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Signal transduction mechanism of interleukin 6 in cultured rat mesangial cells

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Interleukin 6 (IL-6) is one of the potent autocrine growth factors for mesangial cells. We investigated the signal transduction mechanism of IL-6 in cultured rat mesangial cells. IL-6 induced a transient increase of inositol 1,4,5-trisphosphate (Ins 1,4,5-P_3) followed by a transient and sustained increase of intracellular calcium concentration, suggesting that IL-6 stimulates phosphoinositide turnover. IL-6 also stimulated prostaglandin E_2 (PGE_2) production. The IL-6-concentration dependency in PGE_2 production was similar to that in Ins 1,4,5-P_3 production. We concluded that the action of IL-6 on mesangial cells is exerted at least partially through the enhancement of phosphoinositide turnover and PGE_2 production.

Interleukin 6; Mesangial cell; Signal transduction mechanism; Phosphoinositide turnover; Intracellular calcium; Prostaglandin E_2

1. INTRODUCTION

Interleukin 6 (IL-6) was originally characterized as a B cell stimulatory factor-2 [1]. Afterwards it has been clarified that IL-6 has a variety of biological activities including the induction of differentiation or proliferation and the inhibition of proliferation in many types of cells (see reviews [2,3]). Recently, Horii et al. [4] reported that IL-6 stimulated DNA synthesis in cultured rat mesangial cells, and that urinary excretion of IL-6 was increased in patients with mesangial proliferative glomerulonephritis. Furthermore, Ruef et al. [5] reported that IL-6 acted as an autocrine growth factor in cultured rat mesangial cells. Thus IL-6 is now thought as one of the key substances to regulate mesangial cell proliferation. However, the signal transduction mechanism of IL-6 has not yet been elucidated.

Many mesangial growth factors transmit their signals through enhancement of phosphoinositide (PI) turnover. They also stimulate the production of prostaglandin E_2 (PGE_2) which may act as a negative modulator of the actions of these growth factors (see review [6]). So, we investigated the possible involvement of PI turnover and PGE_2 production in signalling mechanisms of IL-6 in cultured rat mesangial cells. In this paper, we measured inositol 1,4,5-trisphosphate (Ins 1,4,5-P_3), one of the hydrolyzed products of phosphatidylinositol 4,5-bisphosphate (PIP_2) to investigate the involvement of PI turnover through activation of phospholipase C (PLC) [7].

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2. MATERIALS AND METHODS

2.1. *Materials*

Human recombinant IL-6 (hrIL-6) is prepared as previously described [8]. Fetal calf serum (FCS) was purchased from Cell Culture Laboratories (Cleveland, OH). Fura-2 acetoxymethyl ester (Fura-2 AM) was purchased from Dojin Chemical (Kumamoto, Japan). Other chemicals were of highest purity available.

2.2. *Preparation of cultured rat mesangial cells*

Cultured rat mesangial cells were obtained from isolated glomeruli prepared from male Sprague–Dawley rats as described in [9]. Cells were maintained in RPMI 1640 supplemented by 20% FCS, and only first-subcultured mesangial cells were used in this study.

2.3. *Measurement of Ins 1,4,5-P_3*

Intracellular levels of Ins 1,4,5-P_3 were determined as described in [9]. In brief, subconfluent mesangial cells seeded on 12-well culture plates were preincubated in RPMI 1640 without FCS for one hour and in HEPES-buffered Hank's balanced salt solution, pH 7.4, for 20 min. HrIL-6 or vehicle was added to the cells and, at the indicated time, the reaction was terminated by addition of ice-cold 15% trichloroacetic acid (TCA). TCA was eliminated by washing with 4 vols. of water-satured diethylether 4 times and the samples were assayed for Ins 1,4,5-P_3 using Amersham's specific binding assay kit, TRK.1000.

2.4. *Measurement of intracellular calcium ion concentration*

As described in [9], intracellular calcium ion concentration (iCa^{2+}) was measured using fura-2-loaded mesangial cells. The cells seeded on 10 × 40 mm glass cover slides were incubated with Fura-2 AM (4 μM) for 60 min, and then with HEPES-buffered Krebs–Henseleit solution, pH 7.2, for an additional 20 min, for cleavage. One cover slide

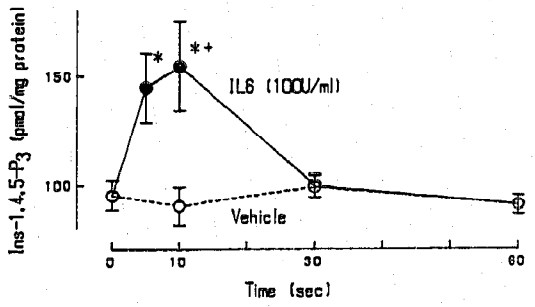

Fig. 1. Time course of Ins 1,4,5-P_3 production by 100 U/ml IL-6 in cultured rat mesangial cells. Data are shown as mean ± SE of 3 to 4 determinations. Statistical analysis was done by unpaired Student's *t*-test for non-multiple comparison and by Dunnett's multiple comparison procedure for multiple comparisons. $^{*}P < 0.05$ vs time 0, $^{+}P < 0.05$ vs vehicle.

was placed in a quartz cuvette with HEPES-buffered Krebs–Henseleit solution and the Ca^{2+}-fura-2 fluorescence was measured by fluorescence spectrophotometer (Hitachi F4000) with wavelengths of 340 and 380 nm for excitations and of 505 nm for emission. iCa^{2+} was calculated using Grykiewicz's formula [10].

2.5. *Measurement of PGE_2*

Mesangial cells seeded on 12-well culture plates as in section 2.3 were used. The cells were preincubated in RPMI 1640 without FCS for 3 days and hrIL-6 or vehicle was added. After 30 min of reaction time, the medium was assayed for PGE_2 using NEN's RIA kit, NEK.020.

3. RESULTS

In cultured rat mesangial cells, 100 U/ml IL-6 rapidly increased Ins 1,4,5-P_3. The intracellular Ins 1,4,5-P_3 level reached its peak at 10 s (0 s: 95.1 ± 7.4, 10 s: 154.6±20.8 pmol/mg protein, $P < 0.05$). After 10 s, Ins 1,4,5-P_3 gradually decreased and returned to its basal value by 30 s (30 s: 99.8 ± 11.5) (Fig. 1). The IL-6-induced increase in Ins 1,4,5-P_3 at 10 s was dose-dependent and a significant increase was observed in the concentrations at and above 0.5 U/ml with a half maximal effect at 1 U/ml (Fig. 2). 100 U/ml IL-6 also increased iCa^{2+} in a pattern with an initial transient peak at 15 s followed by a rapid decrease and again a sustained increase above its basal value, which is so-called 'transient and sustained pattern' (Fig. 3). PGE_2 production was stimulated by IL-6 dose-dependently. A significant increase was observed in concentrations at and above 0.1 U/ml with a half maximal effect at about 5 U/ml (Fig. 4).

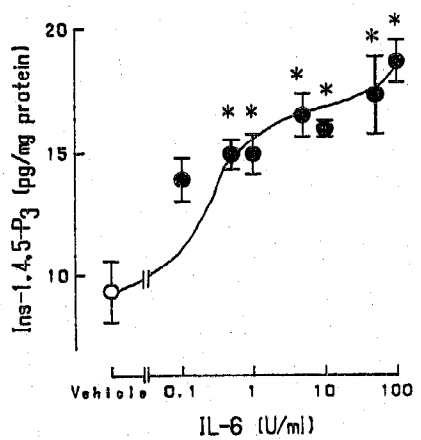

Fig. 2. Dose-dependency of Ins 1,4,5-P_3 production by IL-6 at 10 s in cultured rat mesangial cells. Data are shown as mean ± SE of 3 to 4 determinations. Statistical analysis was done by Dunnett's multiple comparison procedure. $^{*}P < 0.05$ vs vehicle.

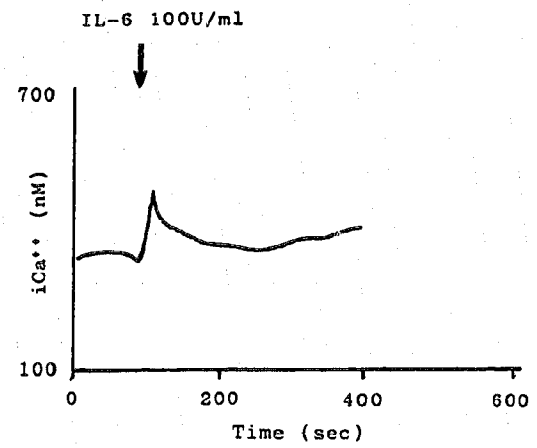

Fig. 3. Change in iCa^{2+} by 100 U/ml IL-6 in fura-2-loaded cultured rat mesangial cells. This figure shows a typical trace from representative experiments.

4. DISCUSSION

Ins 1,4,5-P_3 hydrolyzed from PIP_2 stimulates intracellular calcium mobilization which induces several biological actions [7]. In this study, 100 U/ml IL-6, which had been reported to stimulate the proliferation of rat mesangial cells [2–4], could induce a rapid increase in Ins 1,4,5-P_3 followed by the increment of iCa^{2+} in so-called 'transient and sustained' pattern. These results indicate that IL-6 enhances PI turnover in cultured rat mesangial cells.

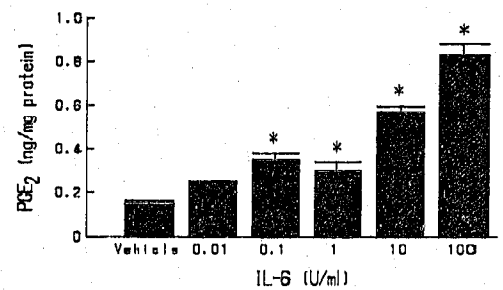

Fig. 4. Effects of IL-6 on PGE_2 production in cultured rat mesangial cells. Data are shown as mean ± SE of 3 to 4 determinations. Statistical analysis was done by Dunnett's multiple comparison procedure. $^{*}P < 0.05$ vs vehicle.

IL-6 also stimulated PGE_2 production. The stimulation of PGE_2 production is a common characteristic of many other growth factors that enhance PI turnover in mesangial cells. Both the dose–response relationship and the concentration to induce a half maximal effect of the stimulation of PGE_2 production by IL-6 were similar to those of the increase in Ins 1,4,5-P_3 by IL-6. These results suggest that PGE_2 production is stimulated by some mechanism coupled to the PI signaling pathway, for example, (1) the activation of phospholipase A_2 (PLA_2), a Ca^{2+}-dependent enzyme, by an Ins 1,4,5-P_3-induced increase in iCa^{2+}, with a resultant release of arachidonic acid from membrane phospholipids, or (2) sequential activations of PLC and diacylglycerol (DAG) lipase with a resultant release of arachidonic acid from DAG. Alternatively, PLA_2 may be activated by the independent mechanism on PI turnover [6]. It remains to be elucidated which mechanism(s) may be mainly involved in PGE_2 generation by IL-6.

Recent works on the molecular cloning of IL-6 receptor and signal transducer, gp130, have revealed that both molecules belong to the cytokine receptor superfamily and have no tyrosine kinase domain in their own intracellular regions [11,12]. The existence of such receptor protein and signal transducer protein in mesangial cells, and any other possible signaling pathways of IL-6 independent of PI turnover should be next studied.

In conclusion, IL-6 may exhibit some biological actions on cultured rat mesangial cells at least partially through the enhancement of PI turnover and the simultaneous stimulation of PGE_2 production.

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