Inhibitory Effects of a Nitric Oxide Synthase Inhibitor, N^G -Nitro-L-arginine Methyl Ester (L-NAME), on 2-Deoxy-D-glucose-Induced Hyperglycemia in Rats

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The inhibitory effects of a potent nitric oxide (NO) synthase inhibitor, N^G -nitro-L-arginine methyl ester (L-NAME), on 2-deoxy-D-glucose (2-DG)-induced hyperglycemia were investigated in rats. L-NAME significantly inhibited 2-DG-induced hyperglycemia, although N^G -nitro-D-arginine methyl ester (D-NAME) did not affect it. A similar NO synthase inhibitor, N^G -monomethyl-L-arginine (L-NMMA), also inhibited 2-DG-induced hyperglycemia. The antagonistic effects of L-NAME are unrelated to the cholinergic system, since the muscarinic receptor antagonist scopolamine did not affect 2-DG-induced hyperglycemia. The neuronal NO synthase inhibitor 7-nitroindazole (7-NI) did not reduce 2-DG-induced hyperglycemia, but rather enhanced it. Our results suggest that NO may be involved in glucose homeostasis and that the inhibitory effects of L-NAME on 2-DG-induced hyperglycemia are not related to muscarinic receptors or neuronal NO synthase.

Key words nitric oxide; N^G -nitro-L-arginine methyl ester (L-NAME); 2-deoxy-D-glucose; blood glucose; rat

Nitric oxide (NO) is released from the endothelium and is related to the relaxation of blood vessels.¹⁾ NO is also synthesized in the central nervous system and it plays an important role in several central functions such as appetite, pain and memory.¹⁾

It is well known that 2-deoxy-D-glucose (2-DG), a glucose analogue, is known to induce hyperglycemia, since it elicits neuroglycopenia which is associated with the hypothalamus.²⁾ 2-DG-induced hyperglycemia is considered useful for investigating the central control of blood glucose homeostasis. Recently, it was reported that a NO synthase inhibitor, N^G -nitro-L-arginine methyl ester (L-NAME), inhibits 2-DG-induced hyperglycemia in mice.³⁾ This suggests that NO also participates in glucose regulation. A recent report indicated that 2-DG-induced hyperglycemia may be closely associated with central cholinergic activity. Takahashi *et al.* demonstrated that acetylcholine release in the hypothalamus is elevated following the administration of 2-DG by microdialysis.⁴⁾ They also indicated a correlation between the hyperglycemic responses of 2-DG and acetylcholine release.⁴⁾ It has been reported that L-NAME has anti-muscarinic activity,⁵⁾ so there is a possibility that the muscarinic receptor may be related to the effects of L-NAME. Therefore, we examined the effects of the muscarinic receptor antagonist scopolamine on 2-DG-induced hyperglycemia.

It has been suggested that L-NAME can inhibit endothelial, inducible and neuronal NO synthase.¹⁾ Since 2-DG elicits neuroglycopenia, neuronal NO may be related to the inhibitory effects of L-NAME. To clarify the involvement of the neuronal NO synthase in 2-DG-induced hyperglycemia, we examined the effects of the neuronal NO synthase inhibitor 7-nitroindazole (7-NI) on the hyperglycemic effects of 2-DG in rats and compared with those of L-NAME.

MATERIALS AND METHODS

Male Sprague-Dawley rats (180—230 g) were pur-

chased from SLC Japan, Inc. (Shizuoka, Japan). Rats were maintained under a controlled 12 h/12 h light-dark cycle (light from 7:00 a.m. to 7:00 p.m.), with a room temperature of $24 \pm 1^\circ\text{C}$ and $55 \pm 5\%$ humidity. All animals were given free access to food and water before the experiments.

2-DG (Wako, Japan), L-NAME (RBI, U.S.A.), N^G -nitro-D-arginine methyl ester (D-NAME, RBI), N^G -monomethyl-L-arginine acetate (L-NMMA, Wako, Japan) and scopolamine hydrochloride (Nacalai Tesque, Japan) were dissolved in saline, while 7-NI (Dojindo, Japan) was suspended in arachis oil. 2-DG was injected i.p. at 250 mg/kg. L-NAME, D-NAME, L-NMMA, scopolamine and 7-NI were injected i.p. 30 min before 2-DG.

Blood samples were taken from the caudal vena cava under light ether anesthesia. Only one sample was removed from each rat. Plasma glucose levels were determined by the method previously described.⁶⁾

Statistical significance was evaluated using Student's *t*-test for comparisons of the two groups. The effects of several drugs on 2-DG-induced hyperglycemia were analyzed by two-way ANOVA followed by Tukey's test.

RESULTS

Figure 1 demonstrates time course changes in glucose levels following the injection of 2-DG at a dose of 250 mg/kg. 2-DG apparently increased plasma glucose levels 15, 30 and 60 min after the injection. Maximum elevation was observed 30 min after the injection of 2-DG.

As shown in Fig. 2, pretreatment with a NO synthase inhibitor, L-NAME (50 mg/kg), significantly inhibited 2-DG-induced hyperglycemia. However, D-NAME, a less active enantiomer of L-NAME, did not affect it. Furthermore, another NO synthase inhibitor, L-NMMA (50 mg/kg), reduced hyperglycemia elicited by 2-DG. The effects of a muscarinic receptor antagonist scopolamine at 10 mg/kg were also studied. As shown in the results, scopolamine did not affect the hyperglycemia induced by 2-DG.

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Fig. 1. Effects of 2-DG on Plasma Glucose Levels of Rats

Results are shown as the means $\pm$ S.E. ($n=6$). 2-DG at 250 mg/kg was injected i.p. *** $p<0.001$.

Fig. 2. Effects of NO Synthase Inhibitors and Scopolamine on 2-DG-Induced Hyperglycemia in Rats

Results are shown as the means $\pm$ S.E. ($n=5-6$). 2-DG was injected i.p. at 250 mg/kg. Plasma glucose levels were determined 30 min after injection of 2-DG. ** $p<0.01$, *** $p<0.001$ vs. saline control for respective group. ## $p<0.01$ vs. saline + 2-DG.

Fig. 3. Effects of 7-NI on 2-DG-Induced Hyperglycemia in Rats

Results are shown as the means $\pm$ S.E. ($n=5-7$). 2-DG was injected i.p. at 250 mg/kg. Plasma glucose levels were determined 30 min after injection of 2-DG. *** $p<0.001$ vs. saline control for respective group. ### $p<0.001$ vs. vehicle + 2-DG.

Figure 3 shows the effects of neuronal NO synthase inhibitor 7-NI (25 mg/kg) on 2-DG-induced hyperglycemia. As shown in the results, 7-NI did not decrease 2-DG-induced hyperglycemia but actually enhanced it.

Pretreatment with NO synthase inhibitors and scopolamine did not affect the basal plasma glucose levels.

DISCUSSION

To date, considerable evidence has been accumulated showing that NO plays a significant role in several physiological functions. However, the involvement of NO in glucose regulation remains unclear. L-NAME significantly reduced 2-DG-induced hyperglycemia in rats, while the less active enantiomer, D-NAME, did not affect it. Our results obtained in rats are consistent with those of a previous study using mice.³⁾ In addition, a similar NO synthase inhibitor, L-NMMA, inhibited 2-DG-induced hyperglycemia. Our results further support the hypothesis that NO may participate in 2-DG-elicited hyperglycemia.

Recently, Takahashi *et al.* reported that 2-DG-induced hyperglycemia in rats is concomitant with acetylcholine release from the hypothalamus, implying that the cholinergic mechanism may play a significant role in 2-DG-induced hyperglycemia.⁴⁾ Furthermore, central injection of the choline esterase inhibitor neostigmine induces hyperglycemia which is prevented by the muscarinic receptor antagonist atropine.⁷⁾ These findings show that the central cholinergic activation results in hyperglycemia and it may be essential in the hyperglycemia elicited by 2-DG. Since L-NAME shows anti-muscarinic activity,⁵⁾ its inhibitory effects on 2-DG-induced hyperglycemia may be related to its anti-muscarinic effects. Thus, we studied the effects of the muscarinic receptor antagonist scopolamine on 2-DG-induced hyperglycemia. However, as shown in our results, scopolamine did not affect 2-DG-induced hyperglycemia. Our findings suggest that muscarinic receptors are not strongly related to 2-DG-induced hyperglycemia. Furthermore, L-NMMA, which is devoid of anti-muscarinic effects,⁵⁾ also prevented 2-DG-induced hyperglycemia. These results indicate that the inhibitory effects of L-NAME on 2-DG-induced hyperglycemia result from its inhibition of NO synthase and that muscarinic receptors are not related to its effects.

Recent results demonstrate that NO can modify neurotransmitter release in the brain.¹⁾ Thus, NO may be involved in glucose homeostasis by affecting neurotransmission in the central nervous system. It is well known that L-NAME can inhibit endothelial, inducible and neuronal NO synthase.¹⁾ Although L-NAME inhibited 2-DG-induced hyperglycemia, the involvement of neuronal NO synthase is not clear. Thus, we studied the effects of a selective neuronal NO synthase inhibitor, 7-NI, on 2-DG-induced hyperglycemia. However, 7-NI did not reduce 2-DG-induced hyperglycemia, but rather significantly enhanced it. These results suggest that neuronal NO is not related to the inhibitory effects of L-NAME on 2-DG-induced hyperglycemia in rats, although the involvement of inducible NO is not yet clarified at present.

It has been suggested that 2-DG-induced hyperglycemia

is mediated by the activation of the sympathoadrenal system, in response to the central nervous system, which leads to catecholamine release from the adrenal medulla.⁸⁾ It has been reported that adrenalectomy or the administration of a sympathetic noradrenergic antagonist, guanethidine, can antagonize 2-DG-induced hyperglycemia.^{8,9)} Thus, the inhibitory effects of L-NAME may originate from its effects in the peripheral system, although its action in the central nervous system cannot be excluded. The involvement of NO in the release of the pancreatic hormone, insulin, or that of catecholamine from the adrenal medulla has been reported.^{10,11)} Therefore, the inhibitory effects of L-NAME on 2-DG-elicited hyperglycemia may be connected with the release of these hormones. Furthermore, the result that 7-NI enhanced glycemia suggests that NO in the central nervous system may play a role in the regulation of blood glucose levels.

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