CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 26, No. 11 November 1978

Regular Articles

[Chem. Pharm. Bull. 26(11)3265—3270(1978)]

UDC 615.212.3.011.4.015.4 : 542.98.04

Inhibitory Effect of Difenamizole on Morphine-Induced Straub Tail Reaction with Special Reference to Monoaminergic Agents[1)]

Tsutomu Kameyama, Makoto Ukai, and Toshitaka Nabeshima

Faculty of Pharmaceutical Sciences, Meijo University[2)]

(Received June 6, 1977)

In order to elucidate the mechanism of difenamizole (DFZ)-induced inhibition of Straub tail reaction (STR), the relationship between biogenic amines and DFZ-induced inhibition of STR (DIS) was particularly investigated in this experiment. 10 mg/kg of apomorphine and 2 mg/kg of methamphetamine decreased the DIS (intraperitoneal and intracerebral injection of DFZ) significantly without producing marked STR. Intracerebral injection of 50 μg/mouse of 5-hydroxytryptamine increased the DIS without affecting STR. On the other hand, the DIS was developed markedly, whether or not mice were pretreated with L-dopa, propranolol, disulfiram, L-5-HTP, tetrabenazine, nialamide, isocarboxazid and tranylcypromine. These results suggest that the DIS might at least be modulated by the variation in the activity of either catecholamine or tryptamine in the central nervous system of mice. In addition, the action mechanism of DFZ on STR was different from that on nociceptive reaction reported previously.

Keywords——morphine; Straub tail reaction; difenamizole; intracerebral injection; 5-hydroxytryptamine; apomorphine; methamphetamine

It has been known that difenamizole (DFZ) has analgesic action as well as muscle relaxant action.[3)] Likewise, Yasuhara *et al.*[4)] have reported an electrophysiological study on the action mechanism of DFZ in the central nervous system (CNS). It has been indicated that the mode of analgesic action of DFZ is relevant to biogenic amines in the CNS.[5)] On the other hand, it has been reported that morphine-induced Straub tail reaction (STR) is inhibited by DFZ.[6)] Furthermore, it is suggested that STR is, at least in part, caused by the increase of catecholaminergic activity and/or the decrease of tryptaminergic activity in the CNS of mice.[7)] The present experiment was designed to clarify the mechanism of DFZ-induced inhibition of STR (DIS) in relation to monoamines in the CNS of mice.

1) A part of this research was presented at the 48th Regional Meeting of the Japanese Pharmacological Society in Osaka, November 1975.
2) Location: *Tenpaku-ku, Nagoya 468, Japan.*
3) T. Kameyama, K. Oyanagi, K. Sasaki, and K. Kisara, *Nippon Yakurigaku Zasshi*, **65**, 529 (1969).
4) M. Yasuhara, H. Naito, E. Saito, M. Fukagawa, K. Takahashi, and M. Kobayashi, *Oyo Yakuri*, **6**, 313 (1972).
5) *a*) T. Kameyama and T. Nabeshima, *Nippon Yakurigaku Zasshi*, **72**, 543 (1976); *b*) T. Nabeshima, M. Ina, and T. Kameyama, *ibid.*, **73**, 907 (1977); *c*) T. Nabeshima and T. Kameyama, *ibid.*, **73**, 961 (1977).
6) T. Kameyama, T. Nabeshima, and M. Ukai, *Chem. Pharm. Bull.* (Tokyo), **25**, 1837 (1977).
7) T. Kameyama, M. Ukai, and T. Nabeshima, *Jpn. J. Pharmacol.*, **28**, 249 (1978); T. Kameyama, T. Nabeshima, M. Ukai, and K. Yamaguchi, *Chem. Pharm. Bull.* (Tokyo), **26**, 2615 (1978).

Materials and Methods

Experiments were performed on male albino ddY mice weighing 18—25 g. The animals freely approached food and water before the experiments. The following drugs were used: DFZ, DFZ-HCl and isocarboxazid (Takeda Chemical Industries, Ltd.), L-dihydroxyphenylalanine (L-dopa) and L-5-hydroxytryptophan (L-5-HTP) (Kyowa Hakko Kogyo Co., Ltd.), apomorphine (Sandoz), methamphetamine (Dainippon Pharmaceutical Co., Ltd.), propranolol, tranylcypromine and 5-hydroxytryptamine (5-HT) (Sigma), nialamide (Pfizer Taito Co., Ltd.), tetrabenazine (Hoffmann-La Roche), disulfiram (Wako Pure Chemical Industries, Ltd.) and morphine (Shionogi and Co., Ltd.).

DFZ (15 mg/kg, *i.p.* and 100 μg/mouse, *i.c.*) was given 15 min before administration of morphine (10 mg/kg, *s.c*). For systemic administration, morphine, apomorphine, methamphetamine, propranolol and tranylcypromine were dissolved in 0.9% saline whereas DFZ, L-dopa, disulfiram, L-5-HTP, nialamide, isocarboxazid and tetrabenazine were suspended in 0.3% carboxymethylcellulose dissolved in isotonic saline. Intracerebral (*i.c.*) injection was done according to the method of previous report.[8] DFZ-HCl and 5-HT were dissolved in 0.9% saline immediately prior to use in a volume of 20 μl/mouse and doses were calculated in terms of μg where applicable. 10 mg/kg of morphine was injected into the back of the neck 15 min after the test drug treatment. STR was then scored at intervals of 10, 30 and 60 min after administration of morphine. The tail elevation was graded according to the modified numerical ratings of Juul[9] as follows: 0=0°, 0.5=1—44°, 1.0=45°, 1.5=46—89°, 2.0=90°, 2.5=91—179°, 3.0=180° above the horizontal table plane. Accordingly, the intensity of STR was expressed as score. p values were obtained by Student's t-test. The experiments were programmed in a semi-soundproof room at 23±1° and 55±2.5% relative humidity.

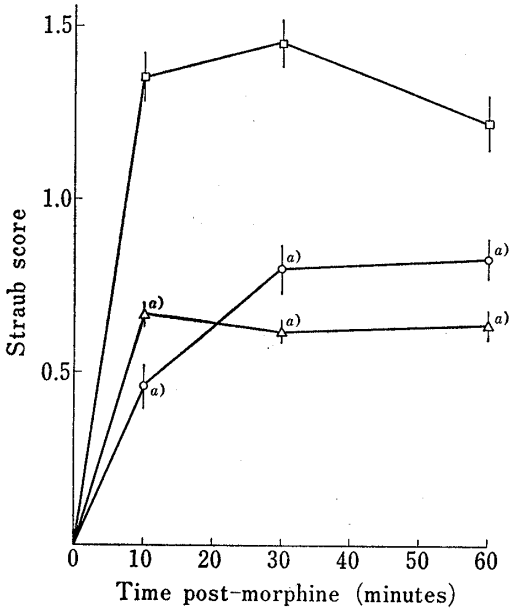

Fig. 1. Effect of Difenamizole (DFZ) on Straub Tail Reaction (STR)

DFZ (15 mg/kg, *i.p.* and 100 μg/mouse, *i.c.*) was given 15 min before administration of morphine (10 mg/kg). Vehicle+morphine 10 mg/kg, *s.c.* (□—□); DFZ 15 mg/kg, *i.p.*+morphine 10 mg/kg, *s.c.* (△—△); DFZ 100 μg/mouse, *i.c.*+morphine 10 mg/kg, *s.c.* (○—○). Each point represents the mean score obtained from twenty mice. Vertical bars show the standard errors of the means. Ordinate: score of STR. Abscissa: time after administration of morphine. Significantly different from vehicle+morphine *a)* $p<0.01$.

Results

Effect of DFZ on Straub Tail Reaction (STR)

As shown in Fig. 1, DFZ (15 mg/kg, *i.p.* and 100 μg/mouse, *i.c.*) inhibited STR at 10, 30 and 60 min after administration of morphine to a considerable extent. In the following experiment STR was observed 30 min after administration of morphine, since DFZ inhibited STR stably from 30 to 60 min after administration of morphine. In order to elucidate the pharmacological mechanism of DFZ, the effect of monoaminergic agents on the DIS was particularly estimated.

Effect of L-Dopa, Apomorphine and Methamphetamine on DFZ-Induced Inhibition of STR (DIS)

As indicated in Fig. 2, 150 mg/kg of L-dopa decreased the DIS and increased STR significantly. 200 mg/kg of L-dopa did not increase the DIS but decreased STR markedly. 10 mg /kg of apomorphine and 2 mg/kg of methamphetamine decreased the DIS significantly without influencing STR. 5 and 10 mg/kg of methamphetamine decreased the DIS as well as increased STR significantly.

Effect of L-Dopa, Apomorphine and Methamphetamine on Intracerebrally Administered DFZ-Induced Inhibition of STR (DIS)

As illustrated in Fig. 3, the effect of L-dopa, apomorphine and methamphetamine on STR was the same described in Fig. 2. 150 mg/kg of L-dopa did not influence the DIS.

8) T.J. Haley and W.G. McCormick, *Brit. J. Pharmacol. Chemother.*, **12**, 12 (1957).
9) A. Juul, *Arch. Int. Pharmacodyn. Ther.*, **62**, 69 (1939).

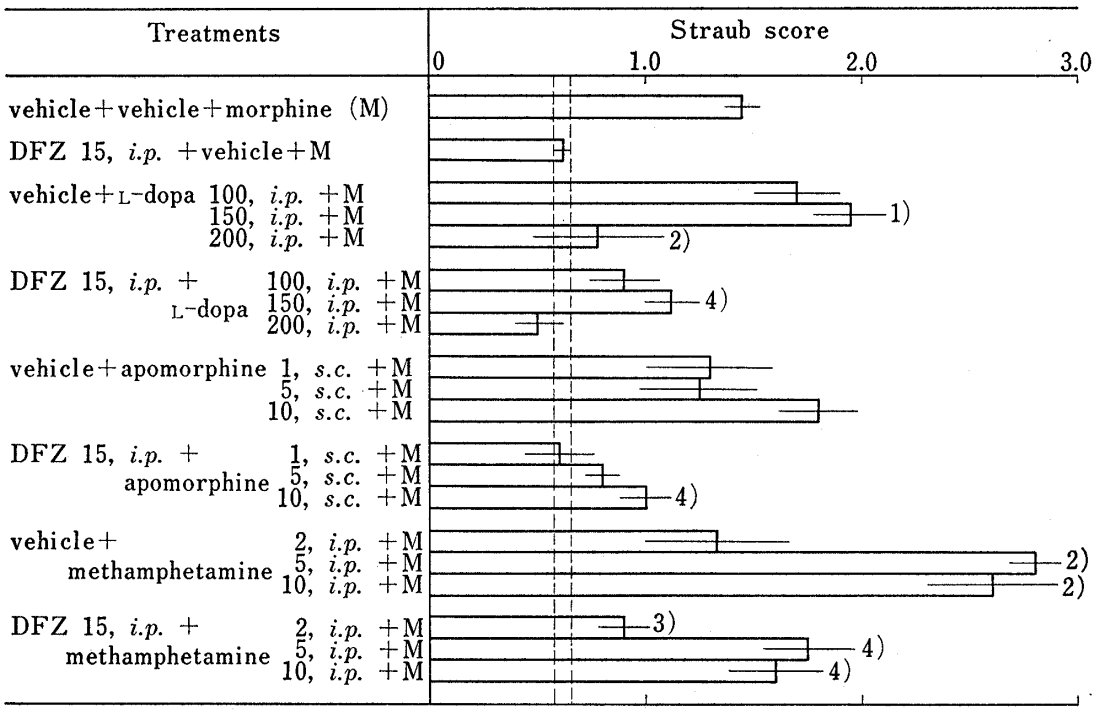

Fig. 2. Effect of L-Dopa, Apomorphine and Methamphetamine on DFZ-Induced Inhibition of STR (DIS)

L-Dopa was given 30 min before administration of morphine. Apomorphine and methamphetamine were given together with DFZ. Each score was obtained from fifteen mice. Horizontal bars represent the standard errors of the means (S.E.M.). The broken lines indicate ±S.E.M. of DFZ+vehicle+morphine. 1) $p<0.05$; 2) $p<0.01$ compared to vehicle+vehicle+morphine. 3) $p<0.05$; 4) $p<0.01$ compared to DFZ+vehicle+morphine.

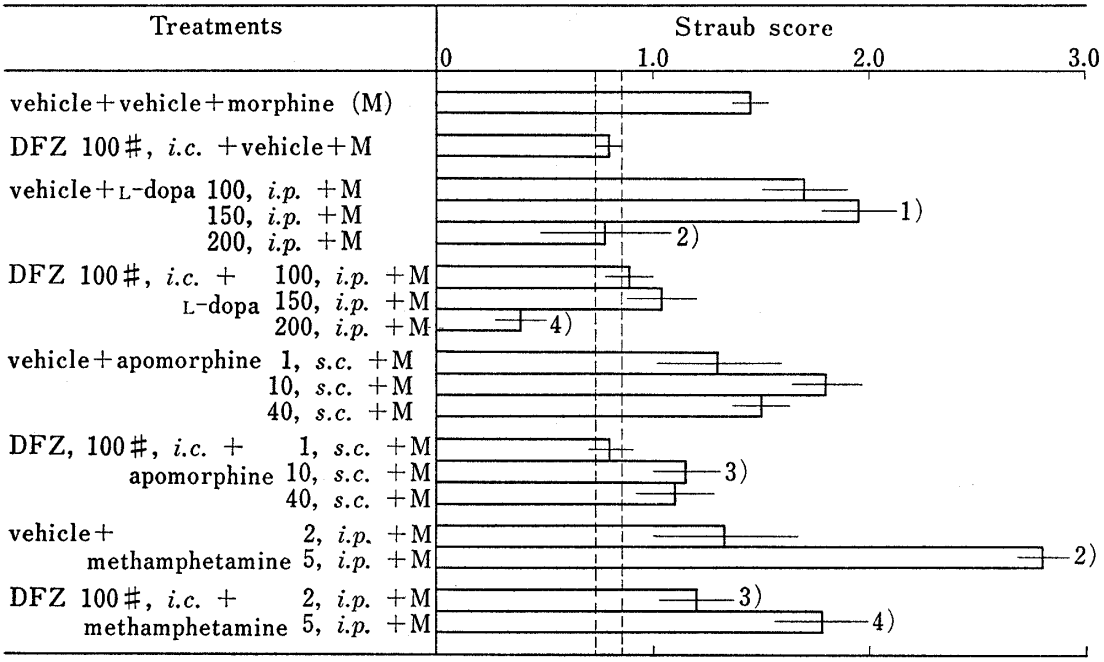

Fig. 3. Effect of L-Dopa, Apomorphine and Methamphetamine on intracerebrally administered DFZ-Induced Inhibition of STR (DIS)

L-Dopa was given 30 min before administration of morphine. Apomorphine and methamphetamine were given together with DFZ. Each score was obtained from fifteen mice. Horizontal bars represent the standard errors of the means (S.E.M.). The broken lines indicate ±S.E.M. of DFZ+vehicle+morphine. 1) $p<0.05$; 2) $p<0.01$ compared to vehicle+vehicle+morphine. 3) $p<0.05$; 4) $p<0.01$ compared to DFZ+vehicle+morphine. # μg/mouse.

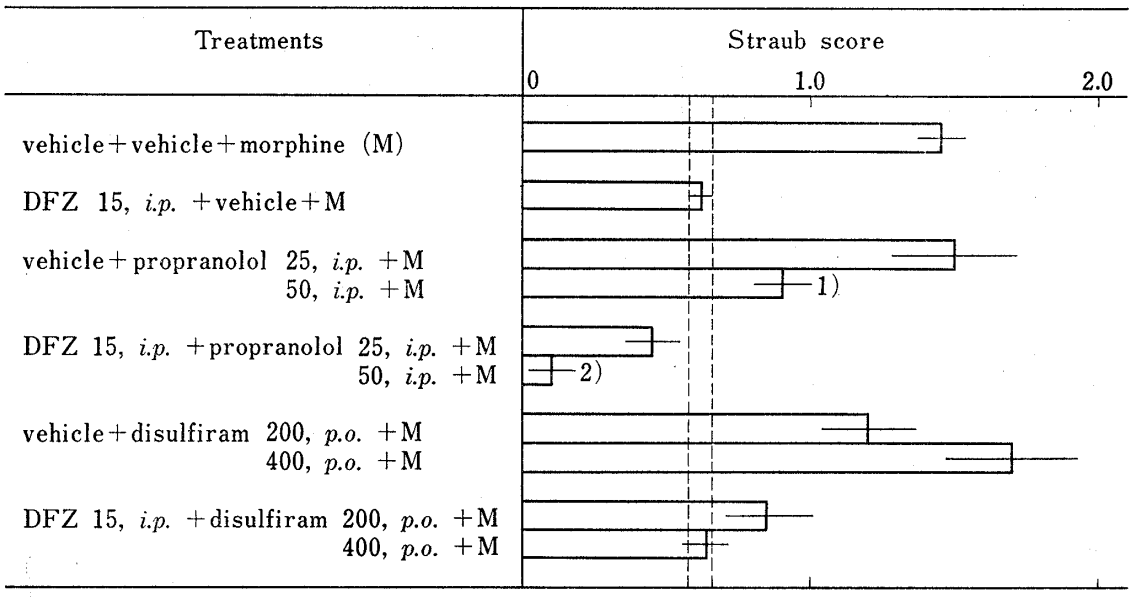

Fig. 4. Effect of Propranolol and Disulfiram on DFZ-Induced Inhibition of STR (DIS)

Propranolol and disulfiram were given 30 min and 6 hr respectively before administration of morphine. Each score was obtained from fifteen mice. Horizontal bars represent the standard errors of the means (S.E.M.). The broken lines indicate ±S.E.M. of DFZ+vehicle+morphine. 1) $p<0.05$ compared to vehicle+vehicle+morphine. 2) $p<0.01$ compared to DFZ+vehicle+morphine.

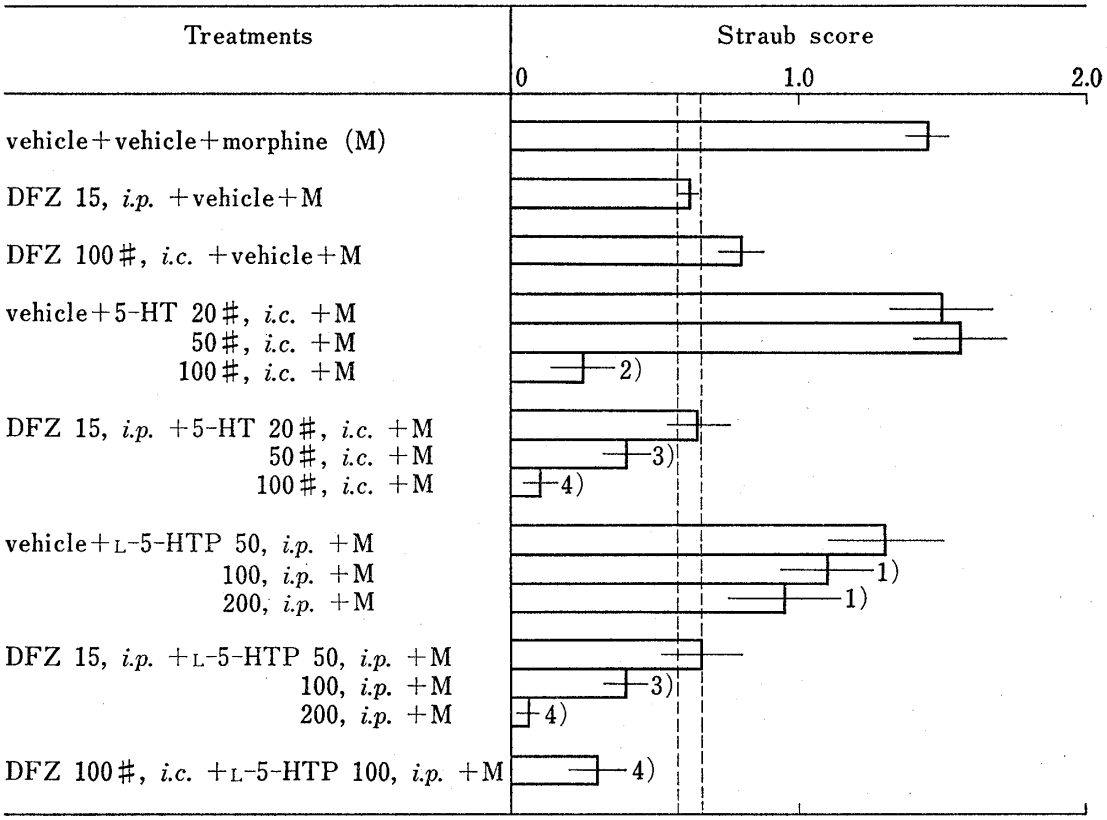

Fig. 5. Effect of 5-Hydroxytryptamine (5-HT) and L-5-Hydroxytryptophan (L-5-HTP) on DFZ-Induced Inhibition of STR (DIS)

5-HT (*i.c.*) and L-5-HTP (*i.p.*) were given 15 and 60 min respectively before administration of morphine. Each score was obtained from fifteen mice. Horizontal bars represent the standard errors of the means (S.E.M.). The broken lines indicate ±S.E.M. of DFZ+vehicle+morphine. 1) $p<0.05$; 2) $p<0.01$ compared to vehicle+vehicle+morphine. 3) $p<0.05$; 4) $p<0.01$ compared to DFZ+vehicle+morphine. # μg/mouse.

200 mg/kg of L-dopa increased the DIS significantly. 10 mg/kg of apomorphine decreased the DIS significantly except 1 and 40 mg/kg of apomorphine. 2 and 5 mg/kg of methamphetamine decreased the DIS markedly.

Effect of Propranolol and Disulfiram on DFZ-Induced Inhibition of STR (DIS)

As indicated in Fig. 4, 25 mg/kg of propranolol had no influence on the DIS and STR. 50 mg/kg of propanolol increased the DIS and decreased STR to a large extent. 200 and 400 mg/kg of disulfiram did not influence the DIS and STR.

Effect of 5-HT and L-5-HTP on DFZ-Induced Inhibition of STR (DIS)

As given in Fig. 5, intracerebral injection of 20 and 50 μg/mouse of 5-HT had no influence on STR but 50 μg/mouse of 5-HT increased the DIS significantly. 100 μg/mouse of 5-HT increased the DIS and decreased STR. 100 and 200 mg/kg of L-5-HTP increased the DIS and decreased STR. Likewise, 100 mg/kg of L-5-HTP increased the DIS (DFZ 100 μg/mouse, *i.c.*).

Effect of Nialamide, Isocarboxazid, Tranylcypromine and Tetrabenazine on DFZ-Induced Inhibition of STR (DIS)

As shown in Fig. 6, 50 and 100 mg/kg of nialamide, 100 mg/kg of isocarboxazid, and 10 and 25 mg/kg of tranylcypromine had no influence on the DIS and STR. 20 mg/kg of tetrabenazine did not influence the DIS but decreased STR significantly.

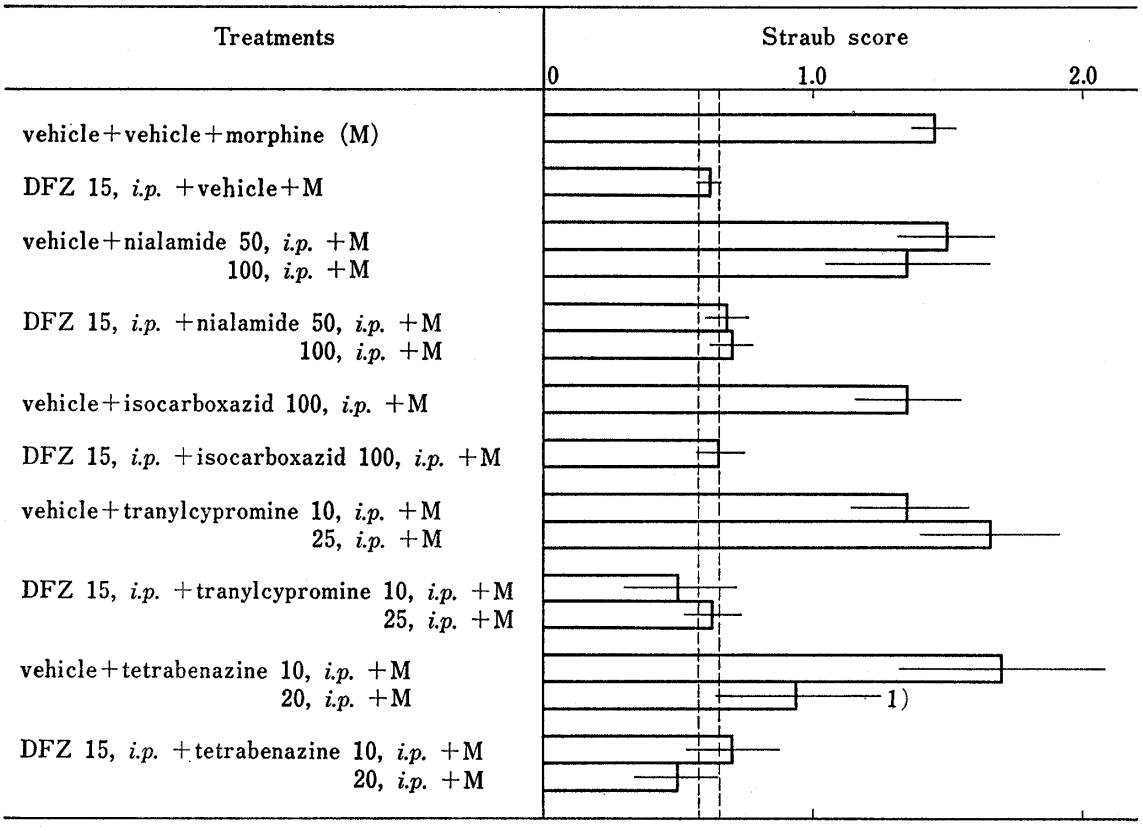

Fig. 6 Effect of Nialamide, Isocarboxazid, Tranylcypromine and Tetrabenazine on DFZ-Induced Inhibition of STR (DIS)

Nialamide, isocarboxazid, tranylcypromine and tetrabenazine were given 19, 16, 16 and 2 hr respectively before administration of morphine. Each score was obtained from fifteen mice. Horizontal bars represent the standard errors of the means (S.E.M.). The broken lines indicate ±S.E.M. of DFZ+vehicle+morphine. 1) $p<0.05$ compared to vehicle+vehicle+morphine.

Discussion

It has been reported that the greater catecholaminergic activity, the greater STR, and the greater tryptaminergic activity the less STR.[7] In addition, it has been indicated that DFZ inhibits STR remarkably.[6] On the other hand, it has been known that the action mechanism of monoaminergic agents on DFZ-induced analgesia[3] is dependent upon the kind of nociceptive stimulation.[5a,b] The present study was done in an attempt to clarify the effect of monoaminergic agents on the DIS. It is shown that apomorphine stimulates dopamine receptors[10] as well as potentiates (as a "pure" dopaminergic agonist) the analgesic effect of morphine[11] and methamphetamine facilitates the release of catecholamine (CA) and blocks the uptake of CA at presynaptic level of CA neuron similarly to amphetamine.[12] 10 mg/kg of apomorphine and 2 mg/kg of methamphetamine decreased the DIS (intraperitoneal and intracerebral injection of DFZ) significantly without producing marked STR. Intracerebral injection of 50 μg/mouse of 5-HT increased the DIS markedly without influencing STR. These results suggest that catecholaminergic activity in the CNS might decrease the DIS, while tryptaminergic activity in the CNS might increase the DIS. On the contrary, the DIS was developed significantly, whether or not mice were pretreated with L-dopa, propranolol, disulfiram, L-5-HTP, tetrabenazine, nialamide, isocarboxazid and tranylcypromine.

Catecholaminergic and tryptaminergic agents decreased DFZ-induced analgesia in a hot plate test in mice.[5a] On the other hand, it is shown that catecholaminergic activity plays an important role in the inhibition of acetic acid-induced writhing movements rather than tryptaminergic activity does.[5b] Additionally, the present investigation suggests that the DIS might be mediated by CA and 5-HT. On the other hand, intracerebral injection of 100 μg/mouse of DFZ inhibits STR[6] and produces analgesia in mice.[5c] It thus appears that the different effect of monoamines in the CNS on the DIS and on the analgesia might be due to individual variation in experimental animal model to evaluate drug action. In addition, Yasuhara *et al.*[4] have suggested that acting point for motor function[13] is considered to be on an upper level than the spinal cord from electrophysiological study. In particular, biochemical data have shown that DFZ increases striatal dopamine and meso-diencephalic norepinephrine contents but decreases striatal 3-methoxytyramine and meso-diencephalic normetanephrine contents.[14] These metabolic alterations of catecholaminergic pathways induced by DFZ seem to be based on the decrement of functional activity of CA in the CNS of mice. Therefore, the inhibitory action of DFZ on STR might be related to the attenuated catecholaminergic activity in the striatum and meso-diencephalon.

10) N.E. Anden, A. Ruberson, K. Fuxe, and T. Hökfelt, *J. Pharm. Pharmacol.*, **19**, 627 (1967); A.M. Ernst, *Psychopharmacologia* (Berl.), **10**, 316 (1967).
11) Z.D. Kovacs and J.I. Szekely, *Psychopharmacol.*, **53**, 65 (1977).
12) J. Glowinski and J. Axelrod, *J. Pharmacol. Exp. Ther.*, **149**, 43 (1965); A. Carlsson, K. Fuxe, B. Hamberger, and M. Lindquist, *Acta Physiol. Scand.*, **67**, 481 (1966); R.P. White, D.B. Cobb, G.R. Breese, and C.B. Nash, *Int. J. Neuropharmacol.*, **5**, 143 (1966).
13) Y. Saji, S. Chiba, Y. Take, and Y. Nagawa, *Takeda Kenkyusho Nempo*, **30**, 406 (1971).
14) T. Nabeshima, K. Yamaguchi, and T. Kameyama, *Jpn. J. Pharmacol.*, **28**, 642 (1978).