Studies on the Role of the Pituitary Gland in Biosynthesis of Corticosteroid-Binding Globulin in the Male Rat*

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Synopsis

The role of the pituitary gland on the biosynthesis of corticosteroid-binding globulin (CBG) was investigated in male albino rats. It took 48 hr after adrenalectomy to record a significant increase in corticosteroid-binding capacity (CBC). Likewise, a significant depression of CBC was observed only beyond 48 hr after the start of 40 μg/day of dexamethasone injection. From these findings it may be interpreted that glucocorticoids exert an inhibitory effect physiologically upon CBG synthesis and that adrenalectomy would abolish such an depressive action by the elimination of endogenous corticosteroids. Daily administration of 20 μg of estradiol resulted in a significant increase in CBC in 5 days. Furthermore, dexamethasone showed inhibiting completely such a stimulatory effect of estradiol on CBC, which was, in contrast, augmented by adrenalectomy. These results suggested that there might exist a competition for the biosynthesis of CBG between the inhibitory action of glucocorticoid and the stimulatory effect of estradiol.

Hypophysectomy did not interfere with the supressive effect of dexamethasone on CBC but abolished the CBC response to adrenalectomy or estradiol treatment, which could not be restored by replenishment with various pituitary hormones, such as ACTH, RGH, RFSH, BTSH or ovine prolactin as well as pituitary autoimplantation. The present results indicate that either unknown pituitary factor(s) or synergic action of multiple pituitary hormones may be needed only when the biosynthesis of CBG is accelerated and elaborated with a mechanism for which the neural and/or vascular connection between the gland and the hypothalamus is indispensable.

Although corticosteroid-binding globulin (CBG) plays an important role in the adrenal cortical function, little information has yet been available as to the mechanism with which the biosynthesis of the CBG is physiologically controlled. In humans, CBG is known to increase during pregnancy (Slaunwhite and Sandberg, 1959) and after estrogen administration (Mills and Bartter, 1959). In rats, a substantial increase was observed in both sexes after bilateral adrenalectomy (Westphal et al., 1962) and in the male animal following estradiol injection (Gala and Westphal, 1965), whereas administration of corticosterone or cortisol decreased CBG activity in adrenalectomized rats (Gala and Westphal, 1966). Furthermore, they revealed that either adrenalectomy or estrogen administration did not induce the increment of CBG activity in hypophysectomized rats, suggesting an involvement of the pituitary gland in such changes in CBG.

The present study was undertaken to attempt to elucidate the mechanism with which biosynthesis of the CBG is regulated by such steroid hormones and also to pursue the unknown pituitary factor.
Materials and Methods

Male Wistar rats weighing from 180 to 220 g were used in all experiments. All animals were kept under conditions of controlled lighting (light 7:30 AM-7:30 PM) and uniform temperature (22 ± 1°C). Hypophysectomy was carried out through outer auricular approach under ether anesthesia and completeness of the operation was assured by macroscopic inspection of the sella turcica at autopsy. Adrenalectomized animals were given 1% saline ad lib, while the hypophysectomized rats were given a 5% glucose solution; rats both adrenalectomized and hypophysectomized were given a solution of 5% glucose in 1% saline. The following preparations for injection were employed. Estradiol: Ovahormone benzonate (Teikokuzohki), Dexamethasone: Dexamethasone phosphate (Banyu), ACTH: β-24 ACTH (Daiichi), RGH (NIH)*, RFSH (NIH)*, BTSH (NIH)** and ovine prolactin (NIH)***. Each preparation was subcutaneously injected once daily with 0.1 ml of vehicle.

The pituitary glands were obtained from donor rats immediately after decapitation. In case of autoimplantation, the glands were removed by suction via the left external auricular canal. They were swiftly embedded in the perirenal adipose tissue in each recipient rat. At autopsy the implanted gland was macroscopically surrounded by blood vessel-rich adipose tissue and maintained its size and pinkish color.

In preparation for blood collection, animals were injected intraperitoneally with pentobarbital (7 mg/100 g BW). Blood was withdrawn from the abdominal aorta within 60 sec. after the start of the laparotomy. Except for time-course study, the sampling was carried out between 9 and 10 a.m.. After immediate separation by centrifugation, the plasma sample from each animal was stored at -20°C.

Plasma corticosterone levels were measured by the fluorometric method of Guilleman et al. (1959). In the measurement of corticosteroid binding capacity (CBC), one ml of the plasma was mixed with 0.2 ml of 4-14C corticosterone solution containing 8 mCi of radioactivity and 2.05 µg of corticosterone. Following the equilibration at 45°C for 5 min, the mixture was abruptly cooled and subsequently incubated overnight at 4°C. One ml of the sample was then subjected to gel filtration at 4°C using Sephadex G-50 column described by Doe et al. (1964). The CBC expresses the amount of protein-bound corticosterone per 100 ml of plasma. The C. V. was calculated as 4.1%.

Results

1. Changes of plasma corticosterone and CBC after bilateral adrenalectomy

Rats were sacrificed 2, 4, 6, 24, 48, 60, 84 hr after adrenalectomy and plasma corticosterone and CBC were determined, comparing with those of untreated control rats. As shown in Figure 1, plasma corticosterone was abruptly decreased to the lowest level just 2 hours after adrenalectomy, whereas CBC began to increase 24 hours after adrenalectomy and a significant rise (P < 0.01) was demonstrated 48 after the operation.

2. Effect of hypophysectomy on CBC response to adrenalectomy

Male rats were divided into four groups; untreated, adrenalectomized, hypophysectomized and both hypophysectomized and adrenalectomized. In the fourth group hypophysectomy was performed immediately before the adrenalectomy. They were sacrificed 3 full days after the operations. CBC of adrenalectomized animals was 56% higher than that of the untreated rats. Hypophysectomy prior to adrenalectomy cancelled the increase in CBC observed in adrenalectomized

Table 1. Response of CBC to adrenalectomy and hypophysectomy

<table>
<thead>
<tr>
<th>Events</th>
<th>CBC (µg/100 ml)</th>
<th>P</th>
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<tbody>
<tr>
<td>Control</td>
<td>59.64 ± 1.19*</td>
<td>#</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>89.05 ± 7.56</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>63.06 ± 5.52</td>
<td>N. S.</td>
</tr>
<tr>
<td>Hypophysectomy + Adrenalectomy</td>
<td>60.48 ± 4.08</td>
<td>N. S.</td>
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*Mean ± S. D.  
#Number of animals
3. Influence of various pituitary hormones on CBC in hypophysectomized-adrenalectomized rats

Started immediately after hypophysectomy plus adrenalectomy, daily injection of one of five pituitary hormones (0.1 mg of ACTH, 10 μg of RGH, RFSH, BTSH and ovine prolactin) was continued for four days. Animals were sacrificed 24 hr after the last injection. There was no significant change in CBC of the hypophysectomized-adrenalectomized rats treated with each pituitary hormone when compared with the control rats. (Fig. 2) Thus, replenishment of these hormones to hypophysectomized rats did not restore the CBC response to adrenalectomy.
4. Changes of plasma corticosterone and CBC following estradiol administration

Male rats were treated with 20 µg/day of estradiol benzonate and sacrificed to determine plasma corticosterone and CBC before and 3, 5, 7, 9, 11 days after the first injection. It was observed that plasma corticosterone did not alter significantly throughout the experimental period. Plasma CBC did not change significantly until 5 days after the initiation of the treatment, when it attained a plateau approximately 39% higher than the control value. (Fig. 3)

5. Influence of hypophysectomy on CBC response to estradiol

Daily injection of 20 µg of estradiol into male intact rats for 10 days resulted in a significant increase in CBC. Hypophysectomy prevented completely such effect of estradiol. Replenishment with ACTH, GH or prolactin did not reproduce the enhancement of CBC following the estradiol administration which was observed in intact animals. Furthermore, there was no change in CBC after estradiol treatment in hypophysectomized rats implanted with their own pituitary gland in the perirenal adipose tissue. (Fig. 4)

6. Effect of pituitary implantation on CBC

Donor rats were divided into two groups; adrenalectomized and estradiol (group). The former was subjected to bilateral adrenalectomy 7 days before the pituitary implantation, the latter was treated with daily 20 µg of estradiol for 10 days. The pituitary glands obtained from two donor rats of each group were instantly implanted into the perirenal adipose tissue of each recipient rat, which was sacrificed 14 days after the implantation. There was no significant difference between CBC of each recipient rat and that of untreated rats. (Fig. 5)

7. Changes of plasma corticosterone and CBC after dexamethasone treatment

Rats were treated with 40 µg/day of dex-
Fig. 4 Influence of various pituitary hormones and pituitary autoimplantation upon CBC response to estradiol in hypophysectomized rats.

Fig. 5 Effect of pituitary implantation from rats treated with estradiol or subjected to adrenalectomy on CBC in recipient rats.

Fig. 6 Responses of the plasma corticosterone and CBC to daily administration of dexamethasone. Each datum represents the mean ± standard deviation of responses for groups of 4 rats.

8. Influence of hypophysectomy on CBC response to dexamethasone

Dexamethasone was injected into intact male rats at a level of 40 μg/day for a period of five days. On the day following the last injection, blood was collected from anesthetized rats. The administration of dexamethasone lowered the CBC by 31% in comparison with the control rats. When the same treatment was applied to hypophysectomized rats, the suppressive effect of dexamethasone...
Table 2. Effect of hypophysectomy on CBC response to dexamethasone

<table>
<thead>
<tr>
<th>Events</th>
<th>CBC (μg/100 ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>49.46 ± 6.38*</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>34.65 ± 7.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>24.47 ± 4.67</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Dexamethasone</td>
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*Mean ± S. D.
#Number of animals

on CBC was not eliminated but the CBC was lowered more markedly. (Table 2)

9. Effect of estradiol upon CBC response to dexamethasone or adrenalectomy

In order to examine the interaction between estradiol and dexamethasone either or both of them were administered into intact male rats at the same level of 40 μg/day for four days. Simultaneous injection of both the steroids lowered CBC by 44% in comparison with the rats treated with estradiol alone. There was no significant difference in CBC between rats treated with both the steroids and those treated with dexamethasone alone. On the contrary, estradiol injection into adrenalectomized rats at a level of 40 μg/day for four days resulted in a significant increase (p < 0.05) in CBC in comparison with the control adrenalectomized rats. (Fig. 7)

Discussion

The present observations confirmed previous reports from other laboratories that either bilateral adrenalectomy or estradiol administration induced an increase in CBC of the male rats (Westphal et al., 1962; Gala and Westphal, 1965; Keller et al., 1966) and that the pituitary gland is necessary for the enhancement of CBC induced by either adrenalectomy or estradiol administration (Gala and Westphal, 1966). Our new finding is that none of the known pituitary hormones (ACTH, GH, FSH, TSH and Prolactin) appear to be responsible for the increase in CBC in male rats, suggesting either an involvement of unknown pituitary factor(s) or necessity of a synergic action of multiple pituitary hormones to raise the plasma CBC level. In this
connection, Westphal (1971) recently suggested that hypophysectomy might remove simultaneously the stimulatory effect of thyroid hormone and the inhibitory action of androgen on CBC in male rats. In addition, the present result showing ineffectiveness of autoimplantation of the pituitary gland upon restoring CBC response to those stimuli would indicate that the neural and/or vascular connection between the pituitary gland and the hypothalamus is indispensable for such response.

As for the influence of glucocorticoid administration upon plasma CBC, the suppressive effect of corticosterone or cortisol on CBG activity was demonstrated in intact rats (Westphal et al., 1963; Keller et al., 1966) as well as in adrenalectomized rats (Gala and Westphal, 1966). Keller et al. (1966) reported that no effect on CBG activity was observed after the treatment of male animals with 25 μg/100 g BW of dexamethasone 24 hours prior to collection of plasma sample. We found that the treatment with similar doses of dexamethasone led to a significant decrease in CBC in male rats as early as 48 hr after the start of the injection. Moreover, a similar depression of CBG was seen as well in hypophysectomized rats following the same treatment with dexamethasone, suggesting that the pituitary gland is not necessary for depressing the effect of corticosteroids on CBC. The foregoing results may be summarized as follows: the presence of the pituitary gland is needed when the CBG activity is enhanced either by estradiol administration or adrenalectomy, but not when CBC is depressed by corticosteroid injection. Then, how does the pituitary gland play a role in elevating CBG activity following those treatments? There are two possibilities to explain the mechanism with which the pituitary gland might be involved in the genesis of CBG. First of all, either estradiol or adrenalectomy may have a direct action on the pituitary gland to produce a "CBG stimulating factor" in the gland, which is subsequently elaborated to prompt the synthesis of CBG in the liver (Guidollet and Louisot, 1969). Such possibility turned out to be unlikely, however, since the implantation of the pituitary glands from rats either treated with estradiol or subjected to adrenalectomy did not result in any increment of CBC in the recipient rats. Alternatively, it might be considered that in response to such stimulus as estradiol or adrenalectomy the pituitary gland is urged to release some pituitary factors, which exert a collaborative action with those stimuli to activate generating CBG in the liver. The fact that the plasma CBC in hypophysectomized rats did not differ from that of intact animals gave evidence of the conception that the pituitary factor is needed and elaborated only when the biosynthesis of CBG is accelerated. Also, this might be the reason why corticosteroid can exert depressive action on CBG without the pituitary gland. The increase in CBC following adrenalectomy and the decrease observed after glucocorticoid administration might interpret that glucocorticoids exert an inhibitory effect physiologically upon CBG and that elimination of endogenous corticosteroids by adrenalectomy would abolish such an inhibitory effect.

There are two alternative mechanisms to be cited with which corticosteroids have a depressive influence upon CBC. One may elucidate the decrease in CBC after corticosteroid administration by occupying the binding sites for corticosteroid with the exogenous steroid. Alternatively, glucocorticoids may have a direct inhibitory action on the biosynthesis of CBG in the liver. The following findings gave us a move possible clue to it. First of all, our data revealed that it took at least 48 hr after the initiation of dexamethasone treatment to exhibit a significant decrease in the plasma CBC. Likewise, it took 48 hr after adrenalectomy to record a significant increase in CBC, although the plasma corticosterone decreased to zero 2 hr after the operation. Finally, according to Gala and Westphal (1966), cortisol was 5 times more potent than corticosterone in decreasing CBG activity in
adrenalectomized rats, in spite of the fact that rat plasma CBG was more extensively bound to corticosterone than to cortisol (Keller et al., 1966). These results are not in agreement with the first hypothesis but in favor of the second conception.

The etiology of the enhancement of CBG following the estradiol administration was still obscure. Westphal and De Venuto reported that estrogen treatment in male rats resulted in changes in corticosteroid-binding globulin concentration with no apparent qualitative alterations (Westphal and De 1969). More recently, Guidollet and Louisol (Guidollet and Louisot, 1969) described their conception that estrogen might play a role in activation of "normally masked molecular sites" for corticosteroids rather than an induction of transcortin system, on the basis of their data that following the injection of 14C-glucosamine the radioactivity of the glucidic fragments of glycoprotein remained constant in the subcellular sites of the rat liver cell. However, it seems rather dangerous to deduce such a conclusion until the incorporation of the fragment is determined specifically in the fraction to which corticosterone binds exclusively.

The present findings that CBC response to estradiol was abolished by cexamethasone and augmented by adrenalectomy would suggest that there might exist a competition between glucocorticoids and estradiol for a common site. In order to clarify the reciprocal actions of such hormones, determination of biosynthetic rate of the plasma CBG in rats has been currently undertaken in this laboratory.

Acknowledgment

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References

Westphal, U., W. C. Williams, Jr., B. D. Ashley, and F. De Venuto (1963.) Hoppe Seyler Z Physiol. Chem. 332, 54.