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# IL-1β, a strong mediator for glucose uptake by rheumatoid and non-rheumatoid cultured human synoviocytes

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Higher basal 2-deoxy-D-glucose uptake in rheumatoid synovial cells than in non-rheumatoid synovial cells, was found to be associated with an increased interleukin-1β (IL-1β) secretion (respectively 850 ± 238 vs. 8.3 ± 2.4 pg/24 h/$10^5$ cells, mean ± S.E.M.). When exogenous human recombinant IL-1β was added to cultures, a marked stimulation of 2-deoxy-D-glucose uptake was performed by both human synovial cultured cells, in a time-dependent and dose-dependent manner (IL-1β 0–100 ng/ml). In non-rheumatoid synoviocytes, stimulation occurred 1–3 h following the addition of 1 ng/ml interleukin-1β and increased up to 24 hours (respectively +150% and +261.4% after 6 and 24 hours association time). Rheumatoid synovial cells were less sensitive to 1 ng/ml IL-1β (respectively +80% and +146.4%). IL-1β increased significantly the $V_{max}$ for 2-deoxy-D-glycose uptake by synovial cells, with no change in the $K_m$. This effect was protein synthesis-dependent, and not secondary to prostaglandin E2 synthesis or cell growth. IL-1β possesses an important effect on glucose homeostasis in synovial cells, which could be indirect and/or regulated by the presence of natural inhibitors.

Human synoviocyte; Glucose uptake; Rheumatoid arthritis; Interleukin-1β

## 1. INTRODUCTION

Inflammation and joint destruction in rheumatoid arthritis are in part mediated by local soluble mediators called cytokines. In this way, IL-1β was demonstrated to induce a wide range of effects in cultured rheumatoid synovial cells, including collagenase, prostaglandin E2 [1], glycosaminoglycans [2] production, as well as DNA synthesis [3]. All of these effects require nutrient and energy supply. Various authors have shown that the rheumatoid synovium is able to metabolize glucose more efficiently than non-rheumatoid synovium (see [4] for a review). In a previous work, we surprisingly found that non-rheumatoid synovial cells (NRSC) were poorly sensitive and RSC-resistant to human insulin, in terms of 2-deoxy-D-glucose (2-DOG) uptake [5], a non-metabolizable analogue of D-glucose. Taylor et al. [6] observed that glycolysis and lactate production in cultured rheumatoid synovial cells (RSC) were increased after 24 h incubation in the presence of IL-1β. These results led us to study the kinetic effects of IL-1β on glucose uptake by NRSC and RSC. In this paper, we also report that the IL-1β-stimulated glucose transport was found to be a protein synthesis-dependent process which was not secondary to cell growth or to the action of prostaglandin E2.

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## 2. MATERIALS AND METHODS

### 2.1. *Chemicals*

Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), trypsin and phosphate-buffered saline (PBS) were from Eurobio (Les Ulis, France). Clostridia collagenase (type Ia), prostaglandin E2 (PGE2), cycloheximide (CHX), indomethacin (INDO), cytochalasin-B (CYT.B) and 2-deoxy-D-glucose were from Sigma (St.-Louis, MO, USA). Human recombinant interleukin-1β (hr-IL-1β) was from Genzyme (Boston, MA, USA). 2-deoxy-D-[1-$^3$H]-glucose (2-DOG) (sp.act 15–25 Ci/ml) was purchased from Amersham International (Bucks, UK) and [$^3$H]6-thymidine ($^3$HTdr) was from CEA (Saclay, France).

### 2.2. *Synovial cell cultures*

Human synovial cells were isolated from surgical samples of synovium obtained during joint surgery from patients with osteoarthritic joint disease ($n$=10) or rheumatoid arthritis ($n$=6), as defined by the American Rheumatism Association criteria [7]. The superficial layer of synovium was dissected, then enzymatically digested with collagenase and trypsin as previously described [8]. Cells were suspended in DMEM containing 15% FCS, penicillin, streptomycin and fungizone and were plated at 5× $10^6$ cells in 75-$cm^2$ culture flasks. The cells were cultured until confluence in DMEM with 10% FCS at 37°C in an atmosphere containing 5% $CO_2$. The medium was changed every 3–7 days. When confluence was attained, a passage was carried out, using trypsin. 7 × $10^4$ cells were suspended in 2 ml of DMEM with 10% FCS and placed in 9.6-$cm^2$ six-well plates at 37°C (5% $CO_2$). Experiments were performed on subconfluent cultures at first passage.

### 2.3. *Immunoassay*

After a 24-h incubation time with fresh medium, culture supernatants were assayed for IL-1β using an enzyme-linked immunosorbent assay (ELISA) (Medgenix, Belgium). Results were expressed as pg/24 h/$10^5$ cells and correspond to IL-1β as a whole (free and bound).

### 2.4. *Treatment with human recombinant IL-1β*

The growth medium of the synoviocyte cultures was replaced by

fresh medium containing 2% FCS and either various concentrations of IL-1$\beta$ (0–100 ng/ml) for 24 h or 1 ng/ml IL-1$\beta$ for 6 h and various other times (1, 3, 6, 9 and 24 h). Uptake assays were then performed.

2.5. *2-DOG uptake*

2-DOG uptake assays were performed as previously described [9]. Briefly, cultures were washed three times with 2 ml of PBS, pH 7.4, and incubated for 5 min with 1 ml PBS containing 0.5 mM 2-DOG (2 $\mu$Ci/ml). The incubation medium was then removed and the dishes rinsed three times ice-cold PBS. The cell monolayers were dissolved in 1 M NaOH and radioactivity was counted in a liquid scintillation counter. Total cell protein was assayed according to Lowry et al. [10] with bovine serum albumin as standard. Results are expressed as $10^3$ cpm/mg protein per 5 min. To estimate kinetic parameters $K_m$ and $V_{max}$, 2-DOG uptake was similarly measured using 0.1–5 mM of unlabeled 2-DOG in the presence of 2 $\mu$Ci/ml [$^3$H]2-DOG. Data have been corrected for non-facilitated diffusion in the presence of 4 $\mu$M CTY.B.

2.6. *Mitogenic assay*

After incubation in the presence of IL-1$\beta$ (6 h), the medium was replaced with 5.4 $\mu$Ci [$^3$H]Tdr for a 0.5 h pulse incorporation. As previously described [3], the cells were then washed with PBS and treated with 5% trichloracetic acid (TCA) (15 min, 4°C) followed by 20% TCA (2 h, 80°C). Radioactivity in the supernatant was counted in a liquid scintillation counter. Resutls are expressed in cpm/well.

2.7. *Statistical analysis*

Values are given as mean ± S.E.M. of triplicate measurements. Each experiment was performed at least twice. Comparisons were made using the Mann and Withney *U*-test. Differences with *P* values of less than 0.05 were considered significant. The kinetic parameters $K_m$ and $V_{max}$ were determined by using a GESTAT package. In this way, the diffusion process was subtracted from velocity data, then a weighted least-square regression method was used for analyzing concentration levels and for correcting velocity data, after linearization according to the usual Lineweaver–Burk technique [11].

## 3. RESULTS AND DISCUSSION

The sensitivity and responsiveness of NRSC and RSC to IL-1$\beta$, studied after a 24 h association time, appeared to be dose-dependent (Fig. 1). 2-DOG uptake in NRSC was significantly increased over basal values at concentrations from 0.1 pg/ml to 100 ng/ml.

In RSC, 2-DOG uptake increased similarly with IL-1$\beta$ from 1 pg/ml to 0.1 ng/ml; no further effect was observed with higher concentrations. The spontaneous production and the release of IL-1$\beta$ by RSC could stimulate glucose uptake, which would therefore lead to a lower sensivity towards the action of exogenous IL-1$\beta$. This hypothesis is supported by the observation that a higher basal 2-DOG-uptake was associated to a cytokine production per day significantly higher by RSC (850 ± 238 pg/24 h/$10^5$ cells, mean ± S.E.M.) than by NRSC (8.3 ± 2.4) (Table I). The time course of IL-1$\beta$-induced 2-DOG uptake in NRSC and RSC was studied using 1 ng/ml IL-1$\beta$. As shown in Fig. 2, 2-DOG uptake stimulation by NRSC and RSC was time-dependent for up to 9 h of incubation. In NRSC, stimulation was significant after a 1-h association time, whereas 3 h were necessary to obtain a similar effect in RSC. In both cultures, 2-DOG uptake stimulation continued to increase strongly up to 9 h and was twice as high in NRSC as in RSC although 2-DOG uptake values were persistently higher in RSC.

Finally, NRSC were more responsive than RSC to 1 ng/ml IL-1$\beta$, regardless of the association time (6 or 24 h) (Fig. 3). The response of NRSC after a contact time of 6 h was similar to that of RSC after 24 h.

There are few data concerning the action of IL-1$\beta$ on glucose uptake in synovial cells. Taylor et al. [6] have shown that glycolysis and lactate production are in-

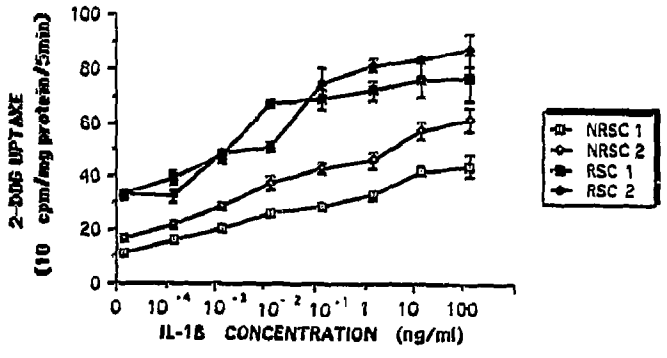


Fig. 1. Effects of increasing concentrations of IL-1$\beta$ on 2-DOG uptake by NRSC ($n$=2) and RSC ($n$=2). Cells were incubated in the presence of various concentrations of IL-1$\beta$ for 24 h. Values represent the mean ± S.E.M. of triplicate measurements. 2-DOG uptake stimulation was not significant at $10^{-4}$ ng/ml in RSC 1 and RSC 2.

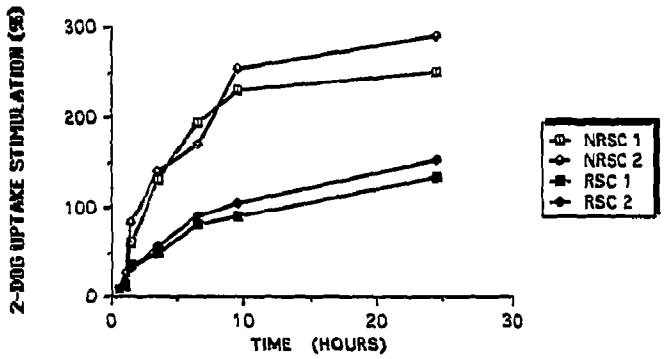


Fig. 2. Time course of IL-1$\beta$ stimulation of 2-DOG uptake. NRSC ($n$=2) and RSC ($n$=2) were incubated in the presence of 1 ng/ml IL-1$\beta$ for the times indicated. Values represent the percentage of stimulation of 2-DOG uptake over basal levels.

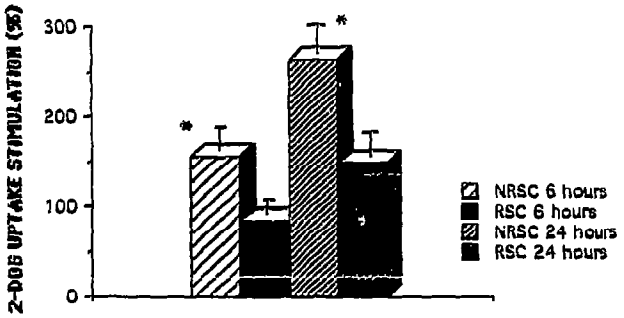


Fig. 3. Effects of IL-1$\beta$ (1 ng/ml) on 2-DOG uptake in NRSC ($n$=6) and RSC ($n$=6) after a 6 and 24 h incubation. Each diagram represents the mean ± S.E.M. of 6 triplicate experiments. *$P$ < 0.05 vs. RSC.

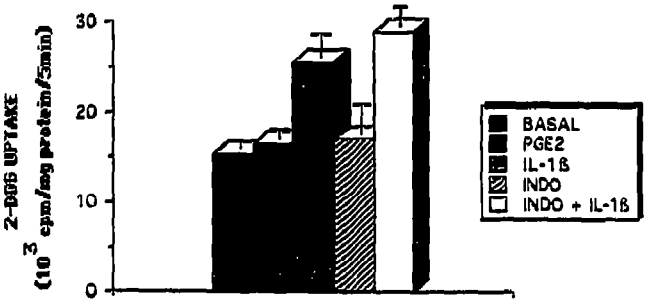


Fig. 4. Effect of exogenous PGE2 ($10^{-6}$ M) on 2-DOG uptake and effect of indomethacin ($10^{-5}$ M) on IL-1$\beta$-mediated 2-DOG uptake by NRSC. Cells were treated for 1 h with PGE2 or for 6 h with IL-1$\beta$, INDO or IL-1$\beta$ + INDO. 2-DOG uptake was then assayed.

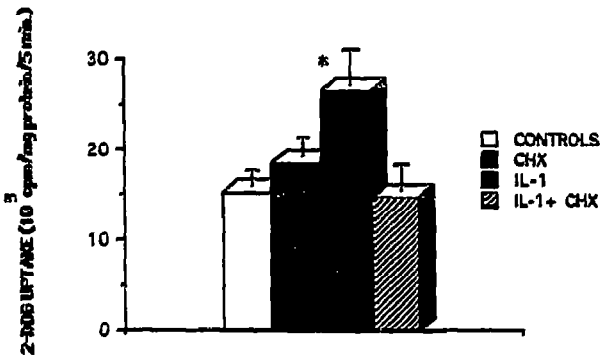


Fig. 5. Effect of cycloheximide (CHX) on basal and IL-1$\beta$-stimulated 2-DOG uptake. NRSC were incubated in the presence or absence of IL-1$\beta$ for 6 h. IL-1$\beta$ treated and untreated cells were also treated with cycloheximide (CHX 100 $\mu$M).

creased after 24 h incubation with IL-1$\beta$. The occurrence and rapidity of the effect on glucose uptake appear to be dependent on the cell type. In effect, in rat adipose cells [12], an increase in glucose uptake is observed after a few minutes incubation with IL-1$\beta$. At the opposite, synovial cells, appear in our study to be more sensitive to IL-1$\beta$ than human gingival fibroblasts [13].

The similarity between the kinetics of IL-1$\beta$-induced production of PGE2 [1], as well as 2-DOG uptake, led us to investigate whether PGE2 was responsible for the glucose-uptake stimulation. As shown in Fig. 4, exogenous PGE2 did not stimulate 2-DOG uptake; moreover, indomethacin, an inhibitor of IL-1$\beta$-mediated PGE2 production, did not modify the effect of the cytokine. In the same way, we investigated if sugar uptake stimulation could be related to possible growth stimulation as demonstrated for human synovial cells during the growth phase [3]; however, our results showed that IL-1$\beta$ did not stimulate thymidine uptake by NRSC after a 6-h association (data not shown).

Since the stimulating action of IL-1$\beta$ could occur via either an increase in the affinity of glucose carriers or an increase in carrier availability, we analysed the kinetics of 2-DOG uptake. Cultures incubated with IL-1$\beta$ for 6 h showed a higher $V_{max}$ than control cultures (4.34 vs. 0.81 nmol/min/mg cell protein), with no variation in the $K_m$ (4.1 vs. 3.4 mM). These results suggest that IL-1$\beta$ might stimulate the synthesis of glucose carriers or increase their recruitment. The possibility of de novo carrier synthesis is reinforced by the results of the experiment with cycloheximide (CHX): as shown in Fig. 5, CHX (100 $\mu$M, 6 h) inhibited the effect of IL-1$\beta$ on 2-DOG uptake.

Table 1

Basal 2-DOG uptake and production of interleukin-1$\beta$ by NRSC ($n$=10) and RSC ($n$=6). Culture medium was changed with a fresh medium; 24 h later, IL-1$\beta$ was measured in supernatants and 2-DOG uptake was performed

| | NRSC | RSC |
|---|---|---|
| $n$ | 10 | 6 |
| IL-1$\beta$ in medium supernatant (pg/24 h/$10^5$ cells) mean ± S.E.M. | 8.3 ± 2.4 | 850 ± 238 |
| Basal 2-DOG uptake (cpm/mg protein/5 min) | 14,630 ± 1,990 | 34,250 ± 2,955 |

Although RSC synthesize more IL-1$\beta$ than NRSC, there was no linear correlation between basal 2-DOG uptake and IL-1$\beta$ concentrations in culture supernatants, suggesting a regulation of IL-1$\beta$ activity. The regulation could be due to the presence of natural inhibitors as soluble receptors [14] which modulate the activity of the cytokine. A second hypothesis is that IL-1$\beta$ is indirectly active on synovial cells by stimulating the synthesis of another substance, which is able to stimulate glucose uptake; this process could explain the long time necessary to observe the action of IL-1$\beta$.

In conclusion, cultured human synovial cells, particularly NRSC are highly sensitive and responsive to the action of IL-1$\beta$ with regard to the stimulation of glucose uptake, suggesting that this cytokine might be a potent regulator of energy supply for synovial cells during inflammatory processes. The IL-1$\beta$-stimulated 2-DOG uptake is quite different from the rapid (30 min) but less potent stimulation induced by insulin in human synovial cells [5,15] and in human fibroblasts (see [16] for a review) Finally, the intensity of glucose uptake stimulation in synovial cells makes them suitable for the study of the effects of IL-1$\beta$ inhibitors which are proposed for the treatment of rheumatoid arthritis [14].

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