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Stimulation by insulin of accumulation and incorporation into protein of L-[^{3}H]proline in the intact levator ani muscle from the rat

The fact that insulin *in vitro* stimulates the incorporation of labelled amino acids into protein of the isolated rat diaphragm has been known for 15 years[1,2], and this effect seems to be independent of the action of insulin on glucose transport[3,4]. In the isolated rat diaphragm, insulin also stimulates the rate of uptake of amino acids across the cell membrane. This enhancement, which was first shown for the non-utilizable amino acid α-aminoisobutyric acid[5], has now been demonstrated for the transport of normal amino acids[6,7]. Neither the mechanism nor the physiological significance of these effects of insulin *in vitro* has been clarified in detail.

In most of these experiments dealing with the effects of insulin on mammalian muscle cells, either the cut or the intact rat diaphragm preparation has been used. The cut diaphragm preparation is not suitable for this type of study since cutting of the muscle fibres during preparation leads to the creation of artificial channels for diffusion of substances into and out of the muscle cells. The intact diaphragm preparation, which was first described by KIPNIS AND CORI[8], is from many points of view a suitable muscle preparation. However, this preparation also has disadvantages as discussed in a previous paper[9]. In addition, we think that it may be dangerous to draw general conclusions from studies on just one mammalian muscle, particularly if this muscle cannot be regarded, either anatomically or physiologically, as a typical skeletal muscle. We have recently reported that it is possible to prepare the levator ani muscle from immature male rats as an intact preparation *in vitro* and that this preparation seems to be useful in experiments on various aspects of mammalian muscle metabolism *in vitro*[9]. In the present paper, the effects of insulin *in vitro* on the rate of uptake and incorporation of L-[^{3}H]proline into protein in the levator ani muscle preparation are described.

The intact levator ani muscles of immature Sprague–Dawley male rats (45–55 g) were prepared and incubated as previously reported[9], *i.e.* the levator ani muscle was incubated in connection with the bulbocavernosus muscles in 1 ml medium in 10-ml flasks at 37° with constant shaking. The medium was Krebs bicarbonate buffer (pH 7.4) gassed with 95 % O_2 and 5 % CO_2 and containing glucose (2.5 mg/ml) and 0.05 mM uniformly labelled L-[^{3}H]proline (292 mC/mmole). Crystalline pig insulin (Novo 1562, 20 I.U./mg) was added to the medium to a final concentration of 0.01 I.U./ml. At the end of the incubation period the levator ani muscle was washed in cold buffer and blotted on filter paper. After dissection from the rest of the bulbocavernosus complex the levator ani muscle was weighed and homogenized in 1 ml 10% trichloroacetic acid. Following centrifugation, the radioactivities of aliquots of the supernatant and of the corresponding incubation medium were determined as described previously[9] in a liquid-scintillation spectrometer (Packard Tri-Carb). Tissue-water content was determined by drying to constant weight at 100°. Extracellular space was measured by incubating the tissue in the presence of uniformly ^{14}C-labelled sucrose as described in a previous paper[9]. Accumulation of L-[^{3}H]proline was calculated and reported as the distribution ratio between the concentration of radioactivity in the intracellular water and that in the medium (counts/min per ml intracellular water : counts/min per

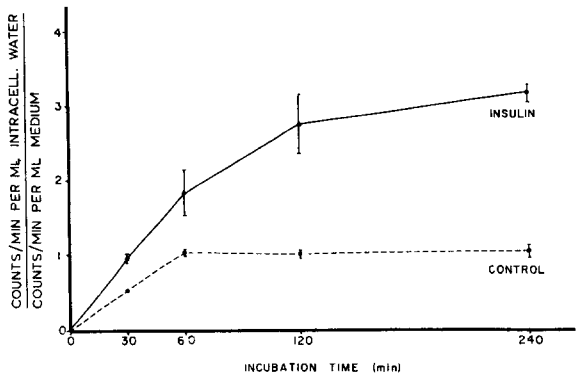

Fig. 1. Effects of insulin *in vitro* on the accumulation of L-[^{3}H]proline in the intracellular water of the intact levator ani muscle from the rat. The muscles were incubated in Krebs bicarbonate buffer containing glucose (2.5 mg/ml) and 0.05 mM L-[^{3}H]proline for various incubation times with and without insulin (0.01 I.U./ml) in the medium. Each point represents the mean of 3 observations and the standard errors are indicated by vertical lines. In the absence of such lines, the standard error was too small to be indicated. The effect of insulin became significant after 30 min of incubation ($P < 0.02$; P values based on t test).

ml medium). The trichloroacetic acid precipitate of the muscle was washed repeatedly with cold trichloroacetic acid and then heated for 15 min at 90° in trichloroacetic acid. The insoluble fraction was extracted twice with ethanol–ether–chloroform (2:2:1) and then dissolved in 0.4 M NaOH. The protein content of the NaOH solution was determined as described by LOWRY *et al.*[10], and the radioactivities of aliquots of the solution were determined by a modification of the SCHÖNIGER combustion technique[11] and liquid-scintillation counting. The samples were counted initially without standard and again after addition of a known internal standard. From the recovered radio-

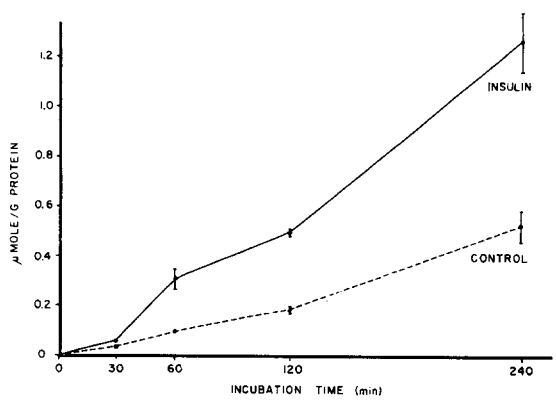

Fig. 2. Effects of insulin *in vitro* on the incorporation of L-[^{3}H]proline into the protein of the intact levator ani muscle from the rat. The muscles were incubated in Krebs bicarbonate buffer containing glucose (2.5 mg/ml) and 0.05 mM L-[^{3}H]proline for various incubation times with and without insulin (0.01 I.U./ml) in the medium. Each point represents the mean of 3 observations and the standard errors are indicated by vertical lines. When such lines are not given, the standard error was too small to be indicated. The effects of insulin after 60, 120 and 240 min were all significant ($P < 0.025$ or less; P values based on t test).

activity (disint./min), the amount of radioactivity was calculated and expressed as μmoles L-[^{3}H]proline incorporated per mg muscle protein.

The results are shown in Figs. 1 and 2. In the experiments without insulin in the medium the accumulation of L-[^{3}H]proline in the intracellular water (Fig. 1) increased during the first hour of incubation and then reached a steady-state level, while the increase of radioactivity in the protein fraction (Fig. 2) continued nearly linearly for at least 4 h. This behaviour is of interest particularly in relation to similar changes reported for various types of isolated cells[12]. It can also be seen from the figures that insulin *in vitro* increased the intracellular accumulation of L-[^{3}H]proline and the rate of incorporation of this amino acid into the protein. For the accumulation the effect was significant after 30 min of incubation, and for the incorporation the effect was significant after 1 h of incubation.

The results show that insulin *in vitro* stimulates the intracellular accumulation and incorporation of L-[^{3}H]proline into protein in the isolated intact levator ani muscle from immature male rats. They also show that this preparation is suitable for further analyses of the mechanism and the physiological significance of the effects of insulin *in vitro* on amino acid transport and protein biosynthesis in mammalian muscle tissue.

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