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Effect of insulin on aminoisobutyric acid uptake by human non-rheumatoid and rheumatoid synovial cells

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The biological sensitivity of cultured non-rheumatoid human synovial cells (NRSCs) and rheumatoid synovial cells (RSCs) was examined in terms of the ability of insulin to stimulate the uptake of α-aminoisobutyrate (AIB). NRSCs, like numerous fibroblastic lines, were sensitive to physiological concentrations of the hormone: half-maximal stimulation was obtained with (4×10^{-10} M) insulin, while maximum transport was found with a 60–90 min association time. On the contrary, although the basal transport was similar in RSCs, insulin was totally unable to accelerate AIB transport in these cells. Inflammatory processes lead to an insulin resistance which most likely involves a post-receptor step at the cellular level.

Insulin; Amino acid transport; Insulin response; Rheumatoid arthritis; (Synovial cell)

1. INTRODUCTION

Synoviocytes are fibroblastic cells which might undergo metabolic modifications during inflammatory processes such as rheumatoid arthritis, through a stimulation by numerous soluble factors or local hormones [1–3]. In a recent paper we described modifications in the initial step of rheumatoid cell culture, i.e. attachment and spreading [4]. On the other hand, the specific ligands which arrive at the synovial membrane via the blood stream have largely been ignored apart from the work of Goldring et al. [5] on the parathyroid hormone. Insulin was chosen with a view to investigating synoviocyte sensitivity to circulating hormones. Insulin is known to stimulate amino acid transport in fibroblasts [6,7] through system A [8]. The α-aminoisobutyrate (AIB) uptake process was thus studied in synoviocytes and the influence of acute inflammation states on AIB transport with regard to insulin was investigated.

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2. MATERIALS AND METHODS

2.1. *Materials*

Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), glutamine, bicarbonate, trypsin, phosphate buffered saline (PBS) were purchased from Eurobio (Paris, France). Clostridia collagenase (type II) and bovine pancreas insulin were from Sigma (St. Louis, USA). α-[1-^{14}C]Aminoisobutyrate (spec. act. 57 mCi/mmol) was obtained from CEA (Saclay, France).

2.2. *Cell cultures*

Synovial cells were isolated from samples of knee synovium obtained at synovectomy from patients with either osteoarthritic joint disease or clinically and biologically confirmed rheumatoid arthritis. Patients presented neither diabetes nor clinical insulin resistance. Synovial cell cultures were prepared using a proteolytic procedure as described [4]. When cellular confluence was attain-

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ed, a passage was carried out using trypsin. 7×10^4 cells were suspended in 2 ml of DMEM plus 4.8% bicarbonate and 2% glutamine supplemented with 10% FCS and placed in 9.6 cm^2 plastic dishes. Sub-confluent cultures were used for cell experiments.

2.3. *AIB uptake*

The experiment was carried out as described [9]. Briefly, to reduce the intracellular pool of amino acids and thus minimise possible trans-effects [10], the cells were placed for 1 h in depletion medium, i.e. PBS alone or with effector. Cells were then incubated for 10 min in PBS containing 8 μM (0.5 μCi/ml) [^{14}C]AIB. Cells were dissolved in 1 M NaOH, radioactivity was counted, and protein content measured by the Lowry method [11]. The results were expressed as cpm/mg protein per 10 min.

2.4. *Statistics*

Data are given as means ± SD of triplicate measurements. Comparisons were made by use of a non-parametric one-way analysis of variance according to Kruskall-Wallis test [12].

3. RESULTS

To evaluate the sensitivity and responsiveness of human non-rheumatoid (NRSCs) and rheumatoid synovial cells (RSCs) to insulin, the biological response to the hormone was analyzed by performing dose-response curves and time courses of insulin action.

3.1. *Dose-response curves for insulin*

Sub-confluent cultures of human synovial cells were incubated at 37°C with various concentrations of bovine insulin (1.7×10^{-10} to 1.7×10^{-7} M). The association time in the presence of insulin was 1 h, since we have previously found that this time allows the maximal stimulation effect of AIB uptake in fibroblasts [13]. The data for AIB uptake by NRSCs are given in fig.1. The transport rate was significantly higher than in controls from 1.7×10^{-9} to 1.7×10^{-7} M insulin and from 1.7×10^{-8} to 1.7×10^{-7} M insulin in the two cell cultures, GE.M. and TE.J., respectively. The maximum stimulation was obtained between $1.7 \times$

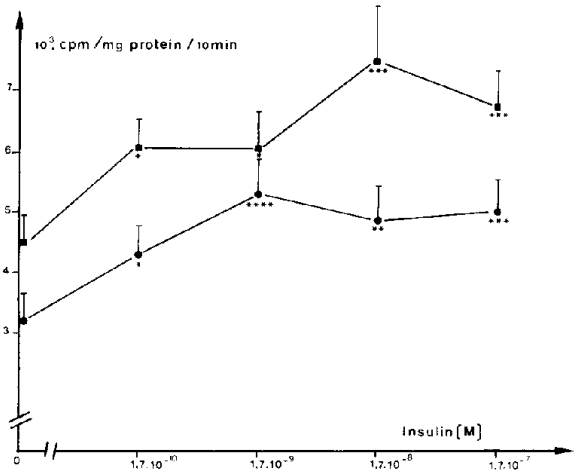

Fig.1. Dose responses of insulin stimulation of AIB transport in donor GE.M (●) and donor TE.J (■) of NRSCs. Each point is the mean ± SD of triplicate determinations. * $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$ and **** $p < 0.001$ compared to basal uptake.

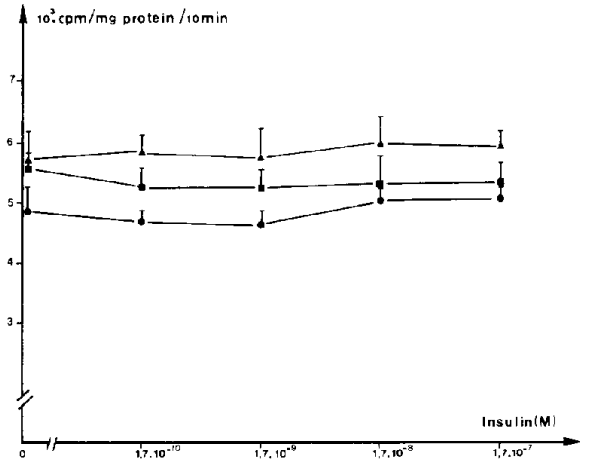

Fig.2. Dose responses of insulin stimulation of AIB transport in donor MO.J (●), donor MI.A (▲) and donor VU.C (■) of RSCs. Each point is the mean ± SD of triplicate determinations.

10^{-9} M and 1.7×10^{-8} M insulin, corresponding to a 60% increase over basal uptake. The NRSCs were sensitive to the action of insulin with half-maximal stimulation at a hormone concentration of approx. 4×10^{-10} M. In the cultures from three rheumatoid donors, AIB uptake was not stimulated whatever the concentration of insulin used (fig.2).

Table 1

Time course of insulin effect on AIB uptake in NRSCs and RSCs

Cell cultures	Time (min)				
	0	30	60	90	120
NRSC	5505 ± 455	6763 ± 343	8058 ± 576**	8488 ± 451**	7940 ± 450*
RSC	3754 ± 390	4566 ± 620	3168 ± 502	3412 ± 416	3230 ± 426

Each point is the mean ± SD of triplicate determination. * $p < 0.01$ and ** $p < 0.001$ compared to control. NRSCs were from donor PA.C and RSC from donor RS.A

3.2. *Time courses of insulin effect*

For these experiments, a physiological stimulating insulin concentration (1.7×10^{-9} M) was used. Results are given in table 1.

In NRSCs, AIB uptake stimulation was obtained after 60 min, but the insulin effect gradually increased to a maximum at 90 min (+52% of basal uptake) when a steady state was reached.

In RSCs, insulin treatment at various association times had no effect on AIB uptake.

4. DISCUSSION

The present study provides evidence that insulin at concentrations within physiological values stimulates the uptake of AIB in NRSCs. This observation is in agreement with previous reports on the insulin effect on AIB uptake in human fibroblasts [14,15]. The hormone-mediated stimulatory effect was detected after a 60 min exposure of the synoviocytes to the hormone, but was only fully expressed 1½ h later. It is well known that insulin enhances amino acid uptake through mRNA and protein synthesis-dependent processes [7]. It appears that the sensitivity and responsiveness of synoviocytes to the hormone (half-maximum response 4×10^{-10} M, stimulation +60%) were quite similar to numerous reports in the literature [14–16].

The basal transport of AIB varied in the different experiments. However, the mean basal transport in NRSCs and RSCs was not significantly different. The AIB transport system can be considered as thus similar in NRSCs and RSCs. Our results are in disagreement with those of Byers et al. [17] who showed a decrease in amino acid transport induced by inflammation in chondrocytes. However, their experiments were performed using rabbit articular cartilage tissue, not cell cultures after in vivo treatment with carrageenin.

We have shown impaired insulin action in cultured synovial cells from patients with rheumatoid arthritis. These results cannot be related to any clinical state of insulin resistance such as leprechaunism [15], diabetes [18] or pregnancy [19]. Moreover, the interference of drugs such as aspirin or glucocorticoids which induce insulin resistance [20,21] appears unlikely; in effect, it was removed during the various steps of the culture. In RSCs, we observed a combination of a lack of sensitivity and responsiveness to insulin, whatever the rheumatoid patient. Insulin resistance at the cellular level is due to an alteration at a pre-receptor, receptor or post-receptor level. As insulin resistance at the pre-receptor level would involve a factor reducing the free insulin concentration, we verified that RSC culture medium did not release an insulin degrading proteolytic enzyme (not shown). At the membrane level, alterations in receptor affinity or concentration could also result in a decreased sensitivity to the hormone. We have demonstrated (submitted) that the number and the affinity of insulin receptors were similar in NRSCs and RSCs. This implies that the anomaly exists at the post-receptor step, preventing the hormone from increasing either the velocity of translocation of amino acids across the membrane or the turnover of carriers [22].

In conclusion, these findings indicate a normal biological effect of insulin in NRSCs and a specific defect in RSCs involving a decreased sensitivity

and an unresponsiveness to the hormone. These data will be of use in the interpretation of future work on RSCs with regard to the biological effects of polypeptide hormones.

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