INSULIN RESISTANCE IN OVINE SKELETAL MUSCLE; INSULIN BINDING AND INSULIN ACTION

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Introduction

It has been known that ruminants is resistant to insulin. This evidence has been supported by the numerous reports that the stimulatory effects of insulin to lipogenesis from glucose are markedly lower in ruminant adipose tissues compared with rats (Vernon, 1979). However, the glucose disposal by adipose tissue is unlikely to be more important in ruminants because acetate rather than glucose is the principal carbon precursor for fatty acid synthesis in this species, whereas muscle utilizes a large proportion of the glucose disposal, as muscle glycogen and glucose oxidation. It is, however, still unknown whether in ruminants, insulin resistance seen in adipose tissue exist in skeletal muscle, and for the role of insulin in ruminant skeletal muscle.

The aim of the present study is to systematically characterize insulin binding to the isolated plasma membrane from ovine gastrocnemius muscle and to investigate as an index of the post receptor events the effect of insulin to glycogen synthesis and glycogen synthase activity to clarify the mechanism of insulin resistance in ruminants.

Materials and Methods

Six adult female Suffork x Corriedale crossbred sheep weighing 48-59 kg were used. They were fed 800 g of hay cube and 200 g of concentrate once daily at 08:00 h, but they were allowed free access to water. On the day of experiments, animals were not fed until the end of sampling skeletal muscle. Female Wistar strain rats weighing 170-180 g, 10 weeks of age, were used and fed Oriental rat chow.

Ovine gastrocnemius muscle (4-5 g) were obtained by open biopsy after an incisal area was locally anesthetized with 2% procain hidrochloride. The plasma membranes were prepared from ovine and rat gastrocnemius muscle according to miner modifications of Kidwai's subcellular separation techniques (Kidwai et al., 1973).

Binding studies were carried out by the method of Saucier et al. (1981) with minor modification. Briefly, plasma membranes were incubated with 0.05 nM ^{125}I-insulin in containing 0.2% bovine serum albumin and varying concentration of unlabeled porcine monocomponent insulin. Nonspecific binding was determined with 8.7 μM unlabeled insulin. All incubations were performed for 4 h at 20°C. At the end of the incubation, the samples were centrifuged at 13,000 x g for 10 min, and the radioactivity of the precipitate was counted.

The glycogen synthesis and glycogen synthase activity were used as an index of the post receptor events. Gastrocnemius muscles were incubated for 2 h at 37°C into Krebs – Ringer bicarbonate HEPES buffer containing U-^{14}C-glucose (5.5 mM, 0.5 μCi) and varying concentration of insulin. The formation of glycogen from labeled glucose was estimated by alkaline hydrolysis of muscle as described previously (Le Marchand-Brustel et al., 1979). Glycogen synthase activity was measured with a modification of the filter paper method of Thomas et al. (1968) in extracts (16,000 x g supernatant) of the gastrocnemius muscle incubated with or without 17 nM insulin. Protein was measured by the method of Lowry using bovine serum albumin as a standard.

Results and Discussion

Because hormone binding to receptors represents the initial step of insulin action, we postulated that a decrease in insulin binding to its receptors could explain the tissue resistance to insulin. In rodents, insulin binding characteristics in muscle have been performed with plasma membrane fractions derived from skeletal muscle. However, to date, the techniques have not been employed to make insulin binding studies in ruminant skeletal muscle. By introducing miner modifications of Kidwai's subcellular separation techniques and by evaluating the purity of plasma membranes by enzymatic markers, we were able to isolate the plasma membranes from ovine gastrocnemius muscle, and compared the characteristics of insulin binding with rats.

As shown in figure 1, the specific binding of ^{125}I-insulin to ovine and rat plasma membranes was 1.04 ± 0.04%/100 μg membrane protein and

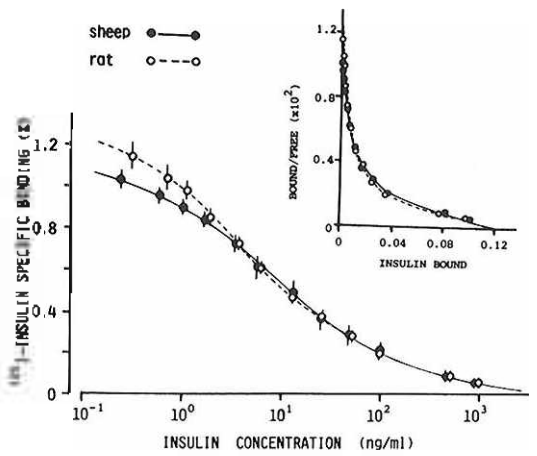

Figure 1. Specific binding of ^{125}I-insulin to plasma membranes of skeletal muscle and inhibiting effect of unlabeled insulin. Vertical bars represent standard error. Inset: Scatchard plot of the binding results.

1.15 ± 0.07, respectively. Half-maximal inhibition of ^{125}I-insulin occurred at 1.8 nM in sheep, no significant difference with rats. Scatchard analyses suggested the presence of more than one type of receptor, or a homogeneous type of receptor in which negative cooperativity occurs. Assuming that there are negative cooperativity, the affinity of empty receptor and filled receptor, and the total number of binding sites did not differ significantly between sheep ($0.90 \times 10^8 M^{-1}$, $0.25 \times 10^8 M^{-1}$, and 0.12 pmol/100 μg membrane protein) and rats. In view of the insulin binding characteristics alone, these results indicated that insulin resistance seen in adipose tissue did not exist in ovine skeletal muscle. However, there is some evidence to suggest that hormone binding is not necessarily an index for the expression of hormone action and that changes in events subsequent to binding may be rate-limiting in the biological response.

We, therefore, investigated as an index of the post receptor events the effect of insulin to glycogen synthesis and glycogen synthase activity, playing key roles in intermediary metabolism. Glycogen synthesis from glucose by ovine skeletal muscle was significantly lower than by rat skeletal muscle in both the basal state (sheep; 135.1 ± 9.0 nmol/g tissue per hr vs rats; (187.0 ± 7.9) and at all insulin concentrations. The percent increase above basal level of glycogen synthesis to the maximal insulin stimulation (8.7 nM) was significantly less in sheep (136%) as compared to rats (189%), indicating a decreased responsiveness, and while insulin concentration to half-maximal effect (ED_{50}), insulin sensitivity, was similar (2.2 nM). Basal glycogen synthase activities assayed in the absence (active form; I) or in the presence (total activity; I+D) of 10 mM glucose-6-phosphate, and %I (I/I+D) were less for ovine muscle than for rat muscle. Insulin significantly stimulated active form and %I of glycogen synthase in both groups, but not total activity. %I did not differ significantly between sheep and rats, but the responsiveness to insulin was higher in sheep rather than rats. These results suggest that insulin resistance in ovine skeletal muscle is not presumably located at an enzymatic site, but may be caused by a defect in receptor-effector coupling or glucose transport system itself.

Although skeletal muscle of ruminants exhibit insulin resistance, the resistance degree is very smaller in muscle than in adipose tissue, so that insulin appears to play a major role in intracellular glucose metabolism in ruminant muscle, probably including protein and lipid metabolism.

(**Key Words**: Insulin Resistance, Skeletal Muscle)

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