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ScienceDirect

General and Comparative Endocrinology 155 (2008) 288–297

GENERAL AND COMPARATIVE
ENDOCRINOLOGY

www.elsevier.com/locate/ygcen

Intensity and duration of corticosterone response to stressful situations in Japanese quail divergently selected for tonic immobility

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Received 6 December 2006; revised 26 April 2007; accepted 7 May 2007

Abstract

Two genotypes of Japanese quail have been divergently selected since the 1980s for long (LTI) or short (STI) duration of tonic immobility [Mills, A.D., Faure, J.M., 1991. Divergent selection for duration of tonic immobility and social reinstatement behavior in Japanese quail (*Coturnix coturnix japonica*) chicks. J. Comp. Psychol. 105(1), 25–38.], an unlearnt catatonic state characteristic of a behavioral fear response ([Jones, R.B., 1986. The tonic immobility reaction of the domestic fowl: a review. World's Poult. Sci. J. 42(1), 82–97.]; [Mills, A.D., Faure, J.M., 1991. Divergent selection for duration of tonic immobility and social reinstatement behavior in Japanese quail (*Coturnix coturnix japonica*) chicks. J. Comp. Psychol. 105(1), 25–38.]). The results of several behavioral tests conducted in LTI and STI quail have led to the conclusion that LTI quail are more fearful than STI quail [Faure, J.M., Mills, A.D., 1998. Improving the adaptability of animals by selection. In: Grandin, T. (Eds.), Genetics and the behavior of domestic animals. Academic Press, San Diego, pp. 235–264.]). However, few studies to date have focused on the Hypothalamic-Pituitary-Adrenal (HPA) axis response to stressful situations in LTI and STI quail, although the HPA axis is involved in fear responses [Siegel, H.S., 1971. Adrenals, Stress and the Environment. World's Poult. Sci. J. 27, 327–349.]. The corticosterone (CORT) response to various putatively stressful situations was therefore assessed in LTI and STI genotypes of quail in order to investigate their HPA axis reactivity to stress.

Repeated induction of TI or 1 min manual restraint induced significant and comparable increases in CORT levels in both genotypes as a TI response. On the other hand, higher CORT responses were found in STI than in LTI quail when the manual restraint period lasted for 2 min or after restraint in a crush cage. Maximum CORT responses and genotype differences were maintained throughout the latter test even when it lasted for 120 min. Investigation of the CORT response to a single TI episode showed that CORT levels at the end of TI were negatively correlated with TI duration. Other experimental contexts consisting of isolation in a familiar or novel environment or the presentation of a novel object induced slight but significant and comparable increases in CORT response in both genotypes, whereas change of cagemates did not.

In conclusion, the present findings indicate that differences in HPA axis response are observed between LTI and STI genotypes when quail are submitted to intense stressors, resulting in a high and prolonged CORT response. By contrast, plasma corticosterone concentrations do not differ between STI and LTI quail in response to stressful situations of lower intensity, which evoke responses limited in amplitude and duration. Genetic selection for divergent duration of TI has thus affected the HPA axis response to stress, and LTI and STI quail constitute an interesting model to investigate genetic variability of HPA axis activity in birds. More specifically, these genotypes of quail could be used to investigate the occurrence of functional differences at different levels of the HPA axis in order to explain the present findings.

Keywords: HPA axis; Corticosterone; Stress; Quail; Tonic immobility

1. Introduction

Several genetic selection programs have been set up for different fear–related behavioral traits in mammals (rats,

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doi:10.1016/j.ygcen.2007.05.009

Bignami, 1965; mice, DeFries et al., 1978) and in birds (chickens (Faure and Folmer, 1975); pheasants (Boyer et al., 1973); quail (Mills and Faure, 1991). The results of these selection programs indicate that animals from divergent genotypes have different behavioral and physiological responses to fearful situations. Moreover, studies in rodents have reported coherent sets of behavioral and physiological responses which are consistent over time and across situations, better known as coping styles (Koolhaas et al., 1999). Thus, rats may be classified as proactive or reactive, depending on the way they respond to stressors. Similar classifications have been established in other species, notably in pigs (Hessing et al., 1994), hens (Korte et al., 1997) and great tits (Groothuis and Carere, 2005). Some animals react in an active fashion (fight or flight) while others are more likely to show inhibited behavior during the acute fear response (Koolhaas et al., 1999). The latter cease all ongoing activities and immediately freeze when a source of danger is detected, thereby reducing the likelihood of detection and attack from a predator. In such situations, they may show a tonic immobility (TI) response if caught (feigning death) (Jones, 1986; Gentle et al., 1989). TI has therefore been described as an unlearnt catatonic state which is thought to be the final stage in a chain of anti-predator behavior patterns (Jones, 1986; Mills and Faure, 1991).

Two genotypes of Japanese quail have been divergently selected since the 1980s for long (LTI) or short (STI) duration of tonic immobility (Mills and Faure, 1991). Several behavioral tests conducted in LTI and STI quail demonstrated that quail which show a long duration of TI response are more fearful than quail which show a short duration of TI response, and that duration of the TI reaction is a reliable indicator of underlying fearfulness (Jones and Mills, 1983; Mills and Faure, 1986; Jones et al., 1991; Faure and Mills, 1998). LTI and STI quail have therefore been widely used as a biological model to investigate fear-related behavioral responses in birds, but these quail also constitute an appropriate model to study the putative relationship between fearfulness and Hypothalamic–Pituitary–Adrenal (HPA) axis reactivity to stress. Indeed, elicitation of instant and adequate fear responses requires activation of the HPA axis and in the final stage release of corticosteroids (corticosterone in birds) from the adrenal glands (Siegel, 1971, 1980; Harvey and Hall, 1990), which are required to re-establish homeostasis via feedback mechanisms and to facilitate behavioral adaptation. Moreover, corticosteroids have been shown to influence the consolidation and potentiation of fear as well as the facilitation or extinction of avoidance in mammals (Schulkin et al., 1994, 1998).

A line of Japanese quail divergently selected for their high corticosterone (CORT) response to restraint (Satterlee and Johnson, 1988) has been shown to exhibit the longest duration of TI (Jones et al., 1992; Satterlee et al., 1993). On the other hand, STI quail have been reported to exhibit higher CORT levels than LTI quail in response to restraint stress (Jones et al., 1994; Rémignon et al., 1998; Hazard et al., 2005a). However, only partial and rudimentary investigations into the CORT response to potentially stressful situations have been performed to date in LTI and STI quail (Launay, 1993; Jones et al., 1994; Faure et al., 1996; Rémignon et al., 1998; Hazard et al., 2005a) and the results have been inconclusive. This is partly due to the lack of consistency in the experimental designs used (generation number, age, sex, rearing conditions, sampling interval...). This can be a very important issue, since CORT levels are very sensitive to any variation in physiological and environmental factors (Hazard et al., 2005a,b). It is therefore impossible from previous studies to reach firm conclusions about differences in the CORT response to stress between LTI and STI genotypes. The aim of the present study was to asses CORT responses in LTI and STI genotypes of quail submitted to a number of putatively stressful situations under careful and standardized experimental conditions in order to characterize HPA axis reactivity to stress in these genotypes and to explore further the possible effects of genetic selection for a fear-related behavioral response on HPA axis reactivity to stress.

Duration of TI has been reported to be significantly prolonged when circulating CORT concentrations are increased by administration of CORT in domestic fowl (Jones et al., 1988). Moreover, greater TI responses have been reported to be associated with high endogenous levels of CORT in mammals (Carli et al., 1979; Kalin et al., 1998). Therefore, we first investigated the putative relationship between TI responses (i.e. the selection parameter) and CORT responses in LTI and STI genotypes.

Then, CORT response patterns to various physical and social stressors were investigated in LTI and STI quail. The stressors consisted of restraint in a crush cage, presentation of a novel object, isolation in a familiar or a novel environment and change of cagemates. These situations were chosen because they have previously been shown to induce CORT release in birds and mammals (Seggie and Brown, 1975; Launay, 1993; Buijs et al., 1997; Ruis et al., 2001; Carobrez et al., 2002; Hazard et al., 2005a). Moreover, it was anticipated that studying the responses to isolation and placing in a novel environment might provide greater understanding of responses to restraint, since both of these stressors are components of the restraint test.

2. Materials and methods

2.1. Animals and rearing conditions

Six-week-old Japanese quail (*Coturnix japonica*) from the 36th generation of two genotypes divergently selected for short (STI) or long (LTI) duration of tonic immobility (TI) were used in the study (Mills and Faure, 1991). Eight successive hatches were necessary to provide the quail used in the present study and the quail used for each specific test were from a single hatch. Quail were identified by wing-banding on the day of hatching. Quail were exposed to continuous light until 3 weeks of age, then to a 16 h light/8 h dark (16L/8D) (light on 6.00 am) rhythm. The quail from the different genotypes and experimental groups were reared in different collective battery cages. The caretaker checked the quail daily in the