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SHORT COMMUNICATIONS

Distinct forms of monoamine oxidase expressed in hepatoma and HeLa cells in culture

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Two functional forms of monoamine oxidase (MAO, monoamine:O_2 oxidoreductase EC 1.4.3.4) can be identified by their drug sensitivity and substrate specificity. Monoamine oxidase of the A type is inhibited by low concentrations of clorgyline [1] and preferentially deaminates 5-hydroxytryptamine and norepinephrine [2]. The B type is inhibited by low concentrations of deprenyl [3] and deaminates phenylethylamine and benzylamine [2]. Physiologic studies *in vivo* with clorgyline and deprenyl support the biochemical evidence obtained *in vitro* that there are at least two functional forms of MAO [4]. The proportions of these two forms of MAO vary in tissues from different species [3, 5, 6]. Dose–response curves with inhibitors have been obtained primarily using homogenates of tissues which contain a number of different cell types. Tipton *et al.* [7], however, assayed homogenates of a homogeneous population of parenchymal cells from rat liver and found an equal distribution of A and B types of MAO activity, indicating that both types of activity can exist in the same cell. Homogenates prepared from cultured rat glioma [8] and mouse neuroblastoma cells [9] contain only type A activity, while those from human HeLa cells contain only type B activity [10]. In previous studies [11, 12], we have looked at the deamination of tryptamine in homogenates from several different cultured cell lines. Most cells were found to have predominantly type A activity, with the exception of a rat hepatoma line MH_1C_1 with both A and B types of activity [11, 13].

The method of preparing tissue samples for assays *in vitro* may affect the characteristics of monoamine oxidase [6, 14, 15]. We have studied monoamine oxidase activity in living cells in culture in order to minimize alterations which may occur secondarily to cell damage. In the present investigation we have used specific substrates and inhibitors to describe the co-existence of A and B types of MAO activity in living rat hepatoma cells and the presence of B activity in living human HeLa cells. We have confirmed this distribution of MAO activity in homogenates and crude mitochondrial preparations from these two cell types.

Rat hepatoma line MH_1C_1 was obtained from the American Type Culture Collection (Cat. No. CCL 144). Human HeLa line Bu25 was obtained from Dr. Saul Kit (Baylor University College of Medicine, Houston, TX). Cells were grown as monolayers on plastic tissue culture dishes (100 mm or 150 mm, Falcon, Oxnard, CA) in Dulbecco's modified Eagle Medium (DMEM, No. H21, Grand Island Biological Co., Grand Island, NY) supplemented with 10% (v/v) fetal calf serum (Flow Laboratories, Rockville, MD) without antibiotics. Cultures were maintained at 37° in a humidified atmosphere of 5% CO_2 and 95% air. Cells were fed every 3–4 days and subcultured (1:3 ratio) every 14 days by treating for 3–5 min with 1X Viokase (Grand Island Biological Co.) and then resuspending by gentle trituration.

Cell homogenates were prepared in 0.1 M potassium phosphate buffer, pH 7.4, as described previously [11]. Homogenates were frozen and stored in liquid nitrogen or assayed immediately after homogenization at 0° in a Potter–Elvehjem glass homogenizer. Crude mitochondrial fractions were prepared by resuspending cells in a swelling buffer (1.5 mM calcium chloride, 10 Mm sodium chloride and 10 mM Tris–HCl, pH 7.5) using 10–15 ml buffer/g wet weight of cells. The suspension was stored on ice for 30 min and then homogenized as above. The tonicity of the suspension was increased by adding one-seventh volume of 7X TES buffer (2 M sucrose, 35 mM EDTA, 50 mM Tris–HCl, pH 7.5). Unbroken cells and nuclei were separated by centrifugation at 1160 *g* for 5 min. This pellet was resuspended in 1X TES buffer and the centrifugation repeated. The two supernatant solutions were pooled and centrifuged at 8100 *g* for 15 min. The pellet containing mitochondria was resuspended in 0.1 M potassium phosphate buffer, pH 7.4 (5–10 mg protein/ml) and assayed immediately.

Monoamine oxidase activity was measured using 5-hydroxy[side-chain2-^{14}C]tryptamine creatinine sulfate (54 mCi/m-mole), [G-^{3}H]tryptamine (1 Ci/m-mole) from Amersham-Searle, Arlington Heights, IL, 5-[1, 2-^{3}H(N)]hydroxytryptamine creatinine sulfate (25.6 Ci/m-mole), or β-[ethyl-2-^{14}C]phenylethylamine HCl (51 mCi/m-mole) from New England Nuclear, Boston, MA. Activity in cell homogenates was assayed in triplicate by incubating 40-μl aliquots with 0.1 M potassium phosphate buffer, pH 7.4, containing 250 μM [^{14}C]5-hydroxytryptamine (13 μCi/μmole) or 10 μM [^{14}C]phenylethylamine (5.0 μCi/μmole), in a final volume of 400 μl, as described previously [13]. Blank values were established by adding 50 μl of 0.4 N HCl to the reaction mixture at time zero. When inhibitors were used, the samples were preincubated for 15 min at 25° with clorgyline (Dr. Sabit Gabay, V. A. Hospital, Boston, MA) or deprenyl (Professor J. Knoll, Sammelweis University of Medicine, Budapest, Hungary). (Similar results were obtained with 10–20 min preincubation periods.) Reactions were run for 1 hr at 37° and stopped by adding 50 μl of 0.4 N HCl. Following incubation, 0.3-ml aliquots were applied to a 0.5 × 24 cm cation exchange resin column (Bio-Rex 70, 100–200 mesh Na^+) and the reaction products were eluted from the column with 2.5 ml of distilled water. Monoamine oxidase activity in mitochondrial fractions was measured as described previously [11] using 40 μM [^{3}H]tryptamine (250 μCi/μmole). Activities were assayed within the range of linearity for protein and time.

When analyzing activity in living cells, cultures were grown to 100 per cent confluency on 150 mm dishes. At the time of assay, cells were gently scraped off the dish into 3–4 ml of conditioned medium with serum. An atmosphere of 5% CO_2 and 95% air was maintained throughout. One hundred μl portions of the cell suspension were incubated with 10 μl of isotope diluted 1:10 in medium containing 1 mM ascorbic acid. MH_1C_1 cells were incubated with 25 μM [^{3}H]5-hydroxytryptamine for 60 min or with 20 μM [^{14}C]phenylethylamine for 20 min; with Bu25 cells the incubation with [^{14}C]phenylethylamine was for 60 min. Inhibitors were preincubated with cells for 10 min. All incubations were at 37°. Activities were assayed within the range of linearity for protein and time. The incubation was terminated by adding 10 μl of 1 N acetic acid containing 1 mM

ascorbic acid. Samples were held on ice for 30 min and stored frozen. Twenty μl portions (50,000–150,000 cpm) were chromatographed with authentic standards on thin-layer cellulose (Eastman 6064) in a solvent of 1-butanol–1 N acetic acid–95% ethanol (3.5:1:1, v/v) [16]. The distribution of radioactivity was determined as described previously [17]. The radioactivity recovered from the chromatograms represented approximately 50 per cent of the amount applied. In both this solvent system and another one consisting of n-propanol–water–concentrated ammonia (64:8:8, v/v) [18], only two peaks of radioactivity were observed, one migrating with 5-hydroxytryptamine or phenylethylamine and one migrating with the deaminated products, 5-hydroxyindole acetic acid or phenylacetic acid respectively.

Protein was determined by a modification of the method of Lowry *et al.* [19] using bovine serum albumin as a standard.

Monoamine oxidase activity was assayed in homogenates of rat hepatoma and human HeLa cells using 5-hydroxytryptamine and phenylethylamine (Table 1). In hepatoma cells activity was greater against 5-hydroxytryptamine than phenylethylamine. In HeLa cells, activity was observed only against phenylethylamine.

In living hepatoma cells, deamination of 5-hydroxytryptamine is 400-fold more sensitive to inhibition by clorgyline (pI_{50} 7.8) than deprenyl (pI_{50} 5.2) (Fig. 1). (The pI_{50} value is the log of the inverse concentration of the drug which produces 50 per cent inhibition.) A similar pattern of inhibition is seen in homogenates with pI_{50} values of 8.1 for clorgyline and 5.2 for deprenyl. These monophasic inhibition curves show that 5-hydroxytryptamine is metabolized almost exclusively by MAO type A activity in living cells as in homogenates.

Table 1. Monoamine oxidase activity in homogenates of rat hepatoma (MH_1C_1) and human HeLa (Bu25) cells*

Substrate	MH_1C_1 (nmoles/min/mg protein)	Bu25 (nmoles/min/mg protein)
5-Hydroxytryptamine	1.4 ± 0.3	<0.0005
Phenylethylamine	0.023 ± 0.001	0.007 ± 0.002

* Values represent the mean of three to five determinations ± S.E.M.

Figure 2 shows a dose–response curve of clorgyline and deprenyl inhibition of monoamine oxidase activity in living MH_1C_1 cells and homogenates using phenylethylamine as the substrate. In living cells phenylethylamine is 400 times more sensitive to inhibition by deprenyl (pI_{50} 6.8) than by clorgyline (pI_{50} 4.2). In homogenates, activity is also more sensitive to deprenyl (pI_{50} 5.8) than to clorgyline (pI_{50} 4.8). The characteristics of inhibition suggest that phenylethylamine is metabolized only by type B activity in these cells.

In living Bu25 cells monoamine oxidase activity against phenylethylamine is more sensitive to inhibition by deprenyl (pI_{50} 7.1) than by clorgyline (pI_{50} 4.8) (Fig. 3). In homogenates, activity is also more sensitive to deprenyl (pI_{50} 9.6) than to clorgyline (pI_{50} 5.1). Phenylethylamine appears to be metabolized by type B activity in these cells.

As a further confirmation that both forms of MAO are functionally active in hepatoma cells, mitochondrial preparations were incubated with tryptamine, a substrate for both MAO A and B activity. A biphasic curve of inhibition was observed with varying concentrations of clorgyline, indicating the presence of 80 per cent type A and 20 per cent type B activity (Fig. 4). With deprenyl we did not observe a distinct biphasic curve, probably due to the similar inhibition kinetics of type A and B activities with this drug. These findings agree with previous observations using tryptamine with living cells and homogenates [11]. Drug inhibition of tryptamine metabolism was also analyzed using mitochondria prepared from HeLa cells. Monoamine oxidase activity was more sensitive to inhibition by deprenyl than by clorgyline (Fig. 4). Curves were monophasic and suggest all type B activity. However, given the low levels of MAO activity in the HeLa cells, we would not have been able to detect type A activity comprising less than 10 per cent of the total.

The present study was undertaken to verify the presence of both A and B types of monoamine oxidase activity in living homogeneous cell populations. Our observations indicate that two types of MAO activity co-exist in living rat hepatoma cells, as well as in homogenates and mitochondrial preparations, and support the current A-B model of activity [1]. Tipton *et al.* [7] report that both A and B types of MAO

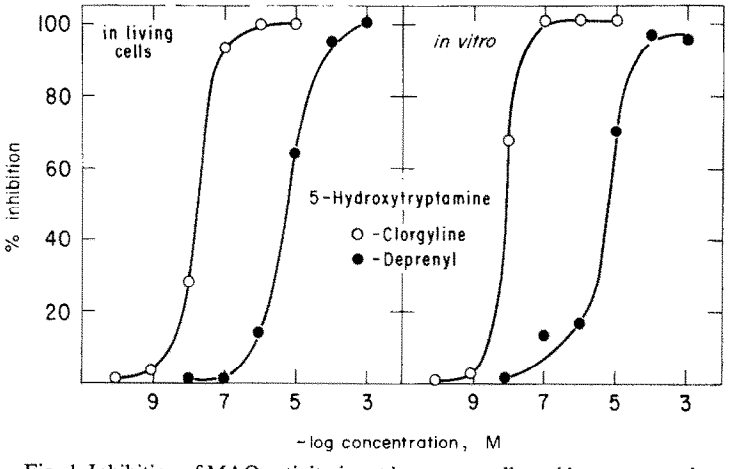

Fig. 1. Inhibition of MAO activity in rat hepatoma cells and homogenates by clorgyline and deprenyl using 5-hydroxytryptamine. In living cells, 100-μl portions of cell suspension (0.5 mg of cellular protein) were incubated for 60 min at 37° without drugs, and 40 per cent of the radioactivity was recovered from the chromatogram as 5-hydroxyindole acetic acid. In homogenates assayed *in vitro*, control activity corresponds to 1.1 nmole/min/mg of protein. Data are shown from one of two to three similar experiments. In other experiments, pI_{50} values were 7.7 for clorgyline and 5.7 for deprenyl in living cells, and 8.8 and 9.7 for clorgyline and 4.9 ± 0.23 (mean ± S.E.M.) for deprenyl in homogenates.

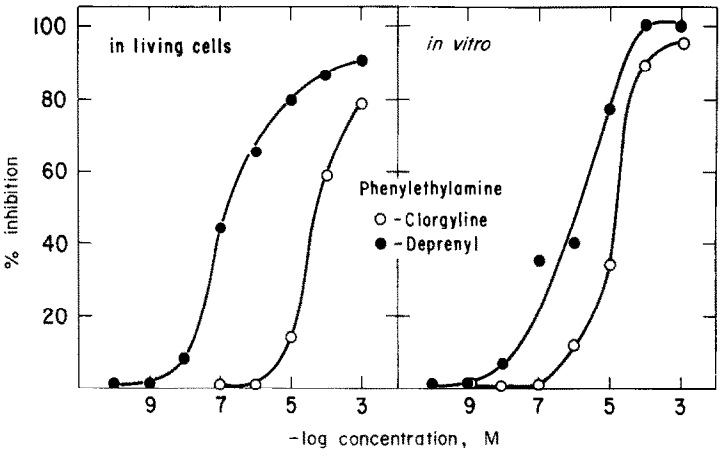

Fig. 2. Inhibition of MAO activity in rat hepatoma cells and homogenates by clorgyline and deprenyl using phenylethylamine. In living cells, 100-μl portions of cell suspension (0.4 mg of cellular protein) were incubated for 20 min at 37° without drugs, and 30 per cent of the radioactivity was recovered from the chromatogram as phenylacetic acid. In homogenates assayed *in vitro*, control activity corresponds to 21 pmoles/min/mg of protein. Data are shown from one of two to three similar experiments. In other experiments, pI_{50} values were 4.3 for clorgyline and 8.0 for deprenyl in living cells, and 4.9 ± 0.16 (mean ± S.E.M.) for clorgyline and 5.2 ± 0.16 (mean ± S.E.M. for deprenyl in homogenates.

activity are present in rat liver parenchymal cells, thought to be the precursor cell type of this hepatoma line. In human HeLa cells only type B activity was detected in living cells, homogenates and mitochondrial preparations.

The presence of two types of MAO activity may reflect isozymic forms of the enzyme arising from expression of different gene loci, post-transcriptional modification, or localization within particular microenvironments.

Cultured cell lines have now been described which contain varying types of MAO activity; all type A (neuroblastoma and glioma) [8, 9, 11], all type B (sarcoma) [10], and both types A and B (hepatoma) [11]. These lines provide the opportunity to study the nature of isozymes and the effects of drugs and environmental conditions on regulation of MAO activity in living cells.

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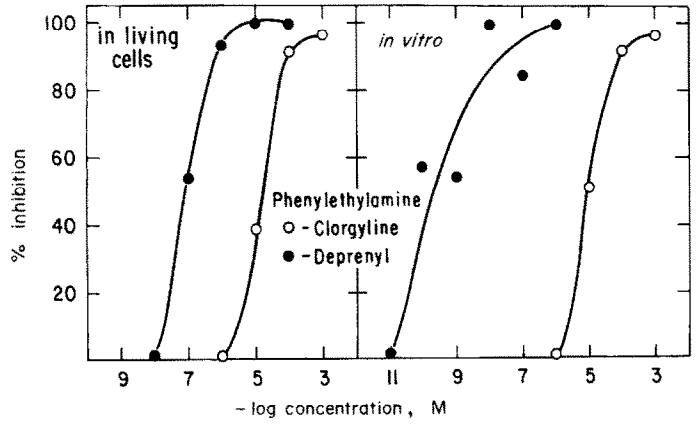

Fig. 3. Inhibition of MAO activity in human HeLa cells and homogenates by clorgyline and deprenyl using phenylethylamine. In living cells, 100-μl portions of cell suspension (0.4 mg of cellular protein) were incubated for 20 min at 37° without drugs, and 13 per cent of the radioactivity was recovered from the chromatogram as phenylacetic acid. In homogenates assayed *in vitro*, control activity corresponds to 2 pmoles/min/mg of protein. Data are shown from one of two similar experiments.

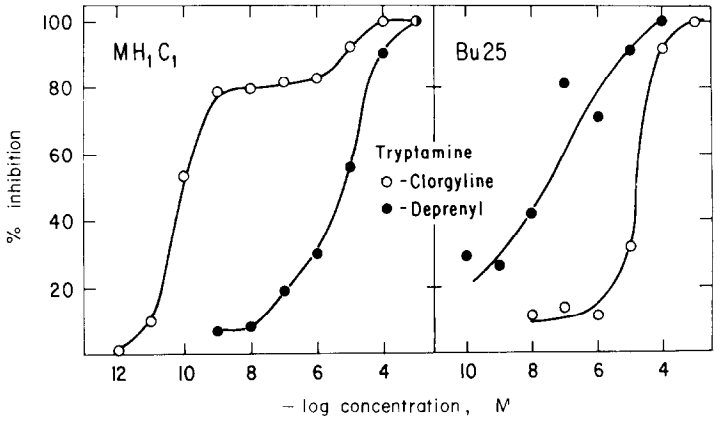

Fig. 4. Inhibition of MAO activity in crude mitochondrial preparations of rat hepatoma MH_1C_1 and human HeLa Bu25 cells using [^{3}H]tryptamine. Control activities were 1.6 and 0.008 nmoles/min/mg of protein in MH_1C_1 and Bu25 respectively. Data are shown from one of two similar experiments.

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Inhibition *in vitro* of rabbit adrenal norepinephrine *N*-methyltransferase by 2,3,4,5-tetrahydro-1H-2-benzazepines

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Previously we reported that phenylethylamine and benzylamine analogs with rigid conformation were inhibitors of norepinephrine *N*-methyltransferase (NMT) (EC 2.1.1.28), the epinephrine-forming enzyme. For example, 2-aminotetralins [1] and 1-aminoindans [2] were more potent inhibitors of norepinephrine *N*-methyltransferase than were their non-cyclized analogs, phenylethylamines [3, 4] and benzylamines [5]. The compounds described here, 2,3,4,5-tetrahydro-1H-2-benzazepines, can be viewed as benzylamine analogs with the amino group connected to the ortho position of the ring via a propyl group. The chlorinated compounds in this series are generally more active inhibitors of norepinephrine *N*-methyltransferase than are the corresponding benzylamines.

Rabbit adrenal glands were purchased from Pel-Freez Biologicals, Inc., Rogers, AR. Norepinephrine *N*-methyltransferase was prepared by ammonium sulfate fractionation of the supernatant fluid after high speed centrifugation of adrenal homogenates and assayed as described previously [6]. Enzyme activity was assayed radiometrically with L-norepinephrine bitartrate (Winthrop) as the methyl-accepting substrate (40 μM unless otherwise indicated) and *S*-adenosyl-L-methionine[methyl-^{14}C] (New England Nuclear) as the methyl donor (20 μM unless otherwise indicated). The formation of radioactive epinephrine was measured after precipitation of the unreacted methyl donor with Reinecke salt (ammonium tetrathiocyanoammonochromate). Inhibitors were tested at four to six concentrations and