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Nitric oxide synthase inhibitor attenuates the anorexigenic effect of corticotropin-releasing hormone in neonatal chicks

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Abstract

Nitric oxide (NO) is known as an orexigenic factor in the brain of mammals and mediates the feeding-stimulatory effect of other factors such as neuropeptide Y (NPY). In neonatal chicks, however, we recently reported that NO might have an anorexigenic effect and suggested that the feeding-regulatory mechanism in chicks might be different from that in mammals regarding NO. In the present study, we investigated the involvement of NO in the effect of other orexigenic and anorexigenic factors in neonatal chicks. Intracerebroventricular co-injection of N^G-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, did not affect NPY- and prolactin-releasing peptide-induced feeding behavior. On the other hand, the co-injection of L-NAME significantly attenuated the anorexigenic effect of corticotropin-releasing hormone (CRH). The anorexigenic effects of glucagon-like peptide-1, alpha-melanocyte-stimulating hormone and ghrelin were not affected by the L-NAME treatment. These results suggest that NO might mediate the anorexigenic effect of CRH in the brain of neonatal chicks.

Keywords: Corticotropin-releasing hormone; Feeding; Intracerebroventricular injection; N^G-nitro-L-arginine methyl ester; Neonatal chicks; Neuropeptide Y; Nitric oxide

1. Introduction

Nitric oxide (NO) is recognized as one of feeding-regulatory factors in the brain of mammals. Since central administration of NO synthase (NOS) inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), consistently decreased food intake in rats (De Luca et al., 1995), NO was expected to stimulate feeding behavior in the brain. In addition, several studies revealed that neuropeptide Y (NPY) (Morley et al., 1999), ghrelin (Gaskin et al., 2003) and orexin-A (Farr et al., 2005) stimulated feeding behavior via NO system in mammals. Similar results were also observed in avian species: intracerebroventricular (ICV) or intraperitoneal administration of L-NAME decreases food intake (Choi et al., 1994) and L-NAME attenuated the orexigenic effect of clonidine and neuropeptide Y (Choi et al., 1995; Bungo et al., 2000). Thus, NO is likely to play an important role in the stimulation of feeding beyond the animal species.

In neonatal layer chicks, however, ICV injection of 400 nmol L-NAME stimulated the feeding behavior (Khan et al., 2007). In the case of neonatal broiler chicks, this dose was not sufficient to stimulate feeding behavior (Khan et al., 2007), but our preliminary experiment revealed that increased level of L-NAME (800 nmol) stimulated the feeding behavior (not published). These results suggested that NO might inhibit feeding behavior in neonatal chicks in contrast to mammals and older chickens. These results also suggested that NO might be related to anorexigenic factors rather than orexigenic factors such as NPY. However, little is known about the relationship between NO and other feeding-regulatory factors in neonatal chicks.

The purpose of the present study was to examine whether NO was related to the effect of orexigenic or anorexigenic neuropeptides in neonatal chicks. We used NPY and prolactin-releasing peptide (PrRP) as orexigenic neuropeptides (Kuenzel et al., 1987; Tachibana et al., 2004), and corticotrophin-releasing hormone (CRH), glucagon-like peptide-1 (GLP-1), alpha-melanocyte-stimulating hormone (alpha-MSH) and ghrelin were used as anorexigenic neuropeptides (Furuse et al., 1997a,b; Kawakami et al., 2000; Saito et al., 2002).

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2. Materials and methods

2.1. Animals

Day-old male broiler chicks (Chunky, *Gallus gallus*, Mori Hatchery, Kagawa, Japan) were kept in a windowless room maintained at 28 °C and provided 24 h lighting. The chicks were given free access to water and a commercial diet (Toyohashi Feed Mills Co. Ltd., Aichi, Japan). They were maintained in accordance with the recommendations of the National Research Council (1996). Chicks were placed in individual cages one day prior to the experiments. They were weighed and divided into experimental groups as uniform as possible in each treatment.

2.2. Drugs and ICV injection

L-NAME, D-NAME (Sigma Aldrich, St. Louis, MO, USA), bovine NPY, rat PrRP, chicken CRH, chicken GLP-1, alpha-MSH and rat ghrelin (Peptide Institute, Osaka, Japan), were dissolved in a saline containing 0.1% Evans Blue. This solvent was used as the control treatment for each experiment. The doses of neuropeptides and L-NAME (or D-NAME) were 21 pmol and 400 nmol, respectively. These doses were decided according to the previous studies (Saito et al., 2002; Tachibana et al., 2004; Tomonaga et al., 2005; Tachibana et al., 2006, 2007; Khan et al., 2007).

The ICV injection was performed according to the method reported previously (Davis et al., 1979). Briefly, the head of the chick was inserted into an acrylic box which had a hole at the top plate. A microsyringe was then inserted into the lateral ventricle through the hole and the drug solution was injected at a volume of 10 μL. This procedure had been defined as a non-stressful method for neonatal chicks (Furuse et al., 1999; Saito et al., 2005). The ICV injection was done under an ad libitum feeding condition. The food intake was determined at 60 min after the injection by measuring the reduction of diet from a pre-weighed feeder. The feeders were weighed using an electric digital balance of precision ±1 mg.

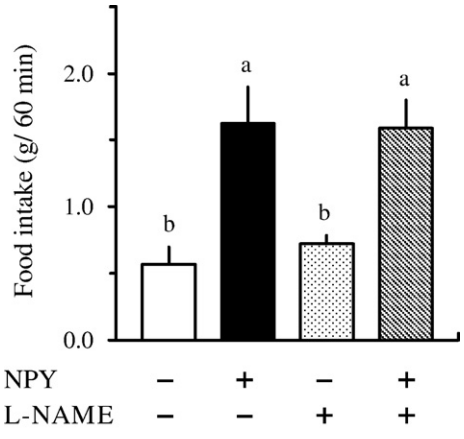

Fig. 1. Effect of ICV co-injection of L-NAME on NPY-induced feeding behavior in neonatal chicks. The injected doses of NPY and L-NAME are 21 pmol and 400 nmol, respectively. Data are expressed as means±SEM. The number of chicks in each group was as follows: control, 9; NPY, 7; L-NAME, 9; NPY plus L-NAME, 9. Groups with different letters are significantly different ($P<0.05$).

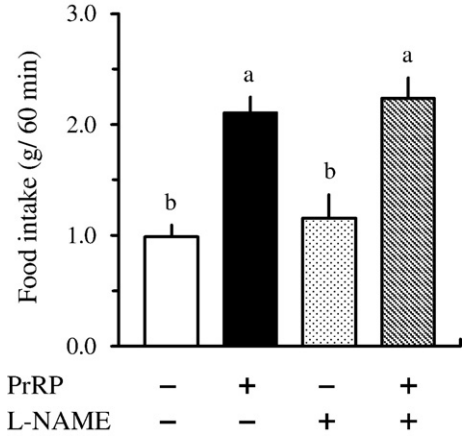

Fig. 2. Effect of ICV co-injection of L-NAME on the orexigenic effect of PrRP in neonatal chicks. The injected doses of PrRP and L-NAME are 21 pmol and 400 nmol, respectively. Data are expressed as means±SEM. The number of chicks in each group was as follows: control, 7; PrRP, 8; L-NAME, 8; PrRP plus L-NAME, 9. Groups with different letters are significantly different ($P<0.05$).

At the end of each experiment, chicks were sacrificed with overdose of pentobarbital. The brain was then removed to confirm the accuracy of drug injection. Chicks which did not show Evans Blue dye in the ventricle were not used for further analyses.

2.3. Effect of L-NAME on the orexigenic effects of NPY and PrRP

In the NPY study, 2-day-old chicks were injected with saline (control), NPY, L-NAME or NPY plus L-NAME. The number of chicks in each group was as follows: control, 9; NPY, 7; L-NAME, 9; NPY plus L-NAME, 9.

In the PrRP study, 3-day-old chicks were injected with PrRP instead of NPY. The number of chicks in each group was as follows: control, 7; PrRP, 8; L-NAME, 8; PrRP plus L-NAME, 9.

2.4. Effect of L-NAME on the anorexigenic effects of CRH, GLP-1, alpha-MSH and ghrelin

In the CRH study, 2-day-old chicks were injected with saline (control), CRH, L-NAME or CRH plus L-NAME. The number of chicks in each group was as follows: control, 6; CRH, 6; L-NAME, 5; CRH plus L-NAME, 7.

In the GLP-1 study, 4-day-old chicks were injected with GLP-1 instead of CRH. The number of chicks in each group was as follows: control, 8; GLP-1, 6; L-NAME, 7; GLP-1 plus L-NAME, 8.

In the alpha-MSH study, 2-day-old chicks were injected with alpha-MSH instead of CRH. The number of chicks in each group was as follows: control, 7; alpha-MSH, 6; L-NAME, 8; alpha-MSH plus L-NAME, 9.

In the ghrelin study, 2-day-old chicks were injected with ghrelin instead of CRH. The number of chicks in each group was as follows: control, 9; ghrelin, 9; L-NAME, 10; ghrelin plus L-NAME, 10.

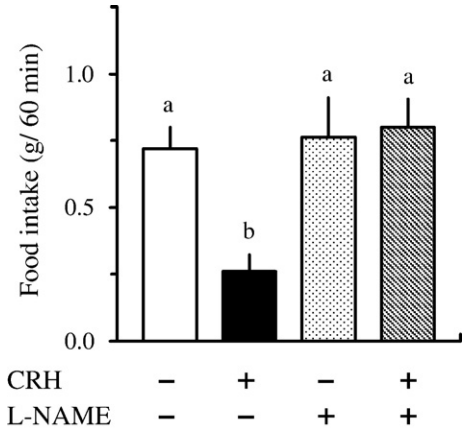

Fig. 3. Effect of ICV co-injection of L-NAME on the anorexigenic effect of CRH in neonatal chicks. The injected doses of CRH and L-NAME are 21 pmol and 400 nmol, respectively. Data are expressed as means±SEM. The number of chicks in each group was as follows: control, 6; CRH, 6; L-NAME, 5; CRH plus L-NAME, 7. Groups with different letters are significantly different ($P<0.05$).

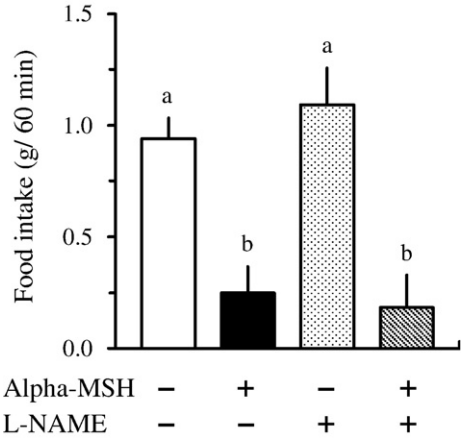

Fig. 5. Effect of ICV co-injection of L-NAME on the anorexigenic effect alpha-MSH in neonatal chicks. The injected doses of alpha-MSH and L-NAME are 21 pmol and 400 nmol, respectively. Data are expressed as means±SEM. The number of chicks in each group was as follows: control, 7; alpha-MSH, 6; L-NAME, 8; alpha-MSH plus L-NAME, 9. Groups with different letters are significantly different ($P<0.05$).

2.5. Effect of D-NAME on the anorexigenic effects of CRH

D-NAME, an inactive form of L-NAME (Moroz et al., 1996) was used to evaluate whether the attenuation of the anorexigenic effect of CRH by L-NAME was due to NOS inhibition. Two-day-old chicks were injected with saline (control), CRH, D-NAME or CRH plus D-NAME. The number of chicks in each group was as follows: control, 5; CRH, 8; D-NAME, 6; CRH plus D-NAME, 6.

2.6. Statistical analysis

Data were statistically analyzed with two-way analysis of variance (ANOVA) with respect to neuropeptides and L-NAME or D-NAME. The Tukey–Kramer test was then performed as a post-hoc test. Significant difference was set at $P<0.05$. Data are expressed as means±SEM.

3. Results

3.1. Effect of L-NAME on the orexigenic effects of NPY and PrRP

Figs. 1 and 2 show the effects of L-NAME on NPY- and PrRP-induced feeding, respectively. Central administration of NPY or PrRP alone significantly increased food intake while L-NAME alone did not affect. The co-injection of L-NAME did not alter the orexigenic effects of NPY and PrRP.

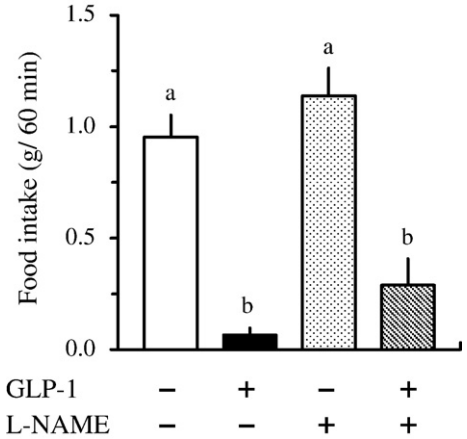

Fig. 4. Effect of ICV co-injection of L-NAME on the feeding-inhibitory effect of GLP-1 in neonatal chicks. The injected doses of GLP-1 and L-NAME are 21 pmol and 400 nmol, respectively. Data are expressed as means±SEM. The number of chicks in each group was as follows: control, 8; GLP-1, 6; L-NAME, 7; GLP-1 plus L-NAME, 8. Groups with different letters are significantly different ($P<0.05$).

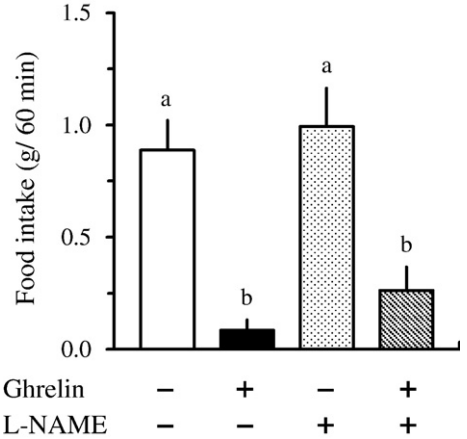

Fig. 6. Effect of ICV co-injection of L-NAME on the anorexigenic effect of ghrelin in neonatal chicks. The injected doses of ghrelin and L-NAME are 21 pmol and 400 nmol, respectively. Data are expressed as means±SEM. The number of chicks in each group was as follows: control, 9; ghrelin, 9; L-NAME, 10; ghrelin plus L-NAME, 10. Groups with different letters are significantly different ($P<0.05$).

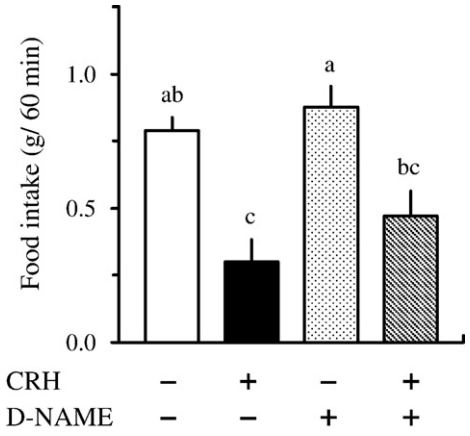

Fig. 7. Effect of ICV co-injection of D-NAME on the anorexigenic effect of CRH in neonatal chicks. The injected doses of CRH and D-NAME are 21 pmol and 400 nmol, respectively. Data are expressed as means ± SEM. The number of chicks in each group was as follows: control, 5; CRH, 8; D-NAME, 6; CRH plus D-NAME, 6. Groups with different letters are significantly different ($P<0.05$).

3.2. Effect of L-NAME on the anorexigenic effects of CRH, GLP-1, alpha-MSH and ghrelin

The effect of ICV co-injection of L-NAME on the anorexigenic effect of CRH is shown in Fig. 3. CRH alone significantly decreased food intake and the effect was significantly attenuated by the co-injection of L-NAME while L-NAME itself did not affect food intake. There was a significant interaction between CRH and L-NAME.

Figs. 4–6 show the effect of ICV co-injection of L-NAME on the anorexigenic effects of GLP-1, alpha-MSH and ghrelin, respectively. ICV injection of these neuropeptides significantly decreased food intake, while L-NAME alone had no effect. The anorexigenic effects of GLP-1, alpha-MSH and ghrelin were not affected by the co-injection of L-NAME.

3.3. Effect of D-NAME on the anorexigenic effects of CRH

Fig. 7 shows the effect of D-NAME on the anorexigenic effect of CRH. Central administration of CRH alone significantly decreased food intake while D-NAME itself did not affect. In contrast to L-NAME, there was no significant difference between CRH alone and CRH plus D-NAME.

4. Discussion

Recently, we reported that ICV injection of L-NAME stimulated feeding behavior of neonatal layer chicks (Khan et al., 2007). This result was opposed to those obtained from rats (De Luca et al., 1995), suggesting that NO might act as an anorexigenic factor in neonatal chicks. NO mediates the orexigenic effect of NPY in rats (Morley et al., 1999). ICV injection of NPY stimulated feeding behavior of neonatal chicks, but the orexigenic effect was not attenuated by NOS inhibition (Fig. 1). This result suggested that NPY-induced feeding in neonatal chicks was not related to NO in contrast to mammals. In mice, NO was reported to mediate the orexigenic effects of orexin-A (Farr et al., 2005) and ghrelin (Gaskin et al., 2003) as well as NPY (Morley et al., 1999). In neonatal broiler chicks, however, ICV injection of orexin-A did not stimulate feeding behavior (Furuse et al., 1999). In addition, ghrelin inhibited feeding behavior of neonatal broiler chicks (Fig. 6; Saito et al., 2002) in opposed to mammals (Nakazato et al., 2001). PrRP is thought to be one of orexigenic neuropeptide in neonatal chicks because ICV injection of this peptide consistently stimulates the feeding behavior (Fig. 2; Tachibana et al., 2004, 2005). The orexigenic effect of PrRP was not affected by co-injection of L-NAME (Fig. 2) as well as in the NPY study. Collectively, it is likely that NO is not related to the orexigenic mechanism in neonatal chicks.

In 4-week-old chickens, ICV injection of L-NAME decreased the food intake (Choi et al., 1994) in contrast to neonatal chicks (Khan et al., 2007). In addition, NO is expected to be related to the orexigenic effect of NPY in 4-week-old chicken because the co-injection of L-NAME attenuated the effect of NPY (Choi et al., 1995). These results are opposed to those of neonatal chicks, indicating that the role of NO on the feeding regulation is different depending on age. This idea is supported by the result that the effect of L-NAME on food intake was dependent on age in mice: it was effective in aged mice rather than young mice since the hypothalamic NOS activity was higher in aged mice than young mice (Morley et al., 1996). Similar difference was also observed in the case of leptin: ICV injection of leptin decreased food intake of chickens (Denbow et al., 2000) but not neonatal chicks (Bungo et al., 1999). The further research will clarify the difference in the role of NO between neonatal chicks and chickens in the future.

CRH is a peptide hormone and stimulates hypothalamic–pituitary–adrenal (HPA) axis as the hypothalamic signal (Vale et al., 1981). CRH is also recognized as one of anorexigenic neuropeptide in several animal species including neonatal chicks (Furuse et al., 1997b; Furuse, 2002). In the present study, we found that the co-injection of L-NAME significantly attenuated CRH-induced decrease in food intake (Fig. 3). The NOS inhibition attenuated the anorexigenic effect of CRH, indicating that CRH decreased feeding behavior via NO system. This observation was also supported by the result of D-NAME: ICV co-injection of D-NAME, an inactive form of L-NAME (Moroz et al., 1996) could not fully attenuated the anorexigenic effect of CRH (Fig. 7). In mammals, NO is demonstrated to be involved in the stimulation of HPA axis. For example, NOS inhibition suppressed the releases of adrenocorticotropic hormone and corticosterone induced by adrenergic and histaminergic agonists (Bugajski et al., 1999, 2000). From this fact, it is possible that NO is also related to HPA axis in neonatal chicks. We also examined the involvement of NO on the effects of GLP-1, alpha-MSH and ghrelin which are the anorexigenic neuropeptides in neonatal chicks (Furuse et al., 1997a; Kawakami et al., 2000; Furuse, 2002; Saito et al., 2002). The co-injection of L-NAME did not affect the anorexigenic effects of these peptides (Figs. 4–6 suggesting that NO was not involved in the anorexigenic effect of these neuropeptides. Collectively, it could be hypothesized that NO might be related to only anorexigenic effect of CRH in neonatal chicks.

In conclusion, the present study revealed that NO might mediate the anorexigenic effect of CRH in neonatal chicks. To our knowledge, this physiological role of NO is only proposed in neonatal chicks. There is evidence that the feeding-regulatory mechanism in the brain of neonatal chicks was different from mammals and chickens (Furuse, 2002). NO might play a key role for the unique feeding-regulatory mechanism in neonatal chicks.

Acknowledgements

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