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KINETIC STUDIES AND EFFECTS *IN VIVO* OF A NEW MONOAMINE OXIDASE INHIBITOR, *N*-[2-(*o*-CHLOROPHENOXY)-ETHYL]-CYCLOPROPYLAMINE

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Abstract—*N*-[2-(*o*-chlorophenoxy)-ethyl]-cyclopropylamine (51641) and related compounds inhibited the oxidation by monoamine oxidase (MAO) of serotonin, tyramine, phenethylamine and tryptamine *in vitro*. The inhibition by 51641 was noncompetitive. By using kynuramine as substrate to study reaction rates, it was found that inhibition by 51641 was maximum at pH 7·5, increased with time of incubation of enzyme and inhibitor, was not reversible by dialysis and was independent of enzyme concentration. *In vivo*, 51641 increased the amount of serotonin in the whole brain of the rat. Urinary excretion of tryptamine by rats, dogs and human subjects was increased after administration of 51641. Compound 51641 thus is a potent inhibitor of MAO *in vitro* and *in vivo*.

SINCE the time of the initial discovery of inhibition of monoamine oxidase [EC 1.4.3.4.monoamine:O_2 oxidoreductase (deaminating)] by certain hydrazines and hydrazides, there have been a number of other compounds discovered that are effective inhibitors of the enzyme. We have recently reported a new series of inhibitors.[1] These compounds, *N*-substituted cyclopropylamines, were shown to inhibit monoamine oxidase (MAO) *in vitro* and to have dopa-potentiating activity *in vivo* typical of MAO inhibitors in mice. One of the most interesting compounds in the new series was *N*-[2-(*o*-chlorophenoxy)-ethyl]-cyclopropylamine (51641). We undertook to study further, *in vitro* and *in vivo*, the properties of 51641 and other new MAO inhibitors in comparison with known inhibitors. This paper describes some of the results.

METHODS

Tranylcypromine sulfate (Parnate) and *d*-amphetamine sulfate (Dexedrine) were obtained from Smith, Kline & French; pargyline hydrochloride (Eutonyl) from Abbott Laboratories; and pheniprazine hydrochloride (Catron) from Lakeside Laboratories. The other MAO inhibitors, as hydrochloride or hydrobromide salts, were synthesized in the Lilly Research Laboratories.

The MAO preparation for studies *in vitro* was a mitochondrial fraction isolated from rat liver by the method of Hogeboom.[2] The final suspension (50%, w/v in 0·25 M sucrose) was lyophilized and stored at −15°. In two experiments reported, a similar preparation from rat brain was used.

Spectrophotometric determination of MAO activity with kynuramine as substrate[3] was measured as described previously.[4] When *m*-iodobenzylamine was used as substrate in this assay, measurements of absorbance at 250 mμ were made. The

incubation temperature was 37° in all cases, and the pH was 7·4 except in the experiment reported in Fig. 1.

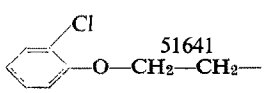
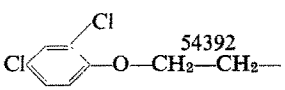
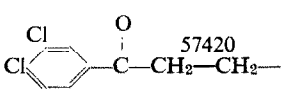
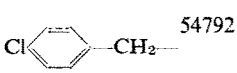
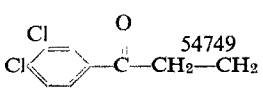
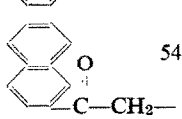
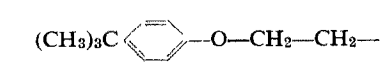
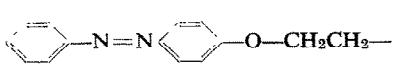
For the study of MAO activity on tryptamine, the radioisotope method of Wurtman and Axelrod[5] was used. In addition, three other substrates, ^{14}C-phenethylamine, ^{14}C-tyramine and ^{14}C-serotonin, were studied. With the latter two, ethyl acetate instead of toluene was used as the extracting solvent.

The method of Mead and Finger[6] was used to determine levels of serotonin in whole brains. The brains were removed from decapitated rats, frozen immediately on dry ice, and stored at −15° prior to analysis. Urinary levels of tryptamine were measured by the method of Sjoerdsma *et al.*[7]

RESULTS

MAO inhibition in vitro. The inhibition of oxidation of four MAO substrates by 51641 and related compounds is shown in Table 1. With 51641, the first compound in the table, approximately a 400 times higher concentration was required to block phenethylamine as compared to the concentration needed to inhibit serotonin

TABLE 1. pI_{50} VALUES WITH FOUR SUBSTRATES

Inhibitors* R—NH—◁ R =	pI_{50}†			
	Serotonin	Tryptamine	Phenethylamine	Tyramine
 51641	9·0	7·7	6·4	6·5
 54392	9·1	8·1	6·7	6·7
 57420	7·6	7·0	6·5	5·8
 54792	8·2	7·7	6·9	7·0
 54749	7·8	7·6	6·8	7·1
 54832	5·5	5·7	5·6	5·5
	7·9	7·7	7·2	7·6
	7·3	7·1	7·5	7·0

* All inhibitors were preincubated with enzyme for 30 min prior to substrate addition. Four to six concentrations of each inhibitor were studied.

† Negative logarithm of the molar concentration of inhibitor required for 50 per cent inhibition of enzyme activity.

oxidation. With some other inhibitors shown in the table, the pI_{50} values* varied little with substrate.

To study some of the properties of these compounds as MAO inhibitors *in vitro*, the spectrophotometric assay with kynuramine as substrate was used so that actual rates of enzymatic activity could be monitored.[3, 4]

The effect of pH on enzyme activity and on inhibition by 51641 is shown in Fig. 1. Both were maximum at pH 7·4 The pH curves were very similar throughout the pH range studied.

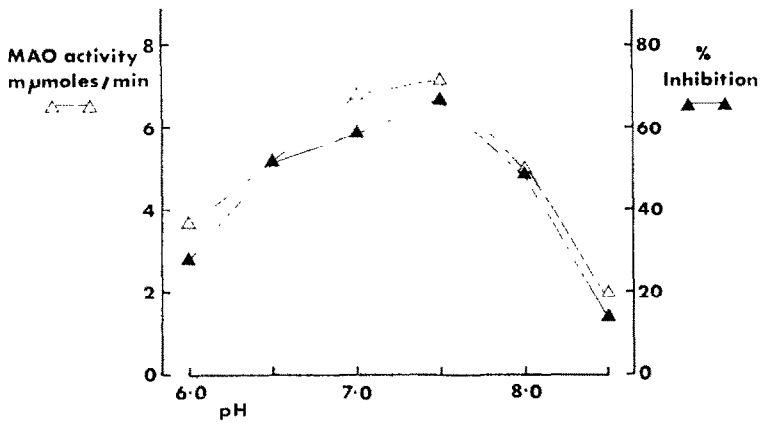


FIG. 1. Effect of pH on MAO activity and on 51641 inhibition. The enzyme was incubated at 37° for 30 min prior to substrate addition, with inhibitor (▲) or without (△). Enzyme activity was then measured and the per cent inhibition was calculated. The final concentration of inhibitor (when present) was 1×10^{-6} M.

The degree of inhibition by 51641 increased with time of incubation with enzyme prior to kynuramine addition. A concentration of 2×10^{-6} M produced 24, 61, 72 and 73 per cent inhibition with 0, 10, 20 or 30 min of preincubation The enzymatic rate of kynuramine oxidation in each case was linear with time.

After 51641 had been added to the enzyme preparation, MAO activity could not be restored by dialysis. In these experiments, the inhibitor and the enzyme were combined in buffer and held at 37° for 30 min. The mixture was then dialyzed at 5° for 48 hr against several changes of buffer. The volume of buffer was approximately 500 times the volume inside the dialysis bag. After dialysis, MAO activity was measured and found to be only 23 per cent of the activity of enzyme dialyzed in a comparable manner, but without inhibitor. Thus, there was 77 per cent inhibition as compared to 90 per cent inhibition produced when 51641 was added after dialysis to the same final inhibitor concentration (10^{-5} M). The inhibitor was not removed to any extent by dialysis, characteristic of an irreversible inhibitor.

Fig. 2 shows the inhibition of varying amounts of the rat liver enzyme by 51641 in comparison with amphetamine, harmaline, tranylcypromine, pargyline, pheniprazine and other inhibitors in the current series. In these experiments, enzyme and inhibitor were preincubated for 30 min; after addition of substrate, the rate of enzyme activity

* pI_{50} = The negative logarithm of the molar concentration of inhibitor required for 50 per cent inhibition of enzyme activity.

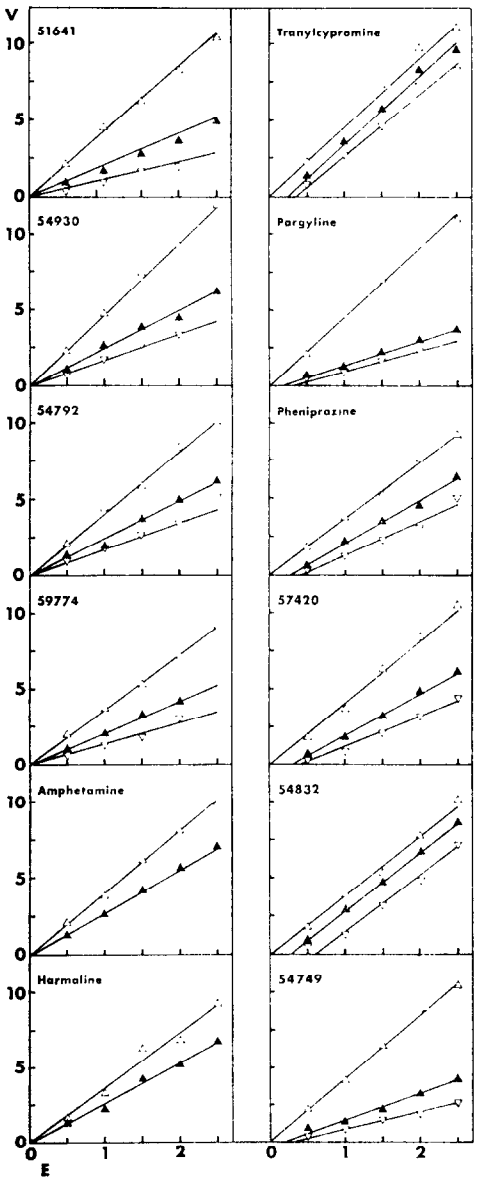


FIG. 2. Inhibition of rat liver MAO. The ordinate shows enzyme velocity (v) expressed as mμmoles kynuramine oxidized/min. The abscissa shows the milligrams of enzyme preparation (E) added. The final molar concentrations of inhibitors were: 51641, 1 and 2 × 10^{-6}; 54930, 2 and 4 × 10^{-7}; 54792, 1 and 2 × 10^{-7}; *d*-amphetamine, 1 × 10^{-4}; harmaline, 2 × 10^{-5}; tranylcypromine, 5 × 10^{-8} and 1 × 10^{-7}; pargyline and pheniprazine, 1·67 and 3·33 × 10^{-7}; 57420, 1 and 2 × 10^{-6}; 54832, 1 and 5 × 10^{-6}; 54749, 5 × 10^{-7} and 1 × 10^{-6}. Structural formulas for numbered compounds are in Table 1, except for 59774, 2-chlorophenylthioethyl cyclopropylamine. In each case, the control velocities are represented by △, the velocities at the lower inhibitor concentration by ▲, and the velocities at the higher inhibitor concentrations by ▽.

was linear with time at all levels of enzyme. With 51641 and the other compounds on the left side of Fig. 2, the per cent inhibition did not vary with enzyme level. With those compounds on the right side of the figure, per cent inhibition varied inversely with amount of enzyme. Data obtained for 51641 with brain mitochondria as enzyme or *m*-iodobenzylamine as substrate for the liver enzyme (Fig. 3) likewise showed a constant relationship between per cent inhibition and enzyme level.

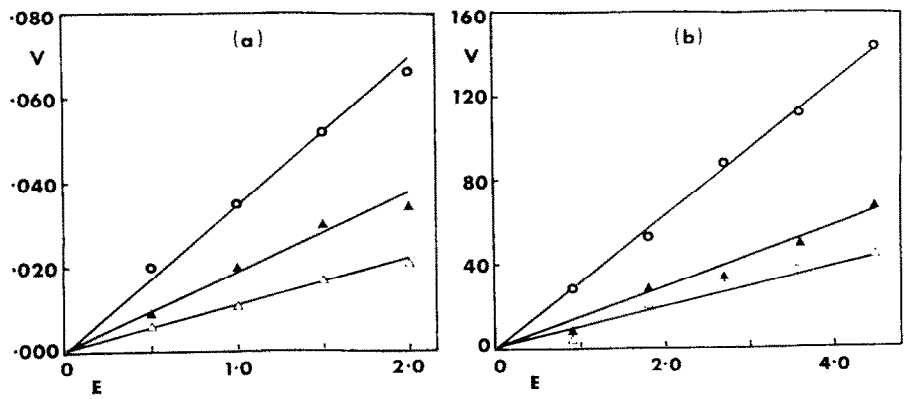


FIG. 3. Inhibition of MAO by 51641. (a) Rat liver mitochondria with *m*-iodobenzylamine as substrate. Enzyme velocity (V) in change in absorbance per minute is plotted vs. mg of enzyme preparation present (E). Final concentrations of 51641 were 1 and 2 × 10^{-6} M. (b) Rat brain mitochondria with kynuramine as substrate. Enzyme velocity (V) in mμmoles/hr is plotted vs. mg of enzyme preparation present (E). Final concentrations of 51641 were 1 and 4 × 10^{-7} M

Fig. 4 is a Lineweaver–Burk plot showing that 51641 is a noncompetitive inhibitor. With serotonin as substrate, a relatively low inhibitor concentration was required. Statistical analysis of the data by the method of Wilkinson[8] revealed that the V_{max} value in the presence of inhibitor (117 ± 5 μμmole/min) was significantly lower ($P < 0{\cdot}01$) than that in the absence of inhibitor (234 ± 22 μμmole/min). On the other hand, the K_m value was not significantly increased. In the absence of inhibitor, the K_m value was 23·9 ± 3·2 μM, while the value determined in the presence of inhibitor was 25·1 ± 1·6 μM. Similar results were obtained with phenethylamine as substrate, in which case a higher concentration of 51641 was necessary for inhibition. The K_m value in the presence of inhibitor was actually lower (64·3 ± 22·6 μM) than in the control reaction (93·2 ± 47·5 μM), although the difference was not statistically significant. The V_{max} value was lowered by the inhibitor from 320 ± 127 to 89 ± 20 μμmole/min. Other experiments with the liver enzyme also indicated that the inhibition by 51641 was noncompetitive in nature. Similar conclusions were reached regardless of whether inhibitor and enzyme were preincubated before substrate addition.

MAO inhibition in vivo. Since MAO inhibition *in vivo* produces a rise in tissue concentrations of amines that are normally destroyed by MAO, elevation of brain amines can be taken as a measure of MAO inhibition in the brain. The levels of serotonin in whole rat brain were increased by 51641 or 54930 (Fig. 5). Serotonin levels reached a maximum 4–8 hr after a single injection of drug; higher serotonin levels were produced by 54930. Serotonin had not returned to normal values 24 hr later.

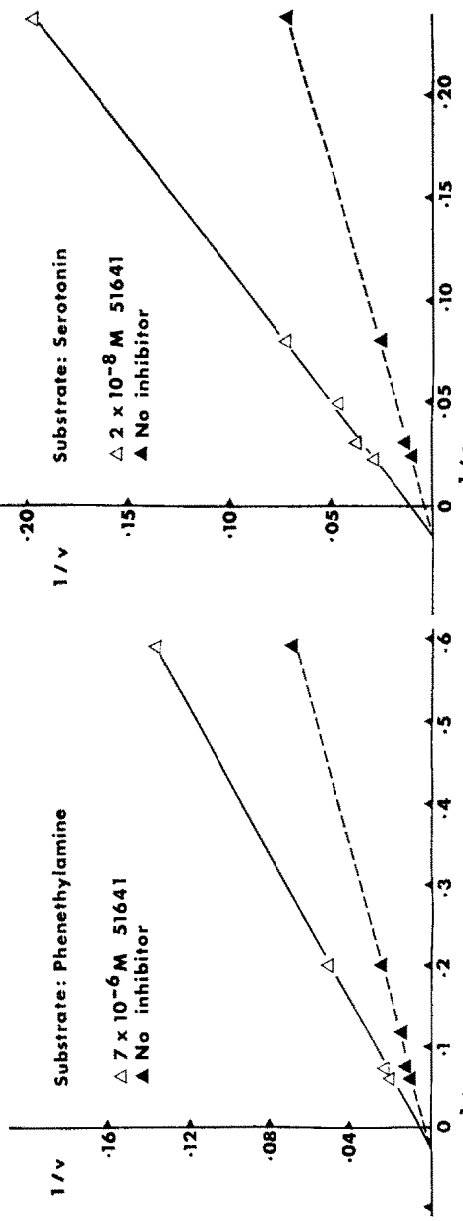


FIG. 4. Noncompetitive inhibition of MAO by 51641. Rat brain mitochondria was the source of enzyme. Enzyme velocity (v) is in $\mu\mu$moles/min, and substrate concentration (s) is μM.

A comparison of the effects of various doses of 51641, tranylcypromine and pheniprazine is shown in Fig. 6. At lower doses, 51641 produced increases in brain serotonin levels as great as those with tranylcypromine or pheniprazine. At higher doses, however, the increase in brain serotonin was not as great with 51641 as with other MAO inhibitors. The animals treated with 40 mg/kg of pheniprazine in three doses did not survive.

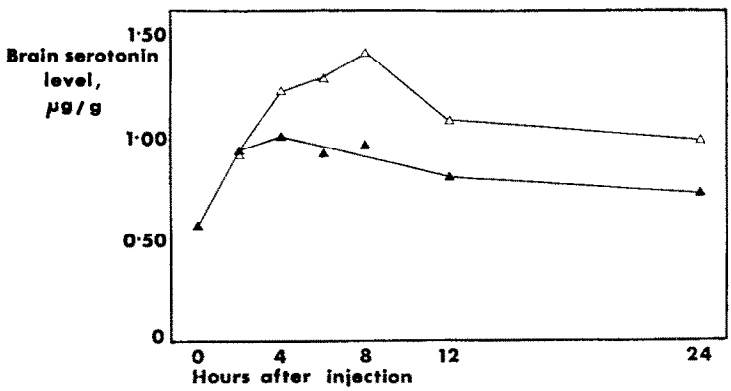


FIG. 5. Increase in serotonin levels in whole brain of rats after 51641 (▲) or 54930 (△). Male albino Harlan rats, body wt. 130–150 g, were used. Drugs were injected i.p. in aqueous solution at a dose of 40 mg/kg. There were 3 rats per group; tissues within a group were combined for analysis.

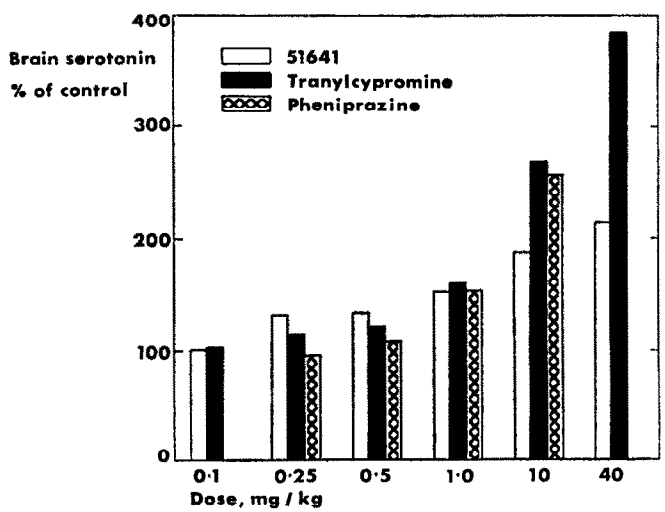


FIG. 6. Serotonin levels in whole brain of rats after 51641, tranylcypromine or pheniprazine. Male albino Harlan rats, body weight 100–120 g, were used. Drugs were injected i.p. in aqueous solution. Three identical doses, each at the level indicated, were given 28, 20 and 4 hr prior to sacrifice. There were 2 groups of 3 rats at each dose. For analysis, tissues within a group were combined and the values shown are averages for the 2 groups. The serotonin level in untreated rats was 0·54 μg/g of brain in this experiment.

Urinary excretion of tryptamine is another criterion for MAO inhibition *in vivo*.[7] Table 2 shows tryptamine excretion by rats treated with 51641 or other MAO inhibitors. Tryptamine output was increased most by tranylcypromine and substantially by 51641. Surprisingly, pargyline did not cause elevated tryptamine excretion in this

TABLE 2. URINARY EXCRETION OF TRYPTAMINE BY RATS*

Dose (mg/kg)	Daily tryptamine excretion (μg/rat)		
	51641	Tranylcypromine	Pargyline
0	4·6 ± 0·3	4·8 ± 0·6	5·1 ± 0·5
0·3	5·0 ± 0·4	6·2 ± 0·8	3·6 ± 0·2
1·0	6·4 ± 0·2	7·5 ± 0·5	3·7 ± 0·3
3·0	7·0 ± 0·4	10·6 ± 0·8	3·9 ± 0·3
10·0	8·6 ± 0·7	23·0 ± 5·7†	4·3 ± 0·4

* Male Sprague–Dawley rats (200–250 g) were used. Drugs were given p.o. twice daily for 7 days; each dose was at the level indicated. Urine was collected after each 24-hr period. There were 10 rats per group. The values shown are averages for the entire period and are expressed on the basis of excretion by each rat for a single day.

† Nine of the 10 rats died; this value is for one rat only.

experiment. The effect of 51641 on tryptamine excretion in dogs is shown in Fig. 7. Daily urinary output of tryptamine increased after the first day to a level by the third day near which it remained relatively constant within groups for 12 weeks of treatment (data not shown). The degree of elevation of tryptamine excretion was related to the dose of 51641. An increased output of urinary tryptamine has been found in human subjects during preliminary clinical studies with 51641. The excretion of tryptamine by one patient is shown in Fig. 8 as a typical example. In this patient, a marked elevation of urinary tryptamine occurred during the first period of drug administration (days 5–9). A similar, but less pronounced, effect was seen during subsequent drug periods.

DISCUSSION

Most of the data indicate that the inhibition of MAO by 51641 *in vitro* is noncompetitive and irreversible. The latter is shown by the failure of enzyme activity to be restored by dialysis and the increase in per cent inhibition with time of incubation of 51641 with enzyme. In a third test for irreversibility, however, 51641 differed from irreversible inhibitors such as pargyline, tranylcypromine and pheniprazine (Fig. 2). The plot of velocity versus enzyme amount was suggested by Ackermann and Potter[9] to distinguish reversible and irreversible inhibitors. Reversible inhibition should produce a line passing through zero, but with a decreased slope compared to the uninhibited reaction. Irreversible inhibitors that "titrate" the enzyme should produce a line that intercepts the x axis at some point greater than zero, corresponding to the amount of enzyme combined by the inhibitor. Although this line would in theory have a slope equal to that for the uninhibited reaction, the actual data show that the line often has a reduced slope. Nevertheless, the failure of the line to intercept zero was a well defined criterion that was used to separate the inhibitors into two groups. Those compounds in the cyclopropylamine series of inhibitors with a keto function in the side chain produced irreversible plots, i.e. the per cent inhibition was clearly

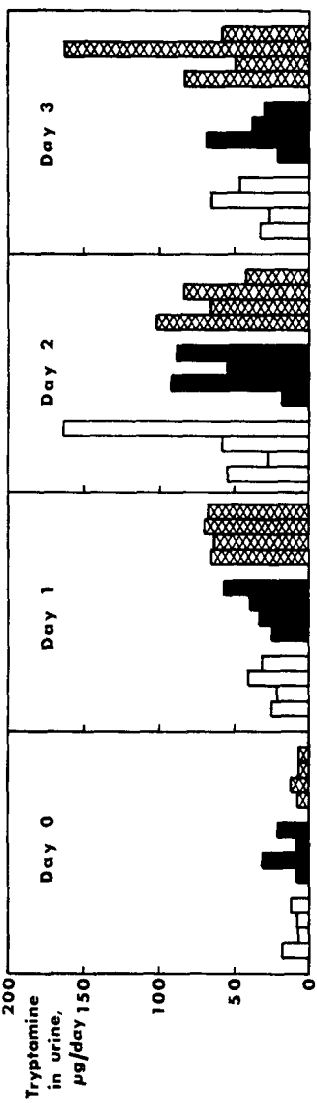


FIG. 7. Urinary excretion of tryptamine by dogs treated with 51641. Four dogs per group received daily oral doses of 51641. Twenty-four-hr urine collections were made and stored frozen prior to analysis. Tryptamine values for individuals shown in the same order for each day.

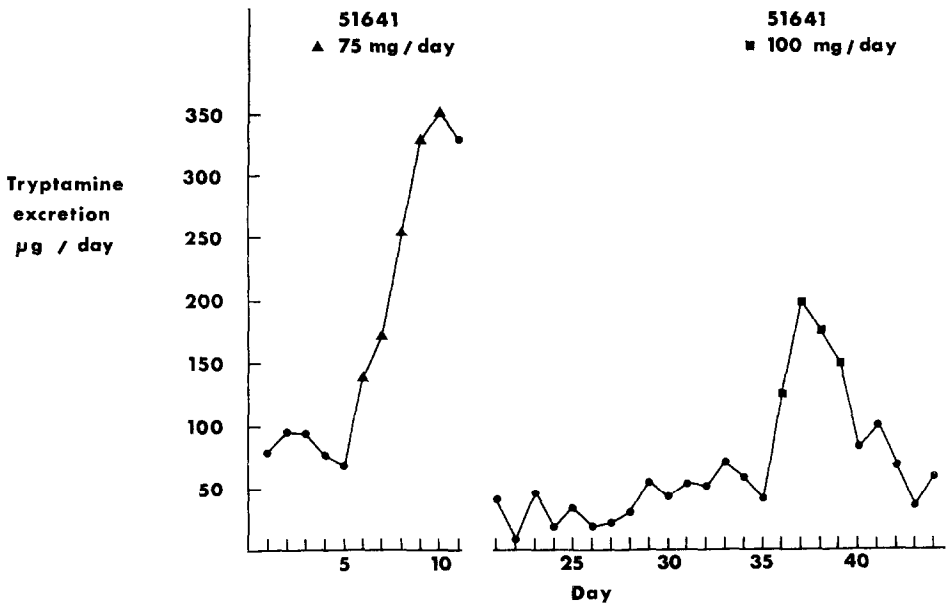


FIG. 8. Urinary excretion of tryptamine by a human subject receiving 51641. The dots show tryptamine during periods when the drug was not given, and the triangles and squares are for days on which drug was administered. Twenty-four-hr urine collections were made and stored frozen prior to analysis.

related to enzyme concentration. On the other hand, 51641 and others in the series gave plots similar to those for the reversible inhibitors, harmaline and amphetamine.

Another interesting property of several inhibitors in the 51641 group is that they block more effectively the oxidation of certain substrates, notably indolealkylamines, as compared to phenylalkylamines.

The effective inhibition of MAO *in vivo* by 51641 was evident from the increase in rat brain serotonin concentration and the increased urinary excretion of tryptamine in three species. One clinical report on 51641 as a potential therapeutic agent was encouraging.[10]

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