EFFECTS OF DEXAMETHASONE ON THE LEVELS OF PLASMA CORTICOSTEROID BINDING GLOBULIN IN RATS AND MONKEYS

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SUMMARY: Consecutive administration of dexamethasone by adding it to drinking water caused decrease of plasma corticosteroid binding globulin in rats even after hypophysectomy. Other synthetic glucocorticoids also decreased corticosteroid binding globulin levels while chlormadinone-acetate had no effect. The decreasing activity of each synthetic steroid was correlated well with its anti-inflammatory action. Successive administration of cycloheximide simulated the effect of dexamethasone. Replenishment of plasma corticosteroid binding globulin occurred after the cessation of dexamethasone treatment and was prevented by cycloheximide administration. These results suggested that dexamethasone specifically reduced corticosteroid binding globulin by directly inhibiting its synthesis in rats. However dexamethasone failed to manifest its action in monkeys. A clear cut species difference was observed.

INTRODUCTION:

It is a well known fact that the binding activity of corticosteroid binding globulin (CBG) in rats is influenced by various endocrine factors. In rats CBG activity is increased after adrenalectomy (1) and decreased by administration of natural glucocorticoids (2). Gala, et al. suggested that part of the depressing effect of cortisol on CBG was related to its action on the TSH-thyroid system (3) (4). However, precise mechanisms have not been elucidated yet. We observed that consecutive administration of dexamethasone, a potent synthetic glucocorticoid which has no binding affinity for CBG (5) (6), caused a decrease of plasma CBG levels in rats, although dexamethasone failed to decrease plasma CBG levels in monkeys. The mechanisms of the CBG reducing effect of dexamethasone and species differences in susceptibility to it are discussed here.

MATERIALS AND METHODS:

Rats Male and female Wistar-Imamichi and Wistar strain rats (10 - 20 weeks of

age) were used in all the experiments. All the rats were kept in air conditioned and light controlled rooms in which the temperature was set at 22 ± 1°C, humidity at 55 - 65%, and lights on between 08:00 and 20:00. They were given rat chow (CA-1; CLEA Japan Inc.) ad lib. Hypophysectomy and adrenalectomy were performed 5 - 7 days prior to use. Thyroidectomized rats were used 19 days after the excision was performed. Adrenalectomized rats and hypophysectomized animals received 0.9% saline solution and 5% glucose solution respectively.

Monkeys Adult female Japanese monkeys (Macaca fuscata) were used. They were kept singly in cages in an air conditioned (temperature: 24 ± 2°C, humidity: 65 ± 5%), and light controlled room (lights on between 07:00 and 19:00). They were fed monkey chow (CLEA Japan Inc.) and fruit.

Reagents and Chemicals All the reagents used were of analytical grade. All the steroids were obtained commercially.

Drug administration All the steroids used (dexamethasone, betamethasone, triamcinolone, paramethasone and chlormadinone-acetate) were administered to the rats by adding them to drinking water at a concentration of 0.1 - 1.0 μg/ml. Cycloheximide was administered intraperitoneally (50 - 100 μg/100g body weight) once or twice a day. Five hundred micrograms or two thousand micrograms of dexamethasone were parenterally administered to adult female Japanese monkeys twice a day at 09:00 a.m. and 17:00 p.m. for 9 days.

Blood samples All the blood samples of rats and of monkeys were obtained at 09:30 - 10:30 and at 16:30 respectively.

Steroid assay Plasma corticosteroids were measured by competitive protein binding assay (7).

Measurement of CBG in plasma Specific binding of [^{3}H]corticosterone (40 Ci/m mole, New England Nuclear) to rat CBG or [^{3}H]cortisol (39 Ci/m mole, New England Nuclear) to monkey CBG was measured by the competitive binding technique. Plasma (0.2 - 5.0 μl) was diluted up to 500 μl with 10 mM tris-HCl buffer ($_p$H 7.5) containing 1 mM EDTA. Five hundred microliters of diluted plasma were incubated with 2 or 8 × 10^{-10}M of [^{3}H]steroid and 0 to 2 × 10^{-8}M of non-radioactive steroid at 2°C for 120 min. After the incubation was terminated, free and bound steroids were separated by gel filtration on sephadex G-50. Bound fractions were counted in a liquid scintillation counter (Aloka LSC 502). Under these experimental conditions, nonspecific binding was negligible. CBG concentrations in plasma were extrapolated by Scatchard plots (8).

Plasma protein analysis Electrophoretic analysis was performed to estimate the concentration of plasma protein fractions using cellulose acetate membrane. Total plasma protein concentration was determined by the method of Lowry et al. (9).

RESULTS AND DISCUSSION:

Consecutive administration of dexamethasone, one of the most potent synthetic glucocorticoids, by adding it to drinking water at concentrations of 0.1 μg/ml, 0.5 μg/ml and 1.0 μg/ml, caused reduction of the plasma CBG levels to 14 - 57%, 4.7 - 7.2% and 3.8 - 4.9% of pretreatment levels, respectively, within 9 days after the start of the administration (Table 1). As shown in Table 1, reduction of CBG also occurred following the consecutive administration of other synthetic halogenated glucocorticoids, i.e. triamcinolone, paramethasone, and betamethasone, while chlormadinone-acetate, a synthetic

Table 1. Effects of synthetic steroids on the CBG levels in rat plasma

Steroids administered	Pre-treatment (A) (μM)	Post-treatment (B) (μM)	B/Ax100 (%)
* Triamcinolone (1 μg/ml)	2.89	0.50	17.3
	3.23	0.93	28.8
	3.00	1.53	51.0
* Paramethasone (1 μg/ml)	3.10	0.17	5.5
	3.15	0.50	15.9
	3.08	0.34	11.0
* Betamethasone (1 μg/ml)	2.92	0.26	8.9
	3.66	0.33	9.0
	3.03	0.22	7.3
* Dexamethasone (1 μg/ml)	3.03	0.14	4.6
	3.15	0.12	3.8
	3.45	0.17	4.9
* Dexamethasone (0.5 μg/ml)	4.00	0.22	5.5
	4.50	0.21	4.7
	3.05	0.22	7.2
* Dexamethasone (0.1 μg/ml)	2.50	0.73	29.2
	4.66	0.65	13.9
	3.10	1.77	57.0
** Chlormadinone-acetate	1.18	1.19	99.2
	1.34	1.36	98.5

* Adrenalectomized female Wistar strain rats were used.

** Adrenalectomized female Wistar-Imamichi strain rats were used. Each steroid was administered to rats for 9 days as described in the text.

halogenated progesterone, showed no effect. No strain difference was observed on the effect of dexamethasone between Wistar and Wistar-Imamichi strain rats. All of these synthetic halogenated steroids have negligible binding affinity for CBG (5) (6). Gala, et al. reported that administration of natural glucocorticoids, which strongly bind to CBG, for several days also caused decreases in CBG activity in rats (2) (4). These experimental results suggested that the CBG reducing activity of each synthetic steroid was correlated well with its glucocorticoid activity, and was not related to its binding affinity for

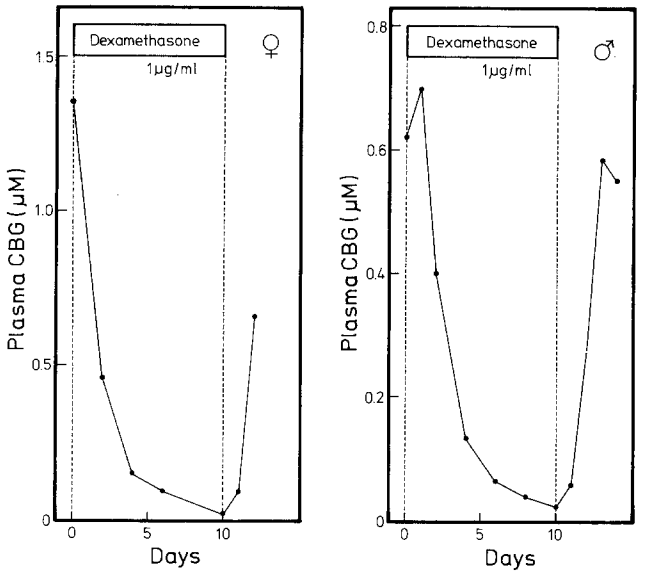

Fig. 1. Effects of dexamethasone on plasma CBG levels in rats. Each line represents the change in CBG levels in one animal. Adrenalectomized Wistar-Imamichi strain rats were used for these experiments. Dexamethasone was administered to rats as described in the text.

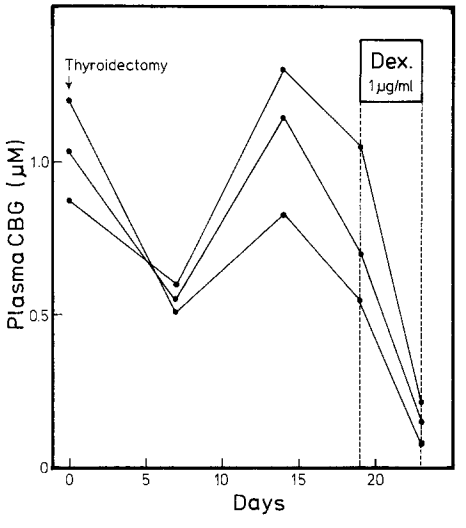

Fig. 2. Effects of dexamethasone on plasma CBG levels in thyroidectomized rats. Female Wistar-Imamichi strain rats were used for these experiments. Thyroidectomy was performed at day 0. Dexamethasone was administered to rats as described in the text.

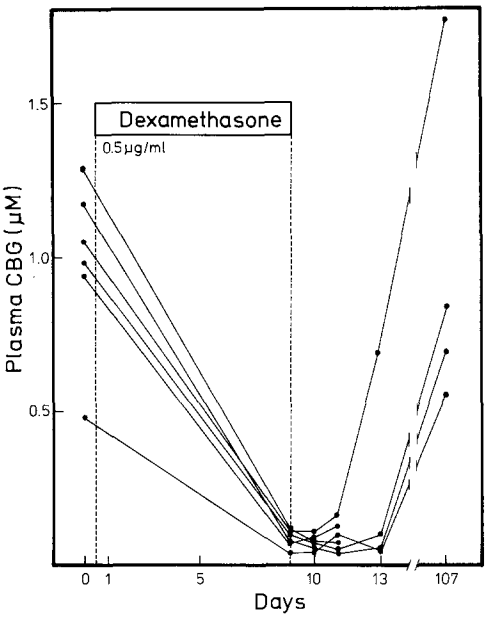

Fig. 3. Effects of dexamethasone on plasma CBG levels in hypophysectomized rats. Wistar-Imamichi strain rats were used for these experiments. Dexamethasone was administered to rats as described in the text.

CBG. The effect of dexamethasone on CBG was reversible. As shown in Fig. 1, replenishment of plasma CBG occurred after cessation of dexamethasone administration. Gala, et al. reported that hypophysectomy caused a decrease in plasma CBG activity in female rats (3), and concluded that the pituitary factor which controls plasma CBG activity in female rats is TSH and the TSH-thyroid system (4). In our experiments, about 40% depletion of plasma CBG was observed in hypophysectomized female rats, while thyroidectomy caused temporary falls in CBG levels (Fig. 2). In the case of hypophysectomized rats, it took longer to recover plasma CBG levels after the termination of dexamethasone treatment than in the case of adrenalectomized rats (Figs. 1 and 3). Our data also indicated that there is a pituitary factor or factors, possibly TSH or something else, which regulate(s) plasma CBG levels in female rats. However as shown in Figs. 2 and 3, the effect of dexamethasone was demonstrated both in thyroidectomized and hypophysectomized rats. These findings suggest that dexamethasone does not

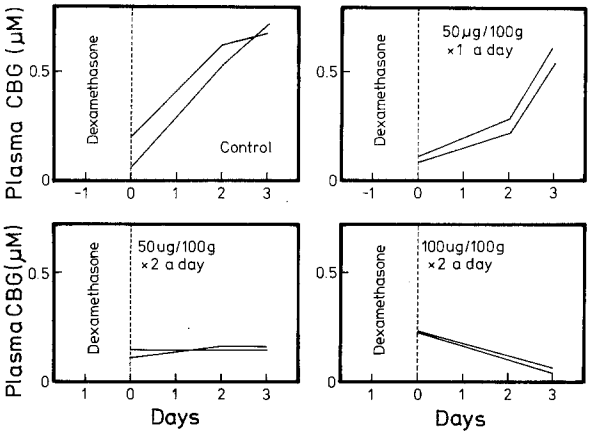

Fig. 4. Effects of cycloheximide administration on plasma CBG levels after cessation of dexamethasone treatment. Intraperitoneal injection of cycloheximide with doses illustrated above was started on the day of cessation of dexamethasone administration. All the animals used were adrenalectomized female Wistar-Imamichi strain rats.

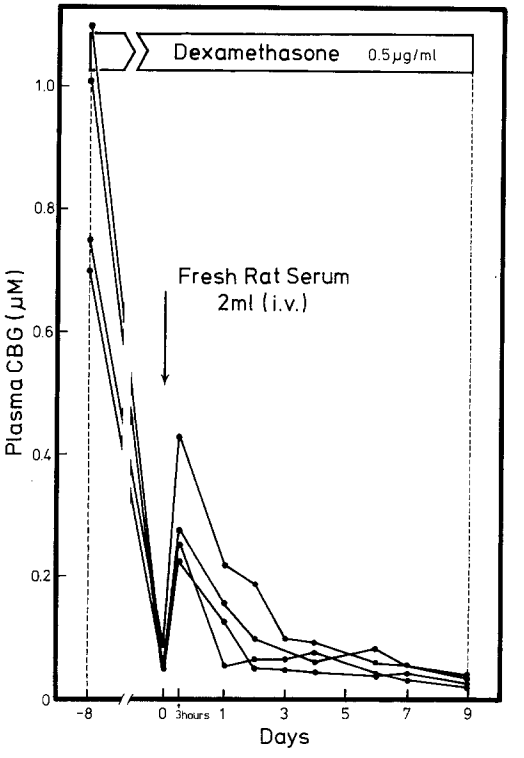

Fig. 5. Disappearance of exogeneously administered CBG from plasma of rats under dexamethasone suppression. Adrenalectomized Wistar-Imamichi strain female rats were used for these experiments. Two ml of fresh rat serum was taken from a normal rat and injected intravenously.

manifest its action through pituitary suppression, or pituitary-thyroid suppression.

In order to elucidate the mechanism of CBG depletion by glucocorticoids, the following experiments were performed. Intraperitoneal administration of cycloheximide which was started on the day of cessation of dexamethasone administration prevented the recovery of CBG in a dose dependent manner (Fig. 4). Intraperitoneal injection of 50 μg of cycloheximide twice a day diminished plasma CBG levels with almost the same decreasing rate as that with dexamethasone treatment. Intravenous injection of fresh rat serum caused an increase in plasma CBG levels in dexamethasone treated rats whose own CBG levels had already decreased to about 0.05 μM or less, and the exogeneously injected CBG disappeared from plasma at almost the same rate as that of intrinsic CBG under the consecutive administration of dexamethasone (0.5 μg/ml) (Fig. 5). These observations strongly suggest that dexamethasone diminishes plasma CBG in rats by directly or indirectly inhibiting its synthesis. According to Gala, et al. (4), CBG may be produced in the liver. It has been widely accepted that glucocorticoids increase the content of RNA and protein in the liver (10) (11), and induce specific enzymes in the liver (12) (13) (14). However Kim, et al. recently reported that pronounced inhibition in liver protein synthesis was observed in rats receiving glucocorticoid over a period of few days (15). They also suggested that glucocorticoid has no effect on the rate of protein degradation in rat liver (15). Their data (15) are based on the analysis of total liver protein synthesis, and not on the analysis of some specific protein. Electrophoretic analysis of plasma protein revealed that consecutive administration of dexamethasone for a period of 9 days resulted in the elevation of liver generated protein fractions such as albumin, α_2 and β globulins except α_1 globulin (Table 2). Chader, et al. (16) reported that the rat CBG migrates as α_1 globulin in paper-and immuno-electrophoresis. Administration of dexamethasone induced 18.8 to 43.9% reduction in α_1 globulin fraction, while CBG was decreased to less than 5% of the pretreatment level (Table 1). These facts

Table 2. Effects of dexamethasone on plasma protein fractions in rats

Rat No.		Plasma concentration (mg/ml) of					
		Alb.	α_1-G	α_2-G	α_3-G	β-G	γ-G
1	A	42.2	11.2	5.1	5.3	11.3	3.5
	B	68.8	9.8	7.8	5.4	17.8	5.2
2	A	43.7	13.2	4.5	4.0	12.1	2.3
	B	55.0	7.4	5.7	3.8	16.2	7.1
3	A	41.8	10.1	3.6	4.8	13.5	10.5
	B	63.5	8.2	7.6	3.4	15.8	3.9

A: Before the start of dexamethasone treatment.
B: After 9 days of dexamethasone treatment.

Adrenalectomized female Wistar strain rats were used.

Dexamethasone was administered to rats by adding it to drinking water at a concentration of 1 μg/ml for 9 days.

Table 3. Changes in plasma CBG levels in Japanese monkeys during dexamethasone treatment

	Plasma CBG concentration (μM)			
	day 0*	day 3*	day 7*	day 9*
Monkey (A) (2mg x 2/day)	0.265	0.237	0.242	0.255
Monkey (B) (0.5mg x 2/day)	0.347	—	—	0.375

* : The number of days after the start of administration

Dexamethasone was administered parenteraly twice a day for 9 days.

indicated that the extent of diminution of α_1 globulin fraction after dexamethasone treatment does not coincide quantitatively with that of CBG depletion. These findings suggested that dexamethasone may inhibit the synthesis of CBG

specifically. (However we do not know if there are some other liver generated specific proteins whose synthesis is also inhibited by dexamethasone administration.) All the data discussed above indicated that dexamethasone exerted its CBG reducing effect through the inhibition of the biosynthesis of CBG. It should be noted, however, that the possibility of acceleration of CBG degradation due to glucocorticoid treatment is not completely excluded yet. All the data discussed above are based on experiments using rats as experimental animals. It is important to know the effect of dexamethasone on plasma CBG occurs in species of animals other than rats, since the remarkable species differences in susceptibility of lymphoid tissue to glucocorticoid have already been reported (17) (18) (19). Such experiments suggested that there are glucocorticoid sensitive species (such as the mouse, rat and rabbit), and glucocorticoid resistant species (such as the monkey, man and guinea pig). In order to study the species difference of glucocorticoid action on plasma CBG levels, Japanese monkeys were used. Contrary to the results obtained in rats, no changes in plasma CBG levels were observed even after 9 days of treatment with an excessive amount of dexamethasone, as shown in Table 3. The above results clearly indicate that there is a distinct species difference in susceptibility to glucocorticoid effect on CBG between rats and monkeys. Whether the animal species can be divided into glucocorticoid sensitive and resistant animals based on the CBG reducing effect of dexamethasone as in the case of lymphoid depletion remains to be elucidated.

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