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Epidermal growth factor as a new regulator of induction of tyrosine aminotransferase and tryptophan oxygenase by glucocorticoids

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Epidermal growth factor (EGF) dose-dependently enhanced the induction of tyrosine aminotransferase and tryptophan oxygenase by glucocorticoids in primary cultures of adult rat hepatocytes without itself having any effect on these enzymes in the absence of glucocorticoids. The amplifications were observed even with dexamethasone at high concentrations (10^{-6} M–10^{-5} M) that had a maximal effect. EGF had no effect on induction of tyrosine aminotransferase by glucagon or Bt_2cAMP. The effect of EGF was also observed in adrenal-ectomized and submaxillar gland-ectomized rats. These results suggest that EGF is an endogenous amplifier of the action of glucocorticoids.

Glucocorticoid; Epidermal growth factor; Enzyme induction; Hepatocyte; Adrenalectomy

1. INTRODUCTION

The activities of tyrosine aminotransferase (EC 2.6.1.5) (TAT) and tryptophan 2,3-dioxygenase (EC 1.13.11.11) (TO) are known to be regulated by glucocorticoids [1,2], insulin [3] and glucagon [3]. Recently, we found several compounds that specifically amplified the induction of TAT by glucocorticoids without themselves having any glucocorticoid action and named these compounds 'glucocorticoid action biomodulators'. Several of these compounds, such as diacylglycerol and 12-*O*-tetradecanoylphorbol 13-acetate, are activators of protein kinase C [4,5].

In order to find endogenous glucocorticoid action biomodulators, we examined the effects of a number of polypeptide growth factors, such as EGF, fibroblast growth factor, platelet-derived growth factor and 7S-nerve growth factor, on induction of TAT and/or TO by glucocorticoids in primary cultures of adult rat hepatocytes and in adrenal-ectomized plus submaxillar gland-ectomized rats. Here we show that EGF specifically amplified the induction of liver enzymes by glucocorticoids in vitro and in vivo.

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Abbreviations: TAT, tyrosine aminotransferase; TO, tryptophan 2,3-dioxygenase; EGF, epidermal growth factor

2. MATERIALS AND METHODS

2.1. *Materials*

Male Wistar strain rats, weighing 180–200 g were used. Dexamethasone sodium phosphate was obtained from Merck. Glucagon and Bt_2cAMP were from Sigma. EGF (mouse) from Toyobo, platelet-derived growth factor (human) and fibroblast growth factor (bovine) from Collaborative Research and 7S-nerve growth factor (mouse) from Wako.

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2.2. *Assay of biological activity*

The biological activities of polypeptide growth factors in vitro were measured in primary cultures of adult rat hepatocytes as reported [5,6]. Isolated hepatocytes were suspended at a density of 5×10^5 cells/ml in Williams medium E containing 10% calf serum, 10^{-6} M dexamethasone and 10^{-7} M insulin in 10-cm dishes at 37°C under 5% CO_2 in air. After 36 h, the medium was replaced by hormone-free medium containing 10% calf serum. One-day cultures were washed twice with serum-free medium and then dexamethasone in saline, Bt_2cAMP in saline, or glucagon in dimethyl sulfoxide and/or each polypeptide growth factor in saline was added to the cultures in serum-free medium for assay of TAT activity. For assay of TO activity, 2.5 mM tryptophan was added in serum-free medium. The final concentration of dimethyl sulfoxide did not exceed 0.05%. At the indicated times, the hepatocytes were washed three times with ice-cold Tyrode buffer but without divalent cations, harvested with a rubber policeman in 2.5 ml of homogenization buffer (0.25 M sucrose containing 0.05 M potassium phosphate buffer, pH 7.5, 1 mM 2-oxoglutarate and 48 μM pyridoxal phosphate) for the assay of TAT, or in 2.5 ml of homogenization buffer (20 mM potassium phosphate buffer, pH 7.0, containing 2.5 mM tryptophan and 2 μM methohemoglobin) for the assay of TO, and then sonicated for 10 s. The sonicate was used for the TO assay or was centrifuged at 100500 × g for 30 min to obtain the supernatant, which was used for the TAT assay. The biological activity of EGF in adrenal-ectomized plus submaxillar gland-ectomized rats was determined by measuring its amplification of the induction of TAT in liver by dexamethasone as reported [4,5].

2.3. *Enzyme and protein assays*

TAT activities in the liver homogenate and the supernatant of hepatocytes were measured by the methods of Rosen et al. [7] and Granner and Tomkins [8], respectively. TO activity was determined by the method of Seglen and Jervell [9]. The protein concentrations of the cytosolic fractions used for the assay of TAT activity and of sonicates used for the assay of TO activity were determined by the methods of Lowry et al. [10] and Bradford [11], respectively.

3. RESULTS AND DISCUSSION

3.1. *Effects of polypeptide growth factors on induction of TAT and TO in vitro*

The effects of polypeptide growth factors on the induction of TAT and TO by dexamethasone were studied in primary cultures of adult rat hepatocytes. When dexamethasone (10^{-7} M) was added to the medium, the specific activity of TAT increased rapidly to a plateau after incubation for 4–8 h and then remained constant unless the dexamethasone was removed. When hepatocytes were incubated with polypeptide growth factors plus dexamethasone (10^{-7} M) for 8 h, TAT induction was markedly amplified by EGF but not by the other polypeptide growth factors tested, such as fibroblast growth factor, 7S-nerve growth factor and platelet-derived growth factor, as shown in table 1. In the absence of dexamethasone, these polypeptide growth factors including EGF had no effect on TAT. EGF amplified not only TAT induction but also TO induction by dexamethasone. The specific activity of TO was enhanced by EGF, increasing to a plateau after incubation for 10–14 h and then remaining constant for 24 h in

Table 1

Effects of polypeptide growth factors on induction of TAT by dexamethasone (10^{-7} M) in primary cultures of hepatocytes

Treatment (ng/ml)	Specific activity of TAT (mU/mg protein)
None	12.1 ± 1.4
EGF (20)	12.3 ± 1.1
Platelet-derived growth factor (5)	12.1 ± 1.8
Fibroblast growth factor (20)	12.3 ± 1.2
7S-Nerve growth factor (20)	11.8 ± 1.3
Dexamethasone	52.6 ± 4.1
Dexamethasone + EGF (20)	153.4 ± 6.8
Dexamethasone + platelet-derived growth factor (5)	52.9 ± 2.4
Dexamethasone + fibroblast growth factor (20)	52.4 ± 3.0
Dexamethasone + 7S-nerve growth factor (20)	51.8 ± 3.4

Experimental conditions are described in section 2. Values are means ± SD for four dishes

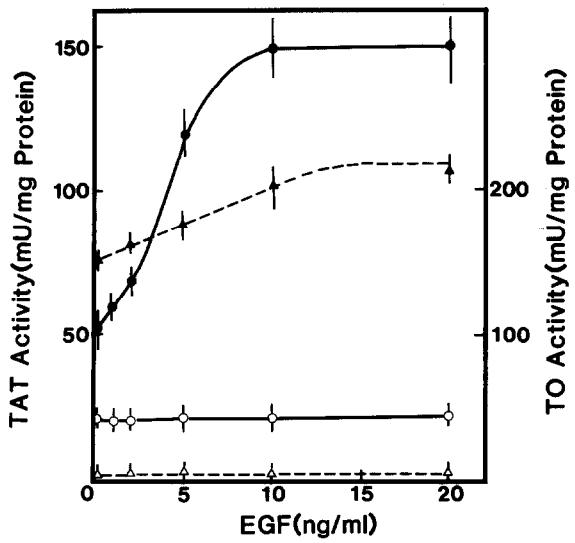

Fig.1. Dose-response curves of EGF for amplifications of TAT and TO induction in rat hepatocytes. TAT activities at the indicated concentrations of EGF with (●) or without (○) 10^{-7} M dexamethasone were measured after 8 h, and TO activities at the indicated concentrations of EGF with (▲) or without (△) 10^{-8} M dexamethasone were measured after 20 h. Values are means from three dishes. Bars indicate SD values.

the presence of 2.5 mM tryptophan (not shown). TAT and TO were not activated directly by addition of EGF in vitro (not shown).

The effects of EGF on TAT and TO induction by dexamethasone were concentration-dependent, as shown in fig.1. EGF was effective at concentrations of above 2 ng/ml and was maximally effective at 10–20 ng/ml. The amplifications of induction of TAT and TO induced by EGF (10 ng/ml) were reproducible, but the extents of the amplifications were variable: 2–3-fold amplification of TAT induction and 1.2–1.6-fold amplification of TO induction were observed. The effects of EGF on the induction of TAT and TO were studied as a function of the concentration of dexamethasone. As shown in fig.2, EGF significantly amplified the induction of TAT (fig.2A) and TO (fig.2B) not only by low concentrations of dexamethasone (10^{-8} M–10^{-7} M) but also by higher concentrations of dexamethasone (10^{-6} M–10^{-5} M) that had a maximal effect. These modes of amplification show that EGF is a 'glucocorticoid potency amplifier' [4], like diacylglycerol [4] and 12-*O*-tetradecanoylphorbol 13-acetate [5]. Recent studies have shown that EGF in A-431 cells rapidly generates

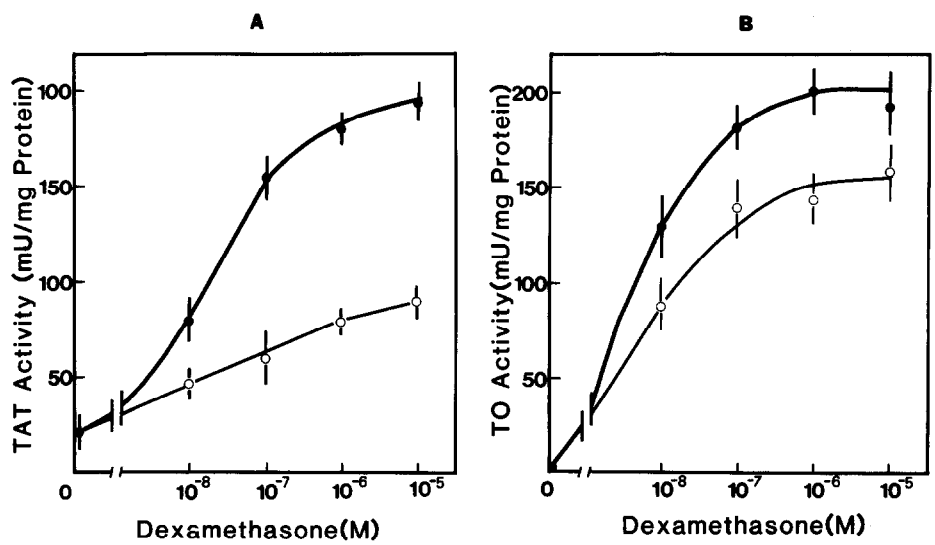

Fig.2. Effect of EGF on the induction of TAT (A) and TO (B) as a function of the dose of dexamethasone in rat hepatocytes. In A, TAT activities at the indicated concentrations of dexamethasone with (●) or without (○) EGF (10 ng/ml) were measured after 8 h. In B, TO activities at the indicated concentrations of dexamethasone with (●) or without (○) EGF (10 ng/ml) were measured after 20 h. Values are means for three dishes. Bars indicate SD values.

Table 2

Effects of EGF on induction of TAT by dexamethasone in adrenal-ectomized plus submaxillar gland-ectomized rats

Treatment (dose/100 g body wt)	Adrenal-ectomy + submaxillar gland-ectomy
	Specific activity of TAT (mU/mg protein)
None	8.1 ± 1.5
EGF (5 μg)	7.9 ± 1.8
Dexamethasone (2 ng)	14.8 ± 2.3
Dexamethasone (2 ng) + EGF (5 μg)	24.2 ± 3.0

EGF and/or dexamethasone in saline was injected intraperitoneally and the rats were killed by cervical dislocation 5 h later. The livers were homogenized in 6 vol. of homogenization buffer and TAT activity in the homogenate was measured as described in section 2. Values are means ± SD for 12 rats

diacylglycerol, resulting from the degradation of phosphoinositide, and that the diacylglycerol may activate protein kinase C [12].

TAT is known to be induced not only by glucocorticoids but also by glucagon and Bt_2cAMP in vivo [3] and in vitro [13]. But EGF (2–20 ng/ml) had no effect on the induction of TAT by glucagon (10^{-10} M–10^{-6} M) or Bt_2cAMP (10^{-6} M–10^{-3} M) during incubation for 4 h (not shown). Thus, the effect of EGF on TAT induction is apparently specific for glucocorticoids.

3.2. *Effects of EGF on induction of TAT in vivo*

In adrenal-ectomized plus submaxillar gland-ectomized rats, EGF (5 μg/100 g body wt) amplified TAT induction by dexamethasone (2 μg/100 g body wt) but it had no effect on the enzyme activity in the absence of dexamethasone, as shown in table 2. These results show that EGF from the submaxillar gland may be an endogenous glucocorticoid potency amplifier and may regulate glucocorticoid actions. The extent of the amplification in vivo was lower than that observed in vitro. One possible explanation for this was that endogenous EGF was not completely removed by submaxillar gland-ectomy, because low concentrations of EGF may be formed in other tissues, such as the kidney [14], brain [15] and intestine [16].

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