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Originals

Effects of two 5-hydroxytryptamine agonists on head-weaving behaviour in streptozotocin-diabetic mice

E. Fujii, T. Nomoto and T. Muraki

Department of Pharmacology, Tokyo Women's Medical College, Tokyo, Japan

Summary. The potencies of 5-methoxy-N, N-dimethyltryptamine (central 5-hydroxytryptamine 1 receptor agonist) and 8-hydroxy-2-(di-n-propylamino) tetralin (central 5-hydroxytryptamine 1A receptor agonist) in eliciting head-weaving behaviour were studied in streptozotocin-diabetic mice and a group of control animals. Both drugs induced head-weaving behaviour in the streptozotocin-diabetic mice and control animals, but the potencies of these 5-hydroxytryptamine 1 agonists were reduced in the streptozotocin-diabetic mice. The numbers of head weaves elicited in the streptozotocin-diabetic and control animals by the two drugs were suppressed by pre-treatment with propranolol (5-hydroxytryptamine 1A receptor antagonist) and methysergide (5-hydroxytryptamine 1 and 2 receptor antagonist), but not by ketanserin (5-hydroxytryptamine 2 receptor antagonist), confirming the involvement of the 5-HT_{1A} receptor. Pre-treatment with nicotinamide before administering streptozotocin prevented streptozotocin-induced hyperglycaemia and restored the inhibition of head-weaving behaviour observed in streptozotocin-diabetic mice. Insulin injection, which partially prevented streptozotocin-induced hyperglycaemia, completely prevented reduction of the number of head weaves elicited by 5-methoxy-N, N-dimethyltryptamine in streptozotocin-diabetic mice. These results suggest that the reduced response to 5-HT_1 agonists in streptozotocin-diabetic mice may be caused by the depletion of insulin.

Key words: 5-MeODMT, 8-OH-DPAT, head-weaving behaviour, 5-HT_{1A} receptors, STZ, diabetic mice.

We have previously reported that responses to centrally acting drugs, such as morphine, pentobarbital and ketamine, may be altered in streptozotocin (STZ)-diabetic mice [1–4]. It has been reported that after administration of 5-HT agonists, rats showed signs of the 5-HT behavioural syndrome, such as resting tremours, rigidity, reciprocal forepaw treading, Straub tail, hindlimb abduction and side-to-side movements of the head (head weaving) [5]. Many studies have been carried out on the 5-hydroxytryptamine 1 (5-HT_1) receptor-mediated responses elicited by administering 5-methoxy-N, N-dimethyltryptamine (5-MeODMT) and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in rats [6–10], but few in mice [11–15]. Thus, it is unknown whether the 5-HT_1 receptor-mediated behaviour can be altered in STZ-diabetic mice. Therefore, we studied whether the 5-HT syndrome induced by 5-MeODMT and 8-OH-DPAT differs between STZ-diabetic mice and control animals. We chose the head-weaving behaviour as the 5-HT behavioural syndrome because a preliminary study showed head-weaving to be the most conspicuous sign of the behavioural 5-HT syndrome in mice. To elucidate the role of insulin, we studied the effects of nicotinamide (NicA) and insulin treatment on the head-weaving behaviour elicited by 5-MeODMT and 8-OH-DPAT in STZ-diabetic mice.

Materials and methods

Experimental animals

Male ddY mice (25–30 g) were housed in an air-conditioned room (temperature 22 ± 2°C, humidity 55 ± 5%) with a controlled light-dark cycle (light on 06.00–20.00 hours) and freely available food and water. To induce diabetes, STZ (170 mg/kg), freshly dissolved in 0.1 mol/l citrate buffer (pH 4.6), was injected i.p. in the mice 2 weeks before the experiment. The control animals received citrate buffer i.p. instead of STZ. Two weeks later, the STZ-treated mice showed signs of diabetes such as reduced growth rate, glucosuria and hyperglycaemia. NicA (500 mg/kg, i.p.) was administered 15 min before STZ. Some STZ-diabetic mice were injected with a daily dose of 10 IU/kg of Isophane (NPH)-insulin for 3 days from the eleventh day following STZ injection. The NPH-insulin was injected s.c. twice a day, once in the morning and once in the evening at different body sites [3]. Experiments were performed about 20 h after the last insulin injection.

Table 1. Changes in body weight and serum glucose level in streptozotocin (STZ), nicotinamide (NicA)-STZ and STZ-insulin treated mice and control animals

		Body weight (g)		Serum glucose (mmol/l)
		Initial	Final	
Control	(11)	31.4 ± 0.39	36.6 ± 0.77	8.4 ± 0.44
STZ	(14)	31.8 ± 0.25	32.3 ± 0.75[a]	33.3 ± 2.16[a]
NicA-STZ	(10)	31.4 ± 0.37	36.3 ± 0.49	7.7 ± 0.55[b]
STZ-insulin	(19)	31.9 ± 0.39	32.9 ± 0.54[a]	24.3 ± 0.33[a,b]

STZ (170 mg/kg) was injected i.p. in 6-week-old mice 2 weeks before killing (STZ group). The control animals received i.p. injections of citrate buffer instead of STZ. NicA (500 mg/kg, i.p.) was administered 15 min before administering STZ (NicA-STZ group). A daily dose of 10 IU NPH-insulin/kg was s.c. injected for 3 days from the eleventh day of the STZ injection (STZ-insulin group). The mice were weighed when the STZ or citrate buffer (initial) was administered and 2 weeks later (final) when the mice were killed, and the serum glucose was measured. Values represent the mean ± SEM. Numbers in parenthesis indicate the number of mice. [a] $p < 0.01$ vs control animals, [b] $p < 0.01$ vs STZ-diabetic mice (Student's t-test).

Blood, collected when the mice were killed, was used for glucose determination by the o-toluidine-boric acid method using a glucose test kit (Wako, Tokyo, Japan) [3].

Behavioural observations

Mice were placed in individual plastic cages and the numbers of head weaves were counted after i.p. injection of 5-MeODMT or 8-OH-DPAT. The appearance of head weaving was observed for 20 min after administering 5-MeODMT. Two-minute observation sessions were repeated every 5 min for 20 min and the sum of the head weaves occurring in the four sessions was taken as the total number of head weaves. In the case of 8-OH-DPAT, the most head weaves occurred within 7 min, therefore we counted the number of head weaves for 7 min after administering 8-OH-DPAT as the total number for 8-OH-DPAT unless otherwise stated.

Drugs and treatment

Propranolol and ketanserin were administered i.p. 15 min before, and methysergide was administered i.p. 5 min before administration of 5-MeODMT or 8-OH-DPAT.

The sources of the drugs used are as follows: STZ and 5-MeODMT (Sigma Chemical Co., Mo, USA), 8-OH-DPAT (8-OH-DPAT HBr, Research Biochemicals Inc., Mass., USA). NicA (Tokyo Kasei Kogyo Co., Tokyo, Japan), NPH-insulin (NPH-ISZILIN, 40 IU/ml, Shimizu Pharmaceutical Co., Shizuoka, Japan), methysergide (Sandoz Pharmaceuticals, Feltham, UK), propranolol (Inderal, Sumitomo Pharmaceutical Co., Osaka, Japan) and ketanserin tartrate (Janssen Kyowa, Tokyo, Japan). 5-MeODMT was suspended in 0.5% carboxymethylcellulose-Na. Methysergide was dissolved in several drops of methanesulphonic acid and diluted in 154 mmol/l NaCl. Other drugs were dissolved in 154 mmol/l NaCl. All drugs were administered to the mice in dosages of 0.1 ml/10 g body weight.

Statistical analysis

Results are expressed as mean ± SEM and were statistically evaluated by Student's t-test.

Results

Changes in body weight and serum glucose in STZ, NicA-STZ and NPH-insulin treated STZ-diabetic (STZ-insulin) mice and control animals

Body weight increases were reduced by STZ treatment ($p < 0.01$) and the mean serum glucose level was 33.3 ± 2.16 mmol/l in STZ-diabetic mice, which was higher than that in the control animals (Table 1). Pre-treatment with NicA completely prevented body-weight reductions and STZ-induced hyperglycaemia. Insulin did not affect body-weight reductions in STZ-diabetic mice, but significantly prevented STZ-induced hyperglycaemia, although the serum glucose levels in STZ-insulin treated mice were higher than in the control animals.

Effects of 5-MeODMT on head-weaving behaviour in STZ, NicA-STZ and STZ-insulin treated mice and control animals

The head-weaving behaviour elicited by 6–10 mg/kg doses of 5-MeODMT in STZ-diabetic mice and control animals was dose-dependent. 5-MeODMT also induced resting tremours in the STZ-diabetic mice and control animals, but we did not study resting tremours in this experiment. The total number of head weaves was lower in the STZ-diabetic mice than in the control animals (Fig. 1). Figure 2 a and b shows the time course for the number of head weaves in each 2-min observation session and the total number of head weaves elicited in STZ, NicA-STZ and STZ-insulin treated mice and control animals by 5-MeODMT (10 mg/kg). The number of head weaves peaked in all mice groups 10 to 12 min after administering 5-MeODMT, however, the peak effect of 5-MeODMT was again less in the STZ-diabetic mice than in the control animals. NicA pre-treatment or insulin treatment almost completely prevented the reduction in the head weaves elicited in STZ-diabetic mice by 5-MeODMT.

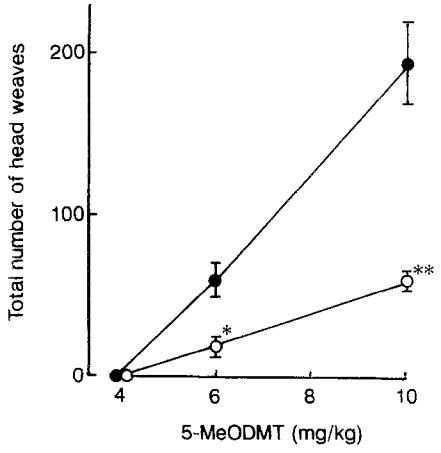

Fig. 1. Effects of increasing doses of 5-methoxy-N, N-dimethyltryptamine (5-MeODMT) on the total number of head weaves in STZ-diabetic (○) mice and controls (●). Values represent the mean ± SEM ($n = 10$). * $p < 0.05$, ** $p < 0.01$ vs control animals

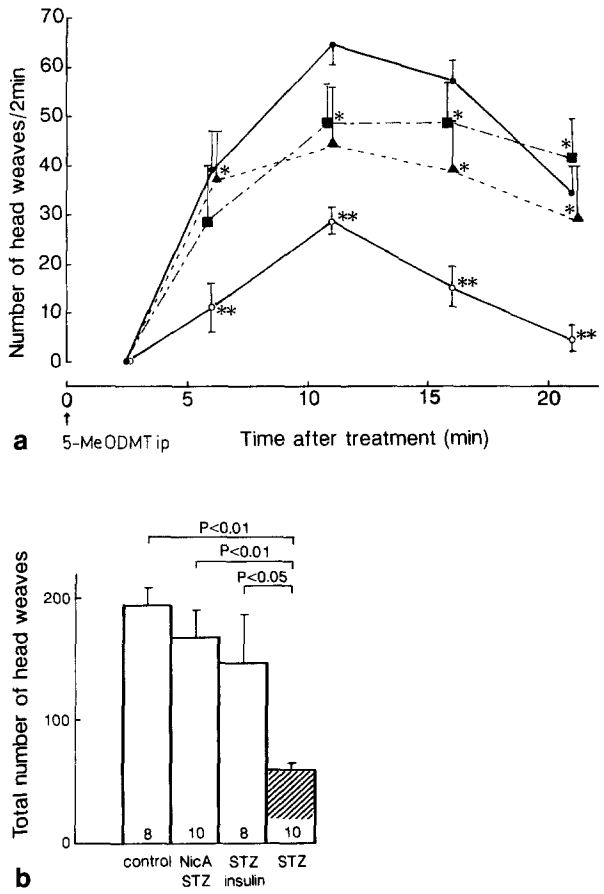

Fig. 2 a, b. Time course of the effect of 5-MeODMT (10 mg/kg, i.p.) on the number of head weaves (**a**) and total number of head weaves elicited by 5-MeODMT (10 mg/kg, i.p.) (**b**) in STZ (○), NicA-STZ (■) and STZ-insulin (▲) treated mice and control animals (●). Treatment of mice was the same as for those listed in Table 1. Values represent the mean ± SEM ($n = 10$). ** $p < 0.01$ vs control animals, * $p < 0.05$ vs STZ-diabetic mice

To show the subtype of 5-HT receptors involved in eliciting the head-weaving behaviour, we examined the effects of three 5-HT receptor antagonists on the 5-MeODMT-induced head-weaving behaviour. The basal number of head weaves by STZ-diabetic mice (60 ± 6, $n = 10$) significantly differed from that of the control animals (195 ± 13, $n = 10$). Pre-treatment with propranolol (20–40 mg/kg) increased mortality only in the 5-MeODMT-treated control animals when administered 5 min previously (data not shown), but propranolol (40 mg/kg) administered 15 min before the 5-MeODMT significantly reduced the number of head weaves in the STZ-diabetic mice and control animals without killing the mice (Fig. 3). Methysergide (10 mg/kg) reduced the number of head weaves in the STZ-diabetic and control animals, with the reduction being lower in the STZ-diabetic mice than in the control animals. Ketanserin (0.5 mg/kg) did not affect the head-weaving behaviour elicited by 5-MeODMT in either the STZ-diabetic mice or control animals. These results suggest that the head-weaving behaviour elicited by 5-MeODMT may be mediated by the postsynaptic 5-HT_{1A} receptor.

Effects of 8-OH-DPAT on head-weaving behaviour in STZ-diabetic mice and control animals

After administering 8-OH-DPAT, the 5-HT syndrome, including resting tremours, hyperactivity, Straub tail and head weaving, was evident in the mice. 8-OH-DPAT induced a short clonic seizure within 7 min, immediately followed by the head-weaving behaviour. The maximum head-weaving rate was observed within 7 min of administering 8-OH-DPAT, and the head-weaving behaviour ceased within 15 min. Figure 4 a and b show the time course for the number of head weaves elicited in STZ-diabetic and control animals by 40 and 60 mg/kg doses of 8-OH-DPAT. The 40 mg/kg dose of 8-OH-DPAT induced head-weaving behaviour in the control animals, with the peak effect observed 5 to 7 min after administering 8-OH-DPAT, but this dose did not induce head-weaving behaviour in the STZ-diabetic mice (Fig. 4 a). A higher dose of 8-OH-DPAT (60 mg/kg) elicited head-weaving behaviour in the STZ-diabetic mice, but not in the control animals. Figure 5 shows the dose-dependent effects of 8-OH-DPAT in inducing head-weaving behaviour in STZ-diabetic and control animals. The dose response curve is bell-shaped, indicating that a higher dose of 8-OH-DPAT inhibited the head-weaving behaviour. The peak effect dose of 8-OH-DPAT for eliciting head-weaving behaviour in STZ-diabetic mice (60 mg/kg) was larger than that for the control animals which have been (40 mg/kg). A larger dose (60 mg/kg or more) of 8-OH-DPAT induced generalized convulsions and death in some control animals which have been excluded from the experiment. The dose response curve for 8-OH-DPAT shifted to the right in STZ-diabetic mice, indicating reduced sensitivity of STZ-treated mice to 8-OH-DPAT.

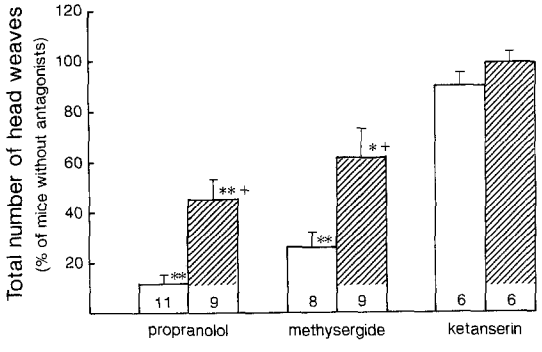

Fig. 3. Effects of 5-HT antagonists on the total number of head weaves elicited in STZ-diabetic mice and control animals by 5-MeODMT. Values (% of total number of head weaves of mice without antagonists) represent the mean ± SEM ($n = 6$–11). STZ-diabetic (▨) mice and control animals (□) received 10 mg/kg doses of 5-MeODMT. Propranolol (40 mg/kg, i.p.) and ketanserin (0.5 mg/kg, i.p.) were administered 15 min before, and methysergide (10 mg/kg, i.p.) was administered 5 min before administering 5-MeODMT. The effects of 5-HT antagonists are expressed as % of the total number of head weaves of STZ-diabetic mice (60 ± 6, $n = 10$) and control animals (195 ± 13, $n = 10$) treated with 154 mmol/l NaCl instead of 5-HT antagonists. * $p < 0.05$, ** $p < 0.01$ vs 154 mmol/l NaCl treated STZ-diabetic mice or control animals. + $p < 0.05$ vs control animals

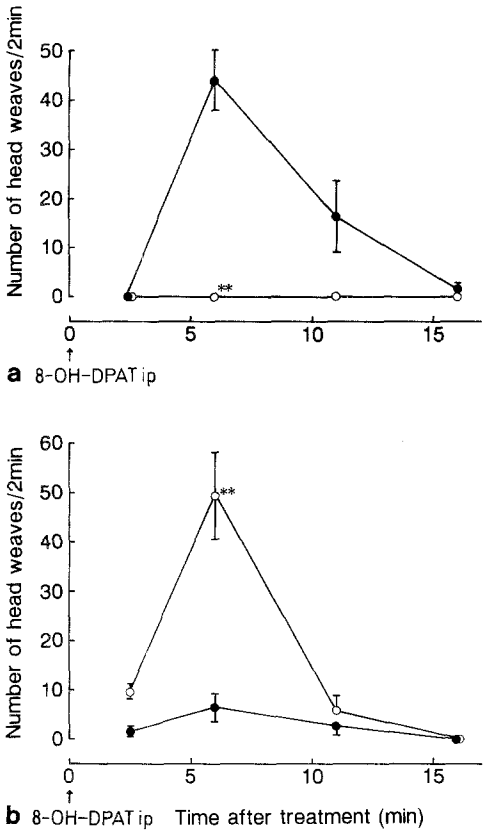

Fig. 4 a, b. Time course of the number of head weaves elicited by 40 mg/kg, i.p. (**a**) and 60 mg/kg, i.p. (**b**) doses of 8-OH-DPAT in STZ-diabetic (○) mice and control animals (●). Values represent the mean ± SEM ($n = 10$). ** $p < 0.01$ vs control animals

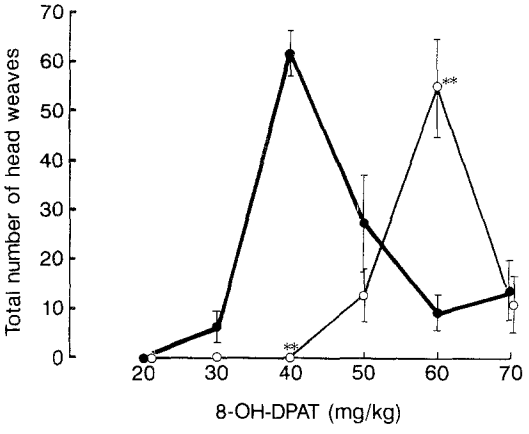

Fig. 5. Effects of the dose of 8-OH-DPAT on the total number of head weaves in STZ-diabetic (○) mice and control animals (●). Values represent the mean ± SEM ($n = 10$). ** $p < 0.01$ vs control animals

Similar to the results with 5-MeODMT, Figure 6 shows that the total number of head weaves elicited in STZ-diabetic mice (47 ± 6, $n = 10$) and the control animals (59 ± 10, $n = 10$) by 8-OH-DPAT is reduced by pre-treatment with propranolol (1 mg/kg) and methysergide (10 mg/kg), but not by ketanserin (0.5 mg/kg), which again suggests the involvement of the 5-HT_{1A} receptor in the STZ-diabetic and control animals.

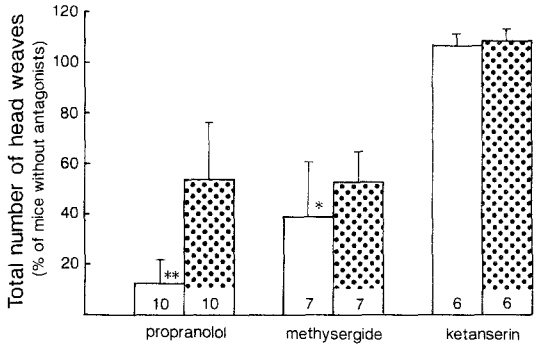

Fig. 6. Effects of 5-HT antagonists on the total number of head weaves elicited in STZ-diabetic mice and control animals by 8-OH-DPAT. Values (% of total number of head weaves of mice without antagonists) represent the mean ± SEM ($n = 6$–10). Control animals (□) and STZ-diabetic mice (▨) respectively received 40 mg/kg and 60 mg/kg doses of 8-OH-DPAT. Propranolol (1 mg/kg, i.p.) and ketanserin (0.5 mg/kg, i.p.) were administered 15 min before, and methysergide (10 mg/kg, i.p.) was administered 5 min before administering 8-OH-DPAT. The effects of 5-HT antagonists are expressed as % of the total number of head weaves of control animals (59 ± 10, $n = 10$) and STZ-diabetic mice (47 ± 6, $n = 10$) treated with 154 mmol/l NaCl instead of 5-HT antagonists. * $p < 0.05$, ** $p < 0.01$ vs 154 mmol/l NaCl-treated control animals

Discussion

We observed that head-weaving behaviour elicited in normal ddY strain mice by 5-MeODMT and 8-OH-DPAT is dose-dependent and that the head-weaving behaviour is prevented by propranolol and methysergide, but not by ketanserin. These results confirm previous studies showing that 5-MeODMT and 8-OH-DPAT induces head-weaving behaviour in rats and normal mice of other strains by activating the central 5-HT_{1A} receptor [5–11, 14, 15].

Previous studies show that s.c. and i.p. injections of lower doses (around 0.1 mg/kg) of 8-OH-DPAT can induce the 5-HT syndrome in rats [9, 10]. Goodwin and Green [11] reported that 8-OH-DPAT (10 mg/kg, s.c.) does not induce the 5-HT syndrome in C57BL mice. However, Yamada et al. [15] reported that in doses exceeding 20 mg/kg, i.p., 8-OH-DPAT induces mild, short-duration 5-HT syndrome in C57BL mice and suggested a species difference in the sensitivity to 8-OH-DPAT between rats and mice. In agreement with previous studies on mice, we found that 8-OH-DPAT (20–70 mg/kg, i.p.) induces the head-weaving behaviour in ddY mice, and we consider that for some unknown reason this mouse strain is particularly insensitive to 8-OH-DPAT.

Previous workers have not reported the inhibition of head weaving by large doses of 8-OH-DPAT, probably because they have not studied the effects of an 8-OH-DPAT dose as high as 40 mg/kg. (We did not study whether high doses of 5-MeODMT inhibit head weaving either.) The mechanism of head-weaving-behaviour inhibition by high doses of 8-OH-DPAT is unclear at present.

We proved that STZ-induced diabetic mice are hyposensitive to 5-MeODMT and 8-OH-DPAT in eliciting head-weaving behaviour, although the difference between STZ-diabetic mice and control animals is only quantitative. The mechanism of STZ-induced hyposensi-

tivity to 5-HT_{1A} agonists is unknown at present. Trulson and MacKenzie [16] reported that there are no significant changes in specific [^{3}H]-5-HT receptor binding in the brains of STZ-diabetic rats compared with control values, however, the regional distribution pattern of the 5-HT_{1A} receptor in the brains of STZ-diabetic mice may differ from that of normal mice. There are several possible causes for the hyposensitivity of STZ-diabetic mice to 5-HT_{1A} agonists, namely, the penetration rate into the brain of injected 5-HT_1 agonists may be affected by STZ pretreatment or hyperglycaemia or abnormal pentose metabolism in STZ-diabetic mice may indirectly affect the responsiveness of the brain to 5-HT_{1A} agonists. It is also unknown whether diabetes reduces the uptake of 5-MeODMT or 8-OH-DPAT by the brain.

To elucidate the role of insulin in the hyposensitivity of STZ-diabetic mice to 5-MeODMT and 8-OH-DPAT, we studied whether the effects of STZ are prevented by insulin and NicA. NicA, an inhibitor of poly (ADP-ribose) synthetase, maintains the intracellular level of NAD, thereby preventing the selective cytotoxicity of STZ on pancreatic Beta cells [17]. In this study, NicA completely and insulin partially prevented hyperglycaemia in STZ-diabetic mice, and NicA and insulin completely recovered the effect of STZ treatment in reducing the number of head weaves elicited by 5-MeODMT. Although we did not study whether NicA and insulin reduce the effect of 8-OH-DPAT in inducing head-weaving behaviour in STZ-diabetic mice, the results suggest that the depletion of insulin may have a causal relationship with the hyposensitivity of STZ-diabetic mice to 5-HT_{1A} agonists.

This study proves that 5-MeODMT and 8-OH-DPAT induce head-weaving behaviour in STZ-diabetic mice and control animals through the 5-HT_{1A} receptor, however, the 5-HT responses of the STZ-diabetic mice were less than those of normal mice, indicating the hyposensitivity of STZ-diabetic mice to 5-HT_{1A} agonists. The clinical importance of the reduced 5-HT syndrome in STZ-diabetic mice is unknown. However, this study suggests that the pharmacological effects of 5-HT agonists or antagonists in diabetic patients may differ from that in normal man.

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References

1. Fujii E, Nomoto T, Tsukahara F et al. (1983) Antinociceptive potency of morphine in streptozotocin-diabetic mice. Tokyo Women's Med Coll 53: 1039–1045 (Abstract)
2. Fujii E, Tsukahara F, Nomoto T (1984) Influences of drugs in streptozotocin (STZ)-diabetic mice (III): alterations in the sensitivity to ketamine anesthesia and anticociceptive potency of thalamonal. Jpn J Pharmacol 36 [Suppl.]: 334
3. Fujii E, Tsukahara F, Nomoto T (1987) Changes in pentobarbital hypnosis and hepatic metabolism in streptozotocin-diabetic mice. Folia Pharmacol Japon 90: 83–89
4. Fujii E, Nomoto T (1987) Influences of drugs in streptozotocin (STZ)-diabetic mice (V): Anticonvulsant activity of ketamine on pentylenetetrazol (PTZ)-induced seizures. Jpn J Pharmacol 43 [Suppl.] 230
5. Jacobs BL (1976) An animal behavior model for studying central serotonergic synapses. Life Scie 19: 777–786
6. Hjorth S, Carlsson A, Lindberg P et al. (1982) 8-Hydroxy-2-(di-n-propylamino) tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. J Neural Transm 55: 169-188
7. Lucki I, Nobler MS, Frazer A (1984) Differential actions of serotonin antagonists on two behavioral models of serotonin receptor activation in the rat. J Pharmacol Exp Ther 228: 133–139
8. Tricklebank MD, Forler C, Middlemiss DN, Fozard JR (1985) Subtypes of the 5-HT receptor mediating the behavioural responses to 5-methoxy-N, N-dimethyltryptamine in the rat. Eur J Pharmacol 117: 15–24
9. Tricklebank MD, Forler C, Fozard JR (1985) The involvement of subtypes of the 5-HT_1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino) tetralin in the rat. Eur J Pharmacol 106: 271–282
10. Smith LM, Peroutka SJ (1986) Differential effects of 5-hydroxytryptamine$_{1A}$ selective drugs on the 5-HT behavioral syndrome. Pharmacol Biochem Behav 24: 1513–1519
11. Goodwin GM, Green AR (1985) A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT_1 and 5-HT_2 receptors. Br J Pharmacol 84: 743–753
12. De Souza RJ, Goodwin GM, Green AR, Heal DJ (1986) Effect of chronic treatment with 5-HT_1 agonist (8-OH-DPAT and RU24969) and antagonist (ipsapirone) drugs on the behavioural responses of mice to 5-HT_1 agonist and 5-HT_2 agonists. Br J Pharmacol 89: 377–384
13. Nabeshima T, Ishikawa K, Yamaguchi K, Furukawa H, Kameyama T (1987) Phencyclidine-induced head-weaving observed in mice after ritanserin treatment. Eur J Pharmacol 139: 171–178
14. Sugimoto Y, Yamada J, Horisaka K (1988) Behavioural effects of 5-HT-1A receptor agonists in mice. Jpn J Pharmacol 46 [Suppl.]: 172
15. Yamada J, Sugimoto Y, Horisaka K (1988) The behavioural effects of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in mice. Eur J Pharmacol 154: 299–304
16. Trulson ME, MacKenzie RG (1981) Subsensitivity to 5-hydroxytryptamine in agonists occurs in streptozotocin-diabetic rats with no change in [^{3}H]-5HT receptor binding. J Pharm Parmacol 33: 472–474
17. Okamoto H (1981) Regulation of proinsulin synthesis in pancreatic islets and a new aspect to insulin-dependent diabetes. Mol Cell Biochem 37: 43–61

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Dr. E. Fujii
Department of Pharmacology
Tokyo Women's Medical College
8-1 Kawada-cho
Shinjuku-ku
Tokyo 162
Japan