Antidepressants upregulate messenger RNA levels of the neuroprotective enzyme superoxide dismutase (SOD1)

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Objective: To investigate the effect of amitriptyline, bupropion, doxepin or venlafaxine on the gene expression of the neuroprotective enzyme superoxide dismutase (SOD1) in a catecholamine cell in vitro model. **Design:** Molecular study of a cultured cell line. **Interventions:** Rat pheochromocytoma (PC12) cells were incubated in 1 and 10 µmol/L of various antidepressant medications for 24 or 48 hours. **Outcome measures:** Northern blot analysis. **Results:** Amitriptyline up-regulated SOD1 messenger RNA in a time- and dose-dependent manner. The greatest up-regulation was following incubation with 10 µmol/L amitriptyline for 48 hours. The addition of bupropion, doxepin or venlafaxine to PC12 cell cultures also up-regulated SOD1 mRNA. **Conclusions:** These findings suggest that some antidepressants have the ability to positively regulate neuroprotective genes.

Objectif : Étudier l'effet de l'amitriptyline, du bupropion, de la doxépine ou de la venlafaxine sur l'expression génique de la superoxyde dismutase (SOD1), enzyme neuroprotectrice, dans une cellule de catécholamine dans un modèle in vitro. **Conception :** Étude moléculaire d'une lignée de cellules cultivées. **Interventions :** On a incubé des cellules de phéochromocytome (PC12) de rat dans 1 et 10 µmol/L de divers antidépresseurs pendant 24 ou 48 heures. **Mesures de résultats :** Analyse par la méthode Northern. **Résultats :** Régulation à la hausse de l'ARN messager de la SOD1 provoquée par l'amitriptyline d'une façon liée à la durée et à la dose. La régulation à la hausse la plus importante a suivi l'incubation avec 10 µmol/L d'amitriptyline pendant 48 heures. L'addition de bupropion, de doxépine ou de venlafaxine aux cultures de cellule PC12 a aussi haussé la régulation de l'ARNm de la SOD1. **Conclusions :** Ces constatations indiquent que certains antidépresseurs peuvent provoquer une régulation positive de gènes neuroprotecteurs.

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Introduction

Clinically efficacious antidepressants act on many different neurotransmitter systems and receptors. Although some antidepressants act by blocking primarily serotonergic, noradrenergic, or dopaminergic reuptake, others block selected serotonergic receptors or inhibit the enzyme monoamine oxidase (MAO).[1] Since clinical improvement of depression is not seen for at least 2 to 3 weeks following initiation of antidepressant administration, the therapeutic efficacy of antidepressants must be related to phenomena occurring downstream from neurotransmitter reuptake inhibition, receptor blockade or enzyme inhibition.[2] Such mechanisms likely include long-term changes in gene regulation in the affected neurons, and resulting changes in the amount of protein expressed by these genes.[3,4]

Recent studies indicate that a number of antidepressants have the ability to regulate the expression of several genes linked to the survival, protection and repair of neurons, including those of the hippocampus.[4,5] Both stress[6] and dysregulation of the hypothalamic–pituitary–adrenal axis (HPA)[7–9] have long been implicated in the etiology and exacerbation of clinical depression. In addition, stress[10,11] and glucocorticoid injections in animals[12,13] have both been found to cause dendritic atrophy in the hippocampus. This led to the proposal that hippocampal atrophy in clinical depression might be due to such factors, and that this process of neuronal atrophy continues throughout the course of depression.[14] Thus, neuroprotective approaches to treatment have been proposed to prevent further clinical deterioration in depression.

The copper/zinc-dependent enzyme superoxide dismutase (SOD1, E.C.1.15.1.6) helps reduce the oxidative stress of a cell and thus prevents premature aging and death of neurons.[15–17] In vivo studies have demonstrated that up-regulation of this enzyme is neuroprotective in ischemia[18] and glutamate neurotoxicity,[19] whereas reductions in SOD1 activity induce apoptotic cell death of cultured neurons.[20,21] Glucocorticoids have not only been implicated in the etiology of depression, but have also been shown to down-regulate SOD1 activity.[22] If at least some cases of clinical depression are accompanied by progressive hippocampal atrophy throughout the course of the illness, antidepressants that up-regulate SOD1 gene expression may prevent further deterioration of clinical symptoms related to hippocampal degeneration. Therefore, we tested the ability of amitriptyline, buproprion, doxepin and venlafaxine to regulate SOD1 messenger RNA in rat pheochromocytoma (PC12) cells.

Methods

The PC12 cell line was obtained from American Type Culture Collection (Rockville, Md.) and cultured in RPMI 1640 medium (Media Laboratory, College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Sask.) containing 5% fetal calf serum and 10% horse serum plus 100 units/mL penicillin and 100 µg/mL streptomycin, as described in protocols provided by the supplier. Two doses of amitriptyline (1 and 10 µmol/L) were added to the PC12 cultures. Cells were harvested after 24 and 48 hours of incubation. In a second experiment, PC12 cells were incubated with 10 µmol/L of either amitriptyline, buproprion, doxepin or venlafaxine for 48 hours. In both experiments, control cultures receiving vehicle only (saline solution) were also harvested at all time points, for comparison.

SOD1 complementary DNA was kindly provided by Dr. Joseph T. Coyle (Harvard Medical School, Boston, Mass.). The cDNA probe was labelled by random primer synthesis with [a-^{32}P]dCTP as described previously.[23,24] Total cellular RNA was prepared from treated cells by extraction in GITC buffer and collected by ultracentrifugation through a 5.7 mol/L cesium chloride. The RNA was chloroform-extracted, ethanol-precipitated, resuspended in diethylpyrocarbonate (DEPC)-treated water, and stored at –70°C until use. RNA was measured spectrophotometrically by absorbance at 260 nm, and 20 µmol/L of the extract was used for Northern blot analysis. The total RNA was denatured at 65°C for 15 minutes in 3-(*N*-morpholino) propane sofonic acid (MOPS) buffer containing 50% formamide and 2.2 mol/L formaldehyde, and separated by electrophoresis in a 1.0% agarose gel containing MOPS buffer and 2.2 mol/L formaldehyde. Following electrophoresis, the RNA was transferred to nylon membranes and the membranes were cross-linked in a UV Stratalinker 2400 (Stratagene, Aurora, Ont.).

Filters were prehybridized at 65°C for 2 hours with prehybridization solution containing 10% dextransulfate, 5 × SSPE (sodium chloride, sodium biphosphate, EDTA), 5 × Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), and denatured salmon sperm DNA (200 µg/mL). Hybridization was performed at 65°C for 18 hours. After hybridization, membranes were washed at

room temperature twice in 2 × SSPE-0.1% SDS, once in 0.1 × SSPE-0.1% SDS at 60°C and once in 0.1 × SSPE-0.1% SDS at 60°C. The membranes were then exposed to X-Omat AR film (Mandel Scentific, Guelph, Ont.) with intensifying screens at –70°C to obtain autoradiograms. The autoradiograms were scanned with a computerized densitometer (Du 640, Beckman) for quantitative estimates, and the signals were adjusted according to the signals of rehybridization with a cyclophilin probe.

Statistical analysis

Results were analyzed by one- or two-way analysis of variance performed using the CLR ANOVA program (Clearlake Research, Houston, Tex.). In the presence of significant *F* values, individual comparisons between means were made using Newman–Keuls test.

Results

The PC12 cell cultures were treated with 1 or 10 µmol/L of amitriptyline and incubated over 24 or 48 hours at 37°C; at these times and doses, there were no apparent signs of cell death or neurotoxicity. Treatment with amitriptyline produced significant increases in SOD1 gene expression in a dose-dependent manner at 24 hours ($F_{2,9} = 22.4, p < 0.0003$) and 48 hours ($F_{2,9} = 45.2, p < 0.00001$), as revealed by one-way analysis of variance. The increases reached 25.5% (for 1 µmol/L) and 35% (for 10 µmol/L) above control levels after 24 hours of incubation, and 36% (for 1 µmol/L) and 47% (for 10 µmol/L) above control levels after 48 hours (Fig. 1). Two-way analysis of variance revealed an effect of amitriptyline treatment ($F_{1,18} = 63.3, p < 0.00001$) and time ($F_{1,18} = 6.7, p < 0.0188$), but no significant association between dose and time ($F_{1,18} = 1.6, p < 0.2362$).

The addition of 10 µmol/L doses of bupropion, doxepin or venlafaxine to the PC12 cell cultures affected SOD mRNA levels ($F_{4,15} = 15.0, p < 0.00001$), as revealed by one-way analysis of variance. In the Northern blot analysis, the cultured PC12 cells contained a single species of mRNA for SOD1 (Fig. 2). The autoradiograms showed the increase in SOD1 mRNA after 48 hours' incubation with 10 µmol/L of amitriptyline, bupropion, doxepin or venlafaxine (Fig. 2). Multiple comparisons of drug-treated samples demonstrated significantly increased SOD mRNA at 48 hours compared with controls ($p < 0.01$). The increases rose 47% above control levels for amitriptyline, 37% above controls for bupropion, 39% above controls for doxepin and 48% above controls for venlafaxine (Table 1). There were no significant differences in the extent of the increases produced in SOD1 mRNA expression between antidepressants.

Discussion

PC12 cells have been widely used as a model for the study of catecholamine synthesis, release and metabolism, as well as neuronal differentiation and cell death.[25,26] SOD1 activity has been demonstrated in PC12 cultures, and its activity has been shown to be reduced by treatment with antisense oligonucleotides; the decrease in SOD1 activity occurs concomitantly with an increase in apoptotic cell death.[27] The present investigation shows for the first time that several antidepressants increase SOD1 gene expression in PC12 cells. This effect has been demonstrated for amitriptyline (a classic tricyclic antidepressant), bupropion (a second-generation antidepressant), doxepin (a norepinephrine reuptake inhibitor) and venlafaxine (a new serotoninergic/noradrenergic reuptake inhibitor). Thus, the results support the hypothesis that antidepressants could protect neurons by up-regulating the expression of a gene coding for a neuroprotective enzyme (i.e., SOD1). Recent experiments have shown that L-deprenyl and olanza-

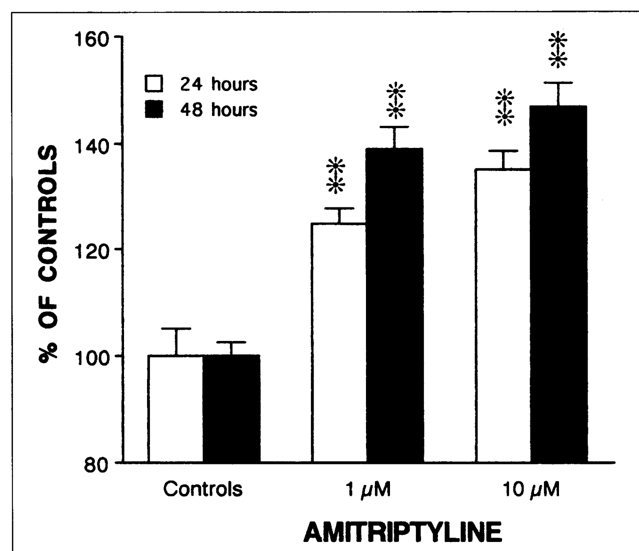

Fig. 1: Effect of amitriptyline on SOD1 gene expression in PC12 cells. The cells were incubated with 1 or 10 µmol/L amitriptyline for 24 or 48 hours. Values are percent means (with standard error bar) obtained from 4 observations. **p < 0.01 (Newman–Keuls test) compared with the control group.

pine both increase the gene expression of SOD1.[24,27] Though the mechanism of their effects is different, both have antidepressant and neuroprotective actions.[24,28–30]

The etiology of depression is only partially understood. Although there have been many reports of hippocampal cell loss in depression, it is difficult to ascertain if the atrophy occurred during neurodevelopment, at the time of onset, or throughout the course of the illness. However, the notion of ongoing neuronal atrophy in depression is supported by the finding that decreases in hippocampal volume are directly proportional to the duration of the illness.[14] In addition, exacerbators of clinical depression such as stress and glucocorticoids have been found to cause hippocampal neuronal atrophy. For example, chronic stress has been shown to cause atrophy of hippocampal neurons in non-human primates,[31] but glucocorticoids, which are thought to be dysregulated in stress[10,13] and depression,[7,8] have also been found to cause hippocampal dendritic atrophy when injected into animals.[32]

It is not known if this volumetric decrease reflects permanent cell loss (via apoptosis or necrosis), or is due to reversible atrophy of neuronal processes. Since antidepressants can reverse many symptoms of clinical depression, and hippocampal atrophy caused by both stress and glucocorticoids can be reversible,[33] it is quite possible that much of this atrophy is transient and therefore state dependent. SOD1 is a ubiquitous enzyme and is widely distributed in the central nervous system, including regions purported to be atrophied in depression, such as the hippocampus.[34] It is possible that up-regulation of this enzyme by antidepressants may prevent further free-radical-induced neurotoxicity in depression caused by dysregulation of the HPA or stress. The up-regulation of SOD1 may occur by an induction of cyclic adenosine monophosphate (cAMP) and cAMP-response element binding protein.[35,36]

Thus, although a common mechanism of action of antidepressants has eluded researchers for years, and since antidepressants act on many different transmitter systems and receptors, it is proposed that one of the shared mechanisms of action of antidepressants is the up-regulation of antioxidant enzymes such as SOD1. In at least those cases of depression that are accompanied by stress or glucocorticoid-induced neurotoxicity, this disorder may need to be treated neuroprotectively throughout the lifetime of the patient. Further studies will be performed in vivo to determine regional differences in SOD1 regu-

Table 1: Effects of antidepressants on SOD1 mRNA levels in PC12 cells, following 48 hours of incubation

Treatment (10 μmol/L)	SOD1 mRNA*, mean (standard error) $n = 4$
Amitriptyline	147† (4.4)
Bupropion	137† (5.1)
Doxepin	139† (7.0)
Venlafaxine	148† (5.6)

*Values expressed as percentage of controls
† $p < 0.01$, one-way analysis of variance analysis and the Newman–Keuls test for multiple comparisons

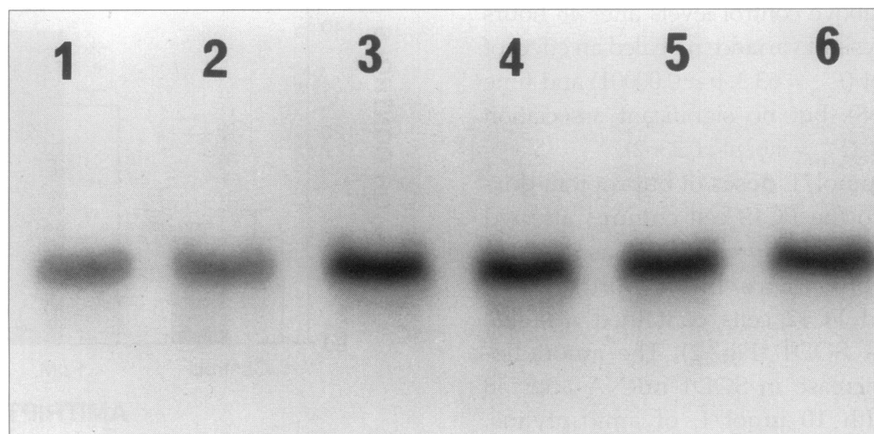

Fig. 2: Effect of antidepressants on SOD1 gene expression. Autoradiogram obtained by Northern blot analysis. Total RNA was obtained from cultured PC12 cells. Lanes 1 and 2 are controls; lane 3 was treated for 48 hours with 10 μmol/L amitriptyline; lane 4 was treated for 48 hours with 10 μmol/L bupropion; lane 5 was treated for 48 hours with 10 μmol/L doxepin; and lane 6 was treated for 48 hours with 10 μmol/L venlafaxine.

lation by antidepressants, including regions such as the hippocampus purported to be atrophied in depression.

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