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Interleukin-1β-induced changes in the kinetic constants of L-proline uptake in human skin fibroblasts

Madeleine FÉNÉANT-THIBAULT,*§ Philippe GALERA,† Jean MACCARIO,‡ Audrey BOUTRON,* Jean-Pierre PUJOL† and Nicole MOATTI‡

*Laboratoire Central de Biochimie, Hôpital de Bicêtre, 94275 Le Kremlin-Bicêtre, †Laboratoire de Biochimie du Tissu Conjonctif, CHU Côte de Nacre, 14033 Caen Cedex, and ‡Faculté des Sciences Pharmaceutiques et Biologiques de l'Université Paris-Sud, 92290 Chatenay-Malabry, France

The effects of interleukin-1β (IL-1) on L-proline uptake in human skin fibroblasts were investigated. Exposure of the fibroblasts to IL-1 (5, 10 or 50 pg/ml) for 2 h did not change L-proline uptake. In contrast, inhibition was observed after 6 h of IL-1 treatment, and only 60 % of the control uptake remained after incubation for 24 h with 10 pg of IL-1/ml. IL-1 depressed the activity of both transfer systems: the low-affinity system inhibited by α-(methylamino)isobutyric acid (Me-AIB), corresponding to system A, and a high-affinity transfer system which is unaffected by Me-AIB. The inhibitory effect increased as the L-proline concentration decreased. To determine whether IL-1-induced prostaglandin release influences proline uptake, indomethacin (14 μM) was added as a cyclo-oxygenase inhibitor. Indomethacin itself decreased L-proline uptake but to a lesser extent than did IL-1. When IL-1 was tested in the presence of indomethacin, the inhibition of L-proline uptake was still observed, with values between those obtained with each substance in isolation. This suggests that the inhibitory effect of IL-1 on proline uptake by skin fibroblasts does not only involve the prostaglandins that accumulate in the medium, but no firm conclusion can be drawn, due to the fact that the inhibition by the two agents was not statistically independent. Kinetic analyses for 1 min combined with inhibition experiments showed that IL-1 induced a decrease in the K_m and $V_{max.}$ values of the high-affinity transport system, whereas it increased the K_m of system A. Therefore the two systems of proline uptake in skin fibroblasts are probably inhibited by IL-1 via different mechanisms.

INTRODUCTION

A number of hormones and growth factors have been shown to modulate the uptake of amino acids by cultured connective cells such as fibroblasts (Guidotti *et al.*, 1978; Lerner, 1985; Saier *et al.*, 1988). However, up to now, the effect of interleukin-1 (IL-1) on the amino acid transport system has not been investigated. This multifunctional monokine (Dinarello, 1988) has been implicated in the catabolism of connective tissue via its ability to stimulate the release of metalloproteinases such as collagenase and stromelysin (Mizel *et al.*, 1981; Murphy *et al.*, 1986). In addition, IL-1 can modulate the production of proteoglycans (Hamermann & Wood, 1984; Bocquet *et al.*, 1985; Tyler, 1985; Langris *et al.*, 1987) and collagen in several cell types. Regarding collagen synthesis, it has been demonstrated that IL-1 increases the levels of mRNA coding for procollagens I and III in both dermal fibroblasts and synovial cells (Goldring & Krane, 1987; Kähäri *et al.*, 1987; Mauviel *et al.*, 1988*a*; Postlethwaite *et al.*, 1988). However, this transcriptional effect of IL-1 is not systematically correlated with an increase in the amount of collagen produced in the culture medium, suggesting that post-transcriptional events may be induced by the monokine which sometimes lead to decreased levels of secreted collagen (Mauviel *et al.*, 1988*a*, 1990). Several potential mechanisms could account for this IL-1-induced inhibition of collagen production. One possibility is that the cell availability of proline, one of the major amino acids in collagen, could be decreased by IL-1 treatment. This would decrease the intracellular pool of proline and negatively affect collagen synthesis. We therefore searched for a possible inhibitory effect of IL-1 on the uptake of proline in cultured skin fibroblasts, combining kinetic and inhibition studies in experimental conditions that permitted the estimation of the activity of the low-affinity transport system (system A) as well as that of the high-affinity system (Fénéant-Thibault *et al.*, 1987). In addition, we tried to estimate whether prostaglandins, which are increased in the medium of IL-1-treated fibroblasts (Mauviel *et al.*, 1988*b*), influence the effect of the monokine on proline uptake.

MATERIALS AND METHODS

Cell cultures

Human fibroblasts were obtained from skin biopsies taken from children during abdominal surgery. Different cell strains were grown at 37 °C in Eagle's minimum essential medium containing non-essential amino acids and supplemented with 10 % (v/v) human serum and antibiotics (penicillin, 200 units/ml; streptomycin, 200 μg/ml) in a CO_2/air (1:19) mixture. All of the experiments were performed on subcultures between the third and eighth passages.

Uptake measurement

Uptake was measured as previously described (Fénéant-Thibault *et al.*, 1987) using confluent fibroblast monolayers in 2 cm^2 multiwell trays (Costar). About 3×10^4 cells were seeded in each well and were then incubated for 72 or 96 h in 1 ml of growth medium, which was replaced 24 h before the experiment by a growth medium supplemented or not with IL-1. The procedure for measurement of amino acid uptake was adapted with some modifications from that described by Gazzola *et al.*

Abbreviations used: IL-1, interleukin-1; Me-AIB, α-(methylamino)isobutyric acid; PBS, phosphate-buffered saline; PGE_2, prostaglandin E_2; PKC, protein kinase C.

§ To whom correspondence should be addressed.

(1981). It began with 2 h of preincubation in phosphate-buffered saline solution (Dulbecco's), pH 7.45, containing 0.1 % glucose (PBS-G) with or without IL-1, after which excess liquid was discarded and the experimental cover was immediately placed over the drained monolayers. The 24 tubes adapted to the multitray cover each contained 0.3 ml of PBS-G plus 0.5 μCi of L-[U-^{14}C]proline or α-[1-^{14}C](methylamino)isobutyric acid (Me-AIB) plus the desired concentration of the corresponding non-radioactive amino acid. The contents of the 24 tubes were simultaneously transferred to the monolayers in the 24 wells by vigorous horizontal shaking of the whole tray and cover, and were then incubated for 1 min at 37 °C. Uptake assays were stopped by dumping the medium in a single motion and immediately washing the 24 wells three times with 2 ml of ice-cold 0.154 M-NaCl (total washing time 10 s). The cluster trays were then drained and the monolayers were solubilized with 250 μl of 1 M-NaOH by incubation with gentle shaking for at least 2 h at room temperature. Samples (150 μl) of the extract were then counted for radioactivity in 10 ml of counting liquid (Picofluor), and 50 μl samples were used for protein determination by the method of Lowry *et al.* (1951).

Materials

Eagle's minimum essential medium (MEM 2111 plus 2 mM-glutamine) was purchased from Eurobio (Paris, France) and PBS (Dulbecco's) was from Gibco (Paisley, Scotland, U.K.). α-[1-^{14}C]Me-IAB (48.4 mCi/mmol) and L-[U-^{14}C]proline (260 mCi/mmol) were obtained from Dupont (NEN Products, Boston, MA, U.S.A.) and from CEA (Gif-sur-Yvette, France) respectively. The unlabelled amino acids and indomethacin were obtained from Sigma (St. Louis, MO, U.S.A.).

Human recombinant IL-1β (sp. activity 10^8 units/mg) was purchased from Genzyme (Boston, MA, U.S.A.). To simplify the presentation of data, manufacturers' units were converted to pg/ml, based on the specific activity stated by manufacturer.

Kinetic analysis

The calculations were performed as previously described (Fénéant *et al.*, 1984; Fénéant-Thibault *et al.*, 1987) using a non-linear regression analysis program. Weighting was by $1/v^2$, where v = rate of uptake. Different models may be fitted, such as:

$$v = V_{max.} \frac{[S]}{K_m + [S]} + K_d \times [S] \quad (1)$$

or

$$v = V_{max.1} \frac{[S]}{K_{m_1} + [S]} + V_{max.2} \frac{[S]}{K_{m_2} + [S]} + K_d \times [S] \quad (2)$$

corresponding respectively to one and two saturable systems plus a diffusion process. When an inhibitor (Me-AIB) specifically inhibits one saturable system, the data in the presence and in the absence of the inhibitor were taken into account. We first obtained the uptake values in the presence of Me-AIB (one saturable system plus a diffusion process). We then subtracted velocities attributable to this system from the observed velocities in the absence of Me-AIB. We thus obtained approximate kinetic constants of the inhibitable system (i.e. system A). Both of the kinetic constants so calculated were considered as the initial estimates in the final program which eventually modelled two saturable systems plus a diffusion process.

Statistical and mathematical analyses

Statistical comparisons between the different proline (or Me-AIB) uptake rates were performed using Student's t test; $P < 0.05$ was considered to be statistically significant.

For repeated tests, we performed 'protected t tests' according to the Bonferroni modification (Schontz, 1986). Each of the N tests is performed with a significance level of $P < 0.05/N$, which ensures an overall level of 0.05. The comparisons of parameters for various kinetic models were performed using F tests. When comparing two values we fitted two models; the first was a saturated model in which the parameters may vary independently, and the second was a restricted model in which the parameters studied are set at equal values. If the parameters differ significantly, the F ratio obtained by dividing the mean change of the residual sum of squares by the residual mean square is also significant, and vice versa.

RESULTS

Effects of IL-1 on L-proline uptake

Cell cultures were exposed to 10 pg of IL-1/ml for time periods of 2–24 h followed by uptake measurements as described in the Materials and methods section. Fig. 1 shows that monokine caused a decrease in proline (0.05 mM) uptake that became statistically significant at about 6 h (24 % inhibition) and reached a maximum at 10 h (48 % inhibition). The effect was still observed up to 24 h (approx. 38 % inhibition).

The fact that no IL-1 effect was observed after a 2 h period (Fig. 1) could be due to the experimental conditions used. We therefore designed new protocols, using different concentrations of IL-1 (5, 10 and 50 pg/ml) and proline (0.01, 0.1 and 1 mM). Furthermore, Me-AIB at a concentration 10 times that of proline ($10 \times [S]$) was added in order to inhibit system A, and this allowed us to estimate the effect of IL-1 on the more specific transport system. Comparisons between incubations in the presence and the absence of IL-1 are based on 9 results in the presence of Me-AIB and 9 results in its absence, obtained under various experimental conditions (Table 1). As the aim was to study the overall effect of IL-1 in the presence of Me-AIB on the

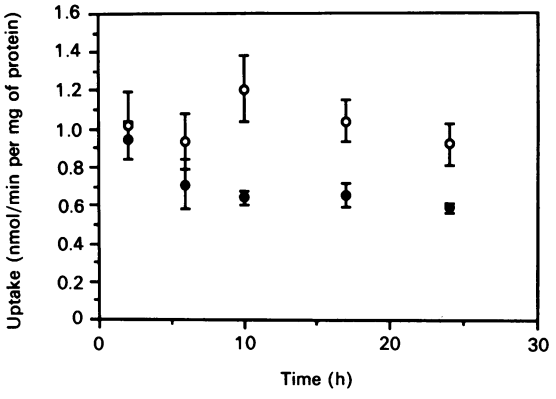

Fig. 1. Effects over time of IL-1 on L-proline uptake in cultured skin fibroblasts

The cells were plated at a density of 3×10^4 per well (2 cm^2) in Eagle's minimum essential medium supplemented with 10 % human serum and antibiotics as described in the Materials and methods section. After reaching confluency, IL-1 (10 pg/ml) was added when changing the culture medium in half of the plate at a given time before the experiment. The medium in the other half of the plate was changed simultaneously, but without IL-1. An identical procedure was performed when the culture medium was replaced by the preincubation buffer (PBS with 0.1 % glucose). The 2 h incubation with IL-1 was performed during preincubation time only. Proline uptake was measured for 1 min at a substrate concentration of 0.05 mM. Results are expressed as nmol/min per mg of protein. The experimental points represent means (±S.D.) of 12 determinations. ○, Control; ●, +IL-1.

Table 1. Effects of 2 h of treatment with IL-1 on L-proline uptake

The experimental conditions were as described in the Materials and methods section. Preincubation for 2 h in PBS containing 0.1 % glucose (PBS-G) was performed in the absence or presence of IL-1 (5, 10 or 50 pg/ml). Cells were incubated for 1 min in 0.3 ml of PBS-G containing 0.01, 0.1 or 1 mM-L-[U-^{14}C]proline ([S]) with or without 10 × [S] of Me-AIB. Each plate contained 12 control wells and 12 experimental wells. Each result is expressed as nmol/min per mg of protein and is the mean ± S.D. of six experimental values.

	Proline uptake (nmol/min per mg of protein)					
	[S] = 0.01 mM		[S] = 0.1 mM		[S] = 1 mM	
	−Me-AIB	+Me-AIB	−Me-AIB	+Me-AIB	−Me-AIB	+Me-AIB
Control	0.132 ± 0.011	0.103 ± 0.011	1.19 ± 0.15	0.47 ± 0.12	5.48 ± 0.31	1.31 ± 0.38
IL-1 (5 pg/ml)	0.134 ± 0.015	0.088 ± 0.012	1.03 ± 0.13	0.39 ± 0.004	5.28 ± 0.73	1.58 ± 0.98
Control	0.137 ± 0.028	0.096 ± 0.007	1.13 ± 0.16	0.37 ± 0.03	7.91 ± 1.36	1.89 ± 0.36
IL-1 (10 pg/ml)	0.109 ± 0.014	0.089 ± 0.012	1.03 ± 0.17	0.33 ± 0.02	7.08 ± 0.43	1.91 ± 0.23
Control	0.155 ± 0.012	0.124 ± 0.015	1.39 ± 0.14	0.45 ± 0.05	8.05 ± 0.43	2.91 ± 0.36
IL-1 (50 pg/ml)	0.130 ± 0.020	0.108 ± 0.014	1.45 ± 0.12	0.51 ± 0.05	8.15 ± 0.55	2.38 ± 0.15

Table 2. Effects of 24 h of treatment with IL-1 (10 pg/ml) on uptake by the proline-specific system and system A

The experimental conditions were as described in the Materials and methods section. IL-1 (10 pg/ml) was added or not to the culture medium 24 h before the experiment and to the preincubation medium (PBS-G) 2 h before the experiment. Each well was incubated in 0.3 ml of PBS-G containing 0.05 mM-L-[U-^{14}C]proline and 0.5 mM-Me-AIB or 0.05 mM-α-[^{14}C]Me-AIB for 1 min. Each plate contained 12 control wells and 12 experimental wells. Results are expressed as nmol of amino acid uptake/min per mg of protein.

	Proline uptake (nmol/min per mg of protein)		Me-AIB uptake (nmol/min per mg of protein)	
	Control	+IL-1	Control	+IL-1
n	19	24	23	24
Mean	0.1609	0.1257	0.597	0.398
S.D.	0.0101	0.0090	0.091	0.078
t	12.1		8.06	
P	$\leqslant 0.001$		$\leqslant 0.001$	

one hand and in its absence on the other, we performed each test for $P < 0.006$ (0.05/9). No IL-1 effects were significant at the protected level of $P < 0.006$.

To gain further insight into the effect of a 24 h incubation period with IL-1 on proline uptake, we investigated this effect on the Me-AIB-non-inhibitable part of uptake. IL-1 (10 pg/ml) was added or not for 24 h, and proline (0.05 mM) uptake in the presence of 0.5 mM-Me-AIB was observed (Table 2). IL-1 caused a significant decrease (22 % inhibition) in specific proline uptake. On the other hand, the influence of IL-1 on system A (i.e. the Me-AIB-inhibitable part of uptake) was directly studied by using Me-AIB as a substrate. The uptake of Me-AIB itself (0.05 mM) was measured in the presence or absence of IL-1 (10 pg/ml). Significant inhibition (33 %) was observed with 24 h of exposure (Table 2). We therefore chose this 24 h incubation period as a standard protocol to further investigate the effects of IL-1 on L-proline uptake.

Contribution of prostaglandins to the IL-1-induced inhibition of L-proline uptake

IL-1 is known to elicit an increase in prostaglandin (PG) release (mainly PGE_2) in its target cells, including fibroblasts (Mauviel *et al.*, 1988*b*). Given that PGE_2 has been reported to inhibit proline uptake in fibroblasts (Goldstein *et al.*, 1986), it was of interest to carry out experiments in the presence of indomethacin, a cyclo-oxygenase inhibitor. Table 3 shows the effects of IL-1 (10 pg/ml) and/or indomethacin (14 μM) on L-proline uptake ([S] = 0.01, 0.1 and 1 mM) with or without Me-AIB (at concentrations 10 times those of the substrate). Concerning the influence of Me-AIB, we can confirm our previous observation that the greater the substrate concentration the

Table 3. Effect of 24 h of treatment with IL-1 (10 pg/ml) and/or indomethacin (14 μM) on L-proline uptake

The experimental conditions were as described in the Materials and methods section and in the legend to Fig. 1. Proline uptake was measured in the same plate in the presence of indomethacin (14 μM), IL-1 (10 pg/ml), or both, and in control wells. The results are expressed as nmol of proline uptake/min per mg of protein (means ± S.D.). Three concentrations of proline ([S] = 0.01, 0.1 and 1 mM) were tested in the presence or absence of 10 × [S] of Me-AIB.

	Proline uptake (nmol/min per mg of protein)					
	[S] = 0.01 mM		[S] = 0.1 mM		[S] = 1 mM	
	−Me-AIB	+Me-AIB	−Me-AIB	+Me-AIB	−Me-AIB	+Me-AIB
Control	0.175 ± 0.007	0.135 ± 0.009	1.119 ± 0.159	0.641 ± 0.022	8.128 ± 0.477	3.557 ± 0.218
+Indomethacin (14 μM)	0.122 ± 0.009	0.097 ± 0.006	0.953 ± 0.093	0.572 ± 0.029	6.366 ± 0.721	3.291 ± 0.117
+IL-1 (10 pg/ml)	0.095 ± 0.005	0.077 ± 0.004	0.722 ± 0.110	0.497 ± 0.031	5.611 ± 0.137	3.242 ± 0.193
+Indomethacin (14 μM) +IL-1 (10 pg/ml)	0.111 ± 0.005	0.089 ± 0.007	0.795 ± 0.037	0.509 ± 0.026	5.886 ± 0.300	2.876 ± 0.825

greater the inhibition (33% inhibition for [S] = 0.01 mM to 56% for [S] = 1 mM).

Indomethacin alone (14 μM) inhibited proline uptake. In the absence of Me-AIB, the inhibition ranged between 15 and 30%. Inhibition also occurred in the presence of Me-AIB, especially for the lowest concentration of proline (0.01 mM). This inhibition was similar to that observed without Me-AIB (about 30%). The inhibitory effect was smaller when the substrate concentration was increased.

IL-1 alone inhibited proline uptake. In the absence of Me-AIB, inhibition ranged from 31% with a concentration of 1 mM to 46% for 0.01 mM-proline. When the uptake was partially inhibited by Me-AIB, IL-1 also induced a decrease in proline uptake. This was particularly evident for the lowest concentration of proline; the inhibition was about 43%, which is approximately the value obtained in the absence of Me-AIB. The inhibitory effect of IL-1 became smaller as the substrate concentration was increased.

When both IL-1 and indomethacin were present, the level of inhibition was between that with indomethacin alone and that (slightly greater) with IL-1 alone. Uptake in the presence or absence of Me-AIB was significantly decreased in the presence of IL-1 plus indomethacin, compared with indomethacin alone, for substrate concentrations of 0.01 and 0.1 mM. No significant effect was seen at higher proline concentrations. Nevertheless, the effects of the two drugs were not statistically independent (tests not shown), so that the results in the presence of both inhibitors are very difficult to interpret.

Taken together, these findings suggest that part of the effects of IL-1 on L-proline uptake in skin fibroblasts, at least at low substrate concentrations, are not due to accumulation of prostaglandins in the medium during IL-1 treatment.

Effects of IL-1 on the kinetic parameters of L-proline uptake

Kinetic studies were performed using 12 concentrations of proline ([S]) in the presence or absence of Me-AIB (10 × [S]) and of IL-1 (10 pg/ml during a 24 h incubation period). As shown in Fig. 2, IL-1 induced a decrease in the total L-proline uptake (measured in the absence of Me-AIB), whatever the substrate concentration used. The percentage inhibition varied from about 15 to 33%. For the Me-AIB-non-inhibitable part of L-proline uptake, a similar inhibitory effect was exerted with IL-1 treatment, and the percentage inhibition varied between 6 and 27%.

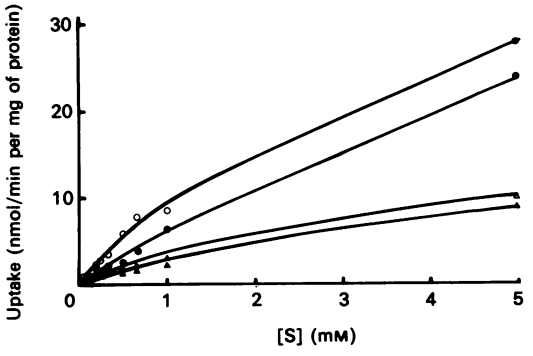

Fig. 2. Effects of IL-1 on the kinetic parameters of L-proline uptake

Experimental conditions were as described in the Materials and methods section. IL-1 (10 pg/ml) was added (●, ▲) or not (○, △) in the culture medium 24 h before the experiment and in PBS/0.1% glucose during the preincubation period. Initial rates of uptake (1 min) were measured for 12 proline concentrations ([S]) ranging from 0.01 to 5 mM in the presence (△, ▲) or absence (○, ●) of 10 × [S] of Me-AIB. Each point is the mean of six results.

Table 4. Kinetic parameters of L-proline uptake in the presence and absence of IL-1

The experimental conditions were the same as described in the Materials and methods section. IL-1 (10 pg/ml) was present or not for 24 h before the experiment. Cells were incubated for 1 min in 0.3 ml of PBS-G containing [U-^{14}C]proline with or without Me-AIB. Twelve concentrations of proline ([S] = 0.01–5 mM) were used in the presence or absence of 10 × [S] of Me-AIB. For each concentration, six measurements were performed. K_m values (±S.D.) are expressed as mmol/l. $V_{max.}$ values (±S.D.) are expressed as nmol/min per mg of protein (see the text for the calculation of the kinetic constants). The kinetic parameters were determined in either the presence or the absence of IL-1. $V_{max.1}$ and K_{m_1} represent the kinetic constants of the high-affinity transfer system, whereas $V_{max.2}$ and K_{m_2} are those of the low-affinity transfer system.

Parameter	Control	+IL-1
$V_{max.1}$	1.66±0.21	0.65±0.20
K_{m_1}	0.27±0.06	0.098±0.036
$V_{max.2}$	43.8±8.8	33.7±5.1
K_{m_2}	6.9±2.0	7.2±1.5
K_d	1.70±0.07	1.64±0.07

Table 5. Choice of the best model for pooled kinetic observations of proline uptake in the presence or absence of Me-AIB and of IL-1

The experimental procedure was as described in Table 4. The 48 results (4 per proline concentration), each being the average of 6 experimental values, were mathematically treated together. Different models including 5–10 kinetic parameters were tested (see text). The sums of the square residuals represent the criterion of the adequacy of the fitting. The constants obtained with the two best models are very similar. $V_{max.1,1}$ and $K_{m_{1,1}}$ represent the constants of the high-affinity transfer system in control cultures, whereas $V_{max.1,2}$ and $K_{m_{1,2}}$ are those in IL-1-treated cultures. For the low-affinity transport system, $V_{max.2,1}$ and $K_{m_{2,1}}$ are for control cultures and $V_{max.2,2}$ and $K_{m_{2,2}}$ are for IL-1-exposed cultures.

	Model with 8 parameters	Model with 9 parameters
$V_{max.1,1}$	1.46±0.34	1.46±0.35
$K_{m_{1,1}}$	0.26±0.07	0.26±0.07
$V_{max.1,2}$	0.69±0.18	0.69±0.19
$K_{m_{1,2}}$	0.12±0.04	0.12±0.04
$V_{max.2,1}$	42.6±12.9	43.4±14.8
$K_{m_{2,1}}$	6.25±2.10	6.37±2.40
$V_{max.2,2}$	Set equal to $V_{max.2,1}$	42.2±25.3
$K_{m_{2,2}}$	9.43±3.13	9.33±6.06
K_d	1.67±0.20	1.66±0.20

Two types of calculations were used to analyse the experimental data. The first method interpreted the kinetics observed with and without IL-1 as described in the Materials and methods section. The kinetic parameters reported in Table 4 clearly show that IL-1 reduced the K_m and the $V_{max.}$ of the high-affinity system. To further analyse the influence of IL-1 on the low-affinity system, another mathematical analysis was introduced. This second type of calculation took the total results (in the presence and absence of IL-1) in order to see how many systems were implicated. We took into account the 48 experimental values (with and without Me-AIB, with and without IL-1), with each being the mean of 6 results, and tested several hypotheses about the number of kinetic parameters potentially implicated. One system is indeed characterized by its two parameters (K_m and $V_{max.}$), but the influence of IL-1 on the uptake may modify one or both parameters. We successively fitted the experimental values with

models incorporating the presence of 5–10 parameters. Several hypotheses were therefore tested. The simplest was the presence of 5 parameters (absence of modification of the systems by IL-1 so that the experimental values in the presence of IL-1 are correctly fitted by the model defined in terms of its absence). The most sophisticated hypothesis included 10 parameters, assuming that IL-1 affects both the parameters of the two systems as well as K_d. The suitability of the adjustment was evaluated by calculating the sum of the squared residuals. The sums of the squared residuals for 5, 7, 8, 9 and 10 parameters were 24.2, 13.7, 5.3, 5.5 and 5.9 respectively. The best adjustment is obtained for the lowest value of the sum of the squared residuals, and we therefore adopted the 8-parameter model as the saturated model. The 7-parameter model did not give a satisfactory fit because of an increase of $(13.7-5.3) = 8.4$ in the residual sum of squares, which corresponded to an F ratio of $8.4/0.1325 = 63.4$ with $(1, 40)$ degrees of freedom, which is highly significant. The main consequence is that, although tenuous compared with rather large standard deviations, the difference between $K_{m_{2,1}}$ and $K_{m_{2,2}}$ (see Table 5) is significant. Standard deviations are frequently overestimated in non-linear regression analysis. The 9- and 10-parameter models do not give a better fit than the 8-parameter model. The slight increase in the observed residual sum of squares is a common consequence of the over-parametrization of non-linear models.

As the initial calculation already indicates, the K_m and $V_{max.}$ values of the high-affinity system were lowered in the presence of IL-1 (Table 5). Concerning the low-affinity system, the fact that the best fit was obtained with 8 parameters as shown in Table 5 implies the existence of a modified low-affinity system in the presence of IL-1. The $V_{max.}$ of the low-affinity system was not altered, but its K_m was increased.

We can conclude that IL-1 alters L-proline uptake in skin fibroblasts in two ways: by decreasing the K_m and $V_{max.}$ of the high-affinity specific system and by increasing the K_m of system A.

DISCUSSION

The purpose of this study was to define the effects of IL-1 on L-proline uptake in cultured human skin fibroblasts. We have shown an inhibitory effect of IL-1 on proline uptake. This inhibition presented three characteristics: it needed several hours to take place, it occurred at all substrate concentrations used, and it altered the kinetic constants of the two systems of proline uptake.

IL-1 is a peptide (M_r 17500) and therefore it is not in direct competition with the amino acid for uptake. The fixation of IL-1α and IL-1β on a specific high-affinity cell receptor, which is down-regulated, has been well-documented (Dinarello, 1988). Optimal binding of IL-1 on chondrocytes was observed at 4 h (Chandrasekhar & Harvey, 1989). The subsequent mechanisms inside the cell have not yet been clarified. In Swiss-3T3 fibroblasts, IL-1 has not been degraded in the lysosomes 6 h after its internalization, and may reach the nucleus and directly act at this cellular level (Mizel *et al.*, 1987). In the light of this, amino acid uptake may be influenced through the regulatory mechanisms of uptake, similar to different models of regulatory genes which have been reported to account for the regulation of system A in different cell types (Saier *et al.*, 1988).

Phospholipids (other than phosphatidylinositol) have been shown to act as second messengers of IL-1 (Rosoff *et al.*, 1988; Kester *et al.*, 1989). This might induce an increase in protein kinase C (PKC) production which, in turn, would be expected to lead to an increase in amino acid uptake (Dawson & Cook, 1987). Moreover, system A synthesis shows an apparent dependency on PKC (Saier *et al.*, 1988). However, the involvement of such mechanisms is not yet fully understood, and the very important processes of feedback regulation may reverse the expected effects. As an example, PKC is a feedback regulator of phosphoinositide metabolism induced by PGE_2 (Yokohama *et al.*, 1988).

In so far as they are Na^+-dependent, the inhibition of the uptake processes we observed is also surprising, given the effects of IL-1 on cellular ionic concentrations. IL-1 has been described as stimulating the Na^+/H^+ antiport (Stanton *et al.*, 1986). Furthermore, incubating proximal tubular monolayers with IL-1 for 12 h resulted in a stimulation of Na^+ influx and of Na^+-dependent transport processes such as α-methylglucoside and aspartate uptake (Kohan & Schreiner, 1988). Nevertheless, *in vivo*, IL-1 decreased the blood concentrations of the amino acids transported by system A and the uptake of AIB was shown to be decreased by IL-1 in some tissues (intestine, heart) and increased in the liver (Argiles *et al.*, 1989).

We have previously demonstrated that L-proline uptake occurs via two systems: one system of high affinity which is not inhibited by Me-AIB, and system A, which is inhibited by Me-AIB and has a weaker affinity (Fénéant-Thibault *et al.*, 1987). The inhibition of the first system by IL-1 was shown in the present study by examining proline uptake in the presence of Me-AIB. The sensitivity of system A to IL-1 was directly observed by studying Me-AIB uptake. We ascertained that 2 h of incubation with IL-1 did not produce any effect. Further experiments were carried out with a 24 h incubation time. The 1 min kinetic studies, in the presence or the absence of IL-1 and Me-AIB, confirmed that both systems were inhibited by IL-1 and indicated that inhibition occurred through different mechanisms. With IL-1 treatment, the K_m and $V_{max.}$ of the high-affinity system were decreased, whereas the $V_{max.}$ of the low-affinity system was not altered but its K_m was increased.

The decrease in the affinity of proline uptake by system A may be related to the increase in the apparent K_m of system A observed in embryonic lung fibroblasts due to PGE_2 (Goldstein *et al.*, 1986). IL-1 is known to lead to the release of PGE_2 in different cell lines, including fibroblasts. This release may take place as soon as 1 h after the addition of IL-1 in Swiss 3T3 fibroblasts (Burch *et al.*, 1989). The PGE_2-induced decrease in the uptake of AIB in embryonic lung fibroblasts was first observed at 4 h after the addition of the effector molecule to the cultures (Goldstein *et al.*, 1986). The IL-1-induced release of PGE_2 may therefore account for the influence of IL-1 on system A and would explain the delay observed.

Nevertheless, in human dermal fibroblasts, some effects of IL-1 may be independent of the release of PGE_2, such as the induction of hyaluronic acid synthesis (Postlethwaite *et al.*, 1988) or the effects on cellular proliferation and the production of collagen and collagenase (Postlethwaite *et al.*, 1989). In an attempt to differentiate the PGE_2-independent effects of IL-1 on proline uptake, we carried out experiments in the presence of indomethacin, a cyclo-oxygenase inhibitor. The inhibitory effect observed in the presence of both IL-1 and indomethacin was greater than that in the presence of indomethacin alone, suggesting that a part of the IL-1 effect is PGE_2-independent. However, there is statistical evidence for the non-independence of the two inhibitory effects, which makes these results difficult to interpret. The inhibitory effect of indomethacin on amino acid uptake has already been described in different cell types (Bayer *et al.*, 1980, 1981), including fibroblasts (Owen & Villereal, 1983; Bannai & Kasuga, 1985). Indomethacin induces a decrease in the $V_{max.}$ of system A (Bayer *et al.*, 1981), so it is quite different from the action of IL-1 that we observed on system A (increase in K_m). The inhibition caused by IL-1 and by indomethacin may thus be

attributed to different mechanisms, both of which may exist in the presence of the two molecules. On the contrary, the action of IL-1 is similar in kind (increase in K_m of system A) to that of PGE_2. Moreover, we also found evidence for an effect of IL-1 on the more specific system of proline uptake (decrease in $V_{max.}$). Such an inhibition involving two uptake systems through different kinetic parameters is worth noting.

Whatever the mechanisms involved, the inhibitory effects of IL-1 on proline uptake in fibroblasts reported here could have some consequences for collagen synthesis in these cells, since proline is a major constituent of this protein. It is tempting to speculate that IL-1 may lower the availability of proline and therefore decrease its intracellular pool, at least at low concentrations of external amino acid where the IL-1 effect is maximal. If such a mechanism could lead to the depression of collagen synthesis, it might contribute to the absence of correlations observed in some studies between IL-1-induced increases in procollagen mRNA and the corresponding amounts of collagen actually produced, which remained similar to or smaller than those in controls (Mauviel *et al.*, 1988*a*, 1990). Further research is required to clarify this point.

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