Studies on the Functions of Central Neuropeptide Y in the Regulation of Peripheral Immune Organs with Special Emphasis to the Spleen in Heat-Exposed Chicks

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論文題名　：　Studies on the Functions of Central Neuropeptide Y in the Regulation of Peripheral Immune Organs with Special Emphasis to the Spleen in Heat-Exposed Chicks
(暑熱暴露ニワトリヒナの末梢免疫器官、特に脾臓の制御における中枢神経ペプチドYの役割に関する研究)

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論　文　内　容　の　要　旨

The global warming is one of the serious worldwide issues which causes numerous problems including high ambient temperature (HT) leading to heat stroke problems for living organisms. Livestock and poultry farming is adversely affected by HT. It is well known that chickens are very sensitive to HT. Our previous study showed that neuropeptide Y (NPY) mRNA expressions were increased in chick brain due to HT. Furthermore, when chicks were centrally injected with NPY and exposed to HT then it was found that some adverse effects of heat stress were attenuated in chicks. Brain NPY is well known for its involvement in the regulation of feeding behavior. However, there was no report on the central functions of NPY in the regulation of peripheral immune organs under heat stress. Heat stress induces oxidative stress, decreases anti-oxidative functions and immunity in chickens. The present study aimed to elucidate the functional role of central NPY in the regulation of peripheral immune organs, particularly spleen under heat stress in chicks.

First, I focused on the effects of NPY in the spleen and liver functions, which are important peripheral organs in the chick related to immune functions. A single dose of NPY (375 pmol) was injected into the chick (6 days old) brain and exposed to either control thermoneutral temperature (CT: 30 ± 1℃) or HT (35 ± 1℃) for 1 h. The rectal temperature was significantly declined in chicks by central injection of NPY under CT, but not HT, as reported elsewhere (Bahry et al., 2017). On the other hand, the mRNA expressions of heat shock protein-70, which is a heat stress marker, were attenuated and glutathione synthetase, which is the antioxidative stress marker, were increased in the chick spleen under HT due to the central injection of NPY. These results suggested that the central injection of NPY attenuated the splenic heat stress response and enhanced its antioxidative status under HT.

Second, the possible mechanism of masking the hypothermic role of NPY under HT was investigated. A very high dose of NPY (1 nmol) was injected into the chick (6 days old) brain and exposed to either CT (30 ± 1℃) or HT (40 ± 1℃) for 2 h. The rectal temperature was significantly reduced in chicks under CT by central NPY; however, the high dose caused a further increment of rectal temperature under HT. When the high dose of NPY was injected into the chick's brain then the mRNA expressions of melanocortin receptor 4 (MC-4R) were significantly increased, and corticotropin-releasing hormone receptor 2 (CRH-2R), γ-aminobutyric acid (GABA) receptor Aβ2 and Aγ were tended to increase under CT and HT. Furthermore, diencephalic dopamine (DA) concentration was significantly increased under CT and HT and DA conversion to 3,4-dihydroxyphenylacetic acid (DOPAC) tended to decrease under HT by central injection of NPY. To investigate the regulation of DA and DOPAC metabolism from amino acid due to central NPY, an experiment was conducted additionally under HT. Two doses of NPY (47 pmol and 188 pmol) were injected into the chick (6 days old) brain and exposed to HT (35 ± 1℃) for 1 h. The rectal temperature and plasma corticosterone

concentrations were not changed by central NPY injection compared to the saline injected chicks. However, diencephalic tyrosine concentration was significantly increased by the moderate dose of NPY (188 pmol) injection under HT. Furthermore, diencephalic 3-methyl histidine concentration tended to increase by high dose of NPY under HT. These results suggest that central NPY might have stimulated brain tyrosine concentration and DA synthesis as we as enhanced protein breakdown. The increased brain DA, and some central food intake and stress regulatory receptors, namely MC-4R, CRH-2R and GABA-Aβ2 and Aγ may contribute to induce thermoregulatory functions and mask the hypothermic role of NPY under HT.

Third, the mRNA expression of NPY receptors (Y1, Y2, Y4, Y5, Y6 and Y7) were examined in the spleen, thymus, and bursa of Fabricius. Chicks (6 days old) were exposed to acute (40 ± 1℃ for 3 h) or chronic (40 ± 1℃ for 3 h/d for 3 days) HT to examine the NPY receptors expression in peripheral immune organs mentioned above. Rectal temperatures were significantly increased by both acute and chronic exposure to HT. Interestingly, NPY-Y4 receptor mRNA expression was found only in the spleen, which was increased by acute ($P<0.05$) and chronic ($P<0.0005$) exposure to HT. Furthermore, immunohistochemical localization of NPY-Y4 was also found in the spleen. These results indicate that NPY-Y4 may be a vital receptor to convey the action of NPY or its related peptides (peptide YY and/or pancreatic polypeptide) to regulate splenic functions under heat stress.

Fourth, the highest dose of NPY (1 nmol) was injected into the chick (6 days old) brain and exposed to either CT (30 ± 1℃) or HT (40 ± 1℃) for 2 h to examine the effect of central NPY on monoamine metabolism in the spleen. Serotonin and its metabolite, 5-hydroxyindolacetic acid were decreased in the spleen by HT. Epinephrine (E) concentration in the spleen was declined by the central injection of NPY under CT, but reverse was true under HT. Splenic 3-methods-4-hydroxy-phenyl-ethylene glycol, which is a metabolite of norepinephrine (NE), concentration increased under HT, which was further enhanced by central injection of NPY. These results suggest that splenic E and NE metabolism may be enhanced by central injection of NPY under HT possibly to regulate splenic immune functions as NE contributes to regulate immune functions in rat spleen (Vida et al., 2011).

In summary, this thesis showed that the central NPY can protect and regulate peripheral immune organ, spleen under HT, and provided valuable achievement contributing to avian physiology and avian immunology. Therefore, this thesis deserves a Ph.D. (Doctor of Agriculture) degree.