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Monoamine oxidase gene transcription in human cell lines: treatment with psychoactive drugs and ethanol

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Summary. In the present study transcriptional activities has been measured with different fragments of the 5′-flanking sequence of the human monoamine oxidase (MAO) genes linked to human growth hormone which was used as a reporter gene. SH-SY5Y neuroblastoma cells and 1242 MG glioma cells were compared under basal conditions as well as after treatments with different drugs. Under basal conditions, the relative reporter activities of the different promoter fragments were similar for both cell lines. No changes in promoter activities, were observed when cells were treated with L-deprenyl, lithium chloride or raclopride. In contrast, increases (2–3-fold) in both reporter gene expression and enzyme activity were observed after ethanol treatment of cells transfected with MAO-B fragments. Gel retardation analysis showed that ethanol caused changes in transcription factor binding to the MAO-B core promoter in both the SH-SY5Y and 1242 MG cell lines in a cell-type specific fashion.

Keywords: Alcohol, growth hormone, neuroblastoma, promoter, reporter gene.

Abbreviations

CNS central nervous system, *GH* growth hormone, *5-HT* 5-hydroxytryptamine, *MAO* monoamine oxidase, *PEA* phenylethylamine.

Introduction

One important pathway for enzymatic deactivation of monoamines is oxidative deamination brought about by monoamine oxidase (MAO; E.C.1.4.3.4.). MAO deaminates a number of endogenous as well as dietary monoamines to their corresponding aldehydes. The endogenous substrates in the central nervous system (CNS) include neurotransmitters such as dopamine, noradrenaline, 5-hydroxytryptamine (5-HT) and trace amines such as phenylethylamine

(PEA), tyramine and octopamine. Two pharmacologically different forms of MAO are expressed in mammals, termed MAO-A and MAO-B. These two forms are encoded by two distinct genes (Bach et al., 1988; Grimsby et al., 1991; Shih et al., 1988) which both are located on the short arm of the X chromosome (Lan et al., 1989). Moreover, the two forms of MAO have different substrate specificities and inhibitor sensitivities. However, the substrate specificities are overlapping rather than absolute (for a review see, Weyler et al., 1990).

The core promoter of both genes has been well characterized by Zhu and coworkers (1992 and 1994). In both cases it appears that a 140–150 bp portion of the immediate 5′-flanking sequence corresponds to the highest promoter activity when linked to the human growth hormone (GH) reporter gene. Notably, both genes display potential binding sites for the transcription factor Sp1 in this portion of their respective 5′-flanking regions. Furthermore, these 0.15 kb fragments are in the region where the MAO-A and MAO-B 5′-flanking sequence share the highest identity. It has been shown that the transcriptional activity of MAO-A is chiefly governed by Sp1-like transcription factors (Zhu et al., 1994), whereas, the factors that regulate the MAO-B gene transcription are currently under investigation.

There is a body of literature showing that certain psychoactive drugs influence MAO activity. For example, MAO-B activity in platelets is significantly altered in psychiatric patients as a consequence of treatment with neuroleptics (Baron et al., 1982; Chojnacki et al., 1981) and lithium salts (Bockar et al., 1974; Chojnacki et al., 1981; Huang et al., 1984). Moreover, an association between MAO activity and ethanol consumption has been proposed by several investigators (Aliyu and Upahi, 1988; Anokhina et al., 1990; Nevo and Parvez, 1994; Tabakoff et al., 1985). Presently, nothing is known about the mechanism for these changes in enzyme activity. Nonetheless, several possibilities exist e.g. changes at the levels of gene transcription, translation or post-translational modification(s), direct effects on the active enzyme protein or alternatively, changes in the cell number in the particular cell populations that were investigated.

The aim of the present investigation was two-fold: a) to compare the transcriptional activity in two different CNS-derived cell lines and b) to study the effect of various drugs on the level of promoter activity. The drugs included were: L-deprenyl, a selective inhibitor of MAO-B, lithium chloride, commonly used in the treatment of bipolar depression, raclopride, a neuroleptic-like drug which is a dopamine receptor antagonist, as well as ethanol in doses that are attained during alcohol intoxication.

Materials and methods

Cell lines

One neuroblastoma cell line, SH-SY5Y, and a glioma (astrocytoma) cell line, 1242 MG, were used for the transfection assays. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 g/L glucose and 10% fetal calf serum (Gibco-BRL, Gaithersburg, MD). Twenty-four hours before transfection experiments, the

glioma cell line (1242 MG) was seeded at a density of 5×10^5 cells per 100 mm dish and the neuroblastoma cell line, SH-SY5Y, at 1×10^6 cells per dish.

Reporter constructs and transfection experiments

DNA fragments containing the immediate 5′ flanking sequences of the MAO-A and MAO-B gene were isolated and cloned into a promoterless transient expression vector, pOGH (Selden et al., 1986). The plasmid constructs used in the present study have been described previously (Zhu et al., 1992). The preparation of plasmid DNA was performed with two consecutive cesium chloride centrifugations at 180,000 g according to the procedure of Sambrook and coworkers (1989). The purified plasmid DNA was stored in TE buffer (10 mM Tris-HCl; 1 mM EDTA), pH 8.0 at −20°C.

Transient transfections were performed using the low pH calcium phosphate coprecipitation procedure described by Chen and Okayama (1986). All values correspond to measurement of GH concentration 24 hours after change of medium and all points were analyzed at least in duplicates in order to reduce dish-to-dish variations. For treatment studies, 5 ml of medium containing the final concentrations of the different drugs was added to the culture dishes and incubated for 24 h before medium samples were taken for GH analysis. Lithium chloride and ethanol were added directly to the medium. Deprenyl and raclopride were added as a 100 X solution (1% ethanol in 0.05 PBS, pH 7.4) and in control cells, the same volume of PBS/ethanol was added without drug.

The amount of GH secreted by transfected cells was measured by a double antibody immunoassay, using a kit purchased from Nichols Diagnostics (San Juan Capistrano, CA). All steps in the radioimmunoassay were carried out according to the instructions of the manufacturer.

Enzyme activity assays

For estimation of MAO catalytic activity, cells were mechanically detached from the dishes, sonified and assayed in a mixture containing 50 mM sodium phosphate buffer, pH 7.4 and 100 μM ^{14}C-labelled serotonin or 10 μM ^{14}C-PEA for the determination of MAO-A and MAO-B activity, respectively. The radioactive substrates were purchased from Amersham (Amersham, UK). The assay was performed at 37° for 20 min and the reaction was terminated by the addition of 3 M HCl. Subsequently, the reaction products were extracted in water-saturated ethyl acetate/toluene (1:1), centrifuged at 1,000 g, and thereafter, the organic phase was removed and mixed with scintillation fluid. The radioactivity of the reaction product was determined by liquid scintillation spectroscopy. The samples were standardized by the estimation of protein concentration in the cell lysates, using the protocol of Lowry and coworkers (1951).

Gel retardation assay

Nuclear proteins were extracted from 1242 MG and SH-SY5Y cells treated with 50 mM of ethanol according to the procedure of Dignam and coworkers (1983), except that the cells were ruptured in buffer A with a polytron (Brinkman Homogenizer PCU 11, Lüzern, Switzerland). As a control, nuclear proteins were extracted from cells without prior ethanol treatment. The MAO-B 0.15 kb *PstI/NaeI* fragment which contains the core promoter, was end-labelled with ^{32}P-dNTP and Klenow polymerase [19]. For the gel retardation assay, 2.5 μg of poly[dA-dT]-poly[dA-dT] and 1.0 μg of poly[dI-dD]-poly[dI-dC] were preincubated with 3 μg of nuclear proteins from ethanol-treated and untreated SH-SY5Y cells for 10 minutes at room temperature in a mixture of 19 μl containing Hepes-NaOH, pH 7.8, 1 mM $MgCl_2$, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT, and 10% glycerol. One μl of the end-labelled DNA (approximately 2 fmol) was added and the incubation was continued for another 20 minutes at room temperature. After mixing with 2.5 μl of loading buffer (250 mM Tris-HCl, pH 7.8, 0.2% xylene cyanol, 0.2% bromophenol

blue, 40% glycerol), the mixture was loaded on a 5% nondenaturing polyacrylamide gel (acrylamide:bisacrylamide, 37.5:1) in 90mM boric acid, 0.1mM EDTA, prerun 10mA for 1 hour. Gel electrophoresis was carried out at 10mA in the same buffer for 1.5 hours. The temperature was below 30°C, thus the protein-DNA complexes were not dissociated. The bands were visualized by autoradiography. The optical density of the bands was estimated using a quantitative image analysis system, Leica Quantimet 500.

Statistical analysis

Statistical significance of differences between different groups was analysed using Students unpaired t-test.

Results

The two cell lines that were used for the experiments in the present study exhibited a varying degree of transfectability. The positive control clone, pXGH, which contains a portion of the metallothionein promoter, resulted in a significant GH secretion into the culture medium from both cell lines. In experiments where both cell lines were analyzed in parallel, transfection of the 1242 MG human glioma cell line resulted in a relatively higher GH secretion than that observed with the SH-SY5Y cell line. Moreover, none of the cell lines exhibited any detectable GH secretion without transfection, e.g. there was no evidence for any endogenous GH biosynthesis and secretion.

MAO-A promoter fragments

In all experiments performed the fragment showing the highest promoter activity was the A0.14 construct which contains a 142bp digest of a proximal segment of the 5′-flanking sequence (−62 to −203). This fragment resulted in at least three-fold higher promoter activity than any other construct investigated (Table 1). In a previous investigation it was concluded that constructs containing increasing length of the MAO-A promoter resulted in a successive lowering of activity (Zhu et al., 1992). In the present study, data is shown for

Table 1. Transcriptional activity of promoter fragments from the 5′-flanking region of the human MAO-A gene as estimated by using growth hormone as a reporter gene. The figures represent net cpm ± standard deviation and data points given within brackets were analyzed as single samples

Fragment	1242 MG		SH-SY5Y	
	net cpm	relative activity	net cpm	relative activity
pXGH	21,221 ± 2,877	–	4,541 ± 883	–
A 0.14	1,109 ± 66	100%	1,544 ± 329	100%
A 0.52	–	–	411 ± 64	26.6
A 1.4	388 ± 54	35.0	(216)	(14.0)
A 1.7	346 ± 9	31.2	419 ± 77	27.1
A 2.6	(193)	(17.4)	196 ± 28	12.7
A 3.5	210 ± 82	18.9	185 ± 11	12.0

two constructs that have not been investigated before, the A2.6 and A3.5 plasmids, respectively. Both these longer constructs result in very low reporter gene expression.

MAO-B promoter fragments

Similar to the MAO-A promoter, the shortest fragment used in the present investigation exhibited the highest reporter gene expression (Table 2). This fragment which is a 150 bp digest is located 99 bp upstream of the start codon. In this study, the longer B0.19 fragment resulted in a reduction of the promoter activity as compared with the B0.15 fragment of 77% and 66% in 1242 MG and SH-SY5Y cells, respectively. In accordance with previous results, the B0.44, and the B1.0 plasmids showed a lower reporter activity as compared with the B0.15 plasmid (Table 2).

Treatment with lithium chloride, L-deprenyl, and raclopride

In order to examine if lithium chloride exposure of the cells could change MAO transcription, the A0.14, A1.4, B0.15 and B1.0 plasmids were used. Despite previous reports about changes in MAO activity after treatment with these drugs in clinical practice, we found no evidence for changes after 24 hours of treatment that would support the concept of regulation at the transcriptional level (Table 3). When the MAO activities were assayed no changes were observed after treatment with lithium chloride or raclopride (data not shown). Similarly, no changes in reporter gene expression were observed after treatments with the selective MAO-B inhibitor L-deprenyl or the dopamine-2-receptor antagonist raclopride.

Treatment with ethanol

Ethanol exposure was applied to the cultures in two different doses, 50 and 100 mM, respectively. No changes were observed when we investigated the growth hormone secretion in cultures that were transfected with MAO-A promoter fragments after ethanol treatment (data not shown). In contrast, in cell cultures transfected with MAO-B promoter fragments, B0.15 and B1.0,

Table 2. Transcriptional activity of promoter fragments from the 5′-flanking region of the human MAO-B gene as estimated by using growth hormone as a reporter gene. The figures represent net cpm ± standard deviation

Fragment	1242 MG		SH-SY5Y	
	net cpm	relative activity	net cpm	relative activity
pXGH	19,888 ± 106	–	4,521 ± 412	–
B 0.15	5,437 ± 1,006	100%	944 ± 125	100%
B 0.19	4,186 ± 16	77.0	626 ± 92	66.4
B 0.44	2,601 ± 87	47.8	–	–
B 1.0	1,433 ± 103	27.3	474 ± 138	50.2

some effects were observed (Table 4). The most pronounced effect was observed in 1242 MG glioma cells. An increase in reporter activity was evident at both ethanol concentrations (approximately 2.5 times the level observed in non-treated cells). Moreover, in transfected SH-SY5Y cells, an increase was observed only for the long fragment at 50 mM, while both fragments caused an increase at the higher ethanol concentration. In none of the experiments did cells transfected with the control plasmids, pOGH or pXGH, show any increase in GH secretion, indicating that the effect was relatively specific. Also, MAO-B enzyme activity was significantly enhanced in the 1242 glioma cells after treatment with the 100 mM ethanol (Fig. 1). However, no significant change in catalytic activity was observed after treatment of the SH-SY5Y cells. Serotonin (5-HT) oxidation which is predominantly catalyzed by MAO-A remained unchanged in both cell lines after ethanol exposure (Fig. 1).

Table 3. Transcriptional activity of promoter fragments from the 5′-flanking region of the human MAO-A and MAO-B genes as estimated by using growth hormone as a reporter gene after 24 h treatment with lithium chloride, L-deprenyl and raclopride. The reporter gene activities are expressed as percent (%) of the activity in non-treated cells ± SEM

cell line	constructs	lithium chloride (10 μM)	L-deprenyl (1 μM)	raclopride (10 μM)
1242 MG	pXGH	91 ± 6	108 ± 5	100 ± 4
	A 1.4	100 ± 10	103 ± 4	–
	B 1.0	106 ± 45	113 ± 11	97 ± 14
SH-SY5Y	pXGH	93 ± 18	95 ± 3	106 ± 7
	A 1.4	117 ± 7	115 ± 24	–
	B 1.0	112 ± 16	95 ± 5	106 ± 11

Table 4. Transcriptional activity of promoter fragments from the 5′-flanking region of the human MAO-B gene as estimated by using growth hormone as a reporter gene. Treatment of cell cultures with ethanol (50 and 100 mM) added 24 hrs after transfection. The cultures were exposed to the drug for 24 h before estimation of GH concentration. The figures represent net cpm. * $P < 0.05$ (Students t-test)

Treatment	1242 MG		SH-SY5Y	
Control				
pXGH	74,858 ± 2,996	(100%)	3,807 ± 620	(100%)
B 0.15	2,636 ± 558	(100%)	178 ± 99	(100%)
B 1.0	1,790 ± 505	(100%)	55 ± 39	(100%)
Ethanol (50 mM)				
pXGH	72,798 ± 1,733	(97%)	–	
B 0.15	6,402 ± 1,069*	(243%)	176 ± 87	(99%)
B 1.0	4,976 ± 1,842*	(278%)	78 ± 39	(142%)
Ethanol (100 mM)				
pXGH	69,235 ± 726	(92%)	3,296 ± 1,210	(87%)
B 0.15	4,426 ± 1,166	(168%)	326 ± 177	(183%)
B 1.0	4,391 ± 428*	(245%)	116 ± 72	(210%)

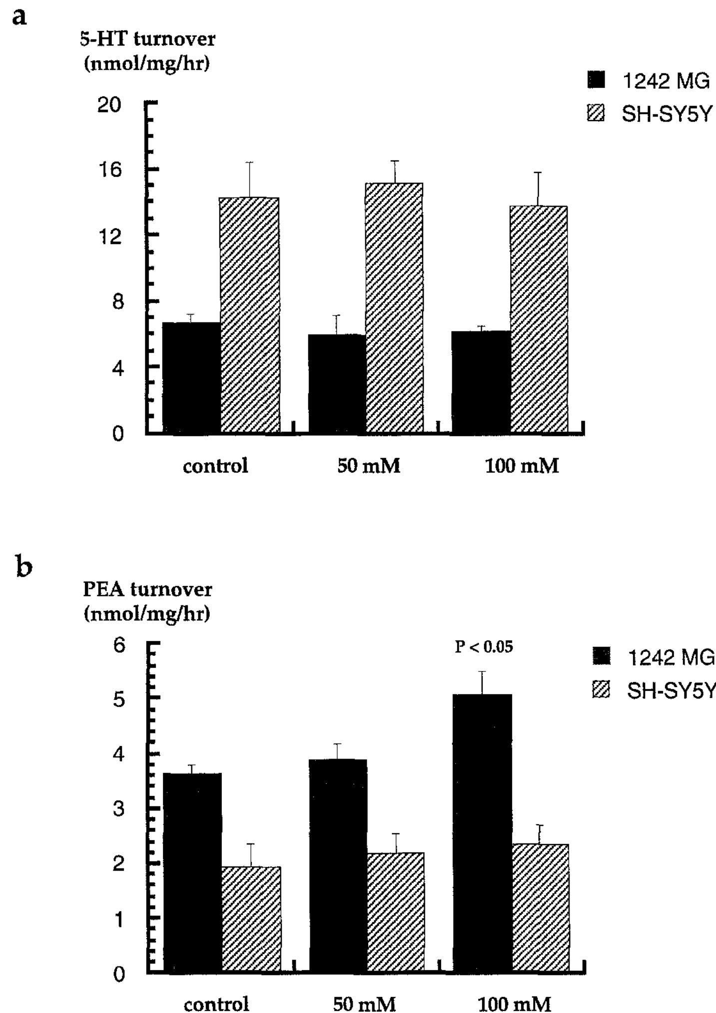

Fig. 1. Estimation of **a** MAO-A and **b** MAO-B enzymatic activity in 1242 MG and SH-SY5Y cells after treatment with 50 and 100 mM ethanol. The determinations were carried out by using the selective substrates 5-HT (**a**) and PEA (**b**), respectively. Each bar represent the mean activity ± standard deviation of three independent estimations

Gel retardation assay

The protein-DNA complexes, due to their higher molecular weight, moved slower (retarded) and were separated from unbound (free) DNA during electrophoresis. Nuclear extracts from both 1242 MG and SH-SY5Y cells resulted in a similar banding pattern in the gel shift assay. Three clearly distinguishable DNA-protein bands were observed on the autoradiographs as shown in Fig. 2, indicating that the B0.15 fragment bound several nuclear proteins. Inclusion of Sp1 consensus oligonucleotide sequences removed the binding of the largest protein to the B0.15 fragment, whereas, band *a* and *b* were not removed, indicating these two remaining proteins were not Sp1-like transcription factors. With regard to the *a* band, no differences were observed

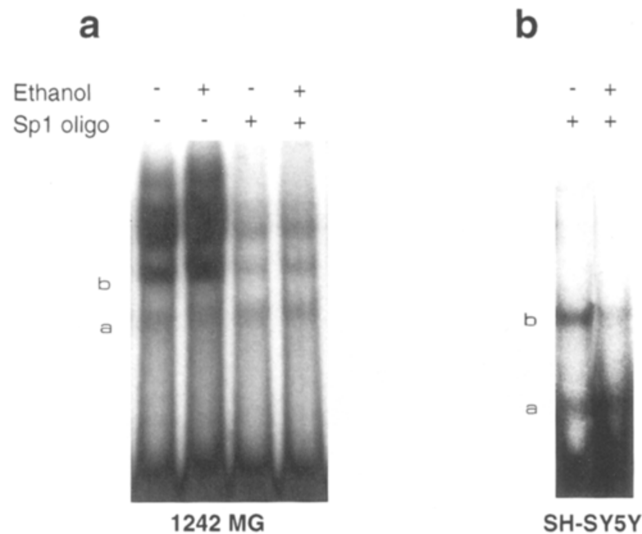

Fig. 2. The effect of ethanol on transcription factor binding to the B0.15 MAO-B promoter fragment determined by gel retardation analysis. The B0.15 fragment was [^{32}P]-labelled and incubated with **a** 1242 MG and **b** SH-SY5Y nuclear extracts. Extracts from nontreated and ethanol treated cells were analyzed. In lanes 3 and 4 of figure a and in both lanes of figure b Sp1 oligonucleotides were added to remove Sp1-like binding. In these lanes the remaining binding of the non-Sp1 bands, corresponds to *factor a and b*, respectively

between extracts from ethanol-treated cells and control cells of both cell lines. An increase in Sp-1-like binding was evident when analyzing extracts of ethanol treated 1242 MG cells as compared with control cells (Fig. 2a). The other two factors did not seem to be affected by ethanol treatment of 1242 MG cells. On the other hand, densitometrical analyses of several independent gel retardation assays showed that *factor b* DNA binding was significantly reduced in ethanol treated SH-SY5Y cells when compared with untreated cells (Fig. 2b). The Sp1-like binding seemed to remain unchanged after ethanol treatment of the SH-SY5Y cell line. The difference in *factor b* binding was most apparent when the Sp1 site was blocked (Fig. 2b). Since ethanol treatment increased the promoter activity (Table 4) in both cell lines, these results suggest that ethanol may increase the transcriptional activity by independent mechanisms in the two cell lines, e.g. by increasing a Sp1-like factor in 1242 MG cells and by lowering a possible silencer (*factor b*) binding to DNA in SH-SY5Y cells.

Discussion

It has previously been shown that the reporter plasmid vector containing the human GH gene for transient transfections of eukaryotic cells is an accurate system with high sensitivity (Selden et al., 1986). Most of the constructs used in the present study have been characterized in SH-SY5Y and other cell lines before (Zhu et al., 1992). Both types of cell lines that were chosen for the present study (glioblastoma and neuroblastoma) represent tumor cell clones

of neural crest-derived cells. These cell lines have been shown to exhibit several characteristics of neurons and astrocytes in the mature nervous system (for a review on SH-SY5Y, see Biedler et al., 1978). Both cell lines express both MAO-A and MAO-B (Fig. 1).

The conclusion of the transfection studies performed under standard conditions (without drug treatment) is that there are no substantial differences between the different cell lines with regard to the relative contribution of the various fragments investigated on the MAO-A and MAO-B promoter activity, e.g. there are no data that would support the idea of celltype-specific elements within the studied sequences. This is in good accordance with previous studies from this laboratory (Zhu et al., 1992) and others (Denney et al., 1994). Interestingly, in transgenic mice the expression patterns of the lacZ gene, when put under the control of the MAO-A or MAO-B 5′-flanking sequences (>3 kb), did not show any celltype specificity (Shih et al., 1995, unpublished results). One might speculate that longer promoter constructs may be required or that other segments of the MAO genes are important for tissue-specific transcription, e.g. it has been shown that intronic regions may contain tissue-specific regulatory elements (Vidal et al., 1990).

It is not known whether selective inhibition of MAO-B, for example, by means of treatment with L-deprenyl affects the transcription of the MAO-B gene. It may be speculated that the biosynthesis of MAO protein could be regulated by substrate and/or product concentrations, e.g. that the transcription is modulated according to the requirement of enzyme. In this report no changes were observed in the reporter gene expression after treatment with 1 μM L-deprenyl, a concentration that is sufficient to completely inhibit MAO-B. These data are in line with another study from our laboratory (Shih et al., unpublished results) where it was shown that the transcript levels, as demonstrated by Northern blotting, were unaffected in mouse brains after chronic administration of L-deprenyl.

In several studies it has been shown that oral treatment with lithium carbonate causes an increase in platelet MAO (MAO-B) (Bockar et al., 1974; Huang et al., 1984). However, it is controversial whether any changes in the MAO activity occur in the brain after lithium treatment. Only one animal study reports an increased MAO activity after chronic lithium chloride administration to female rats (Dawood and Welch, 1979). However, the results of that study have according to our knowledge never been repeated. Our data does not suggest any induction of MAO transcription in the CNS. Yet, it is possible that transcriptional regulation of megakaryocytes, which give rise to platelets, differs from the cell lines used in the present study, e.g. that these cells induce the expression of a transcription factor or enhancer that is important for MAO transcription. On the other hand, it has been shown that the platelet number increases after treatment with lithium salts (Medina et al., 1980) and it has been proposed that platelet maturation is affected by lithium treatment (Jackman et al., 1980). Therefore, one may hypothesize that a larger proportion of younger platelets, relatively rich in MAO, could explain the increase in platelet MAO after lithium therapy. One may argue that the time-frame was too narrow in the present study (24 hrs) as compared with the

longer treatment periods that were reported in the clinical studies. With the present method it is difficult to interpretate data obtained from transfected cell cultures over longer times since dish-to-dish variations with regard to cell growth will generate considerable standard variations. Nevertheless, we also studied the GH expression after 4 days of lithium chloride treatment (data not shown). No alterations in the levels of reporter gene expression were observed after this longer incubation.

Moreover, it has been shown that MAO activity in blood cells from chronic schizophrenics is significantly lower as compared with a control group (DeLisi et al., 1980). However, in most studies the patient group was treated with neuroleptic drugs. Therefore, it has been debated whether the reduction of MAO activity in schizophrenics is caused by the drug treatment per se. In the present study, we investigated if raclopride treatment was able to induce any changes in MAO transcription. This drug, which is a specific dopamine-2-receptor antagonist, did not seem to change the transcriptional activity in our reporter system. According to our knowledge it has never been shown if these two cell lines express dopamine receptors. Nevertheless, mRNA transcripts for several dopamine receptor subtypes (D2, D3, and D4) have been observed by RT-PCR in the SH-SY5Y cell line (Welton, personal communication). We did not observe any changes in MAO catalytic activities after treatment of the cells with raclopride. Our results are therefore in line with the results of other investigators who suggest that low MAO activity in blood cells in schizophrenics may be trait-dependent rather than a consequence of pharmacological treatment (DeLisi et al., 1980; Sullivan et al., 1978).

The effects of ethanol treatment were assayed since several reports indicate that chronic alcohol intake might result in quantitative changes in MAO catalytic activity (Aliyu and Upahi, 1988; Anokhina et al., 1990; Nevo and Parvez, 1994; Tabakoff et al., 1985). Two concentrations of ethanol were used in these transfection experiments, 50 and 100 mM, respectively. These concentrations are frequently used in *in vitro* assays since they represent attainable tissue concentrations of ethanol during alcohol intoxication. In a report by Anokhina and coworkers (1990) it was concluded that hepatic MAO-B was elevated in the offspring of alcoholized rats. This finding may be relevant since the cells used in the present study represent immature cell clones with similarities to undifferentiated cells in the developing CNS. The results of the present study indicate that this effect may, at least in part, be explained by an enhancement of MAO-B transcription. Consistent with the elevated MAO-B promoter activity, Sp1-like binding was increased to the MAO-B core promoter in 1242 MG cells after ethanol treatment. Interestingly, the two cell lines under study respond independently to ethanol treatment. In SH-SY5Y cells, the binding of transcription *factor b* (a possible silencer) to the B0.15 core promoter fragment was decreased by ethanol treatment, suggesting that ethanol may modify MAO-B expression by changing the concentration of *factor b* involved in the transcription control of MAO-B. The induction of *factor b* binding is most apparent in the presence of non-labelled antisense oligonucleotide corresponding to the Sp1 consensus site. It is a common observation in gel shift assays that displacement of one DNA binding protein

may enhance protein binding to other sites. It would be interesting to futher explore the mechanism(s) for the changes in transcription factor binding to the B0.15 MAO-B promoter fragment after ethanol treatment, e.g., to investigate if ethanol interacts directly with these proteins or if it is a regulation for example by phosphorylation or at the transcriptional level.

We also estimated the catalytic activity of MAO in parallel with the transfection assay. However, the changes in enzyme activity was not as pronounced as the increases in reporter gene transcription, e.g., only the higher ethanol concentration resulted in an increase in MAO catalytic activity that reached statistical significance. There could be several factors that cause this discrepancy, e.g. i) it is conceivable that the efficacy of gene transcription may be different for a short plasmid construct as compared with a chromosomal gene. ii) all estimations were carried out after 24 hours of drug treatment and the post-translational events are presumably more complex in the expression of a functional MAO enzyme protein as compared with a shorter polypeptide as GH. This might involve steps of enzymatic modification, co-factor availability and membrane targeting. iii) The transcription of the endogenous MAO genes is most likely influenced by silencers and enhancers in other regions of the MAO genes, whereas the plasmid constructs only contain a portion.

In conclusion, the present study provides data that indicate that ethanol in doses attained during intoxication may influence MAO-B transcription in a cell-type specific fashion.

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