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A neuronal nitric oxide synthase inhibitor 7-nitroindazole reduces the 5-HT_{1A} receptor agonist 8-OH-DPAT-elicited hyperphagia in rats

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Abstract

The effects of the neuronal nitric oxide (NO) synthase inhibitor 7-nitroindazole on 8-hydroxy-2-di-*n*-(propylamino)tetralin (8-OH-DPAT)-induced hyperphagia, which is mediated by the 5-HT_{1A} autoreceptor, were investigated in rats. 7-Nitroindazole suppressed 8-OH-DPAT-elicited increases in food intake. The inhibitory effects of 7-nitroindazole on 8-OH-DPAT-induced feeding were prevented by the NO precursor L-arginine. Although 8-OH-DPAT decreases 5-hydroxytryptamine (5-HT) synthesis, 7-nitroindazole did not reverse the 8-OH-DPAT-elicited decrease in 5-HT synthesis. Therefore, these results indicate that NO formed in the brain is involved in 8-OH-DPAT-induced hyperphagia and that the hypophagic effects of 7-nitroindazole are not dependent on 5-HT synthesis.

Keywords: 8-OH-DPAT (8-hydroxy-2-di-*n*-(propylamino)tetralin); Nitric oxide (NO); 7-Nitroindazole; 5-HT_{1A} receptor; 5-HT (5-hydroxytryptamine, serotonin); Hypothalamus

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is known as a neurotransmitter which suppresses food intake. The postsynaptic 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors are involved in the appetite-depressant properties of 5-HT (Curzon, 1990; Dourish, 1995). It has been reported that a 5-HT_{1A} receptor agonist, 8-hydroxy-2-di-*n*-(propylamino) tetralin (8-OH-DPAT), elicits hyperphagia in rats (Dourish et al., 1986; Curzon, 1990). 8-OH-DPAT activates the somatodendritic 5-HT_{1A} autoreceptor located in the dorsal raphe nuclei, which leads to inhibition of 5-HT neurons, resulting in a decrease in 5-HT synthesis and 5-HT release from the nerve terminal (Hutson et al., 1986; Curzon, 1990; Dourish, 1995).

Nitric oxide (NO) is involved in vasodilator effects and regulates the tension of blood vessels (Moncada et al., 1991). NO in the central nervous system plays a role in several functions such as memory and pain (Moncada et al., 1991; Moore et al., 1993a). NO also participates in food intake, and inhibition of NO formation by NO synthase inhibitors causes hypophagia in several hyperphagic animal models. NO synthase inhibitors inhibit hyperphagia in genetically obese mice and rats (Squadrito et al., 1993; Morley and Flood, 1994). They also inhibit morphine-, chlordiazepoxide- or 2-deoxy-D-glucose-induced feeding (Calignano et al., 1993; Czech, 1996; Yamada et al., 1997). We previously reported that a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), inhibited 8-OH-DPAT-induced hyperphagia in rats (Yamada et al., 1996). NO synthase is divided into three NO synthase types: endothelial, neuronal and inducible NO synthase (Moncada et al., 1991). However, L-NAME shows affinity for all NO synthase types and it alters blood pressure and gastric motility (Moncada et al., 1991; Lefebvre et al., 1992; Plourde et al., 1994). Since 8-OH-DPAT-induced hyperphagia is connected to its inhibition of 5-HT neurons, the inhibitory effects of L-NAME on 8-OH-DPAT-induced hyperphagia may be related to neuronal NO. 7-Nitroindazole is a selective neuronal NO synthase inhibitor which does not inhibit endothelial NO synthase (Moore et al., 1993a,b). In the present paper, therefore, we investigated the effects of 7-nitroindazole on 8-OH-DPAT-elicited feeding and decrease in 5-HT synthesis in the hypothalamus.

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2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 200–240 g were obtained from SLC Japan. They were housed in individual cages and 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 at 23 ± 1°C and humidity at 55 ± 5%. Rats were allowed free access to food and water.

2.2. Drug treatment

8-Hydroxy-2-di-*n*-(propylamino)tetralin hydrobromide (8-OH-DPAT) and 7-nitroindazole were purchased from RBI (USA). 3-Hydroxybenzylhydrazine HCl (NSD 1015) was obtained from Nacalai Tesque (Japan). 8-OH-DPAT was dissolved in saline and injected s.c. 7-Nitroindazole was suspended in arachis oil and given i.p. 30 min before 8-OH-DPAT. NSD 1015 was dissolved in saline and administered i.p. Drugs were injected in a volume of 0.2 ml/100 g.

2.3. Measurement of food intake

Preweighed food was placed in the cage and the amount of food remaining was weighed 1 h and 2 h after the injection of 8-OH-DPAT.

2.4. Determination of 5-HT synthesis in the hypothalamus

5-HT synthesis was assessed by the determination of accumulated 5-hydroxytryptophan (5-HTP) levels following injection of the aromatic amino acid decarboxylase inhibitor NSD 10155. NSD 1015 was injected i.p. at 100 mg/kg 30 min before death. Rats were decapitated and the brain was removed. The hypothalamus was dissected and frozen on dry ice and stored at −40°C until analysis. 5-HTP was determined by high performance liquid chromatography (HPLC) with electrochemical detection.

2.5. Statistics

Results were analyzed by two-way analysis of variance (ANOVA) followed by Tukey's test.

3. Results

3.1. Effects of 7-nitroindazole on 8-OH-DPAT-induced hyperphagia

The effects of 7-nitroindazole on 8-OH-DPAT-induced hyperphagia are shown in Fig. 1. 8-OH-DPAT at a dose of 0.5 mg/kg induced hyperphagia in freely feeding rats. Pretreatment with 7-nitroindazole apparently inhibited 8-OH-DPAT-induced hyperphagia in rats.

3.2. Effects of L-arginine on the inhibitory effect of 7-nitroindazole on 8-OH-DPAT-induced hyperphagia

The effects of L-arginine at 1 g/kg on the inhibitory effect of 7-nitroindazole (25 mg/kg) on 8-OH-DPAT-induced hyperphagia are demonstrated in Fig. 2. Coadminis-

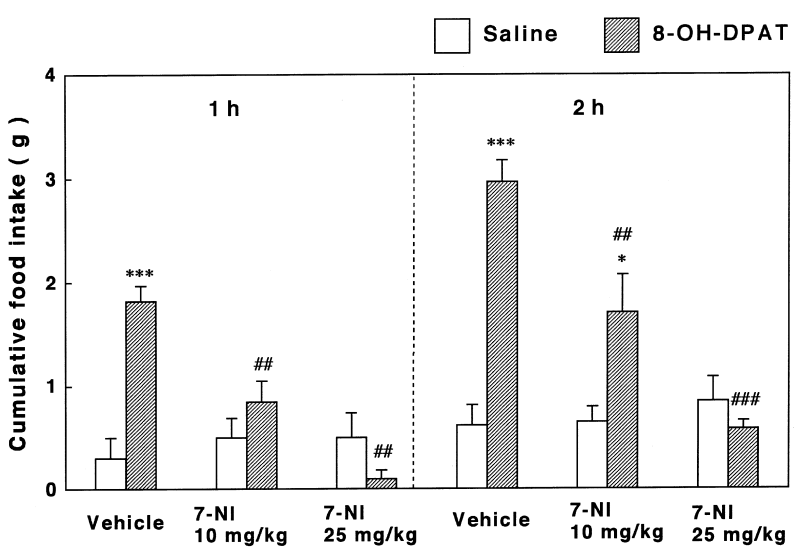

Fig. 1. Effects of 7-nitroindazole (7-NI) on 8-OH-DPAT-induced hyperphagia in rats. Results are shown as the means ± S.E. ($N = 5$–7). 8-OH-DPAT at 0.5 mg/kg was injected s.c. and 7-NI was injected i.p. 30 min before the injection of 8-OH-DPAT. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. saline in the respective group. $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs. vehicle + 8-OH-DPAT-treated group.

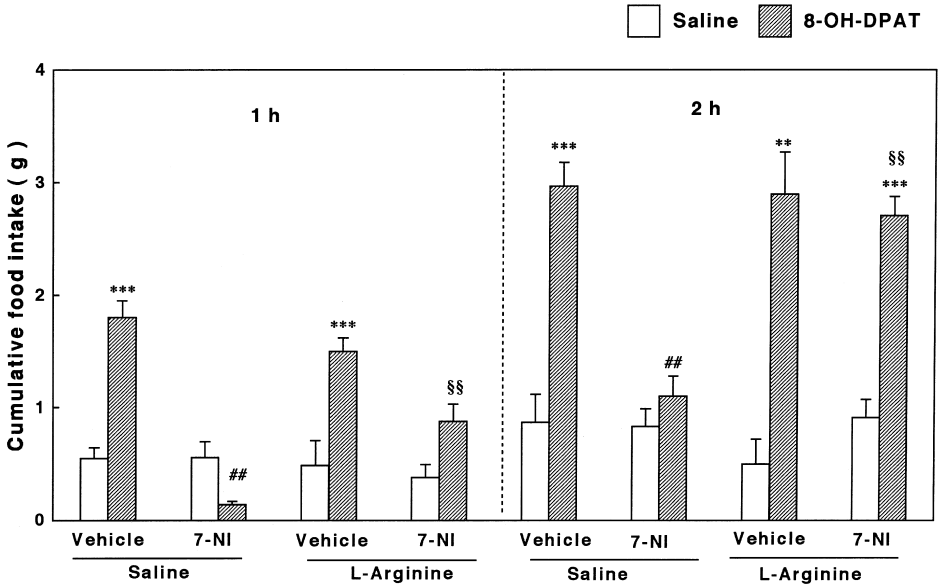

Fig. 2. Effects of 7-nitroindazole (7-NI) plus L-arginine on 8-OH-DPAT-induced hyperphagia in rats. Results are shown as the means ± S.E. ($N = 5–7$). 7-NI at 25 mg/kg and L-arginine at 1 g/kg were given i.p. 30 min before 8-OH-DPAT. $^{***}P < 0.001$, $^{**}P < 0.01$ vs. saline of respective groups, $\#\#P < 0.01$ vs. 8-OH-DPAT of saline + vehicle-pretreated group. §§$P < 0.01$ vs. 8-OH-DPAT of saline + 7-NI-pretreated group.

tration of L-arginine reversed the inhibitory effects of 7-nitroindazole on 8-OH-DPAT-induced hyperphagia.

3.3. Effects of 7-nitroindazole on 8-OH-DPAT-induced decreases in 5-HTP accumulation in the hypothalamus

Fig. 3 demonstrates the effects of 7-nitroindazole on 8-OH-DPAT-induced decreases in 5-HTP accumulation in the hypothalamus. 8-OH-DPAT significantly inhibited 5-HTP accumulation. Pretreatment with 7-nitroindazole (25 mg/kg) did not change 5-HTP accumulation in the saline-treated control group. In addition, 7-nitroindazole did not affect 8-OH-DPAT-induced decreases in 5-HTP accumulation.

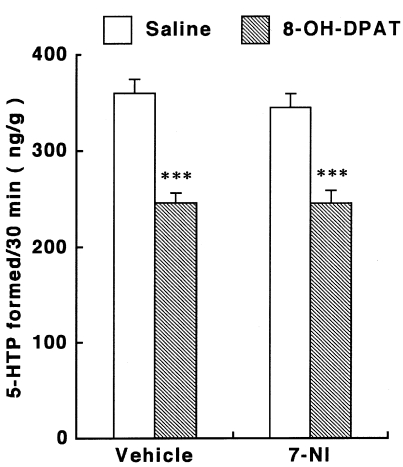

Fig. 3. Effects of 7-nitroindazole (7-NI) on 8-OH-DPAT-induced decreases in 5-HTP accumulation in the hypothalamus. Results are shown as the means ± S.E. ($N = 5–7$). 8-OH-DPAT at 0.5 mg/kg was injected s.c. 7-NI at 25 mg/kg was injected i.p. 30 min before the injection of 8-OH-DPAT. NSD 1015 at 100 mg/kg was injected i.p. 30 min after the injection of 8-OH-DPAT. Rats were decapitated 30 min after NSD 1015. $^{***}P < 0.001$ vs. saline in the respective group.

4. Discussion

NO synthase inhibitors are known to decrease food intake in animals including mice and rats (Squadrito et al., 1993; Morley and Flood, 1994; Yamada et al., 1997). Although NO synthase inhibitors such as N^G-nitro-L-arginine or L-NAME suppress food intake, they non-selectively inhibit NO synthase and can inhibit NO synthase in both the brain and peripheral systems. Since NO regulates relaxation of the stomach and the NO synthase inhibitor N^G-nitro-L-arginine abolishes relaxation of the stomach (Lefebvre et al., 1992; Plourde et al., 1994), appetite suppressant effects of NO synthase inhibitors may be related to their effects on gastric motility. 7-Nitroindazole is a selective brain NO synthase inhibitor that does not affect endothelial NO synthase (Moore et al., 1993a, Moore et al., 1993b). However, little is known about the effects of 7-nitroindazole on food intake. Our findings demonstrate that 7-nitroindazole reduces hyperphagia induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT. The suppressive effects of 7-nitroindazole on 8-OH-DPAT-induced hyperphagia were reversed by coadministration of the NO precursor L-arginine. The antagonistic effects of 7-nitroindazole on 8-OH-DPAT-induced hyperphagia are consistent with our previous results obtained using the non-selective NO synthase inhibitor L-NAME (Yamada et al., 1996). The doses of 7-nitroindazole used in this study

were similar to those used in other tests. 7-Nitroindazole 10–50 mg/kg inhibited pilocarpine-induced seizures (Van Leeuwen et al., 1995). It has also been reported that 25–50 mg/kg of 7-nitroindazole antagonizes methamphetamine-elicited neurotoxicity or cocaine-induced kindling (Di Monte et al., 1996; Itzhak, 1996). As 7-nitroindazole selectively inhibits neuronal NO synthase, our results indicate that neuronal NO is involved in 8-OH-DPAT-induced hyperphagia.

Squadrito et al. (1994a,b) reported that brain 5-HT may be involved in the hypophagic effects of N^G-nitro-L-arginine in rats. Administration of 8-OH-DPAT elicits hyperphagia mediated by the activation of somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nuclei (Dourish et al., 1986; Hutson et al., 1986; Dourish, 1995), resulting in decreased serotonergic activity. Since the hypophagic effects of 7-nitroindazole may be related to its effects on the serotonergic system, we investigated the effects of 7-nitroindazole on the decreases in 5-HT synthesis elicited by 8-OH-DPAT, which is an exclusive index of 5-HT_{1A} autoreceptor activation (Hamon et al., 1984; Hjorth and Magnusson, 1988).

5-HT synthesis was assessed by the determination of 5-HTP levels following the injections of the aromatic amino acid decarboxylase inhibitor NSD 1015. Hypothalamic 5-HTP accumulation was determined because this area plays a significant role in feeding control. As shown in the results, 8-OH-DPAT at a dose of 0.5 mg/kg significantly decreased 5-HTP accumulation in the hypothalamus. This finding supports previously reported data for other brain areas (Hjorth and Magnusson, 1988). However, 7-nitroindazole did not affect 5-HTP accumulation in the saline control group or the 8-OH-DPAT-elicited decreases in 5-HTP accumulation. This indicates that the inhibitory effects of 7-nitroindazole on 8-OH-DPAT-elicited hyperphagia are not dependent on 5-HT synthesis, and that its effects are not derived from stimulation of 5-HT synthesis. Squadrito et al. (1994a,b) reported that a NO synthase inhibitor N^G-nitro-L-arginine can suppress food intake in food-deprived and obese rats by enhancing 5-HT metabolism in the brain. However, our results did not show evidence that 7-nitroindazole increases 5-HT metabolism in the hypothalamus. Squadrito et al. (1994a,b) demonstrated that the levels of the 5-HT precursor tryptophan, 5-HT, and of the 5-HT metabolite, 5-hydroxyindoleacetic acid, in the diencephalon decreased in food-deprived and obese rats and that N^G-nitro-L-arginine modified these changes. However, Knott and Curzon (1974) and Loullis et al. (1979) showed that starvation increased brain tryptophan, 5-HT and 5-hydroxyindoleacetic acid levels in rats, including the hypothalamus. The reason for this discrepancy is unclear but the nutritional condition, age or strain of rats used may be different. We used the 8-OH-DPAT-elicited hyperphagic model in which serotonergic neurons are inhibited. Thus, although Squadrito et al. (1994a,b) reported that the anorectic effects of N^G-nitro-L-arginine in starved rats were mediated by the serotonergic system, the status of brain 5-HT metabolism in their hyperphagic models may be different from that in our experiments. Moreover, the inhibitory effects of N^G-nitro-L-arginine on endothelial NO synthase or its effects in peripheral systems may be related to its hypophagic effects.

It has been suggested that NO may be involved in neurotransmitter release or uptake at nerve terminals (Lorrain and Hull, 1993; Montague et al., 1994). Dopamine and serotonin release have been reported to be enhanced by NO (Lorrain and Hull, 1993). Whether 7-nitroindazole affects 5-HT uptake or release at 5-HT neurons is not clear, and further investigation is required. Since NO synthase inhibitors can inhibit opioid- or neuropeptide Y-mediated feeding (Morley and Flood, 1992; Calignano et al., 1993), it has been speculated that these neurotrasnmitters may participate in the anorectic effects of NO synthase inhibitors. Thus, the hypophagic effects of 7-nitroindazole may be associated with these neurotransmitters.

In summary, our results demonstrate that a selective neuronal NO synthase inhibitor 7-nitroindazole attenuated hyperphagia elicited by the 5-HT_{1A} autoreceptor agonist 8-OH-DPAT. However, 7-nitroindazole did not affect the decrease in 5-HT synthesis in the hypothalamus induced by 8-OH-DPAT. These results indicate that neuronal NO is involved in 8-OH-DPAT-induced hyperphagia but does not affect 5-HT synthesis in the hypothalamus.

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