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GLUCOCORTICOID-INDUCED REDUCTION OF POLY(ADP-RIBOSE) SYNTHETASE IN NUCLEI FROM CHICK EMBRYO LIVER

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SUMMARY

The effect of glucocorticoid hormone administration on the nuclear poly(ADP-ribose) synthetase activity of chick embryo-liver was investigated. Compared with the values obtained with control nuclei, the enzyme activity was markedly reduced in the nuclei of liver prepared from chick embryo treated with 0.1 mg hydrocortisone for 12 hours or longer. The possible relationship between the reduction of poly(ADP-ribose) synthetase activity and decrease in DNA synthesis is discussed.

INTRODUCTION

It is well established that glucocorticoid treatment of normal and neoplastic tissues results in an inhibition of various metabolic functions including DNA synthesis and cell growth(1-5). On the basis of the cytotoxic action, glucocorticoids are clinically used in the therapy of certain neoplasms, however, the mechanisms by which glucocorticoids inhibit these metabolic functions have yet to be defined.

Concerning the participation of poly(ADP-ribose) in the regulation of DNA synthesis, we recently reported that the for-

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The abbreviations used are: ADP-ribose, adenosine diphosphate ribose; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene-glycerol-bis (β-aminoethylether) N,N'-tetraacetic acid; NAD, nicotinamide adenine dinucleotide; dTTP, deoxythymidine triphophate; dTMP, deoxythymidine monophosphate; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

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mation of this polymer bring about an increase in [^{3}H]dTMP incorporation into the acid-insoluble fraction of chick-embryo-liver-nuclei, and that the stimulation of DNA synthesis by poly-(ADP-ribose) formation is due to the increased template activities as a result of an increase in accessibility of nuclease to template DNA(6,7). Our next step was to determine whether glucocorticoids decrease in poly(ADP-ribose) synthetase activity in vivo.

During studies on the regulation of poly(ADP-ribose) formation, we found that administration of glucocorticoid into chick embryo decreased the poly(ADP-ribose) synthetase activity in liver nuclei.

In the present communication, we report evidence that glucocorticoid induces the reduction of poly(ADP-ribose) synthetase activity concomitant with the decrease in DNA synthesis in the nuclei from chick embryo liver.

MATERIALS AND METHODS

Fertilized eggs of the Rhode Island Red species were obtained from Yamasaki Farms, Shimane. The following compounds were purchased: [adenine-2,8,-^{3}H]NAD, [methyl-^{3}H]thymidine, and [thymidine methyl-^{3}H]dTTP from New England Nuclear; DNA polymerase prepared from M. Lysodeikticus from Miles Laboratory; hydrocortisone sodium phosphate from Nippon Merck Banyu Pharmaceutical Co.,LTD.; testosterone propionate and progesterone from Mochida Pharmaceutical Co.,LTD.; all other reagents were from Miyata Chemicals Co.,LTD, Shimane.

Preparation of nuclei

The preparation of the nuclei was as described elsewhere (8). The final nuclear pellet prepared from chick embryo liver was suspended in medium containing 30% glycerol, 50 mM Tris-Cl$^-$ buffer, 1 mM EDTA, 0.5 mM EGTA, and 2 mM 2-mercaptoethanol(pH 8.0).

Poly(ADP-ribose) synthetase assay

The standard assay mixture of poly(ADP-ribose) synthetase activity containing 10 μM [^{3}H]NAD (0.5 μCi), 50 mM Tris-Cl$^-$ buffer (pH 8.0), 1 mM dithiothreitol, and appropriate amounts of the enzyme in a total volume of 0.2 ml was incubated at 25° for 5 min. The reaction was stopped by the addition of 2.5 ml of ice-cold 10% trichloroacetic acid. The insoluble material was collected on a glass fiber filter and washed with a total of 30 ml of 10% trichloroacetic acid. The radioactivities of the sample were determined using a Packard liquid scintillation spectrometer.

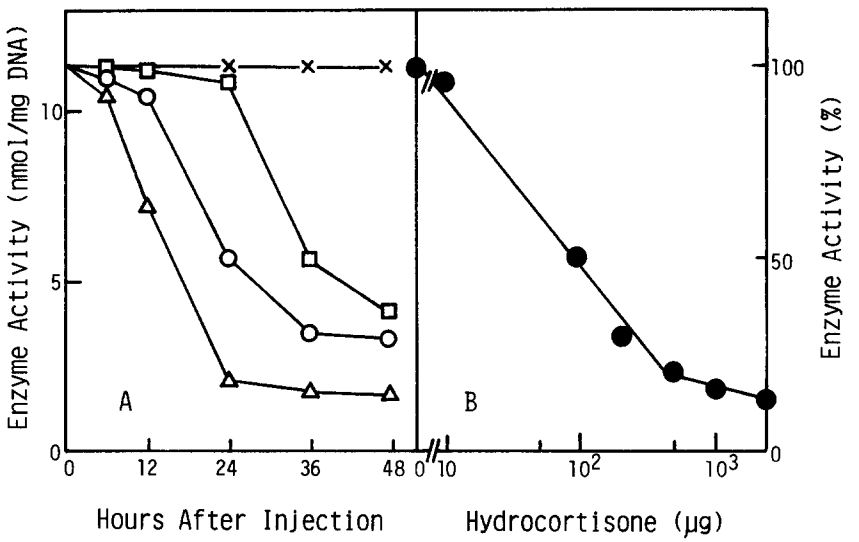

Fig. 1. A: Time course of reduction of poly(ADP-ribose) synthetase by hydrocortisone. 10 μg, 100μg and 1 mg of hydrocortisone sodium phosphate were injected into the fertilized eggs incubated for 13 - 15 days, respectively. For control, 0.2 ml of saline solution was administrated. At the time indicated, the liver nuclei were prepared and the assays were carried out as described under "Materials and Methods". The liver was removed from the 15th-day chick embryo. ×—×; control, □—□; 10 μg hydrocortisone, ○—○; 100 μg hydrocortisone, and △—△; 1 mg hydrocortisone.

B: Dose-response curve for the effect of hydrocortisone on poly(ADP-ribose) synthetase. Various amounts of hydrocortisone sodium phosphate were injected into fertilized eggs incubated for 14 days. After 24 hours, the livers were removed. Experimental conditions were as described in the legend to Fig. 1 A. The values are the mean of three experiments. Ten embryos were used for each experiment.

RESULTS AND DISCUSSION

To investigate the effect of glucocorticoid hormone on nuclear poly(ADP-ribose) synthetase, different amounts of hydrocortisone were injected into eggs at the time indicated. The results are shown in Fig. 1A. There was a pronounced diminution of poly(ADP-ribose) synthetase activity in liver nuclei from the 16 day chick-embryos treated with 100 μg of glucocorticoid for 24 hours or longer, while there was little or no effect within the first 12 hours. With 1 mg or more of glucocorticoid, a re-

duction in the enzyme activity similar to that which occurred within 12 hours of hormone treatment became more pronounced with time up to 24 hours, and the low levels were retained during further treatment. Although, little or no effect on the enzyme activity was observed with 10 μg hydrocortisone treatment for 24 hours, 50 and 65 percent reductions were detected at 36 and 48 hours after injection, respectively. Fig. 1B shows the effect of treatment for 24 hours with various amounts of hydrocortisone on poly(ADP-ribose) synthetase in nuclei. Doses of 0.01, 0.1, 0.2, 0.5, 1.0, and 2.5 mg produced 5, 50, 70, 80, 85, and 89% reduction of enzyme activities, respectively, as compared with values obtained from nuclei not given the hormone-treatment. In addition, a mixture of equal volumes of control and hormone-treated (0.2 mg for 24 hours) nuclei showed the precise poly(ADP-ribose) synthetase activity expected from the mixture (data not shown). These experiments indicate the lack of an inhibitor in the glucocorticoid-treated preparation and that the low poly(ADP-ribose) synthetase activity in the hormone-treated preparation is not due to depressed levels of an enzyme activator.

To confirm that the hormone action on the nuclear poly(ADP-ribose) synthetase was not due to the stimulation of the polymer degradation, the following experiment was carried out. [^{3}H]ADP-ribosylated nuclei were prepared from chick embryo liver treated with or without glucocorticoid and aliquots of the respective nuclei were incubated in the reaction mixture used for poly(ADP-ribose) synthetase assay, except that here NAD was omitted. As shown in Fig. 2, the enhancement of [^{3}H]poly(ADP-ribose) degradation was not apparent in the nuclei obtained from the hormone-treated chick embryo. These findings would indicate that hydrocortisone virtually reduces the poly(ADP-ribose) synthetase ac-

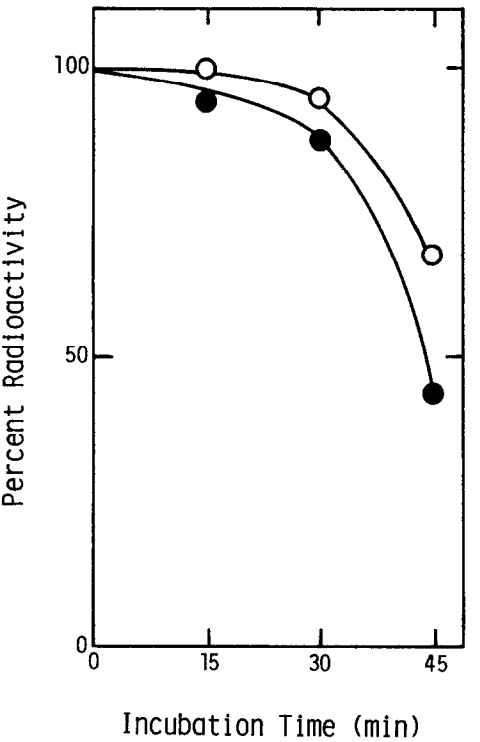

Fig. 2. Degradation of poly(ADP-ribose) in nuclei from chick embryo treated with or wothout hydrocortisone. The nuclei, used for Fig. 1 B experiment, prepared from chick embryo administered 0.2 mg of hydrocortisone or 0.2 ml saline solution, were employed for preparation of poly ADP-ribosylated nuclei. Aliquots of the respective nuclei were incubated in complete poly(ADP-ribose) synthesis system containing [^{3}H]NAD (0.2 µmole, 1.0 µCi) in a total volume of 0.4 ml. After 30 min incubation at 25°, nuclei were collected by centrifugation and resuspended in buffer containing 25 mM Tris-Cl$^-$ (pH 8.0), 10 mM $MgCl_2$, 1 mM dithiothreitol. The nuclei suspensions were incubated at 25°. Aliquots of this preparation were removed at the indicated time points and precipitated with 10% trichloroacetic acid. The radioactivities of acid-insoluble fractions were determined. The values found in the nuclei before incubation were 3237.6 cpm in control (●——●) and 968.0 cpm in hormone-treated (o——o) and set at 100%, respectively.

tivity in the chick-embryo-liver-nuclei, however, the possibility of decrease in the concentration and accessibility of the acceptors for ADP-ribosylation cannot be ruled out.

The effect of other steroids such as progesterone and testosterone was also determined. The data in Table I show that

TABLE I

RELATIVE EFFECT OF VARIOUS STEROIDS INJECTION ON POLY(ADP-RIBOSE) SYNTHETASE ACTIVITY

Steroids	Activity(%)
None	100.0
Hydrocortisone	16.8
Testosterone	103.2
Progesterone	56.6

One mg of hydrocortisone sodium phosphate, testosterone, or progesterone was injected into 14 day chick-embryos. After 24 hours, the nuclei were isolated and the enzyme activity was measured as described under "Materials and Methods". Other conditions are described in the legends to Figs. 1 and 2. The enzyme activity obtained from control nuclei was 11.44 nmoles/mg DNA. This value was set at 100%.

testosterone did not alter the nuclear poly(ADP-ribose) synthetase activity. Progesterone was about half as potent as hydrocortisone when 1 mg of the respective steroid was used.

Glucocorticoid has an effect on many biological systems involved in the synthesis of RNA and proteins. Using actinomycin D and cycloheximide we confirmed that the action of hydrocortisone was not blocked by 5 µg cycloheximide and 25 µg actinomycin D, respectively (data not shown). Thus it would appear that the RNA and protein synthesis are not involved in the generation of hormone actions.

We observed that poly(ADP-ribose) formation in the nuclei of chick embryo liver stimulated the [^{3}H]dTMP incorporation into acid-insoluble fraction and we investigated the effect of glucocorticoid on DNA synthesis and on the template activity for DNA synthesis. For this purpose, [^{3}H]thymidine was injected into fertilized eggs incubated for 15 days, and into which 0.2 mg of

TABLE II

EFFECT OF HYDROCORTISONE ON DNA

SYNTHESIS AND ITS TEMPLATE ACTIVITY

Treatment	[^{3}H] thymidine incorporation(in vivo) dpm/mg dry liver	Template activity (in vitro) dpm/mg DNA
None	117.1 ± 12.4*	3933
Hydrocortisone	49.7 ± 11.3*,**	1787

For in vivo experiment of [^{3}H]thymidine incorporation, 2 μCi [^{3}H]thymidine was injected into fertilized eggs incubated for 15 days and into which 0.2 mg hydrocortisone sodium phosphate was injected 24 hours before removal of the liver. After 6 hours-labelling, the liver was removed and homogenized with 10 vol of 5% perchloroic acid. The precipitate was obtained by clinical centrifuge, followed by two more washings under the same conditions. The precipitate was oxidized using a Packard sample oxidizer and the radioactivity was measured using a Packard liquid scintillation spectrometer. *Mean ± S.D. of three experiments. **Decreased significantly at 1% level. For determination of template activity, the DNA polymerase assay was used with liver nuclei as template, prepared from 15th-day chick embryo treated with or without 0.2 mg hydrocortisone for 24 hours. The reaction mixture contained 30 mM Tris-Cl^- buffer (pH 8.0), 10 mM $MgCl_2$, 25 mM KCl, 2 mM 2-mercaptoethanol, 1 mM EDTA, 1 mM ATP, 20 μM each of dATP, dGTP, and [^{3}H]dTTP (0.5 μCi per 4 nmoles), and 1 unit of M. Lysodeikticus DNA polymerase in a total vol of 0.2 ml. The reaction was started by addition of adequate amounts of nuclei as template and by incubation at 25°. After 15 min the reaction was stopped by the addition of 2.5 ml 10% trichloroacetic acid. The acid-insoluble materials were collected on a glass fiber filter and counted in a Packard liquid scintillation spectrometer.

hydrocortisone had been injected. As shown in Table II, DNA synthesis was clearly depressed by glucocorticoid as demonstrated in a variety of cell types(9-11). Furthermore, we observed that the nuclei isolated from glucocorticoid-treated chick embryo liver displayed a decrease in the template activity for DNA synthesis (Table II). These data further support our previous findings of participation of poly(ADP-ribose) formation on DNA synthesis in chick embryo(6,7).

It has been reported that exposure of rat thymus cells to glucocorticoids leads to a decrease in the ability of nuclei to

survive the lysis of whole cells by hypotonic shock(12). Giddings and Young found much the same result when they used P1798 mouse lymphosarcoma cells(13). As the hormone-dependent nuclear fragility results in a release of DNA from the nuclei, we are now investigating the transfer of poly(ADP-ribose)synthetase by glucocorticoid treatment, the degradation of this enzyme, and changes in the acceptor proteins for ADP-ribosylation.

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