Research Notes

Sex Differences in Central Benzodiazepine Receptor Densities and Circulating Corticosterone Release After Acute Stress in Broiler Chicks[1]

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ABSTRACT The purpose of the study was to determine the effect of sex on central benzodiazepine receptor (CBR) and serum corticosterone (CS) responses to an acute stressor in broiler chicks. Birds were housed in ten mixed-sex groups of eight chicks per cage. At 15 d of age, chicks were taken from a randomly selected cage and blood was immediately sampled (undisturbed controls), or they were taken from the same cage and immersed up to their necks in warm water (partial water immersion, PWI) for 15 min before blood was sampled. After blood sampling, forebrains were dissected for preparation of membranes, and bird sex was determined by gonadal inspection. Serum CS levels were determined by a competitive protein-binding assay. CBR densities were determined by radiolabeled receptor binding assay. There were no sex differences in serum CS levels or benzodiazepine receptor densities in controls. Exposure to PWI significantly increased ($P < 0.01$) circulating CS levels in both sexes, and this elevation was more pronounced ($P < 0.01$) in males than in females. Male, but not female, chicks also showed a significant stressor-induced increase ($P < 0.01$) in CBR densities. These findings showed sexual differences in acute, stressor-induced benzodiazepine and adrenocortical responses that suggest broiler males are more stress-susceptible than females.

(*Key words*: broiler chick, acute stress, benzodiazepine receptor, serum corticosterone)

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INTRODUCTION

The central-type benzodiazepine receptor (CBR) is an allosteric modulatory site localized in the γ-aminobutyric acid, subtype A ($GABA_A$) receptor-chloride channel complex in neuronal cells (Burt and Kamatchi, 1991). These receptors bind selectively and with high affinity to the ligands clonazepam, diazepam, and flunitrazepam (Drugan and Holmes, 1991). The CBR has been implicated in an organism responses to physical (Drugan and Holmes, 1991; Martijena et al., 1992) and psychological stress (Drugan and Holmes, 1991; Marin and Arce, 1996).

In mammals of different ages, CBR responses during stress are considered controversial because exposure to a variety of stressful stimuli has been reported to increase, decrease, or to have no effect on this receptor complex (Braestrup et al., 1979; Medina et al., 1983; Motohashi et al., 1993; Hogg et al., 1996). However, in juvenile fowl, exposure to an acute stressor has consistently produced an increase in the number of [^{3}H]flunitrazepam receptors in synaptosomal membranes within the forebrain without changing binding affinity (Martijena et al., 1992; Martijena and Arce, 1994; Salvatierra et al., 1994, Marin and Arce, 1996), and it has been suggested that the increase in the density observed after acute stressor exposure is due to an exposition of pre-existing receptors (Martijena et al., 1992). On the other hand, serum corticosterone (CS) concentrations were consistently elevated in chickens, quail, and rats following the imposition of a wide range of known stressors, such as crating, handling, restraint, loud noise, or social disturbance (Freeman, 1976; Beuving and Vonder, 1978; Jones and Harvey, 1988; Jones et al., 1994; Natelson et al., 1987; Satterlee and Johnson, 1988).

Because males have been shown to be more fearful (Banks et al., 1979; Jones, 1987) and, in turn, more stress susceptible than females, the purpose of the study was to determine the effect of sex on CBR and CS responses to a partial water immersion (PWI) acute exposure. The acute stressor used resembles the swim-stress test routinely employed for laboratory rodents (Medina et al.,

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Abbreviation Key: CBR = central benzodiazepine receptor; CS = serum corticosterone; PWI = partial water immersion.

1983; Motohashi et al., 1993; Baez and Volosin, 1994; Martijena et al., 1997), except that the chicks were allowed to stand with their heads above water.

MATERIALS AND METHODS

Birds

Eighty, newly hatched, mixed-sex Cobb chicks were obtained from a commercial hatchery.[3] They were housed in 10, white, wooden cages (eight chicks/cage). The cages were 45 × 85 × 50 cm (length × width × height). A controlled temperature of 28 to 32 C and a 12:12 h light:darkness schedule (lights on at 0700 h) was used. Feed (Cargill, broiler BB, 23% CP, 2,950 kcal/Kg) and water were supplied ad libitum.

PWI Stress

At 15 d of age, a chick was randomly removed from a randomly selected cage by an experimenter, carried to a separate room, and placed in a cylindrical basin (22 cm in diameter x 30 cm high) containing water (38 C) approximately 18 cm deep. Thus, when the bird stood upright in the basin, the water reached only up to its neck. A test period of 15 min was used, and water was changed after each trial. None of the birds exhibited signs of exhaustion during the testing. At the end of a trial, the test chick was removed from the basin, immediately decapitated, and bled into a chilled (0 C) tube. Its forebrain was dissected on ice for preparation of synaptosomal membranes.

Whenever an experimental chick was caught, a second experimenter also randomly captured a control chick from the same cage. Controls were carried to a separate room, immediately decapitated, and bled as described above. The blood collection procedure was completed within a maximum of 15 s. After blood collection, samples were immediately centrifuged (4 C) for 10 min at 1,000 × *g*. Serum was then harvested and stored at −30 C.

Only four of the eight birds within each of the 10 cages were individually sampled (two control and two PWI-stressed birds per cage) in order to minimize the stress induced by systematic reductions of group size (Jones and Harvey, 1988). Thus, in total, 20 control and 20 PWI-stressed birds were examined. All birds selected for testing were further dissected to determine their sex by visual inspection of the gonads.

CS Determination

Serum CS concentrations were determined using a competitive protein-binding method (Murphy, 1967). Each sample was from one bird only. Briefly, 50 μL of a serum sample was mixed with 50 μL of distilled water, shaken for 5 s, and placed in boiling water for 90 s. After being cooled, the samples were incubated with a corticosteroid-binding globulin tracer solution [2% horse serum containing 0.5, 1, 2, 6, 7-^{3}H-corticosterone[4] (88 Ci/mmol) as tracer]. Unbound steroid was removed using Florisil[5] (Mesh: 60-100). Radioinert CS used for standards were obtained from Sigma Chemical Co.[5] The intra-assay coefficients of variation were less than 10%. All samples were assessed in the same assay.

TABLE 1. Mean (± SEM) central benzodiazepine receptor density (fmol/mg protein) following partial water immersion (PWI) stress in male and female broiler chicks

Treatment	Male	Female
Control	808.5 ± 43.6 (12)[1,b]	877.9 ± 69.0 (8)[b]
PWI	1,049.3 ± 54.4 (11)[a]	797.0 ± 45.3 (9)[b]

[a,b]Means with no common superscript differ significantly ($P < 0.03$) by Newman-Keuls test.

[1]The number in parentheses indicates the number of central benzodiazepine receptor assays.

CBR Density Determination

All the procedures for preparing forebrain synaptosomal membranes were carried out at 4 C, essentially as described by Awad and Gavish (1987). Tissue from each sample was individually homogenized in 50 volumes of 50 m*M* Tris-HCl buffer (pH 7.4) and then centrifuged at 35,000 × *g* for 15 min. Each pellet was resuspended in 150 volumes of this buffer. Binding assays were conducted in 50 m*M* Tris-HCl buffer (pH 7.4) at 4 C. The binding assay mixture contained 400 μL of membrane suspension at a final concentration of 300 μg of protein/mL and 50 μL of 25 n*M* [^{3}H]flunitrazepam[4] (86.4 Ci/mmol) solution in the absence (total binding) or presence (nonspecific binding) of 10 μM Diazepam.[6] After incubation for 60 min at 4 C, samples were filtered under vacuum over GF/B filters[7] with a M-24R filtering manifold.[8] Samples were washed three times with 4 mL of ice-cold Tris buffer, and radioactivity was counted in a LKB-1219-Rack-Beta counter[9] at 47% efficiency.

Statistical Analysis

Corticosterone data were subjected to a log transformation to minimize heterogeneity of variance before analysis. Transformation was not required for benzodiazepine data. Analyses were performed using a two-way ANOVA that examined the main effects of gender (male, female) and treatment (control, PWI stress). Posthoc group comparisons were conducted using the Newman-Keuls test. A P-value ≤ 0.05 was considered to represent significant differences.

RESULTS

The effects of PWI stress on CBR density in male and female broiler chicks are given in Table 1. Analysis of

[3]INDACOR, Córdoba, 5000, Argentina.
[4]NEN Inc., Boston, MA 02118.
[5]Sigma Chemical Co., St. Louis, MO, 63178.
[6]Hoffmann-La Roche Inc, Nutley, NJ 07110.
[7]Whatman Inc., Clifton, NJ 07014.
[8]Brandel, Gaithersburg, MD 20877.
[9]Wassac-oy, SF-20101, Turku, Finland.

TABLE 2. Mean (± SEM) serum corticosterone concentrations (ng/mL) following partial water immersion (PWI) stress in male and female broiler chicks

Treatment	Male	Female
Control	1.2 ± 0.1 (12)[1,C]	1.2 ± 0.1 (8)[C]
PWI	8.3 ± 1.2 (11)[A]	5.4 ± 0.8 (9)[B]

[A-C]Means with no common superscript differ significantly ($P < 0.01$) by Newman-Keuls test.

[1]The number in parentheses indicates the number of corticosterone assays.

the CBR density data revealed a significant interaction between gender and treatment ($F_{1,36} = 6.75$; $P < 0.015$). The CBR density of male birds exposed to PWI was significantly higher ($P < 0.02$) than those of the male controls, whereas no differences were found between control and stressed female groups. The CBR density of male and female chicks subjected to PWI were also significantly different (males > females; $P < 0.03$).

The effects of PWI stress on CS response in male and female broiler chicks are given in Table 2. Analysis of CS data revealed a significant main effect of gender (males > females; $F_{1,36} = 5.20$; $P < 0.03$) and treatment (PWI > control; $F_{1,36} = 177.08$; $P < 0.01$) as well as significant interactions between these two factors ($F_{1,36} = 4.08$; $P < 0.05$). Post-ANOVA testing showed that serum CS values differed ($P < 0.01$) according to the following order: males exposed to PWI > females exposed to PWI > control males = control females.

DISCUSSION

No differences were apparent in CBR densities or in serum CS levels between male and female control chicks. These results suggest that there are no basal differences between the sexes in these parameters for broiler chicks. Control CS results support our findings that no differences exist in CS levels between males and females in broiler chicks that were first T-maze classified and then served as controls in a PWI-stress study (Marin and Jones, 1999).

Exposure to stressful stimulation is known to increase circulating concentrations of CS in mammalian (Natelson et al., 1987) and avian species (Freeman, 1976; Beuving and Vonder, 1978; Dantzer and Mormede, 1983; Jones and Harvey, 1988; Jones et al., 1994; Hemsworth and Coleman, 1998). Thus, the finding that serum CS levels were significantly higher in chicks that had been exposed to a 15 min of PWI was expected. However, the observation of greater adrenocortical responses to PWI in male than in female juvenile chicks suggests sex differences exist in stress susceptibility. Given that adrenocortical activation and fearfulness are thought to be positively associated in birds (Faure, 1981; Dantzer and Mormede, 1983; Jones et al., 1988; Jones and Satterlee, 1996; Jones, 1996; Hemsworth and Coleman, 1998), our finding that exposure to PWI elicited higher levels of serum CS in males than in females is consistent with reports that show males are more fearful (Banks et al., 1979; Jones, 1987) and, in turn, more stress-susceptible than females.

Exposure to an acute stressor also produces an increase in the CBR in synaptosomal membranes from pooled forebrains of unsexed birds (Martijena and Arce, 1994; Marin and Arce, 1996). In the present study, there were clear sex differences in CBR stress responses between male and female birds; male, but not female, birds showed a significant stress-induced increase in CBR densities. When considered together, the CS and CBR results suggest that male broiler chicks are more stress-susceptible than their female counterparts. Interestingly, in base populations of genetically unremarkable broilers, male chickens exhibit a higher incidence of ascites (Wideman and French, 2000) than females. In addition, this disease condition has been shown to be more prevalent during times of stress (Wideman and French, 1999). Thus, the heightened stress susceptibility of male broiler chicks demonstrated herein may provide an underlying explanation as to why more males suffer from ascites.

In addition, in turkeys, sex differences in the resistance to *Escherichia coli* challenge after immunosuppression with dexamethasone have been reported (Huff et al., 1999). These workers suggested that males were more susceptible to colisepticemia than females, especially under severe stress. It is not known whether the chick sex differences in adrenocortical activation and CBR responses found herein also exist in broiler or turkey breeders. Indeed, a study that evaluates the simultaneous consequences of an acute stress exposure on fear behavior, adrenocortical activity, and CBR responses in male and female breeder birds afflicted with ascites or other sex-related pathologies would be useful. Sex differences in stress-susceptibility might also help explain why male broiler chicks classified in a T-maze as high performers (as opposed to low performers) had a more pronounced body weight advantage than did females classified as high performers when compared to their low performer counterparts (Marin et al., 1997; 1999). Finally, because of our findings, we recommend that in studies in which treatments may affect CBR and CS responsiveness, the sex of the birds should be considered (i.e., sexes should be equally represented or either sex studied independently).

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