of protein in the lysosomal fraction. These are low concentrations resulting from very small doses of Δ^9-THC. It has been estimated that a chronic user of marihuana or hashish may ingest several hundred mg of Δ^9-THC/day [9]. We would expect from the present study that substantial quantities of cannabinoid metabolites would accumulate within tissue lysosomes in these individuals. Our previous studies have indicated that Δ^9-THC and related cannabinoids have a disruptive effect on rat liver lysosomes *in vitro* at concentrations from 25 μM to 1·0 mM [3].

These observation may explain some of the clinical evidence that chronic marihuana use leads to hepatotoxicity and cirrhosis [10]. Damage to lysosomes by cannabinoids may also be the basis of the reduced cellular immunity seen in chronic users of marihuana and hashish [11], since cellular immunity is mediated in part through lysosomal involvement in the immune response [12].

Department of Chemistry,
University of Guelph,
Ontario N1G 2W1, Canada

JEAN E. IRVIN
ALAN MELLORS

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Comparison of the inhibitory effects of propargylamine and pargyline on brain and liver monoamine oxidase activity*

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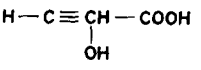
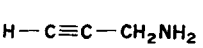
Studies on the mechanism of action of flavoproteins have led to the conclusion that substrate analogs containing an acetylenic function α,β to the carbon which undergoes oxidation are irreversible inactivators [1, 2]. We have shown that α-OH-butynoic acid (I) inactivates lactate oxidase [1], and that propargylamine (II) inactivates beef mitochondrial amine oxidase. These compounds form a covalent complex between the inactivator and the enzyme-bound flavin.

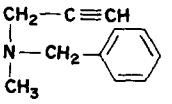
Pargyline (III) is a well-established inactivator of mitochondrial amine oxidase [3], and it has recently been shown that this compound forms an adduct with flavin [4]. It is very probable that propargylamine and pargyline act through the same mechanism and that this mechanism involves the reactivity of the acetylenic group. Our previous studies have shown that propargylamine is a potent inhibitor of monoamine oxidase (MAO) activity in intact pituitary cells in culture [5]. The purpose of the present investigation was to extend these observations on propargylamine to the intact animal, and to determine if the structural differences between pargyline and propargylamine affect their activities *in vivo* on mouse brain and liver MAO.



(I)



(II)



(III)

Albino mice (Swiss Webster strain) of both sexes, weighing 23–30 g, were obtained from Charles River Laboratory. Propargylamine or pargyline was injected intraperitoneally at doses of either 250 or 500 μg/mouse. Nine separate experiments were performed, and similar results were obtained in

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each experiment. At various time intervals (1 hr to 21 days) after injection, groups of mice were sacrificed and liver and brain were rapidly removed. The tissues were either extracted immediately for assay of MAO, or they were frozen at −20° and were extracted within 72 hr. There was no detectable loss of MAO activity in the frozen tissues. Samples of left temporal cortex and of liver were homogenized in a glass tissue grinder with ice-cold 0·1 M Na^+/K^+ phosphate buffer, pH 7·8 (10 ml/g wet wt). The whole homogenate was used without centrifugation. MAO activity was determined by measuring the rate of oxidation of [^{14}C]tyramine [6]. Each determination was performed in duplicate.

Propargylamine HCl obtained from Aldrich Chemical Co. was recrystallized from ethanol–ether. Pargyline was a gift of Abbot Laboratories, North Chicago, Ill. Tyramine hydrobromide-[1-^{14}C] was purchased from New England Nuclear Corp.

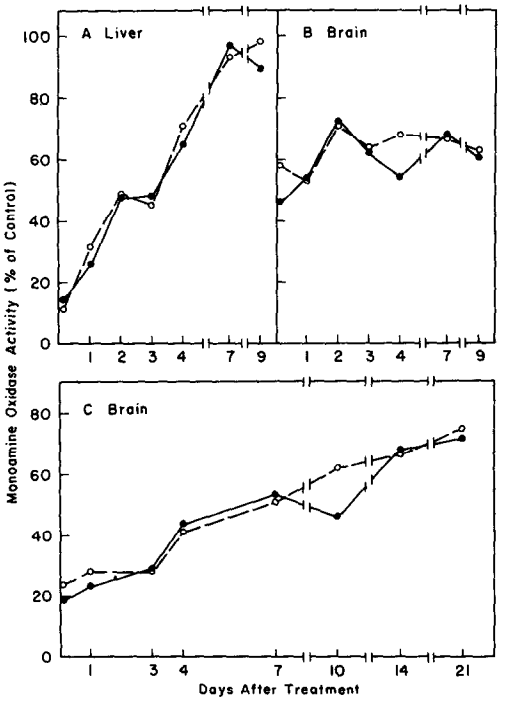

Fig. 1. Monoamine oxidase activity in mouse liver and brain at various time intervals after treatment with propargylamine (○---○) or pargyline (●——●). Results of two experiments are given. In the first experiment (panels A and B), propargylamine and pargyline were each administered at 250 μg/mouse. In the second experiment (panel C), both drugs were given at 500 μg/mouse. The drugs were given at zero time, and the first data points shown were at 1 hr. In experiment one, the mean ± S.E. control MAO activity was 8·9 ± 0·38 × 10^5 cpm/g of liver and 1·8 ± 0·07 × 10^5 cpm/g of brain; all mean experimental values were significantly less than control ($P < 0·01$) except the 7- and 9-day results with liver (panel A). In experiment two, the mean ± S.E. control MAO activity was 1·6 ± 0·03 × 10^5 cpm/g of brain; all experimental values were less than control ($P < 0·001$).

The results presented in Fig. 1 show that propargylamine and pargyline are equally effective in inhibiting MAO activity in mouse liver (panel A); they are also equally effective in inhibiting enzyme activity in brain (panel B). However, at the dose level used in this experiment, 250 μg/mouse, the liver enzyme was inhibited to a greater extent than brain MAO. This experiment also revealed another difference between the liver and brain enzymes. Liver MAO activity returned fully to the control level by 7 days after a single dose of inhibitor, while the brain enzyme showed no appreciable recovery during this period (Fig. 1, panels A and B). In the experiment shown in panel C (Fig. 1), a larger dose (500 μg/mouse) of each inhibitor was used, and the experiment was performed over a more extended time period. Even 21 days after a single dose of inhibitor, the brain enzyme activity had not fully returned to control levels. In these long-term experiments, propargylamine and pargyline produced the same effects. This finding would be expected if both compounds act through covalent modification of the enzyme-bound flavin. Thus, the inhibitory effects of propargylamine and pargyline *in vivo* on both liver and brain MAO are consistent with the mechanism proposed from studies *in vitro* of isolated enzyme [2].* The prolonged inhibitory effects observed with brain MAO confirm previous experiments [7] which suggested that the brain enzyme is renewed at a rate which is considerably slower than that of liver MAO.

Knowledge of the time-course and dose-response characteristics of the inhibition of MAO activity in liver and brain *in vivo* makes it possible to design experiments in which, at short-time intervals, liver MAO activity is preferentially inhibited and, at long-time intervals (7–10 days after treatment), brain MAO activity is inhibited about 50 per cent, while liver enzyme appears to have recovered fully.

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Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154, *and* ROBERT H. ABELES
ARMEN H. TASHJIAN, JR.
Laboratory of Pharmacology, Harvard School of Dental Medicine and Department of Pharmacology, Harvard Medical School, Boston, Mass. 02115, *U.S.A.*

* A. Maycock, W. H. Salach, T. P. Singer and R. H. Abeles, unpublished observations.

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