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Glucose transporter localization in rat skeletal muscle

Autoradiographic study using ATB-[2-^{3}H]BMPA photolabel

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Abstract

Surface glucose transporters of intact muscles were photolabeled with the membrane impermeant ATB-[2-^{3}H]BMPA reagent and localized by autoradiography. We found sparse labeling of the glucose transporters by ATB-[2-^{3}H]BMPA on the sarcolemmal membrane around the muscle fiber. The majority of label was on the interior of the muscle fiber, at a discrete site which matched the distribution of AI junctions and which was presumed to be on the exterior surface of T-tubules. The amount of photolabel on the T-tubule was increased in response to insulin and wasblocked by cytochalasin B. These results support the concept that glucose transport may occur predominately across the T-tubule membrane under basal and insulin-stimulated conditions.

Key words: GLUT4; Glucose transport; Skeletal muscle; Bis-mannose; Photoaffinity label

1. Introduction

We previously reported using ultrastructural immunolabeling that GLUT4 was specifically localized at the T-tubule ; little GLUT4 labeling was observed on the surface sarcolemma of insulin-stimulated human skeletal muscle [1]. The fact that we found GLUT4 labeling predominately on the T-tubule seemed inconsistent with our previous report (using membrane fractionation) that translocation of glucose transporters to the 'plasma membrane' occurred in response to insulin [2]. However, we subsequently measured nitrendipine binding (a marker for Ca^{2+} channel of T-tubules) in 'plasma membranes' isolated by the methods of both Grimditch et al. [3] and Klip et al. [4] and found substantial contamination by T-tubules. We then isolated sarcolemmal membranes and triad membranes by the method of Burdett et al. [5] and demonstrated GLUT4 in the triad membranes but not in the sarcolemma [1]. Based on these observations, we proposed that insulin-stimulated glucose transport into the muscle cell might occur across the T-tubule [6].

To further resolve the site of glucose transport during insulin-stimulation, we felt it was essential to use an entirely different methodology other than immunocytochemistry. ATB-[2-^{3}H]BMPA photoaffinity label has been previously used to identify cell surface glucose transporters in isolated rat adipocytes [7]. In addition, this photoaffinity label has been used to demonstrate that the increase in glucose uptake in skeletal muscle after insulin treatment is associated with increased levels of GLUT4 on cell surface membranes [8–10]. Autoradiographic localization of the photoaffinity label in skeletal muscle should thus provide information concerning which cell surface membrane compartments are involved in glucose transport.

2. Experimental

2.1. Materials

Cytochalasin B and cytochalasin E were purchased from Aldrich (Milwaukee, WI). Insulin was obtained from Eli Lilly (Indianapolis, IN). Male Sprague–Dawley rats, 60–80 g, were purchased from Charles River (Boston, MA) and housed 5 days prior to use. The ATB-[2-^{3}H]BMPA (specific activity 10 Ci/mmol) was prepared from 1-azi-2,2,2-triflurorethylbenzoic acid and [2-^{3}H]BMPA as previously described by Clark and Holman [11].

2.2. ATB-[2-^{3}H]BMPA photolabeling and autoradiography

Rat soleus muscles were isolated from 80–100 g male rats anesthetized with a gas mixture of 70% CO_2/30% O_2 and decapitated. Soleus muscles were equilibrated in 95%:5%, O_2/CO_2-gassed Krebs–Henseleit bicarbonate buffer with Ca^{2+} concentration adjusted to 1.8 mM and containing 1 mM pyruvic acid and 0.1% bovine serum albumin at 29°C for 30 min. Muscles were incubated for an additional 20 min in fresh

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medium with one of the following additions: none for basal conditions; 130 nM insulin, insulin plus 8 μM cytochalasin B and 2.5 μM cytochalasin E. Individual soleus muscles were then transferred to 1 ml of gassed Krebs buffer containing 1 mCi/ml ATB-[2-^{3}H]BMPA for 5 min (or 2.5 mCi/ml for high concentration experiment). Muscles were then irradiated for 2 × 1 min with a 80-W Mercury ARC lamp employing a 20% $CuSO^4$ filter. Soleus muscles were manually turned over in between irradiation intervals. Following irradiation, muscles were blotted on wet 1M Whatman filter paper, trimmed of their tendons and fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, dehydrated, and embedded in LR White resin. Thick sections were cut (1–2 μm) and mounted on gelatinized slides. The slides were coated with Kodak NTB-3 emulsion and exposed for 2–3 weeks at 4°C. After exposure, slides were developed, fixed, and counterstained with 1% Toluidine blue.

2.2.1. Insulin stimulation. Three separate experiments were performed comparing muscle incubated under basal conditions versus 130 nM insulin. For each experiment, three blocks from each treatment group were sectioned at three randomly chosen areas producing a total of nine slides per treatment group for each experiment (18 total slides for each experiment). To quantify cell-surface labeling, all muscle tissue on the slide was counted. The variance between the three blocks from each treatment group was negligible.

2.2.2. Cytochalasin B competition. Three separate experiments were performed comparing muscle incubated with 130 nM insulin versus 130 nM insulin plus cytochalasin B. For each experiment, three blocks from each treatment group were sectioned at three randomly chosen areas producing a total of nine slides per treatment group for each experiment (18 total slides for each experiment). To quantify cell-surface labeling, all muscle tissue on the slide was counted. The variance between the three blocks from each treatment group was negligible.

Quantitation was done by a video-based computer-assisted image analysis system (Bioquant, R&M Biometrics). The skeletal muscle fiber was inscribed and all picture elements (pixels) above a gray-level threshold depicting the silver grains were counted. The ratio of above-threshold pixels (A) to total pixels (B) in the sampled region of muscle reflects the degree of labeling between conditions. Data was statistically analyzed using the unpaired Student's *t*-test and differences were accepted as significant at the $P < 0.05$ level

3. Results

Fig. 1A–C illustrates representative autoradiographs of the various treatment groups taken from the same slides that were quantitated in Fig. 2. One can qualitiatively appreciate the differences between the treatment groups in the number of silver grains: (A) low number under basal conditions, (B) increased number in response to insulin stimulation, and (C) low number with

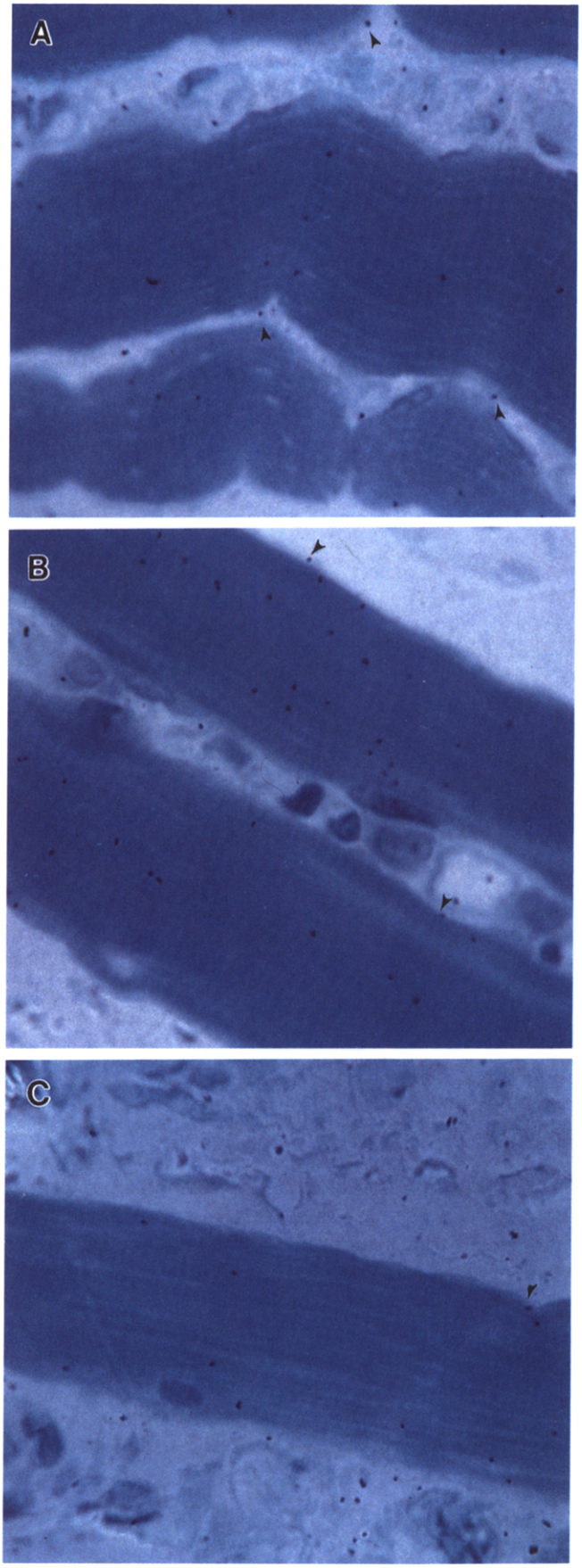

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Fig. 1. Autoradiographs of rat soleus muscle incubated with ATB-[2-^{3}H]BMPA photolabel under (A) basal conditions, (B) insulin stimulation, and (C) insulin plus cytochalasin. One can appreciate the increased number of silver grains due to insulin stimulation (B) versus (A) and (C). At first glance, silver grains appear to be inside the cell. However, the photolabel does not cross the cell membrane during short incubation times so that only glucose transporters on the surface exposed to the extracellular space are labeled. Therefore, our interpretation is that these silver grains are labeling glucose transporters on the external surface of the T-tubule membrane as the T-tubule extends perpendicular to the surface sarcolemma. Labeling of the surface sarcolemma is minimal and discrete (arrowheads). Labeling of the sarcolemma may indicate the location where T-tubules open to the surface of the muscle cell. In addition, silver grains associated with capillaries can be observed. Magnification: 825 ×, oil immersion.

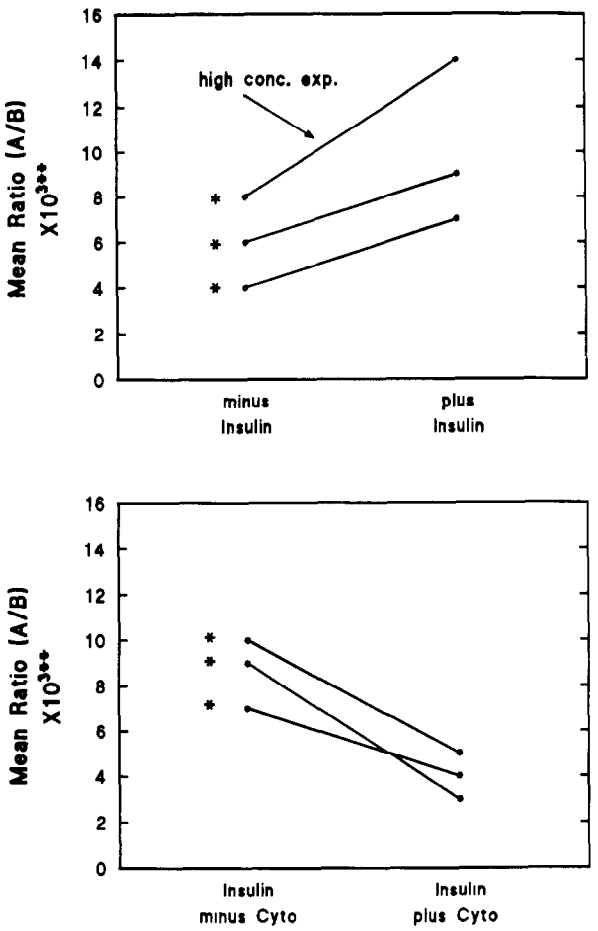

Fig. 2. Quantitation of autoradiographic slides showing the effect of insulin and cytochalasin on ATB-[2-^{3}H]BMPA photo-labeling of soleus muscles. Upper panel: soleus muscles were incubated as described in section 2 in the absence (basal state) or presence of 130 nM insulin and then were photolabeled. Three separate experiments were performed. All experiments showed that insulin significantly increased the amount of labeling. One experiment indicated by the arrow was done with a high concentration (2.5 mCi/ml) of photolabel. Lower panel: soleus muscles were incubated in the presence of 130 nM insulin and 130 nM insulin plus cytochalasin. Three separate experiments were performed. All experiments showed that cytochalasin significantly decreased the amount of labeling. *Statistically significant at $P < 0.05$ level; unpaired Student's t-test. **Mean ratio of above-threshold pixels (A) to total pixels (B).

insulin plus cytochalasin B. In addition, the autoradiographs show that the majority of the photoaffinity label was localized on the interior of the muscle fiber predominately at the A–I myofibrillar junction. Since the photolabel does not cross cell membranes to attain an intracellular location, our interpretation of the silver grain labeling pattern is that the photolabel is localized on the exterior surface of the T-tubules which run along the A–I myofibrillar junction. Photolabel was also observed on red blood cells, endothelial cells of capillaries, and the surface sarcolemma. However, photoaffinity labeling on the surface sarcolemma was scattered and greatly reduced in comparison to the A–I myofibrillar junction and could in fact be associated with T-tubules opening on the cell surface.

Since the photolabeling of the sarcolemma was low, we decided to do one experiment in which soleus muscles were incubated with a high concentration (2.5 mCi/ml) of photolabel. Even with a high concentration of photolabel, the labeling of the sarcolemma was minimal (autoradiograph not shown).

As shown in Fig. 2, quantification of silver grains due to photoaffinity labeling was performed on soleus muscle from each treatment group. In three separate experiments, the number of silver grains at the T-tubule increased significantly in the insulin-treated versus basal condition as indicated by the mean ratio of above-threshold pixels to total pixels (Fig. 2, upper panel). Interestingly, insulin treatment did not appear to cause an increase in the scattered labeling of the surface sarcolemma. In three different experiments, the number of silver grains at the T-tubule decreased significantly ($P < 0.05$) in cytochalasin-treated muscle versus control (Fig. 2, lower panel). There was still considerable labeling in the cytochalasin treated muscle which is probably due to non-specific binding of the photolabel [10]. Considering this background, the increase in surface glucose transporters in response to insulin shown in the upper panel of Fig. 2 underrepresents the full magnitude of the change.

4. Discussion

Using ATB-[2-^{3}H]BMPA photoaffinity label, we have confirmed that glucose transporters are present on the surface of the T-tubule membrane capable of interacting with extracellular environment. Most importantly, we found that insulin stimulation causes an increase in the number of glucose transporters (most likely GLUT4) in the T-tubule and that the increase could be competed by cytochalasin. The lack of labeling of the surface sarcolemma may be due to a detection limit where radioactive signals may represent 'clusters' of glucose transporters whereas single glucose transporters may not be strong enough emitters to expose the radioautographic emulsion. However, we think this is rather unlikely since there is no data to indicate that the GLUT4 intramembranous protein arranges itself in two different patterns.

Recent reports have confirmed T-tubule involvement in glucose transport. Using ultrastructural immunocytochemistry, other investigators have observed GLUT4 immunolabeling in the triad region; but, they also reported GLUT4 localization in subsarcolemmal vesicle structures [12,13]. Although GLUT4 vesicles were found in the triad region, these investigators reported that translocation occurred only at the surface sarcolemma

upon insulin [12] or insulin plus exercise [13] stimulation. This is in contrast to a recent report using a purified T-tubule membrane preparation [14] indicating that acute insulin stimulation causes GLUT4 translocation to predominately the T-tubule (180% increase) in comparison to the sarcolemma (90% increase). Since the method reported here covalently labels the cell surface glucose transporters of the intact soleus before the muscle is fixed, sectioned and quantitated, it is the closest morphological analysis of the transporter in vitro. The methodolgies used in other studies localize the transporter after tissue fixation, and in most cases using labels (antibodies) which bind surface active transporters and intracellular transporters. Even though the issue of GLUT4 localization and/or translocation remains controversial, it seems clear from our results that the T-tubule plays a role in the inulin response.

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