ELSEVIER

European Journal of Pharmacology 316 (1996) 23–26

ejp

Short communication

Effects of a nitric oxide synthase inhibitor on 5-HT_{1A} receptor agonist 8-OH-DPAT-induced hyperphagia in rats

Jun Yamada *, Yumi Sugimoto, Tomoko Yoshikawa, Kazuyoshi Horisaka

Department of Pharmacology, Kobe Pharmaceutical University, Motoyamakita-machi, Higashinada-ku, Kobe 658, Japan

Received 23 May 1996; revised 12 August 1996; accepted 16 August 1996

Abstract

We investigated nitric oxide (NO) involvement in the hyperphagia induced by the 5-HT_{1A} receptor agonist 8-hydroxy-2-di-*n*-(propylamino)tetralin (8-OH-DPAT). A NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), dose dependently inhibited 8-OH-DPAT-induced eating in freely feeding rats. However, the inactive isomer D-NAME was without effect. The inhibitory effects of L-NAME on 8-OH-DPAT-induced hyperphagia were reversed by simultaneous administration of L-arginine. These results suggest that NO participates in the 8-OH-DPAT-induced hyperphagia which is elicited by activation of the 5-HT_{1A} receptor.

Keywords: 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); 5-HT_{1A} receptor; Nitric oxide (NO); N^G-Nitro-L-arginine methyl ester (L-NAME); Food intake

1. Introduction

The 5-HT_{1A} receptor agonist 8-hydroxy-2-di-*n*-(propylamino)tetralin (8-OH-DPAT) causes several pharmacological effects such as 5-HT syndrome, hypothermia, sexual behavior or activation of the hypothalamus-pituitary-adrenal axis in rodents (Fletcher et al., 1993). These effects elicited by 8-OH-DPAT are considered to be mediated by the post-synaptic 5-HT_{1A} receptor. It has been reported that 8-OH-DPAT induces eating in freely feeding rats (Dourish et al., 1986; Dourish, 1995; Fletcher, 1991; Muscat et al., 1989). The 5-HT_{1A} somatodendritic autoreceptor is recognized to be involved in the hyperphagia elicited by 8-OH-DPAT (Dourish et al., 1986; Dourish, 1995). Thus, the activation of 5-HT_{1A} autoreceptors results in inhibition of the firing of serotonergic nerves and in hyperphagia. The 5-HT_{1A} receptor partial agonists like buspirone and gepirone also elicit eating in rats (Dourish, 1995; Gilbert and Dourish, 1987).

Nitric oxide (NO) is produced in the endothelial cells and induces vascular smooth muscle relaxation (Moncada et al., 1991). NO synthase is present in the brain and recent studies suggest that NO is involved in several brain functions such as pain and memory (Moncada et al., 1991). In recent years, the possible role of NO in feeding has been suggested. It has been reported that NO synthase inhibitors inhibit food intake in food-deprived and genetically obese animals (Morley and Flood, 1994; Squadrito et al., 1993). In recent studies, it was demonstrated that the effects of NO synthase inhibitors are related to serotonergic mechanisms (Squadrito et al., 1994a,b). These previous findings raise the possibility that NO may participate in 8-OH-DPAT-induced eating behavior in rats. Therefore, in this study, we studied the effects of a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), on 8-OH-DPAT-induced hyperphagia in rats to clarify the involvement of NO in the regulation of food intake.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 200–240 g were purchased from SLC Japan. They were housed in individual cages and maintained under a controlled 12 h-light/dark cycle (light from 7:00 a.m. to 7:00 p.m.), with room temperature at 24 ± 1°C and humidity at 55 ± 5% for at least 7 days prior to experiments. Rats had free access to food and water. Each rat was used once.

* Corresponding author. Tel.: (81-78) 441-7573; Fax: (81-78) 441-7574.

PII S0014-2999(96)00657-7

2.2. Drug treatment

8-Hydroxy-2-di-*n*-(propylamino)tetralin hydrobromide (8-OH-DPAT), N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) and N^G-nitro-D-arginine methyl ester hydrochloride (D-NAME) were obtained from Research Biochemicals (USA). L-Arginine hydrochloride was purchased from Wako (Japan). All drugs were dissolved in saline. 8-OH-DPAT was injected s.c. L-, D-NAME and L-arginine were injected i.p. 30 min before the injection of 8-OH-DPAT. All drugs were given 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 remaining was weighed 2 h and 4 h after the injection of 8-OH-DPAT. Drugs were injected between 1:00 and 2:00 p.m.

2.4. Statistical analysis

Dose-related effects of 8-OH-DPAT on feeding were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results obtained with L-NAME were analyzed by two-way ANOVA followed by Tukey's test.

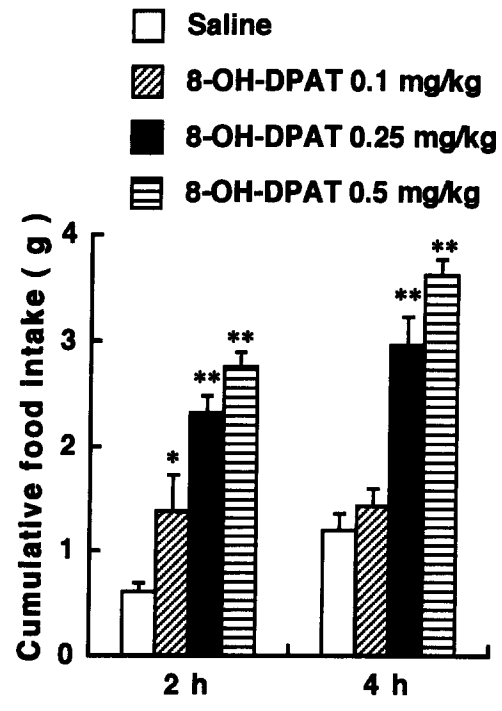

Fig. 1. Effects of 8-OH-DPAT on food intake in freely feeding rats. Results are shown as the means ± S.E. ($n = 5–8$). 8-OH-DPAT was injected s.c. * $P < 0.05$, ** $P < 0.01$. F values: 2 h, $F(3,20) = 23.1$, $P < 0.0001$; 4 h, $F(3,20) = 24.1$, $P < 0.0001$.

3. Results

The effects of 8-OH-DPAT on food intake in freely feeding rats are shown in Fig. 1. 8-OH-DPAT induced significant hyperphagia in a dose-related manner.

The effects of L-NAME and D-NAME on 8-OH-DPAT-induced eating are demonstrated in Fig. 2A. Pretreatment with L-NAME apparently inhibited 8-OH-DPAT-induced eating. However, the inactive isomer of L-NAME, D-NAME (50 mg/kg), did not affect 8-OH-DPAT-induced hyperphagia. The effects of L-arginine at 1 g/kg on the inhibitory effect of L-NAME on 8-OH-DPAT-induced hyperphagia are demonstrated in Fig. 2B. Coadministration of L-arginine prevented the L-NAME-induced inhibition of 8-OH-DPAT-elicited eating.

4. Discussion

Our results demonstrate that 8-OH-DPAT induces apparent hyperphagia in freely feeding rats in a dose-dependent manner, which is in agreement with previous results (Dourish et al., 1986; Fletcher, 1991). We found that pretreatment with a NO synthase inhibitor, L-NAME, significantly inhibited 8-OH-DPAT-induced hyperphagia, although L-NAME itself did not affect eating in freely feeding rats. D-NAME, the inactive isomer of L-NAME, did not affect 8-OH-DPAT-induced hyperphagia even at a high dose of 50 mg/kg. These data suggest that 8-OH-DPAT-induced hyperphagia depends on NO. Furthermore, coadministration of the precursor of NO, L-arginine, reversed the inhibitory effects of L-NAME on 8-OH-DPAT-induced hyperphagia. These results indicate that hyperphagia induced by 8-OH-DPAT is linked with NO.

Previous reports demonstrated that NO participates in eating behavior in rodents. In genetically obese mice and rats, L-NAME or N^G-nitroarginine inhibited increases in body weight and the amount of food consumed (Morley and Flood, 1994; Squadrito et al., 1993). A recent report showed that NO synthase and its mRNA levels are increased in the hypothalamus of genetically obese (*ob/ob*) mice (Morley et al., 1995). Squadrito et al. (1994a) also demonstrated that food deprivation-induced eating in normal rats can be prevented by N^G-nitroarginine. As demonstrated by our present results, we found that L-NAME inhibited 8-OH-DPAT-induced eating. Therefore, under hyperphagic conditions, formation of NO may be enhanced. In our study, L-NAME itself did not affect food intake in normal freely feeding rats, which is in agreement with the results of Squadrito et al. (1994a). Moreover, L-NAME apparently suppresses the food intake of obese rats but its effects are weak in lean rats (Stricker-Krongrad et al., 1996). Thus, it is suggested that competitive inhibition of NO synthesis by L-NAME may strongly reduce enhanced eating behavior in animals and that NO may play a more important role in the hyperphagic state.

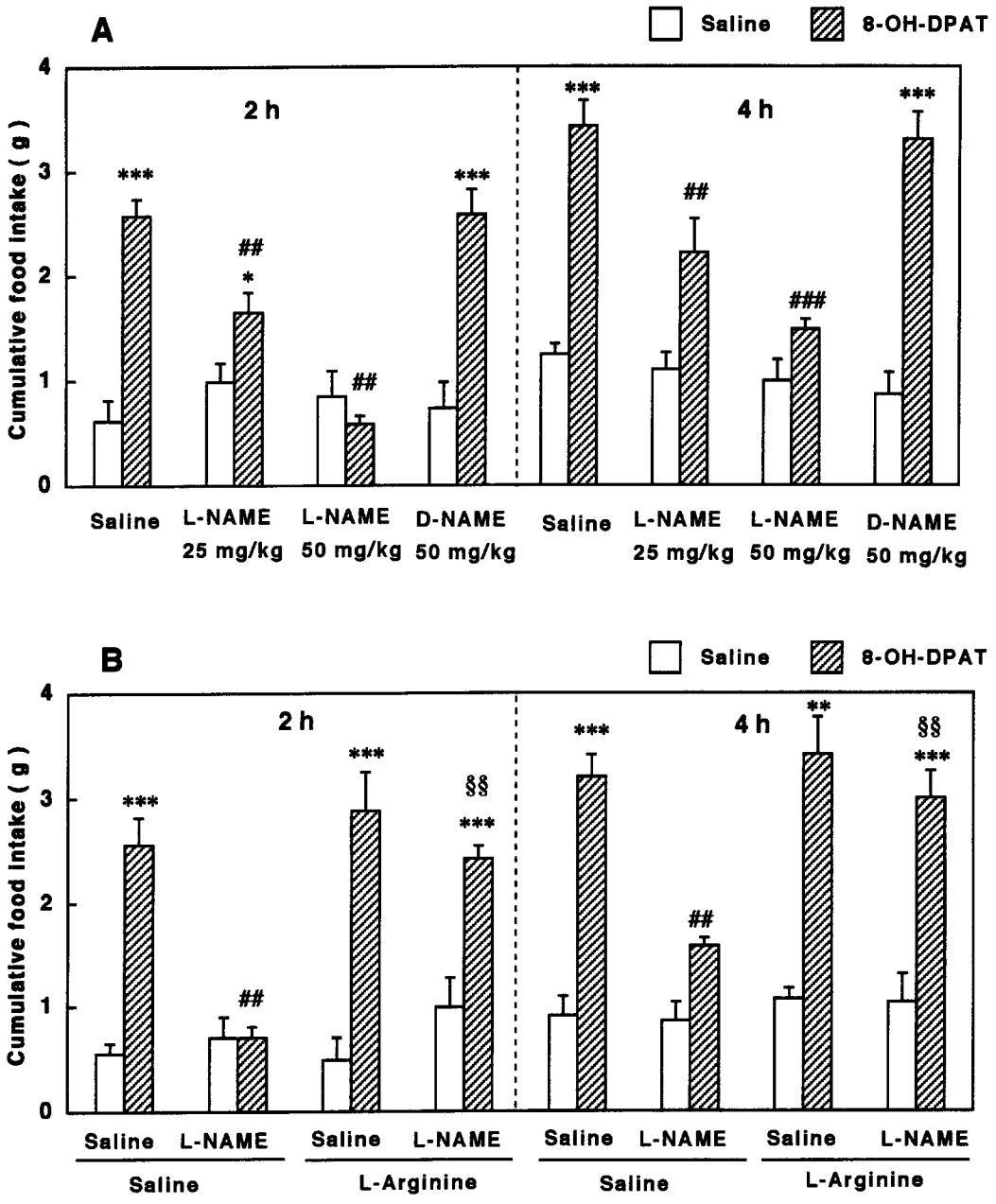

Fig. 2. Effects of L- or D-NAME and L-NAME plus L-arginine on 8-OH-DPAT-induced hyperphagia in rats. Results are shown as the means ± S.E. (n = 5–8). 8-OH-DPAT at 0.5 mg/kg was injected s.c. (A) Effects of L- and D-NAME on 8-OH-DPAT-induced hyperphagia in rats. L- and D-NAME were injected i.p. 30 min before 8-OH-DPAT. * $P < 0.05$, *** $P < 0.001$ vs. saline of respective group. $^{\#\#}$ $P < 0.01$, $^{\#\#\#}$ $P < 0.001$ vs. saline + 8-OH-DPAT-treated group. F values are as follows. 2 h: L- and D-NAME $F(3,41) = 10.5$, $P < 0.0001$, 8-OH-DPAT $F(1,41) = 57.8$, $P < 0.0001$, interaction $F(3,41) = 15.6$, $P < 0.0001$. 4 h: L- and D-NAME $F(3,41) = 11.3$, $P < 0.0001$, 8-OH-DPAT $F(1,41) = 109.9$, $P < 0.0001$, interaction $F(3,41) = 9.87$, $P < 0.0001$. (B) Effects of L-NAME plus L-arginine on 8-OH-DPAT-induced hyperphagia in rats. L-NAME at 50 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 + saline-pretreated group. §§ $P < 0.01$ vs. 8-OH-DPAT of L-NAME + saline-pretreated group. F values are as follows. 2 h: L-arginine $F(1,37) = 9.57$, $P < 0.01$, L-NAME $F(3,37) = 33.4$, $P < 0.0001$, interaction $F(3,37) = 4.52$, $P < 0.01$. 4 h: L-arginine $F(1,37) = 9.22$, $P < 0.01$, L-NAME $F(3,37) = 49.3$, $P < 0.0001$, interaction $F(3,37) = 3.75$, $P < 0.05$.

Previous reports suggested that 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) levels in the brain of obese rats are lower than those in lean rats and that inhibition of hyperphagia in obese rats by a NO synthesis inhibitor is concomitant with an increase in brain 5-HT metabolism (Squadrito et al., 1994b). It was also demonstrated that N^{G}-nitroarginine inhibits enhanced eating and increases brain 5-HT levels in food-deprived rats (Squadrito et al., 1994a). It is well known that 8-OH-DPAT-induced hyperphagia is elicited by the activation of

5-HT_{1A} autoreceptors (Dourish et al., 1986; Dourish, 1995). The stimulation of 5-HT_{1A} autoreceptors by 8-OH-DPAT in somatodendritic nuclei results in the decreased release and turnover of 5-HT. Our results revealed that L-NAME prevented 8-OH-DPAT-induced hyperphagia. Thus, it is implied that L-NAME inhibits hyperphagia by modifying the lowered brain 5-HT turnover induced by 8-OH-DPAT. Therefore, NO may be involved in eating behavior mediated by decreases in brain 5-HT levels and it may contribute to the physiological effects of 5-HT in the regulation of food intake.

It is well known that opioid is involved in the control of feeding behavior (Morley et al., 1983). It is indicated that morphine-induced hyperphagia is mediated by increased dopamine release in the nucleus striatum and nucleus accumbens (Calignano et al., 1993; Morley et al., 1983). Furthermore, morphine-induced eating could be prevented by L-NAME, showing the participation of NO (Calignano et al., 1993). This suggests that both opioidergic and dopaminergic regulation of food intake are closely linked with NO. It was demonstrated that 8-OH-DPAT-induced hyperphagia is blocked by dopamine or opioid receptor antagonists (Fletcher, 1991; Muscat et al., 1989). Taken together with the present results that NO inhibition reduces 8-OH-DPAT-induced hyperphagia, it is indicated that the inhibitory effects of opioid and dopamine receptor antagonists on 8-OH-DPAT-induced eating may be derived from the results of their inhibition of the NO pathway. A previous report demonstrated that L-NAME inhibits neuropeptide Y-induced eating (Morley and Flood, 1992). Thus, the effects of L-NAME may be mediated by neuropeptide Y.

In summary, our results demonstrate that the hyperphagia induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT is inhibited by the NO synthase inhibitor L-NAME. Our results reveal that the hyperphagia elicited by the activation of the 5-HT_{1A} autoreceptor is related to NO. Although the precise mechanism by which L-NAME inhibits 8-OH-DPAT-induced hyperphagia is unknown, L-NAME may modify 5-HT release in the brain. Our present results showing the involvement of NO in 8-OH-DPAT-induced eating further support the significant role of NO in controlling food intake.

References

Calignano, A., P. Persico, F. Mancuso and L. Sorrentino, 1993, Endogenous nitric oxide modulates morphine-induced changes in locomotion and food intake in mice, Eur. J. Pharmacol. 231, 415.

Dourish, C.T., 1995, Multiple serotonin receptors: opportunities for new treatments for obesity?, Obes. Res. 3, 449S.

Dourish, C.T., P.H. Hutson and G. Curzon, 1986, *para*-Chlorophenylalanine prevents feeding induced by the serotonin agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), Psychopharmacology 89, 467.

Fletcher, P.J., 1991, Opiate antagonists inhibit feeding induced by 8-OH-DPAT: possible mediation in the nucleus accumbens, Brain Res. 560, 260.

Fletcher, A., I.A. Cliffe and C.T. Dourish, 1993, Silent 5-HT_{1A} receptor antagonists: utility as research tools and therapeutic agents, Trends Pharmacol. Sci. 14, 441.

Gilbert, F. and C.T. Dourish, 1987, Effects of the novel anxiolytics gepirone, buspirone and ipsapirone on free feeding and on feeding induced by 8-OH-DPAT, Psychopharmacology 93, 349.

Moncada, S., R.M.J. Palmer and E.A. Higgs, 1991, Nitric oxide: physiology, pathophysiology and pharmacology, Pharmacol. Rev. 43, 109.

Morley, J.E. and J.F. Flood, 1992, Competitive antagonism of nitric oxide synthetase causes weight loss in mice, Life Sci. 51, 1285.

Morley, J.E. and J.F. Flood, 1994, Effect of competitive antagonism of NO synthetase on weight and food intake in obese and diabetic mice, Am. J. Physiol. 266, R164.

Morley, J.E., A.S. Levine, G.K. Yim and M.T. Lowy, 1983, Opioid modulation of appetite, Neurosci. Biobehav. Rev. 7, 281.

Morley, J.E., V.B. Kumar, M. Mattammal and D.T. Villareal, 1995, Measurement of nitric oxide synthase and its mRNA in genetically obese (*ob/ob*) mice, Life Sci. 57, 1327.

Muscat, R., A.M.J. Montgomery and P. Willner, 1989, Blockade of 8-OH-DPAT-induced feeding by dopamine antagonists, Psychopharmacology 99, 402.

Squadrito, F., G. Calapai, D. Cucinotta, D. Altavilla, B. Zingarelli, M. Ioculano, G. Urna, A. Sardella, G.M. Campo and A.P. Caputi, 1993, Anorectic activity of N^G-nitro-L-arginine, an inhibitor of brain nitric oxide synthase, in obese Zucker rats, Eur. J. Pharmacol. 230, 125.

Squadrito, F., G. Calapai, D. Altavilla, D. Cucinotta, B. Zingarelli, G.M. Campo, V. Arcoraci, L. Sautebin, G. Mazzaglia and A.P. Caputi, 1994a, Food deprivation increases brain nitric oxide synthase and depresses brain serotonin levels in rats, Neuropharmacology 33, 83.

Squadrito, F., G. Calapai, D. Altavilla, D. Cucinotta, B. Zingarelli, V. Arcoraci, G.M. Campo and A.P. Caputi, 1994b, Central serotonergic system involvement in the anorexia induced by N^G-nitro-L-arginine, an inhibitor of nitric oxide synthase, Eur. J. Pharmacol. 255, 51.

Stricker-Krongrad, A., B. Beck and C. Burlet, 1996, Nitric oxide mediates hyperphagia of obese Zucker rats: relation to specific changes in the microstructure of feeding behavior, Life Sci. 58, PL9.