BINDING OF UDP-GLUCOSE TO HOMOGENATES OF NORMAL AND DENERVATED SKELETAL MUSCLE

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1. Introduction

This laboratory has been investigating various aspects of glycoconjugate metabolism in normal and denervated rat skeletal muscle [1,2]. Recent experiments aimed at demonstrating UDP-glucose: glycoprotein glycosyltransferase activity in muscle homogenates were encouraging, and seemed to show that one week after denervation transferase activity had greatly increased over control muscle values. In these preliminary experiments radioactive UDP-glucose was incubated at 37°C with muscle homogenate and the reaction terminated some time later with the addition of phosphotungstic or trichloracetic acid. Acid-insoluble material was then quantitated by liquid scintillation spectrometry. But it was subsequently determined that if the reaction mixture was incubated at 0–2°C rather than at 37°C far greater amounts of radioactivity were present in the acid insoluble material. These results were not obtained when UDP-glucose was replaced by UDP-galactose, UDP-N-acetylglucosamine, GDP-fucose, GDP-mannose, CMP-sialic acid, or glucose. Similarly prepared homogenates of rat brain or kidney did not substitute for muscle in this regard; radioactivity in acid insoluble material of liver was much reduced, but significant. Most surprising was that the effects depended on the use of *normal* muscle and was nearly absent in homogenates of denervated muscle. This report summarizes many characteristics of this unusual phenomenon.

2. Materials and methods

Adult male Holtzman rats (250–325 g) were killed by a sharp blow on the neck and the extensor digitorum longus muscle (EDL) of each hind leg was removed and placed in a beaker of ice-cold saline. Each muscle was then minced with scissors in 15 vol of cold distilled water and homogenized for 30 strokes with a TenBroeck homogenizer held in an ice–water bath. This homogenate was used directly for experiments.

When required, the EDL of the left or right leg was denervated as described [1]. A sham operation was always performed on the opposite leg and its EDL served as the control. The animals were maintained for one week on a regular laboratory diet with free access to water.

Unless stated otherwise, the usual reaction mixture contained in a final volume of 0.10 ml: UDP-[^{14}C]glucose (236 Ci/mol, New England Nuclear), 0.3 nmol; homogenate protein, 0.30 to 0.50 mg; and 2.5 μmol Tris-maleate buffer to a final pH of 7.0 (23°C). Mixtures were incubated with frequent shaking in an ice–water bath for 0.5 h and terminated by the addition of 10% (w/v) trichloracetic acid (TCA). After an additional 0.5 h in the ice–water bath, the tubes were centrifuged for 10 min at 2000 *g*. Pellets were then washed twice with 10% TCA, dissolved in 1 N NaOH, and plated onto glass fiber discs. The discs were dried and placed in vials containing 0.5% PPO and 0.03% POPOP in toluene; radioactivity was measured in a

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liquid scintillation spectrometer. A final wash with ethanol: diethyl ether (2:1, v/v) removed negligible amounts of radioactive material and was omitted. Data given are counts per minute above background levels. Authentic UDP-[^{14}C]glucose was counted with an efficiency of 88% in this system. Experiments were performed in triplicate.

Protein was determined by a slight modification of the method of Lowry et al. [3] using bovine serum albumin as a standard.

3. Results and discussion

The data in fig.1 demonstrate that at 0–2°C radioactivity in the acid insoluble material of muscle homogenate rapidly approached a high, maximum value. After 0.5 h of incubation nearly 40% of the added radioactivity was recovered in the acid insoluble material. Quantitatively similar results were obtained when the incubated reaction mixtures were terminated by the addition of 1% (w/v) phosphotungstic acid (PTA) or methanol in place of TCA. In other experiments the usual reaction mixture was scaled up 10-fold, incubated at 0–2°C, terminated by centrifugation (5000 *g* for 10 min at 0–2°C), and the pellet washed twice with cold saline. The pellet contained

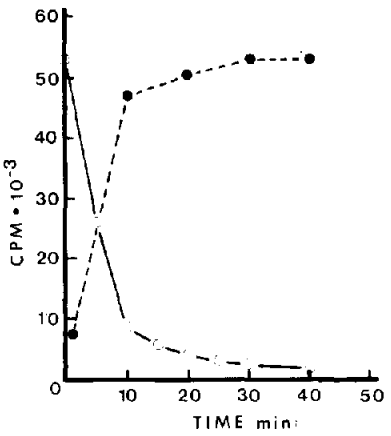

Fig.1. Time course of the increase (•) in radioactivity in acid insoluble material of normal muscle when incubated at 0–2°C and of the decrease (○) upon warming the reaction mixtures to 37°C after a 0.5 h preincubation at 0–2°C. Conditions of the experiment were as given in Materials and methods. The reaction mixtures contained 0.46 mg protein.

greater than 80% of the radioactivity found in comparable TCA terminated reaction mixtures. These experiments indicate that the effect is not merely a consequence of TCA precipitation. Boiling the homogenate prior to its addition to the reaction mixture completely eliminated the effect, as did prior precipitation with TCA or PTA.

When similarly prepared homogenates of rat brain, kidney, or liver replaced muscle in the reaction mixture, the amount of radioactivity in acid insoluble material on a wet weight basis was reduced 99.5%, 99.2% and 96%, respectively. Results similar to those presented for normal muscle were found using homogenates of human gastrocnemius muscle obtained from biopsy material. Quite different results were obtained, however, when homogenates of denervated muscle containing an equivalent amount of protein were substituted for the homogenates of contralateral control muscle. Slightly less than 1% of the radioactivity found in the acid insoluble material of the control muscle was present in the denervated muscle.

Fig.1 also demonstrates the progressive reduction in the amount of radioactivity found in the acid insoluble material of normal rat muscle when reaction mixtures preincubated at 0–2°C for 0.5 h were warmed to 37°C. The radioactive material released appeared to be entirely UDP-glucose as judged by paper chromatographic analysis.

Radiolabeled UDP-glucose was replace in the reaction mixture by equivalent amounts of other radiolabeled nucleotide monosaccharides (UDP-galactose, UDP-*N*-acetyl-glucosamine, GDP-fucose, GDP-mannose, and CMP-sialic acid) and by glucose. In no case did radioactivity in the acid insoluble material exceed 0.2% of that found with UDP-glucose. When unlabeled UDP-glucose (0.5 nmol) was added to the reaction mixture prior to the addition of radiolabeled UDP-glucose, radioactivity in the acid insoluble material was decreased 15%; radioactivity could be nearly eliminated by the addition of greater amounts of unlabeled UDP-glucose.

Acid-insoluble radioactive material could also be reduced if NaCl were added to the reaction mixture. Final concentrations of NaCl reduced radioactivity as follows: 0.01 M (0%), 0.09 M (17%), 0.15 M (50%), 0.23 M (79%), and 0.69 M (99.8%). Disodium EDTA (20 mM) increased radioactivity in the acid insoluble material by 27%. NaCl added after the standard incuba-

tion period and prior to termination was without effect.

The effects of reaction mixture pH on the amount of radioactivity in acid insoluble material after the usual 0.5 h incubation at 0–2°C was studied using Tris-maleate buffers. The results suggested a biphasic relationship and were as follows: pH (5.8) 67241; pH (6.2) 85444; (6.6) 73443; (6.8) 65567; (7.0) 64213; (7.4) 63184; (7.8) 88413; and (8.4) 93857.

The results of these experiments suggest that labile material is present in the homogenates of normal skeletal muscle which can bind UDP-glucose. The binding was greatly reduced in homogenates of denervated muscle, indicating that the material is reduced or altered after denervation. Some properties of this 'binding material' are similar to those described for a 'protein–glycogen complex' isolated from rabbit skeletal muscle [4]. As the glycogen content of denervated rat EDL has been reported to markedly decrease after denervation [5], it is possible that the UDP-glucose 'binding material' and the 'protein–glycogen complex' are one and the same. This possibility was supported by experiments in which rabbit liver glycogen was added to either normal or denervated muscle reaction mixtures. Acid insoluble radioactivity was in each case greatly increased. As UDP-glucose and glycogen alone were not precipitated by TCA, the indication is that some type of glycogen-muscle interaction was required for the effect. Whether a 'protein–glycogen complex' is the UDP-glucose 'binding material' of muscle homogenates and whether this interaction is important in the in vivo situation remains to be established.

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