Relationship Between Hypothalamic-Pituitary-Adrenal Axis Responsiveness and Age, Sexual Maturity Status, and Sex in Japanese Quail Selected for Long or Short Duration of Tonic Immobility

D. Hazard, M. Couty, J. M. Faure, and D. Guémené

Institut National de la Recherche Agronomique, Station de Recherches Avicoles, Centre de Tours-Nouzilly 37380 Nouzilly, France

ABSTRACT The influences of age (4 to 12 wk), sexual maturity status, and sex on hypothalamic-pituitary-adrenal (HPA) axis responsiveness were investigated by measuring changes in peripheral basal levels of corticosterone (B) and responses to 10 min of physical restraint in a crush cage or injection of 1-24 adrenocorticotropic hormone (ACTH) in 2 genotypes of Japanese quail divergently selected for long (LTI) or short (STI) duration of tonic immobility (TI). Although gonad development was more advanced in male STI quail, most birds were still immature at 4 wk of age, but sexual maturity was fully acquired by 6 wk of age in both sexes and genotypes. This change was associated with increases in basal B levels in both genotypes and sexes. On the other hand, HPA axis responsiveness to restraint and adrenal responsiveness to 1-24 ACTH injection remained stable in STI quail, whatever the age. Conversely, significant responses to restraint compared with basal B levels were only observed at 4 and 6 wk of age, and adrenal responsiveness increased with age in LTI quail. Moreover, higher B levels were measured in response to restraint in STI than in LTI quail, whereas similar adrenal responses were measured at 9 and 12 wk of age.

We concluded that an increase in basal B levels is associated with the stage of sexual maturity acquired, but it did not affect HPA axis responsiveness or adrenal B response capacity. On the other hand, age affected HPA axis responsiveness and adrenal B response capacity in LTI quail of both sexes but not in STI quail. It is hypothesized that functional HPA axis maturity occurs after 6 wk of age in the LTI genotype, but it is reached by 4 wk of age in the STI genotype. In conclusion, the divergent selection program for TI conducted on quail resulted in changes in HPA responsiveness that probably resulted from differences in development rate and function of the adrenal glands or other upstream structures of the HPA axis.

(Key words: tonic immobility, Japanese quail, adrenocorticotropic hormone)

INTRODUCTION

Behavioral and physiological responses, including elevated glucocorticoid secretion, enhance the ability of an animal to cope with stressors and other challenges to homeostasis (Tsigos and Chrousos, 2002). In birds, corticosterone (B) is the major and primary adrenal steroid present in the plasma and is released following activation of the hypothalamic-pituitary-adrenal (HPA) axis by various stressful situations (Siegel, 1971; Scott et al., 1983; Jones et al., 1994; Canoine et al., 2002). As in other species, numerous studies have reported changes in circulating B levels that depend upon the effect of physiological factors such as age (Schmeling and Nockels, 1978; Hocking et al., 2001; Sockman and Schwabl, 2001) and the acquisition of sexual maturity (Schmeling and Nockels, 1978). However, the reported results are somewhat conflicting. Decreases in basal B levels with age have been reported for different species of ducks (Faure et al., 2003). On the other hand, basal B levels have been reported to successively increase and decrease with increasing age to reach a stable level before sexual maturity in White Leghorn chicks (Schmeling and Nockels, 1978). Thus, depending upon the study, sexually mature chickens have lower basal B levels than immature birds (Schmeling and Nockels, 1978) or the reverse (Hocking et al., 2001). In rats, the effect of sex also depends upon the genotype. Thus basal B levels are higher in old and adult Wistar males than in young ones, and basal B levels are higher in young females than in old females (Kizaki et al., 2000; Casolini et al., 2002). On the other hand, no significant difference was found

©2005 Poultry Science Association, Inc.
Received for publication June 6, 2005.
Accepted for publication August 20, 2005.
1To whom correspondence should be addressed: guemene@tours.inra.fr.

Abbreviation Key: ACTH = adrenocorticotropic hormone; B = corticosterone; HPA = hypothalamic-pituitary-adrenal; LTI = long tonic immobility; STI = short tonic immobility; TI = tonic immobility.
among young, adult, and old Long Evans male or female rats (Ait-Chaoui et al., 1995; Brudieux et al., 1995). Furthermore, these various factors have been reported to affect HPA axis reactivity. Response in B to handling was shown to increase with age in Sprague-Dawlay male rats (Johnston et al., 1974), and response to 1-24 adrenocorticotropic hormone (ACTH) injection was attenuated in Long Evans rats (Ait-Chaoui et al., 1995). Similarly, the response to ACTH challenge is attenuated with age in male chicks (immature vs. mature; Schmeling and Nockels, 1978) and immature ducks (Faure et al., 2003). In quail, it was not possible from a previous study (our unpublished data) to firmly conclude the respective effects of photoperiod and the acquisition of sexual maturity on HPA axis and adrenal responsiveness. Although the available data appear somehow contradictory, it seems that age and sexual maturity can affect HPA axis function differently depending on the species and genotype. The aim of the present study was therefore to assess the respective effects of age and acquisition of sexual maturity on HPA axis and adrenal responsiveness in both sexes of quail selected for divergent duration of tonic immobility [TI; long tonic immobility (LTI) or short tonic immobility (STI) genotypes; Mills and Faure, 1991].

**MATERIALS AND METHODS**

**Birds and Rearing Conditions**

Japanese quail (Coturnix japonica) from the 36th generation of 2 divergent genotypes selected for STI or LTI were used in the study (Mills and Faure, 1991). Quail were identified by wing-banding on the day of hatching. Quail were exposed to continuous light until 3 wk of age and then to a 16L:8D (light on 0600 h) rhythm. The quail from the different genotype and group of age were reared in different collective battery cages, and food and water were provided ad libitum. The caretaker checked the quail daily in the morning (from 0830 h) and refilled the feeders whenever necessary. Feed was not provided to the quail on the day of the experiment.

**Experimental Procedures**

Groups of 4 quail of the same genotype and sex from approximately 400 quail were randomly created 1 wk before experimentation, which was performed at 4, 6, 9, or 12 wk of age between 0830 and 1230 h. Seven quail were used on average for each measurement (sex × genotype × age × treatment). Quail were bled by decapitation after being subjected to different treatments. To evaluate HPA axis responsiveness and adrenal response capacity, circulating B levels were measured 10 min after beginning physical treatment or pharmacological challenge, respectively. The physical treatment consisted of a restraint period in a crush cage, which has been shown to induce increases in B levels (Beuving and Vonder, 1986; Jones et al., 1994). After capture in the home cage, quail of both genotypes and sexes were transferred individually to a test room and placed in a wooden box measuring 15 × 5 × 10 cm (length × width × height).

The pharmacological treatment consisted of injection of 1-24 ACTH (Immediate Synacthen, Novartis, France; 1 mg = 100 IU) at 10 μg/kg of BW diluted in physiological serum (0.9% NaCl wt/vol) into the pectoralis major muscle. This peptide is very potent in stimulating the release of B in birds (Beuving and Vonder, 1986; Koelkebeck et al., 1986; Launay, 1993; Noirault et al., 1999; Guémené et al., 2001b). Quail were captured prior to injection, transferred to the test room, and individually weighed to adjust the amount of 1-24 ACTH injected per BW. To check for the effects of capture, transfer, weighing, and injection per se, control groups of quail were similarly manipulated and injected with a representative volume of the vehicle (400 to 700 μL of 0.9% NaCl wt/vol). An additional group of quail was included at each age and bled immediately after capture in the home cage to assess basal B concentrations in our specific experimental conditions.

**Anatomical Study of Ovaries and Testes**

Anatomical study of ovaries and testes was performed after birds were euthanized at 4 and 6 wk of age. Gonad development was classified in 4 stages according to the size of the follicles (diameter) or the testes (length), where 0 = no distinct follicles or small testes, 1 = fully immature (follicles <2 mm; testes <5 mm), 2 = intermediate stage of development (follicles = 2 to 10 mm; testes = 5 to 10 mm), and 3 = fully mature (follicles >10 mm; testes >10 mm). Additional quail from an independent experiment conducted at the same time and raised under the same experimental conditions have been included for the anatomical study at 6 wk of age.

**Blood Sample Collection and B Assay**

Blood samples were collected from each quail directly into tubes containing EDTA (≤2 mg/mL of blood) after killing by decapitation. All samples were temporarily stored on ice. After centrifugation at 2,000 × g for 15 min at 4°C, plasma samples were separated and stored at −20°C until measurement of B using a specific radio immunoassay (Etches, 1976).

**Statistical Analysis**

The B values were subjected to a multifactorial ANOVA to assess the effects of genotype, age, sex, treatment, and their interactions using the Statview IV program (Abacus Concept Inc., Berkeley, CA). Whenever ANOVA reached significance (P < 0.05), post hoc tests were performed using the Fisher test of protected least significant difference. The B values are expressed as means ± SE, and the level of significance is P < 0.05 unless otherwise stated. The chi-squared test was used to compare differences in sexual maturity status between LTI and STI male and female quail.
### RESULTS

**Gonad Development and Sexual Maturity Status**

At 4 wk of age, the majority of the females were classified at stage 1 (LTI: 85%; STI: 97%), and none of them had a fully developed ovary (stage 3; Table 1). Gonad development was at a more advanced stage in STI female quail than in LTI female quail ($P = 0.07$). The majority (60%) of males were classified at stages 1 and 2 for the LTI and STI, respectively. The STI males were at a more advanced stage of sexual maturation than LTI males ($P = 0.007$). Some males of both genotypes (LTI: 4/45, STI: 1/42) had full size testes (stage 3) at the age of 4 wk. At 6 wk of age, most quail of both genotypes had a fully developed ovarian hierarchy or testes (females, STI 94%, LTI 95%; males, STI 98%, LTI 98%, respectively).

**Basal B Levels**

Basal B levels were not significantly affected by genotype ($P = 0.47$), but significant effects of age ($P < 0.0001$) and sex ($P = 0.007$) factors were observed (Figure 1A). In female quail of both genotypes, basal B levels were significantly lower ($P < 0.05$) at 4 wk (STI 0.8 ± 0.2 ng/mL, LTI 0.8 ± 0.3 ng/mL) than at 6 wk of age (STI 3.5 ± 0.2 ng/mL, LTI 3.2 ± 0.9 ng/mL), when they reached maximum levels. At 9 wk (STI 1.5 ± 0.6 ng/mL, LTI 2.5 ± 1.5 ng/mL) and 12 wk of age (STI 2.4 ± 1.7 ng/mL, LTI 1.8 ± 0.5 ng/mL), basal B levels were intermediate and did not differ significantly from any of the previously measured levels. In male quail of both genotypes, basal B levels were lower at 4 wk (STI 1.1 ± 0.3 ng/mL, LTI 1.3 ± 0.5 ng/mL) than at any of the other ages tested (6 wk: STI 4.0 ± 0.7 ng/mL, LTI 2.9 ± 0.7 ng/mL; 9 wk: STI

---

Table 1. Sexual maturity stages in male and female short tonic immobility (STI) and long tonic immobility (LTI) quail at 4 and 6 wk of age

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sex</th>
<th>Index</th>
<th>Age</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTI</td>
<td>Female</td>
<td>0</td>
<td>4wk</td>
<td>27</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>23</td>
<td>85</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>88/93</td>
<td>95</td>
</tr>
<tr>
<td>LTI</td>
<td>Male</td>
<td>0</td>
<td>4wk</td>
<td>0</td>
<td>0</td>
<td>1/64</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>27/45</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>14/45</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>4/45</td>
<td>9</td>
<td>63/64</td>
<td>98</td>
</tr>
<tr>
<td>STI</td>
<td>Female</td>
<td>0</td>
<td>4wk</td>
<td>1/37</td>
<td>3</td>
<td>3/102</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>36/37</td>
<td>97</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>0</td>
<td>0</td>
<td>3/102</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>96/102</td>
<td>94</td>
</tr>
<tr>
<td>STI</td>
<td>Male</td>
<td>0</td>
<td>4wk</td>
<td>2/42</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>14/42</td>
<td>33</td>
<td>1/59</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>25/42</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>1/42</td>
<td>2</td>
<td>58/59</td>
<td>98</td>
</tr>
</tbody>
</table>

1Developmental states of testes and ovaries were classified according to size: 0 = no distinct follicles or testes, 1 = small follicles and testes, 2 = intermediate size, and 3 = full adult size.

---

Figure 1. Corticosterone (B) concentrations (ng/mL of plasma) in LTI and STI quail at 4, 6, 9, and 12 wk of age. A) Basal B level. B) B level in quail following a 10-min restraint period in a crush cage. C) B levels in quail 10 min after i.m. injection of a representative volume of the vehicle (NaCl 9%). D) B levels in quail 10 min after i.m. injection of 1-24 ACTH (Immediate Synacthen, Novartis, France) at 10 μg/kg of BW (n = 6 in average; means ± SE). *Means with common superscripts are not statistically different ($P > 0.05$) for a specific line and sex group. **Means are significantly different from those of the corresponding controls ($P < 0.05$). Only significant interactions are reported in the table.
3.1 ± 1.0 ng/mL, LTI 4.4 ± 0.8 ng/mL; 12 wk: STI 3.4 ± 0.7 ng/mL, LTI 5.0 ± 0.5 ng/mL). On point-to-point comparison, basal B levels only significantly differed between sexes for the LTI genotype at 12 wk of age (P < 0.002).

**Corticosterone Response to Restraint**

With the exception of LTI quail of both sexes at the ages of 9 and 12 wk, restraint in a crush cage for 10 min induced a significant increase (P < 0.0001) in B levels compared with basal B levels in all groups at the different ages (Figure 1B). No significant effect of age (P = 0.42) was observed on B concentrations for this specific response, but the effects of genotype (P < 0.0001) and sex (P = 0.002) were significant. A significant interaction between genotype and sex was also revealed by the analysis (P = 0.005). With the exception of 12-wk-old males, restraint-induced B concentrations were significantly higher in STI quail than in LTI quail. On point-to-point comparison, restraint-induced B levels were significantly higher in female STI quail compared with males of the same genotype at 6 and 9 wk, but the differences did not reach significance at the other 2 ages tested for this genotype or at any age for the other genotype.

**Corticosterone Response to Capture and Saline Injection**

Significant effects of age (P < 0.001) and genotype (P < 0.001) were observed on B levels measured 10 min after a single injection of saline solution (Figure 1C). Although the increases were limited in amplitude, B levels measured after saline injection differed significantly from basal levels in 4-, 6-, and 9-wk-old STI female quail and in 12-wk-old STI males but not in LTI quail.

**Corticosterone Response to 1-24 ACTH Injection**

Quail of all ages responded to injection of 1-24 ACTH at a dose of 10 µg/kg of BW by a marked increase in B levels (P < 0.0001; Figure 1D). The age effect (P = 0.005) on the injection-induced rise in B differed significantly between genotypes, as reflected by the significant interaction observed between these 2 factors (age × genotype, P = 0.006). The B responses to injection did not differ with age in STI quail of either sex (P > 0.72), but B responses increased significantly with age in LTI quail of both sexes (P < 0.01). Thus, injection-induced B responses were significantly higher at 9 and 12 wk of age in female LTI quail than at 4 wk and were intermediate at 6 wk. In male LTI quail, injection-induced B responses were significantly higher at 12 wk compared with those measured at 4 and 6 wk and were intermediate at 9 wk. In females, differences between genotypes were significant (P = 0.01) at 4 and 9 wk; higher B responses were measured for STI quail. A similar significant effect was also observed in males at 6 wk of age, whereas the reverse situation was observed at 12 wk of age, with thus a significantly lower response (P = 0.05) for STI male quail. Adrenal responses to 1-24 ACTH injection were in all cases of higher amplitude than levels measured after restraint. The ratio of B response to restraint over B response to 1-24 ACTH injection at the different ages tested were <50% in LTI quail (4 wk, 27%; 6 wk, 43%; 9 wk, 16%; and 12 wk, 21%) and >50% in STI quail (4 wk, 62%; 6 wk, 52%; 9 wk, 66%; 12 wk, 77%).

**DISCUSSION**

The results from the present study demonstrate that age, sexual maturity status, and sex influence basal B levels and B responses to constraint and to 1-24 ACTH challenge differently in 2 genotypes of Japanese quail selected for LTI or STI (Mills and Faure, 1991). Evaluation of gonad development stages by anatomical study indicated that, under the lighting program used in the present study, most quail had reached sexual maturity by 6 wk of age. However, all females and most males were still sexually immature at 4 wk of age. Interestingly, among the males, the genotype selected for STI showed a more advanced stage of gonad development at 4 wk of age. Follicular development showed no difference in females at this specific age, but it cannot be excluded. Indeed, onset of egg laying had previously been shown to occur earlier in STI compared with LTI quail when raised under a constant 14L:10D lighting program (Minvielle et al., 2002). Similarly, early onset of puberty occurs in male Japanese quail from a genotype with a shorter duration of TI (Satterlee et al., 2002), although this genotype was selected for reduced adrenal responsiveness to brief immobilization (Satterlee and Johnson, 1988; Jones et al., 1992). Reduced adrenal responsiveness is thus associated with earlier acquisition of sexual maturity for this specific genotype. On the other hand, it has previously been shown (Remigngon et al., 1998; our unpublished data) and confirmed by the present results that quail with the STI genotype have a higher B response to constraint at 6 wk of age. Contradictory associations between HPA responsiveness to constraint and sexual maturation have thus resulted from 2 independent selection programs for fearfulness using behavioral (Mills and Faure, 1991) or physiological parameters (Satterlee and Johnson, 1988) as selection criterion. These results, indicating that the level of fearfulness or stress and production are related, are puzzling. Whatever the nature of the existing relationship, the results regarding gonad development will enable us to check for the effects of the combination of age and sexual maturity status factors on HPA axis function between 4 and 6 wk of age and then for the effects of age.

Interestingly, although female and male quail reached full gonad development between 4 and 6 wk of age, increases in basal B levels were also measured between these 2 stages. Hocking et al. also reported higher basal B levels in mature than in immature female broiler breeders. On the other hand, Schmeling and Nockels (1978) showed that sexually mature male and female chickens had lower basal B levels than immature birds. There is
thus no apparent consensus concerning the interrelation between regulation of basal B levels and sexual maturity in avian species. However, our present results strongly suggest that the increases in basal B level observed could probably be explained by the acquisition of sexual maturity in our genotypes. This hypothesis is supported by a previous report indicating the existence of comparable differences in basal B levels in quail of the same age (i.e., 6 wk of age) but sexually immature or mature (our unpublished data). Although gonadal steroid levels were not measured in the present study, we can speculate that circulating levels of estrogens and androgens were higher in mature than in immature quail. It can therefore be further hypothesized that the increases in gonadal steroids resulting from sexual maturity could enhance the production and release of basal B in quail. Evidence of the direct effects of gonadal steroids on adrenal enzyme activities and of the indirect effects on adrenal function via the hypothalamic-pituitary gland axis has been previously reported in mammals (Kim et al., 1980). It has also been shown that treatment with estradiol significantly increased resting levels of ACTH and cortisol in adult rainbow trout (Pottinger et al., 1996) and that treatment with testosterone induced an increase in B levels in male passerine (Deviche et al., 2004) and juncos (Ketterson et al., 1991). Differential sex-dependent age effects were found on basal B levels, with significantly higher levels measured at 9 and 12 wk of age compared with the levels measured at 4 wk in males, whereas they did not differ significantly from levels measured at 4 and 6 wk of age in females. We cannot exclude the possibility that these variations could be directly due to changes in respective circulating levels of gonadal steroids or indirectly due to differences in metabolic regulatory processes.

In contrast to what was observed concerning basal B levels, B adrenal response capacity and HPA axis responsiveness were not significantly affected by the acquisition of sexual maturity occurring between 4 and 6 wk of age. In contrast, increases in plasma cortisol levels in response to a brief period of confinement are reduced in sexually mature male rainbow trout compared with immature trout (Pottinger et al., 1995; Pottinger et al., 1996). This reduced responsiveness of the pituitary-adrenal axis to stress was thought to result from elevated androgen levels. Similarly, androgen treatment induced decreases in ACTH and B levels after stress in rats (Handa et al., 1994) and limited increases in B levels in response to human approach, to a surprising event, and to fear conditioning in heifers (Boissy and Bouissou, 1994). Thus, conversely to what is reported in these species, a putative increase in circulating gonadal steroid levels resulting from the acquisition of sexual maturity did not seem to have any major impact on the HPA axis response to constraint or B adrenal response capacity in our 2 quail genotypes.

This does not exclude the possibility that this contradiction may arise from confusing effects of sexual maturity stage and age. Indeed, although the overall age effect on the response to constraint did not appear to be significant, the B responses differed significantly from respective basal control levels at 4 and 6 wk but not at 9 and 12 wk in LTI quail of either sex. We cannot exclude the possibility that a significant response might have been recorded in less than 10 min as previously reported during manual restraint in laying hens (Korte et al., 1997). However, in such a case, the overall increase in B levels would then have been of lower amplitude for the LTI genotype compared with that of the STI genotype. Furthermore, because the responses remained stable at the different ages in both sexes of STI quail but not for LTI quail, the present results clearly show differences in the evolution of HPA axis reactivity with age, depending upon the genotype. These differences should not have been due to a familiarization or habituation process (Jones et al., 2000; Guémené et al., 2001a, 2003; Servière et al., 2002) with this particular fearful situation because the quail were only subjected to this specific test once during their life span. On the other hand, LTI quail are potentially more sensitive to any stressful event that can occur during the rearing period, and thus any prior stressful event might have increased their subsequent capacity to cope with different stressful situations and have limited their HPA axis activation. Such a hypothesis could also explain the large between-genotype difference in responsiveness to a first constraint observed in the present study, as in previous studies (Remignon et al., 1998; our unpublished data). Indeed, placement in a crush cage for 10 min induced greater increases in B levels in STI quail than in LTI quail. Differences in responsiveness between genotypes were also observed in the present study for milder stress, consisting of the manipulation and the injection of a saline solution. We cannot exclude the possibility that these differences resulted from differences in adrenal sensitivity, but this remains to be explored by establishing specific dose-response curves. Alternatively, these between-genotype differences in responsiveness do not result from differences in B adrenal response capacity because no relationship between overall adrenal capacity and response to constraint was observed in LTI quail. Interestingly, although the overall adrenal response capacity remained high and stable at the different ages tested for the STI genotype, it increased with age in LTI quail. Consequently, B adrenal response capacities to 1-24 ACTH injection were similar for all experimental groups at the ages of 9 and 12 wk but were lower at 4 wk for the LTI genotype. The ratio between responses to physical tests and pharmacological challenges differed significantly between the 2 genotypes at specific ages and with age within a genotype. These findings clearly indicate that age affects adrenal responsiveness differently, depending upon the genotype as shown in rats (Johnston et al., 1974; Ait-Chaoui et al., 1995). Very interestingly, although it remains to be confirmed using different experimental approaches, it can be hypothesized that the HPA axis and the adrenal gland itself are functionally mature at the age of 4 wk in STI quail under our specific experimental conditions but not in LTI quail. The B responses to constraint never reached the maximal adrenal response capacity at any age in quail under our experimental conditions. Similarly, differences
in responsiveness after physical stress and pharmacological challenge have been reported for ducks (Faure et al., 2003), and the resulting responses were of similar amplitudes in broilers (Guémené et al., 2005) and laying hens (D. Guémené, unpublished data). Such specificity may result from differences in the physiological mechanisms underlying HPA axis function. Taking this hypothesis into consideration as well as the results of the present study, it is likely that the differences in HPA axis responsiveness to acute stress and changes with aging observed between the LTI and STI genotypes involve upstream structures of the HPA axis (hypothalamus, pituitary gland, or more central structures) instead of the adrenal glands. To prove such a hypothesis, it would be helpful to explore gene expression and synthesis rates of key peptides potentially involved in the cascade that results in B secretion. However, it would also be necessary to perform complementary studies to extend our knowledge regarding adrenal function itself.

In conclusion, acquisition of sexual maturity appears to be associated with increased basal B secretion, but it has no evident effect on the HPA axis response to acute physical stress and B adrenal response capacity. On the other hand, age influences HPA axis responsiveness in LTI quail but not in STI quail. It was thus hypothesized that functional HPA axis maturity is achieved at 4 wk of age for the LTI genotype, and it occurs later for the STI genotype under our experimental conditions. The divergent selection program undertaken on TI duration in quail (Mills and Faure, 1991) may therefore have resulted in changes in the developmental rate and function of adrenal glands and the HPA axis.

ACKNOWLEDGMENTS

We thank A. D. Mills, who managed the selection program with J. M. Faure and provided the quail used in this study. We also thank D. Raine for a valuable contribution to improving the quality of the manuscript. The authors also thank the many people who contributed to this study, especially J.-M. Hervouet and J.-M. Brigand for expert technical assistance. D. Hazard was supported by grants from Institut National de la Recherche Agronomique and the Conseil Régional de la Région Centre for completion of a Ph.D.

REFERENCES


