Behavioral and Electroencephalographic Effects of a Depot Type Neuroleptic Fluphenazine Decanoate, in Rats

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The behavioral and electroencephalographic (EEG) effects of intramuscular fluphenazine decanoate (FI-D) were investigated in rats and compared with those of fluphenazine enanthate (FI-E) and fluphenazine HCl (FI-HCl). It was clearly observed that 1) these two depot type neuroleptics reduced open-field activity, 2) antagonized methamphetamine-induced hyperactivity, 3) inhibited the conditioned avoidance response, and 4) produced catalepsy, for a substantially long period of time (4—35 d). Although both FI-D and FI-E significantly inhibited muricide in olfactory bulbectomized rats and impaired rotarod performance, these effects were relatively weak in potency and short-lasting (4 h—2 d). The EEG was changed to a drowsy pattern which consisted of high voltage slow waves following the injection of FI-D and FI-E. FI-D significantly inhibited the EEG arousal response to auditory stimulation, but FI-E did not. However, neither FI-D nor FI-E inhibited the EEG arousal response to electrical stimulation of the midbrain reticular formation and posterior hypothalamus. These results indicate that FI-D has the same spectrum of pharmacological activity as FI-E, except for its longer duration of action in antagonizing methamphetamine as well as in inhibiting the EEG arousal response to auditory stimulation.

Keywords — fluphenazine decanoate; fluphenazine enanthate; depot type neuroleptics; behavior; electroencephalogram (EEG); rat

Introduction

One of the recent advances in schizophrenia management is the development of long-acting neuroleptics.^{1,2)} It has come to attain a situation where the long-lasting efficacy can be maintained in the patient by prescribing these drugs. Therefore, these drugs have long been expected to treat the outpatients and especially the patients who refuse prescription. Moreover, these drugs may prevent the recurrence by discontinuation of medication which frequently occurs in the recovery stage.³⁾ Fluphenazine (FI), a phenothiazine derivative, is an excellent neuroleptic to improve the automatic response and the rapport in chronic schizophrenics.⁴⁾

When FI is esterified with heptanoic acid and decanoic acid, they are named as fluphenazine enanthate (FI-E) and fluphenazine decanoate (FI-D) respectively.^{5,6)} These drugs are called as the depot type neuroleptic of long acting property. FI-D is longer acting than FI-E and is reported to cause lesser side-effects such as rigidity and parkinsonism in clinical uses.⁷⁻¹⁰⁾ However, it is

considered that FI-D has the mechanism of action similar to FI-E. Kurland *et al.*¹¹⁾ and Donlon *et al.*¹²⁾ have reported that there is no significant difference in pharmacological property between FI-D and FI-E. However, there are few reports which assess the difference of the mechanisms of action of these two drugs in detail. In this way, these results with respect of FI-D and FI-E are not always the same with each report.

The present study was undertaken in order to elucidate pharmacological activities of FI-D and FI-E by using behavioral and electroencephalographic (EEG) techniques with special reference to the onset of action and long acting property.

Materials and Methods

Drugs — FI-D and FI-E, supplied by Squibb Japan Inc., were dissolved in sesame oil containing 1.6% benzyl alcohol and injected bilaterally into the femoral muscle of both sides. Methamphetamine (MA) hydrochloride (Dainippon)

was dissolved in saline and intraperitoneally injected.

Animals — Male Wistar rats (body weight 150–250 g) obtained from Kuroda Animal Laboratory (Kumamoto, Japan) were used except in the anti-muricide and EEG experiments. In the test of anti-muricide and EEG effects, male Wistar King A strain rats obtained from Kyushu University Institute of Experimental Animals were used. The rats were maintained under standardized conditions of temperature ($22 \pm 1^\circ\text{C}$) and light (7:00 a.m.—7:00 p.m.) throughout the experiment.

Open-Field Test — The open-field apparatus, similar to that used by Hall,¹³⁾ which consisted of a circular floor measuring 60 cm in diameter, bounded by a 50 cm high wall. The inside of the apparatus was painted grey and the floor was divided into 19 blocks of approximately equal width with painted lines. A 100 W lamp was placed 80 cm above the center of the apparatus in order to keep constant illumination. The rat was placed in the center of the open field and observed during a 3-min period thereafter, recording ambulation (the number of blocks traversed by the animal; locomotor activity) and rearing (the frequency of upright standing on the hindlegs). The open-field test was carried out at 6 h, 1, 2, 4, 7, 14, 21 and 28 d after intramuscular injection of the test drugs.

The anti-MA activity was measured using the same apparatus. MA 5 mg/kg was intraperitoneally administered at 6 h, 2 and 7 d after the intramuscular administration of either Fl-D or Fl-E at a dose of 10 mg/kg. Thirty min after MA administration, the open-field tests were carried out.

All rats were used only once in the open-field test after administration of Fl-D and Fl-E.

Conditioned Avoidance Response (CAR)

— Rats were trained to learn the CAR in the two-way type shuttle box. In this shuttle box, the floor levels of two compartments have a 5 cm gap, and electric stimulation can be applied separately to the grid floor of each compartment. As the conditioned stimuli 400 Hz monotone of 70 db was given for 5 s, and then alternating electric current of 1 mA was applied to the grid floor of the compartment where the rat was

located as the unconditioned stimuli (foot shock). When the rat moved into the other compartment within the period of conditioned stimuli, it meant a successful conditioned response and the auditory stimulation was immediately stopped. One session consisted of 20 trials repeated at variable intervals (30, 40 or 50 s). When the rat succeeded to avoid foot shock at 80% or more of the trials in 2 successive sessions, the acquisition of the conditioned avoidance learning was considered to be completed. Only animals which completed this criterion of learning were employed in the experiments for assessing drug effects.

Measurement of Catalepsy — The forepaws of rats were placed on a horizontal metal rod (2 mm in diameter) kept 12.5 cm in height. When the rat maintained this unnatural, extended position for more than 30 s, it was considered to be catalepsy positive.

Anti-muricide Test — Male rats of Wistar King A strain weighing 180–250 g were used. The olfactory bulbs were bilaterally removed by suction pump through a hole made in the skull just above the bulbs under pentobarbital anesthesia. The rats were housed in isolation in individual cages (20 × 20 × 23 cm) after the surgical operation. The muricide was regarded as positive when the rat killed a mouse within 3 min after placed in the rat's cage. The olfactory bulbectomized rats (OB rats) exhibiting muricide were selected and used in the drug experiments.

Rotarod Test — The rotarod was a wooden bar with a diameter of 5.2 cm, rotating 15 times per minute. Rats were placed on the bar in the direction opposite to the bar rotation. The rats which remained on the rotarod for at least 2 min of rotation were selected in a preliminary test. The rotarod test was performed three times after the drug administration. The drug effect was judged to be positive when the rats fell from the rotarod within 2 min for all three times.

Measurement of EEG — Male Wistar King A rats weighing 250–300 g were used. The head of the animal was fixed on a stereotaxic instrument (Takahashi Co.) under sodium pentobarbital (40 mg/kg i.p.) anesthesia. According to the rat brain atlas of De Groot,¹⁴⁾ bipolar elec-

trodes made of insulated stainless steel wire (0.2 mm in diameter; insulation was removed only at the tip; the distance between each tip was set at 0.5–1.0 mm) were chronically implanted in the frontal cortex, dorsal hippocampus (A: 3.2, L: 2.8, H: 3.0), midbrain reticular formation (A: 1.5, L: 1.5, H: -1.5) and lateral hypothalamus (A: 4.6, L: 0.5, H: -2.5). The cortical electrodes were placed on the subdural cortical surface. Each electrode was soldered to the pins of a small connector socket which was fixed to the skull with dental cement together with two metal pins previously screwed into the skull. Thereafter, all exposed parts of the electrodes were also covered with cement. The same electrodes were used for EEG recording and electrical stimulation. Procaine penicillin G (Banyu Pharmaceutical Co.) 50000 units was injected into the femoral muscle after surgery. The experiment was started 15 d or more after the electrode implantation. The EEG were recorded on a polygraph (Nihon Koden) in a freely moving condition. The rat was moved into the recording cage (35 × 30 × 30 cm) which was placed in a soundproof box (75 × 100 × 100 cm) equipped with a plastic window under the constant illumination. Electrical stimulations were applied for 5 s to the midbrain reticular formation and lateral hypothalamus with square pulses (100 Hz, 0.1 ms, 1.2–4 V) generated by an electronic stimulator (model MSE-40, Nihon Koden). The auditory stimulation with 2000 Hz monotone (80 db) was applied for 5 s by a sound/light stimulator (model MS-2PS, Nihon Koden). The EEG was recorded for 30 min at the periods of 1, 2, 4, 6 h, 1, 2, 3, 7, 14 and 21 d after the drug administration.

Results

Effects on Open-Field Activity

The effect of Fl-D on ambulation of rats in the open field is shown in Fig. 1. Ambulation was 45.9 ± 5.2 (average $\pm$ S.E.; $N = 10$) at 6 h and 44.3 ± 3.7 ($N = 10$) at 28 d after sesame oil injection. Fl-D significantly decreased ambulation in a dose-dependent manner at 6 h following the intramuscular (i.m.) administration. However, no difference was found in ambula-

Fig. 1. Effect of Fl-D on Ambulation of Rats in Open-Field Test

Ambulation: the number of squares crossed during a 3 min period. Every rat was used only once in the open field test and 10 rats were used in each test period. Abscissa is the time after drug administration. The same is also true in Fig. 2–7. ○, sesame oil; ●, 1 mg/kg (i.m.); △, 5 mg/kg (i.m.); ▲, 10 mg/kg (i.m.); ×, 20 mg/kg (i.m.). a) $p < 0.05$. b) $p < 0.01$.

tion 1 d after the administration of Fl-D 1–5 mg/kg as compared with sesame oil-treated group. Fl-D at doses of 10–20 mg/kg still significantly reduced ambulation 1 d after the administration. This decreased ambulation tended temporarily to return to the control value 2 d after the administration of Fl-D 5–10 mg/kg, thereafter it was reduced again. Ambulation was significantly decreased even 7 d after administra-

Fig. 2. Effect of Fl-E on Ambulation of Rats in Open-Field Test

○, sesame oil; △, 5 mg/kg (i.m.); ▲, 10 mg/kg (i.m.); ×, 20 mg/kg (i.m.). a) $p < 0.05$. b) $p < 0.01$.

TABLE I. Effects of Fl-D and Fl-E on Rearing of Rats in Open-Field Test

Treatment	Dose (i.m.)	Number of rearing								d
		0.25	1	2	4	7	14	21	28	
Sesame oil	1 ml/kg	7.8±1.3	—	—	—	—	—	—	—	8.6±2.3
Fl-D	5 mg/kg	3.1±0.7 ^{a)}	4.4±0.7	4.2±1.2 ^{a)}	3.6±0.9 ^{a)}	4.4±0.7	—	—	—	
	10 mg/kg	1.1±0.4 ^{b)}	2.3±0.5 ^{b)}	5.1±0.7	2.5±0.5 ^{b)}	2.6±0.7 ^{a)}	6.6±1.1	—	—	
	20 mg/kg	0.9±0.3 ^{b)}	1.2±0.5 ^{b)}	3.2±0.6 ^{a)}	0.6±0.3 ^{b)}	2.5±0.6 ^{b)}	4.3±1.0	4.8±1.0	3.4±0.7 ^{a)}	
	5 mg/kg	2.0±0.7 ^{b)}	0.9±0.4 ^{b)}	2.3±0.5 ^{b)}	3.1±1.0 ^{b)}	2.8±0.7 ^{b)}	—	—	—	
Fl-E	10 mg/kg	1.1±0.6 ^{b)}	1.4±0.4 ^{b)}	0.8±0.4 ^{b)}	1.1±0.3 ^{b)}	2.3±0.7 ^{b)}	3.7±1.0 ^{a)}	6.8±1.1	—	
	20 mg/kg	0.5±0.2 ^{b)}	0.3±0.3 ^{b)}	1.6±0.7 ^{b)}	1.9±0.5 ^{b)}	2.4±0.7 ^{b)}	3.6±0.7 ^{a)}	4.7±0.8	4.7±1.0	

All rats were used only once in the open-field test at various time after administration of Fl-D or Fl-E. Each value represents mean ± S.E. Significant differences from control value at 6 h after sesame oil were determined with Mann-Whitney U test. ^{a)} $p < 0.05$. ^{b)} $p < 0.01$.

tion of Fl-D 10 mg/kg ($p < 0.05$, Mann-Whitney U test), and 4 d after 20 mg/kg ($p < 0.05$).

Ambulation was dose-dependently reduced by the administration of Fl-E at 5, 10 and 20 mg/kg 6 h after the administration (Fig. 2). The significant reduction in ambulation was maintained until 4 d, then ambulation returned to the control level completely in the 7th day after the i.m. administration of Fl-E.

The effects of Fl-D and Fl-E on rearing were shown in Table I. Fl-D 1 mg/kg did not appreciably affect rearing at any stages. However, rearing was significantly reduced after administration of Fl-D 5 mg/kg. In the same manner, Fl-D 10–20 mg/kg more significantly decreased rearing until 7 d except 2 d following Fl-D 10 mg/kg administration.

Fl-E 5 mg/kg significantly decreased rearing until 7 d after administration, and Fl-E 10–20 mg/kg until 14 d.

Fl-D significantly decreased ambulation of rats in the open-field, but its degree was slightly less than Fl-E. The temporary recovery of ambulation at the 2nd day after Fl-D administration differed from that of Fl-E. Fl-D significantly decreased rearing, but its potency and duration of action were slightly less than those of Fl-E.

Anti-MA Activity

The mean number of ambulation in normal rats was 46 ± 3.1 (average ± S.E./3 min). MA at 5 mg/kg, i.p. induced hyperactivity with mean ambulation of 108 ± 10.5 in normal rats. The MA-induced hyperactivity was significantly de-

creased 6 h after Fl-D 10 mg/kg administration (13.9 ± 3.9 , $p < 0.01$; Mann-Whitney U test: Fig. 3). Similarly, it was significantly inhibited after 2 (52.3 ± 10.9) and 7 d (60.4 ± 7.3) after the administration, respectively. On the other hand, the MA-induced hyperactivity was also significantly reduced to 18.6 ± 4.4 ($p < 0.01$) and 74.6 ± 6.5 ($p < 0.05$) 6 h and 2 d after pre-treatment with Fl-E 10 mg/kg, respectively. However, no significant difference was found in ambulation 7 d after the administration of Fl-E 10 mg/kg as compared with MA alone group (Fig. 3).

Fig. 3. Effects of Fl-D and Fl-E on MA-Induced Hyperactivity in Rats

MA 5 mg/kg was intraperitoneally administered at 6 h, 2 and 7 d, respectively, after intramuscular administration of either Fl-D and Fl-E in a dose of 10 mg/kg. ^{a)} $p < 0.05$ and ^{b)} $p < 0.01$: significant difference from the MA group.

Fig. 4. Effect of Fl-D on CAR of Rats in Shuttle Box

Eight rats were used for each dose. ○-○-○ & ○-○-○, sesame oil (i.m.); △-△-△, Fl-D 5 mg/kg (i.m.); ▲-▲-▲, Fl-D 10 mg/kg (i.m.). a) $p < 0.05$. b) $p < 0.01$.

These results indicated that the anti-MA effect of Fl-D was significantly longer in duration than that of Fl-E.

Rearing was also increased with MA 5 mg/kg, i.p. and this increase was significantly inhibited to the same extent only at 6 h after pre-treatment with either Fl-D or Fl-E at a dose of

10 mg/kg.

Effects on CAR

Fl-D 5–10 mg/kg significantly inhibited the CAR of the rat in the shuttle box 6 h after administration (Fig. 4). The inhibition of CAR was more prolonged as the dose of Fl-D was increased. Namely, a significant inhibition lasted until 10 and 21 d after the administration of Fl-D 5 and 10 mg/kg, respectively. As the CAR was significantly inhibited in all rats after 6 h, the escape response was also inhibited in 2 and 4 out of 8 rats at doses of Fl-D 5 and 10 mg/kg, respectively (Fig. 5, upper panel).

Fl-E 5–10 mg/kg also significantly inhibited the CAR and its effect was dose-dependently prolonged until 14 d at 5 mg/kg ($p < 0.05$) and 21 d at 10 mg/kg ($p < 0.05$). In this time, the escape response was also inhibited in a dose-dependent manner (Fig. 5, lower panel).

From these results, the inhibitory effect of Fl-D on the CAR seems to be somewhat weaker in potency than that of Fl-E.

Cataleptogenic Effect

Catalepsy was observed in 6 out of 10 rats 4 h

Fig. 5. Effect on Conditioned Avoidance and Escape Responses of Rats in Shutter Box

Upper panel: Fl-D, lower panel: Fl-E. □, no effect; ▨, inhibition of avoidance response; ■, inhibition of avoidance and escape response.

Fig. 6. Cataleptogenic Effects of Fl-D and Fl-E in Rats

Significant difference from the sesame oil-injected group, determined by the Fisher exact probability test. *a)* $p < 0.05$. *b)* $p < 0.01$. *c)* $p < 0.005$. $\circ$, sesame oil; $\bullet$, 5 mg/kg (i.m.), Δ , 10 mg/kg (i.m.); $\blacktriangle$, 20 mg/kg (i.m.).

after the administration of Fl-D 5 mg/kg ($p < 0.05$; Fig. 6, upper panel). The incidence of catalepsy significantly increased 2 h after the administration of Fl-D 10–20 mg/kg ($p < 0.005$). The cataleptogenic effect was statistically significant until 7 d at 5 mg/kg, 35 d at 10 mg/kg and 14 d at 20 mg/kg of Fl-D.

Fl-E also showed a significant cataleptogenic effect at any doses employed ($p < 0.005$ after 4 h: Fig. 6, lower panel). This effect was significant until 4 d at 5 mg/kg, 14 d at 10 mg/kg and 21 d at 20 mg/kg of Fl-E.

As mentioned above, catalepsy induced by Fl-D was dose-dependently increased. However, the duration of cataleptogenic effects was not always prolonged in a dose-dependent manner.

The effect of Fl-D was not significantly different from that of Fl-E in potency as well as in duration.

Anti-muricide Effect

Muricide of OB rats was significantly inhibited 6 h after the administration of Fl-D 10–20 mg/kg (Table II). However, no significant inhibition was observed after 1 and 2 d at 10 and 20 mg/kg, respectively (Table II).

Fl-E 10 mg/kg significantly inhibited muricide in 5 out of 10 OB rats 6 h after administration ($p < 0.05$), but this significant effect was no more observed even after 1 d as in the case of Fl-D.

No difference was found in this effect between Fl-D and Fl-E.

TABLE II. Effects of Fl-D and Fl-E on Muricide in OB Rats at Various Periods of Time after Administration

Treatment	Dose (i.m.)	Incidence of muricide							
		0	0.25	1	2	4	7	14	d
Sesame oil	1 ml/kg	10/10	10/10	10/10	10/10	10/10	10/10	10/10	
Fl-D	10 mg/kg	10/10	4/10 <i>b)</i>	8/10	10/10	10/10	10/10	10/10	
	20 mg/kg	10/10	4/10 <i>b)</i>	4/10 <i>b)</i>	8/10	7/10	8/10	10/10	
Fl-E	10 mg/kg	10/10	5/10 <i>a)</i>	9/10	10/10	10/10	10/10	10/10	

a) $p < 0.05$. *b)* $p < 0.01$.

Fig. 7. Effects on FI-D and FI-E on Rotarod Performance in Rats

Ten rats were used for each dose. Significant difference from the sesame oil-injected group, determined by the Fisher exact probability test. a) $p < 0.05$. b) $p < 0.01$. c) $p < 0.005$. ○, sesame oil; ●, 5 mg/kg (i.m.); △, 10 mg/kg (i.m.); ▲, 20 mg/kg (i.m.).

Fig. 8. Effect of FI-D (10 mg/kg i.m.) on EEG Arousal Response to Auditory Stimulation in the Rat

Auditory stimulation (2000 Hz) was applied at the point indicated by a horizontal line (5 s). The vertical bars at the right-hand of each tracing in panel F indicate 200 V, and the horizontal bar at the right-hand bottom in panel F 3 s.

Effects on Rotarod Performance

Figure 7 shows the effects of FI-D and FI-E on the rotarod performance in rats. FI-D 10–20 mg/kg markedly impaired the performance 2 h after administration (Fig. 7, upper panel). The peak effect was attained after 4 h and the rotarod performance was impaired in all rats. A significant effect was observed even 1 d after FI-D administration ($p < 0.005$ at 10 mg/kg, $p < 0.01$ at 5 and 20 mg/kg), but no significant difference was present after 2 d in the 5 and 20 mg/kg group as compared with control group, although a significant inhibition of the performance was observed until 2 d after administration at 10 mg/kg.

FI-E showed almost the same effect as FI-D (Fig. 7, lower panel). An impairment of the rotarod performance was observed in all rats 4 h after FI-E at 5–10 mg/kg, and after 2 h at 20 mg/kg. A significant effect was found at all doses 1 d after administration ($p < 0.005$ at 5–10 mg/kg; $p < 0.01$ at 20 mg/kg). No significant

difference was observed in any FI-E group as compared with the control group after 4 d. The impairment of rotarod performance by FI-E was dose-dependently increased, but the duration of effect was not dose-related.

FI-D produced the impairment of rotarod performance, but its potency and duration was not significantly different from that of FI-E.

Effects on Spontaneous EEG Activity

FI-D 10–20 mg/kg produced a drowsy EEG pattern in rats, *i.e.* high-voltage slow waves in the cortex and desynchronization of the hippocampal theta waves. The peak effect was observed at 1–2 h after drug injection, thereafter the EEG pattern returned to the control state.

The effect of FI-E 10–20 mg/kg on spontaneous EEG activity was almost the same as that of FI-D and reached a maximum within 1–4 h after administration of the drug.

Effects on EEG Arousal Responses

The effects of FI-D and FI-E on the EEG

Fig. 9. Effect of FI-E (20 mg/kg i.m.) on EEG Arousal Response to Auditory Stimulation in the Rat. The condition of auditory stimulation and calibrations in panel F are the same as Fig. 8.

arousal responses to auditory stimulation and to electric stimulation of the posterior hypothalamus and midbrain reticular formation were investigated by using 40 rats with chronic electrode implants. When auditory stimulation or electric stimulation of the brain was applied during the period in which the rat was in a state with a drowsy pattern of EEG, the EEG arousal response was elicited without causing any recognizable change in behavior.

Auditory Stimulation — The EEG arousal response to auditory stimulation was inhibited in 4 out of 7 rats within 6 h after FI-D 10 mg/kg administration, although the onset and duration of the inhibition of EEG arousal response to auditory stimulation differed in each animal (Fig. 8). The EEG arousal response recovered between 4–6 h after administration of the drug. However, it was again inhibited 1–2 d after administration. Particularly, in 1 of these 4 rats which were inhibited from responding to audi-

tory stimulation by FI-D, long-lasting inhibition was observed until 14 d and the effect disappeared 21 d later (Fig. 8). The degree of inhibition was not always dose-dependent.

The EEG arousal response to auditory stimulation was not inhibited by FI-E 10 and 20 mg/kg in 6 out of 8 and 4 out of 7 rats, respectively (Fig. 9), though it was slightly inhibited only at 2–6 h after administration in the other rats.

Midbrain Reticular (MR) Stimulation — The mean threshold voltage necessary to elicit the minimal EEG arousal response to MR stimulation was 1.5 ± 0.4 V (0.8–2.3 V; $N = 20$) before the drug administration. In all animals, no significant change in the threshold voltage was observed with the administration of the vehicle sesame oil alone. FI-D and FI-E at doses of 10–20 mg/kg produced no significant change in the EEG arousal response to MR stimulation. No significant change was found in the

Fig.10. Effect of FI-D (20 mg/kg i.m.) on EEG Arousal Response to Electrical Stimulation of the PH at the 5-s Period Indicated by a Horizontal Line in the Rats

Calibrations in panel F are the same as in Fig. 8.

threshold voltage necessary to induce the EEG arousal response after administration of either Fl-D or Fl-E at any dose.

Posterior Hypothalamic (PH) Stimulation

— The average threshold voltage required to evoke the minimal EEG arousal response to PH stimulation was higher than that of MR stimulation and was 2.5 ± 0.7 V (1.2–4.0 V; $N = 20$). The effect of Fl-D on the arousal response to PH stimulation resembled that to MR stimulation. There was no significant change in the threshold voltage necessary to evoke the minimal EEG arousal response to PH stimulation before and after the drug administration (Fig. 10). The effect of Fl-E 10–20 mg/kg was almost the same as that of Fl-D.

As is obvious from these results, Fl-D and Fl-E at doses of 10–20 mg/kg slightly increased a drowsy pattern in spontaneous EEG of unanesthetized, unrestrained rats. No significant effect was observed on the EEG arousal response to electric stimulation of either MR or PH. The EEG arousal response to auditory stimulation was more significantly inhibited by Fl-D than Fl-E. However, the effect was not dose-related and not consistent in duration of action.

Discussion

In the present experiment, it was confirmed that both Fl-D and Fl-E, injected i.m., exerted qualitatively similar effects to Fl HCl and other neuroleptics; *i.e.* 1) the open-field activity, MA-induced hyperactivity and CAR were markedly inhibited, 2) muricide of OB rats was also suppressed and 3) a cataleptogenic effect was observed. EEG was changed to a drowsy pattern including high-voltage slow waves in the cortex and desynchronization of the hippocampal theta waves, and the EEG arousal response to auditory stimulation was inhibited. However, electrical stimulation of either the midbrain reticular formation or hypothalamus was not inhibited after the injection of Fl-D and Fl-E.

The duration of action of these drugs was different depending upon the type of effects in each test items. The antimuricide effect in OB rats was the shortest in duration of action tested in this study. This effect of Fl-D was almost the

same in potency as that of Fl-E and both disappeared within 24 h after i.m. injection. It is known that neuroleptics in general can suppress muricide only at doses causing neurotoxicity, and tolerance to this effect easily develops with repeated administration.^{15,16)} Therefore, the short duration of this effect of Fl-D and Fl-E is not due to the reduction of blood level of these drugs but to the tolerance development to this effect. The same explanation would be possible for our result showing no difference in the duration of this effect between Fl-D and Fl-E.

The rotarod performance was completely impaired by Fl-D and Fl-E. This effect almost disappeared 4 d after i.m. injection of either drug at 5 mg/kg, and 7 d after the injection at such higher doses as 10–20 mg/kg. It is known that tolerance readily develops to this effect of neuroleptics. So no significant difference was detected in the duration of action between Fl-D and Fl-E.

The open-field activity was reduced in the same degree by Fl-D and Fl-E. Although this effect of Fl-D was much longer in duration than Fl-HCl, no significant difference was found in duration of this action between Fl-D and Fl-E. The antagonizing effect of Fl-E on MA-induced hyperactivity in rats completely disappeared 7 d after i.m. injection, while that of Fl-D was maintained even after 7 d, indicating the longer-lasting effect of Fl-D than that of Fl-E. Our results coincide well with the results of Puech *et al.*¹⁷⁾ and Chen.¹⁸⁾

The anti-MA effect is known to be a characteristic of neuroleptics and tolerance to this effect will not develop.^{19,20)} It is therefore suggested that the antipsychotic effect of Fl-D may last longer than Fl-E in clinics.

However, this anti-MA effect of Fl-D and Fl-E was less durable than their effects in suppressing CAR as well as in inducing catalepsy. MA-induced hyperactivity is considered to be mainly caused by the excitation of dopamine receptors in the mesolimbic structure such as the nucleus accumbens.²¹⁾ When the dopamine receptors were blocked by Fl-D and Fl-E for a long period of time, the activity of dopaminergic neurons might be facilitated by a feedback mechanism. Thus the effect of MA was probably

exaggerated and in turn resulted in the amelioration of anti-MA effects of Fl-D and Fl-E.

The inhibitory effect on the CAR is manifested characteristically to neuroleptics. The potency of this effect is considered to be correlated with antipsychotic activity.²²⁾ Furthermore, it has been reported that little tolerance develops to this effect with chronic administration of neuroleptics.²³⁾ Fl-D also inhibited the CAR. This inhibitory effect lasted for 14–21 d. Fl-E exerted a similar effect to Fl-D and no significant difference from Fl-D was found in the duration of action, although Fl-E seemed to be rather more potent than Fl-D. Similarly, Kuribara and Tadokoro²⁴⁾ reported that the discriminated avoidance response in Sidman type operant apparatus was markedly inhibited by either Fl-D or Fl-E at doses of 2–8 mg/kg, i.m. However, concerning the duration of effect on avoidance response, we found that the effect of Fl-D persisted longer than that of Fl-E.

The cataleptogenic effect is well known to be closely related to the extrapyramidal symptom such as parkinsonism induced by neuroleptics in clinics.¹⁹⁾ Fl-D has been reported to develop lesser extrapyramidal side effects than Fl-E in clinics.^{7,8,10)} However, in the present experiment, the cataleptic effect of Fl-D was not significantly different from that of Fl-E in potency as well as in duration.

In unanesthetized and unrestrained rats with chronic electrode implants, Fl-D and Fl-E at doses of 10–20 mg/kg i.m. caused a drowsy pattern in spontaneous EEG. In this point, our results confirmed the EEG changes reported in clinical studies.^{5,25,26)} However, in contrast to clinical EEG findings, the drowsy pattern produced by Fl-D and Fl-E in rats was not long-lasting.

The EEG arousal response to auditory stimulation was markedly inhibited for 2 d after i.m. injection of Fl-D in many of the rats. Similar effects were observed with Fl-E injection in much smaller numbers of rats, and the effect lasted no longer than 1 d. These results seem to indicate that Fl-D has longer-lasting effect than Fl-E. Since it is technically difficult to obtain consistent results in EEG experiments for a long period of time, it is difficult to determine exactly

the duration of the EEG effect. The present experiment suggested that a relatively higher blood level might be required for this effect, because it lasted for only a short period of time.

Both Fl-D and Fl-E are slowly liberated from the muscle tissue into blood in a constant rate after i.m. injection and then Fl penetrates into the brain.^{27–30)} In clinics, Fl-D has been reported to exert a much longer duration of action than Fl-E, because of its slower absorption into blood.³⁰⁾

In the present studies, we could not find such a big difference in the duration of action between Fl-D and Fl-E. It is clear that the duration of action of Fl-D was slightly longer than Fl-E, in regard to the anti-MA and cataleptogenic effects. Moreover, the onset of action of Fl-D seemed in general to be delayed more than that of Fl-E, indicating the slower absorption of Fl-D.

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