Effects of a Nitric Oxide Synthase Inhibitor on Hypophagia Induced by the Peripheral 5-HT Receptor Agonists, α -Methyl-5-hydroxytryptamine and 5-Carboxamidotryptamine in Rats

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We investigated the effects of the nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) on hypophagia in rats elicited by α -methyl-5-hydroxytryptamine (α -methyl-5-HT) and 5-carboxamidotryptamine (5-CT) which are suggested to be mediated by the peripheral 5-HT_{2A} and 5-HT₇ receptor, respectively. Both α -methyl-5-HT and 5-CT apparently inhibited food intake in food-deprived rats. L-NAME significantly enhanced α -methyl-5-HT-elicited hypophagia, while it inhibited 5-CT-elicited hypophagia. These results suggest that NO is differentially related to α -methyl-5-HT and 5-CT-induced hypophagia and that NO may play a role in hypo- and hyperphagia.

Key word 5-HT; NO; food intake; 5-HT_{2A} receptor; 5-HT₇ receptor; α -methyl-5-HT; 5-CT

It is well recognized that serotonin(5-hydroxytryptamine, 5-HT) plays a significant role in the regulation of feeding behaviour.¹⁾ Since the activation of central 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptor induces anorexia in rats, it is implied that these receptors are involved in the hypophagic effects of 5-HT in the central nervous system.^{1,2)} It has been reported that the peripheral 5-HT receptor is also associated with food intake. Peripherally administered 5-HT induces anorexia and this effect is associated with the peripheral 5-HT₂ receptor.^{3–5)} We previously reported that the peripheral 5-HT₂ receptor agonist α -methyl-5-HT, reduces food intake in rats which is mediated by the peripheral 5-HT_{2A} receptor.⁶⁾ 5-Carboxamidotryptamine (5-CT) is peripherally acting 5-HT receptor agonist with a high affinity with 5-HT₇ receptors.^{7,8)} Simansky⁹⁾ reported that 5-CT induces hypophagia in food-deprived rats, which is mediated by the 5-HT₇ receptor.

It was reported that nitric oxide (NO) in the central nervous system participates in food intake from results that NO synthase inhibitors cause hypophagia in several hyperphagic animal models.^{10–13)} We previously found that a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), inhibited 2-deoxy-D-glucose and 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) 8-OH-DPAT-induced hyperphagia in rats.^{14,15)} L-NAME also inhibited enhanced feeding in obese animals.^{11,12)} NO synthase is divided into 3 NO synthase types: endothelial, neuronal and inducible NO synthase¹⁶⁾ and it is well known that L-NAME is a nonselective NO synthase inhibitor. It was reported that L-NAME alters gastric and intestinal motility which can modify food intake.¹⁷⁾ Therefore, NO in the periphery may play a role in food intake. Since α -methyl-5-HT and 5-CT acts in the peripheral system, the anorectic mechanisms of these drugs may be related to NO. In the present study, to clarify the involvement of NO, we investigated the effects of NO synthase inhibitor L-NAME on α -methyl-5-HT and 5-CT-elicited hypophagia.

MATERIALS AND METHODS

Male Sprague-Dawley rats (200–240 g, SLC Japan) were housed in individual stainless-steel wire cages. They were

maintained under a controlled 12 h/12 h light/dark cycle (light from 7:00 a.m. to 7:00 p.m.), at a room temperature of 23±1 °C and humidity 55±5% for at least 7 d prior to the experiments. The rats were allowed free access to food and water.

α -Methyl-5-hydroxytryptamine maleate (α -methyl-5-HT), 5-carboxamidotryptamine maleate (5-CT) and L-NAME were obtained from Research Biochemicals Inc. (U.S.A.). All drugs were dissolved in saline and injected i.p. at a volume of 0.2 ml/100 g. L-NAME was given 30 min before the injection of α -methyl-5-HT or 5-CT.

Food was removed for 20 h before the experiments, although water was allowed. Prewedged food was placed in the cage and the amount remaining was weighed 0.5, 1, 2 and 4 h after the injection of α -methyl-5-HT or 5-CT. Drugs were injected between 1:00 and 2:00 p.m.

Dose-related effects of α -methyl-5-HT or 5-CT on feeding were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. Effects of L-NAME on hypophagia induced by α -methyl-5-HT or 5-CT were analyzed by two-way ANOVA followed by Tukey's test.

RESULTS

Figures 1 and 2 show the effects of α -methyl-5-HT and 5-CT on the food intake of food-deprived rats. In food deprived rats, an apparent hyperphagia was observed. Cumulated food intake of non-food-deprived rats were 0.5 h: 0.18±0.10 g, 1 h: 0.25±0.16 g, 2 h: 0.49±0.18 g, 4 h: 0.95±0.24 g (*n*=5). In food-deprived rats, α -methyl-5-HT (0.5–5 mg/kg) and 5-CT (0.05–0.5 mg/kg) elicited hypophagia in a dose-related manner.

Figure 3 demonstrates the effects of L-NAME on α -methyl-5-HT (1 mg/kg)-induced hypophagia. Pretreatment with L-NAME enhanced hypophagia elicited by α -methyl-5-HT. Effects of L-NAME on 5-CT (0.1 mg/kg)-elicited hypophagia are shown in Fig. 4. L-NAME significantly attenuated 5-CT-induced anorexia.

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Fig. 1. Effects of α -Methyl-5-HT on Food Intake of Food-Deprived Rats

Results are shown as means $\pm$ S.E. ($n=6-9$). α -Methyl-5-HT was injected i.p. * $p<0.05$, ** $p<0.01$.

Fig. 2. Effects of 5-CT on Food Intake of Food-Deprived Rats

Results are shown as means $\pm$ S.E. ($n=6-9$). 5-CT was injected i.p. * $p<0.05$, ** $p<0.01$.

Fig. 3. Effects of L-NAME on α -Methyl-5-HT-Induced Hypophagia in Rats

Results are shown as means $\pm$ S.E. ($n=5-7$). α -Methyl-5-HT at 1 mg/kg was given i.p. L-NAME was injected i.p. 30 min before α -methyl-5-HT. ** $p<0.01$, *** $p<0.001$ vs. L-NAME+saline of respective group. ## $p<0.01$ vs. saline+ α -methyl-5-HT of respective group.

Fig. 4. Effects of L-NAME on 5-CT-Induced Hypophagia in Rats

Results are shown as means $\pm$ S.E. ($n=5-7$). 5-CT at 0.1 mg/kg was given i.p. L-NAME was injected i.p. 30 min before 5-CT. Results are shown as means $\pm$ S.E. ($n=5-7$). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. L-NAME+saline of respective group. # $p<0.05$, ## $p<0.01$ vs. saline+5-CT of respective group.

DISCUSSION

We previously demonstrated that α -methyl-5-HT-induced hypophagia is mediated by the peripheral 5-HT₂ receptor and this receptor is involved in feeding behaviour.⁶⁾ Our present results demonstrated that the α -methyl-5-HT decreases food intake in food-deprived rats which is in agreement with our previous report.⁶⁾ Similarly, the 5-HT_{1/5/7} receptor agonist 5-CT also reduced hyperphagia in starved rats. Simansky has shown the anorectic effects of 5-CT food intake for 2 h in mildly food-deprived rats similar to our present results.⁹⁾ Simansky demonstrated that 5-CT-induced hypophagia is mediated by the peripheral 5-HT₇ receptor based on results using 5-HT receptor antagonists.⁹⁾

L-NAME is a commonly used NO synthase inhibitor and it acts in both central and peripheral system. It was reported that L-NAME alters gastric motility or the tone of the gastric fund and these effects can modify food intake.^{17–19)} This suggests that NO in the periphery may regulate feeding behaviour. Thus, we investigated the effects of a NO synthase inhibitor L-NAME on hypophagia induced by the peripheral 5-HT receptor agonists, α -methyl-5-HT and 5-CT. As shown in the results, L-NAME enhanced α -methyl-5-HT-induced hypophagia, while it significantly inhibited 5-CT-elicited anorexia. Our results indicate that effects of L-NAME on hypophagia were the opposite of those elicited by peripheral 5-HT receptor agonists and that L-NAME demonstrates dual effects on food intake.

α -Methyl-5-HT-induced hypophagia is mediated by the peripheral 5-HT_{2A} receptor, because the 5-HT_{2A} receptor antagonist ketanserin blocked it.⁶⁾ Previous findings suggest that decreases in gastric emptying may be related to the anorectic effects induced by a 5-HT releasing drug fenfluramine^{20–22)} and that effects of fenfluramine are antagonized by the peripheral 5-HT₂ receptor antagonist, xylamidine.¹⁸⁾ It has been suggested that 5-HT contracts the gastric pylorus and this effect is antagonized by ketanserin, which blocks the 5-HT_{2A} receptor.²³⁾ Since α -methyl-5-HT causes anorexia through the peripheral 5-HT_{2A} receptor,⁶⁾ α -methyl-5-HT-induced hypophagia may be connected to gastric emptying. It was reported that L-NAME delayed gastric emptying in rats.¹⁹⁾ Therefore, the enhancement of L-NAME on α -methyl-5-HT-induced hypophagia may be caused by their synergistic effects on gastric emptying.

It is noteworthy that L-NAME significantly counteracted 5-CT-induced hypophagia, because previous findings showed that the decrease in NO formation by NO synthase inhibitors attenuated hyperphagia in animals.^{6,12,14,15,20,24)} It was reported that 5-CT increases intragastric pressure and relaxes intestine mediated by the 5-HT₇(5-HT₁-like) receptor.^{25–27)} However, Lefebvre *et al.*¹⁸⁾ demonstrated that L-NAME at a dose of 10 mg/kg antagonized decreases in gastric pressure elicited by vagal stimulation. It suggests that inhibitory effects of L-NAME on 5-CT-induced hypophagia is unlikely to its effects on increases in gastric pressure. It was reported that 5-CT also relaxes intestine mediated by the 5-HT₇ receptor.^{25,26)} It is well known that endogenous NO mediates relaxation of gut.^{16,17)} Calignano *et al.*²⁰⁾ reported that L-NAME increased intraluminal pressure and initiated phasic intestinal contractions. Therefore, L-NAME may inhibit 5-CT-induced hypophagia by antagonizing relaxation of the intestine caused by 5-CT.

It has been suggested that NO in the brain is associated with feeding behaviour, as NO synthase inhibitors reduce centrally mediated hyperphagia related to several neurotransmitters in the brain.^{13,14,20,24)} Since L-NAME inhibits NO synthase in the central nervous system, the involvement of its central effects cannot be excluded at present.

In summary, the present results demonstrate that the NO synthase inhibitor L-NAME enhanced α -methyl-5-HT-induced hypophagia, which is related to the peripheral 5-HT_{2A} receptor. On the contrary, L-NAME blocked 5-CT-elicited hypophagia mediated by the 5-HT₇ receptor. Our results suggest that NO differentially relates to peripheral 5-HT_{2A} and 5-HT₇ receptor-mediated hypophagia and that NO may play a role in both hyper- and hypophagia in feeding behaviour.

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Science, Sports and Culture of Japan.

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