

Catechol-*O*-methyltransferase (COMT): Biochemistry, Molecular Biology, Pharmacology, and Clinical Efficacy of the New Selective COMT Inhibitors

PEKKA T. MÄNNISTÖ¹ AND SEPPO KAAKKOLA

Department of Pharmacology and Toxicology, University of Kuopio, Kuopio, Finland (P.T.M.); and Department of Neurology, University of Helsinki, Helsinki, Finland (S.K.)

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¹ Address for correspondence: Dr. Pekka T. Männistö, Department of Pharmacology and Toxicology, University of Kuopio, P.O. Box 1627, FIN-70 211 Kuopio, Finland. E-mail: Pekka.Mannisto@uku.fi

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I. Introduction

Axelrod et al. (1958) first described the enzyme-catalyzed *O*-methylation of catecholamines and other catechols in the late 1950s. The enzyme responsible for the *O*-methylation, catechol-*O*-methyltransferase (COMT; EC 2.1.1.6),² was partly purified and characterized by the same group (Axelrod and Tomchick, 1958). The subsequent basic research on COMT and the first COMT inhibitors introduced between 1958 and 1975 have been extensively reviewed by Guldberg and Marsden (1975).

The interest in COMT was rekindled in the late 1980s when the potent and selective second-generation COMT inhibitors were developed (Männistö and Kaakkola, 1989, 1990), and soon the structures of the two isoforms of COMT and the gene were characterized and COMT polypeptide cDNAs were cloned (Salminen et al., 1990; Bertocci et al., 1991; Lundström et al., 1991). Several review articles have recently dealt with this development (Männistö and Kaakkola, 1989, 1990; Männistö et al., 1992b, 1994; Roth, 1992; Kaakkola et al., 1994a; Dingemans, 1997), culminating in the marketing of two new COMT inhibitors. This review concentrates on the recent information on the biochemistry and molecular biology of COMT and on the pharmacology and clinical efficacy of the new selective and relative nontoxic COMT inhibitors.

II. COMT Gene and Proteins

A. One COMT Gene and Two Proteins

The structural organization of the COMT gene has been reviewed in detail by Lundström et al. (1995). There is one single gene for COMT, which codes for both soluble COMT (S-COMT) and membrane-bound COMT (MB-COMT; Salminen et al., 1990; Lundström et al.,

1991). In the application of the in situ hybridization and cell hybrid techniques, the COMT gene in humans was localized to chromosome 22, band q11.2 (Grossman et al., 1992a; Winqvist et al., 1992). The human COMT gene contains six exons with the two first exons being noncoding. The expression of the COMT gene is controlled by two distinct promoters located in exon 3 (Salminen et al., 1990; Lundström et al., 1991). The distal 5' promoter (P2) regulates synthesis of 1.9-kb (rat) and 1.5-kb (human) mRNA species. This mRNA can code for both MB-COMT and S-COMT proteins by using the leaky scanning mechanism of translation initiation (Tenhunen and Ulmanen, 1993; Tenhunen et al., 1993, 1994). The expression of the shorter transcript (1.6 kb in rat and 1.3 kb in human) is regulated by the P1 promoter, which is located between S-COMT and MB-COMT ATG start codons and partly overlaps the MB-COMT coding sequence. MB-COMT AUG translation initiation codon is not included in these transcripts, which therefore can code only for S-COMT polypeptide (Tenhunen et al., 1993, 1994; Tenhunen and Ulmanen, 1993).

In most human tissues, there are both transcripts, but in the human brain, only the longer transcript was found in 16 regions studied (Hong et al., 1998). When S-COMT and MB-COMT polypeptides of various rat and human tissues were quantified through Western blot analysis, S-COMT was usually dominant by a factor of 3 or higher. The only exception was the human brain, where 70% of the total COMT polypeptides was MB-COMT and 30% was S-COMT, demonstrating the bifunctionality of the 1.5-kb transcript (Tenhunen et al., 1993, 1994). The varying expression levels of the human COMT promoters suggest regulation by some tissue-specific transcription factors. In fact, human COMT promoters contain several putative binding sites for such factors that may cause the variability of COMT gene expression in different tissues (Tenhunen et al., 1994).

Both rat and human S-COMTs contain 221 amino acids, and the molecular masses are 24.8 and 24.4 kDa, respectively. The human S-COMT is 81% identical with the respective rat enzyme (Lotta et al., 1995; Lundström et al., 1995). Rat MB-COMT contains 43 additional amino acids, and human MB-COMT contains 50 additional amino acids. The corresponding molecular masses of MB-COMT are 29.6 and 30.0 kDa. Of these extra amino acids, 17 (rat) and 20 (human) function as hydrophobic membrane anchors (Salminen et al., 1990; Tilg-

² Abbreviations: COMT, catechol-*O*-methyltransferase; S-COMT, soluble catechol-*O*-methyltransferase; MB-COMT, membrane-bound catechol-*O*-methyltransferase; AdoMet, *S*-adenosyl-*L*-methionine; AUC, area under the curve; DBA, dihydroxybenzoic acid; C_{max} , maximum drug concentration in plasma; DOPAC, 3,4-dihydroxyphenyl acetic acid; 6-FD, 6-[¹⁸F]fluorodopa; MAO, monoamine oxidase; 3-OMD, 3-*O*-methyl-dopa; 3-OMFD, 3-*O*-methyl-[¹⁸F]fluorodopa; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenyl glycol; DDC, dopa decarboxylase; 3-MT, 3-methoxytyramine; PET, positron emission tomography; UPDRS, Unified Parkinson's Disease Rating Scale; PD, Parkinson's disease; $T_{1/2}$, elimination half-life; T_{max} , time to reach C_{max} ; U-0521, 3',4'-dihydroxy-2-methylpropiphenone; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; levodopa, a drug preparation containing *L*-dopa; *L*-dopa, *L*-3,4-dihydroxyphenylalanine.

mann and Kalkkinen, 1990, 1991; Bertocci et al., 1991; Lundström et al., 1991). The remainder of the MB-COMT molecule is suspended in the cytoplasmic side of the intracellular membranes (Bertocci et al., 1991; Lundström et al., 1991; Ulmanen and Lundström, 1991).

B. Three-Dimensional Structure of COMT

Rat S-COMT has been recently crystallized at 1.7- to 2.0-Å resolution (Vidgren et al., 1991, 1994), and the critical atomic structures have been described in detail (Vidgren and Ovaska, 1997). Therefore, only some of the most important aspects are repeated here.

COMT has as a single domain α/β -folded structure in which eight α -helices are arranged around the central mixed β -sheet. The active site of COMT consists of the S-adenosyl-L-methionine- (AdoMet)-binding domain and the actual catalytic site. The binding motif of the AdoMet site is similar to the Rossmann fold, which is a common feature of many nucleotide binding proteins. The crystal structures of several of the methyl transferases that have been characterized are strikingly similar in the AdoMet-binding regions. The catalytic site is formed by a few amino acids that are important for the binding of the substrate, water, and Mg^{2+} and for the catalysis of O-methylation (Fig. 1). The Mg^{2+} , which is bound to COMT after AdoMet binding, converts the hydroxyl groups of the catechol substrate to be more easily ionizable. Near one of the hydroxyl groups of the substrate, there is a lysine residue (Lys144) in COMT that accepts the proton from that hydroxyl, and subsequently the methyl group from the AdoMet is transferred to the hydroxyl group. Lysine acts as a general catalytic base in this base-catalyzed nucleophilic reaction. Mg^{2+} has

an octahedral coordination to two aspartic acid residues (Asp141 and Asp169), to one asparagine (Asn170), to both catechol hydroxyls, and to a water molecule. Hence, Mg^{2+} ions control the orientation of the catechol moiety. In addition, the “gatekeeper” residues Trp38, Trp143, and Pro174 that form the hydrophobic “walls” and that define the selectivity of COMT toward different side chains of the substrate participate directly in the methylation reaction by keeping the planar catechol ring in the correct position (Fig. 1). They contribute significantly to the binding of the substrates (and inhibitors) of COMT (Vidgren et al., 1991, 1994, 1999; Vidgren and Ovaska, 1997). Although Mg^{2+} ions are crucial for COMT, most other small molecule methyltransferases are not Mg^{2+} dependent (Fujioka, 1992).

C. Kinetic Reaction Mechanism of COMT

COMT catalyzes the transfer of the methyl group of AdoMet to one of the hydroxyl groups of the catechol substrate in the presence of Mg^{2+} (Guldberg and Marsden, 1975). Methylation of the 3'-hydroxyl is much more common than that of the 4'-hydroxyl, for reasons that are discussed later. The mechanism and kinetics of the O-methylation reaction have been studied using partially purified enzyme preparations from various sources, most commonly from rat liver and rat or human brain, and recently also with the use of recombinant enzymes. The stereochemical course of the reaction has shown that the methyl transfer proceeds through a direct nucleophilic attack by one of the hydroxyl groups of the catechol substrate on the methyl carbon of AdoMet in a tight S_N2 -like transition state (Woodard et al., 1980).

A sequential ordered mechanism has been proposed based on product inhibition studies (Jeffery and Roth, 1987), but there is some doubt as to their accuracy. AdoMet does seem to be the first substrate to bind, and S-adenosyl homocysteine is the last product to dissociate from the enzyme (Rivett and Roth, 1982; Tunncliffe and Ngo, 1983). However, now that the crystal structure of S-COMT has been resolved, it is possible to extend the kinetic studies to include recombinant S-COMT and MB-COMT with many different substrates. Thus, Lotta et al. (1995) proposed a reformulation of the kinetic behavior and the ordered mechanism of O-methylation. The active site of COMT, which is located in the outer surface of the enzyme, is a shallow groove on the surface of COMT. This is the same in both S-COMT and MB-COMT (Vidgren et al., 1994; Vidgren and Ovaska, 1997). AdoMet is able to bind even without Mg^{2+} . The binding pocket of the methionine portion of AdoMet is deeper within the protein than the Mg^{2+} site, and it would be impossible for AdoMet to bind after Mg^{2+} . Moreover, the catechol substrate cannot bind before AdoMet because then it would also be impossible for AdoMet to reach its binding site in the narrow groove located deep in the COMT molecule (Vidgren et al., 1994; Vidgren and

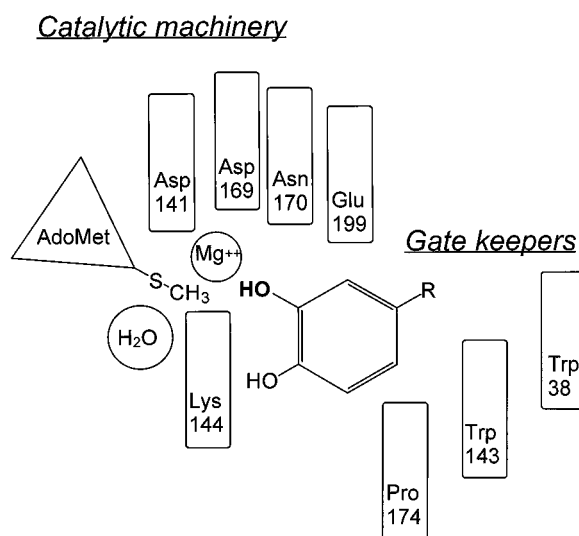


FIG 1. Schematic view of the catalytic site of COMT demonstrating the necessary amino acids (rectangular boxes), Mg^{2+} , and water molecule, with AdoMet and the catechol substrate. Among the amino acids, there are “gatekeepers” and catalytic amino acids, notably Lys144, which acts as a general catalytic base. The activated hydroxyl, to be methylated, is shown in bold. Details are from Vidgren and Ovaska (1997) and Vidgren et al. (1999).

Ovaska, 1997). Therefore, the order in which the compounds bind is as follows: AdoMet binds first, followed by Mg^{2+} and, finally, the catechol substrate. This reaction cycle differs from the previous suggestion that Mg^{2+} binds to COMT in a rapid equilibrium before the addition of AdoMet (Jeffery and Roth, 1987).

Although S-COMT and MB-COMT have identical kinetic mechanisms (Ca^{2+} inhibition, Mg^{2+} requirement, pH optimum, a similar K_m value for AdoMet, recognition by S-COMT antiserum), S-COMT and MB-COMT are certainly different enzymes, and MB-COMT is not a precursor of S-COMT (see above).

Based on early studies with crudely purified enzymes, S-COMT has a high K_m value for dopamine but a very high capacity (V_{max} from 50 pmol/min \times mg protein in skeletal muscle to values as high as 14,690 pmol/min \times mg protein in the liver). MB-COMT has a much lower K_m value but a low capacity (2–40 pmol/min \times mg protein; Guldberg and Marsden, 1975; Roth, 1992). It is noteworthy that the V_{max} values are strongly dependent on the enzyme activities in various tissues rather than on the basic kinetic constants of these enzymes. Physiological substrate concentrations and possible differences in substrate selectivity have to be considered when the relative importance of either enzyme subtype is assessed. Dopamine levels in striatum and hypothalamus of brain homogenates are about 65 and 3 μM , respectively. The striatal and hypothalamic noradrenaline concentrations are 0.8 and 12 μM , respectively. It seems that at the concentrations of catecholamines naturally present, MB-COMT may be more important in their metabolism (Roth, 1992). According to Roth and associates (Rivett et al., 1982; Rivett and Roth, 1982; Roth, 1992), MB-COMT is the predominant enzyme at dopamine concentrations of $<10 \mu M$ and at noradrenaline concentrations of $<300 \mu M$.

In addition, the recombinant human MB-COMT ($K_m = 10 \mu M$) has a higher affinity for catechol substrates than S-COMT ($K_m = 108 \mu M$; Malherbe et al., 1992).

Lotta et al. (1995) also reported that the catalytic number (V_{max}) of rat recombinant S-COMT was slightly higher than that of MB-COMT with all four substrates studied. It is notable that in the study of Lotta et al. (1995), V_{max} values were calculated taking into account the actual enzyme concentration. Thus, V_{max} can be given as U/min, and it represents the catalytic number (k_{cat}). At saturating substrate concentrations, S-COMT functioned two times more efficiently, but the catalytic number for both COMT isoforms was similar for all substrates, nor was there any substrate selectivity. The K_m and k_{enzyme} values varied greatly between the two isoforms and were strongly dependent on the substrate. Malherbe et al. (1992) reported that S-COMT has a 15-fold higher K_m value and a 5-fold higher k_{enzyme} value for catecholamines than MB-COMT. However, this is not the case for dihydroxybenzoic acid (DBA) and L-3,4-dihydroxyphenylalanine (L-dopa; Table 1).

The catalytic sites of S-COMT and MB-COMT have identical amino acid sequences, but the membrane-bound portion of MB-COMT or the charged membrane itself causes more favorable, although poorly characterized, binding interactions even though there is no conformational change in the basic enzyme structure of MB-COMT.

New data generated using recombinant enzyme isoforms have confirmed earlier results but also added further detail to the picture, particularly regarding the binding differences of the various COMT substrates (Lotta et al., 1995; Tables 1 and 2). The atomic structure and the sequence comparison reveal that all residues that are important for the binding of the substrates and for the catalytic activity are similarly conserved in human and rat COMT. Only two amino acids are different in the active site. When the three substrates (L-dopa, dopamine, and DBA), which have different affinities for the active site of COMT, are compared, it is apparent that the kinetic differences are due to interactions of the substrate side chains with COMT residues. Generally,

TABLE 1
Kinetic parameters for 3-O-methylation and 4-O-methylation by human COMT expressed in baculovirus-infected insect cells with four different substrates

	3-O-Methylation			4-O-Methylation		
	V_{max} (catalytic number)	K_m	$V_{max}/K_m \times 100$ (k_{enzyme})	V_{max} (catalytic number)	K_m	$V_{max}/K_m \times 100$ (k_{enzyme})
	1/min	μM	100/min \times μM	1/min	μM	100/min \times μM
L-Dopa						
S-COMT	42.5	613	6.9	2.1	591	0.36
MB-COMT	12.2	266	4.6	8.4	190	4.4
Dopamine						
S-COMT	37.2	207	18			
MB-COMT	16.9	15	112			
Noradrenaline						
S-COMT	34.9	369	9.5			
MB-COMT	18.1	24	75.1			
DBA						
S-COMT	43.4	39	38.9	8.9	35	25.4
MB-COMT	22.2	30	74	1.3	80	1.6

Data are from (Lotta et al., 1995).

TABLE 2
Regioselective parameters for 3-O-methylation/4-O-methylation of human COMT expressed in baculovirus-infected insect cells with three different substrates

Substrate	Relative V_{\max}	Relative V_{\max}/K_m	Relative Rate (at saturating substrate concentrations)
S-COMT			
L-Dopa	20.2	19.5	15.4
Dopamine	4.4	4	4.2
DBA	4.9	4.4	5
MB-COMT			
L-Dopa			88.4
Dopamine			66.3
DBA	17.1	45.5	21.9

Data are from Lotta et al. (1995, their Table 5).

the binding of the catechol ring is similar to binding of enzyme to nitrocatechol-type inhibitors (e.g., OR-486 or OR-1840) when this is viewed with crystallized rat S-COMT (Vidgren et al., 1994). DBA has a charged carboxyl moiety, but the molecule is planar and fits well between the gatekeepers Trp38 and Pro174 (Fig. 1), and thus it has high affinity. Dopamine has a positively charged amino group that, despite its rotational freedom, still makes a repulsive contact with one of the gatekeepers. L-Dopa has the largest, double-charged side chain, and therefore the propulsions are strong and its affinity to COMT is lower.

COMT is able to methylate only one of the two catechol hydroxyls. Both COMT isoforms favor 3-*O*-methylation, and MB-COMT is even more regioselective than S-COMT (Table 2). The *meta/para* ratio is higher, 22 to 88 (depending on the substrate) in MB-COMT than in S-COMT, 4 to 15 (depending on the substrate; Lotta et al., 1995). The reason for favoring 3-*O*-methylation over 4-*O*-methylation may be that as the *p*-hydroxyl group (i.e., 4-*O*-hydroxyl) approaches the AdoMet; this forces the side chain to become orientated in an unfavorable position with the hydrophobic protein residues of the catalytic site. As stated, L-dopa has the strongest repulsive interactions, which are reflected not only in its high K_m value but also as in the high 3-*O*-methylation/4-*O*-methylation ratio (Table 2).

Recently, molecular dynamic simulation studies have been used to explain the preference of *meta-O*-methylation over *para-O*-methylation. The catechol ring has a tilt of about 30 degrees compared with that of the X-ray structure of the active site. This directs any substituent at the *para*-position of the catechol ring into a hydrophobic pocket formed by Trp38 and Tyr200. Hydrophobic substituents are accommodated in this pocket so that *para-O*-methylation is favored, whereas polar substituents are repelled, making *meta-O*-methylation favorable (Lan and Bruice, 1998). In addition, the distances of the hydroxyls to the active methyl group of AdoMet are very different (2.6 versus 4.8 Å), so no competition for the methylation site is possible (Vidgren et al., 1999). Different *meta/para* ratios of substituted catechols are

solely a consequence of their relative ability to bind in two dissimilar orientations to the active site of COMT (Vidgren et al., 1999).

Those catechols that contain electronegative substituents (like NO₂, CN, and F) are potent inhibitors, but poor substrates, of COMT (Bäckström et al., 1989; Borgulya et al., 1989). This point has been recently clarified in a semiempirical study using dinitrocatechol (OR-486) as a model inhibitor (Ovaska and Yliniemelä, 1998). As mentioned, Lys144 acts as a general base in the methylation and can activate one of the catechol hydroxyls before the nucleophilic attack of the methyl group of AdoMet. The electronegative nitro groups reduce the nucleophilicity of the ionized catechol hydroxyls (Fig. 1). Therefore, dinitrocatechol itself is not methylated at all but instead is a potent COMT inhibitor. Among the mononitro catechols, the decrease in nucleophilicity is evidently less than that in dinitrocatechol, and the ultimate effect depends on the side chain. For instance, entacapone is to all intents not *O*-methylated at all, whereas tolcapone is *O*-methylated by about 3% (Dingemans, 1997).

D. Other Enzymological Aspects

COMT is not easily induced or suppressed. The capacity of COMT in the peripheral tissues is probably so high that only a minor fraction of the protein is ever needed. Therefore, general peripheral COMT inhibition may be difficult to achieve. This may be crucial for the safety of COMT inhibitors. Locally, such as in the gastrointestinal mucosa (Schultz and Nissinen, 1989), the new powerful COMT inhibitors can suppress COMT activity sufficiently to cause significant changes in the metabolism of L-dopa.

Some special treatments or situations may increase COMT activity, but most of them cause at best only a doubling of activity. Very early, it was found that long-term treatment with pyrogallol could elevate the COMT activity in the liver (Guldberg and Marsden, 1975). Dipyrindamole (Li et al., 1991), exogenous AdoMet (Baldezarini, 1987), and butylated hydroxyanisole (Lam, 1988) can slightly increase COMT activity.

Pregnancy and progesterone treatment seem to induce COMT activity in the uterus. In the rat, estrogen exposure results in decreased hepatic COMT activity (Cohn and Axelrod, 1971; Parvez et al., 1975). On the other hand, subchronic estrogen treatment increases COMT immunostaining in hamster kidney (Weisz et al., 1998b). There also is a gender difference. The COMT activity in the liver of male subjects is about 30% higher than that in females (Boudikova et al., 1990). During aging, COMT activity in the liver increases by about 10-fold from birth to adulthood (Guldberg and Marsden, 1975). A similar trend occurs in the kidney, where K_m values increase about 5-fold during aging (Vieira-Coelho and Soares-da-Silva, 1996). This could be explained by assuming that the predominant form of COMT is not the

same in newborn rats and adult animals. Some diseases seem to be associated with altered COMT activities. COMT activity in the spinal cord appears to be decreased in Huntington's disease (McGeer et al., 1993), but it is slightly increased in amyotrophic lateral sclerosis where monoamine oxidase (MAO)-B activity was substantially elevated (Ekblom et al., 1993). Chromosomal microdeletion at *22q11-13* (where the human COMT gene is also located) has been associated with a number of defects or syndromes (Lachman et al., 1996a). This phenomenon and genetic COMT polymorphism in various diseases are discussed later (see *Genetic Polymorphism: Variants with Different Thermostability*).

We also made an anecdotal finding that high concentrations of ethanol can reduce MB-COMT activity but increase S-COMT activity in recombinant enzyme forms. However, the concentrations needed are from 50 to 1000 mM, which could scarcely be attained even in cases of ethanol intoxication (Reenilä et al., 1995).

E. Genetic Polymorphism: Variants with Different Thermostability

The level of COMT enzyme activity is genetically polymorphic in human tissues with a trimodal distribution of low ($COMT^{LL}$), intermediate ($COMT^{LH}$), and high ($COMT^{HH}$) activities (Weinshilboum and Raymond, 1977; Boudikova et al., 1990; Jeanjean et al., 1997). This polymorphism, which according to segregation analysis of family studies is caused by autosomal codominant alleles, leads to 3- to 4-fold differences in COMT activity in human erythrocytes and liver (Weinshilboum and Raymond, 1977; Boudikova et al., 1990; Jeanjean et al., 1997). Low COMT activity is associated with enzyme thermostability, even at 37°C (Scanlon et al., 1979; Spielman and Weinshilboum, 1981; Boudikova et al., 1990). Recently, the molecular basis of the thermostability was revealed with the baculovirus expression system; namely, substitution of Val108 by Met108 in the S-COMT (or the corresponding amino acids 158 in the MB-COMT) is caused by transition of guanine to adenine at *codon 158* of the COMT gene (Grossman et al., 1992b; Lotta et al., 1995). Although there are some other mutations in the COMT gene (Lachman et al., 1996b), it seems quite established that the low thermal stability and the low COMT activity go together. Because there is only one COMT gene without any known tissue-specific splice variants (Tenhunen et al., 1994; Lundström et al., 1995), it is likely that the *codon 108/158* polymorphism, causing the change in thermostability, also leads to functional alterations of COMT in all tissues (Lachman et al., 1996b). The catalytic activity per se is the same with the two variants, and the labile enzyme variant is fully stabilized by AdoMet binding. In the following discussion, we do not necessarily go to the methodological details when describing the association of COMT activity with a number of diseases. In publications printed after 1996, the molecular difference may be demon-

strated. Earlier, either the thermostability or only the COMT activity was reported.

Polymorphism of COMT activity could have clinical implications, but the true relationships in about 30 genetic mapping studies with a number of diseases have not been very impressive. There are, however, three or four disorders in which some relationship has been observed. First, there is an obsessive-compulsive disorder in which the affected persons follow various anxiety-reducing rituals, which seems to be correlated to low COMT activity allele (Karayiorgou et al., 1997). Second, low COMT activity allele appears to have some association with aggressive and highly antisocial impulsive schizophrenia (Strous et al., 1997a,b; Lachman et al., 1998). A special velo-cardio-facial syndrome in which the chromosome *22q11* is deleted (including the COMT gene) also carries with it similarly bizarre behavior, but several types of other symptoms, including schizophrenia, may appear (Lachman et al., 1996a,b; Papolos et al., 1996). However, generally there is only a weak (Ohmori et al., 1998) or no association between COMT activity alleles and schizophrenia (Chen et al., 1996; Daniels et al., 1996; Riley et al., 1996; Wei et al., 1996; Karayiorgou et al., 1998). Paradoxically, in one study, the high activity allele was preferentially transmitted from healthy parents to their schizophrenic children (Li et al., 1996).

Recently, Tiihonen et al. (1999) reported a clear indication of the association of the late-onset alcoholism (type 1) and the low-affinity allele of COMT in a Finnish population. When 123 alcoholics were compared with 246 race- and gender-matched controls, the odds ratio for alcoholism of subjects with $COMT^{LL}$ was 2.51 over those with $COMT^{HH}$. The frequency of low allele was also significantly higher in the alcoholics than in 3140 Finnish blood donors representing a general population. The estimate for population etiological percentage of the $COMT^{LL}$ in type 1 alcoholism was 13.3%.

With respect to depression, the results are variable. Several studies do not show any relationship between depression and COMT (BIOMED European Bipolar Collaborative Group, 1997; Gutiérrez et al., 1997; Kunugi et al., 1997b), although some show a low COMT activity allele or a low COMT activity in the erythrocytes in patients with major depression but not in those with the bipolar form (Karege et al., 1987; Ohara et al., 1998b). In some patients, the link seems to be with bipolar manic-depressive illness (Li et al., 1997). Recently it has been shown that the low COMT activity allele is dominant in rapid-cycling (cycle 1–2 days) bipolar manic-depressive disorder (Kirov et al., 1998; Papolos et al., 1998). These findings confirm the results of an earlier report that patients with velo-cardio-facial syndrome, which includes a rapid-cycling bipolar disorder, have low COMT activity (Lachman et al., 1996a; Papolos et al., 1996). Interestingly, polysubstance abusers have been reported to more commonly have the high COMT activity allele than the controls (Vandenbergh et al., 1997). No associ-

ation has been found between anxiety disorders and COMT polymorphism (Ohara et al., 1998a).

Some ethnic differences have been recognized. For instance, the frequency of the low COMT activity allele is lower in Kenyan than in Caucasian or South-West Asian individuals (McLeod et al., 1998). However, black Americans have higher COMT activity than white Americans (McLeod et al., 1994). Low COMT activity is found in a Saami population (Klemetsdal et al., 1994).

The association of the COMT alleles with Parkinson's disease (PD) has been extensively studied, but usually no association has been found (Hoda et al., 1996; Syvänen et al., 1997; Xie et al., 1997). However, some Japanese individuals with the low COMT activity allele may have an increased risk for PD (Kunugi et al., 1997a; Yoritaka et al., 1997). As cited above, different populations have different frequencies of $COMT^{LL}$ and $COMT^{HH}$ alleles and therefore may have variation in individual responses to therapy with a drug preparation containing L-dopa (levodopa; Reilly et al., 1980; Rivera-Calimlim and Reilly, 1984; Klemetsdal et al., 1994). It would be interesting to know whether the confirmed $COMT^{LL}$ PD patients really can benefit more from the levodopa therapy than $COMT^{HH}$ patients, as has been suggested based on COMT activity analysis from erythrocytes (Reilly et al., 1980). It is also known that patients with high COMT activity in their erythrocytes have encountered more adverse effects during levodopa therapy and have had more frequent on-off effects than patients with low COMT activity (Reilly et al., 1983; Rivera-Calimlim and Reilly, 1984).

Finally, low COMT activity allele is found more frequently in patients with breast cancer than in healthy controls (Lavigne et al., 1997), particularly in women with menopausal symptoms (Thompson et al., 1998). This phenomenon may be related to the decreased metabolism of catecholestrogens because COMT also metabolizes these compounds (see *Estrogen Metabolism and Role of COMT and COMT Inhibitors*). However, a recent extensive case-control study on patients with invasive breast cancer in North Carolina (654 cancer patients and 652 controls) did not show any relation of the COMT genotype to the breast carcinoma (Millikan et al., 1998).

In addition to humans, other mammals can have different genetically determined COMT activity. There are two rat strains with different activity levels (Weinshilboum et al., 1979; Roth et al., 1990); however, the rat polymorphism has some other structural basis from the human polymorphism because both rat and pig have Leu108 (not Met108 or Val108; Salminen et al., 1990) in the critical site of the COMT polypeptide, which determines the level of activity and thermal stability (Salminen et al., 1990; Malherbe et al., 1992).

F. Distribution of COMT

In mammals, COMT is widely distributed throughout the organs of the body. COMT is an intracellular en-

zyme. As discussed (see *One COMT Gene and Two Proteins*), the COMT protein in vertebrates appears mostly in a soluble form (as S-COMT), and only a minor fraction is in the particular form (as MB-COMT; Guldberg and Marsden, 1975; Roth, 1992).

1. Brain COMT. The cellular localization of COMT has been studied in several ways. A number of lesion studies (Rivett et al., 1983; Kaakkola et al., 1987) and immunohistochemical studies (Karhunen et al., 1995a; Lundström et al., 1995) have demonstrated that there is no significant COMT activity in presynaptic dopaminergic neurons, but some activity is present in postsynaptic neurons and substantial activity is located in glial cells.

Immunoelectron microscopy studies suggest that COMT resides in astrocytic processes around synapses and capillary walls and in postsynaptic dendritic spines. Some primary neurons also show immunoreactivity to COMT (Karhunen et al., 1995a). Because there are no S-COMT- and MB-COMT-specific antisera available, it has not been possible to separately analyze the tissue distribution of the two enzymes forms. Immunoblotting analysis of the primary cell cultures of the rat brain cells shows that both isoforms of COMT occur in astrocytes, oligodendrocytes, and neurons (Karhunen et al., 1995b).

Some indication of subcellular distribution can be obtained through differential centrifugation. The mammalian S-COMT activity resides in the nonsedimenting, cytoplasmic fractions, and MB-COMT activity resides in the sedimenting fractions, the equivalent of the microsomal fraction (Jeffery and Roth, 1984; Roth, 1992).

With immunoblotting and COMT activity determinations for the analysis of the COMT isoforms, it was shown that both in rat brain and in baculovirus-infected insect cells, the MB-COMT polypeptide resides in the subcellular fractions containing endoplasmic reticulum and cell membranes. These two components could not be separated in this study (Tilgmann et al., 1992). In mammalian cell preparations overexpressing either S-COMT or MB-COMT, the subcellular localization has been studied in detail (Ulmanen et al., 1997). Overexpressed S-COMT was localized in cytosol and in the nucleus, whereas MB-COMT was a microsomal protein. This was determined through double immunostaining against an established marker protein of the rough endoplasmic reticulum. MB-COMT was located in the rough endoplasmic reticulum, facing the cytoplasm. It is worth noting that no MB-COMT has been found in the cell membrane (Ulmanen et al., 1997).

When the cellular distribution of COMT in the rat striatum was studied in an attempt to selectively destroy glial cells with fluorocitrate, we found that initially there was a decrease in COMT activity at 1 and 2 days after lesioning, followed by a marked increase at 3 days. At the same time, a microglial marker (alkaline phosphodiesterase) was increased. Immunohistochemical analysis also revealed a major increase in microglia (OX-42-immunoreactive cells) but not of astroglia (glial fibril-

lary acidic protein immunoreactive cells). No neuronal COMT immunoreactivity was detectable in these studies (Reenilä et al., 1997).

2. *COMT in Other Tissues.* COMT has been found in practically all mammalian tissues investigated. The highest COMT activity in both rat and humans is in the liver, followed by the kidneys and gastrointestinal tract (both stomach and intestine; Nissinen et al., 1988b; Schultz and Nissinen, 1989; Männistö et al., 1992b). These findings have been confirmed and expanded to spleen and submaxillary glands through the use of immunohistochemistry (Karhunen et al., 1994; Lundström et al., 1995). In pancreas, COMT immunoreactivity was found in β and δ cells but not in α cells (Karhunen et al., 1994). The importance of pancreatic COMT activity has also been demonstrated through other means. After the pretreatment of conscious rats with OR-486 (dinitrocatechol), a potent nitrocatechol-type COMT inhibitor, the uptake of radioactivity from [^{13}C]L-dopa into the pancreas was increased by 4-fold. Most of the radioactivity was derived from [^{13}C]dihydroxyphenylalanine ([^{13}C]DOPAC; Bergström et al., 1997). Positron emission tomography (PET) studies have demonstrated high COMT activity in kidney, liver, intestine, stomach, spleen, lungs, and heart of mice (Ding et al., 1996); they also confirmed high COMT activity in baboon liver and kidney.

In kidney, COMT activity is found in proximal tubular epithelial cells, in fact, in the same cells in which dopamine is synthesized. Dopamine acts as a local hormone, exerting diuretic and natriuretic effects (Lee, 1993). COMT mRNA (mostly that of S-COMT) has also been visualized in epithelial cells of proximal tubules, the thick ascending limb of loop of Henle, and the ureter (Meister et al., 1993), where the enzyme is thought to regulate the metabolism of dopamine and other catecholamines. COMT mRNA has been detected in the prenatal kidney at gestational day 18 (Meister et al., 1993). After birth, the K_m values of rat kidney COMT activity appear to increase as a function of age from 3.3 μM at age 3 days to 16.9 μM at age 30 days (see *Other Enzymological Aspects*). V_{max} values (which are dependent on the total amount of enzyme in the tissue, which was not measured) were not much altered (Vieira-Coelho and Soares-da-Silva, 1996). The same authors also claim that the inhibitory activity of tolcapone on kidney COMT would be radically altered as a function of the age. However, because the changes in the COMT protein content were not measured, this conclusion remains uncertain. The same is true for the comparison between kidney and liver COMT inhibition with tolcapone.

Human kidney COMT activity was also assayed by De Santi et al. (1998b), who found it to be 159 pmol/min \times mg protein when DBA was used as the substrate. COMT activity in the liver was three times higher than kidney activity, and in the duodenum, activity about 30% less

than that in kidney was noted. In that study, entacapone was found to be severalfold more active than tolcapone as a COMT inhibitor in all tissues studied. This is an unexpected result that has not been confirmed.

Although it has been demonstrated that the metabolism of the newly formed dopamine in the rat kidney is primarily mediated through MAO, with only a minor involvement of COMT (Fernandes and Soares-da-Silva, 1994), both nitecapone (Eklöf et al., 1997) and entacapone (Hansell et al., 1998) induced a copious diuresis and natriuresis, which in both cases was inhibited by a dopamine D_1 antagonist. However, the amounts of dopamine excreted were only marginally increased by entacapone but much more by *N*-(2-pyridone-6-yl)-*N'*-di-*n*-propylformamide (CGP 28014), which is not at all a COMT inhibitor (see below; Hansell et al., 1998). Nitecapone was shown to enhance Na^+ , K^+ -ATPase inhibition in the proximal tubular cells (Eklöf et al., 1997). Finally, there seems to be a weak but significant correlation of histamine-*N*-methyltransferase and COMT activities in the human renal cortex (De Santi et al., 1998a).

Opossum kidney cells in culture can synthesize and metabolize dopamine (by MAO and COMT) in a manner similar to rat renal tubular cells. Therefore, these cultured cells can be used as an easily available and standardized model system to explore the role of dopamine in kidney function (Guimarães et al., 1997).

In hamster kidney, a 2- or 4-week estrogen treatment induces COMT immunoreactivity, as well as in the nucleus, where only S-COMT was seen (Weisz et al., 1998b), supporting the nuclear localization of S-COMT as discussed. This probably is a compensatory mechanism inducing a metabolizing enzyme to oppose the formation of electrophilic quinones and semiquinones from catecholestrogens, particularly in this animal species (see *Estrogen Metabolism and Role of COMT and COMT Inhibitors*).

Rather high COMT activities have been described in human lung by De Santi et al. (1998b) and in rat lung by Bryan-Lluka (1995). There also is a substantial amount of COMT in the eye, in both the ciliary body and the retinal ganglion cell layer (Karhunen et al., 1994). COMT activity has also been demonstrated in spinal membranes of monkeys and pigs (Kern et al., 1995).

COMT activity is detectable in the skin, with the activity being higher in epidermis than in dermis (Bamsdahl, 1969). COMT is found in keratocytes, where it may metabolize epinephrine, but also in melanocytes, which make up only 3 to 5% of the epidermal cell population (Smit and Pavel, 1995). The epidermis of patients with vitiligo with contains more COMT than does the epidermis from healthy controls (Lepoole et al., 1994). Intermediate indolic compounds, like 5,6-dihydroxyindole-2-carboxylic acid, are formed in the synthesis of eumelanin from tyrosine. These intermediates can be readily oxidized to toxic quinone derivatives. The polymerization of

these potentially toxic intermediates to melanin can be considered a detoxification process. COMT activity may further reduce the amount of dihydroxyindoles and therefore protect normal melanocytes against their own reactive compounds generated during melanogenesis. If the metabolism of dihydroxyindoles by COMT is inhibited, these metabolites can accumulate and become harmful to normal skin (Smit and Pavel, 1995). However, there is a possibility for a novel therapeutic role for COMT inhibition in melanoma treatment (Shibata et al., 1993; Smit et al., 1994). It has been shown that malignant melanocytes contain both S-COMT and MB-COMT. At physiological concentrations of dihydroxyindoles, MB-COMT may be more relevant and functionally more significant than S-COMT (Shibata et al., 1993; Smit and Pavel, 1995). In melanoma cells, the accumulation of reactive dihydroxyindoles after effective COMT inhibition could selectively damage melanoma cells (Smit et al., 1994; Smit and Pavel, 1995).

Some tumors, notably pheochromocytomas, contain high amounts of COMT, particularly MB-COMT. High metanephrine levels in patients with pheochromocytoma are derived from catecholamines produced and metabolized within the tumors. Hence, measurement of plasma-free metanephrine concentrations may be important for the rapid diagnosis of these patients (Eisenhofer et al., 1998).

Human (Keränen et al., 1994), hamster, guinea pig, rabbit, hen, dog and monkey (Zürcher et al., 1996), and rat (Nissinen et al., 1992) erythrocytes contain some COMT activity. The activity varies from species to species, but it is high in rat and quite low in humans. Erythrocyte COMT offers a convenient way of monitoring the COMT inhibition in the body during COMT inhibitor therapy. Several human studies have shown an excellent correlation between the inhibition of erythrocyte COMT activity and the concentration of a COMT inhibitor in plasma or the decrease of 3-*O*-methyl-dopa (3-OMD) levels in plasma (Keränen et al., 1994; Dingemans et al., 1995b). Even human lymphocytes contain a significant amount of COMT, almost half of which appears to be MB-COMT (Sladek-Chelgren and Weinsilboum, 1981).

G. General Importance of COMT

1. Substrates of COMT. The physiological substrates of COMT include L-dopa, catecholamines (dopamine, norepinephrine, epinephrine), their hydroxylated metabolites, catecholestrogens (Ball and Knuppen, 1980), ascorbic acid, and dihydroxyindolic intermediates of melanin. Several dietary and medicinal products are also COMT substrates, such as triphenols and substituted catechols, dobutamine, isoprenaline, rimiterol, α -methyl-dopa, benserazide, carbidopa, dihydroxyphenyl serine (Maruyama et al., 1996), flavonoids, and dihydroxy derivatives of tetrahydroxyisoquinolones. The general function of COMT is the elimination of biologi-

cally active or toxic catechols and some other hydroxylated metabolites. During the first trimester of pregnancy, COMT protects the placenta and the developing embryo from activated hydroxylated compounds formed from aryl hydrocarbons by hydroxylases (Barnea and Avigdor, 1990). COMT also acts as an enzymatic detoxicating barrier between the blood and other tissues shielding against the detrimental effects of xenobiotics (e.g., in the intestinal mucosa and the brain). COMT may also serve some unique or indirect functions in the kidney and intestine tract by modulating the dopaminergic tone; the same may be true in the brain: COMT activity may regulate the amounts of active dopamine and norepinephrine in various parts of the brain and therefore be associated with mood and other mental processes.

2. Quantitative Role of COMT in Metabolism of Catecholamines. The relative importance of enzymes in metabolizing catecholamines and the uptake processes of catecholamines have been clarified. Without exogenous levodopa loading, the high-affinity neuronal reuptake (uptake₁) is an efficient elimination system for the released catecholamines, being responsible for most of their elimination both in peripheral tissues and the brain (Kopin, 1985; Männistö et al., 1992b; Cass et al., 1993). The role of the extraneuronal transport is less clear (Friedgen et al., 1996b). The contribution of metabolism, including COMT, is unimportant, and therefore COMT inhibition does not affect dopamine levels to a detectable degree. It is equally clear that inhibition of MAO (by pargyline) and COMT (by tolcapone), each separately, has little, if any, effect on the removal of norepinephrine, epinephrine, and dopamine on passage through the systemic and pulmonary circulation. The pulmonary, but not the systemic, clearance of catecholamine can be reduced by a combined blockade of MAO and COMT (Friedgen et al., 1996a). Thus, inhibition of COMT does not greatly alter the elimination of infused catecholamines or exercise-induced elevation of catecholamines, which is an important safety-increasing factor in the use of potent COMT inhibitors (see later). However, the elimination pathways are adaptable. When MAO is blocked, both COMT and phenolsulfotransferase activities are increased, but these two pathways do not compete with each other (Buu, 1985).

The situation is dramatically altered when exogenous levodopa is administered. When levodopa is administered alone, it is predominantly decarboxylated to dopamine in the peripheral tissues, and only a small fraction of the dose ever reaches the brain to be used for dopamine synthesis. If dopa decarboxylase (DDC) is inhibited, the majority of surplus L-dopa is metabolized, now preferably by peripheral COMT (Kuruma et al., 1972; Messiha et al., 1972; Fahn, 1974; Da Prada et al., 1984; Männistö et al., 1992b). During the combination therapy, 3-OMD is the major metabolite (Fahn, 1974; Rivera-Calimlim et al., 1977; Reilly et al., 1980; Da

Prada et al., 1984; Hardie et al., 1986), and the role of COMT inhibitors becomes extremely meaningful.

The interplay of catecholamine uptake (uptake₁) and COMT has been explored in several cell lines with COMT activity and artificially expressing the recombinant amine transporters (Eshleman et al., 1997). COMT inhibition by tropolone or a nitrocatechol-type inhibitor, Ro 41-0960, augmented by 4-fold the transport of ligands that were COMT substrates but did not affect substrates that were not metabolized by COMT. The uptake of serotonin was not altered by COMT inhibitors; however, no such potentiation was seen with dopamine uptake into mouse neostriatal synaptosomes. That may be due to the fact that dopamine is protected by intracellular sequestration into synaptic vesicles, organelles that were lacking from the cell lines used in cell culture.

3. COMT Knockout Mice. The ultimate importance of COMT will probably be clarified in a new strain of animals lacking the COMT gene. Such mice were recently produced by Gogos et al. (1998). The mice appeared to be normal, they had only minor changes in their behavior, and the brain neurochemistry of catecholamines was virtually unaltered. The mice were also able to breed normally. Surprisingly, all of the changes were sex and region specific. Mutant male mice, totally lacking COMT activity, had an almost 3-fold increase of dopamine levels in the frontal cortex but not in the striatum or hypothalamus, but females showed no changes. It is noteworthy that despite the complete lack of COMT gene and protein, residual homovanillic acid (HVA) levels were detectable in several brain areas. This points to the possibility that there exists a still-unidentified methylation pathway in the brain. Female knockout mice had impaired emotional reactivity in the black-white box. On the other hand, heterozygous males (but not homozygous males or any females) were more aggressive than their wild-type counterparts. These findings suggest that 1) the importance of COMT in the behavior has probably been underestimated and 2) a complete lack of COMT can be effectively compensated, at least in mice.

III. COMT Inhibitors

A. First-Generation COMT Inhibitors

In 1975, Guldberg and Marsden reviewed early COMT inhibitors. Several of these compounds (e.g., gallates, tropolone, U-0521, 3',4'-dihydroxy-2-methyl-propionophenone) have been used as *in vitro* tools; however, their efficacy *in vivo* is low, and they are short acting. Moreover, they lack selectivity and are rather toxic. For instance, U-0521 depressed the function of the rat vas deferens at 1 μ M and above, which apparently invalidates its use as a COMT inhibitor in smooth muscle preparations (Rice et al., 1997). Also, the limited clinical experiences with gallates, tropolone, ascorbic acid, and U-0521 were disappointing (Ericsson, 1971; Reilly et al., 1983; Reches and Fahn, 1984). It is worth noting that

millimolar concentrations of ascorbic acid may well reduce COMT activity significantly, as was recently shown in primate spinal meninges (Kern and Bernards, 1997).

B. Second-Generation COMT Inhibitors

Three laboratories independently developed very potent, highly selective, and orally active COMT inhibitors. Nitrocatechol is the key structure in most of these molecules (Fig. 2). Only CGP 28014 is a chemically distinct compound (Bäckström et al., 1989; Borgulya et al., 1989; Waldmeier et al., 1990a).

In principle, the new COMT inhibitors can be divided into three groups: 1) mainly peripherally acting compounds, 2) broad-spectrum compounds working in both the periphery and the brain, and 3) atypical compounds, probably acting preferably in the brain. This classification was derived from our comparative studies (Männistö et al., 1992a; Törnwall and Männistö, 1993), where the decrease in the plasma and brain 3-OMD was used as a marker for peripheral COMT inhibition. The decline

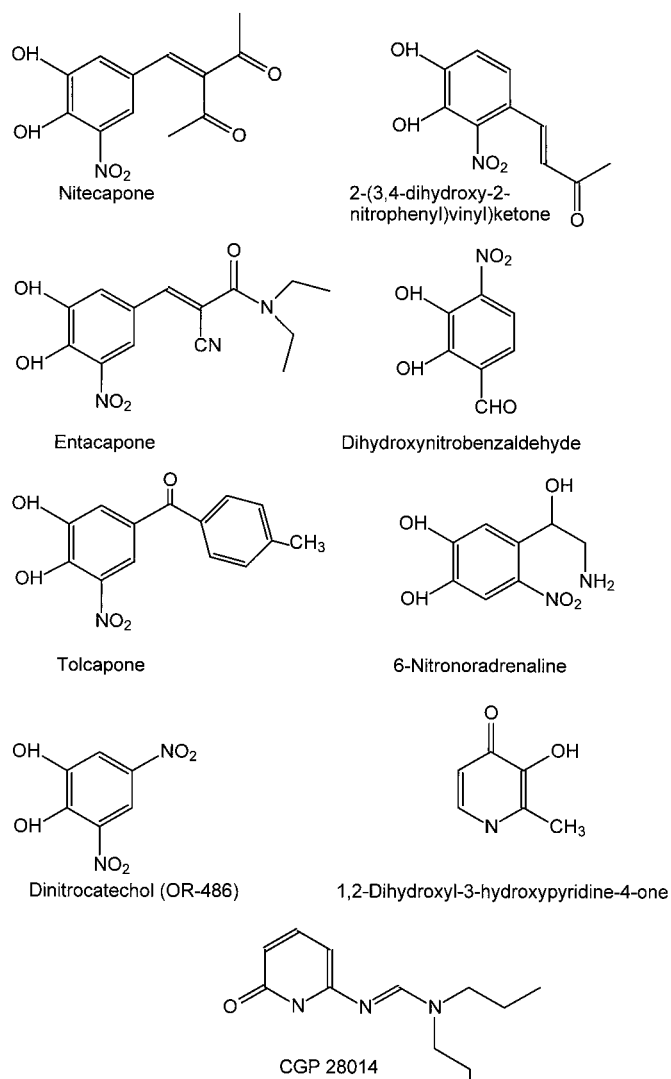


FIG. 2. Chemical structures of some second-generation COMT inhibitors.

of the brain HVA [and 3-methoxytyramine (3-MT) after pargyline treatment] was used as a signal of COMT inhibition in the brain.

Because the amount of COMT is high in the liver, kidney, and intestinal mucosa (Sharpless et al., 1973; Nissinen et al., 1988b), peripherally acting COMT inhibitors would be primarily interesting. The clearest clinical application of these COMT inhibitors would be as adjuncts to levodopa in PD (Männistö and Kaakkola, 1989, 1990). L-Dopa is a catechol, and it is predominantly *O*-methylated to 3-OMD, especially when the peripheral decarboxylation of L-dopa is inhibited by benserazide or carbidopa (Nutt and Fellman, 1984). Although the elevation in 3-OMD levels was not harmful as such, peripheral COMT inhibition should enhance the brain penetration of L-dopa. Those COMT inhibitors that gain access to the brain would provide additional information about the role of metabolism to potentiate brain dopaminergic and adrenergic functions.

C. Properties of New Compounds

1. *COMT Inhibition.* Nitrocatechols are so-called tight-binding inhibitors of COMT, although their binding to COMT is fully reversible (Schultz and Nissinen, 1989; Lotta et al., 1995; Borges et al., 1997). Prolongation of the preincubation time with an inhibitor markedly reduces the IC_{50} values of nitrocatechol-type inhibitors (Schultz and Nissinen, 1989). This particular property has been the source of many problems when the kinetic parameters have been characterized. It is necessary to perform inhibition kinetic studies in a special way to avoid invalid conclusions. In the presence of varying inhibitor concentrations, the reaction velocity of COMT increases progressively with increasing enzyme concentrations and parallels the velocity curve without an inhibitor if sufficiently high amounts of enzyme are used. Also, the effect of the nitrocatechol-type inhibitors can be gradually abolished by dialysis (about 50% in 5 h, fully reversed within 24 h; Schultz and Nissinen, 1989).

Entacapone [OR-611; (*E*)-2-cyano-*N,N*-diethyl-3-(3,4-dihydroxy-5-nitrocinnamamide)] and nitecapone [OR-462; 3-(3,4-dihydroxy-5-nitro-benzylidene)-2,4-pentanedione; Fig. 2] are highly effective inhibitors of rat S-COMT with IC_{50} values of about 150 to 300 nM in the liver and 10 to 20 nM in the brain tissues. K_i values in the rat liver have been 145 and 23 nM for entacapone and nitecapone, respectively (Schultz and Nissinen, 1989; Nissinen et al., 1992). When analyzed with pure recombinant COMT enzyme forms, K_i values of nitecapone and entacapone were around 1 nM or even lower (Lotta et al., 1995). They are also selective because their in vitro IC_{50} values for tyrosine hydroxylase, dopamine- β -hydroxylase, DDC, and MAO-A and -B are in the micromolar range (Männistö et al., 1988; Nissinen et al., 1988a, 1992). They strongly inhibit the COMT activity in a variety of tissues. An oral dose of 10 mg/kg nitecapone or entacapone causes nearly complete inhibition of duodenal COMT activity for 1 to 3 h and highly significant

inhibition in erythrocyte and liver COMT activity for several hours. Full recovery of duodenal COMT activity is achieved at 8 to 12 h after drug administration. The striatal COMT activity is slightly and transiently suppressed by entacapone administration but not at all by nitecapone administration; full recovery is attained at 3 h. Oral ID_{50} values of nitecapone and entacapone have been in a range of about 1 to 5 mg/kg in the liver and duodenum but at least 25 mg/kg or higher in the brain (Schultz and Nissinen, 1989; Zürcher et al., 1990a; Nissinen et al., 1992).

Tolcapone [Ro 40-7592; 4'-methyl-3,4-dihydroxy-5-nitro-benzophenone] resembles chemically entacapone and nitecapone (Fig. 2; Zürcher et al., 1990b). It seems to be slightly more potent at inhibition of liver COMT activity than entacapone and nitecapone, with IC_{50} and K_i values of 36 nM in the rat liver (Zürcher et al., 1990a, 1991; Borgulya et al., 1991). In both recombinant COMT forms, the K_i value of tolcapone was about 0.3 nM (Lotta et al., 1995). Tolcapone can penetrate into the brain and inhibits the brain COMT activity in vivo with an ID_{50} value of 26 to 28 mg/kg (Da Prada et al., 1991; Zürcher et al., 1991). A high oral dose of tolcapone (100 mg/kg) causes virtually complete inhibition of rat heart and kidney COMT activity at 15 to 30 min. Liver and brain COMT activity is less effectively suppressed (maximally by 70% at 30 min). About 50% inhibition is measured at 11 h (heart and kidney), at 8 h (liver), and at 6 h (brain) after dosing. Full recovery of COMT activity occurs by 16 h. The inhibition of COMT activity by tolcapone is fully reversible, as it is with entacapone and nitecapone. Tolcapone does not affect MAO, hydroxyindole-*O*-methyltransferase, histamine-*N*-methyltransferase, or phenylethanolamine-*N*-methyltransferase activities or adrenergic (α , β), serotonergic, or cholinergic receptors (Zürcher et al., 1990a,b).

Nitrocatechols with NO_2 group in positions other than that in the "classic" nitrocatechols have also been described (Fig. 2; Perez et al., 1992, 1993, 1994). These dihydroxyvinyl-type compounds are able to bind to the active site of COMT (Vidgren and Ovaska, 1997), but their efficacy against both human and pig enzymes is generally lower than that of compounds with the NO_2 in the classic site of the catechol ring. The situation is complicated by the fact that the in vitro comparisons have usually been made using pig liver COMT, which differs from rat and human enzymes by having a polar arginine in position 38 instead of hydrophobic Trp38. This difference leads to reduced affinity (higher K_m value for catechol substrates) and higher K_i values for inhibitors. However, even in human COMT, 2-(3,4-dihydroxy-2-nitrophenyl)vinyl phenylketone (vinylphenylketone, or QO IIR) has 4- to 13-fold higher K_i values than nitecapone, entacapone, and tolcapone (Vidgren and Ovaska, 1997). Vinylphenylketone is a tight-binding, reversible COMT inhibitor that resembles the classic nitrocatechols (Perez et al., 1993, 1994).

Also an endogenous COMT inhibitor, 6-nitronorepinephrine (Fig. 2) has been described and quantified in pig and rat brain, where its concentration is around 75 pg/g (Shintani et al., 1996). This compound is generated from the reaction between nitric oxide and norepinephrine. If the synthesis of nitric oxide is inhibited, the amount of 6-nitronorepinephrine is decreased. Perfusion of 6-nitronorepinephrine into the paraventricular nucleus elevated norepinephrine levels and decreased 3-methoxy-4-hydroxyphenyl glycol (MHPG) levels. In vitro, COMT inhibition is modest because the IC_{50} value is as high as 7.5 μ M. However, 6-nitronorepinephrine inhibits also the reuptake of norepinephrine into the synaptosomes, with an IC_{50} value of 31 μ M. In summary, 6-nitronorepinephrine is a potential, although fairly weak, endogenous signal molecule that may link the actions of catecholamines and nitric oxide (Shintani et al., 1996). However, 6-nitronorepinephrine cannot be used as a drug.

CGP 28014, a hydroxypyridine compound (Fig. 2), was first described by Waldmeier et al. (1990a,b). CGP 28014 and its major metabolite, 2-amino-6-hydroxypyridine, are not COMT inhibitors in vitro until millimolar concentrations are reached. CGP 28014 does not affect receptors of the various neurotransmitters and other endogenous substances (Waldmeier et al., 1990a).

An active iron chelator, 1,2-dimethyl-3-hydroxypyridine-4-one (L1, CP20), is a further example of another type of COMT inhibitor that, however, also inhibits tyrosine and tryptophan hydroxylases (Waldmeier et al., 1993).

2. Effects on L-Dopa and Catecholamine Metabolism. In rats, oral administration of entacapone and nitecapone (3–30 mg/kg) in combination with levodopa and carbidopa effectively reduce 3-OMD formation and elevate serum and brain L-dopa, dopamine, DOPAC, and HVA levels (Männistö et al., 1988, 1992a; Nissinen et al., 1988a, 1992). Similar findings have been reported in *Cynomolgus* monkeys in whom the i.v. efficacy of entacapone and nitecapone was equipotent (Cedarbaum et al., 1991). Part of the levodopa saved from COMT is metabolized through alternative pathways because the increase of serum L-dopa is smaller than the decrease in 3-OMD would lead one to expect.

In microdialysis studies, the pharmacokinetics of L-dopa in plasma and gluteal muscle was studied in pentobarbital anesthetized dogs treated with levodopa alone (20 mg/kg i.v.), with levodopa and carbidopa (repeated doses of 100 mg p.o. and 100 mg i.v. at the beginning of anesthesia) and levodopa, carbidopa, and entacapone [15 mg/kg i.v., 1 h before levodopa (Deleu et al., 1995)]. It was found that carbidopa had an L-dopa-sparing effect in both plasma and muscle, and this effect was further enhanced with entacapone. The $T_{1/2\beta}$ of L-dopa was prolonged from 0.76 to 2.66 h by entacapone, whereas it was only 0.4 h without carbidopa and entacapone. A similar shift occurred in the muscle although to a lesser extent.

The $T_{1/2\alpha}$ of L-dopa in plasma was not altered by either treatment. Formation of 3-OMD was greatly reduced in both plasma (by 98%) and muscle (by 85%) with entacapone. Skeletal muscle may be an important L-dopa storage site (Deleu et al., 1995) whose capacity can be significantly increased with entacapone (Ordóñez et al., 1974).

The effective decrease in 3-OMD, HVA, and 3-MT levels, as well as in COMT activity in the brain, can be used as criteria for the classification of tolcapone as a brain-penetrating COMT inhibitor (Zürcher et al., 1990a,b, 1991; Männistö et al., 1992a). In the time course studies, 13 mg/kg tolcapone p.o. decreased several 3-O-methylated metabolites of catecholamines (3-MT, HVA, and MHPG). The formation of 3-MT was suppressed by 90% as early as 1 h after dosing, and that of HVA and MHPG was suppressed more slowly and modestly (80 and 60%, respectively) at about 4 h after tolcapone. Whole brain dopamine was not changed, whereas DOPAC levels were doubled for 6 to 8 h (Zürcher et al., 1991).

After the oral administration of 30 mg/kg tolcapone with 10 mg/kg levodopa and 15 mg/kg benserazide, plasma and whole brain L-dopa levels increased by 4-fold and 3-OMD decreased to very low levels in rats. Dopamine formation in the whole brain increased for at least 6 h (Zürcher et al., 1990a,b).

In a further study on rats, 20 mg/kg levodopa (combined with 15 mg/kg benserazide) has been compared with 10 mg/kg levodopa (and 15 mg/kg benserazide) plus 30 mg/kg tolcapone. The triple treatment approximately doubled the area under the curve (AUC) of plasma L-dopa compared with the conventional double treatment with twice as high a dose of levodopa. Thus, the bioavailability of levodopa was increased 3.5-fold by the addition of tolcapone (Zürcher et al., 1991). However, it is worth noting that the $T_{1/2}$ of levodopa was not markedly prolonged. The plasma levels of 3-OMD, which normally exceed the plasma L-dopa levels by a factor of 2, remained low and near the detection limit for at least 8 h (Zürcher et al., 1991).

In rats, tolcapone (30 mg/kg i.p.) was also able to prolong the elimination half-life (+116%) and area under the plasma apomorphine concentration-time curve (+31%), and apomorphine was present in striatum for a 85% longer time period (Coudoré et al., 1997). Even 2-(3,4-dihydroxy-2-nitrophenyl)vinyl phenylketone (QO IIR, or vinylphenylketone) has recently been proved to act as a peripherally acting COMT inhibitor in in vivo studies in rats (Rivas et al., 1999).

The in vivo effects of CGP 28014 in rats mimicked those of the other COMT inhibitors. The ED_{50} values are 2 to 8 mg/kg p.o. when the endpoints were the decrease in striatal HVA, the decline of striatal 3-MT after clorgyline treatment (both without exogenous levodopa), or the formation of 3-OMD from the exogenous levodopa (without a DDC inhibitor). It was long acting when

administered at high doses of 100 mg/kg (>12 h) or 300 mg/kg (>24 but <36 h). CGP 28014 increased striatal AdoMet levels. However, it unexpectedly increased striatal 5-hydroxyindoleacetic acid and tryptophan levels (Waldmeier et al., 1990a,b).

In our studies in rats, CGP 28014 proved to be a poor COMT inhibitor in the periphery, or at least its inhibitory effect on 3-OMD formation was slow, becoming significant only by 3 h. However, it behaved as an efficient COMT inhibitor-like compound in the brain, preventing both HVA and 3-MT formation (Männistö et al., 1992a). This kind of brain priority has not been described previously for the COMT inhibitors. In fact, in earlier studies, CGP 28014 inhibited both 3-OMD and HVA formation to an equal degree after both p.o. and i.p. administration (Waldmeier et al., 1990a,b). However, in these studies, either CGP 28014 was administered without levodopa or levodopa was administered without the inhibition of the peripheral DDC. Therefore, only moderate amounts of 3-OMD were produced in the periphery because most of the L-dopa was decarboxylated to dopamine. This unusual behavior of CGP 28014 was also seen in rat kidney studies, where it enhanced dopamine and DOPAC secretion but did not affect sodium secretion. This is in contrast to the effect of entacapone, whose action on dopamine secretion was much less but whose action on sodium secretion was much stronger (Hansell et al., 1998).

3. Microdialysis Studies. In vivo microdialysis technique is a widely used method for the estimation of extracellular levels of various substances, such as neurotransmitters and their metabolites. The effects of entacapone, tolcapone, and CGP 28014 alone or in combination with levodopa and DDC inhibitors on brain L-dopa and dopamine metabolism have been investigated in rats. The aims have been, on one hand, to clarify the mechanisms of brain dopamine metabolism and, on the other hand, to confirm the beneficial effect of combination of a COMT inhibitor with levodopa on brain dopamine formation. Only a few studies have dealt with norepinephrine levels and metabolism (Li et al., 1998).

a. Drugs Given Alone. The dose of 10 mg/kg entacapone i.p. alone did not affect the extracellular levels of dopamine and its metabolites in rats, whereas higher doses (30–100 mg/kg) decreased the efflux of HVA and increased the formation of DOPAC (Kaakkola and Wurtman, 1992). Contrary to several other findings in rats, Brannan et al. (1997) reported that 30 mg/kg entacapone had clear central effects, reducing the brain COMT activity at 2 h. Even at a dose as low as 10 mg/kg, brain COMT activity was reduced by 80%, and the only “peripherally selective” doses were 2.5 and 5 mg/kg. The brain-penetrating reference compound OR-486 (dinitrocatechol; Nissinen et al., 1988a) had very similar effects. Central effects of entacapone were seen with levodopa- and carbidopa-treated rats as well (Brannan et al., 1997). We cannot explain these results, which are in

conflict with the general view of other groups (Männistö et al., 1992a; Zürcher et al., 1993; Törnwall et al., 1994). Entacapone did not modify the effects of clorgyline, selegiline, and nomifensine on striatal dopamine metabolism in the rat brain (Kaakkola and Wurtman, 1992).

Tolcapone, at doses of 3 to 30 mg/kg i.p. or p.o., dose-dependently decreased the efflux of HVA and increased levels of L-dopa and DOPAC but did not affect the output of dopamine in the rat striatum (Acquas et al., 1992; Kaakkola and Wurtman, 1993; Napolitano et al., 1995b). In addition, the efflux of 3-MT declined rapidly after tolcapone (40 mg/kg s.c.; Cumming et al., 1992). Tolcapone potentiated the effect of nomifensine on rat striatal dopamine efflux, whereas it did not significantly modify the effects of MAO inhibitors (clorgyline, pargyline, and selegiline; Cumming et al., 1992; Kaakkola and Wurtman, 1993). We did not find in the rat striatum any potentiation by tolcapone on dopamine levels that had been elevated by pargyline or amphetamine (Tuomainen et al., 1996). Neither entacapone nor tolcapone altered the extracellular levels of 5-hydroxyindoleacetic acid in any of the above studies.

Li et al. (1998) thoroughly studied the effect of tolcapone on the extracellular levels of dopamine and norepinephrine and their metabolites in a number of brain areas in anaesthetized rats. Although HVA and MHPG levels were clearly reduced, no increases were seen in dopamine in striatum or nucleus accumbens or norepinephrine in nucleus accumbens, frontal cortex, or two parts of the hippocampus.

Steulet et al. (1993) reported that CGP 28014 (30 mg/kg i.p.) did not significantly change the output of dopamine and DOPAC from rat striatum, whereas Törnwall et al. (1994) found a slight increase in the efflux of dopamine and DOPAC. Both groups reported a significant reduction in the output of HVA. However, when CGP 28014 was administered intrastrially, it induced dose-dependent and severalfold elevation of extracellular dopamine levels but unexpectedly did not alter HVA levels. This tyramine-like action was abolished by nomifensine, indicating that CGP 28014 must be transported into nerve terminals via the dopamine uptake system (Steulet et al., 1993). These findings suggest that CGP 28014 must undergo peripheral metabolism before it can inhibit COMT. No active metabolite has been identified, and therefore other mechanisms of action should be considered (Männistö et al., 1992b; Steulet et al., 1993; Waldmeier et al., 1993).

The microdialysis data show that entacapone is principally a peripherally active COMT inhibitor, whereas tolcapone and CGP 28014 have major effects on the *O*-methylation of brain dopamine. These studies have also confirmed that MAO plays a more important role than COMT in the metabolism of extracellular dopamine. Reuptake (uptake₁) is even more important than either of the metabolizing enzymes in termination of the

actions of catecholamines in the synapse (see *Quantitative Role of COMT in Metabolism of Catecholamines*).

b. Drugs Given with Levodopa. When combined with levodopa and DDC inhibitors, both entacapone and tolcapone caused a significant supplementation of the extracellular levels of L-dopa, dopamine, and DOPAC in the rat striatum (Acquas et al., 1992; Brannan et al., 1992; Kaakkola and Wurtman, 1993; Napolitano et al., 1995a,b). The levodopa-induced increase in HVA output was attenuated by tolcapone but not by entacapone (Törnwall et al., 1994). Both entacapone and tolcapone reduced the levels of 3-OMD (Törnwall et al., 1994; Napolitano et al., 1995a,b).

As described, the effect of entacapone has also been studied in rat skeletal muscle where both carbidopa (100 mg/kg/day p.o.) and entacapone (15 mg/kg i.v.) had distinct levodopa-sparing effects, and when given together, this effect was further enhanced (Deleu et al., 1995).

In conclusion, combination studies with levodopa- and nitrocatechol-type COMT inhibitors have demonstrated in rats that the inhibition of COMT activity results in increased dopamine formation and release in brain after the administration of levodopa and DDC inhibitors. A significant effect can be achieved even if the COMT inhibition is restricted to peripheral tissues.

When combined with levodopa and carbidopa, CGP 28014 did not elevate dopamine levels in the microdialysis fluid of the rats. It transiently decreased HVA formation but did not alter 3-OMD levels (Törnwall et al., 1994), confirming our results with brain homogenates (Männistö et al., 1992a).

4. Voltammetric Studies. Garris and Wightman (1995) studied the effect of various drugs, including tolcapone (40 mg/kg), on the dopamine efflux from the rat caudate-putamen and basolateral amygdaloid nucleus, elicited by electrical stimulation of ascending dopamine fibers at various frequencies. Dopamine efflux was monitored by fast-scan cyclic voltammetry. Tolcapone caused negligible effects in both regions at frequencies of 30 Hz or higher. However, at 20 Hz, these workers did detect a nearly 50% increase in dopamine release. In contrast, the effects of two uptake blockers, nomifensine and cocaine, to stimulate dopamine efflux were robust in both brain regions. Similar results were recently obtained by Budygin et al. (1999) with 30 mg/kg tolcapone and 10 mg/kg GBR 12909, a new dopamine uptake blocker. These results suggest that under normal conditions, uptake₁, rather than transmitter metabolism, regulates extracellular levels of dopamine.

5. Estrogen Metabolism and Role of COMT and COMT Inhibitors. Estrogen metabolism, including the crucial role of COMT in the metabolism of 2- and 4-hydroxylated estrogens (catecholestrogens), was reviewed by Ball and Knuppen (1980) and recently by Zhu and Conney (1998). Some of the highlights and safety aspects are described here. A schematic summary of the main path-

ways, and of the role of COMT in particular, is given in Figs. 3 and 4.

Estradiol (17 β -estradiol), which is reversibly oxidized to estrone, can undergo numerous metabolic routes. Among these, the NADPH-dependent hydroxylation reactions, catalyzed mainly by multiple forms of cytochrome P-450 enzymes, are relevant to this review. There are many hydroxylation products that are formed, but only the catecholestrogens are substrates of COMT, and at high concentrations they may act as competitive COMT inhibitors. Catecholestrogens and their *O*-methylated products, 2- and 4-methoxy estradiols or 2- and 4-methoxy estrones, are not inert metabolites but may possess unique activities that are not necessarily directly associated with the actions of their parent hormones (Zhu and Conney, 1998).

It is difficult to estimate the relative amounts of various hydroxylated estrogens, but in women, 2-OH-estradiol, which is formed mainly in the liver, is the most abundant catecholestrogen detected in urine (50% or more; Dannan et al., 1986; Kerlan et al., 1992; Zhu et al., 1993; Suchar et al., 1995). The other hydroxylated estrogens are much less abundant; 4-OH-estradiol represents about 5% (<15% of that of 2-OH-estradiol) and the 16 α -hydroxylated product represents more than 10% of the total. It is noteworthy that in extrahepatic "target" tissues, 4-hydroxylation is much more common (see below).

There are very low levels of 2-OH-estrogens in the systemic circulation, mainly because they are rapidly further metabolized by COMT and conjugation enzymes (sulfation, glucuronidation; Ball et al., 1978; Fishman and Martucci, 1979; Emons et al., 1983, 1987; Zhu and Liehr, 1996). In addition to liver, 2-OH-estrone and 2-OH-estradiol are also formed locally in target tissues like uterus and breast. 2-OH-estrogens have multiple effects in the body, only a few of which are relevant here. First, they bind to estrogen receptor but only with low affinity, and their hormonal potency is much less than that of their parent compounds. This means that their net effect is antiestrogenic (MacLusky et al., 1983; van Aswegen et al., 1989; Feigelson and Henderson, 1996). Second, they may generate estrogen semiquinones and quinones and highly toxic oxygen radicals (Liehr et al., 1986b; Liehr, 1990; Liehr and Roy, 1990). The latter possibility seems rather theoretical because instead of having tumorigenic activity in routine tests, they tend to inhibit spontaneous tumorigenesis in estrogen-sensitive tissues (Liehr et al., 1986a; Li and Li, 1987; see below). Even a subsequent *O*-methylation product of 2-OH-estradiol, 2-methoxy estradiol, inhibits angiogenesis, and is a strong inhibitor of tumor cell proliferation (Seegers et al., 1989; Lottering et al., 1992; D'Amato et al., 1994; Fotsis et al., 1994; Hamel et al., 1996; Klauber et al., 1997). Interestingly, in patients with depression, the formation of 2-methoxyestrogens is increased, whereas that of 4-hydroxylation is reduced (Banger et al., 1990).

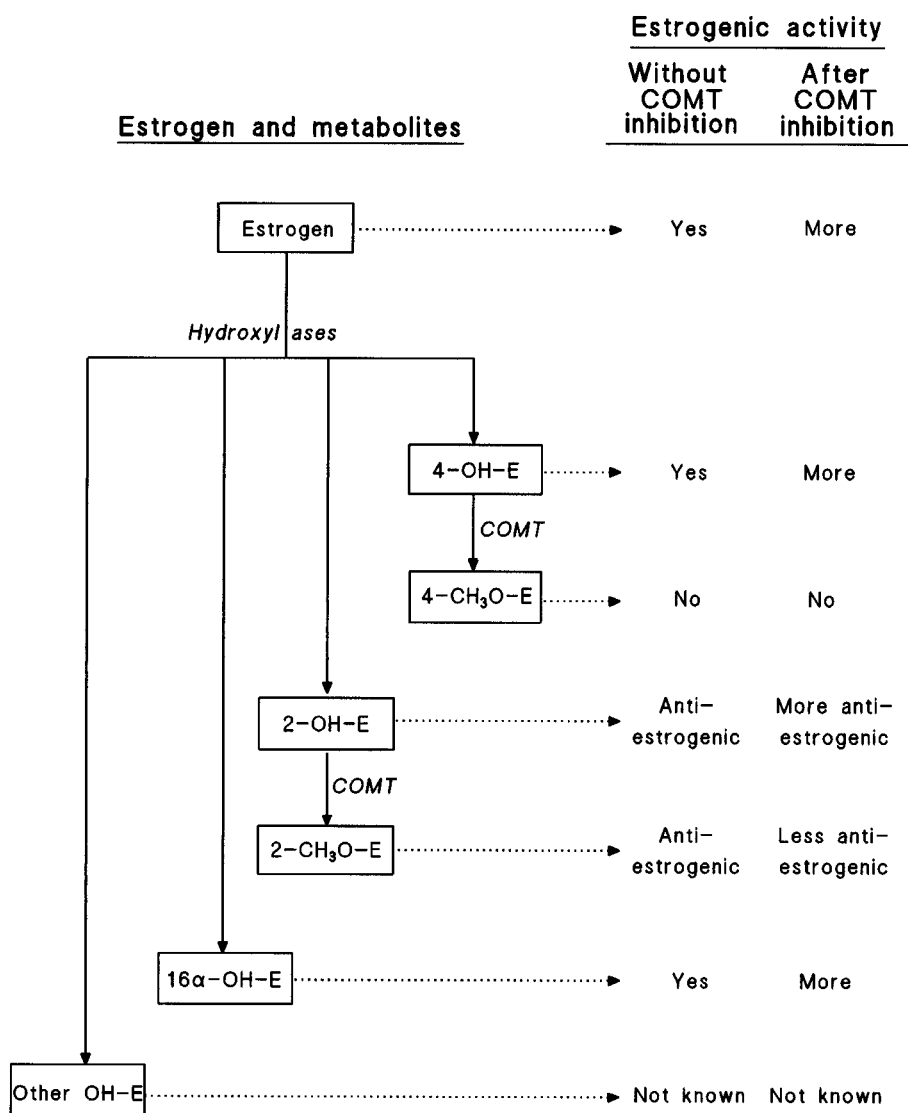


FIG 3. Main estrogen metabolites and their proposed estrogenic (or antiestrogenic) activity according to Zhu and Conney (1998). The theoretical effects of COMT inhibition on the relative amounts of the metabolites, and the subsequent changes in hormonal activities, are also indicated. 2-OH-E, 2-OH-estrogen; 4-OH-E, 4-OH-estrogen; 2-CH₃O-E, 2-methoxyestrogen; 4-CH₃O-E, 4-methoxyestrogen; 16 α -OH-E, 16 α -hydroxy estrogen.

In summary, 2-OH-estrogens and their *O*-methylation products are evidently beneficial and anticarcinogenic (Fig. 3).

4-Hydroxylation of estrogens is not extensive in the liver, but this reaction is much more common in the extrahepatic target tissues of estrogens, such as pituitary (Bui and Weisz, 1988), myometrium and myomal tissues (Lier et al., 1995), and breast (Lier et al., 1995; Hayes et al., 1996). This point has been thoroughly discussed by Weisz (1991, 1994) and recently by Weisz et al. (1998a,b) and Cavalieri's group (Cavalieri et al., 1997; Cavalieri and Rogan, 1998; Stack et al., 1998). The actions of 4-OH-estrogens are completely different from those of 2-OH-derivatives. First, 4-OH-estradiol binds to estrogen receptors as effectively as estradiol itself, and its binding is long lasting (Martucci and Fishman, 1976; MacLusky et al., 1983; van Aswegen et al., 1989). Consequently, 4-OH-estrogens are hormonally very active

(Martucci and Fishman, 1976; Franks et al., 1982). Second, 4-OH-estradiol undergoes a metabolic redox cycling to generate free radicals and chemically very reactive semiquinone and quinone intermediates, which can damage DNA and other cellular components, induce cell transformation, and initiate tumorigenesis (Liehr et al., 1986b; Liehr, 1990; Liehr and Roy, 1990; Fig. 4). Third, 4-OH-estradiol is a strong carcinogen in the hamster kidney. Injections of either 4-OH-estradiol or estrone-3,4-quinone cause kidney and liver tumors but not mammary tumors (Liehr et al., 1986a; Li and Li, 1987).

The above quinone hypothesis, bypassing the importance of pure estrogen receptor stimulation as a key factor in estrogen-dependent cancer, has obtained strong support from recent studies on the abundant appearance of cytochrome P-4501B1, converting estrogens to 4-OH-estrogens in the breast tissue, and apparently also in uterus and ovaries, more effectively than in

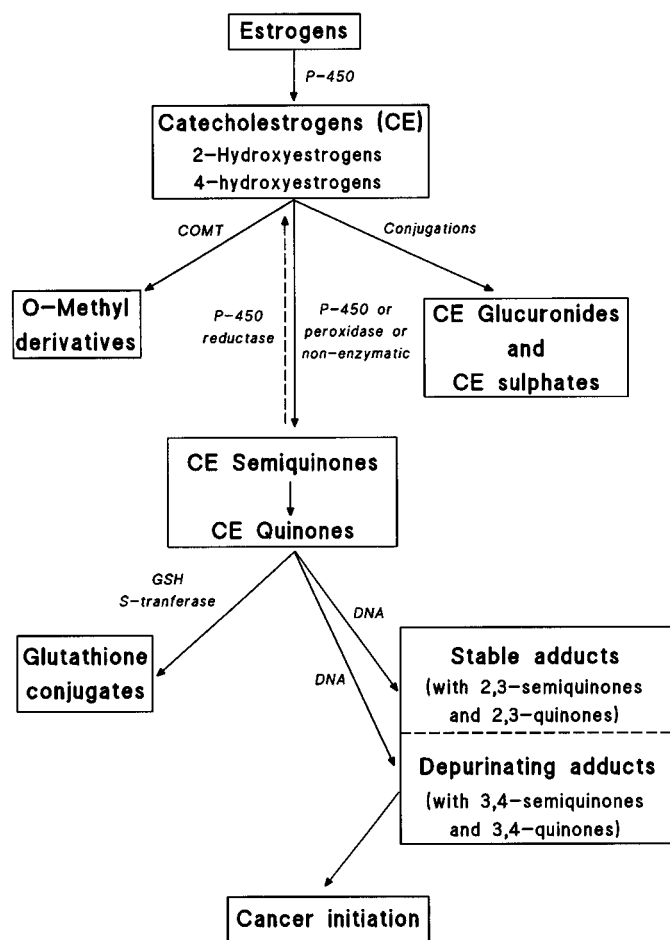


FIG 4. Main metabolic routes of catecholestrogens modified according to Cavalieri et al. (1997) and Cavalieri and Rogan (1998). The formation of catecholesterogen semiquinones and quinones and further formation of stable (with 2,3-semiquinones and 2,3-quinones) and depurinating DNA adducts (with 3,4-semiquinones and 3,4-quinones) are shown. CE, catecholesterogen; P-450; cytochrome P-450; GSH S-transferase; glutathione S-transferase.

tissues not prone to estrogen-linked cancer (Hayes et al., 1996; Spink et al., 1998). Catecholestrogens can be metabolized further by COMT, conjugated to glucuronide or sulfate but also oxidated to semiquinones and quinones. Particularly reactive is estrogen-3,4-quinone, which has been shown to bind to DNA, creating so-called depurinating adducts both in in vitro studies and in animals. These adducts are quickly falling off, taking with them two bases of DNA: adenine and guanine. The gaps formed in the DNA have a strong potential to create gene mutations and eventually cause cancer (Cavalieri et al., 1997). These events are schematically summarized in Fig. 4. Because the formation of 4-OH-estrogens is very low, at least in humans, it is quite unclear how large a risk this metabolic route may have. However, any procedure inhibiting the normal metabolism of estrogens may increase formation of 4-OH-estrogens and can be regarded as potentially dangerous.

Interestingly, after 2 or 4 weeks of estrogen treatment, COMT immunoreactivity was increased in ham-

ster kidneys, and it was seen even in the nucleus (Weisz et al., 1998b). This is unexpected because in contrast to human COMT, hamster kidney COMT lacks nuclear localization signal sequence. The nuclear entry of COMT was also selective because another second phase II enzyme, CuZn-superoxide dismutase, remained extranuclear. The authors suggest that the nuclear translocation of COMT after estrogen treatment is a protective response to the threat of the genome by electrophilic products of catechols, quinones and semiquinones (Weisz et al., 1998b). It can be concluded that 4-OH-estradiol is a potentially harmful compound, a strong tumorigenic substance that may be associated with the genesis of estrogen-dependent cancers (Figs. 3 and 4).

The role of COMT in the *O*-methylation of estrogens and how this is altered by potent COMT inhibitors are areas of concern. Catecholestrogens are generally good substrates of COMT. 2-OH-estrone in particular is rapidly *O*-methylated, and 2-methoxy estrone is one of the most abundant estrogen metabolites in human plasma and urine (Kraychy and Gallagher, 1957; Ball et al., 1979). Both 2-methoxy estradiol and 2-methoxy estrone have very high affinity for the sex hormone-binding protein (Dunn, 1983). Little is known about the role of 4-methoxy derivatives, but their activity is inferior to that of the corresponding 2-methoxy derivatives.

Mono-*O*-methylated estrogens have little or no affinity for estrogen receptors, and they do not exert any estrogenic effect on target tissues (Merriam et al., 1980). It seems, therefore, that *O*-methylation of catecholestrogens is primarily a detoxification pathway. However, 2-methoxy estradiol has a number of effects on its own, not related to estradiol, 2-OH-estradiol or other methoxy derivatives. It inhibits the proliferation of several cancer cell lines in vitro (Seegers et al., 1989; Lottering et al., 1992; Fotsis et al., 1994; Cushman et al., 1995; Klauber et al., 1997). For example, human breast cancer cell lines were particularly sensitive to 2-OH-estradiol, and it depressed the growth of some implanted cancers (Fotsis et al., 1994; Klauber et al., 1997). 2-Methoxy estradiol also disturbs the function of microtubules (D'Amato et al., 1994; Hamel et al., 1996), and it is one of the most potent endogenous inhibitors of angiogenesis known (Fotsis et al., 1994; Klauber et al., 1997). Even the inhibitory effects of some enzyme inducers, like phenobarbital and indole-3-carbinol, on spontaneous breast carcinogenesis in mice may be mediated by enhanced formation of 2-OH-estradiol and its rapid metabolism to 2-methoxy estradiol (Osborne et al., 1990; Bradlow et al., 1991; Jellinck et al., 1991, 1993).

The 16 α -hydroxylation of estrogens does not have any direct connection to COMT. However, this route may be activated if the COMT pathway is inhibited, and therefore the properties of 16 α -OH-estrogens are important (Fig. 3). Both 16 α -OH-estrone and 16 α -OH-estradiol retain most of the hormonal activity of their parent compounds (Fishman and Martucci, 1980). 16 α -OH-

estrone seems to bind covalently to the estrogen receptor and may activate the classic estrogen-mediated oncogene expression and evoke long-term growth stimulation (Hsu et al., 1991). It is fairly well established, based on a number of different experiments, that increased formation of 16 α -OH-estrogens is associated with an increased risk of mammary cancer in mice and humans (Bradlow et al., 1986, 1995; Fishman et al., 1995).

We want to stress that the above summations have not been confirmed in other types of experiments and that they are not universally accepted. Also, other groups have demonstrated that increased urinary excretion of catecholestrogens, but not 16 α -OH-estrogens, can be correlated with an increased risk for nonfamilial breast cancer (Lemon et al., 1992). The same outcome was reached in a Finnish study where catecholestrogens dominated compared with 16 α -OH-estrogens in the population at high risk to develop breast cancer (Adlercreutz et al., 1994a,b). Finally, 16 α -OH-estrone and 16 α -OH-estradiol are quite weak carcinogens in the hamster kidney tumor model (Li and Li, 1987; Seegers et al., 1989; Li et al., 1995). Furthermore, during pregnancy, women excrete large amounts of 16 α -OH-estrogens, but full-term pregnancy does not increase the risk of breast cancer (Merrill, 1958; MacMahon et al., 1970; Fotsis, 1987; Yuan et al., 1988; Henderson et al., 1996). Our conclusion is that there is not sufficiently strong evidence to conclude that 16 α -OH-estrogens are carcinogenic but also that their harmful effects cannot be excluded. It seems that they do not possess any beneficial effects on estrogen-dependent cancers.

The intention of this discussion is to demonstrate that there is a possibility that inhibition of COMT by new drugs may seriously interfere with the metabolism of catecholestrogens. However, to the best of our knowledge, no studies have been conducted on this matter.

6. *Behavior.* Generally, when administered alone, COMT inhibitors have virtually no effect on the motor behavior of rodents (Maj et al., 1990; Männistö et al., 1992b; Männistö, 1998). However, a compound that interferes with adrenergic transmission would be predicted to have important cognition-enhancing effects via improved attention and motivation or secondarily via enhanced cholinergic functions (Levin et al., 1990; Kelland et al., 1993). In fact, we have found that COMT inhibitors affect several phases of learning in a simple passive avoidance paradigm (Khromova et al., 1997). In more sophisticated studies, spatial working memory (radial-arm maze) of intact rats was facilitated after the pretraining i.p. administration of tolcapone (10 mg/kg). Similarly, tolcapone improved the performance of senescent poor performers in a spatial memory task (linear arm maze). However, tolcapone was not able to counteract the performance deficits in rats whose memory had been impaired by scopolamine or bilateral lesions in the nucleus basalis magnocellularis (Liljequist et al., 1997).

In an electric brain self-stimulation model, tolcapone administered alone has shown antidepressant activity (Moreau et al., 1994). In our studies, however, levodopa also was needed to reveal the antidepressant-like effect of tolcapone in two other rat models of depression (Männistö et al., 1995). In addition, it is quite interesting that entacapone (30 mg/kg i.p., a dose that apparently penetrates the blood-brain barrier to some extent), administered with levodopa (100 mg/kg i.p.) induced a place preference in rats (Katajamäki et al., 1998).

Maj et al. (1990) described behavioral effects of tolcapone. It increased exploratory activity of rats at doses of 10 and 30 mg/kg p.o., and the hyperactivity and stereotypy induced by amphetamine and nomifensine were potentiated by tolcapone. Tolcapone slightly antagonized fluphenazine-induced catalepsy but did not protect against pimozide- or haloperidol-induced catalepsy. Also, Himori and Mishima (1994) found that pretreatment with tolcapone (30 mg/kg) slightly potentiated the antagonist effect of levodopa and benserazide on haloperidol-induced catalepsy in the mice administered 1-methyl-4-phenylpyridium intracerebroventricularly. Very high doses of 3-OMD (800 mg/kg) attenuated this effect of tolcapone.

The antiakinetin action of a subthreshold dose of levodopa (5 mg/kg) in the MPTP-treated mice was potentiated by small doses of tolcapone (1 and 3 mg/kg). The same was true with two MAO inhibitors: pargyline (5 mg/kg) and selegiline (L-deprenyl; 1, 5 and 10 mg/kg). When given together, tolcapone and pargyline increased both the peak effect and the duration of the antiakinetin action. In the case of tolcapone plus L-deprenyl, only the duration of action was prolonged (Fredriksson and Archer, 1995).

Entacapone potentiated the turning behavior induced by levodopa and carbidopa in rats with unilateral nigral lesions (Etemadzadeh et al., 1989). Nitecapone resembled entacapone in this respect (Männistö et al., 1988). Comparative studies have confirmed that inhibition of COMT outside the brain (e.g., after entacapone and nitecapone) contributed nearly equally to the levodopa-induced turning behavior as that occurring also within the brain (i.e., after tolcapone; Törnwall and Männistö, 1993). We were not able to show any positive interaction between entacapone (3 mg/kg) and clorgyline (3 mg/kg) on the levodopa/carbidopa-induced turning behavior (Törnwall and Männistö, 1993). Entacapone potentiated the behavioral effects of levodopa also in common marmosets (Smith et al., 1997).

When given with levodopa and benserazide, tolcapone potentiated the increase in motor activity plus the antihypothermic effect and anticataleptic activity of levodopa or levodopa plus pargyline (Maj et al., 1990). Tolcapone potentiated levodopa/carbidopa-induced turning behavior at doses of 3 to 30 mg/kg (Törnwall and Männistö, 1993).

The effects of tolcapone and MAO inhibitors, separately and together, on levodopa-induced turning were thoroughly studied by Heeringa et al. (1997). There was a significant potentiation of levodopa-induced (10 mg/kg levodopa, 15 mg/kg benserazide) contralateral turning by tolcapone (30 mg/kg) or an MAO-A inhibitor, Ro 41-1049, but not with an MAO-B inhibitor, Ro 19-6327. Pretreatment with tolcapone and either of the MAO inhibitors further potentiated the levodopa-induced turning. The blockade of three dopamine-metabolizing enzymes (COMT, MAO-A, MAO-B) at the same time, leaving only conjugation reactions intact, most effectively prolonged the turnings.

In addition, a vinylphenylketone-type COMT inhibitor (QO IIR) was able to potentiate the reversal of reserpine-induced akinesia by levodopa/carbidopa in rats at a dose range of 7.5 to 30 mg/kg i.p. and catalepsy and hypothermia in mice at 30 mg/kg i.p., as did the reference compounds nitecapone and Ro 41-0960 at 30 mg/kg (Rivas et al., 1999). CGP 28104 was as effective as entacapone and tolcapone in potentiating the turning behavior induced by levodopa and carbidopa (Törnwall and Männistö, 1993).

7. S-Adenosyl-L-Methionine-Saving Effect of COMT Inhibitors. It is well known that the levodopa therapy in PD patients depletes their body levels of AdoMet levels, and this depletion also occurs in the brain (Benson et al., 1993). This can be explained by enhanced *O*-methylation of the large doses of L-dopa present, which consumes the methyl groups of AdoMet. Long-term levodopa treatment leads to compensatory enhancement of the synthesis of AdoMet by increasing the activity of methionine adenosyl transferase (Benson et al., 1993). In turn, when high doses of exogenous AdoMet were administered, they depleted nigrostriatal and forebrain tyrosine hydroxylase and brain dopamine (Charlton, 1997). When the COMT-induced *O*-methylation was effectively inhibited by first-generation COMT inhibitors (like tropolone but not pyrogallol; Waldmeier and Feldtrauer, 1987) and second-generation compounds (like tolcapone; Da Prada et al., 1994; Miller et al., 1997; Yassin et al., 1998), AdoMet levels in the brain were increased. Homocysteine levels were restored by tolcapone (Da Prada et al., 1994; Miller et al., 1997). Tolcapone enhanced the activity of methionine adenosyl transferase (Yassin et al., 1998). Even CGP 28014, an atypical inhibitor of *O*-methylation that does not inhibit the COMT enzyme itself, elevated AdoMet levels in the brain (Waldmeier et al., 1990a). Interestingly, MAO inhibition appeared to have just the opposite effect as that observed after COMT inhibition (Yassin et al., 1998).

Enhanced AdoMet levels in the brain during tolcapone therapy may explain the antidepressive (Moreau et al., 1994; Männistö et al., 1995) and cognition-improving (Khromova et al., 1995, 1997) effects of COMT inhibitors. Some studies have shown that the quality of life is increased in PD patients receiving COMT inhibitor ther-

apy (Welsh et al., 1995; Baas et al., 1997; Waters et al., 1997). One report has also described improved cognition during tolcapone therapy (Gasparini et al., 1997). In fact, these findings fit well to the animal data demonstrating both antidepressive and cognition-improving properties of COMT inhibitors (Moreau et al., 1994; Männistö et al., 1995; Khromova et al., 1997; Liljequist et al., 1997).

8. Other Effects of COMT Inhibitors. The nitrocatechols, of which nitecapone and entacapone have been studied in detail, are quite effective antioxidants (Suzuki et al., 1992), nitric oxide radical scavengers (Marocchi et al., 1994), and iron chelators (Haramaki et al., 1995; Orama et al., 1997), and they can protect cells from lipid peroxidation (Haramaki et al., 1995). Nitecapone has also been shown to prevent ischemia-reperfusion injury in experimental heart surgery in rats (Haramaki et al., 1995). In all these studies, the concentrations used (high micromolar or even millimolar) were very much higher than those needed to inhibit COMT. These actions do not appear to be related to COMT inhibition.

We have found that the repeated administration of 3 mg/kg tolcapone, when started before toxin administration, partially restored the memory deficit induced by bilateral infusions of a cholinotoxin, acetylcholine mustard, to the basal magnocellular nuclei of Meynert (Khromova et al., 1995). It is interesting that tolcapone (at 1 or 100 nM), like selegiline (at 1 nM to 10 μ M), can protect aggregation cultures of rat brain exposed to a combination of anoxia and hypoglycemia for 30 min. Tolcapone (10 μ M) was itself toxic. The beneficial effect was seen after only 1 day in culture, not at 2- or 4-day after ischemia (Ekblom et al., 1998).

Nitecapone increased bicarbonate secretion from rat and human duodenum after both i.v. and intraluminal administration (Flemström et al., 1993; Knutson et al., 1993; Flemström and Säfsten, 1994). High concentrations of nitecapone (maximum effect at 225 μ M) increased synthesis and secretion of gastric sulfomucin (Slomiany et al., 1993). Although these findings point to some gastroprotective properties, nitecapone has not been found to be of any use as an antiulcer therapy in humans.

The mechanism of tolcapone-induced dose-dependent diarrhea has been studied in conscious dogs bearing 40-cm jejunal segment loops. Buffered physiological solution was perfused, and the secretions were analyzed at 15-min intervals. Acute oral administration of tolcapone (10 or 30 mg/kg) was well tolerated but induced a dose-dependent efflux of intestinal fluid, sodium, potassium, chloride, and bicarbonate (Larsen et al., 1998). A similar enhancement of bicarbonate excretion has been seen in gastric mucosa studies (Flemström et al., 1993; Knutson et al., 1993; Flemström and Säfsten, 1994). The coadministration of levodopa and benserazide accelerated the onset of tolcapone-induced secretion. No diarrhea developed in animals in these studies, nor did the treat-

ments cause any signs of mucosal damage. The administration of tolcapone or the drug combinations for 7 days reduced secretory responses, demonstrating that tolerance did develop (Larsen et al., 1998). The importance of these findings to the pathogenesis of diarrhea remained open, but it cannot be excluded that the increased secretion may induce diarrhea in some susceptible patients. It is somewhat puzzling that dopamine itself has been reported to have an antisecretory effect in the rat jejunum preparation (stripped epithelial sheets). This action was mediated through α_2 -adrenergic receptors (Vieira-Coelho and Soares-da-Silva, 1998).

Nitrocatechols may inhibit oxidative phosphorylation and uncouple mitochondrial energy production. One study compared entacapone and tolcapone; it was reported that tolcapone was severalfold more effective as an uncoupler than entacapone. The effective concentrations of tolcapone were in the same range as those achieved in plasma during tolcapone therapy (Nissinen et al., 1997). The relevance of this finding to the adverse effects of tolcapone (e.g., to the hepatic failure, rhabdomyolysis, and diarrhea) remains to be clarified.

9. Physicochemical Properties and Animal Pharmacokinetics. Although the COMT-inhibiting K_i and IC_{50} values of nitrocatechols are in the low nanomolar range in vitro, doses as high as 10,000 nM/kg are needed to achieve adequate COMT inhibition in vivo. Evidently, these compounds have poor general intracellular availability. There are very little published data on the physicochemical properties of the new COMT inhibitors. Nitrocatechols are weak acids, and the pK_a values of both entacapone and tolcapone are 4.5. The water solubility of both compounds is low, less than 0.005% ($<0.05 \mu\text{g/ml}$); it is particularly poor in acidic milieu and improved considerably at pH 7.4 (entacapone, 7 mg/ml). The solubility in organic solutions and alcohols is substantially better. The log P (octanol/0.1 N HCl) of entacapone is 2.01; that of tolcapone is 2.6, indicating the better lipid solubility of the latter compound (Roche, 1997; P. T. Männistö and S. Kaakkola, unpublished data).

There is only restricted information about the actual brain and tissue penetration of the nitrocatechols. For instance, Dingemans (1997) mentions unpublished observations that the brain extraction ratio of entacapone is 3% and is not dependent on the concentration in plasma. In contrast, he claims that tolcapone has a concentration-dependent (5–100 $\mu\text{g/l}$) brain penetration of 24 to 46% but does not provide any experimental details to amplify these statements. These values are extremely high compared with our unpublished data generated after the i.v. injection of varying doses of entacapone and tolcapone. The brains were made blood free by saline perfusion before collection. Our brain/plasma ratios were from less than 0.1 to 0.27% for entacapone and from 0.6 to 1% for tolcapone during the first 60 min after i.v. injection of the drugs (P. T. Männistö and S. Kaakkola, unpublished data, 1995). Both

experiments agree on one point: the brain penetration of tolcapone is severalfold higher than that of entacapone.

Studies into the pharmacokinetics of tolcapone in rats have been published (Funaki et al., 1994, 1995). After 10 mg/kg i.v., the $T_{1/2}$ is less than 60 min, the AUC is 21.8 $\mu\text{g} \times \text{h/ml}$, the total clearance is 7.84 ml/min \times kg, and the V_D is 0.291 liters/kg. Oral doses of 20 and 40 mg/kg led to a slightly longer $T_{1/2}$. The maximum drug concentration in plasma (C_{max}) values were 15.9 and 20.2 $\mu\text{g/ml}$ and the AUC values were 21.1 and 49.0 $\mu\text{g} \times \text{h/ml}$ after 20 and 40 mg/kg, respectively. It can be calculated that the oral bioavailability of tolcapone in the rat is 48 to 56%.

Data on entacapone rat kinetics in rats have not been published, but in general, the kinetic values after i.v. administration are close to those of tolcapone. However, after oral administration, the plasma entacapone levels are lower than those obtained with tolcapone. The oral bioavailability appears to be less than 20% in the rat (P. T. Männistö and S. Kaakkola, unpublished data, 1995).

Nitrocatechols are extensively metabolized, and only a small fraction is recovered intact in the urine. The metabolic profile is described in more detail in relation to the human pharmacokinetics.

The reason for the low oral bioavailability of nitrocatechols is still unknown. Their poor water solubility could indicate poor absorption, but also their abundant metabolism may start during the first pass. It seems likely that both factors contribute to their incomplete oral bioavailability.

10. Toxicity. A full preclinical toxicology program has been conducted with entacapone and tolcapone; both drugs have recently been marketed throughout the world. A discussion on human toxicology appears in the clinical section of this review (see *Safety*). The acute toxicity of nitrocatechols is generally low (Borgulya et al., 1989). The acute i.p. LD_{50} value of Ro 41-0960, a structural analog of tolcapone, in mice is about 100 mg/kg, whereas that of nitecapone and entacapone is about 500 mg/kg (Törnwall and Männistö, 1991). Levodopa and carbidopa treatments do not significantly enhance the acute toxicity of COMT inhibitors. Oral LD_{50} values for tolcapone are 1600 to 1800 mg/kg in mice and greater than 2 g/kg in rats (Borgulya et al., 1991). The same is true for entacapone and nitecapone. Because their effective doses are in the range of 3 to 30 mg/kg, the therapeutic index for oral administration in rodents is more than 50. The lethal threshold for tolcapone in rats and dogs was more than 100 $\mu\text{g/ml}$ (Roche, 1997). These values can be compared with the approximate therapeutic plasma levels of 3 to 6 $\mu\text{g/ml}$ attained after 100 and 200 mg of tolcapone delivered in three doses each day.

Some toxicity emerged in long-term toxicity and carcinogenicity studies after fairly high doses of tolcapone (Roche, 1997). Moderate epithelial hyperplasia of the nonglandular part of the rodent stomach (not present in

humans) was seen in rats and mice. In the same studies, 35% of rats developed renal epithelial tumors, depending on the dose. The safety margin of this phenomenon was about 10-fold above the therapeutic plasma concentrations of tolcapone. Finally, an increased incidence of uterine adenocarcinomas was seen at the 250 mg/kg/day dosage level. This finding may be related to the fact that dopamine agonists produced uterine tumors that are specific to rats. In addition, the dopamine-prolactin-estrogen axis may be disturbed. Catecholestrogens are also very good substrates of COMT, and a blockade of their metabolism will lead to accumulation (Ball et al., 1972; Ball and Knuppen, 1980; see *Estrogen Metabolism and Role of COMT and COMT Inhibitors*). It is worth noting that the liver was not a target of tolcapone toxicity in rodents.

We are not aware of the detailed results of the long-term toxicity studies of entacapone. However, genotoxicity and carcinogenicity studies have not revealed any serious toxicity. Some instances of anemia has been noted during repeated administration, probably due to iron chelation by entacapone. In reproduction toxicity studies in rabbits, there was some retardation of fetal growth and bone development (Orion, 1998).

11. Conclusions from Animal Studies. The nitrocatechol-type COMT inhibitors alter L-dopa metabolism and potentiate the action of levodopa plus DDC inhibitors much more effectively than the first-generation COMT inhibitors. Some of the new compounds (entacapone, nitecapone) hardly penetrate the blood-brain barrier but still significantly increase striatal dopamine levels and potentiate the behavioral effects of levodopa in the same way as the brain-penetrating compounds (tolcapone, Ro 41-0960).

The mechanism of action of CGP 28014 is not known. Because it or its known metabolite do not directly inhibit COMT, it might inhibit the transfer of the COMT substrates to the enzyme (i.e., uptake₂). However, in glioma cells, no such inhibition could be seen (M. Törnwall and P. T. M., unpublished results, 1993). A further possibility would be that CGP 28014 forms an unknown metabolite, and this is not formed in vitro, only in vivo. Nevertheless, CGP 28014 mimics the other COMT inhibitors in being an inhibitor of *O*-methylation, preferably that acting in the brain. It is also positive in alleviating the symptoms in animal model of PD.

IV. Positron Emission Tomography Studies

6-[¹⁸F]Fluoro-L-dopa (6-FD) is an analog of L-dopa that is used in PET studies as a tracer of the presynaptic dopaminergic system. Like L-dopa, it is decarboxylated by DDC to [¹⁸F]dopamine and *O*-methylated by COMT to 3-*O*-methyl-[¹⁸F]dopa (3-OMFD). In routine PET studies with 6-FD in combination with DDC inhibitors, 3-OMFD represents a considerable proportion of the radioactivity in both plasma and brain (Firnau et al.,

1987, 1988). Various kinetic models have been used to differentiate the specific from nonspecific activity. Selective COMT inhibition would reduce the formation of 3-OMFD, simplify PET modeling, and improve the quality of PET images.

When given in combination with 6-FD and DDC inhibitors, nitecapone, entacapone, tolcapone, and CGP 28014 substantially reduce the plasma levels of 3-OMFD in monkeys (Guttman et al., 1993; Miletich et al., 1993; Günther et al., 1995; Doudet et al., 1997; Holden et al., 1997; Psylla et al., 1997). The decreased peripheral metabolism of 6-FD after COMT inhibition is reflected as an increased striatal uptake of 6-FD and significantly better PET image contrast. They increased the influx constant $K_{i(o)}$ (using occipital counts as input function) by 45% but did not affect the decarboxylation of 6-FD (Guttman et al., 1993). In comparative studies, tolcapone was most active in improving the availability of 6-FD, followed by CGP 28014 and entacapone. CGP 28014 was more active p.o. than i.v. (Günther et al., 1995; Psylla et al., 1997). Some studies have also been done in rats (Pauwels et al., 1994).

Hartvig et al. (1992) reported that tolcapone, administered in combination with [¹¹C]L-dopa or 6-FD but without a DDC inhibitor to monkeys, did not affect the brain uptake for any of the ligands. Tolcapone did not change the decarboxylation rate of L-dopa in striatum but increased that of 6-FD, probably indicating a significant contribution of 3-OMFD to background activity. Interestingly, the combination of tolcapone plus benserazide significantly inhibited the central decarboxylation of [¹¹C]L-dopa, possibly due to an elevated brain entry of benserazide and its active metabolite (Tedroff et al., 1991). No such effect was observed with tolcapone plus carbidopa.

Quite a few PET studies with COMT inhibitors have been performed in humans, all of them with entacapone or nitecapone. There is no doubt that the quality of PET scans, expressed as a ratio of the activity in the striatum to that in the occipital lobe or cerebellum, is improved. This improvement is seen both in normal volunteers and in patients with PD. With nitecapone, the increase was 22% in both cases (Laihinen et al., 1992). In entacapone-treated volunteers and PD patients, the improvements have varied from 2 to 41% (Sawle et al., 1994; Ruottinen et al., 1995, 1997; Ishikawa et al., 1996). The worst rates were seen in the most advanced cases of PD, for whom the patients had been treated with levodopa for years, and the best results occurred in normal volunteers and in de novo PD patients (Ruottinen et al., 1995, 1997).

To conclude, PET studies with 6-FD have shown that the peripheral COMT inhibitors substantially improve the brain entry of 6-FD; it is likely that such an effect is also obtained in clinical practice with the use of levodopa and DDC inhibitor and with COMT inhibitors. Thus, the peripheral COMT inhibitors can improve the quality of PET imaging with 6-FD.

V. Practical and Theoretical Clinical Uses of COMT Inhibitors

The main clinical application of COMT inhibitors would be as adjunct (or additional adjunct) in the levodopa therapy of PD (Männistö and Kaakkola, 1989, 1990; Männistö et al., 1992b, 1994). As discussed, COMT inhibitors can reduce the formation of 3-OMD from L-dopa. Therefore, the bioavailability of levodopa would be improved, its entry to the brain increased, and possibly the half-life of L-dopa prolonged. Peripherally active inhibitors would also be anticipated to achieve these effects on L-dopa metabolism. A brain-entering compound (e.g., tolcapone) might further potentiate the effect of L-dopa by slowing down the metabolism of dopamine formed from L-dopa in the brain. Thus, a triple therapy (levodopa plus DDC inhibitor plus COMT-inhibitor) would replace the present double therapy in PD.

It is conceivable that a COMT inhibitor could increase the formation of dopamine from L-dopa, even when administered without a DDC inhibitor. This combination may have some beneficial cardiovascular effects, such as increased renal blood flow and improvement in heart function.

COMT inhibitors could also potentiate or prolong the action of compounds with a catechol structure (in addition to levodopa). Such drugs include bronchodilating compounds (epinephrine, isoprenaline, rimiterol), dopamine agonists (dobutamine, fenoldopam, apomorphine), and some antihypertensive drugs (α -methyldopa). There also is the theoretical possibility of increasing the effect of endogenous catecholamines.

Inhibition of COMT in the brain would be beneficial in restoring norepinephrine levels and improving the symptoms caused by the deficit of this transmitter. Norepinephrine, or rather its deficiency, has been thought to be one of the main neurotransmitters implicitly involved in depression. Correction of this deficit with COMT inhibitors, in analogy to tricyclic uptake inhibitors and MAO inhibitors, could represent a novel way to treat depression.

VI. Human Studies with COMT Inhibitors

Because CGP 28014 was not further developed for clinical purposes, there is extremely limited human data on its properties (Bieck et al., 1990, 1993); therefore, the clinical part of this review concentrates on nitrocatechol-type COMT inhibitors.

A. Human Pharmacokinetics of COMT Inhibitors

The COMT inhibitors with nitrocatechol structure are rapidly absorbed after oral administration, and C_{\max} is usually reached in 0.5 to 2 h (see Fig. 5). Oral bioavailability of tolcapone (60%) is about double that of entacapone (32–36%), but the quantitative roles of poor absorption and the first-pass metabolism in the incomplete bioavailability are still unknown. Entacapone and nite-

capone appear to be absorbed slightly more rapidly than tolcapone. Clearly, higher C_{\max} and AUC values are obtained with tolcapone than with entacapone, in part due to better bioavailability and lower clearance of tolcapone (Tables 3 and 4). AUC and C_{\max} values are dose proportional after entacapone (Keränen et al., 1994) and tolcapone (Dingemans et al., 1995a). The volume of distribution at steady state is small for all COMT inhibitors (Table 4). Nitrocatechols are abundantly bound to plasma proteins: tolcapone, about 99.9%, and others, 97 to 98%. At the therapeutic doses, all COMT inhibitors are rapidly eliminated, with an apparent $T_{1/2}$ of 1.5 to 3 h after oral administration. After i.v. administration, entacapone has the shortest $T_{1/2\beta}$, about 0.5 h, and tolcapone has the longest, about 1.2 h (Heikkinen et al., 1994; Keränen et al., 1994; Dingemans et al., 1995a).

All COMT inhibitors are abundantly metabolized, mainly in the liver. The main part of absorbed entacapone is eliminated via the biliary route to feces (Wikberg et al., 1993; Heikkinen et al., 1994), whereas about 40% of tolcapone dose is excreted to feces (Roche, 1997). Only 0.5% or less of an oral dose of entacapone and tolcapone is excreted unchanged in the urine (Wikberg et al., 1993; Heikkinen et al., 1994). The main urine metabolite is the glucuronide of the parent compound, representing 70, 60, and 30% of the metabolites of entacapone, nitecapone, and tolcapone, respectively (Taskinen et al., 1991; Wikberg and Taskinen, 1993; Wikberg et al., 1993; Da Prada et al., 1994). Entacapone [the (*E*)-isomer of the molecule] has one active metabolite, its (*Z*)-isomer. Its AUC accounts for only about 5% of the total plasma AUC of both isomers (Wikberg et al., 1993; Keränen et al., 1994). Entacapone and nitecapone are not *O*-methylated in humans (Taskinen et al., 1991; Wikberg et al., 1993), whereas about 3% tolcapone is converted to 3-*O*-methyltolcapone (Da Prada et al., 1994; Dingemans, 1997; Roche, 1997). This metabolite has a long elimination $T_{1/2}$ of about 35 h (Dingemans et al., 1995a), which may suggest that accumulation could occur. However, during the long-term administration of tolcapone, only minor accumulation of 3-*O*-methyltolcapone was detected due to suppression of its formation by tolcapone itself (Dingemans et al., 1996). In contrast to entacapone and nitecapone, about 3% of tolcapone is oxidized by cytochrome P-450 isoenzymes to active alcohol and carboxyl acid metabolites (Da Prada et al., 1994; Roche, 1997).

During long-term administration at therapeutic doses, neither entacapone nor tolcapone accumulates in plasma (Dingemans et al., 1996; Jorga et al., 1997c; Gordin et al., 1998a). The combination of levodopa and DDC inhibitor with entacapone or tolcapone does not significantly affect the pharmacokinetics (Dingemans et al., 1995b; Gordin et al., 1998a; Jorga et al., 1998b). A dosage reduction for entacapone and tolcapone is recommended in patients with liver impairment because of their increased bioavailability and reduced clearance (Jorga et al., 1997b; 1998c; Gordin et al., 1998b).

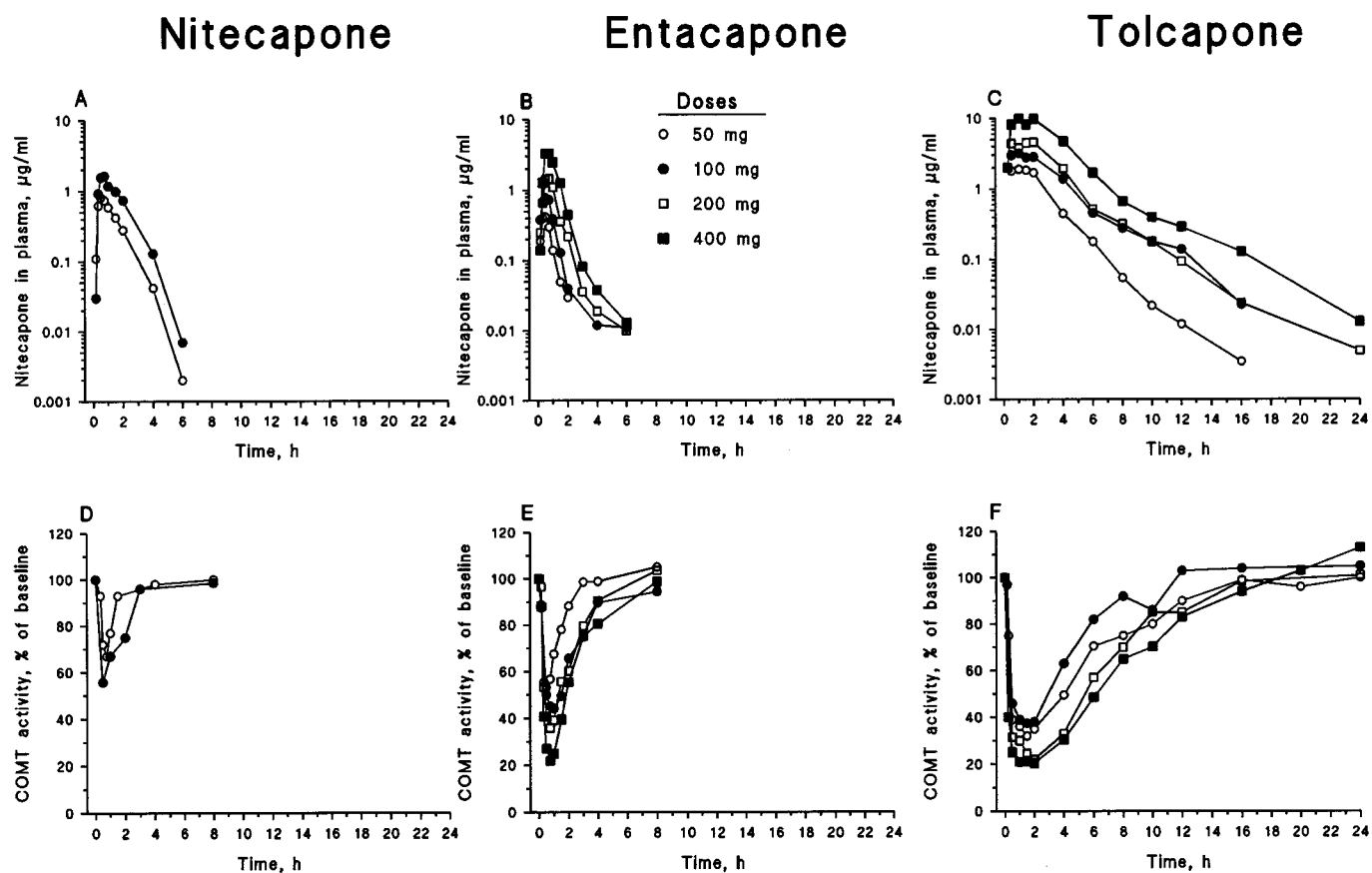


FIG 5. Effects of graded single doses of nitecapone (50 and 100 mg) and of entacapone and tolcapone (25, 100, and 400 mg) on their plasma concentrations and on human red blood cell activity of COMT as a function of time. Mean values of 12 volunteers are given. Data for nitecapone are from Schultz and Nissinen (1989), Kaakkola et al. (1990), and Schultz et al. (1991); those for entacapone are from Keränen et al. (1994), and those for tolcapone are from Dingemans et al. (1995a).

TABLE 3
Pharmacokinetics of COMT inhibitors nitecapone, entacapone, and tolcapone in healthy subjects after oral administration

	Entacapone ^a		Nitecapone ^b	Tolcapone ^c	
	100 mg	200 mg	100 mg	100 mg	200 mg
C_{max} (µg/ml)	1.1	1.8	2.6	4.6	5.7–6.3
T_{max} (h)	0.6	0.7	0.6	1.7	1.8
$AUC_{0-\infty}$ (h · µg/ml)	0.7	1.6	2.7	12.2	17.5–18.5
$T_{1/2\beta}$ (h)	1.6	3.4	N.A.	2	1.8–2.1
Oral bioavailability (%)	32	36	56	N.A.	60

N.A., not available.

^a Keränen et al., 1994.

^b Pentikäinen et al., 1989.

^c Dingemans et al., 1995a; Jorga et al., 1998a.

Values are means.

B. COMT Inhibition

All nitrocatechol-type COMT inhibitors dose-dependently and reversibly inhibit the COMT activity in human erythrocytes (Kaakkola et al., 1990; Keränen et al., 1994; Dingemans et al., 1995a). Tolcapone appears to be the most potent of these inhibitors; it also has the longest inhibitory activity. After a single dose (100–200 mg) of entacapone and nitecapone, the COMT activity has fully recovered within 6 to 8 h, whereas the corresponding time is 13 to 15 h after tolcapone (Fig. 5).

TABLE 4
Pharmacokinetics of COMT inhibitors in healthy subjects after intravenous administration

	Entacapone ^a (20–25 mg)	Nitecapone ^b (9.6–10 mg)	Tolcapone ^c (50 mg)
$T_{1/2\beta}$ (h)	0.4–0.5	0.7–0.8	1.1–1.3
V_{ss} (l)	19.9–29.2	7.7–8.0	8.6–8.8
Clearance (liters/h)	45.0–51.8	17.0–20.4	7.1–8.3
Free fraction (%)	2	3	0.1

^a Heikkinen et al., 1994; Keränen et al., 1994.

^b Pentikäinen et al., 1989, 1990.

^c Jorga et al., 1998a,c.

Values are means.

During repeated administration of entacapone or tolcapone, no tolerance developed to the inhibitory activities (Dingemans et al., 1996; Gordin et al., 1998a). Nitecapone is better at inhibiting human gastric and duodenal than erythrocyte COMT activity, suggesting that it may have greater activity locally in the intestine compared with other peripheral organs, as has also been demonstrated in rats (Nissinen et al., 1988a; Schultz et al., 1991).

C. Effect on Levodopa Pharmacokinetics

Entacapone, nitecapone, and tolcapone dose-dependently increase the AUC values of L-dopa in healthy volunteers, without significantly changing C_{max} values (Table 5, Fig. 6; Kaakkola et al., 1990; Keränen et al., 1993; Dingemans et al., 1995b; Jorga et al., 1997c, 1998b; Sedek et al., 1997; Jorga, 1998). Some tendency to prolongation of T_{max} values of L-dopa has been observed with higher doses of entacapone and tolcapone (Keränen et al., 1993; Dingemans et al., 1995b; Sedek et al., 1997). Tolcapone seems to be the most potent of these COMT inhibitors in increasing the AUC of L-dopa (Fig. 6). Multiple doses of entacapone or tolcapone do not change their effects on L-dopa pharmacokinetics (Dingemans et al., 1996; Jorga et al., 1997c; Gordin et al., 1998a). The effects of entacapone and tolcapone are generally consistent regardless of the decarboxylase inhibitor (carbidopa or benserazide) used with levodopa (Jorga et al., 1997c, 1998b; Myllylä et al., 1997b). However, entacapone increases the AUC of L-dopa 5 to 10% more with levodopa and benserazide than with levodopa and carbidopa (Myllylä et al., 1997b; Orion, 1998). Both entacapone and tolcapone are also effective in combination with controlled-release levodopa preparations (Ahtila et al., 1995; Jorga et al., 1997a, 1998b). In contrast to standard levodopa and carbidopa formulation, when controlled-release levodopa preparations are used, entacapone appears to slightly increase the peak concentration of L-dopa, whereas tolcapone increases the T_{max} value of L-dopa (Ahtila et al., 1995; Jorga et al., 1998b).

Similar to the situation in healthy volunteers, both entacapone and tolcapone significantly increase the

AUC of L-dopa in patients with PD. A 23 to 48% increase in the AUC of L-dopa has been observed after a single 200-mg dose of entacapone (Table 6). The corresponding figures for tolcapone (200 mg) vary from 51 to 58%. There are no data on L-dopa pharmacokinetics in PD patients after a single dose of 100 mg of tolcapone, which is the recommended clinical dose for tolcapone. After 7 to 8 weeks of treatment with tolcapone up to 200 mg t.i.d., AUC values of L-dopa have increased by 33% (Yamamoto et al., 1997). The corresponding value after 8 weeks of treatment with entacapone (1200 mg/day) was 43% (Nutt et al., 1994). The latter study is notable because repeated plasma samples were taken throughout the day. This study demonstrated that during entacapone treatment, the mean daily L-dopa concentration increased despite the reduction in levodopa dose (−27%), and the daily variation in plasma L-dopa levels significantly decreased (Nutt et al., 1994). This would indicate less fluctuation in clinical disability in PD patients, as was found by Nutt et al. (1994). The use of entacapone for 10 days led to a significant increase in AUC of L-dopa in PD patients receiving either standard or controlled-release levodopa formulations (Kaakkola et al., 1995). In this study, entacapone increased L-dopa peak level with a controlled-release levodopa preparation but not with a standard levodopa preparation.

COMT inhibitors reduce the elimination of L-dopa, which equates with an increase in its elimination $T_{1/2}$. This effect has been observed consistently in studies of patients treated with entacapone (see Table 8). The same is true for tolcapone in both volunteer and PD patient studies (Tables 7 and 8).

D. Effect on 3-OMD Levels

As would be expected, all COMT inhibitors reduce the formation of 3-OMD in both healthy subjects and PD patients. Because 3-OMD has a long $T_{1/2}$, about 15 h (Kuruma et al., 1971), the inhibition of 3-OMD formation in PD patients can be observed only after long-term treatment with COMT inhibitors. In healthy subjects, single doses of entacapone, nitecapone, and tolcapone dose-dependently suppressed the formation of 3-OMD,

TABLE 5
Effect of COMT inhibitors entacapone, nitecapone, and tolcapone on pharmacokinetics and metabolism of L-dopa in healthy subjects after single dose administration in combination with levodopa and carbidopa (100 and 25 mg)

Parameter	Entacapone ^a		Nitecapone ^b	Tolcapone ^c	
	100 mg	200 mg	100 mg	100 mg	200 mg
T_{max}	No change	No change	No change	No change	No change
C_{max}	No change	No change	No change	No change	No change
AUC of L-dopa	+29%	+42%	+20%	+50%	+88%
$T_{1/2}$ of L-dopa	No change	No change	N.A.	+32%	+60%
AUC of 3-OMD	−35%	−46%	−48%	−64%	−64%
AUC of DOPAC	+149%	+214%	+120%	+175%	+253%
AUC of HVA	No change	No change	−37%	−52%	−54%

N.A., not available.

^a Keränen et al., 1993.

^b Kaakkola et al., 1990.

^c Sedek et al., 1997.

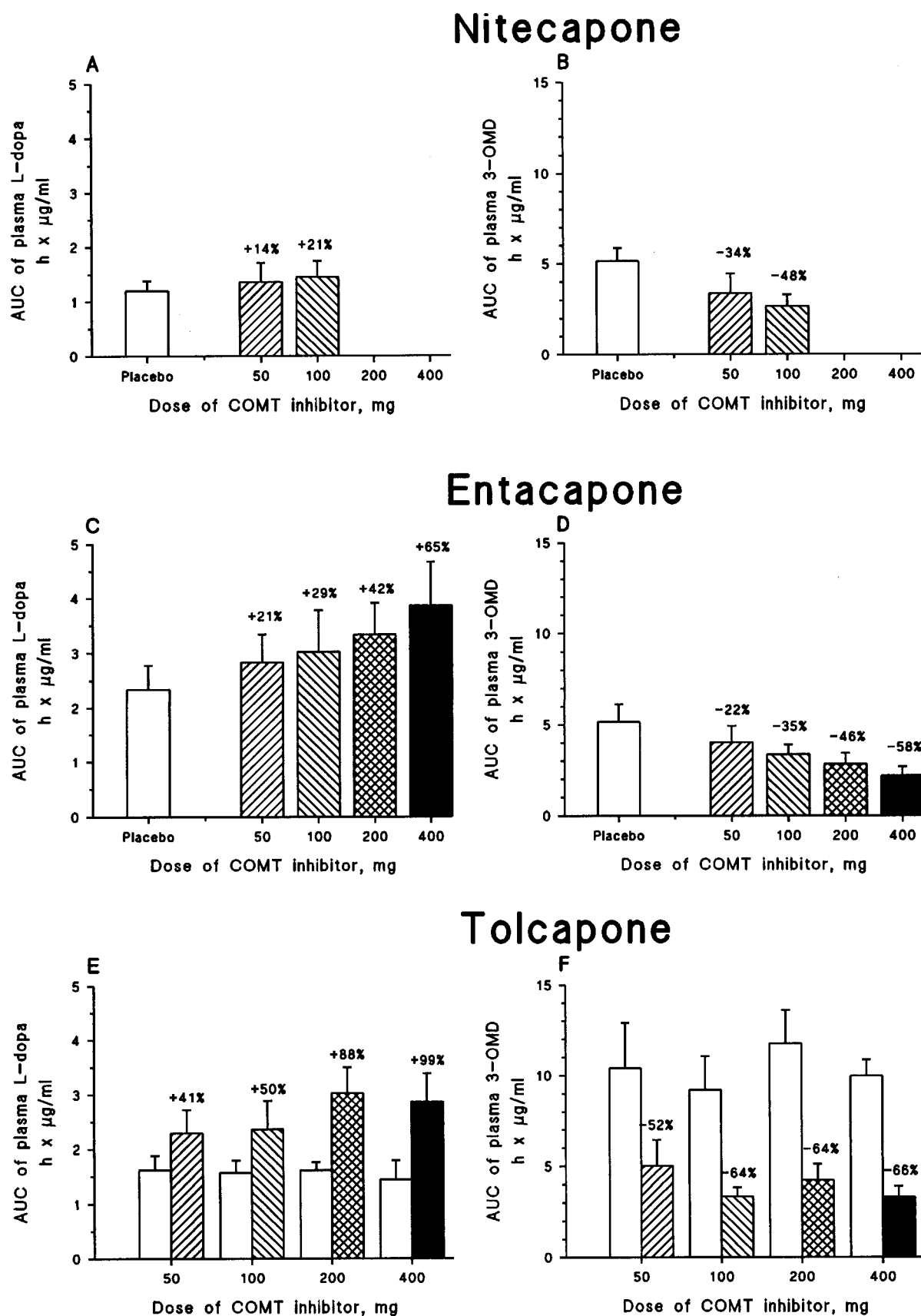


FIG 6. AUC values of L-dopa and 3-OMD after graded doses of nitecapone, entacapone, and tolcapone in healthy human volunteers. Data for nitecapone are from Pentikäinen et al. (1989), those for entacapone are from Keränen et al. (1994), and those for tolcapone are from Sedek et al. (1997). Open columns denote placebo-treated groups. Note that the nitecapone and entacapone groups each have a common placebo, but in the tolcapone groups, each dose has its own placebo.

TABLE 6
Effect of single doses of entacapone and tolcapone on AUC and elimination half-life of L-dopa in patients with PD

Parameter	Entacapone (200 mg)	Tolcapone (200 mg)	Reference
AUC	+23%	+32%	Ruottinen and Rinne, 1996a
	+29%	+51%	Tohgi et al., 1995
	+38%	+53% ^a	Ruottinen and Rinne, 1996b
	+46%	+58%	Davis et al., 1995a
	+48%	+58%	Kaakkola et al., 1994b
	+48%	+58%	Roberts et al., 1993b
	+48%	+58%	Myllylä et al., 1993
	+48%	+58%	Limousin et al., 1995
	+48%	+58%	Nutt et al., 1994
$T_{1/2el}$	+17% ^a	+32% ^a	Ruottinen and Rinne, 1996b
	+32%	+77%	Roberts et al., 1993b
	+32%	+77%	Myllylä et al., 1993
	+32%	+77%	Tohgi et al., 1995
	+39%	+79%	Ruottinen and Rinne, 1996a
	+39%	+79%	Limousin et al., 1995
	+75%	+75%	Nutt et al., 1994
	+75%	+75%	
	+75%	+75%	

^a 400 mg.

with tolcapone being the most potent of the three inhibitors (Table 5, Fig. 6; Kaakkola et al., 1990; Keränen et al., 1993; Dingemans et al., 1995b; Sedek et al., 1997; Jorga et al., 1998b). A similar inhibition of 3-OMD formation has been observed after entacapone or tolcapone in combination with controlled-release levodopa formulations (Ahtila et al., 1995; Jorga et al., 1997a, 1998b). In PD patients, about 50 and 70% inhibition of 3-OMD formation has been demonstrated after entacapone and tolcapone, respectively (Table 7).

E. Effect on Plasma Catecholamine Metabolism

Because COMT is involved in the metabolism of dopamine, norepinephrine, and epinephrine, it is also necessary to consider the actions of COMT inhibitors on peripheral catecholamine metabolism. A single dose (up to 100 mg) or 1-week treatment with nitecapone (100 mg t.i.d.) has not affected plasma dopamine, norepinephrine, and epinephrine levels either at rest or during exercise (Sundberg et al., 1990, 1993b). The same result has been obtained after treatment with a single dose (up to 200 mg) or repeated doses (400 or 800 mg t.i.d. for 1 week) of entacapone (Sundberg et al., 1993a; Illi et al., 1994, 1996a). However, both entacapone and nitecapone significantly changed the metabolic profile of plasma catecholamines. Plasma concentrations of the MAO-dependent metabolites DOPAC and 3,4-dihydroxyphenyl glycol increased, whereas those of the COMT-dependent metabolite MHPG decreased after entacapone and nitecapone administration (Sundberg et al., 1990, 1993a,b; Illi et al., 1994, 1996a). There are no published data available concerning the effect of tolcapone on plasma catecholamines or their metabolites.

In healthy subjects treated with levodopa and DDC inhibitor, COMT inhibitors increased substantially the plasma concentrations of DOPAC and induced a variable decrease in levels of HVA (Table 5; Kaakkola et al., 1990; Keränen et al., 1993; Zürcher et al., 1993; Ahtila et al., 1995; Sedek et al., 1997).

In PD patients, a single dose of entacapone (200 mg) in combination with levodopa and benserazide did not alter the plasma levels of dopamine and norepinephrine but decreased those of MHPG (Lyytinen et al., 1997). In contrast, tolcapone has been reported to elevate significantly plasma dopamine levels (Oechsner et al., 1998). Similar to the situation in healthy subjects, treatment with entacapone or tolcapone decreased the plasma concentrations of HVA and increased those of DOPAC in PD patients (Myllylä et al., 1993; Davis et al., 1995a; Ruottinen and Rinne, 1996a,c; Lyytinen et al., 1997; Oechsner et al., 1998).

These results suggest that supplementation of the present fixed ratio levodopa and DDC inhibitor therapy with a COMT inhibitor may lead to the conversion of some peripheral L-dopa to dopamine and further to DOPAC because the extent of the DDC inhibition may not be sufficient. Oechsner et al. (1998) suggested that the dose of the DDC inhibitor should be increased when combined with tolcapone to avoid the peripheral side effects of dopamine. It would be interesting to evaluate the different possible ratios of levodopa, DDC inhibitor, and COMT inhibitor to identify the optimum dosage schedules.

F. Clinical Efficacy

Only entacapone and tolcapone have been studied in true clinical trials. Both have been effective in several open and double-blind clinical studies. It should be noted that due to serious, although rare, adverse reactions, marketing of tolcapone was suspended in the European Union and Canada in late 1998. In the United States, tolcapone should be used as an adjunct only in patients with PD on levodopa and carbidopa who are experiencing symptom fluctuations and who are not responding satisfactorily to or who are not appropriate candidates for other adjunctive therapies. The reasons for tolcapone withdrawal are discussed in *Safety*. As of early 1999, entacapone is marketed throughout the European Union. It is intended as an adjunct to standard preparations of levodopa and carbidopa or levodopa and benserazide in the treatment of patients with PD and end-of-dose motor fluctuation.

The comparison of the results achieved with these two inhibitors is complicated because of differences in patient materials, study designs, treatment periods, medications, and presentations of the results. With these reservations, some of the clinical results are presented in Tables 8 and 9. All studies with entacapone and most of the studies with tolcapone have been conducted in PD patients who were experiencing clinical fluctuations [i.e., end-of-dose deterioration (wearing off)] and often dyskinesias.

Both entacapone and tolcapone as single doses with levodopa and DDC inhibitor show significant clinical benefits (Table 8). Both drugs prolong the motor re-

TABLE 7
Effect of entacapone and tolcapone on AUC of 3-OMD in patients with PD

COMT Inhibitor	Dose/Day	Treatment Duration	Decrease in 3-OMD AUC	Reference
	mg		%	
Entacapone	600–800	1 wk	44	Kaakkola et al., 1994b
	800–1200	4 wk	45	Ruottinen and Rinne, 1996b
	800–2000	4 wk	63	Ruottinen and Rinne, 1996c
	1200	2 mo	59	Nutt et al., 1994
Tolcapone	800–2000	6 mo	58	Parkinson Study Group, 1997
	300	6 wk	66	Davis et al., 1997
	600	6 wk	79	Davis et al., 1997
	600	7–8 wk	79	Yamamoto et al., 1997

TABLE 8
Clinical efficacy of entacapone and tolcapone in single-dose studies ("levodopa test") in patients with PD

COMT Inhibitor	Patients	Dose	Method	Increase in ON Time	Increase in ON Time	Reference
	<i>n</i>	<i>mg</i>		<i>min</i>	<i>%</i>	
Entacapone	19	200	Motor UPDRS	33	21	Ruottinen and Rinne, 1996a
			Tapping test	62	81	
	12	200	Tapping test	35	27	Merello et al., 1994
	13	200	Tapping test	36	40	
	8		Walking test	72	75	
Tolcapone	12	200	Motor UPDRS	54	39	Ruottinen and Rinne, 1996b
	5–10	100	Motor UPDRS	54	69	
		200	UPDRS	15	15	Roberts et al., 1993a
	4–9	100	Motor UPDRS	62	42	Davis et al., 1995b
		200	UPDRS	51	38	
10	200	Motor UPDRS	62	65	Limousin et al., 1995	

TABLE 9
Clinical efficacy of COMT inhibitors in long-term placebo controlled double-blind studies in fluctuating patients with PD^a

COMT Inhibitor	Patients	Dose of COMT Inhibitor	Duration and Design	On Time Increase ^b	Off Time Decrease ^b	Levodopa Decrease ^b	Reference
	<i>n</i>	<i>mg/day</i>		<i>h</i>		<i>mg</i>	
Entacapone	23	800–2000	4 wk, crossover	2.1	N.A.	140	Ruottinen and Rinne, 1996c
	102 (P)		6 mo, parallel	~1	~1	100	Parkinson Study Group, 1997
Tolcapone	103 (E)	800–2000	6 mo, parallel	1.2	1.3	100	Rinne et al., 1998
	86 (P)		6 wk, parallel	±0	0.9	170	Kurth et al., 1997
	85 (E)	800–2000	6 wk, parallel	1.8	1.7	190	
	42 (P)	600	6 wk, parallel	2.0	2.3	250	Adler et al., 1998
	40 (T)		6 wk, parallel	2.4	1.7	80	
	72 (P)	300	6 wk, parallel	1.8	1.3	80	Baas et al., 1997
	69 (T)	600	3 mo	1.8	0.9	90	
	74 (T)	600	3 mo	0.6	0.9	80	Roche 1997; Rajput et al., 1997
	37 (P)		3 mo	1.5	1.8	220	
	31 (T)	600					
	58 (P)						
	60 (T)	300					
59 (T)	600						
66 (P)							
69 (T)	300						
67 (T)	600						

^a For tolcapone, only the results of clinically recommended doses, 100 or 200 t.i.d., are shown.

^b An overall treatment effect (for tolcapone, no overall treatment effects are given in original publications, thus approximate results were calculated by subtracting the effect of placebo).

P, placebo; E, entacapone; T, tolcapone; N.A., not available.

sponse (the "on time") to levodopa. The dose of 200 mg of entacapone with each levodopa and DDC inhibitor dose has been selected for further clinical use, in part based on the results of a dose-finding study (Ruottinen and Rinne, 1996a) and in part because of compatible pharmacokinetic profiles of levodopa and entacapone. The dose-response relationship for tolcapone has not been as unambiguous in clinical trials (Tables 8 and 9). At

present, the manufacturer recommends that tolcapone should be initiated with 100 mg t.i.d. together with the first levodopa dose and then at 6-h interval with a 12-h nighttime break (Roche, 1997, 1998). The dose can be increased to 200 mg t.i.d., but dopaminergic adverse reactions may be a limiting factor.

The efficacy of entacapone has been elucidated in two double-blind studies of 6 months' duration and that of

tolcapone in several double-blind studies of a maximum of 3 months' duration (Table 9). Both entacapone and tolcapone generally increase the on time and correspondingly decrease the "off time" in advanced PD patients. The actual increase in daily on time has varied from about 1 to 2 h with entacapone and from about 0 to 2.5 h with tolcapone (Table 9). Typically, the clinical efficacy of COMT inhibitors is observed in the early days of treatment, as would be expected because they increase the AUC and half-life of L-dopa already on the first day. In line with their effect on L-dopa pharmacokinetics, COMT inhibitors permit a reduction in daily levodopa dosage by about 100 to 200 mg. After withdrawal of entacapone or tolcapone, a rapid worsening of PD symptoms is observed and a levodopa dose adjustment upward is needed (Parkinson Study Group, 1997; Roche, 1997, 1998; Rinne et al., 1998).

Entacapone has potentiated the magnitude of the levodopa effect; the scores of Unified Parkinson's Disease Rating Scale (UPDRS) have significantly improved (Parkinson Study Group, 1997; Rinne et al., 1998). A similar tendency, although generally nonsignificant, has been observed in long-term studies with tolcapone (Baas et al., 1997; Dupont et al., 1997; Myllylä et al., 1997a; Rajput et al., 1997; Adler et al., 1998). Furthermore, investigators' global measures of disease severity indicate that both entacapone and tolcapone have positive effects on PD symptoms (Baas et al., 1997; Kurth et al., 1997; Myllylä et al., 1997a; Parkinson Study Group, 1997; Rajput et al., 1997; Adler et al., 1998; Rinne et al., 1998). The patients' self-reported global evaluations ("patient's diaries") demonstrate similar positive results in entacapone studies (Parkinson Study Group, 1997; Rinne et al., 1998).

The clinical efficacy of tolcapone has also been investigated in nonfluctuating PD patients. One of these studies included PD patients whose fluctuations were controlled by more frequent levodopa dosing (Dupont et al., 1997). At 6 weeks, tolcapone groups (200 and 400 mg t.i.d.) had moderately greater dose reduction in their

levodopa dose than the placebo group (about 180 mg for tolcapone and 110 mg for placebo). The only statistically significant clinical benefit of tolcapone was observed in UPDRS subscale II (activities of daily living) with 200 mg t.i.d. dosing. In another study, patients with wearing off phenomena were excluded. Thus, this can be considered as more representative of nonfluctuating PD patients (Waters et al., 1997). At 6 months, tolcapone (100 or 200 mg t.i.d.) produced a significant improvement in disability, as assessed by UPDRS and quality-of-life measures. Levodopa doses were slightly but significantly decreased in the tolcapone groups (about 30 mg). The beneficial effects of tolcapone were still maintained at 12 months.

Because there have been no studies carried out directly comparing entacapone and tolcapone, it is not possible to conclude whether there are any significant differences in clinical efficacy.

G. Safety

Overall, the COMT inhibitors have been well tolerated. For instance, the number of premature terminations has not differed significantly between placebo and active treatment groups. In general, the incidence of adverse events appears to be higher in tolcapone-treated PD patients than in entacapone-treated PD patients, although a similar trend is also noticed in the placebo groups (Table 10). The main adverse effects related to entacapone and tolcapone are dopaminergic and gastrointestinal events. In all studies, the most commonly observed dopaminergic adverse event has been a worsening of levodopa-induced dyskinesia. In the majority of cases, this occurs during the first weeks of treatment with the COMT inhibitor. The severity of dyskinesia can be minimized by levodopa dose adjustment. Indeed, this adverse event has rarely lead to treatment withdrawal. Nausea, anorexia, vomiting, orthostatic hypotension, sleep disorders, and hallucinations are other dopaminergic events that may be potentiated by a COMT inhibitor.

TABLE 10

*Most common adverse events reported in placebo-controlled double-blind studies
The adverse events occurring $\geq 5\%$ more frequently in either COMT inhibitor group compared with placebo group are presented.*

	Entacapone Studies ^a		Tolcapone Studies ^b		
	Entacapone (n = 406)	Placebo (n = 296)	Tolcapone 100 mg t.i.d. (n = 296)	Tolcapone 200 mg t.i.d. (n = 298)	Placebo (n = 298)
	<i>% of patients</i>				
Dyskinesia	27.3	13.9	41.9	51.3	19.8
Nausea	11.1	6.4	30.4	34.9	17.8
Insomnia	4.4	3.7	23.6	24.8	18.1
Anorexia	<2	<2	18.9	22.8	12.8
Dystonia	2.7	2.4	18.6	22.1	17.1
Diarrhea	8.4	3.0	15.5	18.1	7.7
Dizziness	7.4	5.4	13.2	6.4	9.7
Urine discoloration	12.6	0	2.4	7.4	0.7
Hallucination	3.4	2.4	8.4	10.4	5.4
Vomiting	<2	<2	8.4	9.7	3.7

^a Orion, 1998.

^b Roche, 1998.

The most common nondopaminergic adverse event is diarrhea. Tolcapone has been associated with diarrhea in about 16 to 18% of cases, and entacapone has been associated in less than 10% of cases (Table 10). It usually occurs within the first 6 to 16 weeks of the treatment. The diarrhea, dose related in the case of tolcapone, may be severe, whereas most cases in entacapone-treated patients have been mild to moderate. Diarrhea has been the most common cause of treatment discontinuation: 5 to 6% of patients treated with tolcapone (Roche, 1997, 1998) and 2.5% of patients treated with entacapone (Orion, 1998). In another study, diarrhea was experienced by 24% of patients treated with tolcapone (200 mg t.i.d.) plus selegiline (5 mg b.i.d.) without levodopa for 1 month (Hauser et al., 1998).

Patients should be warned that their urine may color to dark yellow or orange due to the presence of COMT inhibitors and their metabolites. This harmless event appears to be more common with entacapone than with tolcapone.

Mild decreases in hemoglobin, erythrocyte counts, and hematocrit values have been reported during entacapone therapy, probably due to its iron-chelating property. This adverse effect has been clinically significant in 1.5% of the patients (Orion, 1998).

Elevated liver transaminase levels have been reported in 1 to 3% of patients treated with tolcapone (Roche, 1997), whereas significant transaminase changes have been observed very rarely in patients treated with entacapone (Orion, 1998). Recently, three cases of acute, fatal fulminant hepatitis were described in association with tolcapone treatment (Assal et al., 1998; European Medicine Evaluation Agency, 1998). In addition, potentially fatal neurological adverse reactions, including neuroleptic-like malignant syndrome and rhabdomyolysis, were described. Due to these serious adverse drug reactions, the regulatory authorities in the European Union and Canada decided that marketing of tolcapone should be suspended. At this time, more than 100,000 patients have been treated with tolcapone. In many other countries, including the United States, the use of tolcapone has now been restricted to patients experiencing fluctuations and who were not appropriate candidates for other adjunctive therapies. In these patients, liver function tests should be taken at baseline and regularly every 2 weeks after the initiation of tolcapone treatment. It is not currently considered essential to monitor liver enzyme levels during entacapone therapy.

H. Drug Interactions

There are some pharmacokinetic interactions between COMT inhibitors and other drugs. Many of these interactions are based on the fact that the interacting drugs are also substrates of COMT. Most published information is available for entacapone and nitecapone, but the same general principles probably can be extended to

tolcapone. Some data can be derived from the official product monographs (Roche, 1997, 1998; Orion, 1998).

The poor solubility of entacapone at acidic pH of the stomach could be the reason for its poor bioavailability in rats and humans. A high dose of entacapone seems to decrease the absorption of carbidopa in studies on human volunteers (Ahtila et al., 1995). Entacapone may chelate iron in the gastrointestinal tract, and therefore it is advised to have a 2- to 3-h interval between the administration of iron preparations and entacapone (Orion, 1998). Tolcapone seems to inhibit the *O*-methylation of benserazide, which is an excellent substrate of COMT, to the extent that the brain entry of this compound is increased. If this should occur, then benserazide can start to inhibit dopamine synthesis in the striatum. This is not the case with carbidopa, which is a much less favorable substrate of COMT (Tedroff et al., 1991).

The infusion of catecholamines or isoprenaline, all of which are substrates of COMT, as well as physical exercise, which increases plasma levels of catecholamines, can cause disturbances in heart rhythm. COMT inhibitors have only slightly potentiated this natural activity, but there is some prolongation of the duration of action of catecholamines on pulse rate and blood pressure. Entacapone or nitecapone does not alter cardiovascular responses, hemodynamics, or concentrations of unconjugated catecholamines in plasma of healthy volunteers, but the metabolism profile was clearly shifted toward the MAO-dependent pathways (Sundberg et al., 1990, 1993a; Myllylä et al., 1993; Illi et al., 1994, 1995). Furthermore, the tyramine pressor response was not enhanced by nitecapone (Sundberg and Gordin, 1991). Tolcapone does not modify the action of ephedrine, an indirectly acting sympathomimetic drug (Roche, 1997). Moclobemide, an MAO-A inhibitor (Illi et al., 1996a), or imipramine (Illi et al., 1996b) did not significantly interact with entacapone, nor did desipramine interact with tolcapone (Roche, 1997, 1998).

Because entacapone and tolcapone undergo different routes of metabolism, there could be differences in their interactions with the metabolism of other drugs. In the case of tolcapone, there is no sign of competition for the glucuronidation of desipramine or cytochromal oxidation of tolbutamide or warfarin (Roche, 1997, 1998). Entacapone is not eliminated via cytochrome P-450 enzymes (Wikberg et al., 1993; Wikberg and Vuorela, 1994).

VII. Summary

COMT *O*-methylates catecholamines and other compounds with a catechol structure. The general function of COMT is the elimination of biologically active or toxic catechols and some other hydroxylated metabolites. During the first trimester of pregnancy, COMT present in the placenta protects the developing embryo from

activated hydroxylated compounds. COMT also acts as an enzymatic detoxicating barrier between the blood and other tissues shielding against the detrimental effects of xenobiotics. COMT may serve some unique or indirect functions in the kidney and intestine tract by modulating the dopaminergic tone; the same may be true in the brain: COMT activity may regulate the amounts of active dopamine and norepinephrine in various part of the brain and therefore be associated with the mood and other mental processes.

There is one single gene for COMT, which codes for both S-COMT and MB-COMT using two separate promoters. Both rat and human S-COMTs contain 221 amino acids, and their molecular weights are 24.8 and 24.4 kD, respectively. Rat MB-COMT contains 43 and human MB-COMT contains 50 additional amino acids, of which 17 (rat) and 20 (human) are hydrophobic membrane anchors. The remainder of the MB-COMT molecule is suspended on the cytoplasmic side of the intracellular membranes. Rat S-COMT has been recently crystallized at 1.7- to 20-Å resolution. The active site of COMT consists of the AdoMet-binding domain and the actual catalytic site. The catalytic site is formed by a few amino acids that are important for the binding of the substrate, water, and Mg^{2+} and for the catalysis of *O*-methylation. The Mg^{2+} , which is bound to COMT only after AdoMet binding, improves the ionization of the hydroxyl groups. The lysine residue (Lys144), which accepts the proton of one of the hydroxyls, acts as a general catalytic base in the nucleophilic methyl transfer reaction.

A series of new and highly selective COMT inhibitors have been developed. Entacapone, nitecapone, and tolcapone are nitrocatechol-type potent COMT inhibitors in vitro (K_i in nanomolar range), whereas CGP 28014 is a hydroxypyridine derivative and ineffective in vitro. In animal studies, these compounds effectively inhibit the *O*-methylation of L-dopa, thus improving its bioavailability and brain penetration and potentiating its behavioral effects. Entacapone and nitecapone have mainly a peripheral effect, whereas tolcapone and CGP 28014 also inhibit the *O*-methylation in the brain. In human

volunteers, entacapone, nitecapone, and tolcapone dose-dependently inhibit the COMT activity of erythrocytes, improve the bioavailability of L-dopa, and inhibit the formation of 3-OMD.

In clinical studies in PD patients, both entacapone and tolcapone potentiate the therapeutic effect of L-dopa and prolong the daily on time by 1 to 2 h. The two marketed COMT inhibitors have different treatment strategies, advantages, and disadvantages, which are listed in Table 11.

In the clinic, COMT inhibitors have been well tolerated, and the number of premature terminations has been low. In general, the incidence of adverse events has been higher in tolcapone-treated patients than in entacapone-treated patients. The main events have consisted of dopaminergic and gastrointestinal problems. Dopaminergic overactivity causes an initial worsening of levodopa-induced dyskinesia, nausea, vomiting, orthostatic hypotension, sleep disorders, and hallucinations. Tolcapone has been associated with diarrhea in about 16 to 18% of cases, and entacapone has been associated in less than 10% of cases. Diarrhea has led to discontinuation in 5 to 6% of patients treated with tolcapone and in 2.5% of those treated with entacapone. Urine discoloration to dark yellow or orange is related to the color of COMT inhibitors and their metabolites. Elevated liver transaminase levels are reported in 1 to 3% of patients treated with tolcapone but very rarely, if at all, in patients treated with entacapone. Three cases of acute, fatal fulminant hepatitis have been described in association of tolcapone when more than 100,000 patients have been treated. In addition, a few potentially fatal neurological adverse reactions, including neuroleptic-like malignant syndrome, have described. Because of these serious adverse drug reactions, tolcapone marketing was suspended in Europe and Canada. In early 1999, no restrictions of the use of entacapone have been proposed.

VIII. Future Aspects

Assuming that the safety of entacapone can be substantiated, the next development would be to prepare a combination tablet, containing levodopa, a DDC inhibi-

TABLE 11
Comparison of main properties and treatment strategies for entacapone and tolcapone

	Entacapone	Tolcapone
Oral bioavailability	~35%	~60%
Duration of action (effect of COMT activity in human erythrocytes)	Short	Long
Metabolism	Abundant	Abundant
Brain penetration	Negligible	Marked
Ultimate goal of the therapy	Transient intestinal COMT inhibition while L-dopa is present	Continuous COMT inhibition in the whole body
Method of use	Must be given with each dose of levodopa; between the doses COMT activity will recover	Given every 6 h t.i.d., independently of levodopa dosing to keep COMT continuously suppressed; 12-h break at night
Combination tablet	Possible	Not feasible
Goals of further development	Better absorption	Prolonged duration of action
Means to achieve that goal	Well-soluble and well-absorbed prodrug that rapidly releases entacapone?	Well-absorbed prodrug that slowly releases tolcapone?

tor, and entacapone. However, before that is marketed, it will be necessary to perform extensive pharmacokinetic trials to determine the optimum doses of each component in the tablet. We would propose that the amount of the DDC inhibitor must be increased and the amount of entacapone probably will have to be reduced. Oechsner et al. (1998) reached the same conclusion in the case of tolcapone. A fixed combination tablet would add the patients' compliance but may also complicate the individual dose adjustments.

For safety reasons, it would be necessary to clarify the new directions of the metabolism of L-dopa when its metabolism by both DDC and COMT is inhibited. Also, the consequences of inhibition of the inactivation of catecholestrogens by COMT inhibitors should be studied in detail.

A further point of concern is the action of COMT inhibition in the brain. Because COMT knockout mice exhibited behavioral abnormalities, the central actions of COMT inhibitors require clarification.

With respect to completely new COMT inhibitors, it would be necessary to also develop and study compounds not having a nitrocatechol structure because this may be associated with some risk of side effects. In particular, a compound with good bioavailability would be an improvement on the currently marketed drugs.

Acknowledgments. Studies before 1995 cited in this review were supported by the Academy of Finland, and the newer ones were supported by the University of Kuopio through the Ministry of Education. We are also very grateful for discussions with the research staff of both F. Hoffman-La Roche, particularly Karin Jorga, and Orion-Pharma, particularly Dr. Ariel Gordin.

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