General and Comparative Endocrinology 165 (2010) 345–350

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

How acute is the acute stress response? Baseline corticosterone and corticosteroid-binding globulin levels change 24 h after an acute stressor in Japanese quail

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ARTICLE INFO

Article history:
Received 1 April 2009
Revised 10 August 2009
Accepted 11 August 2009
Available online 15 August 2009

Keywords:
Acute stress response
Corticosteroid-binding globulin
Corticosterone
Artificial selection

ABSTRACT

Changes in plasma corticosteroid-binding globulin (CBG) capacity can alter free plasma concentration and tissue availability of glucocorticoids (GC) and hence alter the organismal response to stress. However, CBG change in response to stress has not been extensively studied. While it is clear that chronic stress can causes CBG decline and in some species acute stressors can reduce CBG during the 30–60 min of the stressor, more long-term changes in CBG following an acute stressor has received less attention. Here we investigated corticosterone (CORT: the primary GC in birds) and CBG levels 24 h after an acute stressor in a unique study system: Japanese quail divergently selected for CORT reactivity to acute stress. Using this model, we examined the interaction of selected CORT reactivity with CBG response to determine if CBG shows a delayed decline in response to an acute stressor and if that decline varies by selected genetic background. We found lowered CBG capacity, elevated total CORT and free CORT 24 h after acute stress in all three quail groups. These results demonstrate for the first time in an avian species that exposure to an acute stressor can affect CBG and CORT 24 h later.

1. Introduction

In vertebrates, rapid elevation of glucocorticoids (GCs) following a stressor initiates changes in behavior and physiology to promote survival (see Sapolsky et al., 2000; Wingfield et al., 1998 for review, see also Breuner et al., 2008). If the stressor is transient, negative-feedback curtails the hormone surge and GCs return to baseline. GC effects on behavior and physiology are mediated by the binding of GCs to corticosteroid receptors on and in target tissues. However, GC receptor binding is modulated by GC access to receptors (bound or not bound to corticosteroid-binding globulins) as well as the number of available receptors (Breuner and Orchinik, 2000). These additional levels of regulation can enhance or subdue GC induced changes in behavior and physiology.

Corticosteroid-binding globulin (CBG) circulates in the blood and binds GCs with high affinity (Westphal, 1971). CBG has the potential to modulate the organismal stress response by altering the potency and tissue specificity of GCs (for greater detail, see Breuner and Orchinik, 2002). The precise role of CBG is debated in the scientific community and evidence supports bioactive roles for GC hormones that are not bound to CBG (free GC hormones), CBG bound to GCs (CBG–GC complexes), and total hormone levels (free GC hormones + CBG–GC complexes). A large body of evidence supports the hypothesis that plasma GCs bound to CBG are inactive and unavailable for uptake by tissues (the free hormone hypothesis; Mendel, 1989); One primary example: binding globulin levels are negatively related to hormone clearance rates, indicating that hormone bound to carrier proteins are unavailable for metabolism and clearance (Siittei et al., 1982).

The relation between GC and stress has been well studied; GCs rapidly increase within minutes of a stressor and return to baseline levels hours later. The CBG response to stress is less well characterized. Several species of birds show CBG decline during a 30–60 min acute handling stress (see Breuner et al., 2006, however see Fokidis et al., 2009) and CBG declines in white-crowned sparrows in response to a chronic stressor (22 h of fasting; Lynn et al., 2003). Surprisingly, there is evidence in mammals that CBG may show a delayed response even to short-term stressors. In pigs, CBG level is stable for 24 h following surgery, then levels decline significantly thereafter (Dalin et al., 1993); in rats, CBG levels decline 6 h after acute tail shock treatment and remain low for 30 h (Fleshner et al., 1995). Furthermore, Fleshner and collaborators reported that baseline GC levels (both total and free) were significantly elevated

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doi:10.1016/j.ygcen.2009.08.003

24 h after the acute stress protocol. These studies suggest that the acute GC response to stress may not be as acute as often presumed.

Here we test the hypothesis that acute stress (a standard handling and serial bleed stress) induces changes in CBG and baseline and free CORT 24 h later. We utilized a unique study system, Japanese quail that are divergently selected for GC reactivity to acute stress (Satterlee and Johnson, 1988). Quail selected for high acute GC reactivity (HS line) have a twofold response to crush cage stress (a form of mechanical restraint and immobilization) as compared to quail selected for low stress reactivity (LS line) (Satterlee and Johnson, 1988) and both lines also differ from a third group of randomly bred Japanese quail (Satterlee and Johnson, 1988). Using this model we can evaluate the interaction of selected stress reactivity with CBG response and determine (1) if acute stress in the previous 24 h leads to changes in CBG capacity, total corticosterone (CORT: the primary GC in brids), and/or free CORT in Japanese quail (as seen in rats; Fleshner et al., 1995) and (2) if a predisposition for stress responsiveness influences CBG capacity, total CORT, and/or free CORT levels differentially. We predict that previous handling stress will lead to an increase in baseline CORT, an increase in free CORT and a decrease in CBG capacity as seen by Fleshner and collaborators in their work with rats (1995). It is unclear what role GCs play in CBG modulation therefore we have no a priori prediction concerning changes in CBG or CORT specific to selected lines.

2. Materials and methods

2.1. Study animals and husbandry

Sixteen LS, HS and random bred male Japanese quail were obtained ($N = 48$). All quail originated from a single base population. The LS and HS quail were divergently selected for 32 generations for either low or high acute CORT responses to brief mechanical restraint, respectively, and a third stock was maintained for the same number of generations to serve as a control (for complete details see Satterlee and Johnson, 1988). All three quail groups were maintained as 12 families containing 15 birds per family with a sex ratio of approximately two females: one male. Each of the 12 families for each quail group was housed in a single cage ($50.8 \times 61.0 \times 26.7$ cm; length × width × height). Birds had access to feed (laying ration containing 21% crude protein and 2750 kcal ME/kg) and water *ad libitum*, and were exposed to an LD14:10 (14 h light:10 h dark) light/dark cycle (more detailed husbandry procedures are described in Satterlee and Marin, 2006). All work with the quail was approved under Louisiana State University animal care protocol AE03–07.

2.2. Acute stress protocol

A subset of quail (12 LS + 12 HS + 12 controls, $N = 36$) were subjected to acute handling stress and serial blood sampling 24 h prior to blood collection for the CBG and CORT assays. In brief, quail were gently lifted, inverted and returned to a box repeatedly for 5 min and blood samples were taken at 0, 15, 30, and 60 min from the start of the handling procedure. Blood samples obtained as part of the stress protocol were assayed in a different lab for an additional study (for detailed analyses see Cockrem et al., submitted for publication). In brief, baseline samples did not differ between LS and HS lines (0.60 + 0.10 ng/ml and 0.70 + 0.10 ng/ml, respectively, $t = 1.46$, $P = 0.152$), but did differ at 60 min post stressor (3.11 + 0.54 ng/ml and 7.88 + 2.24 ng/ml, respectively, $t = 2.38$, $P = 0.022$). Therefore, acute stress increased CORT for at least 60 min in both lines and HS quail responded to stress with higher CORT secretion than LS lines (see Cockrem et al., submitted for publication, for further details).

2.3. Blood collection

Blood samples for CBG and baseline CORT were collected 24 h after acute handling and bleeding stress (handled group: 12 LS + 12 HS + 12 controls; $N = 36$) and from quail that did not experience an acute stressor (naive group: 4 LS + 4 HS + 4 controls; $N = 12$). Blood samples were obtained within 1 min of initial disturbance (removal from cage). Blood was collected in heparinized capillary tubes, stored on ice for a maximum of 2 h, centrifuged to separate plasma, and stored at −20 °C.

2.4. CBG assay

CBG affinity and capacity were determined following the methods of Breuner et al. (2003) but optimized for Japanese quail. In brief, plasma was stripped of endogenous steroids through a 20-min incubation with a dextran-coated charcoal solution and assays were performed at a final plasma dilution of 1:693. All assays were performed at 4 °C and incubated in 50 nM Tris for 2 h. For saturation analysis, plasma was pooled from 11–12 individuals from each quail group. Plasma was incubated 0.15–9 nM [^{3}H] CORT in the presence or absence of 1 μM unlabelled CORT to determine non-specific binding. Non-linear regression of the data for each quail group was best fit by a one-site binding model (LS, $i_d = 2.17 + 0.315$ nM; control, $K_d = 2.70 + 0.448$ nM; HS, $K_d = 2.52 + 0.415$ nM; see Fig. 1). This result is consistent with previous work in this species (see Breuner et al., 2006 for further details). CBG affinity for CORT did not differ between quail groups (one-way ANOVA, $F = 0.4704$, $P = 0.6312$). Based on the results of the saturation analysis, CBG capacity for individual quail samples were determined by incubating plasma stripped of endogenous steroids with 11 nM [^{3}H] CORT in the presence or absence of 1 μM unlabeled CORT. Assays were terminated and CBG bound to [^{3}H] CORT was separated from unbound by rapid vacuum filtration (Brandel Harvester) over glass fiber paper (GF/B, Brandel) that had been soaked for 1 h in 25 mM Tris plus 0.3% polyethylenimine. Filters were rinsed with 9 ml ice-cold 25 mM Tris. Following filtration, radioactivity of filters was determined with standard liquid scintillation spectrophotometry. All samples were run in triplicate. Individual CBG capacity estimates obtained reflected 82.98% occupancy of total binding sites; therefore CBG values were corrected to 100% for all statistical tests and reported values.

2.5. CORT assay

Plasma CORT levels were measured by enzyme immunoassay following the methods of Wada et al. (2007) after extraction with 4 ml of diethyl ether, evaporation under nitrogen gas and re-suspension in phosphate buffer. Samples were run in duplicate aliquots using an enzyme immunoassay kit from Assay Designs® (Ann Arbor, Michigan, USA). All samples were run in a single assay, sensitivity was 4.76 pg/well, and intra-assay coefficients of variation was 3.408%.

2.6. Calculation of free CORT

Free CORT concentrations were calculated using the equation of Barsano and Baumann (1989) as described by Deviche et al. (2001). This equation is commonly used to estimate free hormone levels for several binding globulin systems (e.g., growth hormone, glucocorticoids, androgens, thyroid hormones) and has been shown to correlate highly with direct measures of free CORT in mammals (Taymans et al., 1997; Adcock et al., 2006).

2.7. Statistics

CBG affinity and capacity were determined by fitting untransformed data to appropriate equations using iterative, least-squares, curve-fitting techniques in Prism (GraphPad Software, San Diego,