Biochimica et Biophysica Acta, 628 (1980) 19—25

BBA 29163

POSTNATAL DEVELOPMENT OF GLYCOGEN- AND CYCLIC AMP-METABOLIZING ENZYMES IN MAMMALIAN SKELETAL MUSCLE

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(Received June 13th, 1979)

Key words: Glycogen synthase; Phosphorylase; Lactate dehydrogenase; Phosphodiesterase; Adenylate cyclase; Adrenalin; (Rabbit skeletal muscle)

Summary

Glycogen and cyclic AMP-metabolizing enzymes of rabbit skeletal muscle were examined during postnatal development. Glycogen synthase I, glycogen phosphorylase and lactate dehydrogenase activity increased 7-fold by the 6th—8th postnatal week while glycogen synthase D was present in the neonate at one-half adult levels. Cyclic AMP phosphodiesterase decreased; adenylate cyclase increased 10-fold for both the epinephrine and NaF-stimulated states of the enzyme.

Introduction

The postnatal development of skeletal muscle is characterized by a shift from a mainly oxidative to glycolytic mode of energy metabolism [1]. At birth, most mammalian muscle resembles adult slow-contracting muscle. Those muscles destined to be fast contracting-glycolytic undergo a diminution in oxidative metabolism with a parallel increase in glycogen utilization and anaerobic glycolysis [2]. Piras and Piras [3] showed a postnatal increase in glycogen synthase, glycogen phosphorylase and phosphorylase *b* kinase activity in developing chick muscle. The development of glycogen synthase D activity preceded that of glycogen phosphorylase. Similar patterns of development were observed between chick and rat skeletal muscle. Novak et al. [4] demonstrated an increase in glycogen phosphorylase and phosphorylase kinase up to the 30th postnatal day in developing rat skeletal muscle. Protein kinase activity and cyclic AMP content were highest prenatally, declining 10—30 days after birth.

Enzymes associated with the metabolism of cyclic AMP also display postnatal alterations related to the levels of cyclic AMP and activity of glycogen

synthase and glycogen phosphorylase. Hommes and Beere [5] observed NaF-stimulated adenylate cyclase to increase 10-fold from 2 to 20 nmol cyclic AMP/min per g wet wt. in developing rat. Cyclic AMP phosphodiesterase decreased gradually with postnatal age. Thus, the activity of enzymes which control glycogen metabolism in membrane and cytosolic compartments of skeletal muscle is modified to meet the physiological demands of developing fast muscle.

Materials and Methods

Materials

Malate dehydrogenase and glucose-6-phosphate dehydrogenase were obtained from Boehringer-Mannheim Biochemicals, Indianapolis, IN. Cyclic-[^{3}H]AMP, [α-^{32}P]ATP, and UDP[^{14}C]glucose were from New England Nuclear, Boston, MA.

Methods

Animals. New Zealand white rabbits of postnatal age 0, 1, 2, 3, 4, 6 and 8 weeks served as a source of lower limb skeletal muscle. Animals were killed by intracardiac injection of pentobarbital and the extensor digitorum longus and anterior tibialis muscles removed to ice-cold sucrose buffer (0.25 M sucrose/0.2 mM EDTA/0.1 M Tris-HCl, pH 7.6).

Tissue preparation. Skeletal muscle was homogenized in 5 vols. of ice-cold sucrose buffer with a Polytron homogenizer for 15 s at setting 7.5. The homogenate was passed through four layers of cheesecloth to remove connective tissue fragments. For certain experiments the cytosolic fraction was examined. Cytosol was prepared by centrifugation of the muscle homogenate at 78 000 $\times g$ for 1 h in a Beckman Type 30 rotor, L5-50 ultracentrifuge.

Glycogen determination. After removal, 1 g wet wt. of muscle was extracted with 30% KOH. Glycogen was precipitated by addition of 95% ethanol. Glycogen content was determined by the anthrone method [6].

Triacylglycerol determination. Muscle was extracted with 10 vols. of $CHCl_3$/CH_3OH (2 : 1, v/v). Triacylglycerol was isolated by thin-layer chromatography in one dimension using Silica gel G thin-layer plates and hexane/diethyl ether/formic acid (7 : 3 : 0.15, v/v/v) as the developing solvent. Triacylglycerol content was determined by the method of Sardesai and Manning [7].

Enzyme determinations. a. Lactate dehydrogenase, citrate synthase and hexokinase determinations were performed for skeletal muscle tissue as described by Bass et al. [2].

b. Glycogen phosphorylase activity was determined by the method of Cori et al. [8]. The reaction was initiated by addition of adenosine 5'-monophosphate and inorganic phosphate measured [9].

c. Glycogen synthase activity was measured in a reaction mixture containing 50 mM Tris-HCl, 2 mM Na_2EDTA, 5 mM UDP glucose, 230 000 cpm UDP-[^{14}C]glucose, 8 mg/ml glycogen and 100—200 μg muscle protein (pH 7.4) either in the presence or absence of 10 mM glucose 6-phosphate [10].

d. Cyclic AMP content was determined by the competitive protein-binding

method of Gilman [11] using the kit manufactured by Diagnostic Products Corporation.

e. Cyclic AMP phosphodiesterase activity was measured in a reaction mixture containing 40 mM Tris-HCl, 5 mM $MgCl_2$, 3.75 mM β-mercaptoethanol, 100 μM cyclic AMP, 200 000 cpm cyclic[^{3}H]AMP and 100—200 μg muscle protein (pH 8.0) [12].

Sarcolemmal membrane isolation. Sarcolemma was isolated from rabbit skeletal muscle by a modified procedure previously employed for the membrane isolation from rat skeletal muscle [13]. Lower limb skeletal muscle (10 g) was homogenized in 10 vols. of sucrose buffer by Polytron homogenization for 15 s, setting 7.5. The homogenate was centrifuged at 4300 $\times g$ for 10 min. The resulting pellet was resuspended in 20 vols. (original muscle weight) of LiBr medium (0.5 M LiBr, 0.01 M Tris-HBr, pH 8.5) and stirred 14 h at 4°C. The suspension was centrifuged at 144 000 $\times g$ for 45 min and the pellet washed twice with KCl medium (0.6 M KCl, 0.1 M Tris-HCl, pH 8.0) and deionized water. Crude membrane material was applied to continuous (15—35%) sucrose gradients and centrifuged at 200 000 $\times g$ for 210 min in a SW-41 rotor, L5-50 Beckman ultracentrifuge. Membrane material in the 18—22% zone of the gradient displayed the highest total and specific activity of $(Na^+ + K^+)$/(Mg^{2+})-ATPase and epinephrine-stimulated adenylate cyclase.

Adenylate cyclase determination. Adenylate cyclase determinations were performed by the method of Salomon et al. [14]. Effectors at final concentration included either 10 mM NaF or $1 \cdot 10^{-4}$ M epinephrine bitartrate.

Protein determination. Protein content was determined by the method of Lowry et al. using bovine serum albumin as the standard [15].

Results

Triacylglycerol and glycogen are the major storage forms of fatty acids and glucose in skeletal muscle. Neonatal rabbit skeletal muscle exhibited the highest content of triacylglycerol (9 mg/g wet wt.) and glycogen (12.1 mg/g wet wt.) (Fig. 1). The triacylglycerol content did not decline until the 2nd postnatal week and eventually reached a level of 1.8 mg/g wet wt. by the 8th week. Glycogen content decreased to 7 mg/g wet wt. by the 3rd week and remained at this level. The ratio of glycogen to triacylglycerol content increased during development. Newborn, 1- and 2-week-old animals had a glycogen/triacylglycerol ratio between 1.2 and 1.4. The ratio increased beginning between the 3rd and 4th postnatal weeks reaching values of 3 and 4 at the 6th and 8th weeks, respectively, indicating a relatively greater conservation of glycogen with muscle development. The protein content of the total homogenate increased gradually from 65 mg/g wet wt. in the neonate to 105 mg/g wet wt. in adult skeletal muscle.

Citrate synthase and hexokinase are representative enzymes of the tricarboxylic acid cycle and hexose phosphorylation. Bass et al. [2] have shown these two enzymes to undergo a postnatal decline in activity during the development of the posterior latissimus dorsi muscle of the chick. In our studies citrate synthase and hexokinase activity were maximal at the 1st postnatal week (Fig. 2). Enzyme activity diminished during the 2nd—3rd week and

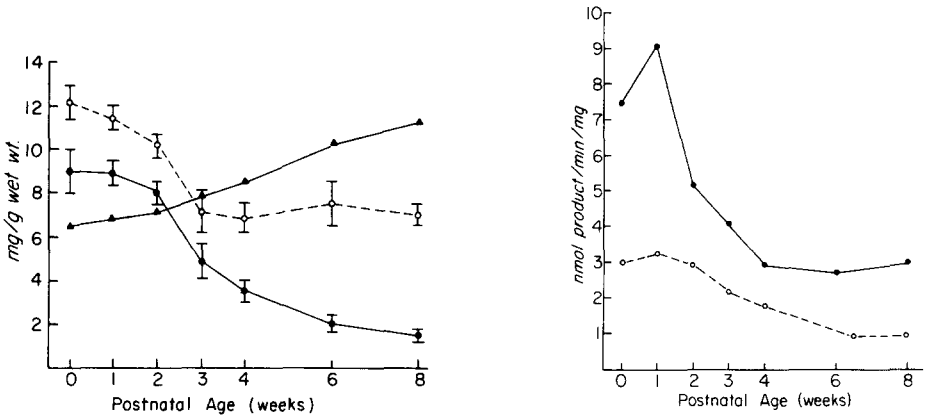

Fig. 1. Triacylglycerol, glycogen and protein content. ●———●, triacylglycerol; ○- - - - -○, glycogen; ▲———▲, protein (mg × 10^{-1}).

Fig. 2. Citrate synthase and hexokinase activity. Muscle homogenate from each developmental age was centrifuged at 78 000 × *g* for 1 h to obtain a supernatant fraction for enzyme activity measurements. ●———●, citrate synthase; ○- - - - -○, extra mitochondrial hexokinase.

remained constant over the 6th—8th week. The parallel loss of triacylglycerol content, citrate synthase and hexokinase activity is consistent with the overall decline in oxidative metabolism in developing muscle.

The decrease in oxidative enzyme markers was accompanied by concomitant increases in enzymes which participate in anaerobic glycolysis (lactate dehydrogenase) glycogenolysis (glycogen phosphorylase, Fig. 3), and glycogen

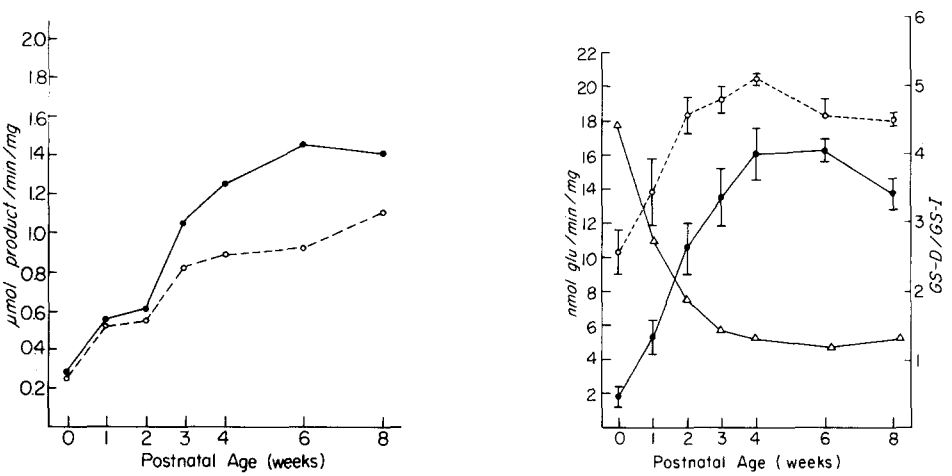

Fig. 3. Glycogen phosphorylase and lactate dehydrogenase activity Glycogen phosphorylase was measured in the total muscle homogenate in the presence of 5 mM AMP. Glycogen phosphorylase (μmol P_i/mg per min, ●———●); lactate dehydrogenase (μmol NAD^+(× 10^{-1})/mg per min, ○- - - - -○).

Fig. 4. Glycogen synthase activity. Glycogen synthase activity in the presence (○- - - - -○) and absence (●———●) of 10 mM glucose 6-phosphate. Ratio of glycogen synthase D to glycogen synthase I activity (△———△).

synthesis (glycogen synthase, Fig. 4). Glycogen phosphorylase, lactate dehydrogenase and glycogen synthase I reached maximal activity between the 6th and 8th postnatal weeks. The increase in glycogen synthase activity was different depending on whether the enzyme activity was measured in the presence (D form) or absence (I form) of 10 mM glucose 6-phosphate. The D form of the enzyme underwent little increase in activity after the 2nd postnatal week whereas the I form reached maximum activity (14—16 nmol Glc/min per mg) by the 4th postnatal week. The increase in glycogen synthase activity was accompanied by a decrease in the proportion of D to I form of the enzyme (Fig. 4). The ratio of glycogen phosphorylase/glycogen synthase D increased during postnatal development approximately 3-fold.

Cyclic AMP is a critical metabolite for the regulation of glycogen synthase and glycogen phosphorylase activity. Cyclic AMP content was 0.95 nmol/g wet wt. at the 1st postnatal week and declined to 0.6—0.7 nmol/g wet wt. by the 6th—8th week. Cyclic AMP phosphodiesterase activity was also maximal at the 1st postnatal week, declining almost 6-fold to 0.2—0.3 nmol 5'-AMP/min per mg between the 4th and 8th weeks.

Adenylate cyclase activity was measured in isolated sarcolemma from 0—1 and 8-week-old skeletal muscle. These postnatal ages represent the pre- and post-transition phases for enzyme development in these studies. The activation of adenylate cyclase showed a potency series typical of that expected for the β_2-adrenergic response; isoproterenol was more effective than epinephrine and norepinephrine with K_m values of $5 \cdot 10^{-8}$, $1.8 \cdot 10^{-7}$, and $1.1 \cdot 10^{-6}$ M, respectively. NaF and epinephrine-stimulated adenylate cyclase increased 10-fold in the 8-week-old compared to neonatal sarcolemma (Table I). The increase in hormonal activity coincided with the increased activity levels of glycogen synthase and glycogen phosphorylase and the overall greater capacity for glycogen metabolism in developing fast muscle. The ratio of F^- to epinephrine-

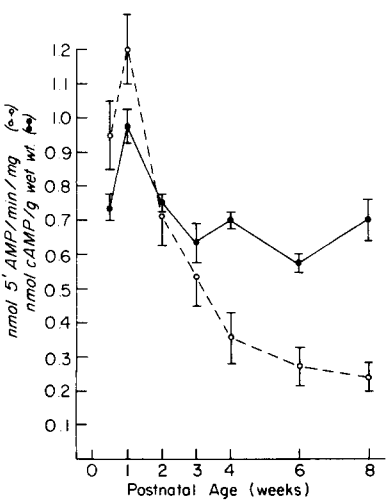

Fig. 5. Cyclic AMP phosphodiesterase activity and cyclic AMP content. Cyclic AMP phosphodiesterase was measured in the total homogenate; cyclic AMP concentration was 100 μM. ○- - - - -○, cyclic AMP phosphodiesterase; ●———●, cyclic AMP content.

TABLE I

ADENYLATE CYCLASE ACTIVITY IN NEONATAL AND ADULT SKELETAL MUSCLE SARCOLEMMA

Results are expressed as the mean ± S.E. for three separate experiments.

	Specific activity (pmol cyclic AMP/min per mg)	
	0—1 week	8 week
Basal	10 ± 3.5	54 ± 8.5
NaF	51 ± 14	657 ± 57
Epinephrine	25 ± 6	289 ± 44
NaF/epinephrine	2	2.3

stimulated activities remained constant, indicating that no selective effect on the epinephrine response occurred with development.

Discussion

The late embryonic and early postnatal phases of skeletal muscle development are characterized by the formation of immature muscle fibers. After birth, the muscle fibers increase in length, diameter, and content of myofibrillar proteins [16]. Muscle groups which ultimately become fast contracting-glycolytic in the adult undergo a postnatal increase in the ability to synthesize and utilize glycogen. This metabolic differentiation has been shown to involve the increased synthesis of the enzymes which mediate glycogen storage, glycogenolysis and glycolysis.

In the present study it was shown that the postnatal development of rabbit skeletal muscle was characterized by an increase in glycogen phosphorylase, glycogen synthase, lactate dehydrogenase and adenylate cyclase activity. The transition for the change in these enzyme activities occurred between the 2nd and 3rd postnatal week.

The sarolemmal membrane is the site of the insulin receptor and β-adrenergic receptor-adenylate cyclase system [17]. Insulin and epinephrine have been shown to control the rate of glycogen synthesis and glycogenolysis in skeletal muscle. One would predict that as muscle differentiates the enzymes for glycogen metabolism, membrane changes would also occur to allow the cell to bind hormones and regulate cyclic AMP concentration. In the studies reported here, cyclic AMP levels declined by 20% comparing 1-week to 8-week-old animals. These measurements were made on resting muscle and do not describe the response of the cell to epinephrine. Further, because the isolation of cyclic AMP from muscle begins with a homogenate, definition of cyclic AMP concentrations in specific muscle cell compartments is not possible. The major enzymes responsible for cyclic AMP metabolism underwent reciprocal changes in activity. The 6-fold loss of cyclic AMP phosphodiesterase activity was accompanied by a 10-fold increase in adenylate cyclase activity in isolated sarcolemma. The increase in both NaF and epinephrine-stimulated adenylate cyclase corresponds with the development of a functional β-adrenergic receptor-

adenylate cyclase system as a membrane control point for the regulation of glycogenolysis by epinephrine.

Acknowledgments

This study was supported by grants from the Muscular Dystrophy Association, Basil O'Connor Starter Research grant of the National Foundation, March of Dimes and NIH grant HD-12562. P.B.S. is a Teacher-Investigator awardee of the National Institute of Neurological and Communicative Disorders and Stroke (NS-00330). The technical assistance of Robert Pearson, Kenneth Crumb and Deborah Kelley is acknowledged.

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