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Neurotoxicity of some MAO Inhibitors in Adult Rat Hypothalamic Cell Culture

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Monoamine oxidase-A (MAO-A) (amiflamine (AMF) and 4-methylthioamphetamine (MTA)) and MAO-B (L-deprenyl) inhibitors were found to be cytotoxic in a concentration-dependent manner for RCHT cells derived from adult rat hypothalamus. The cytotoxic effects were increased when the inhibitors were co-incubated with dicoumarol and especially with 25 μM AMF+100 μM dicoumarol (2.5-fold; $P < 0.001$). The treatment of RCHT cells solely with AMF induced a marked decrease in the expression of DT-diaphorase mRNA.

Keywords: MAO inhibitors; Dicoumarol; Neurotoxicity; DT-diaphorase; Amiflamine; L-deprenyl

A classical metabolic route of dopamine (DA) is catalyzed by MAO to produce 3,4-dihydroxyphenylacetaldehyde and hydrogen peroxide. Hydrogen peroxide in the presence of transition metals is a source of hydroxyl radicals that may lead to cell death. AMF and MTA are two potent and selective phenylisopropylamine inhibitors of the MAO-A isoform (Scorza *et al.*, 1997). MTA is also a potent inducer of serotonin release (Huang *et al.*, 1992). L-deprenyl is a well-known, potent phenylisopropylaminyl MAO-B inactivator, which prevents cell hypoxia induced by dopaminergic neurotoxins (Matsubara *et al.*, 2001). This compound shows antiapoptotic effects in human cell lines (Szende *et al.*, 2000) and is neuroprotective in a mouse model of neurotoxicity (Castagnoli *et al.*, 1999).

DA has been reported to be oxidized enzymatically and nonenzymatically to aminochrome, which can be reduced by one- or two-electron transfer quinone reductases. One-electron reduction of aminochrome produces leukoaminochrome *o*-semiquinone radical, which autoxidizes by reducing oxygen to superoxide radicals (Baez *et al.*, 1995) leading to neurotoxicity (Paris *et al.*, 2001). DT-diaphorase prevents the formation of leukoaminochrome *o*-semiquinone radical by reducing aminochrome by two-electrons to leukoaminochrome (Baez *et al.*, 1995). Dicoumarol is a potent and specific inhibitor of DT-diaphorase (NAD(P)H: (quinone acceptor) oxidoreductase, E.C.1.6.99.2) (Ernster, 1987).

To evaluate the neurotoxic effects of MAO-inhibitors and the possibility that the inhibition of MAO by AMF (donated by ASTRA, Sweden), MTA (synthesized according G.F. Holland with modifications) and L-deprenyl (donated by Prof. J. Knoll) may result in an increase of the oxidative pathway of DA we used the RCHT cloned cell line, derived from the hypothalamus of an adult rat and transformed to a permanent cell line as described (Caviedes *et al.*, 1993). RCHT cells express dopaminergic markers such as tyrosine hydroxylase, DOPA decarboxylase and DA receptors. The cell line grows in monolayers. The cultures were kept in an incubator at 37°C with 100% humidity and an atmosphere of 10% CO_2. The cells were grown in DME/HAM-F12 (1:1), 10% bovine serum, 2.5% fetal bovine serum, and 40 mg/l gentamicine sulphate. MTA, AMF and L-deprenyl proved to be neurotoxic in a concentration-dependent manner (Fig. 1(A)). Comparison between the cytotoxic effects of MAO inhibitors at the same

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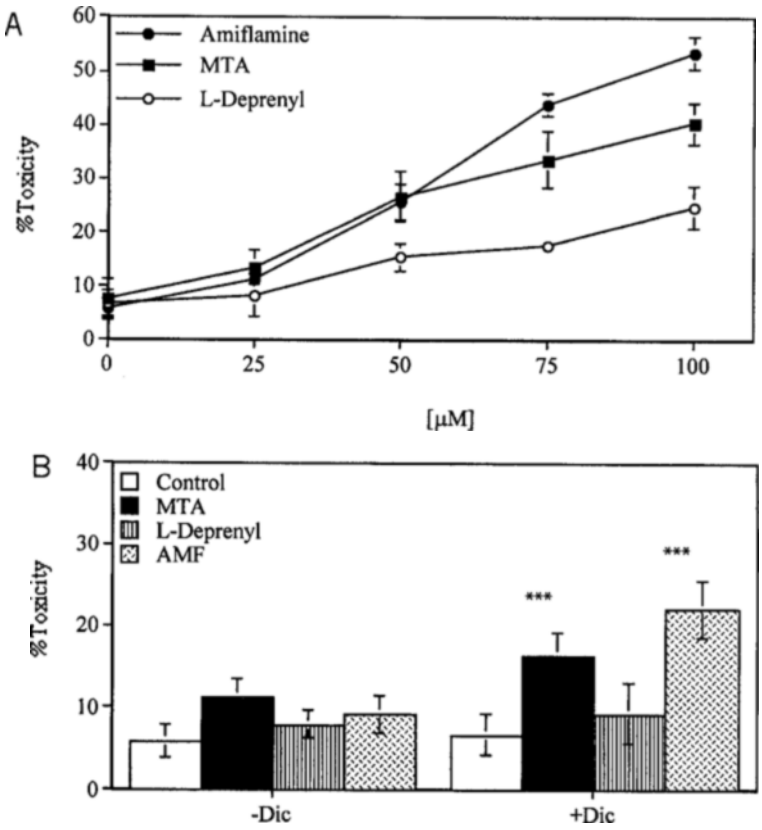

FIGURE 1 The effect of AMF, MTA and L-deprenyl on RCHT cell survival. (A) The effect of AMF, MTA and L-deprenyl on RCHT cell survival at different concentrations. (B) The effect of AMF, MTA and L-deprenyl in the absence and presence of dicoumarol. The values are the mean ± SD ($n = 3$). The significance was determined by using the unpaired Student's t-test.

concentration (25 μM) on RCHT cells showed that MTA was more toxic than other MAO-inhibitors (Fig. 1(B)). However, the cytotoxic effects of MAO-inhibitors changed when co-incubated with dicoumarol. The toxic effects of MTA and AMF were 2.4-fold ($P < 0.001$) and 3.3-fold ($P < 0.001$) higher in the presence of dicoumarol than in the control (Fig. 1(B)). The difference between the effect of MAO-inhibitors in the absence and presence of dicoumarol was 2.5-fold ($P < 0.001$) for AMF and 1.5-fold for MTA ($P < 0.001$), while L-deprenyl produced a non-significant difference (Fig. 1(B)).

We have determined the level of expression of DT-diaphorase mRNA in RCHT cells treated with AMF in the presence or absence of dicoumarol by using RT-PCR as previously described (Dagnino-Subiabre *et al.*, 2000). The treatment with AMF alone induced a marked decrease in the expression of DT-diaphorase mRNA. However, the level of expression of the enzyme returned to control level when the cells were treated with AMF and dicoumarol (Fig. 2).

All three MAO inhibitors tested exhibited neurotoxicity, but this activity appears to depend on the selectivity of the drugs for the two different isoforms of the enzyme, since the MAO-A inhibitors (AMF, MTA) are more toxic than the MAO-B inhibitor (L-deprenyl). The possible mechanism of MAO inhibitor-dependent toxicity may be due to an increase of DA concentration as a consequence of MAO inhibition, resulting in DA autoxidation to form aminochrome. The marked increase in toxicity when AMF and MTA were incubated simultaneously with dicoumarol suggests a neuroprotective role of DT-diaphorase. The decrease of DT-diaphorase mRNA expression when the cells were treated with AMF and the recovery of expression to control levels when the cells were treated with AMF+dicoumarol suggest the existence of two different mechanisms of regulation of DT-diaphorase mRNA.

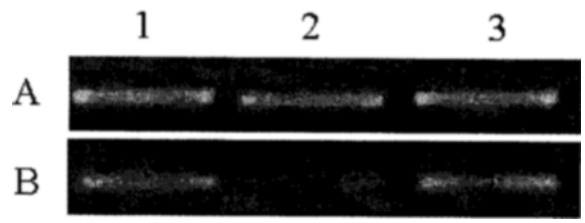

FIGURE 2 The level of expression of DT-diaphorase mRNA in RCHT cells treated with AMF or AMF+dicoumarol. Lane 1 is for control cells, lane 2 is for cells treated with AMF and lane 3 is for cells treated with AMF and dicoumarol. A is for the RT-PCR product of glyceraldehyde-3-phosphate dehydrogenase used as housekeeping gene and B is for DT-diaphorase.

The fact that dicoumarol does not significantly increase the neurotoxicity of L-deprenyl supports the idea that the latter drug exerts its neuroprotective effect by a mechanism independent of MAO-B inhibition.

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