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# Betamethasone Receptor Levels Following Betamethasone Administration*)

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## ABSTRACT

Betamethasone (BM) receptor cncentrations in the liver cytosol from adrenalectomized rats treated with 0.2 and 2 mg BM were measured and correlated with BM concentrations in the serum and liver. The BM levels in the serum, liver cytosol and nuclei changed in a parallel fashion. One hr after BM administration, serum BM reached a peak, then decreased gradually and was undetectable at 24-48 hr. The peak levels of BM in the serum, liver cytosol and nuclei from 0.2 mg BM treated rats were 880.0±96.0 ng/ml, 32.5±8.1 ng/mg protein and 9.6±2.4 ng/mg DNA or 12.1±2.6 ng/g wet liver. Those from 2 mg BM treated rats were 1540.0±942.0 ng/ml, 47.4±38.8 ng/mg protein and 14.2±3.7 ng/mg DNA or 16.6±5.6 ng/g wet liver. In the liver cytosol, there are two types of binding sites for BM, one with high affinity (Kd=$6.0\times10^{-9}$ mol/liter) and low capacity ($6.6\times10^{-13}$ mol/mg protein or 13.0±5.2 ng/g wet liver) and one with low affinity (Kd>$10^{-7}$ mol/liter) and high capacity (>$10^{-11}$ mol/mg protein). The peak levels of BM in the liver nuclei from 0.2 and 2 mg BM treated rats were close to the binding capacity of high affinity binding site in cytosol (12.1 and 16.6 *vs.* 13.0 ng/g wet liver). [$^3$H]BM binding to the liver cytosol from both 0.2 and 2 mg BM treated rats was lost completely at 1 to 6 hr, and recovered at 24 hr in the 0.2 mg BM treated rats and at 48 hr in the 2 mg BM treated rats. Thus, the cytosol and nuclear levels of BM are reciprocally related to [$^3$H]BM binding capacity in the liver cytosol, and the fall of [$^3$H]BM binding to the cytosol is accompanied by the appearence of BM in the nuclei. Therefore, our *in vivo* study suggested that almost all of high affinity receptor bound BM is transferred rapidly to nuclei and remains there in the presence of a sufficient amount of BM in cytoplasm, and thereafter the receptor is released from nuclei to cytoplasm.

## INTRODUCTION

The mechanism of action of glucocorticoid hormones has been studied extensively at the molecular level[1,2,8]. However, the bioavailability of administered synthetic glucocorticoid and its relationship to their own receptors still remain unclear. The purpose of this study was to correlate liver cytosol and nuclear levels of betamethasone (BM) at various time intervals after administration of two doses of BM to rats, with cytosol BM receptor in the liver.

## MATERIALS AND METHOD

*Isotopes and chemicals*

1, 2, 4(n)-[$^3$H]BM (32 Ci/mmol) was obtained from the Radiochemical Centre Amersham (Buckinghamshire, U. K.). Prednisolone and BM were gifts of Shionogi Co. (Osaka, Japan). DNA, cytochrome C, hen egg albumin and

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aldolase were obtained from Baehringer Mannheim GmnH (Mannheim, West Germany). Corticosterone, bovine serum albumin, bovine serum gammaglobulin and trizma base were purchased from Sigma Chemical Co. (St. Louis, U. S. A.). Other chemicals were purchased from Katayama Chemical Co. (Osaka, Japan).

*Animals and preparation of cytosol and nucleus*

Male Wistar rats weighing 200–240 g were used throughout these experiments 3–4 days after adrenalectomy. They were maintained on Oriental brand foods and drinking water supplemented 0.4% saline *ad lib.* A water suspension of 0.2 or 2 mg of BM was administered to these rats through a gastric tube. Before and 1, 3, 6, 24, 48 hr after BM administration, rats were sacrificed, blood was obtained from abdominal aorta, and the liver was removed after perfusion with 50 ml of cold 0.9% saline via the portal vein. The liver was minced with scissors, homogenized in an equal volume of 0.25 M sucrose, 3 mM $MgCl_2$, 50 mM Tris-HCl buffer, pH 7.4, and centrifuged at 105,000 G for 60 min at 4°C. The supernatant was termed cytosol and its protein concentration was determined by the method of Lowry using bovine serum albumin as a standard[15].

To prepare the nuclear fraction, the liver was homogenized in four volumes of the same buffer and centrifuged at 700 G for 20 min. The pellet was suspended in 2.5 M sucrose, 3 mM $MgCl_2$, 50 mM Tris-HCl buffer, pH 7.4 and recentrifuged at 40,000 G for 20 min. The Pellet was resuspended in 0.25 M sucrose, 50 mM Tris-HCl buffer, filtered through several layers of gauze and centrifuged at 700 G for 5 min. The final nuclear fraction was washed three times. The DNA concentration in the nuclear fraction was determined according to the Burton's method[7].

*BM radioimmunoassay*

An appropriate volume of the serum and liver cytosol, or the liver nucleus was extracted with 4 ml of dichloromethane by shaking for one min. These extracts or standards containing 10 pg–10 ng of BM dissolved in ethanol were pipetted into assay tubes. The tubes were then dried under an air stream at 45°C. Antiserum (final dilution 1 : 5,000) and [$^3$H]BM (10,000 cpm) dissolved in 0.1% gammaglobulin in saline were added to each tube in a total volume of 1 ml. After incubation at 4°C for 16 hr, 0.2 ml of the 0.5% dextran coated charcoal (DCC) was added to each tube and the tubes were centrifuged at 3,000 rpm for 15 min. The supernatants were decanted into counting vials which contained 10 ml of Bray's solution and the radioactivity was measured with a liquid schintillation spectrometer. The radioimmunoassay dose-response curves and the sample values were analyzed by the logit-log method presented by Rodbard, Bridson & Rayfort[19]. The validity of this radioimmunoassay method has been described elsewhere[17,18].

*Gel chromatography*

Aliquots of liver cytosol were incubated with [$^3$H]BM in the absence or presence of a 100 fold excess of non radioactive BM at 4°C for 3 hr, and then applied to a column of Sephacryl S-300 (2.5×80 cm) equilibrated with 50 mM Tris-HCl buffer, pH 7.4. The column was eluted with the same buffer and 8 ml aliquot of the eluate was collected, 1 ml of which was then pipetted into the counting vials which contained 10 ml of Bray's solution. The radioactivity was measured by a liquid schintillation spectrometer.

For the evaluation of BM molecules in the cytosol, the liver cytosol from rats 1 hr after 2 mg BM administration was applied to the same column and eluted with 50 mM Tris-HCl buffer, pH 7.4. One ml of 8 ml each fraction was mixed with 4 ml of dichloromethane, and BM or BM like immunoactivity both in aqeous and dichloromethane phase was measured by radioimmunoassay.

*Analysis of BM receptor*

Standards containing 5–500 nM of BM or other glucocorticoids in ethanol were pipetted into the assay tubes. After evaporation under an air stream at 45°C, 3.5 nM of [$^3$H]BM, liver cytosol and 50 mM Tris-HCl buffer, pH 7.4 were added to the tubes in a total volume of 0.5 ml. Following the incubation at 4°C for 3 hr, 0.5 ml of 1% DCC was placed in each tube. The tubes were centrifuged at 3,000 rpm for 15 min and the radioactivity in the supernatant was determined. The inhibition curve was obtained by plotting the percent binding against the logarithm of the dose. The binding capacity and dissociation constant (Kd) of BM receptor were determined from Scatchard plot[22]. The receptor content of the liver cytosol after

BM administration was determined in DCC treated cytosol according to the method mentioned above.

## RESULTS

*Analysis of BM binding in the rat liver cytosol*

The elution pattern from Sephacryl S-300 chromatography of the liver cytosol incubated *in vitro* with [$^3$H]BM contained four radioactive peaks (I, II, III, IV) as shown in Fig. 1. The second peak (II) with the highest binding to [$^3$H]BM completely disappeared in the presence of an excess of unlabelled BM. The molecular weight in this peak was calculated approximately 80,000 daltons from plotting the Kav vs log molecular weight of standard proteins. The first peak (I) situated in the fraction corresponding to void volume was also inhibited by unlabelled BM. Both the third (III) and fourth (IV) peaks were not inhibited by unlabelled BM, which suggests that these fractions consist mainly of non specific binding of [$^3$H]BM to macromolecules and/or of BM metabolites.

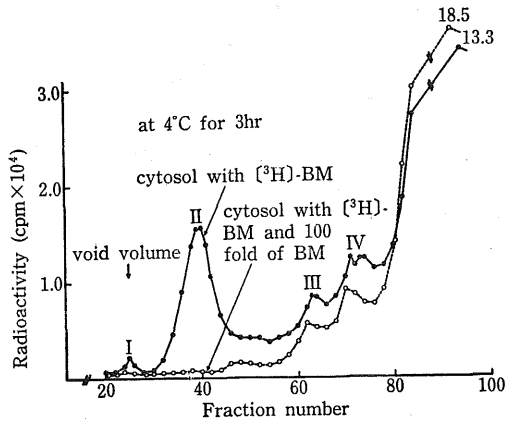


**Fig. 1.** Chromatography of the rat liver cytosol incubated with [$^3$H]betamethasone (BM) on Sephacryl S-300 in the absence (●—●), or presence of a 100 fold excess of BM (○···○). This column was calibrated with standard proteins; cytochrome C (MW: 12,500), hen egg albumin (45,000) and aldolase (158,000).

The competitive inhibition study showed that the liver cytosol had a higher affinity for BM than for prednisolone or corticosterone (Fig. 2) Scatchard plot, calculated from the blnding

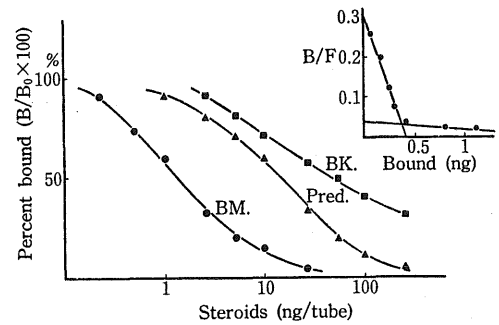


**Fig. 2.** Binding inhibition curve of the rat liver cytosol with [$^3$H]betamethasone in the presence of 0.1–100 ng of betamethasone (BM), prednisolone (Pred) or corticosterone (BK), and Scatchard plot of the rat liver cytosol binding to BM.

inhibition data, demonstrated the presence of two BM binding components with a high and low affinity ($Kd = 6.0 \pm 0.07 \times 10^{-9}$ and more than $10^{-7}$ mol/liter: mean ± S.D.) and with a low and high binding capacity ($6.6 \pm 2.6 \times 10^{-13}$ and more than $10^{-11}$ mol/mg protein).

Double reciprocal plot analysis was performed in order to investigate the degree of competition of other glucocorticoids for the BM high affinity binding site in cytosol as shown in Fig. 3. Both corticosterone and prednisolone markedly increased the slope with essentially no change in the vertical intercept value. These findings are characteristic of competitive inhibition and provide strong evidence that the BM binding site in the rat liver cytosol is common to the binding components of corticosterone and prednisolone.

*BM administration*

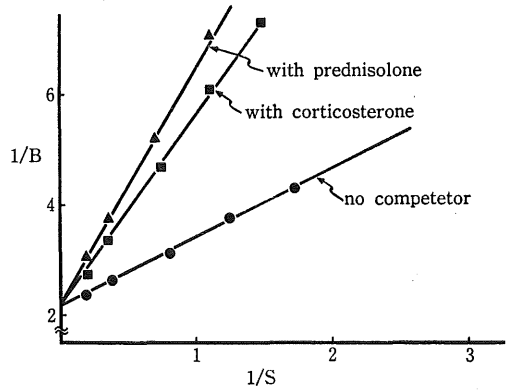


**Fig. 3.** Double reciprocal plot of the liver cytosol binding to betamethasone in the presence of prednisolone or corticosterone.

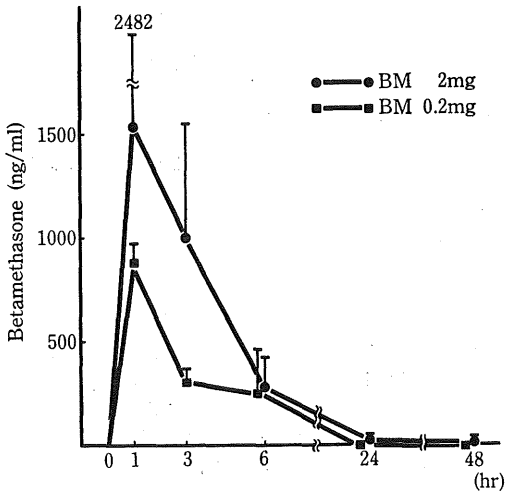


**Fig. 4.** Betamethasone (BM) concentration in the rat serum after administration of BM through a gastric tube. Results are given as the mean± S. D. (n=4).

The peak levels of BM were observed 1 hr after 0.2 or 2 mg BM administration (880.0± 96.0 or 1540±942 ng/ml) as shown in Fig. 4. The serum BM level in the rats treated with 0.2 mg BM became undetectable after 24 hr, while a small amount of BM still remained at 48 hr in the rats treated with 2 mg BM. The concentration of BM in the liver cytosol reached the peak level of 32.5±8.1 or 47.4±38.8 ng/mg protein 1 hr after 0.2 or 2 mg BM administration (Fig. 5). It declined to undetectable at 24 hr in the rats treated with 0.2 mg BM, but in the rats treated with 2 mg BM, it was 0.49± 0.41 or 0.20±0.25 ng/mg protein at 24 or 48 hr. The peak levels of BM in the liver nuclei were 9.6±2.4 ng/mg DNA or 12.1±2.6 ng/g wet liver 1 hr after 0.2 mg BM treatment and 14.2±3.7 ng/mg DNA or 16.6±5.6 ng/g wet liver 3 hr after 2 mg BM administration (Fig. 6). These BM concentrations in nuclei corresponded approximately to the binding capacity of the high affinity BM binding component in the liver cytosol (13.0±5.2 ng/g wet liver).

The [$^3$H]BM binding to the liver cytosol fell

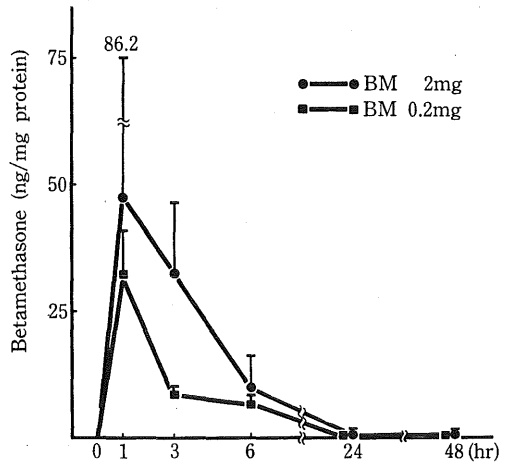


**Fig. 5.** Betamethasone (BM) concentration in the rat liver cytosol after administration of BM through a gastric tube. Results are given as the mean±S. D. (n=4).

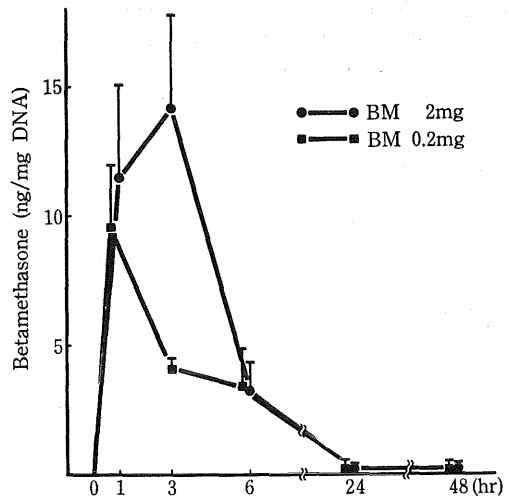


**Fig. 6.** Betamethasone (BM) concentration in the rat liver nuclei after administration of BM through a gastric tube. Results are given as the mean ±S. D. (n=4).

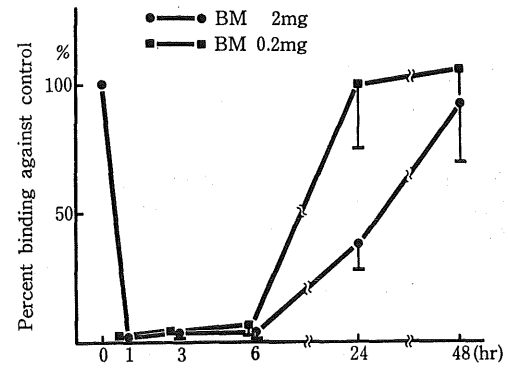


**Fig. 7.** Percent binding of the liver cytosol to [$^3$H]betamethasone (BM) from rats administered with BM against the mean binding of the cytosol from control rats. Results are given as the mean±S. D. (n=4).

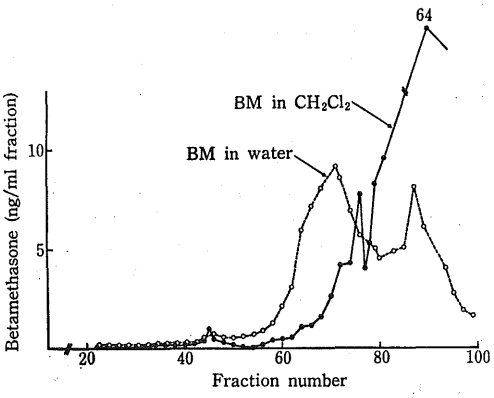


**Fig. 8.** Elution pattern of betamethasone (BM) in the liver cytosol from rats 1hour after 2 mg BM administration on Sephacryl S-300 gel chromatography. The closed (●—●) and open circle (○···○) indicate BM value in dichloromethane and water soluble phase, respectively.

rapidly after 0.2 and 2 mg BM administration, and became undetectable at 1 and 6 hr. The [$^3$H]BM binding recovered towards the control level at 24 hr in the 0.2 mg BM treated rats, while it did not recover until 48 hr in the rats treated with 2 mg BM (Fig. 7).

The elution pattern on Sephacryl S-300 column chromatography in Fig. 8 showed that almost all of BM or BM like immunoactivity was recovered in the fractions corresponding to free [$^3$H]BM peak in Fig. 1 with essentially no immunoactivity in the fractions identical with peak II (BM-receptor complex). The eluate from fractions corresponding to peak IV in Fig. 1 was extractable by dichloromethane but not adsorbed by DCC, suggesting that this eluate probably contained BM or BM metabolites nonspecifically bound to the macromolecules different from BM receptor. Water soluble form of BM like immunoactivity was eluted at fractions 60 to 70 (peak III and IV in Fig. 1), which were adsorbed by DCC, and seemed to be BM metabolites with immunologically similar stractures to BM.

## DISCUSSION

In this study, we clarified the presence of two types of binidng components to BM in rat liver cytosol with Kd of $6.0\times10^{-9}$ and more than $10^{-7}$ mol/liter and with binding capacity of $6.0\times10^{-13}$ and more than $10^{-11}$ mol/mg protein, respectively. The high affinity binding component was shown to have a molecular weight of about 80,000 daltons by Sephacryl S-300 gel chromatography and to possess a high affinity also to corticosterone and prednisolone by the double reciprocal plot studies. These finding indicate the high affinity component may be identical to G protein of Beato et al. which characterizes a specific glucocorticoid receptor[3,4,6].

We studied the tissue BM levels after *in vivo* BM administration to rats in correlation with the quantitative changes of BM receptors in the liver cytosol. When adrenalectomized rats were exposed to 0.2 and 2 mg BM, the serum and liver cytosol levels of BM increased to the peak at 1 hr. The elution profile on Sephacryl S-300 of cytosol BM at 1 hr revealed that almost all of BM existed as free, not as receptor bound form, suggesting that receptor bound BM moved from cytoplasm to nuclei when a high concentration of BM was present in cytoplasm. The elution profile also showed that portion of BM was already transformed to water soluble forms. Beato et al.[3,4] indicated there were many glucocorticoid metabolites in the rat liver cytosol 20 min after [$^3$H]corticosterone injestion.

The [$^3$H]BM binding study to DCC treated cytosol from BM administered rats was carried out at 4°C for 3 hr by which maximal binding might be achieved and the rate of dissociation of this steroid complex slowed[12], suggesting that the [$^3$H]BM binding to cytosol in this study reflects the unoccupied reseptor volume. The specific binding of [$^3$H]BM to cytosol was lost completely within 1 hr after the administration of 0.2 and 2 mg BM to adrenalectomized rats. The recovery of [$^3$H]BM binding was not completed until 48 hr in the 2 mg BM treated rats, but was completed within 24 hr in the 0.2 mg BM treated rats. Thus, the administration of large amounts of BM produces a complete and prolonged BM receptor loss in the liver cytosol. Beato et al.[5] published a similar report of the glucocorticoid receptor regulation by corisol. Fifty percent or all of [$^3$H]dexamethasone binding capacity of the rat liver cytosol was saturated thirty minutes after the injection of 0.2 mg (physiological dose) or 5 mg (pharmacological dose) of cortisol acetate to adrenalectomized rats. Four hr later, the binding capacity was almost recovered in the 0.2 mg treated rats, while in the 5 mg treated

rats, about half of the capacity was not yet recovered. The degree of receptor loss and the receptor recovery was similar to that of our findings. Beato et al.[5] however did not measure the cytosol glucocorticoid levels and could not show the correlation between the glucocorticoid concentrations and the receptor levels in cytosol. This glucocorticoid receptor regulation by glucocorticoid in the liver agrees with that of uterus estrogen receptor by estrogen[16, 21].

It is of interest that peak concentrations of nuclear BM (12.1±2.6 and 16.6±5.6 ng/g wet liver) both in the 0.2 and 2 mg BM treated rats were approximately equal to the binding capacity of the cytosol receptor (13.0±5.2 ng/g wet liver). Several lines of *in vitro* evidences[10, 20, 23, 24] indicated that the cytoplasmic receptor is required for nuclear binding of glucocorticoids, of which process is due to reversible association of the hormone receptor complex with nuclei. Still more, it is observed that the time course of nuclear binding and dissociation occurs simultaneously with those of cytosol receptor depletion and repletion[9, 11, 13, 14], and that receptors do reappear in the cytoplasm when steroid is removed from the incubation medium[20]. These findings could explain our *in vivo* data more rationally. In our data, there is an inverse relationship between the nuclear BM level and the unoccupied receptor volume in the rat liver cytosol after the administration of BM. When the highest value of BM, which was almost equal to the binding capacity of cytosol receptor for BM, was observed in the nuclei, almost all of the receptor was lost from the cytosol. Afterthen, the rapid restoration of glucocorticoid receptor had occurred concomittently with the disappearence of BM from cytosol and nuclei.

In conclusion, our *in vivo* study indicated that almost all of BM receptor is transferred rapidly to nuclei and remains there in the presence of a sufficient amount of BM in cytosol, and the receptor reappears in cytosol when BM disappears from cytosol and nuclei.

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