Rapid communications

Effect of dexamethasone on the synthesis and degradation of insulin receptor mRNA in cultured IM-9 cells

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Summary. The effect of glucocorticoid (dexamethasone) on insulin receptor mRNA was examined in the IM-9 cell line by dot hybridization analysis using insulin receptor c-DNA probe (phINSR 13-1). Insulin receptor mRNA was found in a dose- and time-dependent manner, to increase during a 24-h culture with glucocorticoid. Although there has been some dispute about the effect of glucocorticoid on degradation of the insulin receptor itself, we have found glucocorti-

coid had no significant effect on the degradation rate of insulin receptor mRNA. These results suggest that glucocorticoid has the effect of increasing de novo synthesis of insulin receptor mRNA. But the molecular mechanism of glucocorticoid's effect still needs to be investigated.

Key words: Insulin receptor, dexamethasone, IM-9 cell, dot hybridization analysis.

Numbers of insulin receptors on the surface of various cell membranes have been reported to increase when cultured with dexamethasone [1–3]. This phenomenon was assumed to be the direct effect of glucocorticoid which increased de novo synthesis of insulin receptor [1, 4]. Recently, McDonald et al. [5] have found that glucocorticoid increases insulin receptor mRNA levels in both IM-9 cell and pancreatic acinar cell lines by applying northern blot analysis. However, there has been controversy concerning the effect of glucocorticoid on degradation of the insulin receptor itself [1, 4, 6]. As until now there has been little information about the effect of glucocorticoid on degradation of insulin receptor mRNA, in this study we have conducted experiments using IM-9 cells to evaluate the effect of glucocorticoid (dexamethasone) on synthesis and degradation of insulin receptor mRNA with the aid of dot hybridization method [7].

Materials and methods

Cultured human lymphocyte cell line, IM-9, was used in this study. Guanidine isothiocyanate (GTC) and cesium-chloride (CsCl) were purchased from Sigma (St. Louis, Mo, USA), dexamethasone and actinomycin D from Merck & Co. Inc. (Rahway, NJ, USA), and human insulin from Novo (Copenhagen, Denmark). Nick translation of insulin receptor probe was carried out by using Nick Translation Kit (Amersham, Arlington Heights, USA).

Cells were grown in the medium [RPMI 1640 with supplementation of 10% fetal calf serum (Sigma)]. Cells in the late log phase of growth were centrifuged for 5 min and resuspended in fresh medium. After assessing bioactivity (>90% by trypan blue), effects of dexamethasone were examined as described below.

RNA was extracted from 10⁸ cultured IM-9 cells by ultracentrifugation using GTC/CsCl method [8]. Five, 10, 20, and 40 µg of extracted RNA samples were denatured by 2.2 mol/l formaldehyde at 65 °C for 15 min, then applied to the nitro cellulose filter (S&S, Kassel, FRG). After vacuum baking at 80 °C for 2 h, hybridization was carried out at 42 °C for 24 h by using ³²P d-CTP labelled insulin receptor c-DNA probe⁹ (phINSR 13-1) of which specific radioactivity was over 10⁸ cpm. Hybridized filter was washed several times with 0.1X SSC and 0.1% SDS at 25 °C. Autoradiography was taken at –70 °C for 24 h with an intensifying screen (Dupon, Newtown, USA). After taking autoradiogram, blotted areas were counted separately by liquid scintillation counter to measure insulin receptor mRNA levels.

Experiments were carried out as follows.

Effect of dexamethasone on synthesis of insulin receptor mRNA

Dexamethasone was added to the medium to become final concentrations of 10^{–5}, 10^{–6}, 10^{–7}, and 10^{–8} mol/l respectively. Changes in insulin receptor mRNA levels after 24-h culture with dexamethasone were evaluated by comparing the results with the data obtained without addition of dexamethasone. In addition, in order to investigate the time dependent changes, insulin receptor mRNA levels were examined at 0, 2, 4, 6, 12, and 24 h after addition of dexamethasone at the concentration of 10^{–6} mol/l.

Effect of dexamethasone on degradation of insulin receptor mRNA

In order to evaluate the effect of dexamethasone on the degradation rate of insulin receptor mRNA, actinomycin-D, which is known to inhibit newly synthesised RNA, was added to IM-9 cells already cultured for 12 h with or without 10^{-6} mol/l dexamethasone. Then culture was continued with actinomycin-D ($5 \mu\text{g/ml}$) for another 12 h with or without 10^{-6} mol/l dexamethasone, as before. Changes in insulin receptor mRNA levels were measured at 0, 4, 8, and 12 h by dot hybridization method.

In these experiments described above, mRNA levels of B actin [5, 10], which is known to be stable even after glucocorticoid treatment, were also examined by dot hybridization method and it was demonstrated that the precise amount of RNA samples were applied to the filter in each experiment (data not shown).

Statistical analysis

Results are expressed as mean $\pm$ SD throughout, significance of differences are assessed by using Student's paired t-test.

Results

Effect of dexamethasone on synthesis of insulin receptor mRNA

After culturing IM-9 cells with dexamethasone at various concentrations for 24 h, insulin receptor mRNA levels were found to increase 2–3-fold compared to the levels in IM-9 cells cultured 24 h without addition of dexamethasone; 174% at 10^{-8} mol/l, 187% at 10^{-7} mol/l, 329% at 10^{-6} mol/l, 368% at 10^{-5} mol/l. The mean percentage changes of three experiments in insulin receptor mRNA during 24-h culture with 10^{-6} mol/l dexamethasone were found as follows (assuming 100% at time 0): $130 \pm 7\%$ at 2 h, $121 \pm 18\%$ at 4 h, $179 \pm 36\%$ at 6 h, $291 \pm 37\%$ at 12 h, $344 \pm 12\%$ at 24 h after addition of dexamethasone.

Effect of dexamethasone on degradation rate of insulin receptor mRNA

Dot hybridization analysis, in which the effects of dexamethasone on the degradation rate of insulin receptor mRNA were examined, are shown in Figure 1. At time 0, before addition of actinomycin-D, mRNA levels were found increased 2-fold in IM-9 cells already cultured with dexamethasone for 12 h compared with the 12 h control cultured without dexamethasone.

After addition of actinomycin-D, mRNA levels were found to decrease as shown in Figure 1 in both conditions with or without addition of dexamethasone. Mean percentage changes in insulin receptor mRNA levels at 4, 8, and 12 h were found as follows, when compared to the insulin receptor mRNA levels at time 0 ($n=5$); with dexamethasone insulin receptor mRNA levels were $60 \pm 7\%$ at 4 h, $30 \pm 4\%$ at 8 h, $18 \pm 5\%$ at 12 h, respectively, and without dexamethasone insulin receptor mRNA levels were $58 \pm 4\%$ at

Fig. 1. Evaluation of the dexamethasone's effect on both synthesis and degradation of insulin receptor mRNA. Actinomycin-D ($5 \mu\text{g/ml}$) was added after cultured 12 h with or without 10^{-6} mol/l dexamethasone. Five, 10, 20, and 40 μg of RNA samples extracted from 10^8 cells of IM-9 cultured with or without 10^{-6} mol/l dexamethasone at 0, 4, 8, and 12 h after addition of actinomycin-D, were applied to the nitrocellulose filter. Dot hybridization was carried out using ^{32}P labelled insulin receptor cDNA probe

Fig. 2. Effect of dexamethasone on degradation rate of insulin receptor mRNA. Results are mean of five experiments, respectively. ●—● With dexamethasone, ○—○ without dexamethasone

4 h, $29 \pm 5\%$ at 8 h, $22 \pm 6\%$ at 12 h respectively. The mean half life time of degradation of insulin receptor mRNA was calculated to be 4.7 ± 0.8 h with addition of dexamethasone. There were no significant differences compared with the control (without addition of dexamethasone), in which $T_{1/2}$ was 5.2 ± 0.8 h. (Fig.2)

Discussion

Till now evidence has been accumulated that insulin receptors on various cell membranes were increased by

glucocorticoid treatment in *in vitro* experiments [1–3]. For example, Fantus et al. [1] have found that insulin receptors on cultured human lymphocyte (IM-9) increased in a dose- and time-dependent manner after the addition of glucocorticoid. However, quite interestingly, at the same time they have reported an increased degradation rate of the receptor itself. On the other hand, Knutson [6], Sahlhanick [4] et al. have reported a decreased degradation rate of the insulin receptor.

In the molecular levels, recently McDonald et al. [5] found that the insulin receptor mRNA levels in IM-9 cells increased in a dose- and time-dependent manner by dexamethasone treatment. But there still remains the question of dexamethasone's effect on the degradation of insulin receptor mRNA, in addition to the existing controversies concerning the degradation of the insulin receptor itself as mentioned above [1, 4, 6]. In other words, whether the effect of dexamethasone is to increase *de novo* synthesis of insulin receptor mRNA or not is as yet not clearly known. So we have investigated carefully in IM-9 cells the effects of glucocorticoid on both the synthesis and degradation of insulin receptor mRNA by dot hybridization analysis.

In this study, we have confirmed that dexamethasone increases insulin receptor mRNA levels as reported by McDonald et al. [5]. But in addition, we evaluated the effects of dexamethasone on the degradation of insulin receptor mRNA. We found dexamethasone had no significant effects on the degradation of insulin receptor mRNA.

From this study, we have demonstrated that dexamethasone stimulated *de novo* synthesis of insulin receptor mRNA in IM-9 cells. This effect might be produced via a 'recognition site' of glucocorticoid in the insulin receptor gene. However, in order to investigate the precise molecular mechanism of dexamethasone's effect to increase insulin receptor mRNA synthesis, further studies will be necessary.

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