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BIOCHEMICAL CHARACTERISTICS OF A POTENTIAL ANTIDEPRESSANT, 2-(7-INDENYLOXYMETHYL)MORPHOLINE HYDROCHLORIDE (YM-08054-1)

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Abstract—The effects of potential antidepressant, 2-(7-indenyloxymethyl)morpholine hydrochloride (YM-08054-1), on uptake, release and oxidative deamination of catecholamines and serotonin [5-hydroxytryptamine(5-HT)] were studied *in vitro* with rat brain, and compared with the effects of several tricyclic antidepressants and viloxazine, a structural analogue of YM-08054-1. YM-08054-1, at concentrations of less than 1 μM, considerably inhibited 5-HT uptake by synaptosomes from rat whole brain as well as noradrenaline (NA) uptake by synaptosomes from rat hippocampus, similar to amitriptyline. Viloxazine and iprindole were weak inhibitors in both uptake reactions. In the release of either [^{14}C]-5-HT from [^{14}C]-5-HT-prelabeled hypothalamic slices or [^{14}C]NA from [^{14}C]NA-prelabeled hippocampal slices, YM-08054-1 and amitriptyline were much less potent than such typical potent releasers as methamphetamine and tyramine. YM-08054-1 was also found to be a weak inhibitor of both type A and type B monoamine oxidases, whereas the tricyclic antidepressants preferentially inhibited the type B enzyme. These biochemical results indicate that YM-08054-1 may be an amitriptyline-like antidepressant, but distinct from viloxazine.

The drugs which are being used currently in the management of depression are mainly inhibitors of MAO,* a group of tricyclic compounds, and psychomotor stimulants [1]. Among these chemical groups, the inhibitors of monoamine oxidase have been rarely used because of their side effects. The tricyclic compounds have been widely used as antidepressants, although they also have some clinically adverse effects related to autonomic nervous dysfunction, e.g. dryness of the mouth, constipation, urinary retention and postural hypotension in man [2]. Moreover, 2–3 weeks are commonly necessary for the onset of an antidepressant response after administration of tricyclic compounds or MAOI is initiated [1, 2,]. Thus, it is important to develop antidepressants which lack these adverse effects and which have a rapid onset of action.

During the course of screening a series of indenyloxymethylmorpholines structurally unrelated to the tricyclic compounds, *dl*-2-(7-indenyloxymethyl)morpholine hydrochloride (YM-08054-1; Fig. 1) was found to be particularly effective in a variety of important pharmacological tests predictive of antidepressant activity such as the potentiation of methamphetamine-induced stereotypy, the potentiation of responses to biogenic amines, and the inhibition of reserpine-induced hypothermia [3, 4]. YM-08054-1 was also characterized by the lack of anticholinergic activity [4].

The present paper describes the potent inhibitory effects of YM-08054-1 on the synaptosomal uptake of serotonin and noradrenaline. The effects of YM-08054-1 on the release of some biogenic amines and MAO activity are also described.

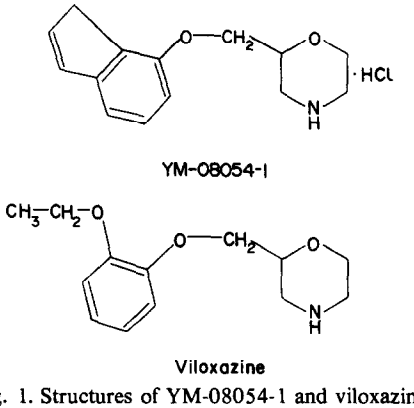

Fig. 1. Structures of YM-08054-1 and viloxazine.

MATERIALS AND METHODS

Chemicals. 5-[2-^{14}C]Hydroxytryptamine (56 mCi/m-mole), *l*-[methylene-^{14}C]noradrenaline *d*-bitartrate (57 mCi/m-mole) and [ethylamine-2-^{14}C]dopamine hydrochloride (50 mCi/m-mole) were purchased from the Radiochemical Centre, Amersham, England. β-[Ethyl-1-^{14}C]phenylethylamine hydrochloride (9.86 mCi/m-mole) was the product of the New England Nuclear Corp., Boston, MA, U.S.A. Clomipramine · HCl, imipramine · HCl and desipramine · HCl were obtained from Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan. Pargyline · HCl and tranylcypromine · HCl were from the Sigma Chemical Co., St. Louis,

* Abbreviations used are; MAO, monoamine oxidase; MAOI, monoamine oxidase inhibitors; 5-HT, 5-hydroxytryptamine; NA, *l*-noradrenaline; DA, dopamine; and β-PEA, β-phenylethylamine.

MO, U.S.A. Iproniazid · H_3PO_4 and tyramine were from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. Methamphetamine · HCl and amitriptyline · HCl were from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan, and Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan, respectively. YM-08054-1, viloxazine · HCl, safrazine · HCl (β-piperonylisopropylhydrazine · HCl), iprindole fumarate and phenelzine acid sulfate were given by Dr. T. Kojima of our laboratories.

Preparation of synaptosomes. Male Wistar rats, 8- to 10-weeks-old, were decapitated, and the whole brain, hippocampus or corpus striatum was immediately dissected, as described by Glowinski and Iversen [5], weighed and homogenized in 4 vol. of cold 0.32 M sucrose in ice with a glass homogenizer fitted with a teflon pestle set for ten up-and-down strokes. The homogenate was diluted to 10% with 0.32 M sucrose and centrifuged at 4° for 10 min at 900 g. The supernatant fluid was further centrifuged for 20 min at 11,500 g. After washing once with cold 0.32 M sucrose the pellet was used as the crude synaptosomes.

Uptake of biogenic amines. The uptake of biogenic amines was determined by the methods of Schacht and Heptner [6], with a slight modification.

The crude synaptosomes were suspended at about 0.6 mg of protein (equivalent to 16 mg wet tissue) per 1 ml of Krebs–Henseleit bicarbonate buffer containing 0.1 mM iproniazid, 11 mM glucose, 1.14 mM ascorbic acid, 0.067 mM EDTA and 1.27 mM calcium chloride. For 5-HT or NA uptake by the whole brain synaptosomes, 2 ml aliquots of the synaptosomal suspension were incubated for 3 min at 37° in a shaking water bath in the presence or absence of test compounds. The uptake reaction was initiated by the addition of 20 μl of [^{14}C]-5-HT or [^{14}C]NA to give a final concentration of 10^{-7} M, continued for 2.5 min, and terminated by quickly cooling the mixture in an ice bath. The incubation mixture was then filtered through a Whatman glass fiber disk GF/F. The disk was washed, dried and transferred to a vial with 10 ml of scintillation fluid consisting of toluene (667 ml), Triton X-100 (333 ml), 2,5-diphenyloxazole (5.5 g) and 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (100 mg) in 1 liter [7]. The radioactivity was measured in a Packard Tricarb scintillation spectrometer (model 3390). For the determination of nonspecific uptake, the incubation was performed at 0° without the addition of the test compounds, as described by Schacht and Heptner [6].

[^{14}C]NA uptake by hippocampal synaptosomes [8] and [^{14}C]DA uptake by the striatal synaptosomes [9, 10] were also determined as described for [^{14}C]-5-HT uptake except for the use of 1 ml of the reaction mixture with about 0.6 mg of protein.

The IC_{50} (the concentration of drugs which inhibits the uptake reaction by 50 per cent) was calculated from the dose-response curves by plotting per cent inhibition on a probit scale as a function of log concentration [11].

Release of biogenic amines. The release of preloaded ^{14}C-amines from brain slices was studied by the perfusion technique described by Raiteri *et al.* [9], with a slight modification. Four slices (0.6 mm in thickness) of hypothalamus (6.0 mg wet tissue), hippocampus (8.5 mg wet tissue) and striatum (8.1 mg wet tissue) were prelabeled by incubating for 20 min at 37° with [^{14}C]-5-HT, [^{14}C]NA and [^{14}C]DA at 10^{-7} M, respectively, in Krebs–Henseleit bicarbonate buffer containing 10 mM glucose, 1.14 mM ascorbic acid, 1.27 mM calcium chloride [12] and 0.1 mM iproniazid. After thoroughly washing with the cold buffer, the slices were placed in a superfusion chamber containing 2 ml of the Krebs–Henseleit bicarbonate buffer with the same composition as described above, and then superfused with the same buffer at 37° at a flow rate of 0.4 ml/min. After 20 min, the superfusion medium was replaced by the same buffer containing the desired concentration of test drug, and the superfusion was further continued for 20 min. Throughout these experiments, the superfusion medium was constantly aerated with 95% O_2–5% CO_2 gas. The superfusate was collected every 5 min directly into liquid scintillation vials and mixed with 15 ml of Bray's scintillation fluid [13]. The radioactivity was determined with a Packard liquid scintillation counter. Since the preloaded radioactivity was not identical among the slices at the onset of perfusion, the radioactivity of superfusate was expressed as the per cent of initial preloaded radioactivity for comparisons of drug potency in releasing biogenic amines.

MAO activity. In the present studies, hypothalamic mitochondria were used as MAO preparations [14]. The substrates were 5-HT and NA for the type A enzyme and β-PEA for the type B MAO [15–17]. MAO activity was determined by the method of Goridis and Neff [18], with a slight modification. Hypothalamic mitochondria equivalent to 30 mg wet tissue were suspended in 1 ml of 0.067 M sodium-potassium phosphate buffer, pH 7.2. The incubation mixture contained 30 μl of the mitochondrial suspension (about 30 μg of protein equivalent to 0.9 mg wet tissue) and various amounts of inhibitors in a total volume of 170 μl of 0.067 M phosphate buffer, pH 7.2. When [^{14}C]NA was used as a substrate, 0.2 μmole ascorbic acid was added to the incubation. After preincubation for 3 min at 37°, 0.5 nmole of [^{14}C]-5-HT, [^{14}C]NA or [^{14}C]β-PEA was added to initiate the reaction and the mixture was further incubated for 20 min, except for the [^{14}C]β-PEA deamination test where the incubation time was 2.5 min. The reaction was stopped by adding 50 μl of cold 60% perchloric acid. The mixture was placed on a Dowex 50W × 8 column in H^+ form with 200-400 mesh (1.5 × 0.5 cm for 5-HT or 2.0 × 0.5 cm for NA and β-PEA). Deaminated products were eluted directly into vials and the radioactivity was determined in 15 ml of the Triton–toluene scintillation fluid. Under the assay conditions, the recoveries of 5-hydroxyindole-3-acetic acid, *dl*-3, 4-dihydroxymandelic acid and phenylacetic acid were 94.0, 100.0 and 94.9 per cent respectively. The IC_{50} values (the concentrations of drugs needed to inhibit the enzyme activities by 50 per cent) were graphically calculated on logarithmic probability paper.

Protein determination. Protein was determined by the method of Lowry *et al.* [19] with bovine serum albumin as a standard.

RESULTS

Inhibition of uptake. As shown in Fig. 2, the rate of uptake of [^{14}C]-5-HT, [^{14}C]NA or [^{14}C]DA by the

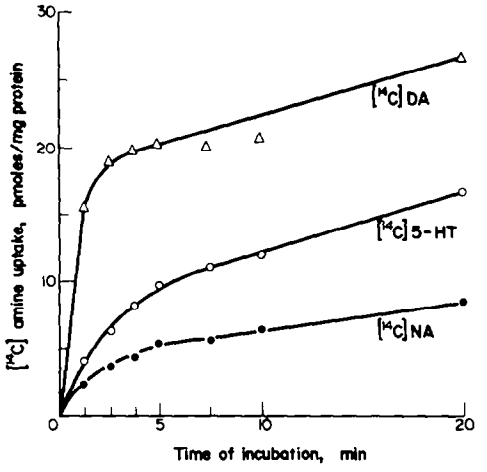

Fig. 2. Time courses of [^{14}C]-5-HT, [^{14}C]NA and [^{14}C]DA accumulation in synaptosomes from whole brain, hippocampus and corpus striatum respectively. For [^{14}C]-5-HT accumulation, synaptosomes of whole brain (about 1.2 mg of protein) were preincubated for 3 min at 37° in 2 ml of Krebs–Henseleit bicarbonate buffer containing 0.1 mM iproniazid, 11 mM glucose, 1.14 mM ascorbic acid, 0.067 mM EDTA and 1.27 mM calcium chloride. The uptake reaction was initiated by addition of 10^{-7} M [^{14}C]-5-HT and terminated by quickly cooling the mixture in an ice bath. The incubation mixture was then filtered through a Whatman glass fiber disk GF/F. The disk was washed, dried and transferred to a vial for the determination of radioactivity. For both [^{14}C]NA uptake by hippocampal synaptosomes and [^{14}C]DA uptake by striatal synaptosomes, the incubation volume was 1 ml with 0.6 mg of protein. The results are means of triplicate experiments.

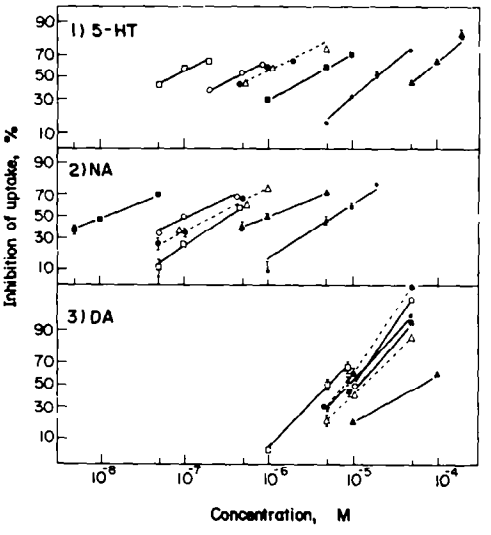

Fig. 3. Uptake of ^{14}C-amines by brain synaptosomes in the presence of various concentrations of YM-08054-1 and other antidepressants. Uptake of ^{14}C-amines by brain synaptosomes was determined as described in the legend of Fig. 2. The incubation mixtures contained the indicated concentrations of test drugs and were incubated for 2.5 min. The results are the means (per cent inhibition) ± S.E. of six experiments for both 5-HT and NA uptake and of four experiments for DA uptake. The values without a S.E. indicate that the S.E. was less than 2.5 per cent. Key: (---△---) YM-08054-1; (—▲—) viloxazine; (—○—) imipramine; (---●---) amitriptyline; (—□—) clomipramine; (—■—) desipramine; and (—●—) iprindole.

crude synaptosomal preparations was apparently biphasic. The uptake increased rapidly during min 1 and at a continuously decreasing rate up to about 5 min. Thereafter, the rate of uptake was constant for at least 15 min. The Lineweaver–Burk plot of uptake revealed that the V_{max} of striatal DA uptake (31 pmoles/mg of protein/min) was much higher than that of either whole brain 5-HT uptake (7.8 pmoles/mg of protein/min) or hippocampal NA uptake (5.3 pmoles/mg of protein/min).

Under these experimental conditions, YM-08054-1, viloxazine, iprindole and some typical tricyclic antidepressants were examined for their inhibitory actions on synaptosomal uptake of [^{14}C]-5-HT, [^{14}C]NA and [^{14}C]DA (Fig. 3). Uptake of [^{14}C]-5-HT was most intensely inhibited in a dose-dependent manner by clomipramine, with an IC_{50} of 7.3×10^{-8} M. Imipramine, amitriptyline and YM-08054-1 revealed similar inhibitions, and were about one-tenth as potent as clomipramine. The IC_{50} values of all the drugs tested are

Table 1. The IC_{50} values of ^{14}C-amine uptake by rat brain synaptosomes*

	IC_{50} (M)			
	5-HT	NA		DA
Drugs	(Whole brain)	(Whole brain)	(Hippocampus)	(Striatum)
YM-08054-1	7.1×10^{-7}	3.2×10^{-6}	2.6×10^{-7}	1.4×10^{-5}
Amitriptyline	7.0×10^{-7}	2.9×10^{-6}	2.3×10^{-7}	8.1×10^{-6}
Clomipramine	7.3×10^{-8}	2.4×10^{-6}	3.6×10^{-7}	5.1×10^{-6}
Imipramine	4.2×10^{-7}	5.8×10^{-6}	1.1×10^{-7}	1.0×10^{-5}
Desipramine	3.3×10^{-6}	3.6×10^{-6}	1.2×10^{-8}	1.2×10^{-5}
Iprindole	2.0×10^{-5}	1.3×10^{-5}	6.3×10^{-6}	9.2×10^{-6}
Viloxazine	6.6×10^{-5}	1.9×10^{-5}	1.0×10^{-6}	6.3×10^{-5}

* The inhibition of ^{14}C-amine uptake by rat brain synaptosomes was determined as described in the legend of Fig. 3. The values for IC_{50} (the concentration of drug which inhibits the uptake reaction by 50 per cent) were calculated from logarithmic probability plots of percent inhibition (Fig. 3).

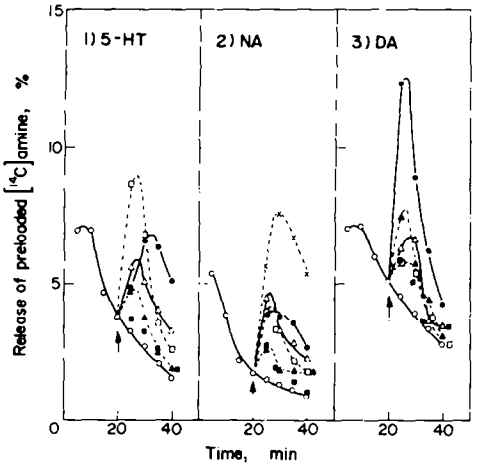

Fig. 4. Effects of methamphetamine, YM-08054-1, viloxazine, amitriptyline and imipramine on ^{14}C-amine release from brain slices. Four slices of hypothalamus, hippocampus and corpus striatum were prelabeled with [^{14}C]-5-HT, [^{14}C]NA and [^{14}C]DA at 10^{-7} M, respectively, in Krebs–Henseleit bicarbonate buffer containing 0.1 mM iproniazid, 10 mM glucose, 1.14 mM ascorbic acid and 1.27 mM calcium chloride. After washing with the buffer, the slices were placed in a superfusion chamber and superfused with the oxygenated buffer at 37°. After 20 min, indicated by an arrow, the superfusion medium was replaced by either indentical medium (controls) or medium containing a test drug at 10^{-5} M, and the superfusion was continued for another 20 min. The radioactivity of the superfusates was measured every 5 min and converted to per cent of the preloaded radioactivity. The results are means of three experiments. Key: (—○—) control; (—●—) methamphetamine; (—△—) YM-08054-1; (---▲---) viloxazine; (--- ---) amitriptyline; (---■---) imipramine; and (--- ×---) tyramine.

depicted in Table 1. Desipramine with an IC_{50} of 3.3×10^{-6} M was the lowest in inhibitory potency among the tricyclic antidepressants tested. It is of interest that viloxazine, a chemical analogue of YM-08054-1, was a far weaker inhibitor of 5-HT uptake than was YM-08054-1.

In contrast, uptake of [^{14}C]NA by hippocampal synaptosomes was most strongly inhibited by desipramine in a dose-dependent manner, with an IC_{50} of 1.2×10^{-8} M. The inhibitions by imipramine, amitriptyline and YM-08054-1 were comparatively similar, and their IC_{50} values were 1.1×10^{-7} M, 2.3×10^{-7} M and 2.6×10^{-7} M respectively (Table 1). Clomipramine, despite being the most potent inhibitor of 5-HT uptake, was the weakest inhibitor of the [^{14}C]NA uptake reaction among the tricyclic antidepressants tested. Viloxazine exhibited a moderate inhibition of NA uptake, and iprindole was the weakest of all the compounds tested. It was noteworthy that, when [^{14}C]NA uptake inhibition was tested with synaptosomes from whole brain, all the drugs appeared to be less active in comparison with the inhibition of hippocampal NA uptake (Table 1). It was also found that all the drugs were rather weak inhibitors of [^{14}C]DA uptake (Table 1). Viloxazine was found to be the least potent of all the drugs tested.

These experiments demonstrate that the inhibitory effects of YM-08054-1 on uptake of the biogenic monoamines are on the whole, very similar to those of amitriptyline.

Stimulation of amine release. Stimulatory effects of YM-08054-1, viloxazine, amitriptyline and imipramine on the release of preloaded biogenic amines, in comparison with such known releasers as methamphetamine and tyramine were investigated. The perfusion

Table 2. Effects of antidepressants on release of preloaded ^{14}C-amines from brain slices*

Drugs	Ratio: Concentration (M) 10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Release of [^{14}C]-5-HT						
Methamphetamine			1.09 ± 0.11	1.18 ± 0.33	2.18 ± 0.21+	3.25 ± 0.57‡
YM-08054-1			1.33 ± 0.21	1.69 ± 0.23§	1.73 ± 0.15‡	1.70 ± 0.24‡
Viloxazine			1.11 ± 0.25	1.21 ± 0.13	1.30 ± 0.29	1.88 ± 0.21‡
Amitriptyline			1.41 ± 0.38	1.47 ± 0.15§	2.16 ± 0.47§	1.99 ± 0.11‡
Imipramine			1.12 ± 0.04§	1.69 ± 0.29§	1.00 ± 0.11	1.41 ± 0.26
Release of [^{14}C]NA						
Tyramine			1.74 ± 0.18§	2.78 ± 0.56‡	4.72 ± 1.28‡	5.58 ± 0.88+
Methamphetamine			1.00 ± 0.18	2.37 ± 0.57‡	2.45 ± 0.36‡	2.27 ± 0.24+
YM-08054-1			1.51 ± 0.35	1.79 ± 0.16‡	2.25 ± 0.47	1.87 ± 0.44
Viloxazine	1.15 ± 0.08	1.36 ± 0.32	2.19 ± 0.25§	1.13 ± 0.20	1.37 ± 0.22	1.66 ± 0.40
Amitriptyline			1.41 ± 0.23	1.05 ± 0.29	2.01 ± 0.31§	2.95 ± 0.58§
Imipramine			1.59 ± 0.42	1.09 ± 0.25	1.40 ± 0.40	1.68 ± 0.26
Release of [^{14}C]DA						
Methamphetamine			1.46 ± 0.37	1.62 ± 0.39	2.07 ± 0.41§	2.42 ± 0.39‡
YM-08054-1			1.37 ± 0.13	1.25 ± 0.15	1.39 ± 0.40	1.26 ± 0.19
Viloxazine			1.12 ± 0.04§	1.15 ± 0.03‡	1.31 ± 0.08‡	1.09 ± 0.03§
Amitriptyline			1.25 ± 0.09	1.10 ± 0.16	1.14 ± 0.09	1.21 ± 0.06§
Imipramine			1.40 ± 0.15§	1.09 ± 0.18	1.16 ± 0.22	1.30 ± 0.21

* Release of preloaded ^{14}C-amines from brain slices in the presence or absence of drugs was determined as described in the legend of Fig. 4. The results are expressed as the ratio of the total released amount of labeled amine in the presence of drug to that in the absence of drug during the entire 20 min period of superfusion. The values are means ± S.E. of three experiments.

+ $P < 0.01$.

‡ $P < 0.05$.

§ $P < 0.10$.

technique, in which no reuptake of released amines occurs, was used for exact determination of the release.

Figure 4 shows the time course of the stimulatory effects of methamphetamine, YM-08054-1, viloxazine, amitriptyline and imipramine at 10^{-5} M on the release of [^{14}C]-5-HT from hypothalamic slices, [^{14}C]NA from hippocampal slices and [^{14}C]DA from striatal slices. The release of [^{14}C]-5-HT was markedly elevated by methamphetamine, amitriptyline and YM-08054-1. In the release of [^{14}C]NA, tyramine was particularly potent, and other drugs such as methamphetamine, YM-08054-1 and amitriptyline were moderately potent. The release of [^{14}C]DA was strikingly stimulated by methamphetamine, but only moderately stimulated by the other agents. Table 2 indicates the effects of various concentrations of drugs on the release of [^{14}C]-amines. Methamphetamine, amitriptyline and YM-08054-1, at concentrations of 10^{-5} M to 10^{-4} M, significantly increased the release of [^{14}C]-5-HT in this order of potency. Imipramine was slightly effective at 10^{-6} M, above this concentration the effect was insignificant. Viloxazine was the least potent of all the drugs tested, and effective only at 10^{-4} M. The release of [^{14}C]NA was greatly stimulated by tyramine and methamphetamine at concentrations of 10^{-6} to 10^{-4} M. YM-08054-1 and viloxazine significantly stimulated the release of [^{14}C]NA only at 10^{-6} M and 10^{-7} M respectively. Imipramine had a very weak effect on [^{14}C]NA release. In the release of [^{14}C]DA, YM-08054-1, viloxazine, amitriptyline and imipramine showed only slight stimulatory effects, in contrast to methamphetamine which exhibited more than 2-fold stimulation.

Inhibition of MAO. Under the experimental conditions, deamination of 5-HT and NA proceeded almost linearly for the first 20 min. On the other hand, the time course of deamination of β-PEA was apparently biphasic. The reaction proceeded linearly for the first 2.5 min, and thereafter at a slower but constant rate. The apparent K_m values for 5-HT deamination under the standard assay conditions were 5.1×10^{-6} M and 5.3×10^{-5} M, as determined by the Lineweaver–Burk analysis, suggesting the existence of two distinct enzymes or of a single enzyme with negative cooperativity. By the same analysis, the apparent K_m values for NA were found to be 5.4×10^{-6} M and 2.2×10^{-4} M, while the K_m for β-PEA was 3.9×10^{-6} M.

Under the standard conditions, YM-08054-1 and some antidepressants were studied for their abilities to inhibit the deamination of 5-HT, NA and β-PEA. The IC_{50} values (the concentrations to inhibit MAO by 50 per cent) were obtained simply from the dose–response curves in Fig. 5 and are presented in Table 3. The inhibitory effect of YM-08054-1 on oxidative deamination of β-PEA was rather weak when compared with tricyclic antidepressants, whereas the compound was as effective in the inhibition of deamination of NA and 5-HT as the tricyclic antidepressants. Viloxazine [20] was the weakest inhibitor of MAO, with any substrate, among the drugs tested. It was also confirmed that phenelzine, tranylcypromine, pargyline and safrazine were all potent MAO inhibitors under these conditions. The nonhydrazine MAOI preferentially inhibited β-PEA deamination, whereas the hydrazine derivatives showed a slight preference for the inhibition of NA and 5-HT deamination. The tricyclic antidepressants tested,

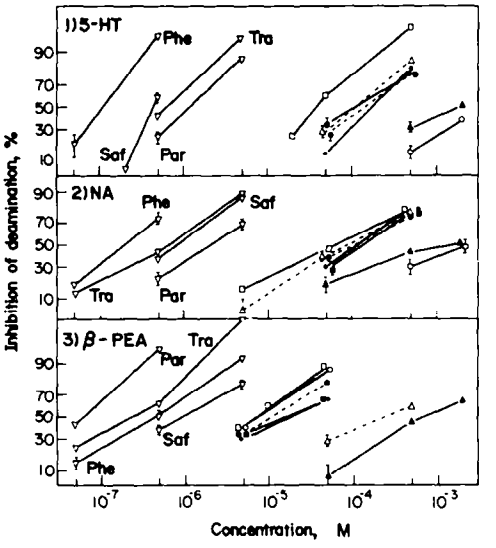

Fig. 5. Oxidative deamination of ^{14}C-amines by hypothalamic mitochondrial MAO in the presence of various concentrations of YM-08054-1 or other antidepressants. The mitochondria (30 μg of protein) were preincubated for 3 min at 37° in a total volume of 170 μl of 0.067 M phosphate buffer, pH 7.2. When [^{14}C]NA was used as a substrate, 0.2 μmole ascorbic acid was present in the incubation. The reaction was initiated by addition of 0.5 nmole [^{14}C]-5-HT, [^{14}C]NA or [^{14}C]-β-PEA and stopped by adding 50 μl of 60% perchloric acid. The incubation time was 20 min except for [^{14}C]-β-PEA deamination where the incubation was carried out for 2.5 min. The mixture was placed on a Dowex 50W × 8 column in H^+ form with 200–400 mesh (1.5 × 0.5 cm for 5-HT or 2.0 × 0.5 cm for NA and β-PEA). Deaminated products were eluted with water and the radioactivity was determined. The results are the means (per cent inhibition) ± S.E. of four to six experiments. The values without a S.E. indicate that the S.E. was less than 3 per cent. Abbreviations: Phe, phenelzine; Saf, safrazine; Par, pargyline; and Tra, tranylcypromine. Key: (---△---) YM-08054-1; (—▲—) viloxazine; (—○—) imipramine; (---●---) amitriptyline; (—□—) clomipramine; (—■—) desipramine; and (—●—) iprindole.

Table 3. The IC_{50} values of ^{14}C-amine deamination by rat hypothalamic mitochondrial MAO*

Drugs	IC_{50} (M) 5-HT	NA	β-PEA
YM-08054-1	1.1×10^{-4}	9.0×10^{-5}	2.6×10^{-4}
Pargyline	1.2×10^{-6}	2.1×10^{-6}	6.4×10^{-8}
Tranylcypromine	6.4×10^{-7}	6.8×10^{-7}	2.6×10^{-7}
Phenelzine	1.1×10^{-7}	2.0×10^{-7}	4.9×10^{-7}
Safrazine	4.5×10^{-7}	8.5×10^{-7}	1.0×10^{-6}
Amitriptyline	1.5×10^{-4}	1.0×10^{-4}	1.1×10^{-5}
Clomipramine	3.9×10^{-5}	6.9×10^{-5}	7.8×10^{-6}
Imipramine	3.6×10^{-3}	2.4×10^{-3}	8.1×10^{-6}
Desipramine	1.1×10^{-4}	1.4×10^{-4}	1.7×10^{-5}
Iprindole	1.8×10^{-4}	1.2×10^{-4}	1.9×10^{-5}
Viloxazine	1.9×10^{-3}	1.6×10^{-3}	7.0×10^{-4}

* ^{14}C-amine deamination by rat hypothalamic mitochondrial MAO was determined as described in the legend of Fig. 5. The values of IC_{50} were calculated from logarithmic probability plots of per cent inhibition (Fig. 5).

like pargyline, showed more potent inhibitory effects on β-PEA deamination than on either 5-HT or NA deamination.

DISCUSSION

It has been suggested that the therapeutic mechanism of antidepressants is closely associated with potentiation of the availability of neurotransmitters to the postsynaptic receptors [21, 22]. At least three distinct mechanisms to increase the availability of neurotransmitters at the synaptic cleft have been suggested for antidepressants currently used, namely, the inhibition of uptake of neurotransmitters, the inhibition of MAO activities, and the potentiation of release of neurotransmitters [1]. It is well known that the clinical effect of tricyclic antidepressants has been generally attributed to the inhibition of 5-HT and NA uptake [21, 22]. The present experiments indicate that YM-08054-1 is particularly potent in the inhibition of both 5-HT and NA uptake by the synaptosomes from rat brain (similar to amitriptyline and dissimilar to such specific NA blockers as desipramine and nisoxetin [23] and to a nonselective blocker, EXP 561 [24]), but rather weak in the stimulation of release of 5-HT and NA from brain slices. Such a weak stimulatory effect on 5-HT release has also been reported with amitriptyline and mianserine (an inhibitor of 5-HT uptake) [9]. Along with the fact that YM-08054-1 was much less potent in the inhibition of MAO than MAO inhibitors, the data described above suggest that YM-08054-1 exerts its pharmacological activities in animals mainly by blocking reuptake of the released 5-HT and NA, thereby increasing the concentration of amines at the synaptic cleft as proposed for the action of tricyclic antidepressants [21, 22].

Regarding the amine selectivity in uptake inhibition, there are some reports indicating that imipramine and amitriptyline tend to inhibit 5-HT uptake more than NA uptake *in vivo* [22, 25–27]. The present experiments in the cell-free system indicate that YM-08054-1 inhibits 5-HT uptake by the synaptosomes from whole brain much more than NA uptake. However, this greater inhibition does not necessarily mean that YM-08054-1 acts selectively on 5-HT uptake in all parts of brain, since the extent of inhibition of synaptosomal NA uptake seems to be dependent on the part of the brain. For instance, all antidepressants tested in the present experiments inhibited NA uptake of hippocampal synaptosomes one order of magnitude more than of whole brain synaptosomes. Our preliminary *ex vivo* experiments, however, have indicated the selective inhibition of hypothalamic synaptosomal 5-HT uptake by YM-08054-1 as compared with NA uptake (unpublished data).

It has been reported recently that antidepressant drugs block stimulation of adenylate cyclase by histamine through a histamine H_2-receptor in homogenates of guinea pig hippocampus [28] and that amitriptyline has greater affinity for the H_2-receptor than does cimetidine [29]. Although the effects of YM-08054-1 on the histamine-sensitive adenylate cyclase in brain have not been examined yet, our preliminary experiments have indicated that, in contrast to cimetidine, both amitriptyline and YM-08054-1 have no effect on the chronotropic response to histamine in isolated guinea pig atria, suggesting no involvement of the H_2-receptor blockade in their antidepressant action.*

There is a great deal of similarity between YM-08054-1 and amitriptyline not only in boichemical, but also in pharmacological characteristics such as antagonistic effects on hypothermia induced by reserpine and potentiation of methamphetamine-induced stereotypy [3, 4]. However, YM-08054-1 can be distinguished from amitriptyline primarily by the pharmacological characteristic that YM-08054-1 is virtually devoid of peripheral anticholinergic activity, a clinically adverse effect especially undesirable in the aged. Although YM-08054-1 and viloxazine, a structural analogue, are potent in the antagonistic effects on hypothermia induced by reserpine in mice and are devoid of peripheral anticholinergic activity, there are a number of dissimilarities between the compounds. YM-08054-1 is a much more potent inhibitor of 5-HT uptake *in vitro* and a much stronger potentiator of behavioral responses of mice to 5-hydroxytryptophane, than is viloxazine [4]. Furthermore, YM-08054-1 potentiates the stereotyped behavior of rats induced by methamphetamine, whereas viloxazine exhibits no such effect. These biochemical and pharmacological properties of YM-08054-1 seem to be novel and distinct from those of other known antidepressants [30].

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