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DIACYLGLYCEROL AMPLIFIES THE INDUCTION IN VIVO OF TYROSINE AMINOTRANSFERASE AND ORNITHINE DECARBOXYLASE BY GLUCOCORTICOID

Hiroshi Kido, Naomi Fukusen, Kazumi Ishidoh
and Nobuhiko Katunuma*

Department of Enzyme Chemistry, Institute for Enzyme Research,
School of Medicine, The University of Tokushima,
Tokushima 770, Japan

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Summary: In adrenalectomized rats, diacylglycerol, a potent activator of protein kinase C, specifically enhanced the induction of tyrosine aminotransferase and ornithine decarboxylase by even maximally effective doses of dexamethasone phosphate, but itself had no effect on these enzyme inductions in the absence of glucocorticoid. The amplifications of enzyme induction by diacylglycerol was dose-dependent and the time courses of the amplified inductions were similar to those of the inductions by dexamethasone phosphate alone. Since diacylglycerol did not affect the induction of these enzymes by glucagon and insulin, its amplifying effect seemed to be specific for induction by glucocorticoids.

Recently the findings of many compounds that modulate biological responses, such as interferon, muramyl dipeptide, thymosin α_1 and various lymphokines, have led to the concept of so-called "Biological Response Modifiers" (1).

Previously Katunuma et al. found a factor from Proteus mirabilis that greatly amplified the action of doses of glucocorticoid that alone had minimal effects, but which was not effective with optimal doses of glucocorticoid (2-7). We named this factor "Glucocorticoid Sensitivity Amplifier" (GSA). We purified this factor from P. mirabilis and found that it was composed of

*To whom correspondence should be addressed.
Abbreviations: GSA, glucocorticoid sensitivity amplifier; GPA, glucocorticoid potency amplifier; 1,2-DG, 1,2-racemic dioctanoyl glycerol; TAT, tyrosine aminotransferase, ODC, ornithine decarboxylase; TPA, 12-o-tetradecanoyl phorbol-13-acetate.

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pseudouridine, oleamide and phosphate (as described in detail elsewhere). We also found another factor that markedly enhanced the effect of glucocorticoid at doses that caused maximal effects, but did not itself have any glucocorticoid-like activity and we named this factor "Glucocorticoid Potency Amplifier" (GPA). We purified a factor with GPA activity from P. mirabilis and identified it as guanosine 3'-diphosphate (8,9). We named the compounds in nature that modulate glucocorticoid actions, such as GSA and GPA, "Glucocorticoid Action Biomodulators".

Recently we found that 1,2-racemic dioctanoyl glycerol (1,2-DG), a potent activator of Ca^{++}/phospholipid-dependent protein kinase C, has strong GPA activity without itself having any glucocorticoid-like action in adrenalectomized rats. In addition, several recent investigations have suggested that the properties of steroid hormone receptors are modulated by phosphorylation and dephosphorylation (10-12). Here we show that 1,2-DG has GPA activity, especially at low concentration of 1,2-DG, and discuss the mechanism of its effect in enhancing the actions of glucocorticoid.

MATERIALS AND METHODS

Materials-----Male Wistar strain rats, weighing 180-200 g were used. All animals were adrenalectomized 7-8 days before experiments and given laboratory chow and saline ad libitum, unless otherwise noted. Dexamethasone sodium phosphate was obtained from Merck & Co. Insulin and glucagon were from Sigma Chemical Co. and DL-[1-^{14}C]-ornithine monohydrochloride (specific activity, 61 mCi/mmole) was from Amersham. 1,2-DG was kindly supplied by Dr. Y. Nishizuka, Kobe University, School of Medicine, Kobe, Japan.

Assay of Biological Activity-----The biological activity of 1,2-DG was determined by measuring its amplification of the induction of tyrosine aminotransferase (TAT) and ornithine decarboxylase (ODC) by dexamethasone, insulin and glucagon. 1,2-DG in saline containing 20% dimethyl sulfoxide and, or dexamethasone in saline or insulin in saline or glucagon in saline containing 20% dimethyl sulfoxide was injected intraperitoneally into adrenalectomized rats. The rats were killed by cervical dislocation 5 h after administration of dexamethasone or 3 h after administration of insulin or glucagon. In experiments with insulin the rats were also given 2 ml of 10% glucose at hourly intervals to prevent hypoglycemic shock. Their livers were homogenized in 6 vol. 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 assay of TAT and were also homogenized in 5 vol. of homogenization

buffer (0.25 M sucrose containing 0.1 M Tris-HCl buffer, pH 7.2, 5 mM dithiothreitol, 0.2 mM pyridoxal phosphate and 1 mM EDTA) for assay of ODC. The homogenate for assay of ODC was centrifuged at 100,000 xg for 30 min and the resulting supernatant was used for ODC assay.

Enzymes and Protein Assays-----Activity of TAT in the homogenate was measured by the method of Rosen et al. (13). ODC activity was determined by measuring release of CO_2 as described by O'Brien and Diamond (14). The liver supernatant in a volume of 75 µl in a 15-ml disposable culture tube was preincubated at 37°C for 5 min. Then 25 µl of a solution containing 75 mµCi of DL-ornithine monohydrochloride [1-^{14}C] and 20 nmoles of L-ornithine was added. Incubations were carried out for 60 min at 37°C and then the reaction was stopped by adding 0.2 ml of 2 M citric acid and released CO_2 was trapped in 0.1 ml of 10% KOH. Protein concentrations were determined by the method of Lowry et al. (15).

RESULTS

Amplification of Induction of Enzymes by 1,2-DG

The time course of amplification of induction of TAT by dexamethasone was studied in adrenalectomized rats (Fig. 1). When a small dose of dexamethasone (4 µg per 100 g body weight) was injected intraperitoneally, the activity began to increase after a lag time of 1-2 h, reaching a peak after 6 h and decreasing to the basal level after 12 h. When 1,2-DG was injected intraperito-

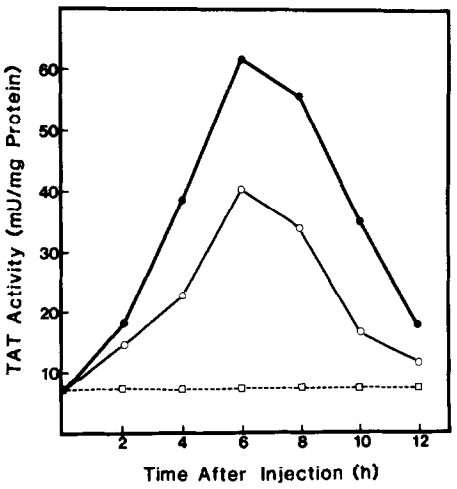

Fig. 1. Time course of amplification of TAT induction. Curves show TAT activities in adrenalectomized rats after administration of dexamethasone (4 µg/100 g body weight) and/or 1,2-DG (80 µg/100 g body weight) at zero time: dexamethasone (○); dexamethasone plus 1,2-DG (●); 1,2-DG (□). Values are mean TAT activities in livers of 4 adrenalectomized rats.

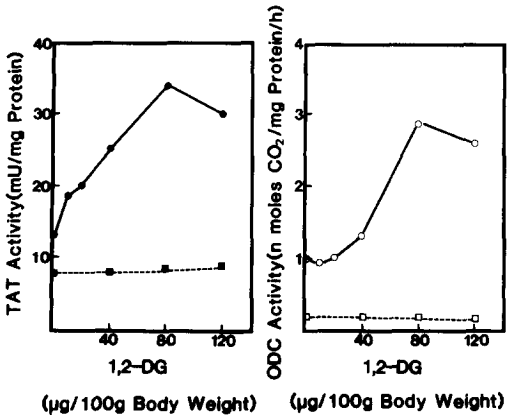

Fig. 2. Dose-response curves for the amplifying effects of 1,2-DG on the induction of TAT and ODC. Dexamethasone (1 μg/100 g body weight) and/or various concentrations of 1,2-DG were injected into adrenalectomized rats and their liver TAT and ODC activities were measured 5 h later as described in Materials and Methods. TAT activity with (●) and without (■) dexamethasone. ODC activity with (○) and without (□) dexamethasone. Values are means for 5 adrenalectomized rats.

neally with the same dose of dexamethasone, the induction was markedly enhanced but showed a similar time course to that with dexamethasone alone. 1,2-DG itself had no effect on TAT activity in adrenalectomized rats.

1,2-DG amplified not only the induction of TAT by dexamethasone but also that of ODC and the effects of 1,2-DG on induction of these activities were found to be dose-dependent, as shown in Fig. 2. In both cases, these inductions by dexamethasone were amplified by 1,2-DG at doses of above 10 to 20 μg, and 1,2-DG was maximally effective at doses above 80 μg. But the intensities of amplification of these enzymes by 1,2-DG were somewhat different: limited amounts of 1,2-DG at doses of 10 to 40 μg amplified the induction of TAT more than that of ODC, but the maximum amplification of ODC by 1,2-DG at doses of above 80 μg was more than that of TAT induction. 1,2-DG itself had no effect on these enzymes. Moreover, the activities of TAT and ODC in a homogenate of rat liver *in vitro* were not directly activated by added 1,2-DG (data not shown).

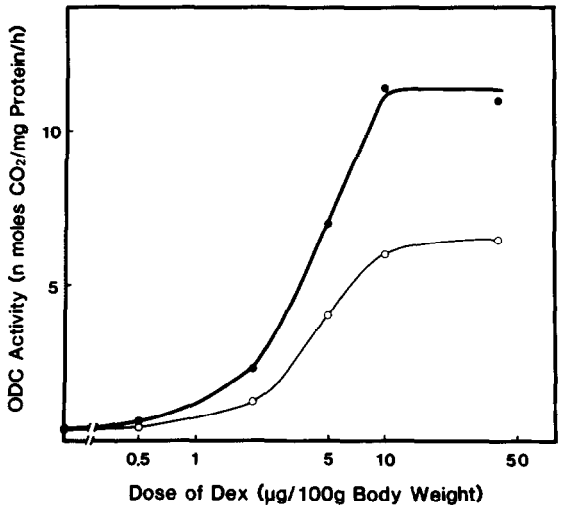

Fig. 3. Dose-response curves for induction of ODC activity by dexamethasone with and without 1,2-DG. Adrenalectomized rats were treated intraperitoneally with various doses of dexamethasone with (●) or without (○) 1,2-DG (80 µg/100 g body weight). The rats were killed 5 h later and their liver ODC activities were measured. Values are means for 4 adrenalectomized rats.

The effect of 1,2-DG on the induction of ODC was studied as a function of the dose of dexamethasone in adrenalectomized rats. As shown in Fig. 3, 1,2-DG amplified the induction of ODC not only at low doses of dexamethasone (2.0 to 10 µg) but also by maximally effective doses (above 10 µg) of dexamethasone. A similar pattern of amplification of TAT induction was also observed (data not shown).

Hormonal Specificity of the Effect of 1,2-DG

Glucagon and insulin are also reported to induce TAT (16) and ODC (17) in adrenalectomized rats. As shown in Fig. 4, insulin and glucagon both induced an approximate 3-fold increase in TAT activity and 1,2-DG did not have any effect on this induction. Insulin and glucagon also induced about 4-fold increase in ODC activity and 1,2-DG did not have any effect on the induction by glucagon, but almost completely inhibited the induction by insulin. The mechanism of inhibition by 1,2-DG of ODC induction by insulin is unknown. These results suggest that 1,2-DG specifically ampli-

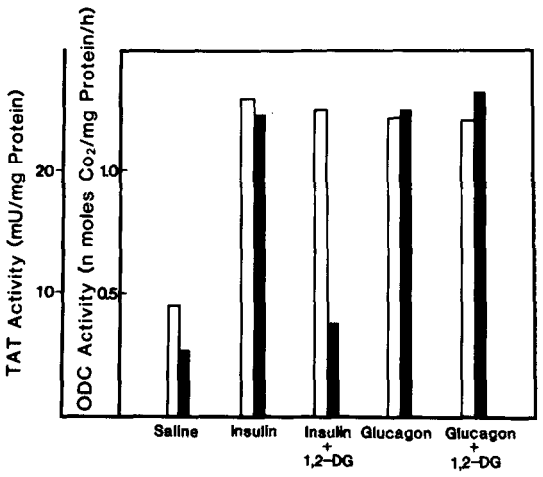

Fig. 4. Effects of 1,2-DG on induction of TAT and ODC activities by insulin and glucagon. Biological activities of 1,2-DG (80 µg/100 g body weight) in adrenalectomized rats treated with insulin (1.5 Unit/100 g body weight) or glucagon (150 µg/100 g body weight) were measured as described in Materials and Methods. Liver ODC (filled columns) and TAT (open columns) activities were measured 3 h after their administrations. Values are means for 5 adrenalectomized rats.

fies the inductions of TAT and ODC by dexamethasone but not those by insulin and glucagon in adrenalectomized rats.

DISCUSSION

The present study shows that 1,2-DG administered to adrenalectomized rats specifically enhanced the effect of even a maximally effective dose of glucocorticoid, without itself having any glucocorticoid-like action. 1,2-DG was effective at very low doses (above 30-60 nmoles/100 g body weight), like a hormone.

The GPA activity of guanosine 3'-diphosphate (8,9) was confirmed by Mukai et al., who synthesized this novel compound using Streptomyces adephospholyticus nucleotide pyrophosphokinase (EC 2.7.6.4) (18,19). Recently we found that a tumor-promoting phorbol ester, 12-o-tetradecanoyl phorbol-13-acetate (TPA), has also GPA activity and that 1,2-DG and TPA are more potent than guanosine 3'- diphosphate (as described in detail elsewhere). Both TPA and 1,2-DG have been reported to be strong activators of Ca^{++}/phospholipid-dependent protein kinase C (20-24).

On the other hand, several recent investigations have suggested that steroid binding as well as transformation of steroid receptors may be influenced by phosphorylation and dephosphorylation (10-12). These investigations suggest that amplification of glucocorticoid action by TPA and 1,2-DG may be due to activation of Ca^{++}/phospholipid-dependent protein kinase C and increased phosphorylation of the glucocorticoid receptor. However, TPA and 1,2-DG do not amplify glucocorticoid action in the absence of glucocorticoid in adrenalectomized rats, whereas they themselves stimulate protein kinase C *in vivo* and *in vitro* (20-24). These results also suggest either that phosphorylation of the glucocorticoid receptor by the activated protein kinase C does not take place in the absence of glucocorticoid or that further processes involved in the mechanism of action of glucocorticoid after phosphorylation of the glucocorticoid receptor may require the presence of glucocorticoid.

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