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European Journal of Pharmacology 402 (2000) 247–250

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Short communication

A nitric oxide synthase inhibitor reduces hyperphagia induced in rats by the 5-HT_{1A} receptor agonist, 8-OH-DPAT, independently of hypothalamic serotonin metabolism

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Received 5 May 2000; received in revised form 14 July 2000; accepted 17 July 2000

Abstract

In rats, a nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) inhibited the hyperphagia induced by the 5-hydroxytryptamine (5-HT)$_{1A}$ autoreceptor agonist, 8-hydroxy-2-di-*n*-(propylamino)tetralin (8-OH-DPAT). 8-OH-DPAT reduced 5-HT metabolism in the hypothalamus, and this was not blocked by pretreatment with L-NAME. L-NAME also did not affect basal hypothalamic 5-HT metabolism or reverse the decreases in 5-HT synthesis in hypothalamus. These results suggest that the hypophagic effects of L-NAME, which inhibits NO formation, are independent of 5-HT metabolism in the hypothalamus.

Keywords: Nitric oxide (NO); 8-OH-DPAT (8-hydroxy-2-di-*n*-(propylamino)tetralin); Food intake; 5-HT (5-hydroxytryptamine, serotonin); N^G-nitro-L-arginine methyl ester (L-NAME); Hypothalamus

1. Introduction

Nitric oxide (NO) is released from the endothelium in blood vessels and regulates the tone of blood vessels (Snyder, 1992). NO also participates in a variety of central functions such as memory, pain or the neurotoxicity elicited by activation of glutamate receptors (Moncada et al., 1991; Moore et al., 1993). There is increasing evidence that NO is involved in food intake. Inhibition of NO formation by NO synthase inhibitors elicits hypophagia in several hyperphagic animal models, such as genetically obese mice and rats (Squadrito et al., 1993; Morley and Flood, 1994). Moreover, NO synthase inhibitors can suppress drug-induced hyperphagia in experimental animals. It has been reported that NO synthase inhibitors, L-N^G-nitro-arginine or N^G-nitro-L-arginine methyl ester (L-NAME), reduce increases in food intake elicited by chlordiazepoxide and morphine (Calignano et al., 1993; Czech, 1996). We previously demonstrated that L-NAME inhibited the feeding induced in rats by a glucose analog, 2-deoxy-D-glucose- and 5-hydroxytryptamine (5-HT)$_{1A}$ receptor agonist, 8-hydroxy-2-di-*n*-(propylamino)tetralin (8-OH-DPAT) (Yamada et al., 1996, 1997). We also reported that a neuronal NO synthase inhibitor, 7-nitroindazole, can suppress 8-OH-DPAT-elicited hyperphagia (Sugimoto et al., 1999). These previous findings reveal that NO is a modulator in the regulation of food intake.

It is well known that 5-HT regulates appetite and the facilitation of serotonergic activity elicited by 5-HT releasing drugs or 5-HT reuptake inhibitors results in anorexia (Dourish, 1995). A 5-HT_{1A} receptor agonist, 8-OH-DPAT, elicits hyperphagia following the activation of the somatodendritic 5-HT_{1A} autoreceptors located in the dorsal raphe nuclei, which leads to inhibition of 5-HT neurons, resulting in decreases in 5-HT turnover or 5-HT release (Hutson et al., 1986; Dourish, 1995). We had reported that a neuronal NO synthase inhibitor, 7-nitroindazole, did not increase 5-HT synthesis in rat hypothalamus, although it reduces 8-OH-DPAT-induced hyperphagia (Sugimoto et al., 1999). However, it is not yet clear whether the hypophagic effects of L-NAME are related to serotonergic

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00507-0

neurons. We now investigated the effects of L-NAME on 5-HT metabolism and synthesis in the hypothalamus, which is important in controlling appetite.

2. Materials and methods

Male Sprague-Dawley rats (200–240 g) were obtained from SLC Japan. They were housed in individual cages and maintained under a controlled 12:12 h light/dark cycle (lights on at 07:00 h), with room temperature at 23 ± 1°C and humidity at 55 ± 5% for at least 7 days prior to experiments. The rats were given free access to food and water. Preweighed food was placed in the cage and the amount of food remaining was weighed 1 h after the injection of 8-OH-DPAT.

L-NAME hydrochloride and 8-OH-DPAT hydrobromide were obtained from Research Biochemicals (USA). Drugs were dissolved in saline and administered in a volume of 0.2 ml/100 g. L-NAME and 8-OH-DPAT were injected i.p. and s.c., respectively. L-NAME was injected i.p. 30 min before the injection of 8-OH-DPAT because of the previous finding that prior injection of L-NAME at that time effectively reduced 8-OH-DPAT-elicited hyperphagia (Yamada et al., 1996). Drugs were injected between 13:00 and 14:00 h.

The rats were decapitated 1 h after the injection of 8-OH-DPAT and the brain was removed. The hypothalamus was dissected out on dry ice and stored at −40°C until analysis. 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were measured by high performance liquid chromatography (HPLC) with electrochemical detection.

The 5-HT synthesis was assessed by the determination of accumulated 5-hydroxytryptophan (5-HTP) levels following injection of the aromatic amino acid decarboxylase inhibitor, 3-hydroxybenzylhydrazine HCl (NSD 10155, Nakarai Tesque, Japan). NSD 1015 was injected i.p. at 100 mg/kg 30 min before death. The 5-HTP was determined by HPLC with electrochemical detection.

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

3. Results

The effects of L-NAME on the hyperphagia elicited by 8-OH-DPAT are shown in Fig. 1. The 8-OH-DPAT at 0.5 mg/kg induced an apparent increase in food intake for 1 h. Pretreatment with L-NAME dose dependently suppressed 8-OH-DPAT-induced hyperphagia.

Fig. 2A shows the effects of L-NAME on 5-HT and 5-HIAA levels in the hypothalamus. L-NAME did not change basal 5-HT, 5-HIAA contents or the 5-HIAA/5-HT ratio in hypothalamus. In saline-pretreated rats, 8-OH-DPAT at a dose of 0.5 mg/kg increased 5-HT. 8-OH-DPAT decreased 5-HIAA levels and the 5-HIAA/5-HT ratio. Pretreatment with L-NAME did not modify the 8-OH-DPAT-induced changes in 5-HT, 5-HIAA contents or 5-HIAA/5-HT ratio.

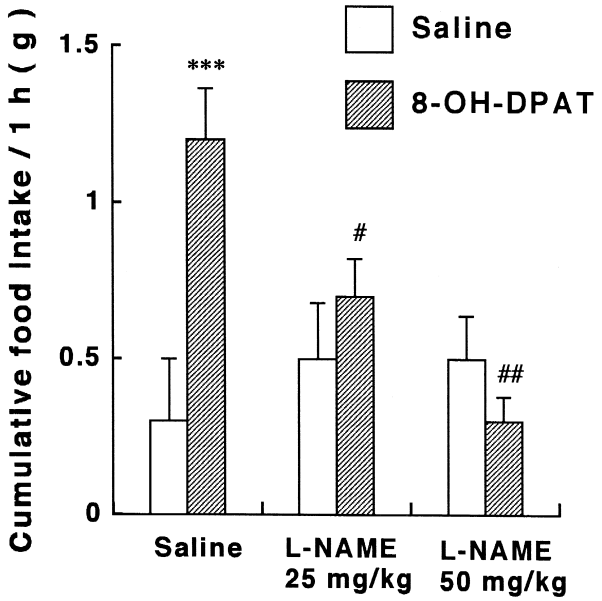

Fig. 1. Effects of L-NAME on 8-OH-DPAT-induced hyperphagia in rats. Results are shown as the means ± S.E. ($N = 5–7$). The 8-OH-DPAT at 0.5 mg/kg was injected s.c. and L-NAME was injected i.p. 30 min before the injection of 8-OH-DPAT. $^{***}P < 0.001$ vs. saline in the respective group. $\#P < 0.05$,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

Fig. 2B shows the effects of L-NAME on 8-OH-DPAT-induced decreases in 5-HT synthesis in the hypothalamus. The 5-HT synthesis was assessed by the determination of 5-HTP levels following the injections of the aromatic amino acid decarboxylase inhibitor, NSD 1015. 8-OH-DPAT significantly inhibited 5-HTP accumulation. Pretreatment with L-NAME (50 mg/kg) did not change the 5-HTP accumulation in the saline-treated control group. In addition, L-NAME slightly enhanced the 8-OH-DPAT-induced decreases in 5-HTP accumulation.

4. Discussion

8-OH-DPAT increases food intake in freely feeding rats and the effects are mediated by the activation of somatodendritic 5-HT_{1A} autoreceptors of dorsal raphe nuclei, resulting in inhibition of 5-HT neural firing (Hutson et al., 1986; Dourish, 1995). As shown in our results, 8-OH-DPAT at a dose of 0.5 mg/kg increased food intake for 1 h. The hyperphagia elicited by 8-OH-DPAT was apparently suppressed by an NO synthase inhibitor, L-NAME, in a dose-related manner, consistent with a previous report (Yamada et al., 1996). It was reported that administration of 8-OH-DPAT also decreases serotonergic activity and causes inhibition of 5-HT release from nerve terminals, as shown by microdialysis (Hutson et al., 1989). It was also

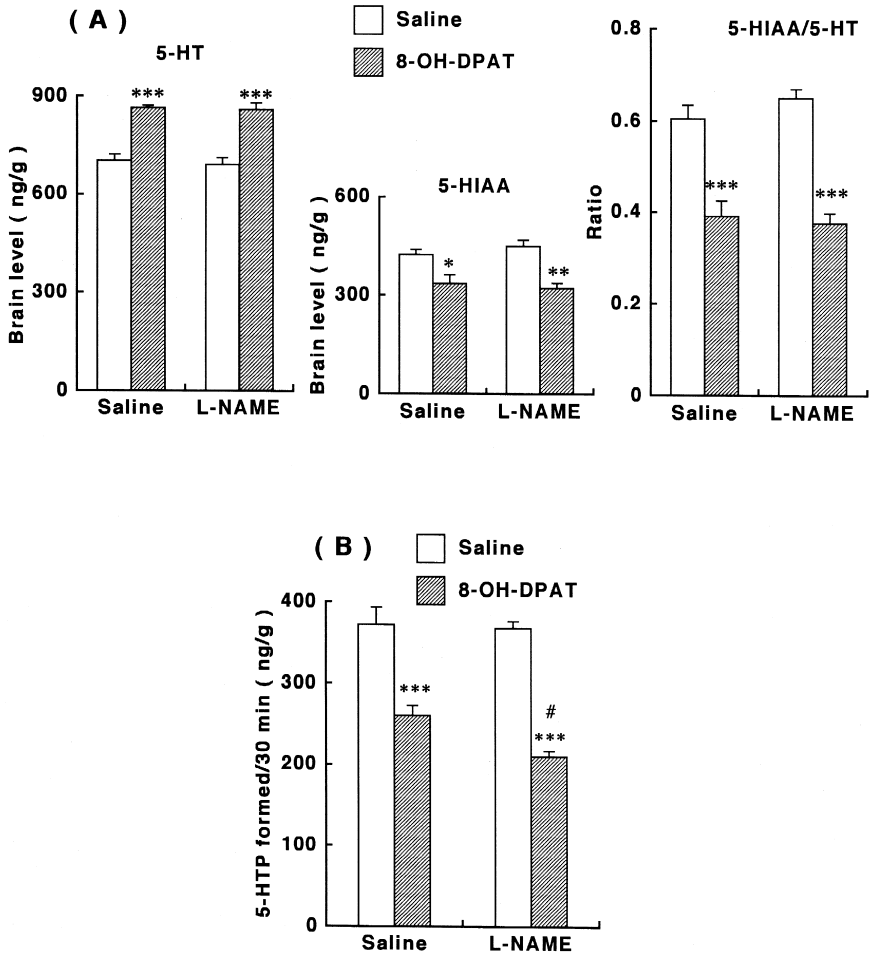

Fig. 2. Effects of L-NAME on hypothalamic 5-HT and 5-HIAA levels and 5-HT synthesis in 8-OH-DPAT-treated rats. (A) Effects of L-NAME on hypothalamic 5-HT and 5-HIAA levels in 8-OH-DPAT-treated rats. Results are shown as the means ± S.E. ($N = 5–7$). L-NAME at 50 mg/kg was given i.p. 30 min before 8-OH-DPAT. The rats were decapitated 1 h after the injection of 8-OH-DPAT. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ vs. saline of respective groups. (B) Effects of L-NAME on 8-OH-DPAT-induced decreases in 5-HTP accumulation in the hypothalamus. Results are shown as the means ± S.E. ($N = 5–7$). The 8-OH-DPAT at 0.5 mg/kg was injected s.c. L-NAME at 50 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. The rats were decapitated 30 min after NSD 1015. $^{***}P < 0.001$ vs. saline in the respective group. $\#P < 0.05$ vs. 8-OH-DPAT in the saline-pretreated group.

reported that both hyperphagic effects and inhibition of 5-HT release appeared after 1 h and lasted for around 4 h after the treatment with 8-OH-DPAT (Yamada et al., 1996; Sugimoto et al., 1999; Auerbach et al., 1989; Hutson et al., 1989). Furthermore, 8-OH-DPAT decreases 5-HT metabolism and synthesis in various regions of the brain including the hypothalamus which controls appetite (Hjorth et al., 1982; Hutson et al., 1986; Hjorth and Magnusson, 1988). Since L-NAME suppresses 8-OH-DPAT-induced feeding, L-NAME may attenuate 5-HT metabolism. Therefore, we investigated the effects of L-NAME on 5-HT metabolism and 5-HT synthesis in rat hypothalamus.

L-NAME did not change either basal 5-HT or a 5-HT metabolite, 5-HIAA, content in the hypothalamus. As shown in Section 3, 8-OH-DPAT increased 5-HT and decreased 5-HIAA and the 5-HIAA/5-HT ratio in the hypothalamus. Our results showing increases in 5-HT concentrations after treatment with 8-OH-DPAT are consistent with the report by Hjorth et al. (1982) that relatively high doses of 8-OH-DPAT elevate 5-HT levels and inhibit utilization of 5-HT. Since it was reported that 8-OH-DPAT reduces 5-HT synthesis (Hjorth and Magnusson, 1988), it is considered that 5-HT turnover in the hypothalamus is decreased. Actually, as shown in the results, 8-OH-DPAT reduces 5-HT synthesis (5-HTP accumulation). L-NAME slightly enhanced the decreases in 5-HT synthesis in the hypothalamus. Therefore, pretreatment with L-NAME did not at all counteract the decreased hypothalamic 5-HT metabolism elicited by 8-OH-DPAT.

Our results indicate that L-NAME did not change basal 5-HT or 5-HIAA levels in the hypothalamus, although the dosage of L-NAME used in our study could decrease the hyperphagia elicited by 8-OH-DPAT. Furthermore, L-NAME did not increase the 8-OH-DPAT-induced de-

creases in 5-HT synthesis in the hypothalamus. These results indicate that the inhibitory effects of L-NAME on 8-OH-DPAT-elicited hyperphagia are not dependent on 5-HT metabolism in the hypothalamus and that its effects are not derived from facilitation of 5-HT turnover. We had previously demonstrated that a neuronal NO synthase inhibitor, 7-nitroindazole, did not alter 5-HTP accumulation in hypothalamus (Sugimoto et al., 1999). Therefore, the hypophagic effects of NO synthase inhibitors on 8-OH-DPAT-elicited hyperphagia are not elicited by facilitation of 5-HT metabolism and synthesis. Squadrito et al. (1994) reported that the anorectic effects of the other NO synthase inhibitor, N^G-nitro-L-arginine, in starved rats were mediated by increases in 5-HT and 5-HIAA levels in brain. However, these authors did not study the effects of N^G-nitro-L-arginine on 5-HT synthesis, and the status of brain 5-HT metabolism in their hyperphagic models may have differed from that in our experiments. Since NO synthase inhibitors can inhibit opioid- or neuropeptide Y-mediated feeding (Morley and Flood, 1992; Calignano et al., 1993), these neurotransmitters may be implicated in the anorectic effects of NO synthase inhibitors. Thus, the hypophagic effects of L-NAME may be associated with these neurotransmitters.

In summary, our results demonstrate that an NO synthase inhibitor, L-NAME, attenuated the hyperphagia elicited by the 5-HT_{1A} autoreceptor agonist, 8-OH-DPAT, and that L-NAME did not increase 5-HT metabolism of the hypothalamus. These results suggest that the hypophagic effects of L-NAME are independent of 5-HT metabolism in the hypothalamus.

Acknowledgements

This work was supported in part by The Science Research Promotion Fund of The Japan Private School Promotion Foundation.

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