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Responses of Serum Corticosterone and Corticosteroid-Binding Globulin to Acute and Prolonged Stress in the Rat

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Responses of serum corticosterone (B) and corticosteroid-binding globulin (CBG) to ether anesthesia (a "classic" acute stress) and to a number of stressors influencing metabolic homeostasis — fasting, physical exercise, cold exposure, and water deprivation — were studied in male and female rats. Metabolic stressors included placing in an ice bath, physical exercise (swimming), fasting for 2 d, swimming after fasting for 2 d, cold-room (4°C) exposure for 2 d, fasting in combination with cold-room exposure for 1 d, and water deprivation for 2 d. The study demonstrated clear differences between males and females in basal B levels and B responses to some stressors. Only ether anesthesia and fasting resulted in similar B levels in males and females whereas in control and all other groups serum B levels were higher in females. Serum CBG was considerably higher in females. In females, ether, swimming, swimming after fasting, fasting, and fasting during cold exposure resulted in a decrease in circulating CBG. Ice bathing and cold exposure did not influence CBG, and water deprivation elevated serum CBG. In males, animals subjected to fasting and fasting during cold exposure had CBG levels lower than control animals. Other groups did not differ from the control. Higher CBG levels in females counterbalanced higher total B in setting circulating free B: significant sex differences in free B were observed only after swimming or fasting during cold exposure. Stress-responsive changes in CBG levels seem to contribute little to changes in free B; the main contributing factor is the rise in total B. However, CBG may play a special role, independent of the functions of corticosteroids. It is proposed that the need for substantial mobilization of spare fuel (as it takes place during physical exercise or fasting) is critical in involving CBG in the stress response.

Key Words: Corticosteroid-binding globulin; stress; glucocorticoid; metabolic stress; fuel mobilization.

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Introduction

Glucocorticoids are a key element of the stress response. The availability of circulating corticosteroids for target tissues is mediated by corticosteroid-binding globulin (CBG), which divides them into the free and the bound fractions. Only the free fraction is considered to be available for uptake in target tissues, and hence is biologically active *(1,2)*. Thus, CBG could be considered as an antiglucocorticoid factor; on the other hand, CBG probably is the main factor for retaining physiologically needed glucocorticoid levels in the circulation. CBG may also protect some tissues from adverse influences of corticosteroids: chronically elevated free corticosteroids are considered to cause cell death in some brain regions *(3)*.

Corticosteroids have been the main object of stress research whereas comparatively little attention has been paid to the roles of CBG. This could lead to poor understanding or underestimation of the functions of CBG. Recent reports suggest that the functions of CBG may be much broader than maintaining circulatory hormone transport. First, immunocytochemical and biochemical studies demonstrated that CBG-bound corticosteroids also are available for uptake by target cells *(1,4)*. Second, although serum CBG levels are relatively constant, CBG was reported to respond to some types of acute stress *(5–8)*.

In rats, both basal and stress corticosterone levels were reported to be higher in females than males; several mechanisms were proposed for explaining these gender differences *(9–12)*. Among other factors, higher CBG levels in females *(6,13,14)* also may be critical, although the role of CBG in this aspect was predominantly ignored. The aim of the present study was to compare responses of corticosterone and CBG to a variety of stressors in male and female rats. It was expected that such experiments may shed some new light on functions of CBG and interactions between CBG and glucocorticoids in the stress response. In addition to ether anesthesia, considered a "classic" experimental acute stress, other treatments (fasting, physical exercise, and cold exposure) were related with challenges for the system of metabolic homeostasis — the system whose regulation is a main function of glucocorticoids. Such stressors have been common for mammalian life, adaptation, and evolution throughout all their history *(15)*. Note that

energy expenditure is substantially different under these conditions: fasting depresses metabolism whereas physical exercise or cold exposure enhances it *(16)*.

Results

Two-way analysis of variance (sex × experimental group) demonstrated that both corticosterone (B) and CBG were sex-dependent parameters (B, higher in females: F = 71.54; df 1, 335; $p = 0.000000$; CBG, higher in females: F = 319.90; df 1,335; $p = 0.000000$). Both B and CBG levels were dependent on experimental group (B: F = 32.7; df 8,335; $p = 0.000000$; CBG: F = 13.47; df 8,335; $p = 0.000000$). Interaction between sex and group had effects on both B (F = 5.69; df 8,335; $p = 0.000001$) and CBG (F = 4.82; df 8,335; $p = 0.000012$).

Calculated free corticosterone was dependent on sex (F = 17.808; df 1,335; $p = 0.000031$) and experimental group (F = 15.08; df 8,335; $p = 0.000000$), with significant interaction between sex and group (F = 3.64; df 8,335; $p = 0.000442$)

Corticosterone

Basal corticosterone levels were higher in females (Fig. 1). Ether stress resulted in equal corticosterone levels in males and females. Response to ice bathing was higher than during ether treatment, with significantly higher corticosterone in females. Swimming as well as swimming after fasting produced the highest corticosterone responses in both sexes, significantly more pronounced in females. Fasting for 48 h had no influence on corticosterone in females but caused an elevation in males. Cold exposure elevated serum corticosterone only in females; fasting during cold exposure resulted in a greater elevation of circulating corticosterone in both sexes. In both cases related to cold exposure, serum corticosterone was higher in females. Response to water deprivation also was greater in females.

Free corticosterone was elevated in both sexes, compared with the control, after ice bathing, swimming, and swimming after fasting (Fig. 2). Fasting during cold exposure produced an elevation in free corticosterone only in females. Ether, fasting, cold exposure, and water deprivation did not change free corticosterone levels in both sexes. Sex differences in free corticosterone were observed only after swimming and fasting during cold exposure.

Corticosteroid-Binding Globulin

CBG levels were considerably higher in females (Figs. 3 and 4). In females (Fig. 3), ether, swimming, swimming after fasting, fasting, and fasting during cold exposure resulted in a decrease in circulating CBG. Ice bathing and cold exposure did not influence CBG, and water deprivation elevated serum CBG.

In males (Fig. 4), animals subjected to fasting and fasting during cold exposure had CBG levels lower than control animals. Other groups did not differ from the control.

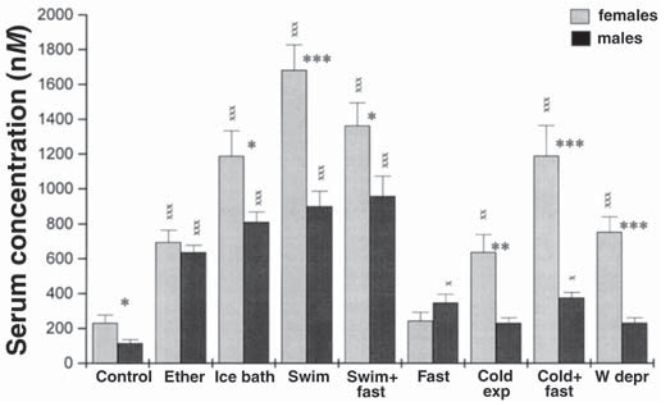

Fig. 1. Serum corticosterone in males and females under conditions of rest (control) and under the action of different factors: ether, ether stress; ice bath, placing in an ice bath; swim, swimming; swim+fast, swimming after fasting; fast, fasting; cold exp, cold exposure; cold+fast, cold exposure in combination with fasting; w depr, water deprivation. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ between males and females; x, $p < 0.05$; xx, $p < 0.01$; xxx, $p < 0.001$ compared with control.

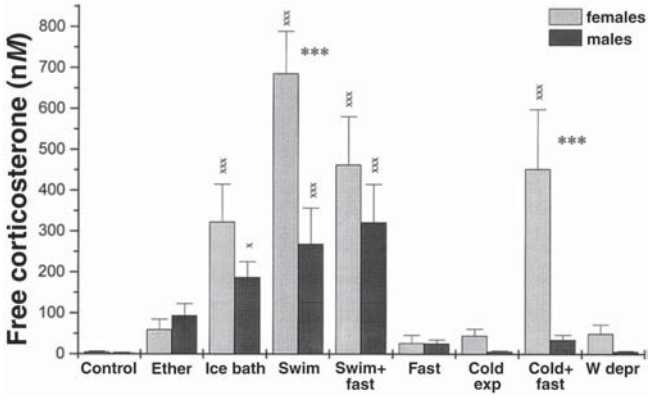

Fig. 2. Calculated free corticosterone in males and females under conditions of rest (control) and under the action of different factors: ether, ether stress; ice bath, placing in an ice bath; swim, swimming; swim+fast, swimming after fasting; fast, fasting; cold exp, cold exposure; cold+fast, cold exposure in combination with fasting; w depr, water deprivation. ***, $p < 0.001$ between males and females; x, $p < 0.05$; xxx, $p < 0.001$ compared with control.

Discussion

Our study demonstrated clear differences between males and females in basal corticosterone levels and corticosterone responses to some stressors. Only ether anesthesia and fasting resulted in similar corticosterone levels in males and females, and all other treatments under study, either acute (ice bathing, physical exercise) or prolonged (cold exposure and water deprivation), produced higher circulating corticosterone in females. Two types of corticosterone responses were also reported in Lewis and Fisher rats: females produced a higher response to ACTH whereas no sex differences were seen in response to novelty, nicotine, lipopolysaccharide, and saline *(17)*. Clearly, higher

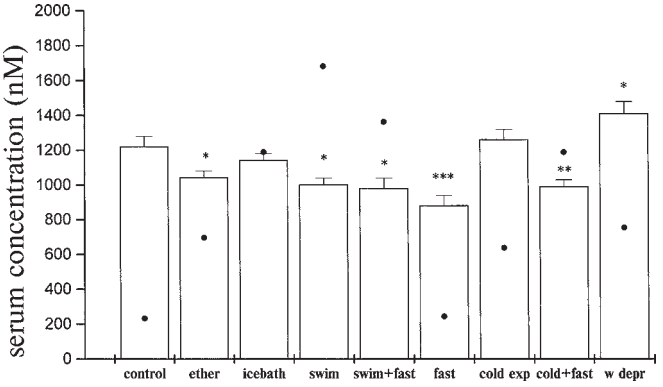

Fig. 3. Serum CBG in females under conditions of rest (control) and under the action of different factors: ether, ether stress; ice bath, placing in an ice bath; swim, swimming; swim+fast, swimming after fasting; fast, fasting; cold exp, cold exposure; cold+fast, cold exposure in combination with fasting; w depr, water deprivation. •, corresponding corticosterone concentration; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared with control.

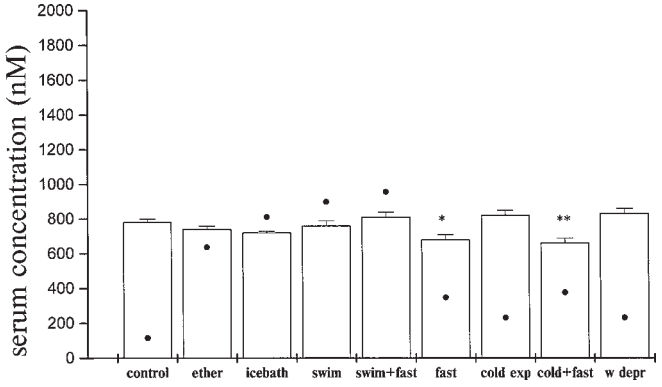

Fig. 4. Serum CBG in males under conditions of rest (control) and under the action of different factors: ether, ether stress; ice bath, placing in an ice bath; swim, swimming; swim+fast, swimming after fasting; fast, fasting; cold exp, cold exposure; cold+fast, cold exposure in combination with fasting; w depr, water deprivation. •, corresponding corticosterone concentration; *, $p < 0.05$; **, $p < 0.01$ compared with control.

female pituitary-adrenal response to forced swimming stress was observed in five inbred strains of rats *(18)*. It was also reported that female rats release more corticosterone in response to alcohol *(11)*, foot shocks *(12)*, and chronic restraint stress *(14)*.

CBG is the main carrier for circulating corticosterone; hence, the substantial sex difference in CBG seems a good candidate responsible for the sex differences in corticosterone levels. Figures 3 and 4 show that binding capacity of CBG was markedly exceeded by circulating corticosterone only after physical exercise in females. Maximal corticosterone responses in males (to ether, ice bath, and physical exercise) were similar to (as if determined by) the binding capacity of CBG. Correlation analysis actually revealed a highly significant correlation between corticosterone and CBG in females after swimming ($r = 0.61$, $p = 0.004$, $n = 20$). However, no significant correlation coefficients were found in any other groups, although the study was specially designed with the hope of revealing more of them. The similar corticosterone levels observed in males and females in response to ether anesthesia and fasting also is against the supposition that the differences in CBG levels could explain sex differences in corticosterone responses. Thus, factors other than CBG seem to play critical roles in determining circulating corticosterone levels, although the role of CBG in the determination of the maximal height of corticosterone response cannot be neglected. Interestingly, it was only swimming in females that resulted in the highest serum corticosterone as well as in the highest excess of CBG capacity by corticosterone.

It was predictable that higher CBG levels in females may counterbalance higher total corticosterone levels in setting free corticosterone levels available for target tissues. The calculation of free corticosterone actually revealed only two sex differences: after swimming and after fasting during cold exposure. All other sex differences as well as the differences between control and ether-treated animals or control and water-deprived females were insignificant. Changes in CBG levels had little influence on free corticosterone: the drop in CBG during ether stress or fasting was unable to enhance free corticosterone substantially. As can be assumed by comparing Figs. 1 and 2, only the degree of elevation of total corticosterone was critical in elevating free corticosterone significantly. It seems that the physiological implications of the calculations of free hormone should be treated carefully. Although the free hormone concept is considered to be generally accepted, some data suggest that CBG-bound hormones are also available for target tissues *(1,4)*. Furthermore, some endogenous factors such as free fatty acids (FFAs) may influence steroid binding by plasma *(19,20)*, probably acting via albumin *(2,21)*.

The results demonstrate that CBG, like corticosterone, responds to stress, both to rather prolonged, such as fasting in both sexes, and to acute, such as ether treatment and physical exercise in females. In a previous study, a significant drop in CBG levels in response to fasting was observed only in females; however, an obvious tendency to lowering CBG activity was also seen in males *(6)*. The drop in CBG activities during fasting seems to be independent of sex, although it is more pronounced in females. The differences in CBG responses observed between males and females may reflect possible sex differences in the time course of the response. The magnitude of CBG responses may also differ in males and females as it does in the case of corticosterone level. Sex difference in CBG response was reported in rats subjected to chronic restraint stress when CBG was suppressed only in females *(14)*.

A previous study *(6)* found only a tendency in both sexes to a reduction in CBG levels in response to ether stress. There is little information on CBG responses to acute

stress. The relevant observations are limited to a reduction in CBG activity during the acute phase of inflammation *(5)* and after foot shocks in foxes *(22)*, and a rise in CBG activities after foot shocks in rats *(23)*. A decrease in CBG was produced by immobilization *(8,24)* or foot shocks *(7)* in rats, but only at least 24 h after the treatments. In clinical studies, we observed a decrease in CBG activity during cardiovascular surgery in response to cardiac arrest; however, there were no changes in CBG levels during closed heart surgery without cardiac arrest *(25,26)*. Therefore, the significant decrease in CBG activity in response to ether stress in this study was an unexpected finding.

Exclusive of ether treatment and water deprivation, all other treatments were related to a direct heavy load on energy-producing systems of the body. A slight elevation of corticosterone level observed during fasting, when the most pronounced decrease in circulating CBG occurred, suggests that the response of CBG to a treatment may be independent of corticosterone response. Note that, in case of prolonged stress (fasting or cold exposure), some corticosterone or CBG responses (including possible peaks) may have occurred before the sacrifice. Fasting in the cold produced a more substantial increase in corticosterone level, compared with starvation alone or exposure to cold with food, but only in females. In females, exposure to cold with food had no influence on CBG activity, although serum corticosterone was elevated. It is obvious that adaptation to the cold with food available ad libitum does not require a large-scale mobilization of spare energy sources of the body, as in the cases of fasting and physical exercise.

Note that swimming after fasting did not produce a further decrease in CBG activities. Moreover, CBG was significantly ($p < 0.05$) elevated, compared with the fasted controls, in fasted males after swimming. Such a response appears paradoxical in comparison with the effects observed in this and previous studies *(6,25,26)* when, in most cases, CBG levels remained unchanged or lowered in response to a stressor. A prompt increase in serum binding of corticosteroids was noted in response to foot shocks in rats *(23)* and after ACTH injections in hares *(27)*.

Another stressor, which caused an increase in CBG activity, was water deprivation in females. In females, this stress resulted in the elevation of serum corticosterone up to levels close to those observed during ether stress, whereas the elevation was markedly less pronounced in males. Water deprivation directly affects water homeostasis, a vitally important function of the body. It is evident that this treatment also affects metabolic homeostasis; it was reported that water deprivation reduced food intake in rats *(28)* and energy consumption in fruit bat *(29)*. These are similar to results of food deprivation; thus, dehydration seems to suppress CBG responses to fasting. Some elevation in effective CBG levels may be explained by blood dehydration; this should mean that some sex differences in maintaining water homeostasis exist in rats.

Thus, CBG responded to both stress that enhances metabolism (physical exercise) and stress that depresses it (fasting). Interestingly, fasting, fasting with cold exposure, and physical exercise (running) were reported to produce high circulating FFA levels, whereas cold exposure (48 h, 4°C) had a minor effect on FFA in rats *(30)*. In this study, the first three above-mentioned treatments had an evident influence on CBG, with cold exposure having no effect. FFAs were shown to stimulate or lower (depending on basal circulating FFA levels) corticosteroid-binding capacity of plasma *(19,20)*; these effects may be responsible for the picture observed in this study. On the other hand, it can be speculated that the critical factor determining CBG response seems not to be the activation or suppression of metabolism *per se*, but the need for mobilization of spare fuel (e.g., FFA) as it takes place during physical exercise or fasting. Acute chilling (ice bath) also demands fuel mobilization; however, the key role in such cases must be played by thermoregulatory mechanisms, including thyroid hormones. Several mechanisms may be proposed for changes in serum CBG during fuel mobilization. CBG, a member of the serine protease inhibitors superfamily *(31)*, was shown to be cleaved by elastase released by activated neutrophils in sites of inflammation; this results in loss of binding activity *(32)*. In case of severe needs for energy, CBG may be similarly involved in interactions with proteases activated to maintain basal catabolic level. Note, however, that the considerably higher CBG levels in males subjected to physical exercise after fasting compared with fasted animals suggests that the decrease in CBG levels during fasting may be accounted for by reversible removal of CBG from circulation rather than by its degradation. The redistribution of CBG between blood and interstitial fluids, according to the idea of Hsu and Kuhn *(33)* explaining circadian variations in circulating CBG levels, or receptor-mediated uptake of CBG by target tissues *(4)*, may be responsible for the changes in CBG levels observed in the present study.

Materials and Methods

Animals

Experiments were performed with Wistar rats weighing 150 g. The stage of estrous cycle in females was not taken into account. Animals were kept in groups of five per cage under a controlled photoperiod (lights on, 8:00 AM to 6:00 PM) at 20°C and were given water and food ad libitum. The protocols were approved by the Institute of Cytology and Genetics Animal Care Committee. Animals were subjected to the following treatments:

1. Ether stress: light ether anesthesia (exposure to ether vapor in special cages) for 10 min followed by a 10-min period of rest; the ether vapor concentration was chosen so that animals successively underwent the state of initial anxiety, excitement, and sleepiness.

2. Ice-bathing (acute chilling): placing in an ice bath (ice and water) with legs partially immersed in water for 15 min followed by a 25-min period of rest.
3. Physical exercise: swimming in a water bath (25°C) for 20 min followed by a 30-min period of rest.
4. Fasting: lack of food for 2 d.
5. Cold exposure: placing a cage in a cold room (4°C) for 2 d.
6. Water deprivation: lack of drinking water for 2 d.

The periods of rest after acute stress were chosen arbitrarily to allow animals to reach some stable emotional and physical state, which was apparently the same for all groups; animals, initially indifferent to external stimuli (sleepy after ether, shivering with cold after ice bathing, or tired after swimming) began to move and demonstrate weak reaction of escape from the experimentor's hand. This was considered as the state of controllable recovery, with CBG and corticosterone being in the equilibrium state. Two groups were subjected to combined treatments: fasting for 2 d followed by swimming, and fasting during cold exposure for 1 d. Rats were sacrificed by decapitation between 9:30 AM and 10:00 AM. Each experimental group consisted of 20 animals.

Corticosterone Assay

Corticosterone was measured by a method of competitive protein binding *(34)* using rat CBG, corticosterone from Sigma (St. Louis, MO, and [1,2,6,7- H^3]corticosterone (specific activity 90 Ci/mmol; Isotop, Sankt-Petersburg, Russia). Intra- and interassay coefficients of variation were less than 5% and 10% respectively.

Measurement of CBG

CBG was measured by a radioligand method as described previously *(6)*. Briefly, examined sera were previously treated with dextran-coated charcoal for 10 min at 37°C to remove endogenous steroids. Charcoal was removed by centrifugation. Diluted serum (0.1 mL, final dilution 1:200) was incubated with 60 n*M* H^3-corticosterone without (total binding) or with (nonspecific binding) the addition of 20 μ*M* nonradioactive corticosterone for 10 min at 37°C and for 1 h on an ice bath. Charcoal-dextran suspension (0.2 mL, at 4°C) was simultaneously added to six samples. Without mixing tubes were centrifuged in 30 s on a microfuge for 20–25 s. The radioactivity of supernatant was counted at 50% efficiency. It was shown that under described conditions, 60 n*M* H^3-corticosterone provides saturation of all specific corticosterone-binding sites.

Free corticosterone was calculated using total serum corticosterone concentration, CBG concentration, and the association constant of corticosterone and CBG as described previously *(35,36)*.

Statistics

Values are given as mean+SEM. Results were analyzed by multivariate analysis of variance followed by post-hoc Newman-Keuls test (STATISTICA for Windows, Statsoft), and values of $p < 0.05$ or less were considered to be significant.

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