sor M. Pulkkinen, and Professor O. Hänninen, and the able technical assistance of Miss Leena Ruokonen is gratefully acknowledged. Grant: U.S Public Health Service (AM. 06018-11).

Department of Physiology,
University of Turku,
Turku, Finland

ANTERO AITIO

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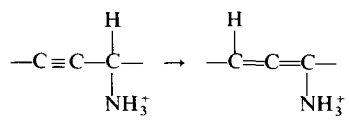
Biochemical Pharmacology, Vol 23, pp 2205–2207 Pergamon Press, 1974 Printed in Great Britain

Inhibition of monoamine oxidase activity by propargylamine in pituitary cells in culture: lack of effect on cell growth or prolactin production*

(*Received* 29 *October* 1973; *accepted* 21 *December* 1973)

A NUMBER of acetylenic compounds, for instance pargyline, inhibit monoamine oxidase (MAO) activity.[1] It has been proposed that the basis of the inhibition is the ability of these compounds to rearrange to allenes upon interaction with the active site of the enzyme.[2] The allene then reacts with functional groups at the active site



to bind covalently and inactivate the enzyme. Accordingly, the simplest compound which should inhibit MAO is propargylamine ($CH \equiv C—CH_2NH_2$). We have found that propargylamine inactivates and covalently labels mitochondrial monoamine oxidase† and plasma amine oxidase *in vitro*.[2] The compound also

* This investigation was supported in part by research grants from the American Cancer Society (BC-13) and the National Institute of Arthritis, Metabolism and Digestive Diseases (AM 11011).

† T. SINGER, H. J. SALLACH, A MAYCOCK and R. H. ABELES, unpublished data.

inactivates monoamine oxidase in cells grown in culture as well as in the liver and brain of intact mice and rats The activity of propargylamine in mice is comparable to that of pargyline * In this communication, we report the effect of propargylamine on a strain of functional pituitary cells in culture.

GH_4 cells are a subclone of the GH_3 strain of rat pituitary tumor cells.[3,4] GH_4 cells produce no measurable growth hormone in the basal unstimulated state, but they synthesize and secrete large quantities of prolactin [5] Cells were grown in plastic tissue culture dishes (Falcon) at 37° in a humidified atmosphere of 5% CO_2 and 95% air. The medium was Ham's[6] F 10 supplemented with 15% horse serum and 2 5% fetal calf serum. All experiments described in this report were performed with GH_4 cells in the logarithmic phase of cell growth [4]

Experiments were performed by growing cells to the desired density (mid-logarithmic phase). Medium was removed and fresh medium alone or fresh medium containing propargylamine (at the concentrations described) was added at zero time, and the cultures were incubated for an additional 3–4 days. Medium was collected for prolactin assay, and the cells were washed with 0 15 M NaCl and harvested for amine oxidase assay (see below) and protein determination by the method of Lowry *et al.*[7]

Prolactin was measured in culture medium by a specific microcomplement fixation immunoassay method [4] Production of prolactin is defined as the amount of prolactin that accumulates in the culture medium per mg cell protein per 24 hr.[4]

The rate of oxidation of [^{14}C]tyramine was taken as a measure of amine oxidase activity.[8] Cells were scraped from the dishes and suspended in 0·1 M Na^+/K^+ phosphate buffer, pH 7·8, at a final protein concentration of 1–2 mg/ml. Cells were broken in a glass homogenizer at 4°, and 0·3 to 0 5 ml of the whole homogenate was used in the enzyme assay. Each determination was performed in duplicate.

Propargylamine HCl obtained from Aldrich Chemical Co. was recrystallized from ethanol–ether. Tyramine hydrobromide[1-^{14}C] was purchased from New England Nuclear Corp.

The data in Table 1 show the activities of MAO in GH_4 cells grown under control conditions and in cells treated for 3–4 days with 200 μM propargylamine. Enzyme activity in cells treated with propargylamine was markedly reduced. Propargylamine and pargyline have the following structural feature in common: $H_3N—CH_2—C\equiv C—$. The efficiency of propargylamine *in vitro* and *in vivo* as an inhibitor of MAO

TABLE 1. MONOAMINE OXIDASE (MAO) ACTIVITY IN CONTROL AND PROPARGYLAMINE-TREATED GH_4 CELLS

MAO activity		% Inhibition of MAO activity (range)
Control* (cpm × 10^{-2}/mg cell protein)	Propargylamine (200 μM)†	
4·0 ± 0·50	<0·10 to 0·40	89 to >95

* Mean value ± S.E.; ten separate experiments.
† Range, four separate experiments.

provides further evidence that this structural feature is responsible for inactivation of the enzyme Evidence that the inhibition of MAO activity was not a nonspecific toxic effect of the drug on the cells was of two sorts. First, propargylamine did not reduce significantly total cell protein per culture dish, a sensitive measure of cell growth and viability.[3,4] In four experiments, the mean (±S.E.) total cell protein in control cultures was 1·55 ± 0 13 mg/dish, and in cultures treated with propargylamine it was 1·38 ± 0·13 mg/dish. Second, the data in Fig. 1 show the relationship between the concentration of propargylamine in medium, MAO activity in GH_4 cells, and prolactin production. It is noteworthy that inhibition of MAO activity by propargylamine was not associated with any decrease in the production of prolactin.

The findings described in this report extend to intact cells results of previous experiments *in vitro* on the inactivation of mitochondrial MAO and plasma amine oxidase by propargylamine.[2] This simple acetylenic compound inhibited, to less than 5 per cent of control, the activity of the MAO that is present in prolactin-producing rat pituitary cells in culture. This model system *in vitro* was chosen for study because of the now substantial body of evidence that indicates that biogenic amines play a role in the control of prolactin secretion by the pituitary gland.[9] That marked inhibition of MAO activity was not associated with any decrease in total protein per culture dish or in the production of prolactin indicates that the activity of the enzyme is not essential for the growth of GH_4 cells or for the synthesis and secretion of prolactin by these cells. Although the GH-cell system has proven useful as a model in a variety of phar-

* A. H. TASHJIAN, JR and R. H. ABELES, unpublished data.

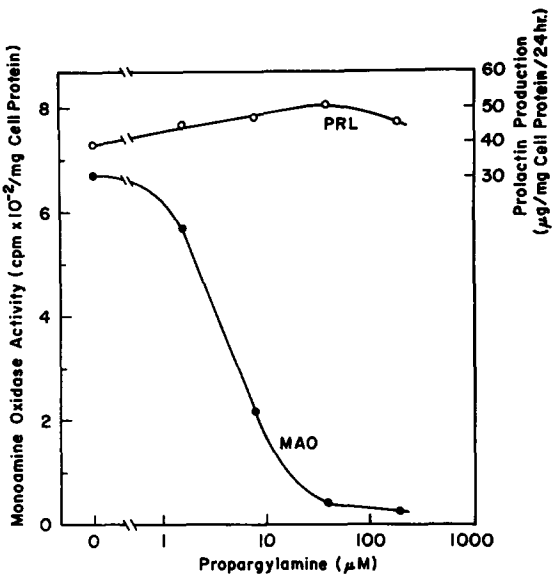


FIG. 1. Effects of propargylamine on monoamine oxidase activity (MAO) and prolactin (PRL) production by GH_4 cells. Each point gives the mean value of duplicate determinations.

macological and biochemical studies,[4,5,10,11] we do not conclude, until it has been tested directly, that inhibition of MAO in lactotrophs will have no effect on prolactin secretion in the intact animal.

Acknowledgement—The authors thank Miss Susan M. Fish for her assistance in these experiments.

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

ROBERT H. ABELES
ARMEN H. TASHJIAN, JR.

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