VI. SUMMARY

Catechol-O-methyltransferase (COMT) is a cellular enzyme localized in the brain mainly in glial cells as well as in many peripheral tissues, including the intestinal tract, liver and kidney (Kaakkola et al., 1994). It catalyses the transfer of the methyl group from S-adenosyl-L-methionine to one of the hydroxyl groups of a catechol substrate in the presence of Mg\(^{2+}\). The physiological substrates of COMT include L-Dopa (levodopa, L-3,4-dihydroxyphenylalanine), catecholamines (dopamine, noradrenaline, adrenaline), their hydroxylated metabolites, catechol estrogens and ascorbic acid.

Inhibitors of COMT, such as entacapone and tolcapone, are used as adjuncts to L-Dopa in the treatment of Parkinson’s disease (PD) because they prevent the metabolism of L-Dopa to its inactive O-methylated derivative 3-O-methyl-dopa (3-OMD) (Spencer and Benfield, 1996; McNeely and Davis, 1997). This is important because 3-OMD is the major plasma L-Dopa metabolite when the peripheral aromatic amino acid decarboxylase (AADC) is inhibited with benserazide or carbidopa. By reducing levels of this inactive metabolite, COMT inhibition improves bioavailability of L-Dopa and increases the duration of clinical response in patients with PD.

Preclinical studies have shown that tolcapone is a dose-dependent inhibitor of both peripheral and central COMT, whereas entacapone acts only peripherally (Brannan et al., 1997); both compounds are approximately equipotent with respect to peripheral COMT inhibition (Zürcher et al., 1991; Kaakkola and Wurtman, 1992; Männistö, 1992a). Central O-methylation of L-Dopa and dopamine can not occur following central COMT inhibition; it is therefore reasonable to assume that treatment with L-
Dopa and an AADC inhibitor in combination with the COMT inhibitor tolcapone leads to an increased central availability of L-Dopa and dopamine, which may subsequently result in an increase in the generation of free radicals and neurotoxicity (Kuhn et al., 1998). The very reactive hydroxyl radicals (OH·) are produced by autoxidation of L-Dopa and dopamine, and by the Fenton reaction, which involves the iron(II)-catalysed breakdown of hydrogen peroxide (H₂O₂), a product of monoamine oxidase (MAO)-catalysed dopamine degradation (Halliwell, 1992; Götz et al., 1994). Indeed, using microdialysis studies in rats, it was demonstrated that a dose of 10 mg/kg intraperitoneally (i.p.) administered tolcapone, in contrast to the same dose of entacapone, increased the extracellular levels of 3,4-dihydroxy-phenylacetic acid (DOPAC, the metabolite of MAO-catalysed degradation of dopamine) and decreased those of homovanillic acid (HVA) and 3-methoxytyramine (3-MT, the metabolite of COMT-catalysed dopamine degradation) (Kaakkola and Wurtman, 1993). This dose was thus used to differentiate between the effects of central and peripheral COMT inhibition in the rat.

Based on experimental studies which have shown deleterious effects of L-Dopa in vivo and in vitro (Fahn, 1997), it has been suggested that L-Dopa itself may contribute to the progression of the disease; this hypothesis is, for many clinicians, the rationale for postponing and sparing L-Dopa and to begin the therapy with dopamine agonists and MAO type B (MAO-B) inhibitors, and N-Methyl-D-Aspartate (NMDA) antagonists (Myllylä et al., 1997; Montastruc et al., 1999). Using [¹²³I]-β-CIT single photon emission computed tomography (SPECT), it was recently demonstrated that patients treated with L-Dopa show a more rapid decrease of dopamine transporter density (Staffen, 2000). This result could be interpreted as an indication for in vivo neurotoxicity of high concentrations of L-Dopa.
The aim of this study was to examine the dose-dependent acute effects of systemically administered entacapone and tolcapone on the extracellular formation of OH\(^{-}\) in \textit{vivo} following treatment with L-Dopa and the AADC inhibitor carbidopa. The formation of extracellular OH\(^{-}\) were determined by the measurement of 2,3-dihydroxybenzoic acid (2,3-DHBA), a reaction product of OH with sodium salicylate (Patty, 1995), using microdialysis in the striatum of anesthetised rats. The COMT inhibitors were administered as 5% gum arabic suspensions intraperitoneally (i.p.) at doses of 0, 0.5, 1.0, and 10 mg/kg body weight to a total of 48 male HAN-Wistars rats. At the same time animals were treated with 50 mg/kg i.p. carbidopa. L-Dopa was injected i.p. 40 min after drugs of interest. Microdialysis samples were collected every 20 min for 280 min at a perfusion rate of 1\(\mu\)l/min. Systemically administered tolcapone but not entacapone induced a dose-dependent increase in OH\(^{-}\) formation in the striatum of anesthetised rats following treatment with L-Dopa and carbidopa. The highest dose of tolcapone used (10 mg/kg i.p.) induced a progressive increase in the dialysate levels of 2,3-DHBA. The dose-dependent increase in OH\(^{-}\) formation was also demonstrated using the area under curve (AUC) value. However, only the highest tolcapone dose used increased the AUC\(0-280\) significantly to 280% compared to the control value. The increase in OH\(^{-}\) formation is most likely caused by the higher enzymatic and non-enzymatic (autoxidation) metabolism of dopamine as a result of increased central availability by reducing the rate of metabolism.