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≪Research Note≫

Comparison of the Effects of Octopamine and Noradrenaline on Feeding and Sleep-like Behaviour in Fasted Chicks

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Octopamine, an important neurotransmitter in invertebrates, has noted several similarities with noradrenaline (NA) in mammals. To compare the effect of octopamine and NA in the central nervous system, food intake and categorized postures of fasted chicks were investigated after intracerebroventricular (ICV) injection. We found that ICV injection of NA significantly inhibited food intake of fasted chicks at 30 min postinjection, but not octopamine. Although chicks treated NA showed a tendency to be sleep-like behavior, octopamine significantly induced hyperactivity when compared with NA. The results presented here suggest that octopamine may also act on nervous system(s) without noradrenergic system in the brain of chicks.

Key words : octopamine, noradrenaline, feeding behavior, sleep-like behavior, chick

Introduction

Octopamine is produced from tyramine, a metabolite of tyrosine, and it has been identified as a naturally occurring biogenic amine in invertebrates and vertebrates (Robertson and Juorio, 1977 ; David and Coulon, 1985 ; Evans, 1985 ; Williams *et al.*, 1987). This amine has been known as an important neurotransmitter in insects (Evans, 1985). In mammals, octopamine is present in very low concentrations in several sympathetically innervated organs (Molinoff and Axelrod, 1972 ; Ibrahim *et al.*, 1985) and in the brain (Buck *et al.*, 1977 ; Danielson *et al.*, 1977 ; David and Delacour, 1980). Similar to mammals, the existence, metabolism and storage of octopamine in the brain of chickens have been reported (Juorio, 1978). Although the role of octopamine in the central nervous system of vertebrates is unknown, there are some reports that octopamine has relation to noradrenergic system (Axelrod and Saavedra, 1977 ; Jones, 1982) and octopamine binds both α_1- and α_2-adrenoceptors in rats and rabbits (Brown *et al.*, 1988). In fact, central injection of octopamine induced eating response in rats (Fletcher and Paterson, 1989) and chicks (Bungo *et al.*, 2002) under *ad libitum* condition. On the other hand, it has generally been assumed that octopamine may

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produce symptoms of hepatic encephalopathy by interference with the release of the true transmitter, NA and dopamine (Lam *et al.*, 1973).

Although the role of octopamine and the differences between NA and octopamine have studied in mammals, there is little information in the chick. Therefore, to determine the difference between NA and octopamine in the central nervous system, food intake and categorized postures of fasted chicks were investigated after ICV administration of them.

Materials and Methods

Animals

Day-old male broiler chicks were purchased from a local hatchery (Fresh Foods, Ehime, Japan). Birds were maintained in a room with 24 h light and at a temperature of 30℃. They were given free access to a commercial starter diet (Nihon Nosan Kogyo Co. Ltd., Yokohama, Japan) and water during the pre-experimental period. Before each experiment, body weight was measured and chicks were distributed into experimental groups so that the average body weight with groups was as uniform as possible

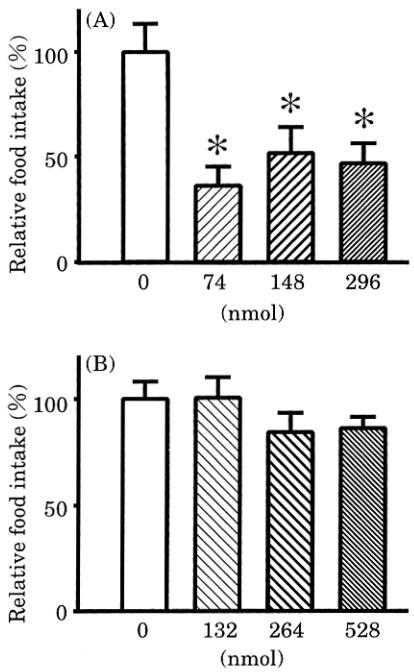

Fig. 1. Relative food intake of chicks following ICV administration of (A) noradrenaline (0–296 nmol) or (B) octopamine (0–528 nmol). The numbers of birds were : (A) control, 8 ; 74 nmol of noradrenaline, 8 ; 148 nmol of noradrenaline, 10 and 296 nmol of noradrenaline, 7 ; (B) control, 9 ; 132 nmol of octopamine, 6 ; 264 nmol of octopamine, 9 and 528 nmol of octopamine, 7, respectively. Values are means±SEM. $^*P<0.05$, compared with saline (control) injection alone. Absolute food intakes for saline control were (A) 1.08±0.14 g and (B) 2.58±0.21 g.

Table 1. Effect of octopamine and noradrenaline on the various behaviorlal categories of the neonatal chick after 5, 10 and 15 min following ICV injection

	Treatment	(n)	Number of chicks with the posture of score#				*P* vs	
			A	B	C	D	Control	Noradrenaline treatment
(5 min)	Control	(9)	4	4	1	0	—	—
	Noradrenaline	(9)	2	3	0	4	NS	—
	Octopamine	(7)	7	0	0	0	NS	$P<0.05$
(10 min)	Control	(9)	7	1	1	0	—	—
	Noradrenaline	(9)	1	5	0	3	$P<0.02$	—
	Octopamine	(7)	6	1	0	0	NS	$P<0.05$
(15min)	Control	(9)	5	1	2	1	—	—
	Noradrenaline	(9)	3	2	0	4	NS	—
	Octopamine	(7)	7	0	0	0	NS	NS

#A : active wakefulness (contained eating), B : standing/sitting with eyes open, C : standing motionless with eyes closed, and D : sitting motionless with head drooped (sleeping posture).
NS : no significant.

within the same experiment. The birds were reared individually in experimental cages.

Intracerebroventricular (ICV) Injections

The birds were intracerebroventricular (ICV) injected with the solutions (10μl) using a microsyringe according to the methods of Davis *et al.* (1979) and Furuse *et al.* (1999). NA (bitartrate salt) and DL-Octopamine (hydrochloride) were purchased from Sigma (St. Louis, MO, USA) and Nacalai Tesque, Inc. (Kyoto, Japan), respectively. Drugs were dissolved in a 0.1% Evans Blue solution, which was prepared in a 0.85% saline. 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 numbers of birds for the data analysis were noted in Fig. 1 and Table 1.

Experimental Procedure

Effect of NA and Octopamine on food consumption of fasted chicks

After being deprived of food for 3 h, birds (NA, 2-day-old ; octopamine, 3-day-old) were given the same diet for 1 h immediately after each treatment. They were injected by the ICV route with NA (0, 74, 148 and 296 nmol) or octopamine (0, 132, 264 and 528 nmol).

Effect of NA and Octopamine on posture of chicks

Birds (3-day-old) with 3 h fasting were divided into 3 groups, i.e., saline, NA (60 nmol) and octopamine (1500 nmol). The reason for the different doses between two amines was due to the fact that octopamine was 25 fold less active than NA on α_2-adrenoceptors in the rat cerebral cortex (Brown *et al.*, 1988). The birds were

observed the posture at 5, 10 and 15 min immediately after injection. Observation was performed by a trained observer who was not informed of each treatment. As referring to van Luijtelaar *et al.* (1987), four behavioral categories were distinguished : (A) active wakefulness (contained eating) ; (B) standing/sitting motionless with eyes open ; (C) standing motionless with eyes closed ; (D) sitting motionless with head drooped (sleeping posture).

Data Analysis

For food consumption, ANOVA was used to determine the overall statistical significance due to the treatment. When each treatment effect was significant, Duncan's multiple range test was used to compare the significance among means and level of significance was set at $P<0.05$. The results are presented as means±SEM. Behavioral data were subjected to t-test for ordered classifications (Snedecor and Cochran, 1967) between each treatment.

Results

Effect of NA and Octopamine on food consumption

Fig. 1 gives the effect of ICV injection of NA or octopamine on food intake in fasted chicks over 30 min postinjection. Food intake was significantly decreased by 74, 148 and 296 nmol NA when compared with control (Fig. 1 A ; $P<0.05$). However, this effect disappeared at 60 min postinjection (data not shown). On the other hand, each level of octopamine failed to suppress food intake of chicks when compared with control (Fig. 1 B ; $P>0.05$).

Effect of NA and Octopamine on posture of chicks

The result on the effect of central injection of NA or octopamine on the posture of chicks is shown in Table 1. Throughout the 15 min experimental period, the ICV injection of NA (60 nmol) tended to induce sleep-like behavior (posture of the score D) compared to saline control. On the other hand, most of chicks with octopamine indicated posture of the score A (active behavior) during experiment but this effect had no significance when compared with control. As compared NA with octopamine, the ICV injection of octopamine significantly induced the posture of the score A (active behavior) while NA produced the score D (sleep-like behavior) without 15 min post-injection.

Discussion

With regard to the dose effect of NA, dose of 74 nmol also inhibit food intake of fasted chicks (Fig. 1 A ; $P<0.05$), but not of satiated chicks (Bungo *et al.*, 2001). It is reported that central NA of food-deprived chicks significantly increased during the first hour of access to food (Tachibana *et al.*, 2000). Thus, it seems that increased endogenous NA after refeeding cooperates the effect of exogenous NA on feeding behavior.

Under an *ad libitum* condition, we found that low dose of NA (6 nmol) significantly stimulated feeding behavior of chicks (unpublished data) but the high doses (148 and 296 nmol) suppressed them (Bungo *et al.*, 2001). Additionally, the

effective level of clonidine (α_2-adrenoceptor agonist ; 50 ng) significantly increased food intake of satiated chicks by ICV injection. However, dose of 100 ng failed to affect feeding behavior and the five-fold level (250 ng) of clonidine produced anorexia in satiated chicks with sleep-like behavior (Bungo *et al.*, 1999). Similar to those, the ICV injection of NA inhibited food intake of fasted chicks at 30 min postinjection (Fig. 1 A ; $P<0.05$). This anorexic effect might be due to sleep-like behavior by NA because central injection of NA (60 nmol) tended to induce sleep-like behavior (Table 1). Denbow *et al.* (1981) also observed that ICV injection of NA failed to stimulate food intake in the domestic fowl, presumably because it induced a narcoleptic response. Noradrenergic mechanisms are important not only in feeding response but also in the sleep-waking cycle, for example it is believed that the locus coeruleus prevents motor activity during rapid eye movement (REM) sleep (Kruk and Pycock, 1991). Harsing *et al.* (1989) also suggested that stimulation of α_2-adrenoceptors with pre- and post-synaptic locations or inhibitor of α_1-adrenoceptors in the central nervous system might shift the depression/vigilance balance to the direction of depression, which might be accompanied by decreased activity of cortical noradrenergic neural transmission.

Similar to the result in rats (Fletcher and Paterson, 1989), we found that ICV injection of octopamine (79 and 158 nmol) stimulated food intake of satiated chicks, through α_2-adrenoceptor (Bungo *et al.*, 2002). From the results of NA and clonidine as mentioned above, if central injection of octopamine should quite acts in the NA manner, the overdosed octopamine might depress feeding behavior of chicks with sleep-like behavior. However, two overdosed octopamine (264 and 528 nmol) failed to affect food consumption of fasted chicks (Fig. 1 B). Additionally, 1500 nmol octopamine that is decuple much than the effective dose (158 nmol) also did not suppress feeding behavior (unpublished data) and induced hyperactivity opposite to the response by 60 nmol NA that is decuple much than the effective dose (Table 1). It follows from this that octopamine-induced hyperphagia of satiated chicks in the previous experiment (Bungo *et al.*, 2002) might be due to octopamine-induced hyperactivity. Moreover, it appeared to be some different effect on the behavior of chicks between octopamine and NA. It is reported that octopamine bound dopamine D_1 receptor (Cheng *et al.*, 1990) and that the octopaminergic system, which was separated from the noradrenergic system, might exist in the brain of rats (Hicks and McLennan, 1978). Thus, it seemed reasonable to think that central octopamine could have an effect on dopaminergic and/or other nervous system(s) so as to induce hyperactivity. The relationship between octopamine and other nervous system remains to be studied.

The results presented here suggest that central octopamine induces hyperactivity and may affect not only noradrenergic system but also other nervous systems in the neonatal chick.

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