[Chem. Pharm. Bull. 24(12)2918—2926(1976)]

UDC 547.786.3.09 : 615.21.076.9

Effect of 1,2-Benzisoxazole-3-acetamidoxime on Caudato-thalamo-cortical System in Cats

Tsugutaka Ito, Kouichi Yoshida, and Masanao Shimizu

Department of Pharmacology, Research Laboratories, Dainippon Pharmaceutical Co., Ltd.[1]

(Received February 19, 1976)

1,2-Benzisoxazole-3-acetamidoxime hydrochloride (PF-257) was found to have a potentiating effect on the β-(3,4-dihydroxyphenyl)-L-alanine response in addition to the imipramine-like effect on behaviors in rodents. In the present experiments, the effects on the caudate spindle and additional several responses of PF-257 were examined to clarify the mode of action of the compound on the central dopaminergic system. In gallamine-immobilized cats, PF-257 depressed the caudate spindle in a dose of 5 mg/kg, *i.v.* which changed the spontaneous electroencephalographic (EEG) arousal patterns to the drowsy ones and had little or no effect on the EEG arousal response induced by stimulation of the sciatic nerve. With 5 mg/kg which caused the EEG changes, PF-257 facilitated both recruiting response and cortical focal seizure elicited by stimulation of the thalamus and visual cortex, respectively. With 10 mg/kg, acceleration of the spontaneous EEG arousal patterns, facilitation of the EEG arousal response, and biphasic actions on both recruiting response and caudate spindle were observed. From these results, it seems possible to conclude that PF-257 has one of sites of action in the caudate nucleus and the depression of this nucleus by the compound probably results in the increased susceptibility of the thalamo-cortical region.

As reported in the previous papar,[2] 1,2-benzisoxazole-3-acetamidoxime hydrochloride (PF-257) possesses an imipramine-like effect on behaviors in rodents. Besides, the compound potentiates the behavioral effect and the increase in brain dopamine content caused by β-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA). Imipramine also enhances the behavioral excitation caused by L-DOPA.[3] This action of imipramine has been elucidated to be mediated through the aminergic mechanisms excluding dopaminergic system.[4] In contrast, it may be conceivable that the action of PF-257 is involved in the function of the central dopaminergic neurons, since the compound enhanced the increase in dopamine content after L-DOPA, though imipramine had no effect. Also, the mechanism of the action of PF-257 seemed to be different from that of imipramine or monoamine oxidase inhibitors as reported previously.[2] These facts let us investigate the action of PF-257 on the central dopaminergic system.

Dopamine in the brain is highly concentrated in the corpus striatum[5] which may participate in regulating the electrical activity of the brain and in regulating the motor functions.[6] In animal experiments, it has been reported that electrical stimulation of the caudate nucleus elicited the spindle bursts (caudate spindle) in the cortex, thalamus and caudate nucleus, probably through the activation of the inhibitory caudato-thalamo-cortical system.[7–9]. The

1) Location: *Enoki-cho, Suita, Osaka 564, Japan.*
2) M. Shimizu, K. Yoshida, T. Karasawa, Y. Masuda, M. Oka, T. Ito, C. Kamei, M. Hori, Y. Sohji and K. Furukawa, *Experientia* (Basel), **30**, 405 (1974).
3) G.M. Everett, "Antidepressant Drugs," ed. by S. Garattini and M.N.G. Dukes, Excerpta Medica, Amsterdam, 1966, p. 164.
4) E. Friedman and S. Gershon, *Europ. J. Pharmacol.*, **18**, 183 (1972).
5) A. Bertler and E. Rosengren, *Experientia* (Basel), **15**, 10 (1959).
6) T. Shimamoto and M. Verzeano, *J. Neurophysiol.*, **17**, 278 (1954).
7) N.A. Buchwald, E.J. Wyers, T. Okuma and G. Heuser, *Electroenceph. Clin. Neurophysiol.*, **13**, 509 (1961).
8) G. Heuser, N.A. Buchwald, and E.J. Wyers, *Electroenceph. Clin. Neurophysiol.*, **13**, 519 (1961).
9) N.A. Buchwald, G. Heuser, E.J. Wyers, and C.W. Lauprecht, *Electroenceph. Clin. Neurophysiol.*, **13**, 525 (1961).

caudate spindle was reported to be enhanced with neuroleptics[10,11] which block dopamine receptor function.[12]

In the present study, the effect of PF-257 on the caudate spindle was examined on the basis of these findings in comparison with that of psychotropic drugs. As the results obtained, PF-257 was found to depress this response. Therefore, in an attempt to have insight into the mechanism of action of PF-257, effects on the nervous system involved in the mechanism eliciting the caudate spindle were studied by examining the effects on the electroencephalographic (EEG) arousal response, thalamic recruiting response and cortical focal seizure (cortical excitability). A preliminary report of these findings has been communicated.[13]

Materials and Methods

Cats of both sexes weighing 2.5 to 4.0 kg were fixed on a stereotaxic apparatus (Todai Noken type) under ether anesthesia, immobilized with gallamine triethiodide (i.m.) and artificially ventilated at the rate of 26 strokes/min. The subsequent surgical procedures were done under local anesthesia with procaine hydrochloride.

For the EEG recording and electrical stimulation, silver ball electrodes were placed on the cortices (anterior suprasylvian, posterior suprasylvian, posterior sigmoid and medialis ectosylvian), and stainless steel concentric electrodes (external diameter 0.8 mm) insulated except the tips were implanted in the head of the caudate nucleus, hippocampus and thalamus according to the atlas of Snider and Niemer.[14] Coordinates of electrode positions in mm were as follows: dorsal hippocampus, anterior (*A*) 0.5, lateral (*L*) 11.0, horizontal (*H*) +7.0; ventralis anterior (thalamus), *A* 11.0, *L* 4.0, *H* +4.0; caudate nucleus, *A* 14.8, *L* 5.0, *H* +9.0. Also, two kinds of stainless steel electrodes (external diameter 200 μ and 400 μ) insulated except at the tips were implanted into the visual cortex (posterior lateralis) for the EEG recording and stimulation, respectively. The reference electrode was inserted into the neck muscle. The EEG recordings were made bipolarly for the spontaneous EEG and the EEG arousal response, and monopolarly for other responses by using a Nihonko- with power units (RB-5). For the electrical hden Recticorder (RJG-3006) stimulation, a Nihonkohden SEN-1101 stimulator with an isolator was used.

1. EEG Arousal Response——The sciatic nerve was isolated from the surrounding tissue and placed on bipolar silver electrodes (the distance 3 mm). The EEG arousal response was obtained by stimulation of the sciatic nerve at 60 Hz square pulses with a duration of 0.03 msec for 5 sec. The stimulation voltage was fixed at which the EEG arousal pattern in the cortex persisted for approximately 20 sec after stimulation (0.6—1.5 V).

2. Caudate Spindle——The stimulating electrode was placed in the head of the caudate nucleus. When the nucleus was stimulated at 0.5 Hz square pulses with a duration of 1.0 msec, the caudate spindle appeared in the cortex. The stimulation voltage was gradually increased until the caudate spindle was produced in the ipsilateral cortex by almost every stimulus, and fixed at this point. This voltage varied from 3.0 to 4.5 V.

3. Recruiting Response——The recruiting response in the cortex was elicited by stimulation of the ventralis anterior of the thalamus at 8 Hz square pulses with a duration of 1.0 msec for 10 sec. As the stimulation was increased in intensity, the response typically showed a waxing and waning in the amplitude of the waves. Then, this condition of stimulation was used (1.0—3.0 V).

4. Cortical Focal Seizure (Cortical Excitability)——The experiment was performed according to the method of Vastola and Rosen[15] with some modifications. The stimulating electrodes (the distance 1 mm) were placed in the upper layer of the visual cortex (posterior lateralis), and four recording electrodes in the ipsilateral cortex in parallel with the midline of the brain. By stimulation of the visual cortex at 70 Hz square pulses with a duration of 1 msec for 2 sec, the electrical seizure was elicited in a restricted area of the cortex. This voltage varied from 14.0 to 20.0 V.

In some experiments, the arterial blood pressure was recorded from the femoral artery by using a transducer (Nihonkohden MP-4T). Exposed neural structures were covered with warm liquid paraffin, and body temperature was maintained constant using an infrared lamp. After completion of the experiments, the location of electrodes was confirmed histologically.

5. Chemicals——Drugs used were PF-257 (1,2-benzisoxazole-3-acetamidoxime-HCl, Dainippon), chlorpromazine-HCl (Wintamine, Shionogi), haloperidol (Serenace, Dainippon), imipramine-HCl (Tofranyl,

10) T. Ito, Y. Masuda, and M. Shimizu, *Folia Pharmacol. Japon.*, **71**, 14p (1975) (in Japanese).
11) G. Stille and A. Sayers, *Experientia* (Basel), **23**, 1028 (1967).
12) J.W. Kebabian, G.L. Petzold, and P. Greengard, *Proc. Natn. Acad. Sci.*, U.S.A., **69**, 2145 (1972).
13) T. Ito, K. Yoshida, and M. Shimizu, *Japan. J. Pharmacol.*, **25**, 71p (1975).
14) R.S. Snider and W.T. Niemer, "A Stereotaxic Atlas of the Cat Brain," Univ. of Chicago Press, 1961.
15) E.F. Vastola and A. Rosen, *Electroenceph. Clin. Neurophysiol.* **12**, 327 (1960).

Fujisawa), pentobarbital Na (Nembutal, Dainippon), and methamphetamine-HCl (Philopon, Dainippon). These were administered by slow *i. v.* injection into a cannulated forelimb vein. In the experiments where an animal was given more than one drug, at least 1 hr was allowed to elapse after the effect of preceding drug had disappeared. In addition, drugs were given to different animals in different orders of administration.

Results

Effect of PF-257 on Spontaneous EEG

The spontaneous EEG patterns of gallamine-immobilized cats were characterized mainly by low amplitude fast waves in the cortex and regular waves (θ waves) in the hippocampus.

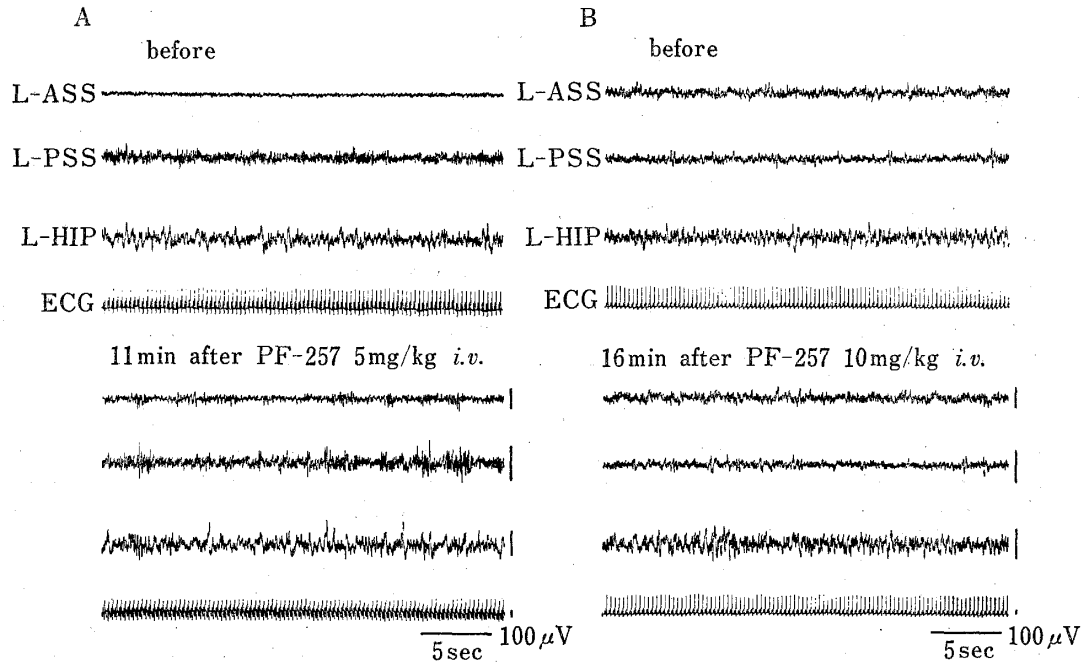

Fig. 1. Effect of PF-257 on Spontaneous EEG Arousal Patterns in Gallamine-immobilized Cats

Abbreviations: L-ASS=left anterior suprasylvian, L-PSS=left posterior suprasylvian, L-HIP=left dorsal hippocampus, ECG=electrocardiogram

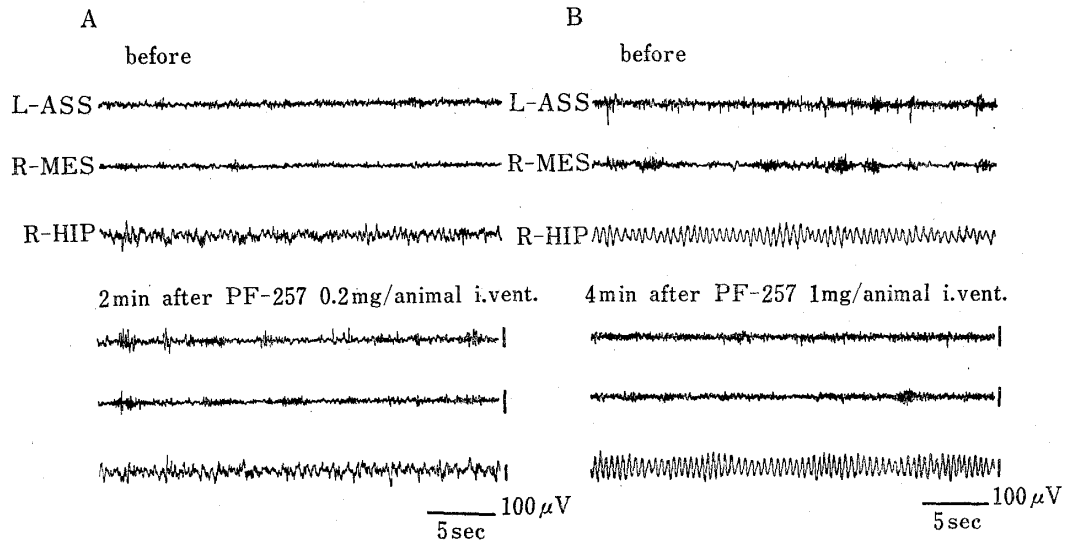

Fig. 2. Effect of Intraventricularly-injected PF-257 on Spontaneous EEG Arousal Patterns in Gallamine-immobilized Cats

Abbreviations: L-ASS=left anterior suprasylvian, R-MES=right medialis ectosylvian, R-HIP=right dorsal hippocampus

Fig. 1 shows the typical effect of PF-257 on the spontaneous EEG arousal patterns. Following the injection of PF-257 (2—5 mg/kg, *i.v.*), the EEG drowsy patterns, *i.e.*, slow waves of high amplitudes, were induced in the cortex. With a high dose of 10 mg/kg, PF-257 slightly accelerated the EEG arousal patterns; reduction in the amplitude, increase in the mean frequency in the cortex and appearance of marked regular waves in the hippocampus. Chlorpromazine-HCl, imipramine-HCl and pentobarbital Na at a dose of 5 mg/kg shifted the EEG arousal patterns to the drowsy ones. Haloperidol (1 mg/kg) had no effect.

In order to ascertain whether the EEG effects induced by PF-257 are due to the direct action on the central nervous system, the effect of PF-257 injected into the ventriculus lateralis was examined on the spontaneous EEG. With the intraventricular injection of 0.1—0.2 mg/animal, PF-257 caused the EEG drowsy patterns which lasted for 15—30 min (Fig. 2). When the dose was increased to 0.5—2.0 mg/animal, a further decrease in amplitude and an increase in frequency were induced in the cortex. The intraventricular dose necessary for causing such EEG changes was approximately one-sixtieth of the intravenous dose necessary for causing similar changes.

Effect of PF-257 on EEG Arousal Response

A typical EEG arousal response was produced in the cortex and hippocampus by stimulation of the sciatic nerve. PF-257 at a dose of 5 mg/kg was without effect on the response (Fig. 3A). In Fig. 3B, the change in the duration of the EEG arousal response is shown as a percentage of the pre-injection value. A high dose (10 mg/kg) of PF-257 facilitated the response: further increase in frequency and decrease in amplitude in the cortex during stimulation and prolongation of arousal time in post-stimulation. Chlorpromazine-HCl, imipramine-HCl and pentobarbital Na significantly shortened the duration at a dose of 5 mg/kg. On the other hand, methamphetamine-HCl (0.5 mg/kg) significantly prolonged it. Haloperidol (1 mg/kg) caused little or no change.

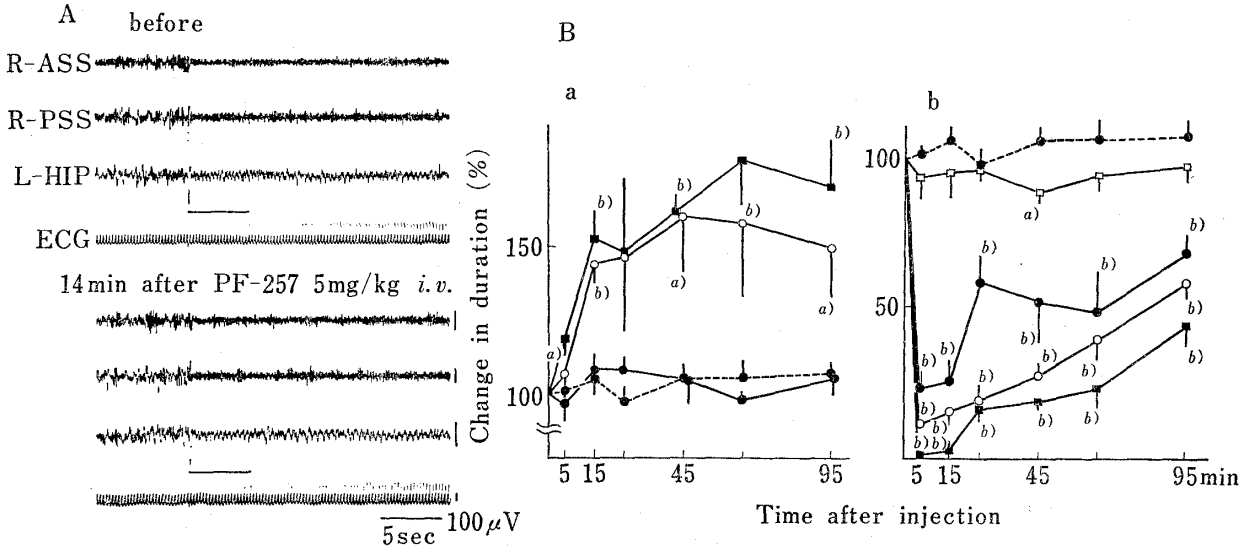

Fig. 3. Effect of PF-257 and Psychotropic Drugs on EEG Arousal Response induced by Stimulation of the Sciatic Nerve in Cats

A: Effect of 5 mg/kg of PF-257. The sciatic nerve was stimulated during the period indicated by a horizontal line. Abbreviations: see in Fig. 1. B: Comparison of the effect caused with PF-257 and psychotropic drugs on the EEG arousal response. Ordinate: the change in the duration of the EEG arousal response caused by fixed-voltage stimulation of the sciatic nerve, shown as a percentage of the pre-injection value. Abscissa: time after the injection. a) ●—● control (saline 0.1 ml/kg, *i.v.*), ●—● PF-257 5 mg/kg, ○—○ PF-257 10 mg/kg, ■—■ methamphetamine-HCl 0.5 mg/kg. b) ●---● control (saline), □—□ haloperidol 1 mg/kg, ○—○ chlorpromazine-HCl 5 mg/kg, ●—● imipramine-HCl 5 mg/kg, ■—■ pentobarbital Na 5 mg/kg. Each point represents the mean obtained in separate four experiments with the S.E. indicated. Differences statistically significant from the control: *a*) $p<0.05$; *b*) $p<0.01$.

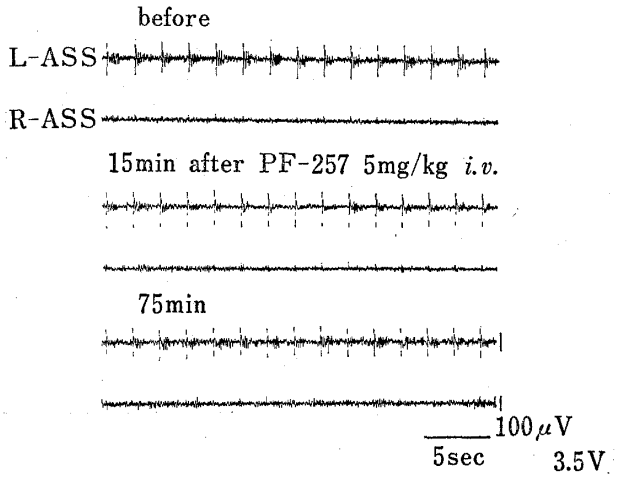

Fig. 4. Effect of PF-257 on Caudate Spindle in Cats

The caudate spindle was induced by stimulation of the head of the caudate nucleus at 0.5 Hz square pulse. Abbreviations: see in Fig. 1.

Effect of PF-257 on Caudate Spindle

The effect of PF-257 on the caudate spindle is shown in Fig. 4. Following the injection of PF-257 (5 mg/kg), the caudate spindle was depressed: decrease of the amplitude and shortening of the duration were observed. In Fig. 5, the change in the average activity (index: duration× amplitude) of the caudate spindle elicited during 15 sec is shown as a percentage of the pre-injection value. PF-257 (5 mg/kg) decreased the caudate spindle activity up to 17.9±3.9%, 16.2±7.0% and 24.7±9.3 % of the control level in 15, 25 and 65 min after the injection, respectively. With 10 mg/kg of PF-257, the caudate spindle was also depressed, but in four of 5 animals an increase of the amplitude and a prolongation of the duration were induced after an initial depression. Chlorpromazine-HCl (5 mg/kg), haloperidol (0.5 mg/kg), imipramine-HCl (5 mg/kg) and pentobarbital Na (5 mg/kg) enhanced the caudate spindle, though methamphetamine-HCl (0.5 mg/kg) depressed the response.

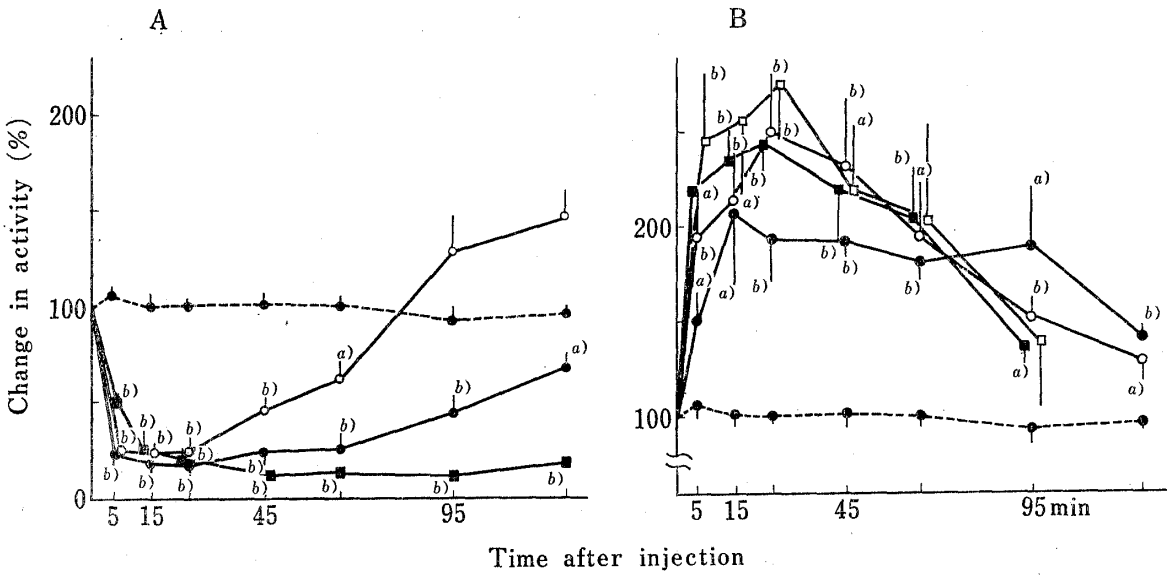

Fig. 5. Effect of PF-257 and Psychotropic Drugs on Caudate Spindle in Cats

Ordinate: the change in the average activity (index: duration×amplitude) of the caudate spindle elicited during 15 sec, expressed as a percentage of the pre-injection value. Abscissa: time after the injection. A: ●---● control (saline), ●—● PF-257 5 mg/kg, ○—○ PF-257 10 mg/kg, ■—■ methamphetamine-HCl 0.5 mg/kg. B: ●·····● control, ●—● haloperidol 0.5 mg/kg, ○—○ chlorpromazine-HCl 5 mg/kg ■—■ imipramine-HCl 5 mg/kg, □—□ pentobarbital Na 5 mg/kg. Each point represents the mean obtained in at least separate three experiments with the S.E. indicated. Differences statistically significant from the control: *a*) $p<0.05$; *b*) $p<0.01$.

Effect of PF-257 on Recruiting Response

The thalamic recruiting response was induced in the cortex by low frequency stimulation of the ventralis anterior. PF-257 (5 and 10 mg/kg) enhanced the recruiting response: an increase of the amplitude and a facilitation of the waxing and waning phenomenon in the ipsi- and contralateral cortices (Fig. 6). In some cases injected with 10 mg/kg, the recruiting

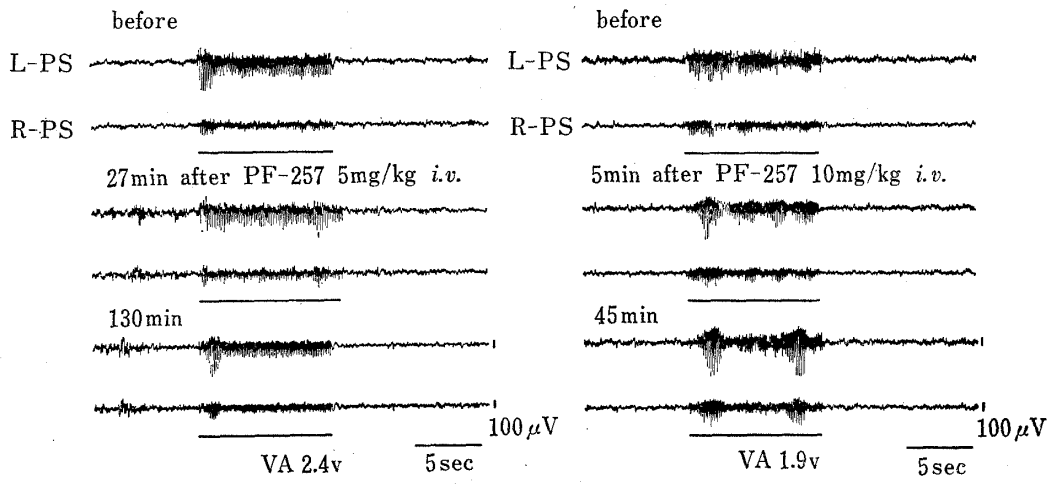

Fig. 6. Effect of PF-257 on Thalamic Recruiting Response in Cats

The recruiting response was induced by stimulation of the left ventralis anterior of thalamus during the period indicated by a horizontal line. Abbreviations: L-PS=left posterior sigmoid, R-PS=right posterior sigmoid.

response was depressed after an initial enhancement. Chlorpromazine-HCl, imipramine-HCl and pentobarbital Na enhanced the response at a dose of 5 mg/kg. On the other hand, methamphetamine-HCl (0.5 mg/kg) markedly depressed it (Fig. 7).

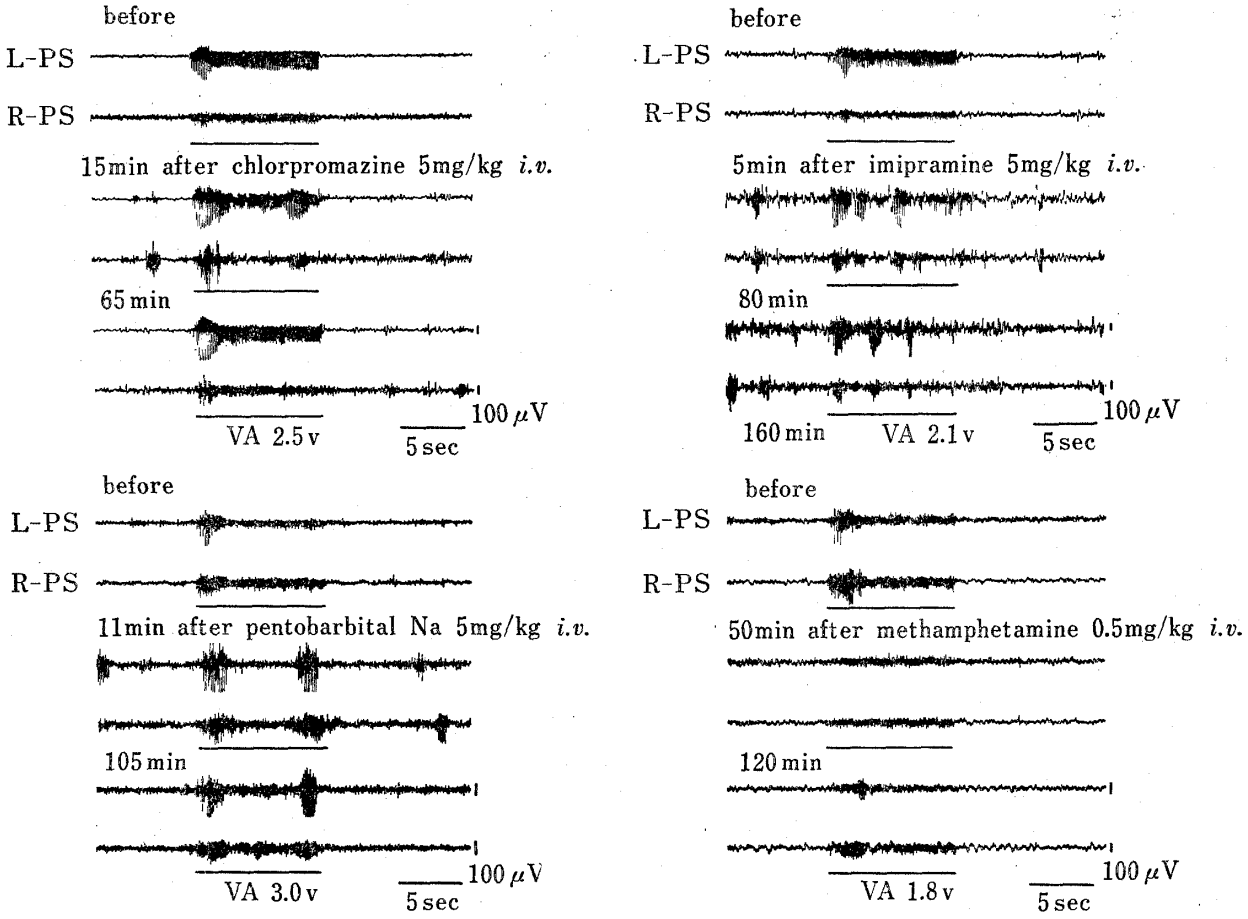

Fig. 7. Effect of Psychotropic Drugs on Thalamic Recruiting Response in Cats

The recruiting response was induced by stimulation of the left ventralis anterior during the period indicated by a horizontal line. Abbreviations: see in Fig. 6.

Effect of PF-257 on Cortical Excitability

The cortical focal seizure was induced by stimulating the visual cortex. PF-257 at a dose of 5 mg/kg facilitated the focal seizure as shown by an increase in the duration and amplitude and a facilitation of the cortical spreading (Fig. 8). In one of 5 animals, the focal seizure was temporarily changed to a generalized seizure 35 min after the injection of 5 mg/kg.

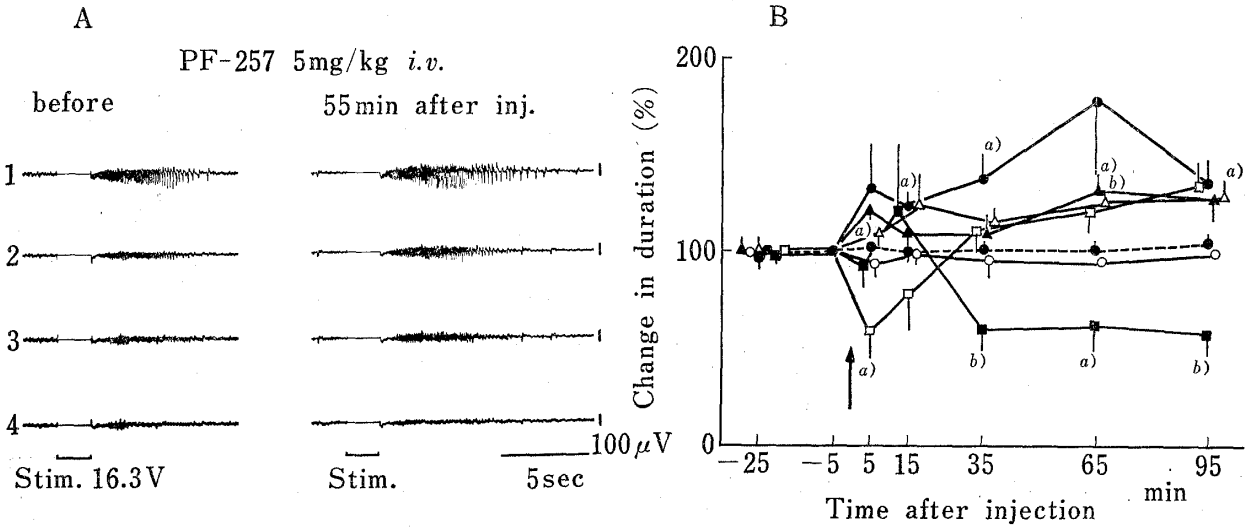

Fig. 8. Effect of PF-257 and Psychotropic Drugs on Cortical Focal Seizure in Cats

A: Facilitation of the focal seizure observed after PF-257. The visual cortex was stimulated during the period indicated by a horizontal line. Interelectrode distances: stimulating electrode-1=3.0 mm, 1—2=3.0 mm, 2—3=1.5 mm, 3—4=3.0 mm. B: Comparison of the effect on the cortical focal seizure. Ordinate: the change in the duration of the seizure (recording position 1), expressed as a percentage of the pre-injection value. The drug was *i.v.* injected at (↑). Abscissa: time after the injection. ●---● control (saline), ●—● PF-257 5 mg/kg, ○—○ PF-257 10 mg/kg, △—△ haloperidol 1 mg/kg, □—□ chlorpromazine-HCl 5 mg/kg, ■—■ imipramine-HCl 5 mg/kg, ▲—▲ methamphetamine-HCl 0.5 mg/kg. Each point represents the mean obtained in at least separate four experiments with the S.E. indicated. Differenes statistically significant from the control: *a*) $p<0.05$; *b*) $p<0.01$.

With 10 mg/kg of the compound, the change suggesting the facilitation of seizure was induced except for the duration which was not increased. Methamphetamine-HCl (0.5 mg/kg) and haloperidol (1 mg/kg) also facilitated the seizure. On the other hand, imipramine-HCl (5 mg/kg) depressed the seizure though a generalized seizure was produced in some cases (2 of 5 animals). Chlorpromazine-HCl (5 mg/kg) showed a biphasic action: an initial depression followed by a gradual facilitation.

Effect of PF-257 on Blood Pressure and ECG

The mean blood pressure was approximately 120 mmHg in immobilized control cats. PF-257 at doses of 5—10 mg/kg caused a fall in blood pressure (approximately 30 mmHg) which returned to the control level within 5 min. No abnormality was observed in the ECG after the injection of PF-257 (2.5—10 mg/kg). Thus, it seemed unlikely that the effects of PF-257 on the EEG responses examined were mediated through the change of blood pressure.

Discussion

PF-257 (5 mg/kg) changed the spontaneous EEG arousal patterns to the drowsy ones. The result of intraventricular injection study (Fig. 2) indicates that this effect of PF-257 may be exerted through the direct action on the central nervous system. Furthermore, it was found that at the same dose PF-257 depressed the caudate spindle without impairment of the EEG arousal response (Fig. 3 and 4).

It has been demonstrated that the caudate spindle is facilitated during sleep or after the administration of pentobarbital.[7] The facilitation of the caudate spindle after pentobarbital

was confirmed in the present study. In contrast, the caudate spindle was depressed after an injection of methamphetamine or by high frequency stimulation of the sciatic nerve,[10] both of which facilitated the spontaneous EEG arousal patterns. From these findings, it seems likely that there is a close relationship between the changes of the caudate spindle activity and the spontaneous EEG patterns. However, the actions of 5 mg/kg of PF-257 on both responses could not be satisfied with this relationship.

As shown in Fig. 5, haloperidol markedly enhanced the caudate spindle without affecting the EEG arousal response. In the present study, chlorpromazine and imipramine induced the enhancement of the caudate spindle with concomitant inhibition of the EEG arousal response. In our report,[10] it was demonstrated that chlorpromazine as well as haloperidol might act on the caudato-thalamo-cortical system, since these drugs enhanced the caudate spindle even in lower dose having no effect on the EEG arousal response induced by stimulation of the sciatic nerve. Imipramine had no effect on the caudate spindle at low dose having no effect on the EEG arousal response.[10] Stille and Sayers[11] reported the similar findings with bulbocapnine and cataleptogenic neuroleptics. Thus, it seems probable that PF-257 also acts on the caudato-thalamo-cortical system since the compound caused the depression of the caudate spindle without impairment of the EEG arousal response.

Despite the depression of the caudate spindle, PF-257 increased the amplitude of the thalamic recruiting response induced by stimulation of the ventralis anterior, a part of the neural circuit producing the caudate spindle in the cortex.[8] It has been reported that the increase of the recruiting response after pentobarbital was probably secondary to the depression of the midbrain reticular formation (release phenomenon).[16] The facilitatory effect of PF-257 (5 mg/kg) on the recruiting response may be due to the same mechanism as that of pentobarbital. However, this possibility is ruled out, since the compound showed little or no effect on the EEG arousal response. Saxena, *et al.*[17] reported from the physiological and pharmacological aspects that the neurons involved in the genesis of the caudate spindle and recruiting response were functionally identical. In the present study, the effects of PF-257 on two responses were different: depression of the caudate spindle and facilitation of the recruiting response. Therefore, it can be considered that PF-257 may have one of its sites of action in the caudate nucleus, closely relating to the dopaminergic mechanism.

From the biochemical observation, it was reported that PF-257 enhanced the increase in brain dopamine after L-DOPA and reduced the rate of disappearance of endogenous dopamine in the α-methyl-*p*-tyrosine-treated rat brain.[2] The reduction of dopamine turnover in the striatum is known to be induced with anticholinergics.[18,19] However, PF-257 had no cholinolytic activity in the central nervous system.[2] Accordingly, the involvement of anticholinergic action of PF-257 is ruled out.

An increase in the activity of the thalamo-cortical region after PF-257 was further supported by an increase in cortical excitability; the compound increased the duration and amplitude of the cortical focal seizure. This effect of PF-257 was similar to that of chlorpromazine, haloperidol and methamphetamine. As described above, PF-257 (5 mg/kg) caused little or no effect on the EEG arousal response while the spontaneous EEG drowsy patterns were induced. Therefore, it is unlikely that the increase in susceptibility of the thalamo-cortical region is due to activation of the reticular formation or the cortex itself. A possibility seems to exist that the increase in susceptibility of this region is the result of depression of the caudate nucleus which gives the inhibitory influence on this region.[8]

At 10 mg/kg, PF-257 accelerated the spontaneous EEG arousal patterns, facilitated the EEG arousal response, and acted biphasically on both recruiting response and caudate spindle.

16) E.F. Domino, *J. Pharmacol. Exp. Ther.*, **115**, 449 (1955).
17) P.N. Saxena, K.K. Tangri, and K.P. Bhargava, *Electroenceph. Clin. Neurophysiol.*, **17**, 506 (1964).
18) N.-E. Andén and P. Bédard, *J. Pharm. Pharmacol.*, **23**, 460 (1971).
19) G. Bartholini and A. Pletscher, *Experientia* (Basel), **27**, 1302 (1971).

Also, an increase in the recruiting response occurred at the time when the spontaneous EEG arousal patterns were accelerated. At the present time, the mode of action induced by 10 mg/kg of PF-257 is difficult to be fully explained. Probably, in addition to the caudate action, other mechanisms of action seem to be related with these phenomena. In this aspect, the problem remains obscure regarding possible noradrenergic or serotonergic action of PF-257 on the central nervous system.

In considering the results obtained in the present experiments, it seems possible to conclude that a low dose of PF-257 causes the depression of the caudate nucleus, which probably results in the increased susceptibility of the thalamo-cortical region associated with the brain higher function. In other words, it is likely that the compound has one of sites of action in the caudate nucleus of which a dysfunction has been suggested as a factor in the production of the psychiatric disorder[20] or parkinsonism syndromes.[21]

20) F.A. Mettler, *Psychiat. Quart.*, **29**, 89 (1955).
21) D. Hornykiewicz, *Pharmac. Rev.*, **18**, 925 (1966).