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Selective *in Vivo* Inhibition of Monoamine Oxidase in Rat Tissues by *N*-[2-(*o*-Chlorophenoxy)-Ethyl]-Cyclopropylamine (40197)

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N-[2-(*o*-Chlorophenoxy)-ethyl]-cyclopropylamine hydrochloride, Lilly 51641, has been described earlier as an inhibitor of monoamine oxidase (MAO) (1, 2). The degree of MAO inhibition by 51641 both *in vitro* and *in vivo* was dependent on the substrate used for MAO assay, e.g. serotonin oxidation was inhibited more than phenylethylamine oxidation. According to the terminology of Johnston (3), 51641 is a selective inhibitor of type A MAO. We are describing here some further studies of 51641 as an inhibitor of MAO in rat tissues *in vivo,* using serotonin oxidation as an index of type A MAO activity and phenylethylamine oxidation as an index of type B MAO activity (4). These studies extend previous observations on 51641 as a selective type A MAO inhibitor and reveal that not only are higher doses of 51641 required to inhibit type B MAO *in vivo* but also that the duration of type B MAO inhibition is much less than that of type A MAO inhibition.

Methods. Male Wistar rats weighing 130–150 g from Harlan Industries, Cumberland, IN, were given food and water *ad libitum.* Lilly 51641 was injected ip in aqueous solution at an injection volume of 1 ml/kg. Rats were decapitated in groups of 5; tissues were rapidly removed, frozen on dry ice, and stored at −15° prior to analysis. MAO activity in whole homogenates of tissues was assayed with 80 μM [^{14}C]phenylethylamine or 100 μM [^{14}C]serotonin as substrate (5). The radiocarbon-labeled substrates were obtained from New England Nuclear Corporation. MAO activity was calculated as μmoles/g tissue/min of incubation at 37°. The tissue MAO activity for each treated rat was expressed as a percentage of the control mean; mean values of these percentages and their SE were calculated for each group of five rats. Comparisons between groups were made by Student's *t* test.

Results. Figure 1 shows the inhibition of types A and B MAO 1 hr and 24 hrs after injection of 51641 at three different dose levels. In all cases there was statistically significant ($P < 0.05$) inhibition of MAO activity. Greater inhibition of type A MAO (serotonin as substrate) than of type B MAO (phenylethylamine as substrate) was observed in brain at both time intervals. A lesser degree of selectivity was observed in liver, and still lower selectivity in heart, though even in heart inhibition of the oxidation of serotonin was greater than that of phenylethylamine at each dose.

The duration of MAO inhibition in brain after injection of 51641 at 30 mg/kg is shown in Fig. 2. The inhibition of type A MAO activity (serotonin as substrate) persisted over a long time period, having a half-life of approximately 6 days. The inhibition of type B MAO (phenylethylamine as substrate) contrasted sharply. Type B MAO decreased biphasically with an initial half-life of only 8 hr followed by a second phase of decline with a half-life of just under 2 days.

The results in liver were generally similar (Fig. 3). Type A MAO inhibition decreased with a half-life of nearly 3 days, whereas type B MAO inhibition decreased with a half-life of only 11 hr. A second phase of type B MAO inhibition was not detected, since after 24 hr there no longer was any statistically significant inhibition of phenylethylamine oxidation in liver.

Figure 4 shows the time-dependence of MAO inhibition in heart. The inhibition of both serotonin and phenylethylamine oxidation lasted for relatively long times, with half-lives of approximately 6 and 4 days, respectively. Thus inhibition of phenylethylamine oxidation in this tissue persisted for much longer times than in brain and liver.

Discussion. These data confirm and extend earlier published results from our own laboratory (1, 2, 5–7) and others (8) showing that 51641 is a selective inhibitor of type A MAO

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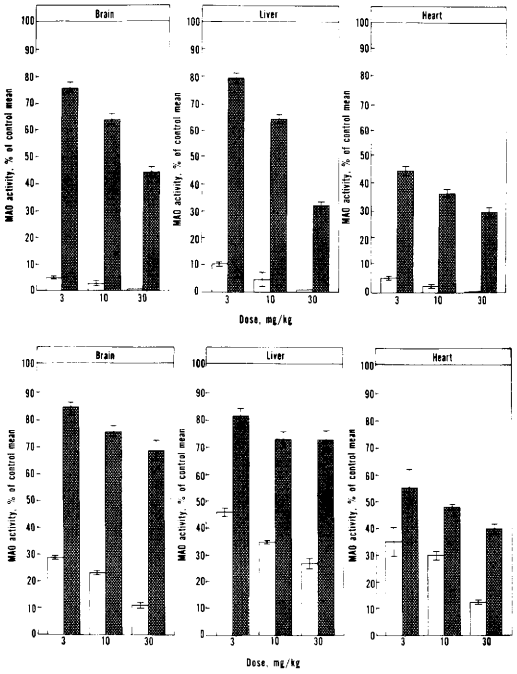

FIG. 1. Dose-dependence of the inhibition of MAO at 1 hr (top) and at 24 hr (bottom). Enzyme activity was determined with serotonin (open bars) or phenylethylamine (cross-hatched bars) as substrate as described in the *Methods* section. Mean values ± SE for five rats per group are shown.

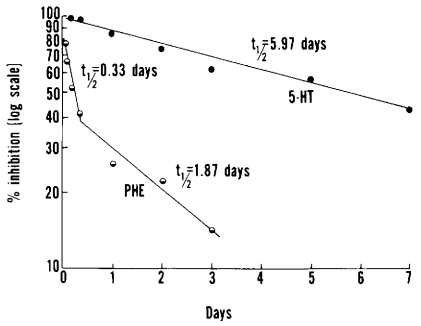

FIG. 2. Duration of the inhibition of brain MAO action on serotonin (5-HT) and phenylethylamine (PHE). Enzyme activity in the control groups was 92 ± 3 μmoles/g/min with 5-HT as substrate and 63 ± 4 μmoles/g/min with PHE as substrate. Percent inhibition values (calculated as percentage of the control mean) are means for five rats per point. SE of these percentages were 5% of the mean value for each group or less. Half-lives were calculated by a least squares method of determining the slope of best fit to the data points.

in rats. Such selectivity was manifest by greater inhibition of MAO action on serotonin than on phenylethylamine in all three

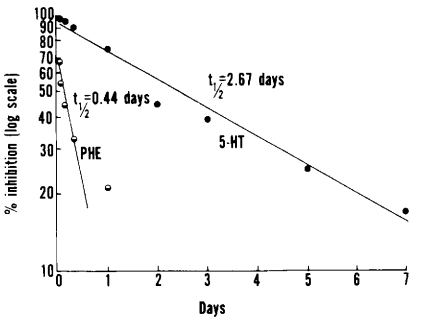

FIG. 3. Duration of the inhibition of liver MAO action on serotonin (5-HT) and phenylethylamine (PHE). Enzyme activity in the control groups was 291 ± 18 μmoles/g/min with 5-HT as substrate and 587 ± 37 μmoles/g/min with PHE as substrate. See Fig. 2 legend for other details.

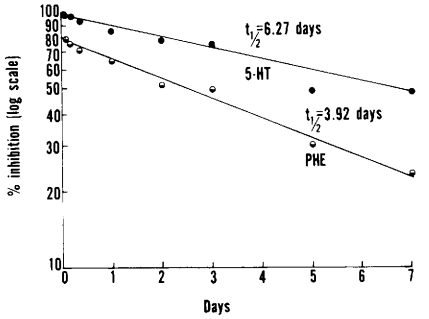

FIG. 4. Duration of the inhibition of heart MAO action on serotonin (5-HT) and phenylethylamine (PHE). Enzyme activity in the control groups was 85 ± 6 μmoles/g/min with 5-HT as substrate and 20 ± 2 μmoles/g/min with PHE as substrate. See Fig. 2 legend for other details.

tissues studied. Not only was serotonin oxidation inhibited at lower doses of 51641 than was phenylethylamine oxidation, but also the duration of inhibition of serotonin oxidation was longer than the duration of inhibition of phenylethylamine oxidation after 51641 injection. Both in brain and in liver, the inhibition of phenylethylamine oxidation had an initial half-life measured in days. Apparently the *in vivo* inhibition of type B MAO by 51641 is not irreversible as is the inhibition of type A MAO. In brain, a second slower phase of type B MAO inhibition was detected and may indicate that a slight degree of irreversible type B MAO inhibition does occur. The inhibition of phenylethylamine oxidation in heart followed a time course much more like that of serotonin oxidation; other work has suggested that phenylethylamine

oxidation in heart may occur by type A rather than type B MAO (9).

Egashira *et al.* (10) had observed that a similar selective inhibitor of type A MAO, clorgyline, reacted rapidly with type A MAO in an irreversible manner *in vitro* whereas it reacted much more slowly with type B MAO. The differences between the degree and duration of inhibition of phenylethylamine and serotonin oxidation that we observed with 51641 may relate to the rate at which it reacts with types A and B MAO *in vivo*.

Summary. Both at 1 and at 24 hr, 51641 inhibited serotonin oxidation to a greater extent than phenylethylamine oxidation in brain, heart and liver when it was injected at different dose levels into rats. After a 30 mg/kg dose, 51641 produced a long-lasting inhibition of serotonin oxidation in all three tissues (half-life 3–6 days). In contrast, the initial half-life of inhibition of phenylethylamine oxidation was only 0.3–0.4 days in brain and liver (4 days in heart). Apparently 51641 does not inhibit type B MAO irreversibly *in vivo*.

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