

## Feed Intake after Infusion of Noradrenalin, Dopamine or its Precursor into the Lateral Ventricles in Neonatal Chicks

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**Abstract:** To clarify the role of catecholaminergic systems in the regulation of feeding behavior in the neonatal chick, we investigated the action of central Noradrenalin (NA), Dopamine (DA), L-DOPA and Tyrosine (Tyr) on feed intake. Each substance or saline was acutely administered into the lateral ventricle (ICV) of chicks. Tyr, L-DOPA and DA did not significantly affect feed intake compared with saline control over the 60 min experimental periods. However, ICV injection of NA significantly stimulated feeding behavior in chicks at 30 min postinjection. These results suggest that the central NAergic system is involved in the control of feed intake, whereas the large increments of Tyr, L-DOPA and DA in the brain of chicks may not be involved in NA induced hyperphagia.

**Key words:** Feed intake, noradrenalin, dopamine, L-DOPA, tyrosine, chicks

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### INTRODUCTION

Many peptides and neurotransmitters are important in the control of feed intake in birds, i.e., insulin (Shiraishi *et al.*, 2008), opioid (Bungo *et al.*, 2004, 2009), serotonin (Denbow *et al.*, 1983; Sashihara *et al.*, 2002) and  $\gamma$ -aminobutyric acid (Denbow, 1990; Bungo *et al.*, 2003). It is well known that catecholamines such as Noradrenalin (NA) or Dopamine (DA) are involved in feed intake control in animals (Wellman, 2005).

However, there are contradictory results for birds. For instance, central injection of NA decreased feed intake in the turkey (Denbow, 1989), whereas in the egg-type chicken (Denbow *et al.*, 1983) this effect on feed intake was not observed.

On the other hand, feed intake was enhanced by NA in the pigeon (Ravazio *et al.*, 1990) and the meat-type chicken (Denbow *et al.*, 1981). These feeding responses to catecholamine treatment is thought to be a consequence of artificial selection to produce fast-growing birds (Barbato, 1994; Kuenzel, 1994; Denbow, 1999) and to be associated with differences in catecholamine circuitry in the hypothalamus of these avian linkages (Kruzlock and Barbato, 1991). However, comparable data in neonatal chicks is controversial and limited. Therefore, to expand the current knowledge regarding the role of the catecholaminergic system in feed intake of neonatal chicks, the present study was designed to assess NA, DA and its precursor, Tyrosine (Tyr) or L-DOPA.

### MATERIALS AND METHODS

Day-old male layer chicks (Kudoh-sha Hatchery, Ehime, Japan; Akita Co. Ltd, Hiroshima, Japan) were kept in a windowless temperature-controlled room with 24 h lighting and maintained at a temperature of 30°C. They were given free access to a commercial starter diet (Nihon Nosan Kogyo, Yokohama, Japan; Nichiwa Sangyo Co. Ltd, Kobe, Japan) and water during the pre-experimental period.

The birds were reared individually in experimental cages and had *ad libitum* access to feed up to the time of experiments.

The handling of birds was performed in accordance with the regulations of the Animal Experiment Committee of Hiroshima University and the recommendations of the NRC (1985). They were distributed into experimental groups based on their body weight so that the average body weight was as uniform as possible for each treatment.

Birds (3-5 days old) fed *ad libitum* were given diet for 30 min immediately after treatment. Feed intake was determined by measuring the reduction of diet from a pre-weighed feeder. The weight of feeders was measured using an electric digital balance of precision  $\pm 1$  mg. All drugs (L-tyrosine methyl ester hydrochloride (Tyr), 3, 4-dihydroxyl-L-phenylalanine (L-DOPA), Dopamine Hydrochloride (DA) and L-noradrenalin

bitartrate salt monohydrate (NA)) were purchased from Sigma (St. Louis, MO, USA). The drugs were dissolved in a 0.1% Evans Blue solution, which was prepared in 0.85% saline. Saline containing Evans Blue was used as a control. The birds were Intracerebroventricular (ICV) injected with the solutions (10  $\mu$ L) using a microsyringe according to the methods used by Davis *et al.* (1979). Each chick was injected once only with saline or drug solution.

The doses of each drug applied in this study were referred to in previous reports (Denbow *et al.*, 1983; Choi *et al.*, 1996; Hagemann *et al.*, 1998; Bungo *et al.*, 2001). At the end of the experiments, birds were sacrificed by decapitation, after which the location of the injection site was confirmed. Data from the individuals that were not verified by the presence of Evans Blue dye in the lateral ventricle were deleted.

The number of birds used for data analysis is shown in each figure. ANOVA was used to determine overall statistical significance due to treatment. When a treatment effect was significant, the Turkey-Kramer test was used to compare the significance among means. Statistical significance was set at  $p < 0.05$ . Data were expressed as means  $\pm$  SEM.

**RESULTS AND DISCUSSION**

The effect of ICV administration of Tyr, L-DOPA, DA or NA on feed consumption in chicks fed *ad libitum* over a 60 min period is shown in Fig. 1-4. Both levels of each of Tyr, L-DOPA and DA had no effect on feed intake compared with saline control during the experimental period (Fig. 1-3,  $p > 0.05$ ). However, feed intake of chicks was significantly increased by NA (20 nmol), when compared with control at 30 min postinjection (Fig. 4,  $p < 0.05$ ) and the effect was short lived, having disappeared by 60 min. The ICV injection of Tyr did not affect feed intake in chicks (Fig. 1), which is in agreement with a previous report, showing that ICV administration of Tyr had no effect on feeding behavior in the chicken (5-weeks old) (Choi *et al.*, 1996).

This is because Tyr levels and catecholamine synthesis in the central nervous system appear to be at an upper limit, when protein intake is at or above requirement levels (Fernstrom and Fernstrom, 1994). In fact, Choi *et al.* (1996) found that central Tyr injection did not affect the concentration of NA in the appetite-related sites of the brain.

There is a significant increase in the amount of DA stored and available for release following L-DOPA application, when L-DOPA is actively taken up into DA neurons in the central nervous system. By comparison with the DAergic system, there is relatively little increase

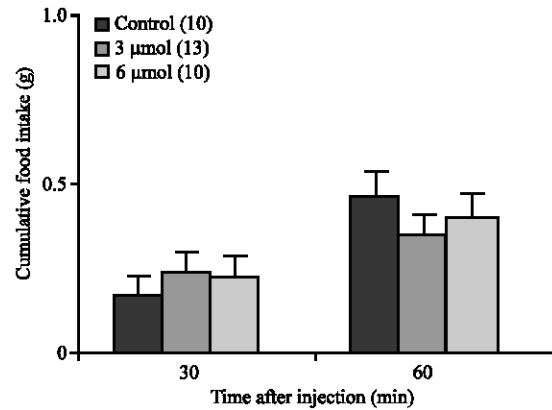


Fig. 1: Cumulative feed intake of the *ad libitum* chick injected intracerebroventricularly with saline or one of two doses of tyrosine (3 or 6  $\mu$ mol). Values are means  $\pm$  SEM of the number of chicks in parentheses

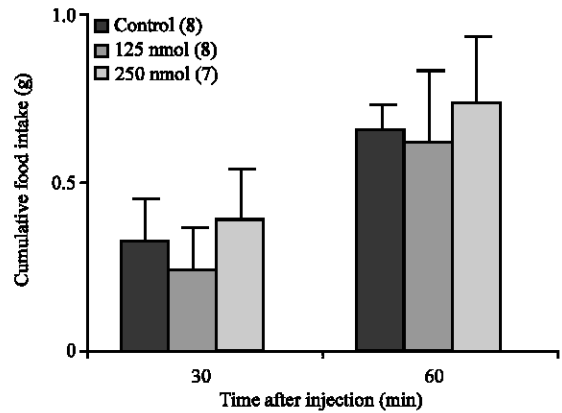


Fig. 2: Cumulative feed intake of the *ad libitum* chick injected intracerebroventricularly with saline or one of two doses of L-DOPA (125 or 250 nmol). Values are means  $\pm$  SEM of the number of chicks in parentheses

in the synthesis of NA following L-DOPA administration (Kruk and Pycock, 1991). Because central DA administration did not change feed intake (Fig. 3), the increment of DA after L-DOPA infusion might fail to alter feeding behavior in chicks (Fig. 2). Kruk (1973) revealed that ICV administration of DA resulted in a 47% suppression of feed intake in fasted rats. In contrast, the act of feeding can elicit increases in DA within the nucleus accumbens shell, which may be associated with the rewarding properties of eating (Swanson *et al.*, 1997). However, the present results, showing that ICV injection of DA failed to change feed intake in chicks over 60 min postinjection (Fig. 3) are in good agreement with other

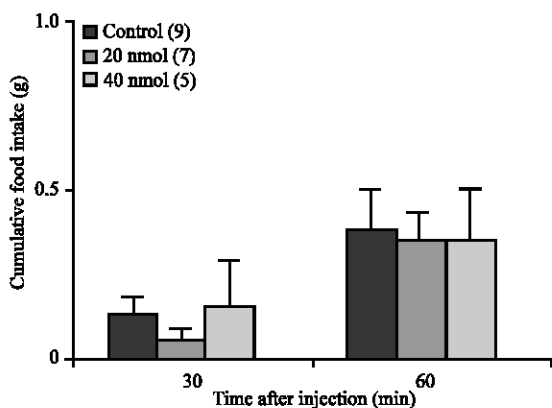


Fig. 3: Cumulative feed intake of the *ad libitum* chick injected intracerebroventricularly with saline or one of two doses of dopamine (20 or 40 nmol). Values are means±SEM of the number of chicks in parentheses

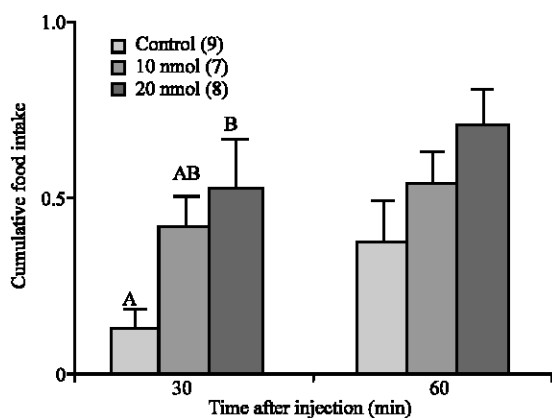


Fig. 4: Cumulative feed intake of the *ad libitum* chick injected intracerebroventricularly with saline or one of two doses of noradrenalin (10 or 20 nmol). Values are means±SEM of the number of chicks in parentheses. Means with different letters are significantly different at  $p < 0.05$

reports on chickens (Denbow *et al.*, 1981, 1983). Thus, it seems reasonable to think that increased DA activity in the brain of chicks might not be directly associated with the control of feeding. The site-specific action of DA remains to be studied.

Feed intake of chicks was significantly increased by NA (20 nmol), when compared with control at 30 min postinjection (Fig. 4,  $p < 0.05$ ) and the effect was short lived, having disappeared by 60 min.

The study of avian feeding patterns has been complicated by the fact that their food intake appears to be particularly sensitive to genetic selection for growth rate (Barbato, 1994; Kuenzel, 1994; Denbow, 1999).

Although, there are many reports supporting a role for NA in the regulation of ingestive behavior in avian species, they contain contradictory results: central NA injections increase feed intake in pigeons (Ravazio *et al.*, 1990) and broiler chickens (Denbow *et al.*, 1981) but have no effect in Leghorn chickens (Denbow *et al.*, 1983) and decrease feeding in turkeys (Denbow, 1989). It can be assumed that an effective dose in birds differs with species and/or age because there are genotype differences in catecholamine concentrations in the hypothalamus (Kruzelock and Barbato, 1991). We found that a low dose of NA (6 nmol) significantly stimulated feeding behavior of broiler chicks under *ad libitum* conditions (unpublished data) but high doses produced anorexia in satiated chicks with sleep-like behavior (Bungo *et al.*, 2001, 2002). Thus, the narcoleptic response to the high dose of NA might have masked a stimulation of feed intake in the previous reports on Leghorn chickens and turkeys.

## CONCLUSION

The present results suggest that the central NAergic system is involved in feed intake control in neonatal chicks. However, central acute loading of Tyr, L-DOPA or DA did not affect feeding behavior and these large increments in the brain of chicks might not be involved in NA-induced hyperphagia.

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