Chem. Pharm. Bull. 24(11)2673—2685(1976)

UDC 547.786.3.09 : 615.31.076.9

Effects of 1,2-Benzisoxazole-3-acetamidoxime on Central Monoamine Neurons in the Rat

Tadahiko Karasawa, Kiyoshi Furukawa, Kouichi Yoshida, and Masanao Shimizu

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

(Received February 19, 1976)

The effects of 1,2-benzisoxazole-3-acetamidoxime hydrochloride (PF-257), a novel psychotropic agent possessing the pharmacological properties similar, in some respects, to those of tricyclic antidepressants or monoamine oxidase inhibitors, on the metabolism of brain monoamines were studied in rats. A single large dose or repeated doses of PF-257 caused a slight but significant increase in brain norepinephrine level without affecting the contents of dopamine and serotonin. Like monoamine oxidase inhibitors, PF-257 prevented and reversed the decrease in brain norepinephrine induced by reserpine but it lacked the property to inhibit monoamine oxidase in the brain. Unlike tricyclic antidepressants, the compound was devoid of the capacity to inhibit norepinephrine uptake *in vitro*. Administration of PF-257 in combination with L-β-3,4-dihydroxyphenylalanine (L-DOPA) enhanced the increase in brain dopamine without influencing the amount of the amino acid incorporated into the brain. At relatively small doses, PF-257 reduced the rate of decline of brain norepinephrine and dopamine levels following α-methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor, and also decreased the levels of brain 3-methoxy-4-hydroxy-phenylethyleneglycol sulfate and homovanillic acid, major metabolites of brain norepinephrine and dopamine, respectively. Thus, it is suggested that the fundamental mechanism underlying the pharmacological and biochemical effects of PF-257 may be its activity to decelerate catecholamine metabolism in the brain without inhibiting the enzymes participating in the metabolic degradation of the amines.

1,2-Benzisoxazole-3-acetamidoxime hydrochloride (PF-257)[2] is a compound acting on the central nervous system with an unique profile[3] in a series of pharmacological tests in animals. A certain similarity of this compound to clinically efficacious antidepressant drugs such as imipramine and monoamine oxidase inhibitors in the pharmacological properties has suggested an involvement of adrenergic mechanism for its action. Tricyclic antidepressants have been characterized by the ability to potently inhibit intraneuronal uptake of norepinephrine or serotonin. This action of tricyclic antidepressants and the inhibition of brain monoamine metabolism by monoamine oxidase inhibitors have been considered as the mechanisms underlying the pharmacological and clinical effects of the two classes of antidepressant drugs. PF-257, however, lacks both of these actions, suggesting its different mode of action.

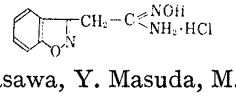
In order to elucidate the action mechanism of PF-257, we have investigated the effects of this compound on biogenic monoamines, *i.e.*, catecholamines and serotonin, in the rat brain.

The present paper describes the results obtained from such investigations.

Materials and Methods

Drugs and Reagents——1,2-Benzisoxazole-3-acetamidoxime hydrochloride (PF-257) was synthesized in this laboratory. Imipramine hydrochloride, chlorpromazine hydrochloride, haloperidol, cocaine hydro-

1) Location: *Enoki-cho, Suita, Osaka, 564, Japan.*

2) H. Uno, K. Kurokawa, K. Natsuka, Y. Yamato (the late), and H. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **24**, 632 (1976). Structure: 

3) M. Shimizu, K. Yoshida, T. Karasawa, Y. Masuda, M. Oka, T. Ito, C. Kamei, M. Hori, Y. Sohji, and K. Furukawa, *Experientia*, **30**, 405 (1974).

chloride, methamphetamine hydrochloride, pargyline hydrochloride and iproniazid phosphate, which were used as reference drugs, were the commercial products. DL-α-Methyl-*p*-tyrosine methylester hydrochloride (α-MpT), DL-α-methyl-3,4-dihydroxyphenylalanine (α-*m*-DOPA), hydroxylamine hydrochloride and pyrogallol which were used as enzyme inhibitors and L-β-3,4-dihydroxyphenylalanine (L-DOPA) were also the commercial products. Reserpine was obtained from ampoules for medical use. All the drugs were dissolved in water or in a minimum quantity of hydrochloric acid, depending on their solubility. The doses of the drugs are expressed as the form presented above.

The reagents used for the biochemical analysis were of highest purity commercially available.

Animals——Male Wistar strain rats weighing 150—200 g were used. They were kept in an air-conditioned room at 22—24° and were allowed free access to food and water throughout the experiments.

Determination of Biogenic Monoamines and L-DOPA——Determination of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in brain tissue and dissection of brain into several discrete regions were performed as described previously.[4] L-DOPA was assayed fluorometrically according to the method of Laverty and Taylor[5] after isolation by double column chromatography on Amberlite CG-50 and aluminum oxide. The isolation procedure was as follows: Brain tissue was homogenized in 0.4 N perchloric acid and centrifuged. The supernatant was adjusted to pH 7.5—8.5 with 0.5 M K_2CO_3 and the $KClO_4$ formed was removed by centrifugation at 0°. The clear supernatant was passed through a column of Amberlite CG-50 (equilibrated in 0.1 M phosphate buffer, pH 6.3), followed by washing with water. The effluent of the sample solution and the wash water were successively passed through a column of aluminum oxide which was placed beneath the Amberlite CG-50 column. L-DOPA flowed out from the Amberlite CG-50 column without being retained and was adsorbed by aluminum oxide in the lower column, while catecholamines including DA which interfered with the fluorometric measurement of L-DOPA were removed by the resin. The L-DOPA adsorbed on aluminum oxide was eluted with 0.2 N HCl and determined fluorometrically.

Determination of Homovanillic Acid, 3-Methoxy-4-hydroxyphenylethyleneglycol Sulfate and 5-Hydroxyindoleacetic Acid——Homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), the major metabolites of DA and 5-HT in the rat brain, respectively, were determined as reported previously.[6] The determination of 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MOPEG-SO_4), a major metabolite of brain NE in the rat, was performed according to the method of Meek and Neff.[7]

Assay of Enzyme Activity——The procedure for assaying L-DOPA decarboxylase activity was essentially similar to the method described by Davis and Awapara,[8] except for the use of fluorometry in place of absorptiometry for measuring formed DA. The activity of monoamine oxidase was assayed as reported previously.[9]

Measurement of NE Uptake into Nerve Endings in Homogenates of Rat Brain——Rat brains were homogenized in ice-cold 0.25 M sucrose solution with a glass homogenizer with a loosely fitted Teflon pestle. The homogenate was centrifuged at $1000 \times g$ for 10 min and the supernatant fluid was used as a crude nerve ending preparation. Incorporation of ^{3}H-NE into nerve endings in this preparation and the effects of drugs thereon were examined *in vitro* as reported elsewhere.[9]

Results

Effects on Brain Monoamine Levels

A single oral dose of PF-257 caused a selective increase in brain NE without affecting the levels of DA and 5-HT. As shown in Table I, a significant increase in brain NE was observed at 3, 6 and 12 hr after a dose of 300 mg/kg *p.o.* Imipramine, a typical tricyclic antidepressant, did not cause any change in the brain monoamine levels at a dose of 100 mg/kg *p.o.*

Table II shows that the increase in brain NE level after PF-257 was dose-dependent.

When PF-257 was repeatedly given to rats, the increase in brain NE became more conspicuous than that observed after single doses. As shown in Fig. 1, administration of PF-257 at daily doses of 30—300 mg/kg *p.o.* for 28 consecutive days caused a significant increase in brain NE. The treatment, however, caused no change in the level of brain DA or 5-HT. Being distinct from the case of single administration, repeated administration of imipramine

4) T. Karasawa, K. Furukawa, K. Yoshida, and M. Shimizu, *Jap. J. Pharmacol.*, **25**, 727 (1975).
5) R. Laverty and K.M. Taylor, *Anal. Biochem.*, **22**, 269 (1968).
6) T. Karasawa, I. Nakamura, and M. Shimizu, *Life Sci.*, **15**, 1465 (1974).
7) J.L. Meek and N.H. Neff, *Brit. J. Pharmacol.*, **45**, 435 (1972).
8) V.E. Davis and J. Awapara, *J. Biol. Chem.*, **235**, 124 (1960).
9) M. Shimizu, T. Hirooka, T. Karasawa, Y. Masuda, M. Oka, T. Ito, C. Kamei, Y. Sohji, M. Hori, K. Yoshida, and H. Kaneko, *Arzneim-Forsch.* (Drug Res.), **24**, 166 (1974).

TABLE I. Effect of a Single Large Dose of PF-257 on Monoamine Levels in the Whole Rat Brain (Time- Course Study)

Treatment	Time (hr) after drug	Brain monoamines (ng/g) NE	DA	5-HT
Control	—	445±32	1110±94	479±66
PF-257	3	489±21[a]	1200±50	566±20[a]
300 mg/kg	6	509±61[a]	1150±59	547±29
p.o.	12	539±18[b]	1150±83	545±66
Imipramine	3	430±12	1130±16	466±12
100 mg/kg	6	428±27	1090±43	461±26
p.o.	12	416±36	1090±43	419±38

Values are means±S.D. of 5 to 6 rats.
a) significantly different from control at $p<0.05$
b) significantly different from control at $p<0.01$

TABLE II. Effect of Single Doses of PF-257 on Monoamine Levels in the Whole Rat Brain (Dose-Response Relationship)

Treatment	Dose (mg/kg, *p.o.*)	Brain monoamines (ng/g) NE	DA	5-HT
Control	Saline	488±40	1160±102	442±38
	33.3	495±30	1290±103	478±45
PF-257	100	538±48	1230±161	484±27
	300	589±43[a]	1300±135	485±36

Values are means±S.D. of 6 rats. Rats were killed 12 hr after PF-257 administration.
a) significantly different from control at $p<0.01$

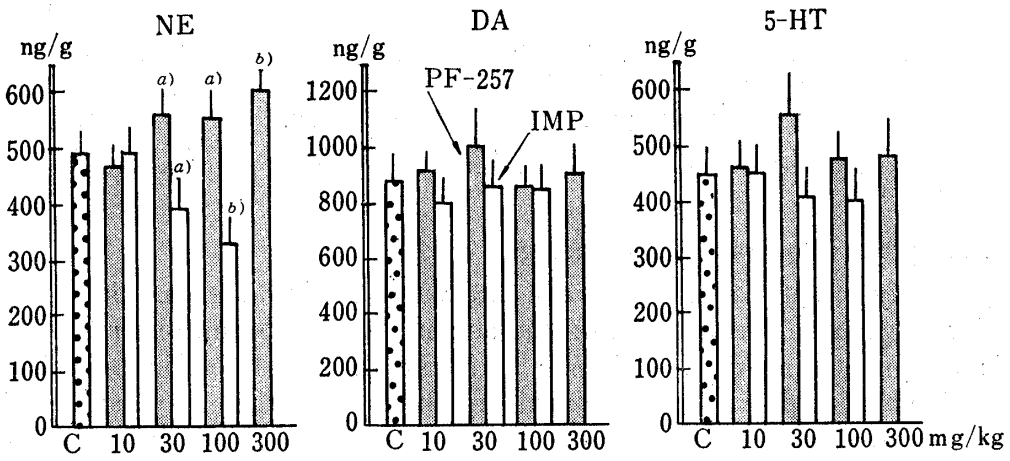


Fig. 1. Effect of Long-Term Administration of PF-257 and Imipramine on Brain Monoamine Levels in Rats

PF-257 and imipramine (IMP) were administered to rats *p.o.* once a day for 28 consecutive days. The rats were killed 2 hr after the last medication. The columns represent means±S.D. (vertical bar) of 5 rats. C: control receiving saline, *a*) $p<0.05$, *b*) $p<0.01$ compared with control

resulted in a remarkable decrease in the level of brain NE at daily doses of 30 and 100 mg/kg *p.o.* without an accompanying change in DA or 5-HT level.

Effect on Reserpine-Induced Depletion of Brain Monoamines

One of the pharmacological properties that PF-257 shares with imipramine and monoamine oxidase inhibitors is antagonism to the syndrome induced by reserpine in rodents. Reserpine is known to exert its pharmacological effects by depleting the brain of biogenic monoamines. To examine whether the reversal of reserpine-induced syndrome by PF-257 was mediated *via* brain monoamines, the interaction of the compound with reserpine was studied with respect to the levels of brain monoamines.

Fig. 2 shows the results obtained from such studies. Three hours after reserpine 1 mg/kg *i.p.*, the levels of brain NE, DA and 5-HT were moderately decreased as shown in the shadowed columns in Fig. 2. Pretreatment of rats with PF-257 300 mg/kg *p.o.* 1 hr prior to reserpine completely prevented the reserpine-induced depletion of brain NE but it failed to prevent the decrease of DA or 5-HT. Imipramine which was as potent as PF-257 in antagonizing the reserpine-induced behavioural syndrome in rats showed a significant enhancement of NE depletion, an effect opposite to that of PF-257. On the depletion of DA or 5-HT imipramine was without effect. Pargyline, a monoamine oxidase inhibitor, was not only effective in antagonizing the depletion of all of the three monoamines but also able to even enhance the levels of NE and 5-HT over the normal values.

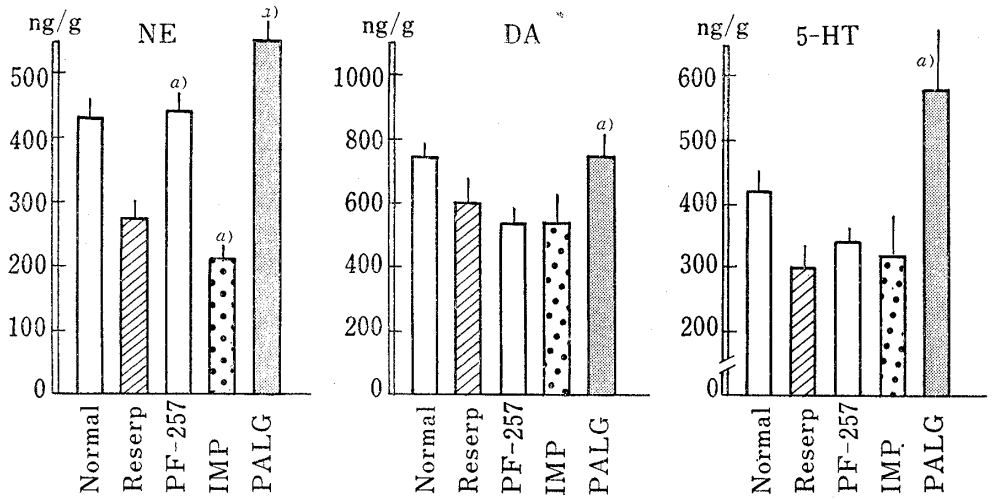


Fig. 2. Effect of PF-257, Imipramine and Pargyline on Reserpine-Induced Depletion of Brain Monoamines in Rats

PF-257 (300 mg/kg), imipramine (IMP, 100 mg/kg) and pargyline (PALG, 100 mg/kg) were administered to rats *p.o.* 1 hr prior to reserpine (Reserp, 1 mg/kg *i.p.*). The rats were killed 3 hr after reserpine. The columns represent means±S.D. (vartical bar) of 5 rats. *a)* $p<0.01$ compared with control

Effect on NE Uptake into Brain Nerve Endings

It is generally accepted that reuptake of catecholamines into presynaptic nerve terminals is the major mechanism for terminating the actions of the transmitters released from the adrenergic presynaptic neurons. Tricyclic antidepressants and certain central stimulants such as cocaine and amphetamines block this uptake process, thereby potentiate the action of catecholamines, especially that of NE, at their receptors in the peripheral and central nervous systems. To examine whether PF-257 possessed such activity, the effect of this compound on the incorporation of ^{3}H-NE into crude nerve ending fractions of rat brain was studied *in vitro*. As shown in Fig. 3, PF-257 showed no inhibition of the uptake of NE even at the concentration as high as 10^{-4}M, whereas imipramine, cocaine and methamphetamine, used as reference drugs, showed potent inhibition. Haloperidol and chlorpromazine, the

typical neuroleptic drugs, also strongly inhibited the NE uptake. The potency of the drug action, expressed as the concentration which would cause a 50% inhibition (ID_{50}), was as follows: imipramine 3.1×10^{-5}M, methamphetamine 3.8×10^{-7}M, cocaine 1.1×10^{-6}M, chlorpromazine 1.8×10^{-5}M and haloperidol 8.2×10^{-6}M.

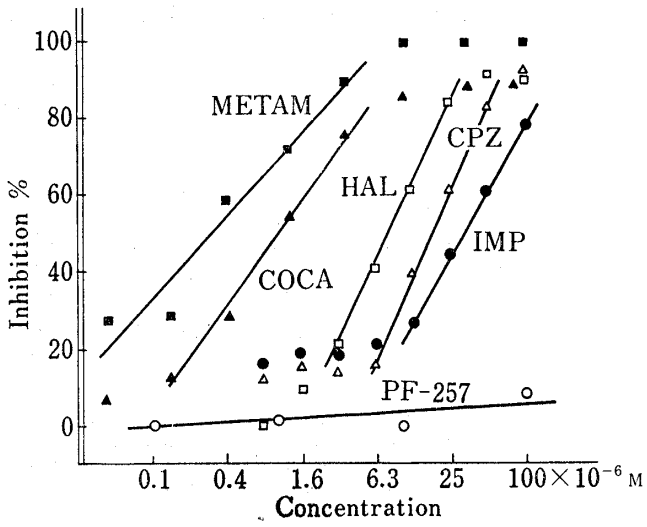


Fig. 3. Effect of PF-257 and Some Reference Drugs on NE Uptake into Nerve Endings in Rat Brain Homogenates

METAM: methamphetamine, COCA: cocaine, IMP: imipramine, CPZ: chlorpromazine, HAL: haloperidol

Potentiation by PF-257 of L-DOPA-Induced Increase in Brain DA

When administered to rats in combination with L-DOPA, PF-257 enhanced the increase in brain DA level caused by the amino acid. Fig. 4 shows the time course of the change in brain DA and L-DOPA levels following L-DOPA alone (100 mg/kg *i.p.*) or in combination with PF-257 (300 mg/kg *p.o.*). After L-DOPA administration (100 mg/kg *i.p.*), the level of brain L-DOPA increased rapidly, reaching a peak at 15 min, and returned to the normal level at 2 hr. Concomitant with the increase in brain L-DOPA level, the level of brain DA also increased. The time course of the change in brain L-DOPA level after administration of the amino acid was not affected by PF-257, while the increase in DA level was pronouncedly enhanced by PF-257.

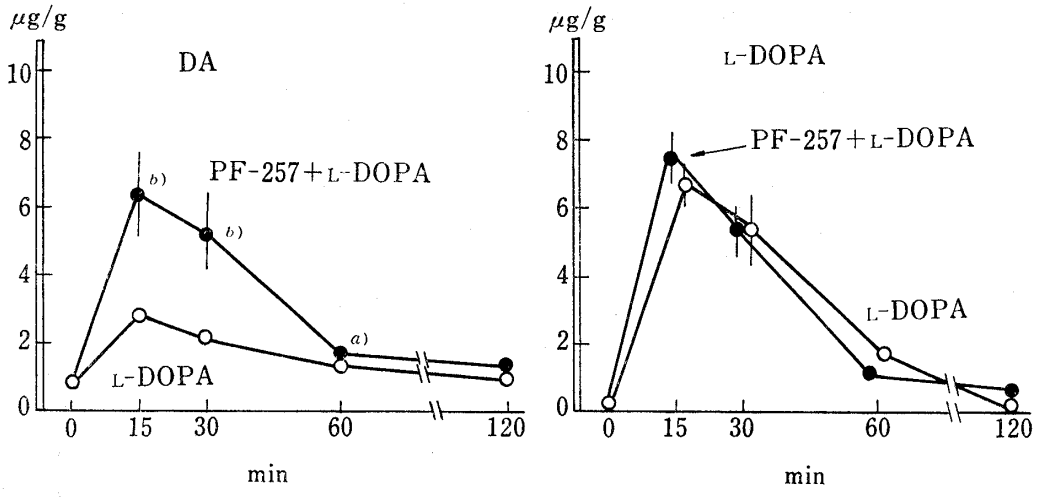


Fig. 4. Effect of PF-257 Combined with L-DOPA on the Levels of Brain DA and L-DOPA in Rats

PF-257 (300 mg/kg *p.o.*) was administered to rats simultaneously with L-DOPA (100 mg/kg *i.p.*). The rats were killed at 15, 30, 60 and 120 min after the drugs. Each point represents the mean ± S.D. (vertical bar) of 5 rats.
a) $p<0.05$, *b*) $p<0.01$ compared with L-DOPA group

Table III shows a dose-response relationship for the L-DOPA potentiating effect of PF-257. At 30—240 mg/kg *p.o.*, PF-257 caused a dose-related enhancement of the increase in brain DA level which was induced by various doses of L-DOPA (50—250 mg/kg *i.p.*).

Table III. Dose-Response Relationship for the L-DOPA Potentiating Effect of PF-257

		Brain DA (ng/g) Dose of PF-257 (mg/kg, *p.o.*)			
		0	30	120	240
Dose of L-DOPA (mg/kg, *i.p.*)	0	864± 61	—	—	—
	50	1480±216	1710± 72	2000± 225[b]	2280± 437[b]
	100	2290± 80	2600±271[a]	3710± 366[b]	4610± 830[b]
	150	3190±205	4840±709[b]	5620± 597[b]	7060± 244[b]
	200	4800±146	5850±404[b]	6570± 609[b]	9590±1450[b]
	250	7350±818	7920±795	10800±1930[b]	11100± 737[b]

Values are means±S.D. of 5 rats. Rats were given PF-257 *p.o.* simultaneously with L-DOPA *i.p.* and killed 30 min later. DA was assayed on the whole brain.
a) significantly different from the group receiving L-DOPA alone at $p<00.5$
b) significantly different from the group receiving L-DOPA alone at $p<0.01$

Effect of PF-257 on L-DOPA-Induced Change in Monoamine Levels in Various Parts of the Brain

In order to examine whether there was a regional difference in the enhancement of L-DOPA-induced brain DA increase by PF-257 and whether PF-257 would modify the L-DOPA-induced change in brain monoamines other than DA, the levels of NE, DA and 5-HT were simultaneously measured in several parts of rat brains after administration of L-DOPA alone or in combination with PF-257.

Groups of rats were given L-DOPA 100 mg/kg *i.p.* alone or together with PF-257 300 mg/kg *p.o.* and they were killed 1 hr later. The concentrations of the three monoamines were simultaneously determined in the dien- and mesencephalon, cerebral cortex and corpus striatum. The results are shown in Table IV. In the rats receiving L-DOPA alone, the level of DA in all the three brain parts was significantly increased with a concomitant decrease in 5-HT level in the same regions. These changes in DA and 5-HT levels were accelerated,

Table IV. Effect of PF-257 on L-DOPA-Induced Changes in Monoamine Levels in Various Parts of Rat Brain

Brain region	Drugs	Dose (mg/kg)	Monoamines (ng/g) NE	DA	5-HT
Diencephalon plus mesencephalon	normal	—	573±58	0	538±119
	L-DOPA	100 *i.p.*	479±46[a]	442± 262[b]	386± 45
	L-DOPA + PF-257	100 *i.p.* + 300 *p.o.*	542±75	1266± 363[b,c]	247± 45[a,d]
Cerebral cortex	normal	—	249±30	108± 66	311± 22
	L-DOPA	100 *i.p.*	252±29	868± 265[b]	252± 7[b]
	L-DOPA + PF-257	100 *i.p.* + 300 *p.o.*	247±41	1850± 275[b,c]	192± 46[b,c]
Corpus striatum	normal	—	148±25	5110± 856	47± 21
	L-DOPA	100 *i.p.*	218±59	6720± 507[a]	0
	L-DOPA + PF-257	100 *i.p.* + 300 *p.o.*	316±25[b,c]	10900±1970[b,d]	0

Values are means ±S.D. of 4 rats. PF-257 was given to rats simultaneously with L-DOPA and the rats were killed 1 hr after the drugs.
a) significantly different from normal at $p<0.05$
b) significantly different from normal at $p<0.01$
c) significantly different from L-DOPA group at $p<0.05$
d) significantly different from L-DOPA group at $p<0.01$

in all brain regions, by administration of PF-257. Particularly, the elevation of striatal DA was most pronouncedly enhanced by PF-257, as can be judged by the net increase per g tissue. In general, however, the enhancement by PF-257 of the change in DA and 5-HT showed no peculiar regional difference in quality. In contrast to DA or 5-HT, little change was observed in the NE level of these brain parts following L-DOPA. Moreover, administration of PF-257 in addition to L-DOPA caused no effect on the NE level of dien- plus mesencephalon and cerebral cortex, though the increase of NE in the striatum was slightly accelerated.

Effect on Brain Enzymes Responsible for Monoamine Metabolism

PF-257 was devoid of activity to inhibit, *in vitro*, L-DOPA decarboxylase which was derived from brain, liver or kidney of rats, as shown in Table V. α-*m*-DOPA and hydroxylamine which are well-known L-DOPA decarboxylase inhibitors showed a concentration-dependent inhibition of the enzyme. It is of interest that pyrogallol, a catechol-O-methyltransferase inhibitor, also inhibited the enzyme. Moreover, PF-257 did not inhibit the enzyme

TABLE V. Effect of PF-257 on L-DOPA Decarboxylase Activity *in Vitro*

Drugs	Concentration (M)	Inhibition %		
		Brain	Liver	Kidney
Control	—	0	0	0
PF-257	10^{-3}	0	0	0
	10^{-4}	0	0	0
	10^{-5}	0	0	0
α-m-DOPA	10^{-3}	58.3	61.2	76.9
	10^{-4}	22.1	18.9	28.8
	10^{-5}	0	7.4	2.7
Hydroxylamine	10^{-3}	98.8	96.8	98.3
	10^{-4}	75.9	88.1	88.5
	10^{-5}	44.4	43.3	20.0
Pyrogallol	10^{-3}	81.7	85.8	82.6
	10^{-4}	57.6	64.2	54.3
	10^{-5}	27.2	26.7	26.4

Five % homogenates of brain, liver, and kidney of rats were used as enzyme sourses.

TABLE VI. Effect of PF-257 on L-DOPA Decarboxylase Activity *in Vivo*

Treatment	DA formed (μmole/g wet tissue/hr)	
	Brain	Kidney
PF-257 300 mg/kg, *p.o.*	2.14±0.26	62.7±6.56
α-m-DOPA 100 mg/kg, *i.p.*	1.77±0.16[a]	50.9±6.18[b]
Normal	2.06±0.21	68.0±5.86

Values are means±S.D. of 5 rats. Rats were given PF-257 *p.o.* or α-*m*-DOPA *i.p.* and sacrificed 1 hr later. The activity of L-DOPA decarboxylase was assayed on brain and kidney homogenates of the rats.
a) significantly different from normal at $p<0.05$
b) significantly different from normal at $p<0.01$

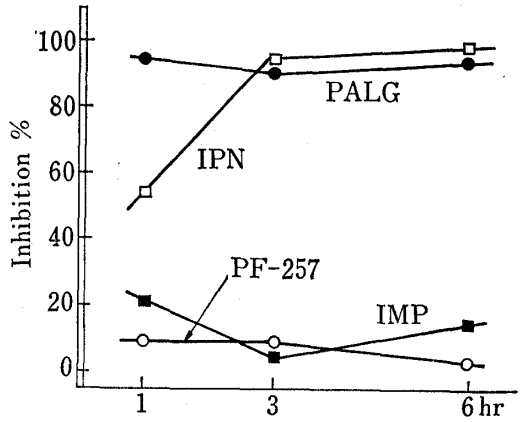


Fig. 5. Effect of PF-257 on Brain Monoamine Oxidase

PF-257 (300 mg/kg), imipramine (IMP, 100mg/kg), iproniazid (IPN, 100 mg/kg) and pargyline (PALG 100 mg/kg) were given to rats *p.o.* The rats were killed 1, 3, and 6 hr after the drugs and brain monoamine oxidase activity was assayed *in vitro*. Each point represents the mean of 5 rats.

activity in the brain and kidney *in vivo* even at a high dose of 300 mg/kg *p.o.* (Table VI). In the same experiment, α-*m*-DOPA 100 mg/kg *i.p.* significantly inhibited the enzyme of both brain and kidney.

The activity of brain monoamine oxidase was not affected by PF-257 *in vivo*; even at a dose as high as 300 mg/kg *p.o.* of the compound, there was no significant inhibition of the enzyme activity over the period of 1—6 hr after its administration, in contrast to the strong and sustained inhibition by pargyline and iproniazid, the typical monoamine oxidase inhibitors (Fig. 5).

Furthermore, PF-257 did not possess the activity to inhibit catechol-O-methyltransferase in the brain and peripheral tissues, though the data are not presented here.

Thus, it appears that the change in brain monoamine levels induced by PF-257 alone or in combination with L-DOPA is not due to its effects on the enzymes responsible for the metabolism of the brain biogenic monoamines.

Table VII. Effect of PF-257 on α-MpT-Induced Disappearance of Brain Monoamines (Dose-Response Relationship)

Treatment	Dose (mg/kg)	Brain monoamines (ng/g)	
		NE	DA
α-MpT (*i.p.*)	250	239±17	482±46
PF-257 (*p.o.*)	10	261±39	479±95
+	30	316± 9[b]	523±34
α-MpT 250 mg/kg, *i.p.*	100	337±44[b]	611±91[a]
	300	386±45[b]	715±77[b]
Normal		402±20	971±49

Values are means±S.D. of 5 rats. Rats were given PF-257 *p.o.* 1 hr prior to α-MpT 250 mg/kg *i.p.* and they were sacrificed 2 hr after α-MpT.
a) significantly different from α-MpT group at $p<0.05$
b) significantly different from α-MpT group at $p<0.01$

Table VIII. Effect of Reference Drugs on α-MpT-Induced Disappearance of Brain Monoamines

Treatment	Dose (mg/kg)	Brain monoamines (ng/g)	
		NE	DA
α-MpT (*i.p.*)	250	239±17	482± 46
Imipramine (*p.o.*)	10	269±26	499± 54
+	30	285±16[b]	565± 13[a]
α-MpT	100	270±13[a]	512± 60
Pargyline (*p.o.*)	10	254±24	524± 12
+	30	288±34[a]	558± 82
α-MpT	100	351±47[b]	728±119[b]
Chlorpromazine (*p.o.*)	10	228±46	371± 44[b]
+	30	228±21	405± 39[b]
α-MpT	100	246±53	384± 43[b]
Haloperidol (*p.o.*)	10	222±22	271± 18[b]
+	30	194±14[b]	260± 24[b]
α-MpT	100	177±17[b]	241± 31[b]
Normal		402±20	971± 49

Values are means ±S.D. of 5 rats. Rats were given drugs *p.o.* 1 hr prior to α-MpT 250 mg/kg *i.p.* and they were sacrificed 2 hr after α-MpT.
a) significantly different from α-MpT group at $p<0.05$
b) significantly different from α-MpT group at $p<0.01$

Effect on the Disappearance of Brain Catecholamines Caused by α-MpT

The effect of PF-257 and reference drugs on the turnover of brain catecholamines was studied by measuring the decline of brain catecholamine levels after tyrosine hydroxylase inhibition with α-MpT. The results are shown in Tables VII and VIII. The levels of brain NE and DA decreased exponentially with time after an *i.p.* injection of α-MpT 250 mg/kg (preliminary experiment). Two hr after the injection, the levels of brain NE and DA were reduced to approximately one-half their initial values as shown in Tables VII and VIII. Oral administration of PF-257 1 hr prior to α-MpT reduced the decline of brain NE and DA levels at doses higher than 30 mg/kg, suggesting its activity to retard catecholamine turnover in the brain (Table VIII). Similar results were obtained with pargyline (Table VIII), as expected from its mode of action. The results of other reference drugs in the same experiment are shown in Table VIII. Imipramine caused a selective retardation of the decline of brain NE. Chlorpromazine and haloperidol, the representatives of phenothiazine and butyrophenone types of neuroleptic drugs respectively, enhanced the decline of DA.

Effect of PF-257 on the Levels of MOPEG-SO_4, HVA and 5-HIAA in the Brain

As shown in Table IX, administration of PF-257 resulted in a dose-related reduction in the levels of brain MOPEG-SO_4 and HVA, major metabolites of NE and DA, respectively, in the rat brain. However, the level of brain 5-HIAA, a major metabolite of 5-HT, was scarcely affected.

Table IX. Effect of PF-257 on the Levels of MOPEG-SO_4, HVA, and 5-HIAA in the Whole Rat Brain

Treatment	Dose (mg/kg, *p.o.*)	Brain acid metabolites (ng/g)		
		MOPEG-SO_4	HVA	5-HIAA
Control	saline	140±11	40± 9	228±18
PF-257	12.5	144± 8	31±10	198±13[a]
	25	126± 6[a]	26± 5[a]	213±14
	50	114± 5[b]	22± 7[b]	209± 9
	100	95±12[b]	21±10[a]	232±13
	200	88± 5[b]	16±10[b]	239± 8

Values are means±S.D. of 5 rats. Rats were given PF-257 *p.o.* and sacrificed either 2 or 6 hr later. Brain HVA and 5-HIAA were simultaneously determined on the rats killed at 2 hr and MOPEG-SO_4 on the rats killed at 6 hr.
a) significantly different from control at $p<0,05$
b) significantly different from control at $p<0.01$

Table X. Effect of PF-257 on Chlorpromazine-Induced Increase in Brain HVA Levels

Treatment		Brain HVA (ng/g)
Normal		46± 5
Chlorpromazine	4 mg/kg, *i.p.*	221±26
+PF-257	30 min before	125±54[a]
(100 mg/kg, *p.o.*)	30 min later	99±21[a]
Chlorpromazine	10 mg/kg, *i.p.*	265±35
+PF-257	30 min before	166±57[b]
(100 mg/kg, *p.o.*)	30 min later	177±56[b]

Values are means±S.D. of 5 rats. PF-257 was given to rats at 100 mg/kg *p.o.* 30 min before or after chlorpromazine 4 or 10 mg/kg *i.p.* The rats were killed 90 min after chlorpromazine.
a) significantly different from the rats receiving chlorpromazine alone at $p<0.05$
b) significantly different from the rats receiving chlorpromazine alone at $p<0.01$

Antagonism of PF-257 to Chlorpromazine with Respect to Brain HVA Level

PF-257 attenuated the increase in brain HVA level caused by chlorpromazine. As shown in Table X, the marked increase in brain HVA level which occurred 1 hr following either 4 or 10 mg/kg *i.p.* of chlorpromazine was significantly reduced by PF-257 100 mg/kg *p.o.* which was administered 30 min prior to or 30 min after chlorpromazine.

Discussion

In several pharmacological tests with experimental animals, PF-257 has shown the properties suggestive of an antidepressant activity.[3] These include a reversal[10–12] of the effect of reserpine in rodents, a potentiation[13,14] of catecholamine effects on the nictitating membrane in cats, a suppression[15,16] of the mouse-killing behaviour of olfactory-ablated rats and so on. Such properties are characteristic of the clinically efficacious tricyclic antidepressant drugs, and also in part, of another class of effective antidepressants, monoamine oxidase inhibitors.

Many lines of biochemical evidence have suggested that the pharmacological and clinical effects of the two types of antidepressants are brought about through potentiation[17] of catecholamines or 5-HT at their receptors within the brain. A number of experiments have demonstrated that imipramine inhibits the uptake[18–20] of NE into presynaptic neurons from which it is released in response to nerve impulses, thereby enhancing the action of NE at the receptors, and that monoamine oxidase inhibitors cause an increase in the levels[20] of brain catecholamines or 5-HT, resulting in a spill-over[21] of these monoamines onto the receptor sites.

The close similarity of PF-257 to tricyclic antidepressants and monoamine oxidase inhibitors in the pharmacological properties led us to investigate the effects of this compound on NE uptake and on monoamine oxidase activity in the brain. In the *in vitro* experiment where the effect on NE uptake was examined using ^{3}H–NE, PF-257 was found to be completely devoid of the activity to inhibit the incorporation of ^{3}H–NE into nerve endings of brain homogenates (Fig. 3). This is in marked contrast to the results of the reference drugs, imipramine, methamphetamine, cocaine, *etc.* which inhibited the NE uptake potently. It is also evident that PF-257 lacks the activity to inhibit monoamine oxidase, since it did not affect the enzyme activity in the brain at a dose as high as 300 mg/kg *p.o.*, in contrast to the complete inhibition by the typical monoamine oxidase inhibitors, pargyline and iproniazid (Fig. 5). Thus, it appears that PF-257 differs from either of the two classes of antidepressant drugs in the underlying mode of action.

10) S. Garattini and A. Jori, "Antidepressant Drugs," ed. by S. Garattini and M.N.G. Dukes, Excerpta Medica, Amsterdam, 1966, p. 179.
11) P.S.J. Spencer, "Antidepressant Drugs," ed. by S. Garattini and M.N.G. Dukes, Excerpta Medica, Amsterdam, 1966, p. 194.
12) J. Metys and J. Metysova, "Antidepressant Drugs," ed. by S. Garattini and M.N.G. Dukes, Excerpta Medica, Amsterdam, 1966, p. 255.
13) E.B. Sigg, *Canad. Psychiat. Ass. J.*, **4**, 75 (1959).
14) E.B. Sigg, L. Sofler, and L. Gyermek, *J. Pharmacol. exp. Ther.*, **142**, 13 (1963).
15) S. Ueki, S. Watanabe, and M. Inoue, *Jap. J. EEG. EMG.*, **1**, 113 (1972). (in Japanese).
16) S. Ueki, S. Nurimoto, and N. Ogawa, *Folia Psychiatrica et Neurologica Japonica*, **26**, 245 (1972).
17) J.J. Schildkraut and S.S. Kety, *Science*, **156**, 21 (1967).
18) J. Glowinski and J. Axelrod, *Nature, Lond.*, **204**, 1318 (1964).
19) U.S.v. Euler, "Handbook of Experimental Pharmacology," ed. by H. Blaschko and E. Muscholl, Springer-Verlag, Berlin, 1972, p. 187.
20) E. Marley and J.D. Stephenson, "Handbook of Experimental Pharmacology," ed. by H. Blaschko and E. Muscholl, Springer-Verlag, Berlin, 1972, p. 463.
21) S. Spector, C.W. Hirsch, and B.B. Brodie, *Intern. J. Neuropharmacol.*, **2**, 81 (1963).

Administration of a large dose (300 mg/kg *p.o.*) of PF-257 to rats resulted in a selective increase in brain NE level without affecting the level of DA or 5-HT (Table I, II). A similar change in brain NE level occurred at lower doses with its repeated administration (Fig. 1). This effect of PF-257 on brain monoamine levels differs from the properties of monoamine oxidase inhibitors since the latter drugs can cause, both by single and by repeated administration, an increase not only in NE level but also in DA and 5-HT levels in the brain of this species. As opposed to the effect of PF-257, imipramine caused a selective decrease in brain NE level by repeated administration (Fig. 1), although it was without effect by a single dose. Such a change in brain NE level with repeated administration of imipramine or other tricyclic antidepressants has been previously reported,[22,23] but the significance or mechanism has not been elucidated.

Reserpine is known to cause marked depletion of brain monoamines as a result of its interference with amine uptake into storage vesicles, and this action is believed to be the cause of the behavioural changes induced by the alkaloid. Though it is a well-known fact that tricyclic antidepressants can potently antagonize the behavioural symptoms induced by reserpine, there have been conflicting data[10,20,23] as to whether these drugs can also prevent the depletion of brain monoamines. In the present experiment, imipramine was found to rather enhance the reserpine-induced depletion of brain NE (Fig. 2). In contrast, PF-257 could reverse the depletion of brain NE induced by reserpine. This effect of PF-257 was reminiscent of the property of monoamine oxidase inhibitors. At the same time, however, there was a distinct difference between PF-257 and monoamine oxidase inhibitors in this regard; the antagonism of the former compound was specific for NE while pargyline, a monoamine oxidase inhibitor, was able to antagonize the depletion of DA and 5-HT as well as of NE (Fig. 2).

Numerous neurochemical and neuropharmacological studies have demonstrated that functional availability of catecholamines at their receptor sites would control, through a feedback mechanism, the activity of presynaptic adrenergic neurons, thereby causing a change in the turnover of the amines.[24,25] Neuroleptic drugs have been known to increase the turnover of catecholamines, especially that of DA, in the brain. This is explained on the basis of compensatory release of DA from presynaptic DA neurons as a result of blockade of DA receptors by the drugs, *i.e.*, a loss of functioning of the transmitter at the receptor sites. In contrast, tricyclic antidepressants have been known to decrease the turnover of brain NE and 5-HT.[26,27] This effect of tricyclic antidepressants has been considered to result from a decreased activity of presynaptic NE or 5-HT neurons which is elicited, *via* a feedback mechanism, by an enhanced function of NE or 5-HT at the receptors due to the inhibition of intraneuronal reuptake of these transmitters.[28,29] The presynaptic reuptake of catecholamines and 5-HT is proved to be the major mechanism for terminating the action of the monoamine transmitters released in response to impulses. Our results for the effect of imipramine, chlorpromazine and haloperidol on catecholamine turnover in the brain are in good agreement with the above mentioned concept, as revealed by the changes in the rate of decline of brain NE or DA level by these drugs following α-MpT, a tyrosine hydroxylase inhibitor (Table VIII). The decreased turnover of brain NE and DA after pargyline (Table VIII) appears a matter

22) J.J. Schildkraut, A. Winokur, and C.W. Applegate, *Science*, **168**, 867 (1970).
23) S. Roffler-Tarlov, *Biochem. Pharmacol.*, **24**, 1321 (1975).
24) A. Carlsson and M. Lindqvist, *Acta pharmacol. et toxicol.*, **20**, 140 (1963).
25) H. Nybäck, F.-A. Wiesel, and G. Sedvall, "Frontier in Catecholamine Research," ed. by E. Usdin and S.H. Snyder, Pergamon Press Inc., New York, 1973, p. 601.
26) H. Corrodi and K. Fuxe, *European J. Pharmacol.*, **7**, 56 (1969).
27) J. Schubert, H. Nybäck, and G. Sedvall, *J. Pharm. Pharmacol.*, **22**, 136 (1970).
28) M.H. Sheard, A. Zolovic, and G.K. Aghajanian, *Brain Res.*, **43**, 690 (1972).
29) H.V. Nybäck, J.R. Walters, G.K. Aghajanian, and R.H. Roth, *European J. Pharmacol.*, **32**, 302 (1975).

of course in view of its mode of action. When PF-257 was tested for its effect on the decline of brain catecholamines induced by α-MpT, the compound was found to retard the turnover of both NE and DA, as shown in Table VII.

In agreement with the above results, PF-257 caused a decrease in the levels of brain MOPEG-SO_4 and HVA, major metabolites of NE and DA in the rat brain, respectively (Table IX). Apomorphine which mimics the effect of DA at DA receptors is reported to decrease the level of brain HVA[30,31] and clonidine, a centrally acting NE receptor agonist, reduces the concentration of brain MOPEG-SO_4.[32] The decreased levels of brain HVA and MOPEG-SO_4 after PF-257, therefore, suggest that the activity of both DA and NE receptors would be enhanced by the compound, either directly by its own action or indirectly through a facilitation of the function of DA and NE.

The level of brain 5-HIAA, a main metabolite of 5-HT, however, was not affected by PF-257 (Table IX). This may imply that PF-257 can neither modify the action of 5-HT at receptors nor does it possess the activity to mimic the function of 5-HT.

PF-257 was found to potently enhance the increase in brain DA level when combined with L-DOPA (Fig. 4, Table III). The level of brain L-DOPA, however, was not altered by PF-257 (Fig. 4). The latter fact is consistent with the result that PF-257 did not inhibit L-DOPA decarboxylase in the brain and peripheral organs both *in vitro* and *in vivo* (Tables V, VI). Inhibition of L-DOPA decarboxylase in the peripheral organs and not in the brain is known to result in an enhancement of brain DA level following L-DOPA.[33] This is due to an increased amount of the amino acid entering the brain tissue.[20] The examples of the drugs possessing such activity are Ro-4-4602[34] and MK 486.[35] The lack of enhancement of brain L-DOPA level with PF-257 excludes the possibility that the compound belongs to this class of drugs.

In the physiological state, DA in the brain is distributed, as a transmitter, in specific areas of the structure such as the corpus striatum, substantia nigra, *etc.*, while the enzyme L-DOPA decarboxylase which converts L-DOPA to DA is widely distributed in various parts of the brain. It is significant, therefore, to examine whether the enhancement by PF-257 of brain DA level following L-DOPA takes place in specific regions of the brain or not. Qualitatively, no regional specificity was observed for the effect of PF-257 among the three brain parts, *i.e.*, cerebral cortex, corpus striatum and dien- and mesencephalon (Table IV). Quantitatively, however, there occurred a more marked enhancement of DA level in corpus striatum, a region rich in DA nerve terminals, than in other parts of brain (Table IV). The decrease in 5-HT level in some regions of brain after L-DOPA and its enhancement by PF-257 (Table IV) seem to be caused by a displacement[36] of 5-HT from its storage sites by large amounts of DA formed from L-DOPA by the action of non-specifically distributed L-DOPA decarboxylase.

The marked increase in brain HVA concentration after chlorpromazine was antagonized by PF-257 irrespective of whether it is administered before or after the neuroleptic drug (Table X). These results indicate that PF-257 has an ability to suppress the release of DA which is caused, through a feedback mechanism, by the blockade of DA receptors.

As a conclusion, it seems that the suppression of catecholamine release from adrenergic presynaptic neurons in the brain may be a fundamental property of PF-257, since all of the biochemical effects of this compound presented in this paper can be consistently explained on the basis of this action.

30) B.-E. Roos, *J. Pharm. Pharmacol.*, **21**, 264 (1969).
31) LAL. Samarthji, T.L. Sourkes, K. Missala, and G. Belendiuk, *European J. Pharmacol.*, **20**, 71 (1972).
32) C. Braestrup, *J. Pharm. Pharmacol.*, **20**, 71 (1972).
33) M. Sandler, "Handbook of Experimental Pharmacology," ed. by H. Blaschko and E. Muscholl, Springer-Verlag, Berlin, 1972, p. 845.
34) W.P. Burkard, K.F. Gey, and A. Pletscher, *Experientia* (Basel), **18**, 411 (1962).
35) C.C. Porter, L.S. Watson, D.C. Titus, J.A. Totaro, and S.S. Byer, *Biochem. Pharmacol.*, **11**, 1067 (1962).
36) K.Y. Ng, T.N. Chase, R.W. Colburn, and I.J. Kopin, *Science*, **170**, 76 (1970).

This suppression of catecholamine release by PF-257 is probably due to its action to enhance the activity of postsynaptic adrenergic receptors which may causally be related with various pharmacological effects of PF-257 in animal tests. It remains, however, to be clarified whether the enhancement of adrenergic receptors by PF-257 is brought about directly by the compound itself or indirectly through the action of endogenous catecholamines, or by other unknown mechanism.