmer, 1986). Thus, there is some agreement between in vitro and in vivo results concerning the PGs and protein turnover.

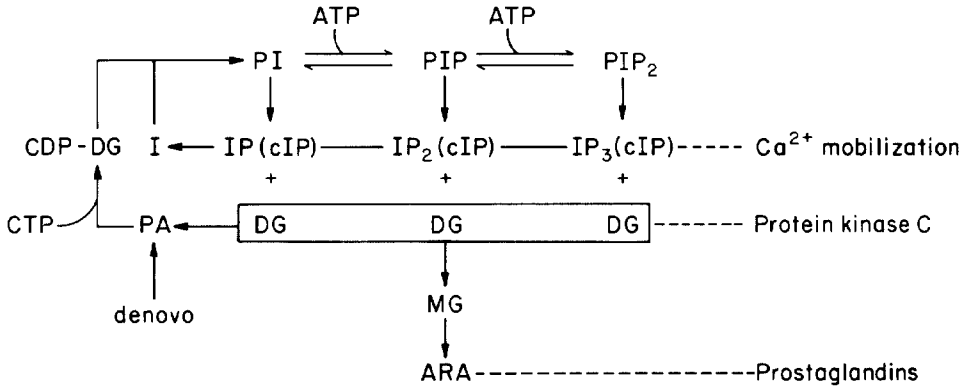
Phosphoinositide Metabolism

The phosphoinositides constitute 2% to 8% of the lipid in cell membranes of eukaryotic cells and are essential for survival (Majerus et al., 1986). In its simplest form, phosphotidylinositol (PI), the polar head group is composed of myo-inositol. The inositol component, through the actions of kinases, may be phosphorylated at the 4 position (phosphotidylinositol 4-monophosphate, PIP) or at the 4, 5 positions (phosphotidylinositol 4,5-bisphosphate, PIP_2). A futile cycle is indicated as the reverse reactions are catalyzed by phosphatase enzymes. The cycle can be interrupted by the phosphodiesterase, phospholipase C which acts on PI, PIP and PIP_2 to yield 1,2-diacylglycerol (DAG) and the inositol phosphates. Degradation of the inositol phosphates, inositol 1-monophosphate (IP), inositol 1,4-bisphosphate (IP_2), and inositol 1,4,5-trisphosphate (IP_3) back to PI is achieved through the action of phosphatases. The general scheme for PI synthesis and degradation is shown in Figure 1. Myoblasts contain more polyphosphoinositides than do other cell types, i.e., a ratio of 20:1:1 for PI:PIP:PIP_2 (Wakelam, 1983) compared to 100:1:1 for hepatocytes (Michell et al., 1981).

The metabolism of the phosphoinositides (for recent review, see Majerus et al., 1986) can lead to the production of at least three different messenger molecules, arachidonic acid, IP_3 and DAG. Once the messengers are formed, a host of intracellular and cell-to-cell reactions (as shown above for arachidonic acid) are evoked in skeletal muscle as well as in other cells. IP_3 has been shown to effect the mobilization of Ca^{2+} from intracellular stores in many cell types. Both Ca^{2+} and DAG are physiological activators of protein kinase C, a serine and threonine specific protein kinase. Ullrich et al. (1986) described the molecular biology of this enzyme which is not discussed herein. The purification of a 40 kDa inhibitor of protein kinase C from bovine brain was recently reported (Hucho et al., 1987). The relation between enzyme activators and inhibitors is not fully understood at this time. This enzyme is activated by tumor-promoting phorbol esters, such as 12-0-tetradecanoyl phorbol-13-acetate (TPA), in a manner analogous to activation by DAG (Nishizuka, 1984). The

Figure 1

Phosphoinositide metabolism and messenger production.



Key: PI, phosphatidylinositol; PIP, phosphatidyl 4-monophosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; cIP_3, inositol 1:2-cyclic 4,5-trisphosphate; IP_2, inositol 1,4-bisphosphate; cIP_2, inositol 1:2-cyclic 4-bisphosphate; IP inositol-1-phosphate; cIP, inositol 1:2 cyclic phosphate; DG, 1,2-diacylglycerol; MG, 2-monoacylglycerol; ARA, arachidonic acid; I, inositol; PA, phosphatidic acid; CDP-DG, cyticine diphosphate diacylglycerol. Adapted from Majerus et al (1986) Science 234:1519.

latter discovery has proved useful in the study of responses associated with activity of protein kinase C.

In addition to production of the inositol phosphates from degradation of polyphosphoinositides, inositol cyclic phosphates can be formed (Wilson et al., 1985). This latter group of metabolites (inositol 1:2 cyclic phosphate, inositol 1:2-cyclic 4-bisphosphate and inositol cyclic 1:2-cyclic 4,5-trisphosphate) has not been tested for activity in any muscle system. But in other cell types, cellular responses seem to be prolonged due to slower rates of degradation (Majerus et al., 1986).

The pathway of PI metabolism is more elaborate than shown as IP_3 can be phosphorylated to yield I 1,3,4,5-P_4. Phosphorylation proceeds by an ATP-dependent reaction that is catalyzed by IP_3 3-kinase. This enzyme appears to have a higher affinity for IP_3 than does the competing 5-phosphomonoesterase which can convert I 1,3,4,5-P_4 to I 1,3,4-P_3. IP_3 and cyclic IP_3. IP_3 and cyclic IP_3 also serve as substrates for 5-phosphomonoesterase. Phosphorylation of the 5-phosphomonoesterase enzyme by protein kinase C results in its activation.

As reviewed by Holub (1987), the myo-inositol-containing phospholipids have a rather restrictive fatty acid composition in mammalian cells. The sn-1-position is predominately stearic acid and the sn-2-position is predominately occupied by arachidonic acid. These attendant fatty acids are released sequentially from DAG through the action of lipases. Arachidonic acid can also be directly released from phosphoinositide by the action of phospholipase A_2 (McKean et al., 1981; Rittenhause, 1984). The relative contribution of these two pathways to release of arachidonic acid in skeletal muscle is not known. In addition to the previously assigned role for arachidonic acid (PG synthesis), this fatty acid can stimulate phospholipase C and protein kinase C activity and Ca^{2+} release from endoplasmic reticulum (Majerus et al., 1986). Recently, Chan and Turk (1987) reported that arachidonate-induced Ca^{2+} release from liver endoplasmic reticulum did not require its metabolism through the lipooxygenase of cyclooxygenase pathways. It also inhibited Ca^{2+} uptake by sarcoplasmic reticulum isolated from pig *longissimus* muscle (Cheah, 1981). But, again, the physiological significance of at least the latter finding is not known.

Receptor Activated Phosphoinositide Metabolism

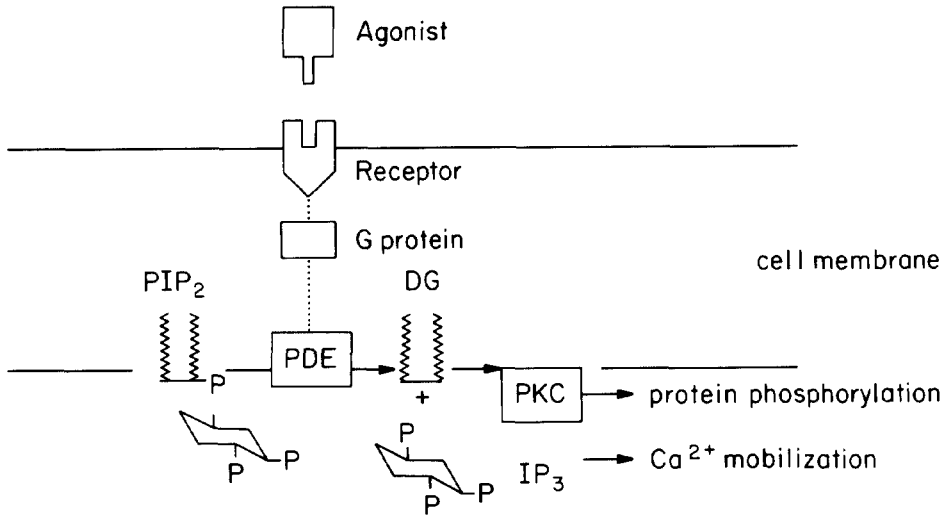
Durell et al. (1969) and Michell et al. (1975, 1981) suggested that accelerated phosphoinositide turnover was a cellular response to signal transduction across the cell membrane. The generalized scheme for receptor activation is shown in Figure 2. The details of this scheme are reviewed elsewhere (Majerus et al., 1986; Cockroft, 1987; Spiegel, 1987). The initial stages of the cascade of events bear striking similarity to those associated with hormone mediated adenylate cyclase activation. Both involve an agonist-receptor complex interacting with a guanidine nucleotide (GTP) binding protein (G protein for phospholipase C activation and G_s or G_i in the case of adenyl cyclase regulation) to activate their respective enzymes. For the otherwise soluble phospholipase C enzyme to be activated, it must be membrane bound. The G protein possibly serves to bind the phospholipase C enzyme to the inner surface of the cell membrane. The G protein appears to be the gene product ($p21^{ras}$) of the ras protooncogene (Wakelam et al., 1986, 1987; Parries et al., 1987).

Inositol 1,4,5-Trisphosphate and Skeletal Muscle Contraction

Michell et al. (1981) observed that the accelerated turnover in receptor mediated phosphoinositide was associ-

Figure 2

Model for activation of phosphatidylinositol degradation.



Key: G protein, GTP binding protein (ras?); PDE, phosphodiesterase or phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein-kinase C; IP_3, inositol 1,4,5-trisphosphate.

ated with Ca^{2+} mobilization. It is now well established that IP_3 can cause release of Ca^{2+} from the endoplasmic reticulum of various cell types (see Berridge, 1986 for review). In 1985, Volpe et al. reported that IP_3 stimulated the release of Ca^{2+} from a skeletal muscle sarcoplasmic reticulum (SR) fraction enriched in feet structures from terminal cisternae. They were the first to propose that IP_3 was the chemical transmitter for excitation-contraction coupling in muscle. An indirect argument can be made in support of the transmitter being chemical in nature as the coupling event does not appear to be electrically mediated. As pointed out by Endo (1985), any depolarization of the SR by direct coupling to depolarization of the plasma membrane cannot account for the several millisecond time lapse between initial stimulation and Ca^{2+} release. Furthermore, the SR is not depolarized during Ca^{2+} release and there is no evidence of an intracellular membrane connection between the t-tubule and the SR.

Further support for the IP_3 coupling hypothesis is available. First, fractions rich in t-tubule membranes from skeletal muscle contained 5% to 6% PI (Rosemblatt et al., 1981; Lau et al., 1979; Sumnicht et al., 1982). Second, all the enzymes of the PI signaling system were present in t-tubule preparations from skeletal muscle (Hidalgo et al., 1986; Varsanyi et al., 1986). However, it was necessary to add exogenous PIP as substrate to detect formation of PIP_2 by t-tubule preparations from rabbit skeletal muscle (Varsanyi et al., 1986). No addition was necessary with t-tubule preparations from frog skeletal muscle (Hidalgo et al., 1986). Third, addition of exogenous IP_3 to skinned fiber preparations from skeletal muscle elicited isometric force generation (Volpe et al., 1985; Vergara et al., 1985; Nosek et al., 1986; Thieleczek and Heilmeyer, 1986). As low as 1 uM IP_3 produced force generation in the presence of 1 mM free Mg^{2+} when IP_3 was injected directly into the myofibrillar space of skinned rabbit skeletal muscle fibers (Donaldson et al., 1987). One-half of the fiber preparations responded to IP_2 and only one to IP, which indicated that IP_3 was the primary mediator of the response. In addition, force generation was also elicited in chemically skinned fibers from rabbit skeletal muscle by GTP-γ-S, a non-hydrolyzable analog of GTP (Di Virgilio et al., 1986). And, fourth, tetanic stimulation of frog skeletal muscle fibers caused the release of IP (Vergara et al., 1985). These results argue favorably for IP_3 coupling.

Of the studies conducted to date, however, none has provided direct proof that IP_3 was the mediator of the excitation-contraction event. To the contrary, other studies have indicated that the issue is not at all clear. In Table 3, I have attempted to categorize the findings of studies on this topic. Mikos and Snow (1987), Palade (1987), and Pessah et al. (1987) all failed to observe an IP_3 elicited release of Ca^{2+} from terminal cisternae enriched in feet structures. These workers utilized a more physiological level (1 mM) of Mg^{2+} than did Volpe et al. (1985) who used 90 uM free Mg^{2+}. Mg^{2+} concentration is important because low concentrations are associated with reduced activity of 5-phosphomonoesterase which would effectively reduce the rate of IP_3 degradation. But low concentrations also favor Ca^{2+} induced Ca^{2+} efflux from the SR. Regardless, differences in Mg^{2+} concentration cannot completely explain the variability in results among laboratories. For example, Volpe et al. (1985) and Vergara et al. (1985) obtained a force generation response to IP_3 using skinned fibers bathed in low Mg^{2+}, a response which Donaldson et al. (1987) also demonstrated at 1 mM mg^{2+}. However, Lea et al. (1986) could not elicit a force response to IP_3 by either skinned frog or crab fibers at either 40 uM or 1 mM Mg^{2+}. And, addition of 50 uM GTP was without effect on force generation. Walker et al. (1987), used skinned frog skeletal muscle fibers to study the effect of rapid release of IP_3 on isometric force generation. They used a photolabile, but biologically inactive, precursor molecule of IP_3 which underwent photolysis to IP_3 when laser pulsed. IP_3 concentrations in excess of 25 uM were required to increase fiber tension. In addition, the time to half-maximal fiber tension ($t_{1/2}$) of greater than 10 seconds was three orders of magnitude greater than the $t_{1/2}$ associated with an electrical stimulus. Results were the same at either 40 uM or 0.3 mM Mg^{2+}. In the original work by Volpe et al. (1985), a $t_{1/2}$ of 3

Table 3. Evidence Concerning Inositol 1,4,5-trisphosphate Induced Calcium Release from the Sarcoplasmic Reticulum of Skeletal Muscle.

Preparation			
Isolated SR fractions	*Skinned fibers*[1]	*Species*	*Source*
Yes	Yes	Rabbit	Volpe et al., 1985
	Yes	Rabbit	Donaldson et al., 1987; Thieleczek and Heilmeyer, 1986.
No		Rabbit	Sherer and Ferguson, 1985: Adunyah et al., 1986; Palade, 1987; Mikos and Snow, 1987; Pessah et al., 1987.
	Yes	Frog	Vergara et al., 1985; Nosek et al., 1986.
	No	Frog	Lea et al., 1986.
Yes	Yes	Barnacle	Rojas et al., 1987.

[1]Fibers were either chemically or mechanically skinned.

seconds for force generation was obtained in the presence of 20 uM IP_3 while the $t_{1/2}$ in response to 10 mM caffeine was 0.58 seconds. This time difference is difficult to resolve, especially if one desires to argue the case for IP_3 as the coupler. In light of the number of studies with skinned fiber preparations, however, that indicated force generation in response to IP_3 addition, it remains attractive to suggest some moderating role for this compound in muscle contraction (Volpe et al., 1986).

Phosphoinositide Metabolism and Myogenesis

The relation between phospholipid turnover and cell proliferation was very recently reviewed (Vincenti and Villereal, 1986; Berridge, 1987). With respect to skeletal muscle, Koomaraie (1987) found evidence of a relationship between cell proliferation and phosphatidylinositol metabolism in rat L_6 myoblasts in cell culture. He reported that inhibitors of phosphatidylinositol biosynthesis inhibited cell proliferation (but not protein deposition per cell) in a reversible manner. A prelabeling of growth-arrested cells with myo-[2-^{3}H] inositol resulted in an approximate ratio of 7.5:1:1.5 for IP, IP_2, IP_3 after 30 minutes of growth stimulation. A rapid breakdown of PI (Wakelam and Pette, 1982), PIP and PIP_2 and a rapid increase in the production of phosphatidic acid (Wakelam, 1983) accompany the fusion of chick embronic myoblasts upon stimulation by Ca^{2+}. Thus, the Ca^{2+}-dependent process of myoblast fusion (van der Bosch et al., 1972) is also associated with PI turnover.

TPA was shown to reversibly inhibit terminal differentiation of myoblasts (Cohen et al., 1977; Sulakhe et al., 1985; Cossu et al., 1983, 1985). But results with satellite cells have been conflicting. Cossu et al. (1983; 1985; 1986) reported that TPA did not influence the differentiation of mice or human satellite cells in culture. They (Cossu et al., 1986) postulated that differences in membrane phospholipid composition at the site of protein kinase C binding and activation may explain the differential response between embryonic myoblasts and satellite cells. In support of this hypothesis, they demonstrated that normal satellite cells could be made sensitive to TPA by modifying the phospholipid composition of the plasma membrane. Addition of phosphatidylserine (PS) containing liposomes and TPA prevented fusion of satellite cells in a reversible manner. Exchanging the liposomes for ones containing phosphatidylcholine did not prevent fusion. Interestingly, distribution of protein kinase C specific activity between cytosolic and membrane fractions was similar for myoblasts and satellite cells. In addition, changes in activities in response to the presence of PS, PS + DAG, or PS + TPA were similar between myoblasts and satellite cells. Also, TPA did not stimulate the turnover of PI. Which protein(s) was phosphorylated by protein kinase C (which presumably was activated by TPA) and how that protein(s) orchestrated the cellular response is not known.

In contrast to the findings of Cossu's group, Fisher et al. (1983) found that TPA caused a dose-dependent (0.01-100 ng/ml) inhibition of myotube formation and of creatine phosphokinase isoenzyme conversion from BB to MM in cultures of satellite cells from adult human skeletal muscle. Consistent with the other studies, TPA had no effect on cell proliferation, which suggests that stimulation of protein kinase C activity is not essential to muscle cell proliferation. Caution must be exercised as other interpretations are possible, e.g., protein kinase C activation may already have been maximal at the time of addition of the TPA.

Phorbol Esters and Acetylcholine Sensitivity

Eusebi et al. (1985) studied acetylcholine (ACh) sensitivity in cultured chick and mouse myotubes. Primary cultures of muscle cells were prepared from embryonic chick muscle and from adult mouse muscle. Application of TPA to the chick myotubes reduced sensitivity to ACh. But sensitivity was not reduced in the mouse myotubes. Addition of PS, but not phosphatidylcholine, to the mouse myotube cultures allowed TPA addition to depress ACh sensitivity. Whether these results reflect species variation or age-associated differences is not known. With respect to the latter possibility, these results are strikingly similar to the differential response of proliferating myoblasts and satellite cells to TPA that was reported by Cossu's group (see above).

At the neuromuscular junction of frog skeletal muscle, TPA increased the quantal transmitter content of the endplate potential (Haimann et al., 1987) and increased the frequency of miniature endplate potentials (Eusebi et al., 1986). Endplate depolarization, however, was reversibly reduced by 20% to 60% upon iontophoretic application of ACh (Caratsch et al., 1986). These effects may be related to down regulation of ACh receptors by TPA (Burztajn and Schneider, 1986). Phorbol esters were also shown to increase the frequency and amplitude of miniature endplate potential in mice (Murphy and Smith, 1987).

Summary

A clearer picture is emerging of fatty acid metabolism in the skeletal muscle of the fetus. Mechanisms regulating the storage and mobilization of triacylglycerol, however, need to be elucidated.

From the studies to date, it can be concluded that PI turnover is associated with proliferation, with fusion and with the modulation of excitatory signals. The studies in which TPA was used as an activator of protein kinase C need to be interpreted with some caution because none proved that observed changes in response to TPA were a direct result of activation of protein kinase C. To illustrate, TPA did not stimulate PI turnover, but did stimulate the turnover of phosphatidycholine in Swiss-mouse 3T3 cells (Takuwa et al., 1987). Thus, other mechanisms of cell activation may be postulated. At present, it is not possible to assign a cause and effect relation between an elevation in phosphoinositide metabolism and PG synthesis. But PG synthesis is always accompanied by an increase in phosphoinositide metabolism.

Thus, lipid metabolism is intimately involved in sustaining and regulating muscle function. The phospholipids, in particular the phosphoinositides, are certainly deserving of a great deal more attention to elucidate their role in myogenesis, protein turnover and in muscle contraction.

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