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Characterization of Phentermine and Related Compounds as Monoamine Oxidase (MAO) Inhibitors

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ABSTRACT. Phentermine was shown in the 1970s to inhibit the metabolism of serotonin by monoamine oxidase (MAO), but never was labeled as an MAO inhibitor; hence, it was widely used in combination with fenfluramine, and continues to be used, in violation of their labels, with other serotonin uptake blockers. We examined the effects of phentermine and several other unlabeled MAO inhibitors on MAO activities in rat lung, brain, and liver, and also the interactions of such drugs when administered together. Rat tissues were assayed for MAO-A and -B, using serotonin and β-phenylethylamine as substrates. Phentermine inhibited serotonin-metabolizing (MAO-A) activity in all three tissues with K_i values of 85–88 μM. These potencies were similar to those of the antidepressant MAO inhibitors iproniazid and moclobemide. When phentermine was mixed with other unlabeled reversible MAO inhibitors (e.g. pseudoephedrine, ephedrine, norephedrine; estradiol benzoate), the degree of MAO inhibition was additive. The cardiac valvular lesions and primary pulmonary hypertension that have been reported to be associated with fenfluramine-phentermine use may have resulted from the intermittent concurrent blockage of both serotonin uptake and metabolism. BIOCHEM PHARMACOL **59**;12: 1611–1621, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. phentermine; MAO-A; MAO-B; serotonin; amines; anorectics

The anorexigen fenfluramine and its dextro-isomer dexfenfluramine have been implicated in the development of echocardiographically defined cardiac valvular disease [1, 2]. Initial open-label observations were interpreted as showing that as many as 30% of patients who took one of these drugs would develop such disease [2]; however, subsequent controlled studies indicate that it occurs much less frequently, and in most cases is asymptomatic [3–6].

Dexfenfluramine, which enhances serotonin-mediated neurotransmission by blocking serotonin reuptake and, through its nordexfenfluramine metabolite, directly releasing the transmitter and activating postsynaptic serotonin-$_{2A}$ and -$_{2C}$ receptors [7], had been used for many years outside the United States, without apparent association with valvular lesions; similarly, fenfluramine had been prescribed for three decades without generating known valvular pathology. Hence, we wondered whether the occurrence of apparent valve damage in the United States might have been related partly to the widespread, peculiarly American practice of taking fenfluramine along with another anorexigen, phentermine [8]. Phentermine, like fenfluramine but not dexfenfluramine, is a generic drug; it is usually described as a "sympathomimetic amine," implying a primary action on noradrenergic neurotransmission [9]. We examined its effects in rats, and found that it releases brain dopamine [10], so we conducted additional studies to see whether it also raised blood dopamine levels in humans [11].

After a single oral therapeutic phentermine dose (15 or 30 mg), plasma dopamine levels did rise significantly; however, levels of serotonin within blood platelets increased by an even greater proportion [11]. Since there was no concurrent rise in plasma serotonin levels, the increase in platelet serotonin most likely reflected a slowed degradation of the amine, and not an increase in its uptake from the plasma. (Platelets are unable to synthesize serotonin.) This degradation is catalyzed in platelets by an enzyme that shares properties with the type of MAO‖ (MAO-B) that, in other organs, preferentially metabolizes β-phenylethylamine and related amines but not serotonin [12, 13]. A literature search then revealed that the ability of phentermine to inhibit MAO had been described in the 1970s [14, 15], but, surprisingly, this property was not mentioned and still is not mentioned on the label of the drug [9]. Thus, even though the labels for fenfluramines and other serotonin reuptake blockers instructed users never to take these compounds in combination with a MAO inhibitor [9], it

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‖ *Abbreviations*: MAO, monoamine oxidase; and fen-phen, fenfluramine-phentermine.

had been possible for millions of Americans to take the "fen-phen" combination.

In early studies that described the inhibition of MAO by phentermine, the characteristics of this inhibition (e.g. its selectivity for MAO-A and MAO-B; its reversibility) were not investigated fully. To understand the mechanism of the pharmacological effects of phentermine and its interactions with other drugs, we have now characterized its inhibition of MAO in the rat brain and liver, and in the lung, the site at which most plasma serotonin is destroyed [16]. The drug inhibits the enzyme in all three tissues, with a potency similar to those of the antidepressant MAO inhibitors iproniazid and moclobemide. Moreover, numerous other widely used drugs also are unlabeled MAO inhibitors, and the effects of administering two or more of them together can be additive.

MATERIALS AND METHODS

Determination of MAO-A and MAO-B Activities

Male Sprague–Dawley rats (Taconic) weighing 250–275 g were decapitated, and their lungs, livers, and brains were removed, rinsed with saline solution (0.9% NaCl), dried on filter paper, weighed, and homogenized in 10 (lung) or 20 (liver and brain) vol. of 0.1 M sodium phosphate buffer. MAO-A and MAO-B activities were assayed as described previously by Lyles and Callingham [17] with some minor modifications. (We assume that the serotonin-metabolizing enzyme in these tissues, unlike that in human platelets, is MAO-A; hence, we use this term and serotonin-metabolizing activity, or MAO-B and β-phenylethylamine-metabolizing activity, interchangeably.) Briefly, 25 μL of homogenate was preincubated in a round-bottom glass culture tube (10–13 × 75 mm) for 20 min at 37° in the absence or presence of various concentrations (typically 6–8 different concentrations) of a compound to be tested, in a total volume of 50 μL. Following preincubation, the reaction was started by adding 50 μL of [^{14}C]serotonin (final concentration 125 μM in experiments for determining IC_{50} values or 32–2000 μM for kinetic studies) or [^{14}C]β-phenylethylamine (final concentration 8 μM in experiments for determining IC_{50} values or 1–128 μM for kinetic studies). Incubation times were 5 or 10 min for studies on [^{14}C]serotonin and 2 min for those on [^{14}C]β-phenylethylamine. Reactions were stopped by the addition of 25 μL of 3 N HC1. Deaminated metabolites were extracted by vigorous vortexing for 20–30 sec with 1 mL of toluene: ethyl acetate (1:1; v/v) saturated with water. Following extraction, the tubes were centrifuged for 10 min (1500 *g*, 4°) to separate the aqueous and organic phases. The aqueous phase was frozen on dry ice, and the organic phase was collected into a small (7 mL) scintillation vial for subsequent counting. After the addition of 5 mL of Optifluor® and vigorous shaking, radioactivity was measured with a liquid scintillation spectrometer (LS-6500, Beckman). Blank values were obtained by adding 25 μL of 3 N HC1 to the enzyme mixture prior to adding the substrate.

In studies on the inhibition of MAO activities by phentermine and other drugs, 25-μL aliquots of rat brain, liver, and lung homogenates were preincubated for 20 min at 37° in the absence or presence of various concentrations of phentermine or other test compounds, in a total volume of 50 μL. Following preincubation, the reaction was started by the addition of 50 μL [^{14}C]serotonin or [^{14}C]β-phenylethylamine, and samples were subsequently treated as above. Enzyme activities observed with each concentration of a test compound were expressed as percents of the activity observed in the absence of the drug. For each compound the concentration–inhibition curve was obtained by plotting the log molar concentration of a test compound against the percent of control enzyme activity remaining. The concentration of a test compound producing 50% inhibition (IC_{50}) was calculated graphically from semi-log plots (the concentration–inhibition curve) of inhibitor concentration against percent inhibition. K_i values (μM) were determined from the equation $K_i = IC_{50}/(1 + S/K_m)$. The S value (substrate concentration as μM) in this equation was 125 μM for [^{14}C]serotonin or 8 μM for [^{14}C]β-phenylethylamine, respectively. K_m values (μM) for serotonin or β-phenylethylamine were obtained for each tissue from the double-reciprocal Lineweaver–Burk plots of data from kinetic experiments. In these experiments, 25 μL homogenate was preincubated for 20 min in the absence of drug in a total volume of 50 μL (25 μL homogenate + 25 μL water). Following preincubation, the reaction was started by the addition of [^{14}C]serotonin or [^{14}C]β-phenylethylamine, as above. K_m (μM) and V_{max} (nanomoles per minute per milligram of tissue) values were calculated by linear regression analyses of the Lineweaver–Burk plots.

In experiments on the interactive effects of several MAO inhibitors, these were mixed prior to incubation with the liver enzyme. Final concentrations of inhibitors examined in combination were the same as those used when the drugs were tested alone. Inhibition of MAO activity was expressed as decimal activity, which equals v_i/v_o, where v_i is activity in the presence of inhibitor(s) and v_o is activity without inhibitor(s). To determine whether the inhibition produced by two or more drugs was additive, the decimal activity of the combination was compared with the product of the decimal activities obtained when each drug was assayed alone.

To examine the time-dependency of the inhibition of MAO by phentermine, 25-μL aliquots of rat brain, liver, and lung homogenates were preincubated at 37° for 0, 5, 10, 15, 20, 25, 30, or 40 min with 100 μM phentermine; [^{14}C]serotonin (final concentration 125 μM) then was added, and the mixtures were incubated for another 5 min. Reactions were stopped by adding 25 μL of 3 N HC1. Deaminated products were extracted and counted as above.

The reversibility of the inhibition of MAO by phentermine was studied using the dilution method. Rat liver was homogenized in 5 vol. of 0.1 M sodium phosphate buffer (pH 7.4), and aliquots (200 μL) of the homogenate were incubated at 37° for 20 min in the absence or presence of phentermine (320 μM). After this preincubation, 50 μL of

the mixture was diluted 50-fold with 0.1 M sodium phosphate buffer, pH 7.4, and the MAO activities in aliquots (50 μL) of the diluted homogenates, taken 5, 10, 15, or 20 min after dilution, were measured by incubating them with [^{14}C]serotonin or [^{14}C]β-phenylethylamine, as above. The degree of inhibition prior to dilution was determined by comparisons with MAO activities in aliquots of the undiluted homogenates.

In competition experiments, aliquots (25 μL) of rat brain homogenates were preincubated for 20 min at 37° in the absence or presence of three concentrations of phentermine (100, 320, and 1000 μM), in a volume of 50 μL. After preincubation, reactions were started by the addition of [^{14}C]serotonin (final concentration: 31.3, 62.5, 125, 250, 500, 1000, or 2000 μM) and incubation for 5 min. Reactions were stopped, and the deaminated products were extracted and counted as above. The enzyme activity (V; nmol/min/mg tissue) observed using each concentration (S; μM) of substrate (serotonin) was calculated; double-reciprocal plots were obtained by plotting 1/S values against 1/V values in the absence or presence of phentermine.

Chemicals and Drugs

[^{14}C]Serotonin creatine sulfate (1.8 to 2.2 GBq/mmol) and [^{14}C]β-phenylethylamine hydrochloride (1.8 to 2.2 GBq/mol) were purchased from Amersham and New England Nuclear, respectively. Serotonin creatine sulfate, β-phenylethylamine hydrochloride, pargyline hydrochloride, clorgyline hydrochloride, iproniazid phosphate, phentermine hydrochloride, *d*- and *l*-ephedrine, *d*- and *l*-pseudoephedrine hydrochloride, *d*,*l*-norephedrine hydrochloride (phenylpropanolamine), and estradiol benzoate were purchased from the Sigma Chemical Co. Nialamide, *R*(-)deprenyl hydrochloride, and tranylcypromine hydrochloride were purchased from Research Biochemicals International.

RESULTS

Inhibition of MAO Activities in Rat Lung, Liver, and Brain by Phentermine

Phentermine inhibited the ability of MAO to deaminate both serotonin and β-phenylethylamine in homogenates of rat lung, liver, and brain. Inhibition occurred at micromolar concentrations, was concentration-dependent, and was of roughly similar magnitude in all three tissues (Fig. 1). The drug was a more potent inhibitor of serotonin metabolism (MAO-A) than of β-phenylethylamine metabolism (MAO-B) (Table 1). Its inhibition of serotonin metabolism was enhanced by preincubation of the tissues (at 37°), and was maximal and stable after 5 min of preincubation (Fig. 2). A similar time-dependence was also evident for the inhibition by phentermine of β-phenylethylamine metabolism.

Preincubation of rat liver homogenates with phentermine (320 μM) for 20 min inhibited the metabolism of both serotonin and β-phenylethylamine, by 87 and 48%, respectively. Diluting the samples 50-fold resulted in total

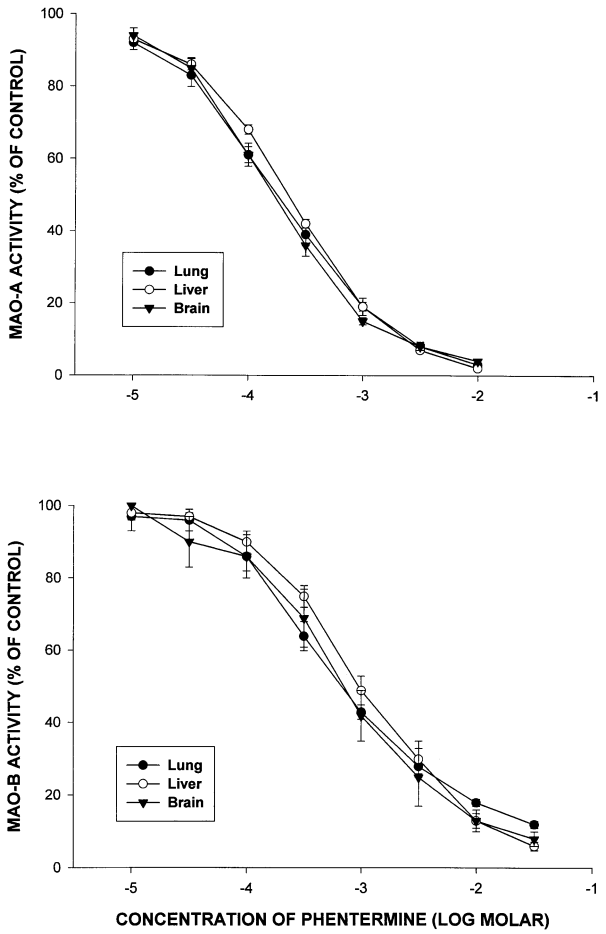

FIG. 1. Concentration–inhibition curves for inhibition by phentermine of MAO activities in homogenates of rat lung, liver, and brain. Aliquots (25 μL) of the homogenates were preincubated for 20 min at 37° in the absence or presence of various concentrations of phentermine [10, 32, 100, 320, 1, 000, 3, 200, 10, 000, and 32, 000 μM] in a volume of 50 μL. After the preincubation period, the reaction was started by the addition of [^{14}C]serotonin (final concentration, 125 μM) or [^{14}C]β-phenylethylamine (final concentration, 8 μM), and the assays continued as described in the text. Enzyme activities in the presence of each drug concentration were expressed as percents of those in homogenates preincubated without phentermine (control). Concentration–inhibition curves were obtained by plotting log molar concentrations of phentermine against percent enzyme activity. Control MAO-A and MAO-B activities were 81 ± 4 and 88 ± 6, 450 ± 32 and 205 ± 7, or 81 ± 4 and 70 ± 6 pmol/min/mg tissue in the lung, liver, and brain, respectively. Each point represents the mean ± SEM of 5–7 experiments.

recovery of MAO activities, indicating that phentermine inhibits MAO activities reversibly (Fig. 3). Moreover, as shown by kinetic studies on brain homogenates, this inhibition was competitive (Fig. 4).

Inhibition of MAO Activities in Rat Lung, Liver, and Brain by D-Amphetamine, Ephedrines, and Moclobemide

MAO activities in rat lung (Fig. 5), liver (Fig. 6), and brain (Fig. 7) were inhibited by *d*-amphetamine, *d*-ephedrine,

TABLE 1. K_I values of phentermine and some related drugs for MAO-A and MAO-B in rat lung, liver, and brain homogenates

	K_i (μM)					
	MAO-A			MAO-B		
Drugs	Lung	Liver	Brain	Lung	Liver	Brain
Phentermine	85 ± 10	85 ± 4	88 ± 9	326 ± 43	416 ± 57	310 ± 90
d-Amphetamine	6.1 ± 0.6	6.5 ± 0.8	4.9 ± 0.3	106 ± 9	119 ± 8	118 ± 21
d,l-Norephedrine	243 ± 33	267 ± 16	222 ± 18	3400 ± 650	3300 ± 850	2600 ± 820
d-Ephedrine	840 ± 60	844 ± 41	644 ± 43	5500 ± 200	5500 ± 650	5500 ± 120
l-Ephedrine	570 ± 50	600 ± 54	520 ± 19	4250 ± 750	4700 ± 560	4600 ± 400
d-Pseudoephedrine	1220 ± 120	1030 ± 110	1020 ± 30	5750 ± 750	5200 ± 600	5200 ± 400
l-Pseudoephedrine	890 ± 20	870 ± 50	970 ± 20	> 6500	> 6500	< 6500
Moclobemide	11 ± 2	10 ± 2	14 ± 3	1250 ± 200	1600 ± 400	1650 ± 150

MAO-A and MAO-B activities were assayed using serotonin and β-phenethylamine, respectively, as substrates, and concentration–inhibition curves were obtained for the drugs indicated in rat lung, liver, and brain homogenates as described in Fig. 1. The concentration producing 50% inhibition (IC_{50}) was calculated graphically from semi-log plots (the concentration–inhibition curve) of inhibitor concentration against percent inhibition. K_i values (μM) were determined from the equation $K_i = IC_{50}/1 + S/K_m$). The S value (substrate concentration as μM) in this equation was 125 μM for MAO-A or 8μM for MAO-B, respectively. K_m values (μM) of serotonin (MAO-A) or β-phenethylamine; (MAO-B) were obtained form double-reciprocal Lineweaver-Burk plots in kinetic experiments for each tissue. Data are given as means ± SEM of 4–7 determinations.

l-ephedrine, *d,l*-norephedrine, *d*-pseudoephedrine, *l*-pseudoephedrine, and moclobemide at micromolar concentrations (Table 1), and in a concentration-dependent manner. In general, the responses of the three tissues were similar (Figs. 5–7), the inhibitory effects were reversible (Fig. 8), and the ability of each drug to inhibit the deamination of serotonin (MAO-A) was greater than its ability to inhibit the deamination of β-phenylethylamine (MAO-B).

Inhibition of MAO Activities in Rat Lung and Brain Homogenates by Other Drugs

To compare the inhibitory potencies of phentermine and other unlabeled MAO inhibitors with those of antidepressant MAO inhibitors, we characterized the concentration–inhibition curves for the irreversible MAO inhibitors clorgyline, nialamide, deprenyl, tranylcypromine, and phenelzine, as well as for iproniazid and pargyline, in rat lung and brain homogenates. All of these compounds inhibited serotonin and β-phenylethylamine metabolism in both tissues, in a concentration-dependent manner (Figs. 9 and 10). Serotonin metabolism (MAO-A) in the lung (Fig. 9) and the brain (Fig. 10) was inhibited by clorgyline, phenelzine, tranylcypromine, pargyline, and deprenyl at nanomolar concentrations; as with phentermine, the inhibition produced by nialamide or iproniazid occurred only at micromolar concentrations. β-Phenylethylamine metabolism (MAO-B) in lung (Fig. 9) and brain (Fig. 10) was inhibited by deprenyl, pargyline, tranylcypromine, and phenelzine at nanomolar concentrations, and by clorgyline, nialamide, and iproniazid at micromolar concentrations (Figs. 9 and 10).

The concentration–inhibition curves of iproniazid and nialamide in rat liver homogenates (data not shown) were similar to those in lung and brain homogenates. The ec_{50} values for the MAO inhibitors, determined graphically from the concentration–inhibition curves, were similar (Table 2).

Interactions of Phentermine and Other MAO Inhibitors

Incubation with phentermine at a concentration, 130 μM, that alone caused 22% inhibition of hepatic MAO (serotonin-metabolizing) activity, caused considerably greater inhibition when the incubation mixture also contained *l*-pseudoephedrine, *d*-pseudoephedrine, *l*-ephedrine, *d*-ephedrine, *d,l*-norephedrine, or estradiol benzoate. In all cases the magnitude of the inhibition observed with the drug combinations was close to, or equal to, the products of the inhibitions produced when the drugs were tested alone (Table 3). Similar additive effects were observed when phentermine was mixed with both *d*-pseudoephedrine and *d*-ephedrine, and when these three drugs were mixed with a fourth, *d,l*-norephedrine or estradiol benzoate. Additive effects were not observed when the above drugs were mixed with irreversible MAO inhibitors; in that situation the degree of inhibition obtained with the drug mixture was about the same as that caused by the irreversible MAO inhibitor alone.

DISCUSSION

These data confirm earlier demonstrations [14, 15] that phentermine is an MAO inhibitor, and show that it reversibly inhibited both the serotonin-metabolizing MAO-A and the β-phenylethylamine-metabolizing MAO-B (Fig. 1, Table 1). Its potency in inhibiting serotonin metabolism is on the same order as those of the antidepressant MAO inhibitors moclobemide and iproniazid (Tables 1 and 2), but less than the potencies of such irreversible MAO inhibitors as pargyline and tranylcypromine (Figs. 9 and 10; Table 2). The effects on serotonin metabolism of combining phentermine with other widely used unlabeled MAO inhibitors such as pseudoephedrine, norephedrine, and estradiol are additive (Table 3); hence, patients who took several of these compounds (and other MAO inhibitors, such as *d*-amphetamine) in "compounded

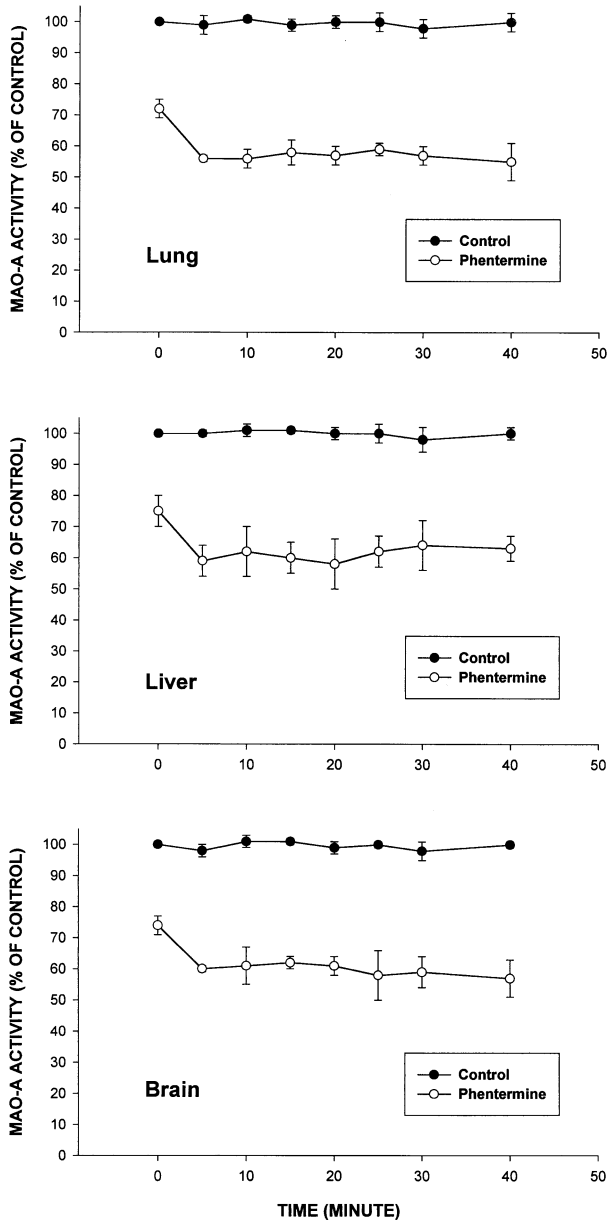

FIG. 2. Time–course of the inhibition by phentermine of serotonin metabolism in rat lung (top), liver (middle), and brain (bottom) homogenates. Assays were performed and analyzed as described in the text. Control MAO-A activity (at time 0) was 94 ± 8, 505 ± 45, or 87 ± 11 pmol/min/mg tissue in the lung, liver, and brain, respectively. Each point represents the mean ± SEM of 3 experiments.

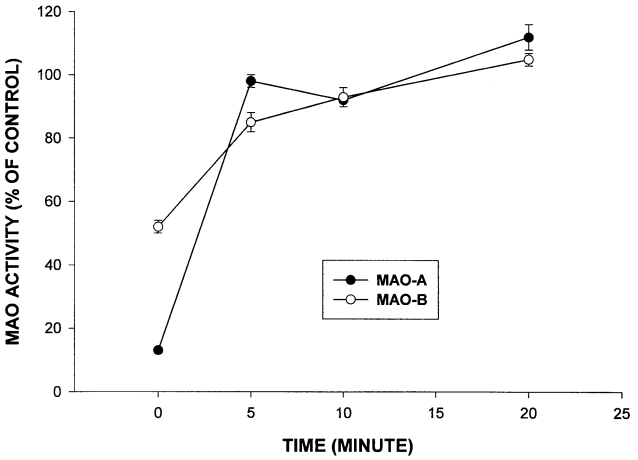

FIG. 3. Reversibility by dilution of the inhibition by phentermine of serotonin and β-phenylethylamine metabolism in rat liver. Rat liver was homogenized in 5 vol. of 0.1 M sodium phosphate buffer (pH 7.4), and aliquots (200 μL) of the homogenate were incubated at 37° for 20 min in the absence or presence of phentermine (320 μM). After the preincubation, 50-μL aliquots of the mixture were diluted 50-fold with 0.1 M sodium phosphate buffer, pH 7.4, and aliquots (50 μL) of the diluted homogenates were taken 5, 10, 15, or 20 min after dilution, and incubated with [^{14}C]serotonin (125 μM) or [^{14}C]β-phenylethylamine (8 μM) as described in the text. Activities were compared with those of undiluted homogenates. Control MAO-A and MAO-B activities were 480 ± 70 and 225 ± 15 pmol/min/mg tissue in liver, respectively. Each point represents the mean ± SEM of 3 experiments.

preparations" [18] may have experienced substantially greater MAO inhibition than that anticipated from the kinetic parameters of phentermine alone. The apparent potency of estradiol (i.e. estradiol benzoate) was low, perhaps reflecting the poor water-solubility of this hormone in the absence of estrogen-binding proteins. The hormone has been shown to exert antidepressant effects, and these have been attributed to MAO inhibition [19].

The observed potency of phentermine for inhibiting the serotonin-metabolizing MAO-A in rat liver, lung, and brain was much greater than that previously reported: The early studies concluded that phentermine inhibits MAO with EC_{50} values of 10 mM or 270 μM for rat liver [15] or brain [14], respectively. These EC_{50} values were clearly different from, and much higher than, the K_i values (and apparent EC_{50} values) observed in the present study. In these previous studies, however, the concentrations of serotonin used as a MAO substrate (5 or 2 mM) were several-fold higher than its concentration (125 μM) used in the present study. It is well known that MAO exists in two forms, MAO-A and MAO-B, and that MAO-A preferentially metabolizes serotonin: the K_m values of serotonin for MAO-A and MAO-B are about 125 μM and 2 mM, respectively [20]. Thus, EC_{50} values of phentermine for the inhibition of MAO reported by Seiler and Wasserman [15] in liver, or by Moller Nielsen and Dubnick [14] in brain are for MAO-A + MAO-B, but not MAO-A alone. Moreover, since the inhibition of MAO by phentermine is competitive (Fig. 4), it is not possible to discern the effect of a competitive inhibitor (phentermine) when the substrate concentration (serotonin) is 8- to 20-fold higher than the K_m value of the competitor. Clearly, the potency of phentermine was underestimated in the previous studies.

Phentermine was second only to *d*-amphetamine, among the unlabeled MAO inhibitors tested in the present study, for its ability to inhibit MAO-A as well as MAO-B. The potencies of phentermine, *d*-amphetamine, and related drugs for inhibiting the serotonin-metabolizing MAO-A

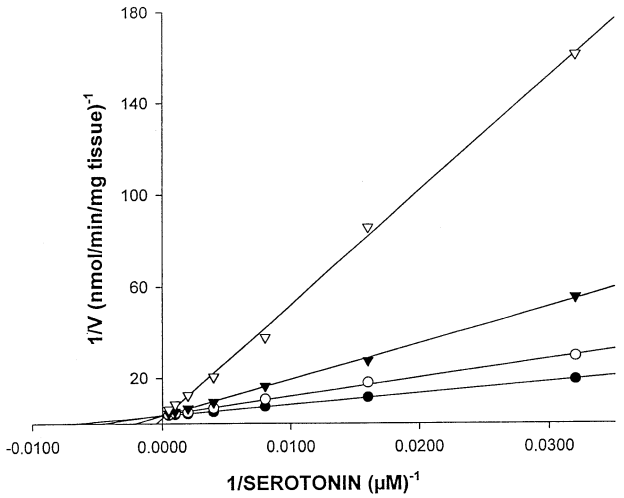

FIG. 4. Double-reciprocal plot of the inhibition by phentermine of [^{14}C]serotonin deamination in rat brain homogenates. Aliquots (25 μL) of rat brain homogenates were preincubated for 20 min at 37° in the absence (●) or in the presence of 100 μM (○), 320 μM (▼), or 1000 μM (▽) phentermine in a volume of 50 μL. After the preincubation, the reaction was started by the addition of [^{14}C]serotonin (final concentration: 31.3, 62.5, 125, 250, 500, 1000, or 2000 μM), and incubated for 5 min. The rest of the assay was performed as described in the text. Double-reciprocal plots were obtained by plotting the 1/S value against the 1/V value in the absence or presence of phentermine. Each point represents the mean of duplicate determinations of one typical experiment.

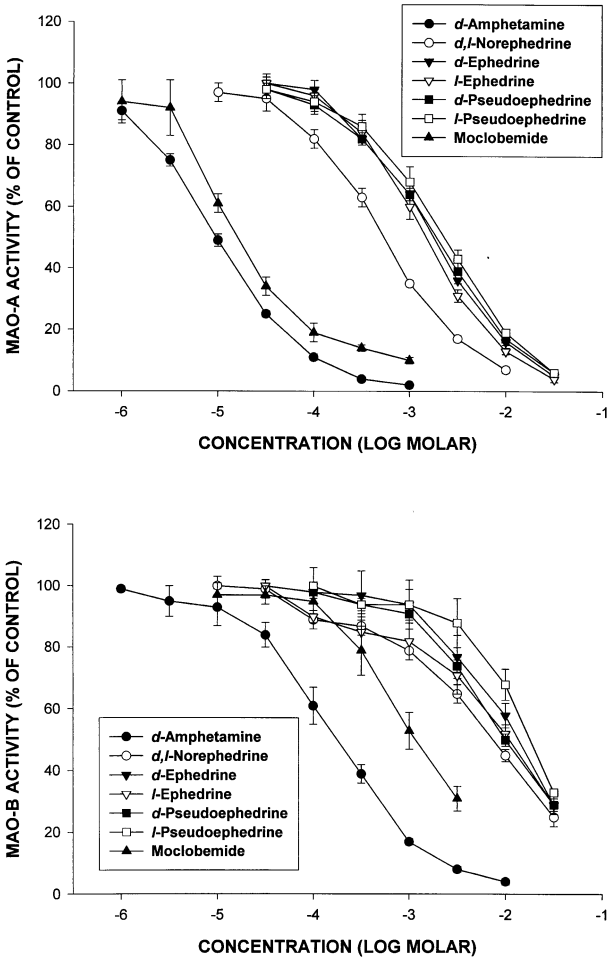

FIG. 5. Concentration–inhibition curves of various drugs in rat lung homogenates. Control MAO-A and MAO-B activities were 104 ± 4 and 98 ± 5 pmol/min/mg tissue, respectively. Each point represents the mean ± SEM of 4 experiments.

were higher than their potencies for inhibiting the β-phenylethylamine-metabolizing MAO-B in each of the rat tissues tested (Table 1). These results indicate that phentermine, *d*-amphetamine, and the other drugs tested are preferentially MAO-A inhibitors. In agreement with the present results, it has been reported previously that *d*-amphetamine [21], various amphetamine analogs [22], and *d,l*-norephedrine [23] preferentially inhibit MAO-A. The reported K_i values for *d*-amphetamine, 20 μM [22] and 11 μM [22], and for *d,l*-norephedrine, 150 μM [23], are also in good accordance with those found in the present study.

What is the contribution of the MAO-A inhibiting activity of phentermine to its overall pharmacological effects (i.e. its biochemical, behavioral, and toxic actions), when given alone or in combination with other drugs? In central and peripheral catecholaminergic synapses, phentermine interacts with noradrenaline and dopamine transporters at about 2–8 μM [24, 25] to increase the levels of these transmitters; it thereby produces "sympathomimetic," "central stimulating," and anorectic effects. The inhibition of MAO-A by phentermine would be expected to enhance these pharmacological effects by slowing the oxidative deamination of intraneuronal catecholamines and of extraneuronal catecholamines released from presynaptic sites. Thus, inhibition of MAO-A by phentermine might contribute to its pharmacological effects; however, because the transmitters it influences through MAO-A inhibition are the same as those released by the drug, qualitative differences would not be expected. The severe side-effects of classical irreversible MAO inhibitors, such as the hypertensive crisis observed after ingestion of tyramine-rich foods (e.g. aged cheeses), are also unlikely to occur with phentermine treatment because the MAO inhibition it produces is reversible and is relatively selective.

When phentermine-induced MAO-A inhibition occurs concurrently with serotonin uptake blockade (caused by fluoxetine) and/or serotonin release (by fenfluramine or dexfenfluramine), the observed effects might differ qualitatively from those of either drug given alone. Package inserts and product labels for fenfluramine and dexfenfluramine—as well as for all of the other serotonin reuptake blockers [9]—have stated unequivocally that these drugs are never to be administered along with an MAO inhibitor, lest free serotonin in the brain or blood rise to toxic levels and produce a "serotonin syndrome" or pathologic changes in vascular tissue. In two important sites, the central nervous system and the lung, the combination of phentermine and a serotonin uptake blocker or releaser might be expected to cause major, if transient, increases in free (i.e.

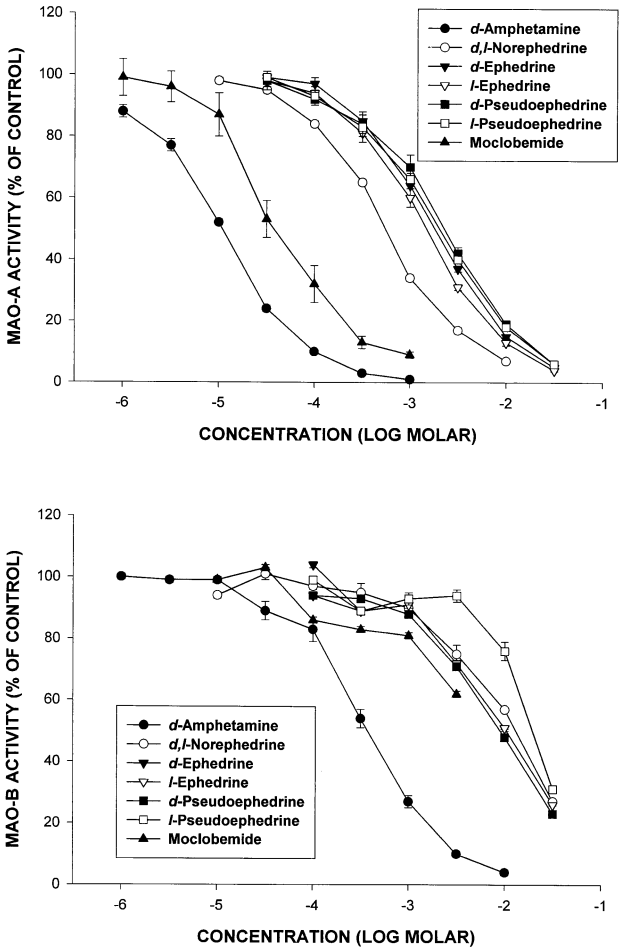

FIG. 6. Concentration–inhibition curves of various drugs in rat liver homogenates. Control MAO-A and MAO-B activities were 460 ± 33 and 310 ± 28 pmol/min/mg tissue, respectively. Each point represents the mean ± SEM of 4 experiments.

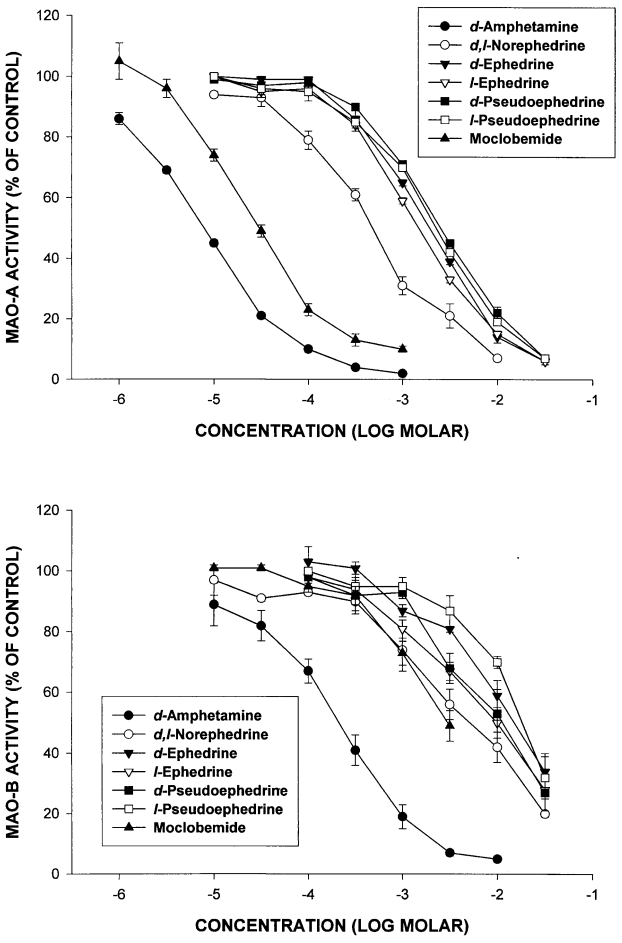

FIG. 7. Concentration–inhibition curves of various drugs in rat brain homogenates. Control MAO-A and MAO-B activities were 102 ± 7 and 79 ± 8 pmol/min/mg tissue, respectively. Each point represents the mean ± SEM of 4 experiments.

plasma or intrasynaptic) serotonin. Neurochemical experiments show that MAO inhibition *per se* can increase extracellular serotonin [26] and enhance such serotonin-mediated effects as anorexia [27]. Similarly, phentermine alone suppresses appetite [28], increases extracellular serotonin levels [29, 30], and, when given in combination with serotoninergic agents such as fenfluramine, enhances their effects on appetite [31] and on extracellular serotonin levels [30]. The effects of phentermine on extracellular brain serotonin levels, when given alone or combined with fenfluramine [29, 30], are compatible with its inhibitory action on MAO-A (Table 1). The reversible and competitive nature of the MAO-A inhibition produced by phentermine may help to explain why the phentermine–fenfluramine combination, while more effective than fenfluramine alone in treating obesity, was associated with relatively few cases of "serotonin syndrome," or with the vascular damage seen when potent and irreversible MAO inhibitors are combined with serotonin uptake blockers or releasers. Perhaps, because phentermine failed to produce severe, acute toxic reactions when taken with tyramine-rich foods or serotonin uptake blockers, its ability to inhibit MAO was thought to be relatively unimportant in its pharmacological actions, and so this inhibition went generally unrecognized after its initial description. Even though this action was initially described and confirmed in the 1970s [14, 15], the drug package insert never mentioned it, and few, if any, physicians were (or are) aware of it. This situation allowed millions of Americans to take phentermine in combination with fenfluramine (as "fen-phen") [8], and also allows some patients to take "pro-phen" (Prozac, or fluoxetine, plus phentermine) right now.

The effect of phentermine as a MAO-A inhibitor on peripheral serotonin metabolism, mainly in the lung, may explain the cardiac valvular lesions [1, 2] and perhaps the primary pulmonary hypertension [18] observed in some patients taking "fen-phen." The lung is uniquely important in peripheral serotonin metabolism in both rats [16] and humans [32]. Most, if not all, circulating serotonin is cleared by the lung, through active uptake into lung tissue followed by oxidative deamination [16, 32]. An important functional consequence of this inactivation is that reduced concentrations of serotonin thus enter the left atrium and

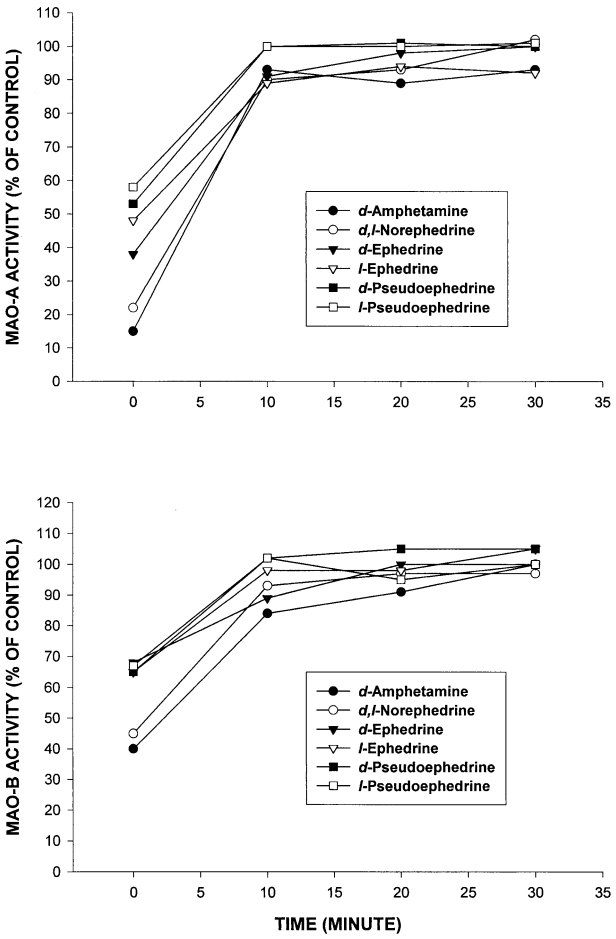

FIG. 8. Reversibility of inhibition by various drugs of serotonin-metabolizing activity in rat liver homogenates. Control MAO-A and MAO-B activities were 520 and 218 pmol/min/mg tissue. Each point represents the mean of duplicate determinations of one typical experiment.

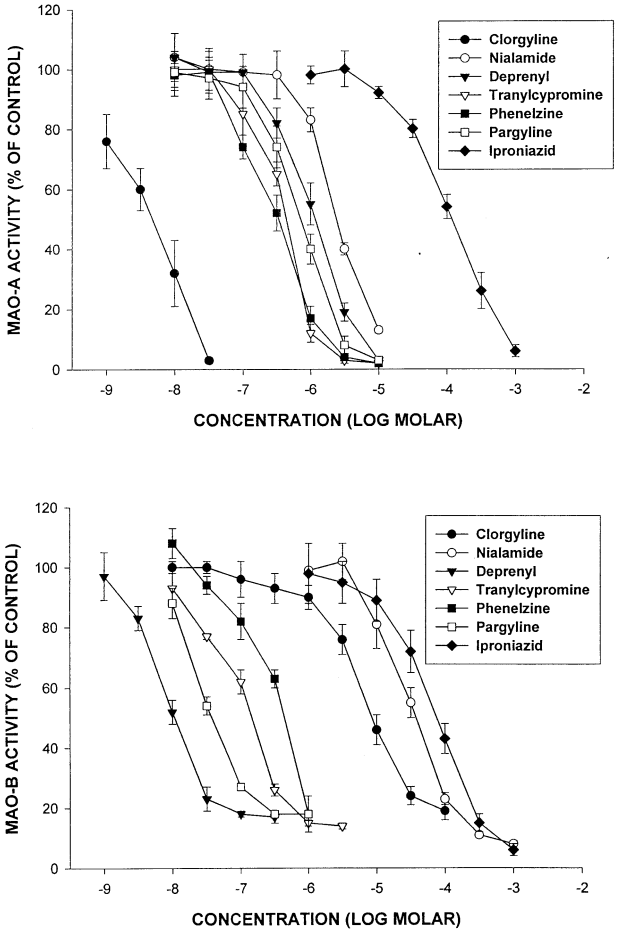

FIG. 9. Concentration–inhibition curves of some MAO inhibitors in rat lung homogenates. Control MAO-A and MAO-B activities were 78 ± 5 and 94 ± 6 pmol/min/mg tissue, respectively. Each point represents the mean ± SEM of 4 experiments.

the systemic circulation. The inhibition of MAO-A in lung tissue by phentermine and related drugs strongly suggests that these drugs thereby inhibit serotonin metabolism, and allow more intact serotonin to enter the left atrium. This would be especially likely when phentermine or other unrecognized MAO inhibitors were given in combination with a serotonin uptake blocker (which would inhibit the uptake of serotonin by lung tissue) or a serotonin releaser. The only way to prove conclusively that giving a serotonin uptake inhibitor with phentermine increases pulmonary plasma serotonin concentrations would be to give the drugs to volunteers and measure serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in plasma samples obtained from pulmonary arterial and venous blood. But at present such a study would be unethical. Data are available, however, showing that giving phentermine to rats, without serotonin uptake blockers, does suppress serotonin metabolism in the lung [33]. (In that study, doses of 0.5 and 5 mg/kg generated calculated lung phentermine concentrations of 63 and 380 μM, respectively.) It would be anticipated that giving phentermine with a serotonin uptake blocker, such as fenfluramine, would amplify this effect, inasmuch as the serotonin in pulmonary plasma would no longer be protected from oxidative deamination by being taken up by platelets. Such an increase in the concentration of blood serotonin leaving the lung would be expected to increase pulmonary vascular resistance, as has been demonstrated in the isolated perfused rat lung [34]. In accordance with this view, at least one case of primary pulmonary hypertension has already been described in a patient chronically taking phentermine along with the serotonin uptake blocker fluoxetine [35].

When investigators at the Mayo Clinic first described surgically confirmed heart valve lesions in people who had been treated with "fen-phen" [1], and when the FDA, analyzing uncontrolled echocardiographic data from five laboratories, concluded that as many as 30% of patients so treated went on to develop such lesions [2], it was speculated that the lesions resulted from pathologic elevations in blood serotonin, similar to those seen in carcinoid syndrome, which had been caused by the fenfluramines [1]. A single group of investigators also presented echocardio-

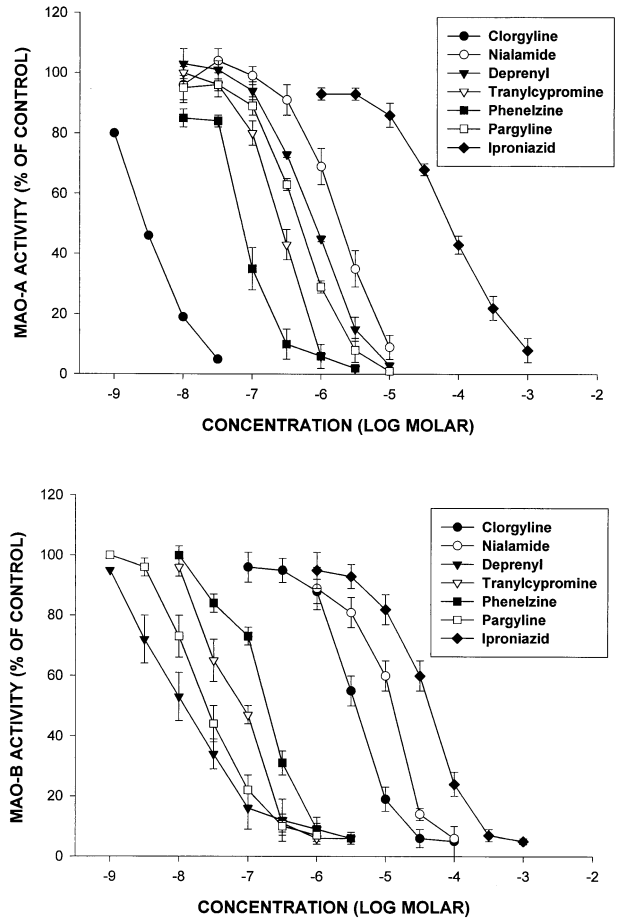

FIG. 10. Concentration–inhibition curves of some MAO inhibitors in rat brain homogenates. Control MAO-A and MAO-B activities were 86 ± 5 and 72 ± 8 pmol/min/mg tissue, respectively. Each point represents the mean ± SEM of 4 experiments.

graphic evidence [2, 36] that heart valve lesions had developed in a few patients who had taken dexfenfluramine without phentermine, and on that basis the companies marketing dexfenfluramine or fenfluramine voluntarily withdrew them. Phentermine—which, by itself, had not been associated with valvular lesions—continued to be available. That fenfluramine or dexfenfluramine, administered alone, could generate such lesions might have been questioned given their apparent absence in the tens of millions of patients outside the United States who had been treated, since the 1960s, with one or the other drug; it might instead have been hypothesized that this new, uniquely American clinical problem was related to the uniquely American way that fenfluramine usually was administered, i.e. in combination with phentermine.

Phentermine was described and continues to be described in its package insert as a sympathomimetic amine [9]. Very few articles have been published on the pharmacologic effects of the drug during the past two decades, and, as far as we can determine, *no* articles have confirmed its safety, in humans or experimental animals, when taken in combination with a fenfluramine. We observed that a number of widely used over-the-counter or prescription drugs besides phentermine also inhibit MAO-A, e.g. pseudoephedrine, norephedrine, amphetamine, ephedrine, and estrogen (Tables 1 and 3). Most of these compounds are also reversible MAO inhibitors, and when two or more are combined, their effects on MAO are additive (Table 3).

The fact that the effects of reversible MAO inhibitors are additive bears on the possible consequences, among obese European patients, of having taken both a fenfluramine and a multi-drug "compounded preparation" that frequently contained numerous MAO inhibitors, including amphetamine-like compounds [18].

The effects of unrecognized MAO inhibitors on pulmonary serotonin metabolism might also explain the tendency of fenfluramine-associated valvular lesions to affect the left side of the heart [1]—in contrast to the right-sided lesions found in patients with the carcinoid syndrome—as well as the reported association between fenfluramine use and primary pulmonary hypertension [18]: Most circulating serotonin is cleared in the lung, through metabolism by MAO [16]. In the carcinoid patient, pulmonary MAO is still active, so plasma serotonin concentrations in the right side of the heart are greater than those in the left side; however, as discussed above, in patients taking an MAO inhibitor the pulmonary clearance of serotonin is inhibited,

TABLE 2. EC_{50} values of MAO inhibitors for MAO-A and MAO-B in rat lung and brain homogenates

	EC_{50} (μM)			
	MAO-A		MAO-B	
Inhibitors	Lung	Brain	Lung	Brain
Clorgyline	0.005 ± 0.001	0.006 ± 0.001	11 ± 3	6 ± 2
Nialamide	3.0 ± 0.2	2.6 ± 0.4	21 ± 5	13 ± 1
Deprenyl	1.5 ± 0.2	1.3 ± 0.2	0.011 ± 0.002	0.013 ± 0.005
Tranylcypromine	0.440 ± 0.030	0.370 ± 0.060	0.156 ± 0.022	0.087 ± 0.016
Phenelzine	0.322 ± 0.050	0.070 ± 0.020	0.380 ± 0.040	0.197 ± 0.016
Pargyline	0.810 ± 0.140	0.700 ± 0.110	0.027 ± 0.003	0.019 ± 0.002
Iproniazid	77 ± 11	59 ± 14	59 ± 14	48 ± 10

MAO-A and MAO-B activities were assayed and the concentration–inhibition curves obtained for their inhibition by known MAO-A and MAO-B inhibitors in lung and brain homogenates. The concentration producing 50% inhibition (IC_{50}) was calculated graphically from semi-log plots (the concentration–inhibition curve) of inhibitor concentration against percent inhibition. Data are given as means ± SEM of four determinations.

TABLE 3. Inhibition of rat liver MAO-A by combinations of inhibitions

Treatment	Inhibitor	Concn (mM)	MAO activity	
			Observed	Predicted
1	Phentermine	0.13	0.78	
2	*l*-Pseudoephedrine	0.89	0.74	
3	*d*-Pseudoephedrine	1.33	0.79	
4	*l*-Ephedrine	0.89	0.74	
5	*d*-Ephedrine	1.00	0.83	
6	*d,l*-Norephedrine	0.33	0.77	
1 + 2			0.57	0.58
1+3			0.63	0.62
1+4			0.58	0.58
1+5			0.67	0.65
1+6			0.61	0.60
1	Phentermine	0.12	0.79	
7	Estradiol benzoate	0.20	0.69	
1+7			0.54	0.54
1	Phentermine	0.13	0.78	
3	*d*-Pseudoephedrine	1.33	0.79	
5	*d*-Ephedrine	1.67	0.73	
1+3+5			0.47	0.45
1	Phentermine	0.075	0.89	
3	*d*-Pseudoephedrine	1.00	0.81	
5	*d*-Ephedrine	1.00	0.83	
6	*d,l*-Norephedrine	0.25	0.77	
1+3+5+6			0.47	0.46

Rat liver homogenates were incubated with serotonin plus one or more drugs, and MAO-A was assayed as described in the text. In studies on estradiol benzoate, the medium contained DMSO to increase the solubility of agent. Predicted MAO activity was obtained from the products of the observed activities for each of the drugs when present alone. Values represent individual experiments.

so the difference between right- and left-sided plasma serotonin levels may disappear, and valvular lesions become as likely to develop in the left side.

Clearly, something must be done to protect patients from using novel drug combinations—such as "fen-phen," or now phentermine plus fluoxetine (Prozac) ("phen-pro")—the safety of which has not been affirmed, and whose components may not even have been labeled accurately. In the interim, steps must be taken to affirm that *all* drugs that inhibit serotonin metabolism by MAO are labeled as such, and that physicians be warned once again not to combine such drugs with serotonin uptake blockers.

NOTE ADDED IN PROOF

In an additional group of 27 women, a single low dose of phentermine (15 mg, p.o.) significantly increased platelet serotonin levels after 2 hr, whether data were calculated using medians or means ($P = 0.0005$ and 0.0007, respectively), or as percent medians or means ($P = 0.0001$ and 0.0005, respectively).

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