Original Article

Differences in the Expression and Sensitivity of Cultured Rat Brain Neuronal and Glial Cells Toward the Monocrotophos

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ABSTRACT

Inducible expressions cytochrome P450s (CYPs) against environmental chemicals in brain tissues of experimental animals is well-documented. However, the precise role of specific brain cell type in the metabolism of different class of xenobiotics has not been explored adequately. We study the expression of selected CYPs (1A1/1A2, 2B1/2B2, 2E1) in primary cultures of rat brain neuronal and glial cell exposed to an organophosphate pesticide-monocrotophos (MCP), a known neurotoxicant. The cultured neurons and glial cells express significant expression of CYP1A1, 2B2 and 2E1 isoenzymes, where the levels were comparatively higher in neuronal cells. Neuronal cells exhibited greater induction of CYP2E1 against MCP exposure, while glial cells were having more vulnerability for CYP1A and 2B isoenzymes. Similarly, cells were showing substrate specific responses against the specific inducers of CYPs, that is, ethanol (2E1), cyclophosphamide (2B1/2B2), 3-methylcholanthrene (1A1/1A2). The altered expression and activity of selected CYPs in cultured neuronal and glial cells could be helpful in explaining the association between MCP-induced neurotoxicity/metabolism and synthesis or transport of the neurotransmitters. The induction of CYPs in glial cells may also have significance as these cells are thought to be involved in protecting the neurons from environmental insults and safeguard them from toxicity. The differential expression pattern of CYPs in neuronal and glial cells exposed to MCP also indicate the selective sensitivity of these cells against the xenobiotics, hence suggested their suitability as tool to screen neurotoxicity potential of variety of xenobiotics.

Key words: Cytochrome P450s, monocrotophos, primary culture, rat brain glial cells, rat brain neuronal cells, Xenobiotic metabolism

INTRODUCTION

Primary cultures of rat brain cells have been shown to exhibit stable expression of xenobiotic metabolizing cytochrome P450s (CYPs). Preceding chapters have shown that the cultured neuronal and glial cells express CYP1A, CYP2B, and CYP2E1 isoenzymes. Cell-specific differences were also observed in their responsiveness to the inducers, known to increase the expression of CYPs in the liver. However, studies are needed to investigate if the responsiveness of these cultures is retained following exposure to environmental chemicals, which will further allow the use of primary cultures of brain cells in neurotoxicological research. Further, the responsiveness of these CYPs to their known inducers is also retained in these primary cultured cells. Thus, primary cultured rat brain neuronal and glial cells were used, as an *in vitro* model in the present study to understand the neurotoxicity of monocrotophos (MCP).

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MCP, a widely used organophosphate pesticide, has been shown to act at multiple sites in the central nervous system (CNS) and produce neurotoxicity in laboratory animals.[1] Studies have shown that the metabolism of MCP plays an important role in its neurotoxicity.[2] Our laboratory have shown that oral administration of deltamethrin produces a marked dose and time-dependent increase in the xenobiotic metabolizing CYP1A, 2B, and CYP2E isoenzyme of CYP in rat brain.[3] This increase in cerebral CYPs could be correlated with the symptoms of neurobehavioral toxicity of deltamethrin.[4,5] Significant regional differences were also observed in the CYP enzyme induction in brain regions which accumulate majority of the pyrethroids, exhibited maximum induction in CYP enzyme activity.[4]

Thus to establish, cultured neuronal and glial cells, as an experimental alternatives to conventional animal toxicity testing, in the present study attempts were made to investigate differences, if any, in the expression of the CYPs involved in its metabolism and toxicity in the primary cultures of rat brain neuronal and glial cells, following exposure to MCP. Attempts were also made to investigate differences, if any, in the sensitivity of the brain cells toward MCP, which might help in explaining the cell-specific vulnerability to the neurotoxicants.

MATERIALS AND METHODS

Reagents and consumables

All the specified chemicals and reagents *viz.,* MCP were purchased from Sigma (Sigma, St. Louis, MO, USA) unless otherwise stated. Culture medium dublecco's modified eagle medium: Nutrient mixture F-12 (DMEM/F-12), antibiotics, fetal bovine serum (FBS), and trypsin- ethylenediaminetetraacetic acid (EDTA) were purchased from Gibco-BRL, USA. All the antibodies used in this study were procured from Chemicon International, USA. Culture wares and other plastic wares used in the study were procured commercially from Nunc, Denmark. Milli Q water (double-distilled deionized water) was used in all the experiments.

Neuronal and glial cell culture

Pregnant albino wistar rats weighing 175-200 g (~8-week-old) were obtained from CSIR-Indian Institute of Toxicology Research breeding colony and raised on a commercial pellet diet and water *ad libitum*. Animals were cared for in accordance to the policy laid down by Animal Care Committee of CSIR-Indian Institute of Toxicology Research and animal experimentation was approved by the ethical committee of the center. For neuronal cell culture 14-day-old embryos[6] and for glial cells 0-1-day-old pups were used.[6] In brief brains from 14-day-old rat fetus or from 1-day-old pups was dissected and kept in Hank's balanced salt solution (HBSS) containing antibiotic and antimycotic. Brain tissue was minced into small pieces, trypsinized at 37°C for 5-7 min, with 0.5 mg/mL trypsin in HBSS containing 0.5% BSA. Following incubation, trypsin was aspirated and tissues were incubated (37°C, 15 min) with DMEM/F-12 media containing 12% FBS. Cells were then dissociated by passing tissue pieces 20 - 30 times through 1 mL pipette tip and then by passing it through 200 µL pipette tip. The resulting cell suspension were filtered through a 50 µm diameter nylon mesh and pelleted by centrifugation. The cell pellet was then suspended in culture medium (DMEM/F-12 with 20 mM KCL, 6 g/L glucose, 0.2% sodium bicarbonate, 100 units/mL penicillin G sodium, 100 µg/mL streptomycin sulphate and 0.25 µg/mL amphotericin B, and 10% FBS) and used further for fractionation into neuronal and glial cells.[6]

Assessment of purity of neuronal and glial cells

Specific monoclonal antibody markers prior to each experiment were used to check the purity of neuronal and glial cultures, following the protocol described earlier by us.[6]

Identification of noncytotoxic dose of MCP

In the present investigations, neurotoxin-MCP, an organophosphate pesticide, was used. MCP was dissolved in DMEM/F-12 medium. Prior using in the expression studies, noncytotoxic dose of MCP was identified in cultured neuronal and glial cells. Cytotoxicity assessment was done using standard endpoint, that is, tetrazolium bromide MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay as described earlier by us.[7] In brief, neuronal and glial cells (1×10^4 cells/well) were seeded in poly-L-lysine (PLL)-coated 96-well tissue culture plates and incubated in the CO_2 incubator for 24 h at 37°C. Then, the medium was aspirated and cells were exposed to medium containing MCP (10^{-7} M-10^{-3} M) for 24-96 h at 37°C in 5% CO_2-95% atmosphere under high humid conditions. Tetrazolium salt [10 µL/well; 5 mg/mL of stock in phosphate buffered saline, (PBS)] was added 4 h prior to completion of respective incubation periods. On the completion of incubation period, the reaction mixture was carefully taken out and 200 µL of culture grade dimethyl sulfoxide (DMSO) was added to each well. The content was mixed well by pipetting up and down several times until dissolved completely. Plates were then incubated for 10 min at room temperature and color was read at 550 nm using Multiwell Microplate Reader (Synergy HT, Bio-Tek, USA). The unexposed sets and sets exposed to $MnCl_2$ (10^{-4} M) were also run parallel under identical conditions that served as basal and positive control, respectively.

Transcriptional changes

Transcriptional changes in the selected CYPs (1A1/1A2, 2B1/2B2, and 2E1) were studied in both neuronal and glial cells exposed to pesticide-MCP. Xenobiotics-induced alterations in the mRNA expression level were expressed in relative quantification by comparing the data obtained from unexposed cells. The quantitative real-time polymerase chain reaction (RT-PCRq) analysis was done following the protocol described earlier by us.[2] In brief, total RNA was isolated from both experimental and unexposed control sets using Gene Elute mammalian total RNA Miniprep Kit (Catalog No. RTN-70, Sigma, USA). Total RNA (1 µg) was reverse transcribed into cDNA by Super Script III first strand cDNA synthesis kit (Catalog No. 18080-051, Invitrogen Life Science, USA). RT-PCRq assay reactions were carried out with 2× SYBR Green polymerase chain reaction (PCR) master mix (Applied Biosystems, USA) using ABI PRISM 7900HT Sequence Detection System having software version 2.2.1 (Applied Biosystems, USA). Results were expressed relative to the housekeeping gene, that is, β-actin. Real-time reactions were carried out in triplicate wells for each sample.

Enzyme activity

Following the respective exposures cells were harvested and processed for microsome preparation following the protocol described earlier by us.[6] In brief, cells were scrapped in PBS at 4°C and pelleted by centrifuging at 500 × g for 10 min. The cell pellet was resuspended in microsomal dilution buffer containing 0.1% (v/v) glycerol, 0.25 mM protease inhibitors cocktail, 0.01M EDTA, and 0.1 mM dithiothreitol. The cells were then sonicated thrice at 15 Hz for 10 s each. Following sonication, the cells were again centrifuged at 9000 × g for 20 min. The supernatant was then further centrifuged at 105,000 × g for 60 min, to isolate the microsomal fraction. The microsomal pellet thus obtained was then resuspended in microsomal dilution buffer and protein estimation was done by Bradford's Reagent (Fermentas Inc., Maryland, USA). The activity of 7-ethoxyresorufin-O-deethylase (EROD) for CYP1A, 7-pentoxyresourfin-O-dealkylase (PROD) for CYP2B, and N-nitrosodimethylamine demethylase (NDMA-d) for CYP2E1 were determined by following the methods described earlier by us[6,8,9] using a Perkin Elmer LS 55 Luminescence spectrophotometer.

Translational changes (immunocytochemical localization)

Immunocytochemical localization of CYPs-specific proteins was carried out by using antiprimary antibodies following the protocol of Kapoor *et al*.[9] Briefly, cells (1×10^4 cells/well) were allowed to adhere on the surface of PLL-coated eight-well chamber slides (Lab Tek, Campbell). Cells were exposed to MCP. Following exposure, cells were fixed by using 4% paraformaldehyde for 20 min and blocked with PBS containing 0.02% triton-X100 and 0.1% BSA for 2 h to block the nonspecific binding sites. The neuronal cells were incubated with primary antibody 1:100 dilution specific for neuronal (β-III tubulin), and CYP isoform-specific antibodies (anti-CYP2E1, anti-CYP2B, and anti-CYP1A) and the glial cells were incubated with monoclonal anti-GFAP (glial fibrillary acidic protein) (specific for glial cells), and CYP isoform specific antibodies (anti-CYP2E1, anti-CYP2B, and anti-CYP1A) (Chemicon International, USA) for 2 h at room temperature followed by washing with PBS. Finally, cells were washed with PBS to remove unbound antibody and incubated with tetramethylrhodamine-5-(and-6)-Isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC) label secondary antibody for 15-30 min. Cells were visualized under fluorescent microscope (Nikon Eclipse 80i equipped with Nikon DS-Ri1 12.7 megapixel camera, Japan) and quantification was done by measuring the change in percent area of protein expression with the help of Leica Qwin 500 Image Analysis Software (Leica, Germany).

Statistical analysis

The results are expressed as mean and standard error of means (mean ± SE) for at least three experiments. One-way analysis of variance followed by *post hoc* Dunnett's test was employed to detect differences between the groups of treated and control. $P < 0.05$ was taken to indicate significant differences.

RESULTS

Assessment of purity of neuronal and glial cells by immunocytochemistry

Simultaneous staining of the neuronal cells with β-III tubulin (neuronal marker) and GFAP (glial marker), gave positive immunofluorescence for β-III tubulin (90-95%), whereas GFAP staining was only 5% in these neuronal-enriched cultures. Similarly, when the glial cells were simultaneously stained with β-III tubulin and GFAP, positive immunofluorescence was seen for GFAP (90-95%), whereas β-III tubulin showed only 5% immunofluorescence in these glial enriched cultures.

Cytotoxicity assessment of MCP

Seven-day-old cultures harvested and plated on 96-well plates, exposed to 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M of MCP for 24, 48, 72, and 96 h and assessed by the MTT assay for cytotoxicity showed that except for 10^{-4} and 10^{-3} M, none of doses used were toxic. A concentration of 10^{-5} M and time interval of 48 h were chosen for immunocytochemical studies [Figure 1].

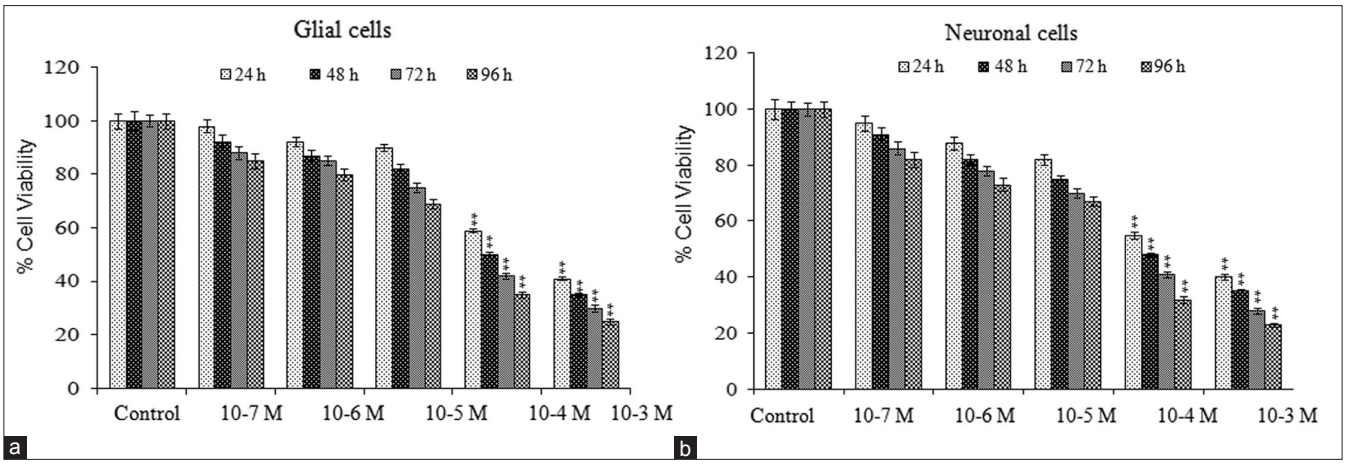

Figure 1: Identification of noncytotoxic doses of neurotoxicant-monocrotophos in cultured neuronal and glial cell. Cells were exposed to MCP (10^{-7}-10^{-3} M) for 24-96 h in (a) neuronal and (b) glial cells. The percent cell viability was assessed using MTT assay. Values are given as mean ± standard error of the mean of the data obtained from three independent experiments. * = $P < 0.05$, ** = $P < 0.01$

Xenobiotics-induced transcriptional changes in CYPs

Neuronal cells show a significant gradual increase in the expression of CYP2E1 following the exposure to MCP (10^{-5} M) for 6, 12, 24, and 48 h, that is, 7 ± 0.6-, 11 ± 0.9-, 18 ± 1.2-, and 25 ± 1.4-fold of control, respectively. The trend of upregulation in the expression of mRNA of CYP2E1 in glial cells was similar to that of neuronal cells; however, the magnitude of expression was little bit lower than neuronal cells [Figure 2a]. Neuronal cells responded significantly against MCP (10^{-5} M) exposure for 6, 12, 24, and 48 h by inducing the expression levels of mRNA of CYP2B1/2B2 (4 ± 0.1-, 7 ± 0.5-, 13 ± 0.6-, 20 ± 0.9- and 5 ± 0.30-, 9 ± 0.5-, 15 ± 1.0-, 22 ± 1.2-fold of control, respectively). The similar trend and magnitude of induction in the expression levels of mRNA of CYP2B1/2B2 was also recorded in glial cells [Figure 2b, c]. Both neuronal and glial cells were also found to induce expression levels of mRNA of CYP1A1/1A2 against the exposure of MCP (10^{-5} M) [Figure 2d, e].

NDMA-d, PROD, and EROD assay

Microsomes from untreated cultured neuronal and glial cells were found to catalyze the CYP2E1 dependent N-demethylation of NDMA-d, CYP2B-dependent PROD, and CYP1A-dependent EROD activities. The specific activity of NDMA-d in control neuronal and glial cells was almost similar, while the specific activity of PROD and EROD in untreated neuronal cells was two-folds greater when compared with the untreated glial cells [Figure 3c-f]. When cultured cells were exposed with 10^{-5} M of MCP for different time periods, a time-dependent increase was observed in the CYP2E1-dependent NDMA-d, CYP2B-dependent PROD, and CYP1A-dependent EROD activity. MCP causes significant ($P < 0.01$) induction in the catalytic activity of CYP2B (31.5 ± 1.5, 38.3 ± 1.8, 44.8 ± 2.5, 46.5 ± 2.1, and 49.8 ± 1.9 pmoles resorufin/min/mg protein at 12 h, 24 h, 48 h, 72 h, and 96 h, respectively) in neuronal cells, while (15.6 ± 1.0, 17.9 ± 1.2, 22.5 ± 1.6, 24.6 ± 1.8, and 30.8 ± 2.0 pmoles resorufin/min/mg protein at 12 h, 24 h, 48 h, 72 h, and 96 h, respectively) in glial cells [Figure 3c, d]. MCP causes significant induction in the catalytic activity of CYP1A (12.5 ± 0.6, 16.7 ± 0.9, 19.4 ± 1.2, 21.8 ± 1.6, and 23 ± 1.9 pmoles resorufin/min/mg protein at 12 h, 24 h, 48 h, 72 h, and 96 h, respectively) in neuronal cells, while (7.4 ± 0.4, 10.2 ± 0.8, 12.2 ± 0.5, 15.8 ± 1.2, and 18.4 ± 1.4 pmoles resorufin/min/mg protein at 12 h, 24 h, 48 h, 72 h, and 96 h, respectively) in glial cells [Figure 3e, f]. Again the induction in the neuronal cells was more than that observed in glial cells for NDMA-d activity, whereas the induction in glial cells was more than that observed in neuronal cells for EROD and PROD activity.

Assessment of CYP2E1, CYP2B, and CYP1A expression in cultured neuronal and glial cells by immunocytochemistry

Both neuronal and glial cells cultured with DMEM/F-12 medium alone or treated with MCP (10^{-5} M), grown on PLL-coated glass slides, when fixed, and stained with isoform specific anti-CYP antibodies and with anti-b-III tubulin or anti-GFAP, and the secondary antibodies labeled with FITC or TRITC showed positive staining for CYP2E1, CYP2B, and CYP1A. As shown in Figures 4A and B, 5A and B, and 6A and B, the neuronal cells exhibiting staining with anti-β III tubulin also expressed CYP2E1, CYP2B, and CYP1A as judged by staining with anti-CYP2E1, anti-CYP2B, and anti-CYP1A, respectively. Likewise, glial cells exhibiting staining with anti-GFAP also expressed CYP2E1, CYP2B, and CYP1A as judged by staining with anti-CYP2E1, anti-CYP2B, and anti-CYP1A, respectively [Figures 4C, D, 5C, D, and 6C, D]. Some of the glial cells which did not exhibit any staining with anti-GFAP were found to be

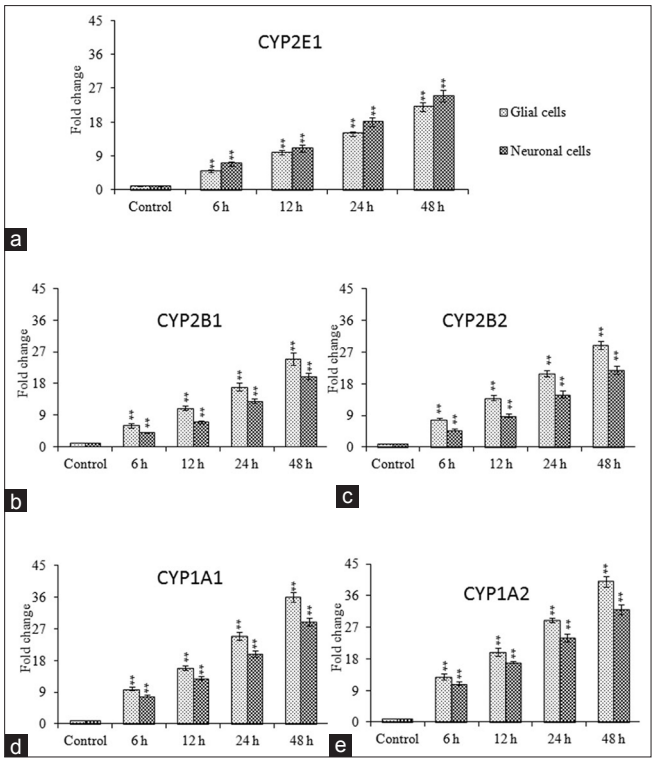

Figure 2: Real-time polymerase chain reaction analysis for xenobiotics-induced transcriptional changes in cytochrome P450s genes in neuronal and glial cells. Fold changes in altered mRNA expression of CYP2E1 in neuronal and glial cells (a), following the exposure of monocrotophos (6-48 h). Fold changes in altered mRNA expression of CYP2B1/2B2 in neuronal and glial cells (b, c) following the exposure of MCP (6-48 h). Fold changes in altered mRNA expression of CYP1A1/1A2 in neuronal and glial cells (d, e), following the exposure of MCP (6-48 h). β-actin was used as endogenous control to normalize the data and xenobiotic exposure induced alterations in transcripts are expressed in fold changes (mean ± standard error of the mean) compared with unexposed controls. * = $P < 0.05$ and ** = $P < 0.01$ in comparison to respective unexposed controls

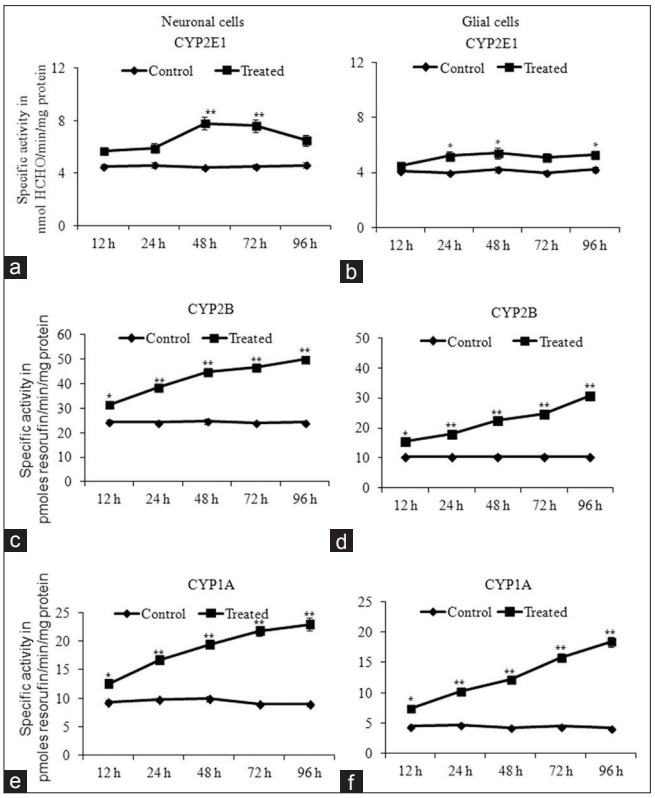

Figure 3: Xenobiotics-induced alteration in cytochrome P450s-specific enzymatic activity in neuronal and glial cells. N-nitrosodimethylamine demethylase (CYP2E1) activity in (a) neuronal cells and (b) glial cells following the exposure of monocrotophos (12-96 h). 7-pentoxyresourfin-O-dealkylase (CYP2B) activity in (c) neuronal cells and (d) glial cells, following the exposure of MCP (12-96 h). 7-ethoxyresorufin-O-deethylase (CYP1A) activity in (e) neuronal cells and (f) glial cells, following the exposure of MCP (6-48 h). Data are expressed in mean ± standard error of the mean of specific activity (in their respective units) of catalytic activity in microsomal fractions. * = $P < 0.05$ and ** = $P < 0.01$ in comparison to respective unexposed controls

immunoreactive for CYP2E, CYP2B, and CYP1A as the primary culture used in the present study was a mixed culture consisting of oligodendrocytes, microglia, and astrocytes and the monoclonal anti-GFAP used recognizes only the astrocytes and Bergman glial cells, thus cells other than above such as microglia and oligodendroctyes could be positive for CYP2E, CYP2B, and CYP1A, but negative for GFAP.[10] Similarly some anti-β-III tubulin negative cells, which were immunopositive for CYP2E, CYP2B, and CYP1A, could be the endothelial or fibroblast cells contaminating the neuronal cultures. Software analysis (Leica Qfluro Standard, Leica Microsystems Imaging Solutions Ltd., Version V1.2.0) revealed that treatment of MCP resulted in an increase in the intensity of FITC fluorescence. The mean intensity in treated glial cells for CYP2E1, CYP2B, and CYP1A was increased (35%, 104%, and 125%, respectively) as compared with the control cells. Likewise, the neuronal cells exhibited an increased intensity of fluorescence for CYP2E1, CYP2B, and CYP1A in treated cells when compared with the control cells (58%, 21%, and 33% respectively).

DISCUSSION

Similar to that observed with *in vivo* studies, real-time PCR, immunocytochemical data, and alterations in the marker CYP enzyme activity in cultured brain cells have demonstrated that MCP is a mixed type of inducer and the increase observed in the different CYP isoforms, activity and expression after exposure to MCP could be due to the involvement of the different isoforms in the metabolism of MCP. Consistent with the *in vivo* studies, a concentration and time-dependent increase in the activity of the CYP-dependent enzymes (NDMA-d, PROD, and EROD) was observed in the cultured rat brain neuronal and glial cells, following exposure to MCP.

Our data have clearly demonstrated that cell specificity exists in the sensitivity toward MCP. As reported with CYP inducers in the previous chapters,[6,8,9] cell-specific differences were observed in the sensitivity of individual CYPs as reflected by alterations in their expression and

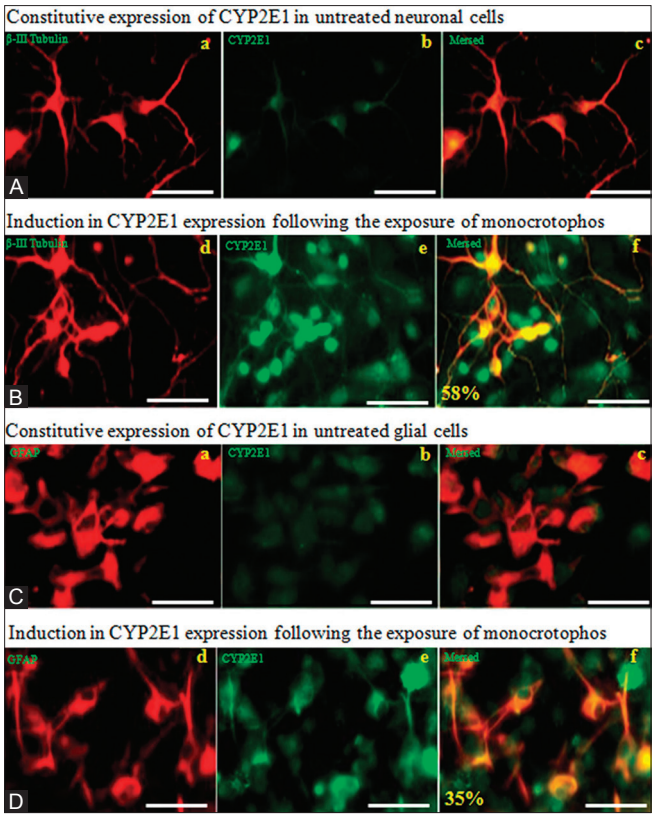

Figure 4: Immunocytochemical localization for relative quantification of xenobiotics induced alteration in protein expression of CYP2E1 in (A, B) neuronal and (C, D) glial cells. In neuronal cells [4 A-B], (a), (b), and (c) represent primary cultures of neuronal cells in DMEM/F-12. (d), (e) and (f) represent cultures of neuronal cells in DMEM/F-12 + MCP. (a) and (d) show cells in culture that are positive for β-III tubulin (red-TRITC), a neuronal marker. (b) and (e) show immunoreactivity in the same neuronal cells with anti-CYP2E1 (green-FITC). (c) and (f) represent an overlay of the two images, control and MCP treated, respectively. In glial cells [4 C-D], (a), (b) and (c) represent primary cultures of glial cells in DMEM/F-12. (d), (e), and (f) represent cultures of glial cells in DMEM/F-12 + monocrotophos. (a) and (d) show cells in culture that are positive for GFAP (red-TRITC), a glial marker. (b) and (e) show immunoreactivity in the same glial cells with anti-CYP2E1 (green-FITC). (c) and (f) represent an overlay of the two images, control and MCP-treated, respectively. Original magnification ×40, scale 20 μm

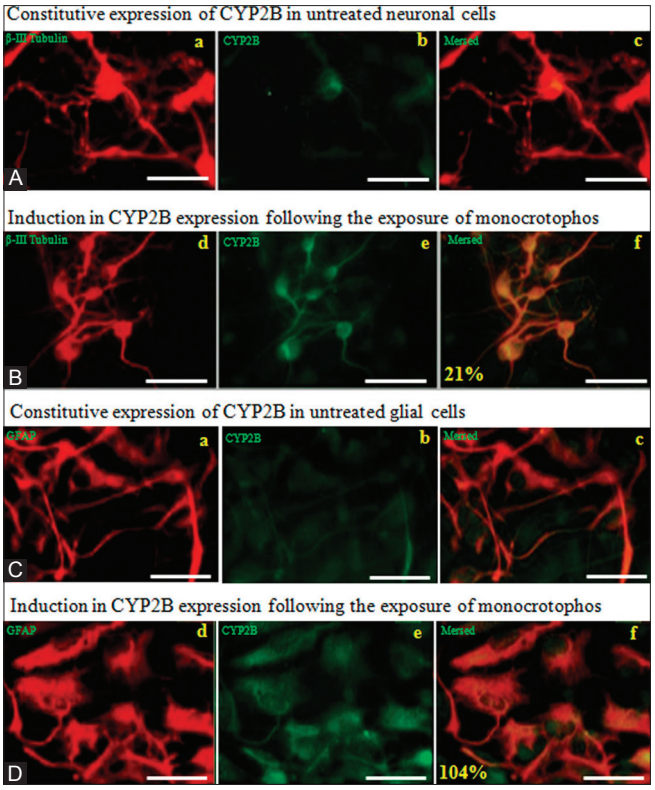

Figure 5: Immunocytochemical localization for relative quantification of xenobiotics induced alteration in protein expression of CYP2B in neuronal (A, B) and glial (C, D) cells**.** In neuronal cells [5 A, B], (a), (b) and (c) represent primary cultures of neuronal cells in DMEM/F-12. (d), (e) and (f) represent cultures of neuronal cells in DMEM/F-12 + MCP. (a) and (d) show cells in culture that are positive for β-III tubulin (red-TRITC), a neuronal marker. (b) and (e) show immunoreactivity in the same neuronal cells with anti-CYP2B (green-FITC). (c) and (f) represent an overlay of the two images, control and MCP treated, respectively. In glial cells [5 C, D], (a), (b) and (c) represent primary cultures of glial cells in DMEM/F-12. (d), (e) and (f) represent cultures of glial cells in DMEM/F-12 + MCP. (a) and (d) show cells in culture that are positive for GFAP (red-TRITC), a glial marker. (b) and (e) show immunoreactivity in the same glial cells with anti-CYP2B (green-FITC). (c) and (f) represent an overlay of the two images, control and MCP treated, respectively. Original magnification ×40, scale 20 μm

catalytic activity following exposure to MCP. In contrast to the greater sensitivity of NDMA-d activity and CYP2E1 expression in neuronal cells, glial CYP2B and CYP1A isoenzymes exhibited greater sensitivity toward MCP as reflected by increased mRNA and protein expression and higher PROD and EROD enzyme activity in these cells when compared with the cultured neuronal cells. These differences in the cellular sensitivity of CYP isoenzymes toward MCP may help in explaining differences in the sensitivity of different brain regions toward MCP. The differences in the sensitivity of different brain regions have been attributed to the differences in the distribution of CYPs in these regions, which may metabolize MCP to different amounts in these regions. Dayal *et al.*,[4] have shown that region-specific induction in the activity of CYP-dependent monooxygenases could be correlated with the ability of different brain regions to metabolize and accumulate MCP. As brain regions differ tremendously in their cellular composition, cell density and function, and the expression pattern of brain CYPs is also extremely varied,[11] these regional differences in the mechanism of MCP have been explained by differences in the sensitivity of the different cell types expressed differently in different brain regions toward MCP. The ability of MCP to induce the expression of neuronal CYPs is of significance as recent studies have indicated a role of CYPs in neurotransmission.[10,12] Studies have also shown that modulation in activity of brain CYPs affects neurotransmission altering either synthesis or transport of neurotransmitters.[13] Possible endogenous substrates for CYP2E1, 1A2, and 2D6 have also been identified

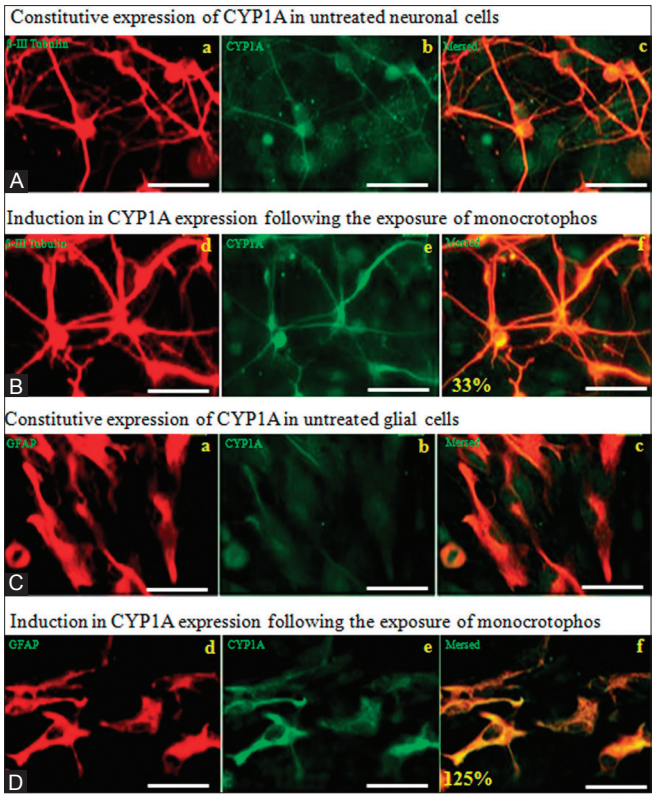

Figure 6: Immunocytochemical localization for relative quantification of xenobiotics induced alteration in protein expression of CYP1A in neuronal (A, B) and glial (C, D) cells. In neuronal cells [6 A-B], (a), (b), and (c) represent primary cultures of neuronal cells in DMEM/F-12. (d), (e), and (f) represent cultures of neuronal cells in DMEM/F-12 + monocrotophos. (a) and (d) show cells in culture that are positive for β-III tubulin (red-TRITC), a neuronal marker. (b) and (e) show immunoreactivity in the same neuronal cells with anti-CYP1A (green-FITC). (c) and (f) represent an overlay of the two images, control and MCP-treated, respectively. In glial cells [6 C-D], (a), (b), and (c) represent primary cultures of glial cells in DMEM/F-12. (d), (e), and (f) represent cultures of glial cells in DMEM/F-12 + MCP. (a) and (d) show cells in culture that are positive for GFAP (red-TRITC), a glial marker. (b) and (e) show immunoreactivity in the same glial cells with anti-CYP1A (green-FITC). (c) and (f) represent an overlay of the two images, control and MCP-treated, respectively. Original magnification ×40, scale 20 μm

in the brain.[14,15] CYP2E1 has been associated with dopaminergic neurotransmission; the enhanced sensitivity of neuronal CYP2E1, in particular could be attributed to the dopaminergic effect of MCP. Kirby *et al.*,[16] have earlier reported that deltamethrin, treated in combination with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a parkinsonian neurotoxin, caused a significant increase in dopamine uptake, consistent with the increased dopamine outflow *in vivo* and suggested an upregulation in dopamine transporter (DAT) expression.

Though, the exact mechanism of pesticides, including organophosphate, in the etiology of Parkinson's diseas (PD) remains to be established. Elwan *et al.*, and Franco *et al.*,[17,18] have reported that lower level exposure to pyrathroids and organophosphate may contribute to PD through upregulation of DAT and increased uptake of endogenous and exogenous neurotoxicants, while increased levels result in apoptotic cell death.[19] Thus, considering that CYP2E1 has a role in dopamine metabolism[13] and PBS the fact that CYP2E1 has been found to be colocalized with tyrosine hydroxylase,[20] one could speculate that the MCP-induced alterations in neuronal CYP2E1 could, in turn, be associated with alterations in the levels of dopamine-induced by MCP.

The increase in the activity of CYP1A and CYP2B isoforms in cultured neurons following MCP exposure could also be of significance, as earlier studies from our laboratory have shown the involvement of these xenobiotic metabolizing CYPs in the neurobehavioral toxicity of deltamethrin.[5] The increase in the expression of these CYPs in cultured neurons on exposure to MCP could be associated with the alterations in the specific brain functions catalyzed by these cells, as well as by these CYP isoforms.

The specific increase in the expression of CYP1A1 in cultured neurons could be associated with the alterations in the levels of catecholamines reported after MCP exposure. Deltamethrin have been earlier reported to alter the levels of various catecholamines in different brain regions.[16] The concentration of acetylcholine was found to be altered in the cerebellum and hippocampus and that of dopamine in the striatum.[16,21] Recent studies have indicated that catecholamines and adrenoreceptors are involved in the regulation of CYP1A1 expression.[22]

Likewise, the increase in the CYP2B expression and dependent catalytic activity in cultured neuronal cells could be associated with the interaction of MCP with the $GABA_A$ receptor complex.[23] A relation between neurological effects of barbiturates mediated via binding with GABA receptor complex, and their capacity to induce CYP2B proteins has been reported.[24] Recent studies using reporter gene protocol have also shown that ligands of peripheral benzodiazipine receptor (PBR) or $GABA_A$ receptor induce CYP2B activity, and this is mediated through the PBRU and the nuclear receptor binding sites NRI/NR2.[24]

As compared to the induction of CYPs in neuronal cells, the greater magnitude of increase in the expression and catalytic activity of CYP2B, CYP1A, and CYP2E1 in glial cells is of toxicological significance, as these cells are the main cellular components of the blood-brain barrier and have an important physiological role in integrating neuronal inputs, neurotransmitter release, and the protection and repair of nervous tissue. Earlier studies have further suggested that astroglial cells play a protective and decisive role in biotransformation of xenobiotics that reach the CNS.[25,26] The role of astrocytes in the defense against reactive oxygen species has also been reported.[27] Glutathione-S-transferase, phase II enzyme has also been reported to be localized exclusively in glial cells, constituting a first line of defense

against toxic substances.[28] The greater responsiveness of CYP1A and CYP2B isoenzymes in glial cells to MCP could be attributed to the involvement of these isoforms in toxication-detoxication mechanisms. However, as CYP1A and 2B enzyme induction has been found to be correlated with the potentiation of the neurobehavioral toxicity of MCP, increase in the expression of these isoenzymes in both glial and neuronal cells could also be involved in the metabolic activation of the MCP at the target site(s).

CONCLUSION

Results of the present study have indicated cell-specific differences in the expression and activity of xenobiotic metabolizing CYPs induced by MCP. Interestingly, glial CYP2B and CYP1A isoenzymes exhibited greater sensitivity to MCP, whereas greater sensitivity of neuronal CYP2E1 was observed in the cultured neuronal cells as reflected by alterations in the mRNA and protein expression and catalytic activity. The increase in the CYP activity and associated expression in cultured neuronal cells induced by MCP could help in explaining the effects of MCP on neurotransmission, as these CYPs are involved in the synthesis or transport of the neurotransmitters. Likewise, as these CYP isoforms are involved in the metabolic activation of MCP the induction of CYPs in glial cells is also of significance as these cells are thought to be involved in protecting the neurons from environmental insults and safeguard them from toxicity. Furthermore, these differences in the induction of CYP isoforms, in the cultured brain cells have further indicated the differences in the sensitivity of these CYPs, expressed may also help in explaining regional differences observed in the sensitivity to MCP.

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