I. INTRODUCTION

The actions of hormones on target cells are controlled not only by the concentrations of hormones in the blood but also by modulators of these hormonal actions on their target cells. In fact we have found several modulators of glucocorticoid action, which amplify or suppress glucocorticoid action on target cells without themselves having any glucocorticoid-like action (1-6). Figure 1 shows the modes of action of the two different groups of amplifiers. One group is "Glucocorticoid Sensitivity Amplifier" (GSA), which specifically enhances the sensitivity of target organs or cells to glucocorticoid but has little effect with doses of glucocorticoid that has maximal effects. The other group is "Glucocorticoid Potency Amplifier" (GPA), which amplifies glucocorticoid actions even with sufficient concentrations of glucocorticoids to have maximal effects. On the other hand, we also found suppressor of glucocorticoid action which inhibits the actions of glucocorticoid in target cells. This paper summarizes the nature and the mechanism of action of these modulators.

II. RESULTS AND DISCUSSION

Nature and Mechanism of Action of GSA

We previously found that administration of a small dose of glucocorticoid induced less liver tyrosine aminotransferase (TAT) in germ-free rats than in conventional rats (7) and isolated a compound from enteric flora of rats that enhanced the glucocorticoid sensitivity of target organs (6). The structure of the compound is assumed to be 5'-pseudouridinyl N-oleoylphosphamate. The effects of the c-nucleotide compound and its-related derivatives on TAT induction by dexamethasone (Dex) in adrenalectomized rats is shown in Table I. Synthetic 5'-pseudouridinyl-N-oleoyl-carbamate also enhanced the TAT induction. The components and the related compounds, such as oleamide, pseudouridine, and pseudouridine-5'-phosphate, had no effect. The effect of GSA is specific for glucocorticoid and it did not amplify the TAT induction by glucagon or insulin in adrenalectomized rats (1).

Effects of GSA on incorporation of [3H]Dex into the liver and its binding to cytosol receptor were studied. As shown in Fig. 2, GSA caused 25% increase in both maximal incorporation of [3H]Dex into the cytosol and maximal binding of [3H]Dex to cytosol receptor. However GSA did not change the total receptor concentration in the liver cytosol, the sedimentation coefficient of the receptor and the dissociation constant of the [3H]Dex binding reaction at 0°C (2).
TABLE 1
Effects of GSA and its related compounds on TAT induction by Dex in adrenalectomized rat

| Treatment (nmol/100g B.W.) | Specific Activity of TAT (mU/mg protein) |
|-----------------------------|----------------------------------------|
| Saline                      | 7.0 ± 1.1                               |
| Dex (1)                     | 15.1 ± 1.4                              |
| 5'-Pseudouridinyl N-oleoylphosphamate (40) | 6.8 ± 1.3 |
| Dex (1)-5'-pseudouridinyl N-oleoyl phosphamate (40) | 38.7 ± 3.2 |
| 5'-Pseudouridinyl N-oleoylcarbamate (80) | 7.0 ± 1.2 |
| Dex (1)+5'-pseudouridinyl N-oleoylcarbamate (80) | 29.7 ± 2.6 |
| Oleamide (80)               | 6.8 ± 1.3                               |
| Dex (1) + Oleamide (80)     | 14.9 ± 1.2                              |
| Dex (1) + Pseudouridine (80) | 14.9 ± 1.5                              |
| Pseudouridine 5'-phosphate (100) | 6.9 ± 1.3                              |
| Dex (1) + Pseudouridine 5'-phosphate (100) | 15.4 ± 1.5   |

*TAT activities were measured 5hr after administration of these compounds.

Fig. 2. Effects of GSA on incorporation of [3H]Dex into the cytosol and its binding to cytosol protein. Adrenalectomized rats were injected intraperitoneally with [3H]Dex (50μCi/100g body weight) with or without GSA (0.25μg / 100g body weight) at zero time. Incorporation of [3H]Dex into the liver cytosol of rats treated with Dex with(●) or without (▲) GSA; protein-bound [3H]Dex in the liver cytosol of rats treated with Dex with (●) GSA. Vertical lines represent standard errors of values in 3 adrenalectomized rats.

Nature and Mode of Action of GPA

Potent activators of protein kinase C (PKC), such as rac-1,2-diocatanylglycerol (DG) and the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA), and epidermal growth factor (EGF) enhanced the induction of TAT by even maximally effective doses of glucocorticoid in adrenalectomized rats and in primary cultures of rat hepatocytes as shown in Table II (3,4,6). These activators of PKC also enhanced the induction of other enzymes, such as tryptophan oxygenase and ornithine decarboxylase, by glucocorticoid but had no effect on induction of TAT by glucagon or insulin and on induction of glucose-6-phosphate dehydrogenase by 17β-estradiol (3). However, these compounds had no effect on glucocorticoid action in the absence of glucocorticoid. Phorbol derivatives and components of TPA, such as 4β-phorbol, phorbol 12-tetradecanoate, phorbol 13-acetate, and 4-0-methylphorbol 12-tetradecanoate 13-acetate, which have no tumor-promoting activity or ability to activate PKC, did not have any effect on TAT induction by glucocorticoid as shown in Table II. In A-431 cells, EGF is reported to induce generation of diacylglycerol formed by degradation of phosphoinositide. These findings provided indirect evidence that PKC participates in the expression of glucocorticoid action and thus that activators of PKC amplify the action of glucocorticoid.
TABLE II
Effects of TPA, its derivatives and 1,2-DG on TAT induction by Dex in adrenalectomized rat

| Compound (mg/100g body weight) | TAT Activity (mU/mg protein) |
|-------------------------------|-------------------------------|
| Saline                        | 7.6 ± 1.2b                    |
| Dex (0.5)                     | 14.8 ± 2.8                    |
| TPA (20)                      | 7.4 ± 1.4                     |
| Dex (0.5) + TPA (20)          | 42.3 ± 6.8                    |
| Phorbol 13-acetate (20)       | 7.6 ± 1.3                     |
| Dex (0.5) + Phorbol 13-acetate (20) | 16.3 ± 2.7               |
| Phorbol 12-tetradecanoate (20) | 7.4 ± 1.1                     |
| Dex (0.5) + Phorbol 12-tetradecanoate (20) | 13.0 ± 2.9               |
| 4β-Phorbol (20)               | 7.5 ± 1.2                     |
| Dex (0.5) + 4β-Phorbol (20)   | 15.8 ± 2.8                    |
| 4-O-Methylphorbol 12-tetradecanoate 13-acetate (20) | 7.4 ± 1.2               |
| Dex (0.5) + 4-O-Methylphorbol 12-tetradecanoate 13-acetate (20) | 14.8 ± 2.7               |
| 1.2-DG (80)                   | 7.8 ± 1.1                     |
| Dex (1.0) + 1.2-DG (80)       | 34.0 ± 3.6                    |

\(^a\)TAT activities were measured 5hr, after administration of these compounds. \(^b\)Values, ± S.D.

Suppressors of Glucocorticoid action and Their Mechanism of Action

We recently found several compounds that significantly suppressed the induction of TAT by glucocorticoid without themselves having any inhibitory activity of TAT. One of them is TGF-β which suppresses the actions of glucocorticoids, in contrast to EGF (8). The other is H-7 (5) and sphingosine, potent inhibitors of PKC.

To determine which process in the induction of TAT by glucocorticoid is inhibited by H-7, we examined the subcellular distribution of glucocorticoid-receptor complexes in primary hepatocytes after their treatment with \(^3\)H]Dex with and without H-7 and the results are shown in Fig. 3 (5). The induction of TAT by Dex and the nuclear accumulation of receptor-bound \(^3\)H]Dex were both inhibited concentration-dependently by H-7 and the radioactivity in the cytosol increased with decrease in the radioactive in the nuclei. The combined radioactivities in the cytosol and nuclei being almost constant, regardless of the concentration of H-7. In addition, the total radioactivity incorporated into the cells was not affected by H-7. These results indicate that H-7 inhibits the translocation of glucocorticoid-receptor complexes into the nuclei or inhibits the binding of glucocorticoid receptor to DNA and consequently inhibits the induction of TAT by glucocorticoid. However, H-7 did not affect the extent of phosphorylation of glucocorticoid receptor both in the absence or in the presence of glucocorticoid (9). This finding rules out the possibility that PKC phosphorylates the glucocorticoid receptor resulting to enhance the nuclear accumulation.

Further experiments are needed to identify some protein(s) which is phosphorylated by PKC and induces translocation of glucocorticoid receptor into nuclei and/or stimulates the binding of glucocorticoid receptor to DNA.

III. SUMMARY

We have found several compounds that specifically modulate the action of glucocorticoid in vivo and in vitro without themselves having any glucocorticoid-like action and have proposed the concept of "Glucocorticoid Action Biomodulators". These biomodulators consist of "Glucocorticoid Sensitivity Amplifier" (GSA), "Glucocorticoid Potency Amplifiers" (GPAs), and suppressors of glucocorticoid action. GSA increased the incorporation of glucocorticoid into
the liver and its binding to cytosol receptor without changing the total receptor concentration in liver cytosol and the equilibrium constant of the glucocorticoid binding reaction. GPAs, potent activators of protein kinase C, markedly enhanced the glucocorticoid action and the glucocorticoid action was inhibited by the inhibitors of protein kinase C. H-7, an inhibitor of protein kinase C, inhibited the translocation of glucocorticoid-receptor complex into nuclei without affecting the extent of phosphorylation of glucocorticoid receptor. These findings suggest that GPA(s) and the suppressors modulate some protein(s) which regulates the translocation of glucocorticoid receptor into nuclei.

Fig. 3. Dose-response curves for inhibition of TAT induction and accumulation of receptor-bound \[^{3}\text{H}]\text{Dex in nuclei of hepatocytes by H-7. Hepatocytes were incubated with}^{10^{-9}}\text{M }^{[3}\text{H}]\text{Dex plus various concentrations of H-7 at 37°C for 4 hr. The rate of induction of TAT (●) is shown as a percentage of the activity of control cells not treated with Dex. The specific binding of}^{[3}\text{H}]\text{Dex to the cytosolic (○), nuclear (Δ) fraction and total radioactivity (□) incorporated. Values are means from three dishes. Bars, S.D.}

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