

Doctoral Thesis

Hormonal and Molecular Study of
Chicken Aggressive Behavior

(Summary)

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Summary

Aggressive behavior is observed in almost all animal species. It is an innate social behavior which is associated with conflict between two individuals. Aggressive behavior is supposed to be essential to winning the competition for limited resources like mate, territory, feed, as well as for getting social dominance position within a group. On the contrary, excessive chicken aggression results in reduced production which leads to great economic losses in poultry industry. Furthermore, as aggressive behavior of chicken causes injury and stress during poultry operations it is also an important issue for animal welfare. For the purpose of higher quality and quantity of poultry productions and animal welfare, it is necessary to elucidate the mechanisms of chicken aggressive behavior and make countermeasures against severe aggression of chickens in the poultry industry. This doctoral thesis makes attempts to elucidate the mechanisms of chicken aggressive behavior in both hormonal and molecular perspectives.

1. Comparison of testosterone-induced aggressive behavior between isolated- and grouped-raised male layer chicks by social interaction (SI) test

Testosterone (T) is known to induce aggressive behavior, mainly in male animals. Subcutaneous implantation of the T-filled silastic tubes, rather than intramuscular injection of T, is generally recommended for the long-term treatment of exogenous T. However, the effect of T implantation on chicken aggressive behavior has not been investigated. In addition, it is not known what concentration of T is sufficient to induce aggressive behavior or whether rearing conditions, like isolated- or grouped-raising, affect T-induced aggressive behavior in chickens. The present study, therefore, aimed to examine the relationship between the lengths of T-filled tubes, blood T concentration, and aggressive behavior in group- and

isolation-raised male layer chicks respectively. The testes were bilaterally removed and silastic tubes of various lengths filled with crystalline T were subcutaneously implanted at 14 days of age. A social interaction (SI) test was performed to quantitatively assess chicken aggressive behavior at 32 days of age. Comb weight and size were used to assess the activation of endogenous androgen receptors. Total aggression frequencies (TAF) and aggression establishment rate (AER) were used to evaluate aggressiveness. In the present study significant positive correlations ($P<0.001$) were observed between the comb parameters and plasma T concentration. In the isolation-raised chicks, the TAF and AER were high regardless of the lengths of the implanted T tubes or the corresponding plasma T concentrations. In the group-raised chicks, however, there was a trend for differences of the AER between the T-implanted aggressors ($P=0.0902$), and the AER significantly increased with implantation of 1.0 cm long T-filled tubes ($P<0.05$), which corresponded to approximately 47 pg/ml of plasma T concentration. These results suggest that both grouped raising and approximately 47 pg/ml of plasma T concentration are needed for the induction of T-dependent aggressive behavior, and that isolation-induced aggressive behavior is T-independent in male layer chicks.

2. The relationship between T and territorial or isolation-induced aggressive behavior in male layer chicks

Our results suggest that isolation-induced aggressive behavior is T-independent. However, the concentration of blood T in the castrated chicks, probably derived from adrenal gland, is approximately 24 pg/ml, it is impossible to exclude completely the effect of lower concentration of blood T on chicken aggressive behavior only by castration. In this chapter, flutamide, a non-steroidal antiandrogen, was subcutaneously implanted in the castrated male

layer chicks to examine the relationship between T and territorial or isolation-induced aggressive behavior. Aggressive behavior between intact, castrated, and flutamide-implanted castrated male chicks was monitored with resident-intruder (R-I) and SI tests, respectively. TAF and AER were used as indices of aggressive behavior of the chicks. Both castration and flutamide implantation did not affect AER in the R-I test, but in the SI test the AER tended to increase in the intact chicks ($P<0.1$) and decrease in the flutamide-implanted castrated male chicks ($P<0.1$). These results suggest that T affects aggression occurrence of isolation-induced aggression, not of territorial aggression, and non-testicular T could play a role in stimulating isolation-induced aggression in male layer chicks.

3. Microarray screening of the genes that are related to aggressive behavior in the hypothalamus of male layer chicks

Plenty of researches strongly indicate that the hypothalamus is one of the most important brain regions associated with aggressive behavior in mammals. Previous studies showed that electrical stimulation of the hypothalamus induced violent attacks in rats, and immunostaining of c-Fos, as a marker of neuronal activation, revealed that aggression resulted in activation of various nuclei of the hypothalamus of rodents. A recent study also showed that the optogenetic stimulation of a specific hypothalamic nucleus, named ventrolateral subdivision of the ventromedial hypothalamus, induced aggressive behavior in mice toward intruders, and optogenetic silencing of the neurons in this nucleus reversibly suppressed aggressive behavior. Hypothalamus is also reported to play an essential role in inducing aggressive behavior in avians, but information about the genes regulating aggressive behavior in the avian hypothalamus is lacking. In this chapter, therefore, the aim of the research was to identify the candidate genes that are related to aggressive behavior in

chicken hypothalamus using microarray technique. Aggressive behavior of the chicks was monitored for 5 min with R-I or SI test, respectively, and after 30 min of the behavioral test the brain blocks including hypothalamus were collected. Total RNA from the blocks were extracted with RNeasy Mini Kit (QIAGEN) according to the manufacturer's instruction, and gene expression between the samples was detected with *Gallus* (chicken) oligo DNA microarray (Agilent). The result revealed an overlap of gene expressions in the hypothalamus between the aggressive residents in the R-I test and aggressor chicks in the SI test. The 38 genes were commonly up-regulated and the 24 genes were commonly down-regulated in both the R-I and SI tests. Among the overlap genes of the two behavior tests, about 16% are related to cellular signaling pathway associated genes, 11% are metabolism, 10% are immunoglobulin superfamily proteins or immunoglobulin receptors, 5% are ubiquitin system, and 3% of the genes are involved in neural activity. The present results suggest that the genes which change their expression by aggression play an important role in the regulation of aggressive behavior in the hypothalamus of male layer chicks. And the aggressive behavior of chickens is supposed to be regulated by complex gene regulation circuits.

CONCLUSION

The present study suggests that in group-raised condition approximately 47 pg/ml of plasma T concentration is sufficient for the induction of T-dependent aggressive behavior. It also suggests that T has no effect on the occurrence of territorial aggressive behavior but affects the occurrence of isolation-induced aggression in male chickens. Furthermore, candidate genes involved in chicken aggressive behavior under R-I and SI behavior tests have been listed up, which may help understanding of the molecular mechanisms in chicken aggressive behavior.