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# No change of brain extracellular catecholamine levels after acute catechol-*O*-methyltransferase inhibition: a microdialysis study in anaesthetized rats

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**Abstract**

Catechol-*O*-methyltransferase inhibitors have been newly introduced as adjunct drugs to the levodopa/dopa decarboxylase inhibitor therapy in Parkinson's disease. When given alone, catechol-*O*-methyltransferase inhibitors seem to affect behaviour. We wanted to determine whether the concentrations of free amine would be increased by catechol-*O*-methyltransferase inhibition with tolcapone and underpin the positive behavioural effects. To this end, dopamine and noradrenaline levels were analyzed in the microdialysis perfusion fluid collected from several brain regions in chloral hydrate anaesthetized rats. We also analyzed the turnover rate of catecholamines in the brain after single doses of tolcapone and entacapone using the α-methyl-*p*-tyrosine method. On their own, tolcapone (at 10 or 30 mg/kg) did not elevate dopamine or noradrenaline levels in any brain region studied although the formation of catechol-*O*-methyltransferase-dependent metabolites was strongly reduced. Neither tolcapone nor entacapone (at 30 mg/kg) affected the turnover rate of catecholamines. It seems that catechol-*O*-methyltransferase inhibitors do not alter behaviour by elevating extracellular levels of free catecholamines levels but other explanations are needed. 

*Keywords:* Catechol-*O*-methyltransferase inhibitor; Entacapone; Tolcapone; Microdialysis, brain; Catecholamine turnover

## 1. Introduction

Catechol-*O*-methyltransferase inhibitors have been newly introduced as adjunct drugs to the levodopa/dopa decarboxylase inhibitor therapy used in Parkinson's disease. Catechol-*O*-methyltransferase-inhibition improves the bioavailability of L-dopa and its transport into the brain and subsequently enhances dopamine synthesis in the brain (Kaakkola et al., 1994; Männistö et al., 1992b). There are two such drugs available. Tolcapone inhibits brain catechol-*O*-methyltransferase activity at moderate doses (3–10 mg/kg) suppressing homovanillic acid (HVA) levels and increasing dihydroxyphenyl acetic acid (DOPAC) levels in the striatum and in the microdialysis fluid (Männistö et al., 1992a; Zürcher et al., 1990). In contrast, entacapone at doses of 30 mg/kg and higher only temporarily suppresses striatal catechol-*O*-methyltransferase activity (Nissinen et al., 1992). Doses up to 100 mg/kg have been needed in in vivo microdialysis studies to significantly decrease the striatal HVA efflux and to increase DOPAC efflux (Kaakkola and Wurtman, 1992).

Generally, when given alone, catechol-*O*-methyltransferase inhibitors have virtually no effect on the motoric behaviour of rodents (Männistö, 1998; Männistö et al., 1992b; Maj et al., 1990). However, a compound which interferes with adrenergic transmission would be predicted to have important cognition enhancing effects via improved attention and motivation or by regulation of cholinergic functions (Kelland et al., 1993; Levin et al., 1990). In fact, we have found that catechol-*O*-methyltransferase 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 following pretraining i.p. administration of tolcapone (10 mg/kg). Similarly, tolcapone improved the performance of senes-

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cent poor performers in the spatial memory task (linear arm maze). However, tolcapone was not able to counteract the performance deficits in rats in which the memory had been impaired by scopolamine or bilateral lesions in the nucleus basalis magnocellularis (Liljequist et al., 1997). In a special brain stimulation model, tolcapone given alone has shown antidepressant activity (Moreau et al., 1994). In our studies, however, also levodopa was needed to reveal the antidepressant-like effect of tolcapone in two other rat models of depression (Männistö et al., 1995).

It is documented that the new catechol-*O*-methyltransferase inhibitors, when combined with levodopa and dopa decarboxylase inhibitors, lead to an increase in brain (Männistö et al., 1992a; Zürcher et al., 1990) and extracellular L-dopa and dopamine levels (Acquas et al., 1992; Kaakkola and Wurtman, 1993; Törnwall et al., 1994). Similar neurochemical studies with catechol-*O*-methyltransferase inhibitors given alone are rather rare. However, we (Törnwall et al., 1994) and others (Acquas et al., 1992; Kaakkola and Wurtman, 1992) have found that tolcapone alone does not alter striatal extracellular dopamine levels.

In the present study, noradrenaline levels were analyzed in the microdialysis fluid collected from the frontal cortex, nucleus accumbens and dentate gyrus and dopamine levels collected from the striatum and nucleus accumbens in chloral hydrate anaesthetized rats to determine whether the concentrations of free amine would be increased by catechol-*O*-methyltransferase inhibition with tolcapone in some of the brain regions involved in learning and memory. We also analyzed turnover rate of catecholamines in the brain after single doses of tolcapone and entacapone. The latter drug was used as a negative control reflecting mainly peripheral catechol-*O*-methyltransferase inhibition.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats from B&K, Sollentuna, Sweden or from the National Animal Center, University of Kuopio, Finland, weighing 280–350 g, were used. Water and food were available ad libitum. All procedures were reviewed by the Animal Ethics Committee at the University of Kuopio and approved by the local Provincial Government.

### 2.2. Microdialysis

Rats were anaesthetized with chloral hydrate (350 mg/kg) dissolved in 0.9% NaCl, and given intraperitoneally (i.p.) as a dilute solution to avoid irritation in volume of 1 ml/100 g. Additional doses were given on demand several times a day. Body temperature was maintained at 37°C with a homeothermic blanket unit (Harvard Apparatus, Edenbridge, UK).

Each rat was placed in a Kopf stereotaxic apparatus and a dialysis probe was implanted through a burr hole with the center of the membrane part extending to the final coordinates of various brain regions as follows: striatum: AP 0.5, L 3.2, DV −5.8; nucleus accumbens: AP 1.5, L 1.5, DV −7.2; frontal cortex: AP 3.5, L 1.2, DV −5.3; dentate gyrus of hippocampus; CA3 region: AP −3.8, L 2, DV −3.8; hippocampus; lower CA3 region: AP −5.0, L 5.0, DV −5.0 (Paxinos and Watson, 1982).

Custom-constructed probes (Kaakkola and Wurtman, 1992; Parry et al., 1990; Törnwall et al., 1994), having 210 μm o.d. and usually 4 mm exposed membrane, were used. In the dentate gyrus region, 2 mm exposed membrane was used. The probe was perfused with artificial cerebrospinal fluid (147 mM $Na^+$, 3.5 mM $K^+$, 1.0 mM $Ca^{2+}$, 1.2 mM $Mg^{3+}$, 129 mM $Cl^-$, 1.0 mM $PO_4^{3-}$ and 25 mM $HCO_3^-$, gassed with $O_2/CO_2$ (95%/5%) to pH 7.35) with a flow rate of 2 μl/min. In some studies in hippocampus, 10 μM of nomifensine was added to the perfusion fluid to elevate noradrenaline levels. After a 120 min wash-out period, the dialysate samples were then collected for 20 min periods in vials containing 10 μl of 0.5 M acetic acid to reduce decomposition of amines. This wash-out period is sufficient to stabilize major fluctuations in the release of various neurotransmitters.

The use of chloral hydrate anaesthesia requires some comments. Chloral hydrate induces a deep short-term anaesthesia and analgesia in rats and causes cardiovascular and respiratory depression (Field et al., 1993). Although any anesthesia may affect transmitter levels, the functionality of dopamine and noradrenaline receptors is not abolished. Electrophysiological studies have shown that in the rats anaesthetized with chloral hydrate the burst-firing activity of midbrain dopamine neurons was suppressed to about one-half of that in the awake rats (Hamilton et al., 1992; Kelland et al., 1989). It has also been found that in the chloral hydrate anaesthetized rats the basal dopamine levels were also about 50% of those in the conscious rats. However, responses to various manipulations such as $K^+$-depolarization, tetrodotoxin treatment (Kask et al., 1997; Tuomainen et al., 1996) and the dopamine and noradrenaline releases induced by amphetamine were not altered (Chen and Kandasamy, 1996; Hamilton et al., 1992; Pan et al., 1996; Warenycia and McKenzie, 1988). Any anaesthesia forms a sort of background noise which cannot be ignored. There are also differences between the anaesthetics. As an example, Pan et al. (1995) reported that chloral hydrate did not affect the noradrenaline responses to cocaine although those were suppressed by pentobarbital. Interestingly, the extraction fraction of noradrenaline (decreased) and dopamine (not altered) in the frontal cortical microdialysis were differently affected by chloral hydrate anaesthesia (Pan and Lai, 1995). Collectively, these findings demonstrate that it is possible to modify dopaminergic and noradrenergic transmission during anaesthesia. This was also demonstrated in the present study (Fig. 1).

Recoveries of the probes at a flow rate of 2 μl/min were 12.6 ± 1.0% (mean ± S.E.M.) for L-dopa, 11.3 ±

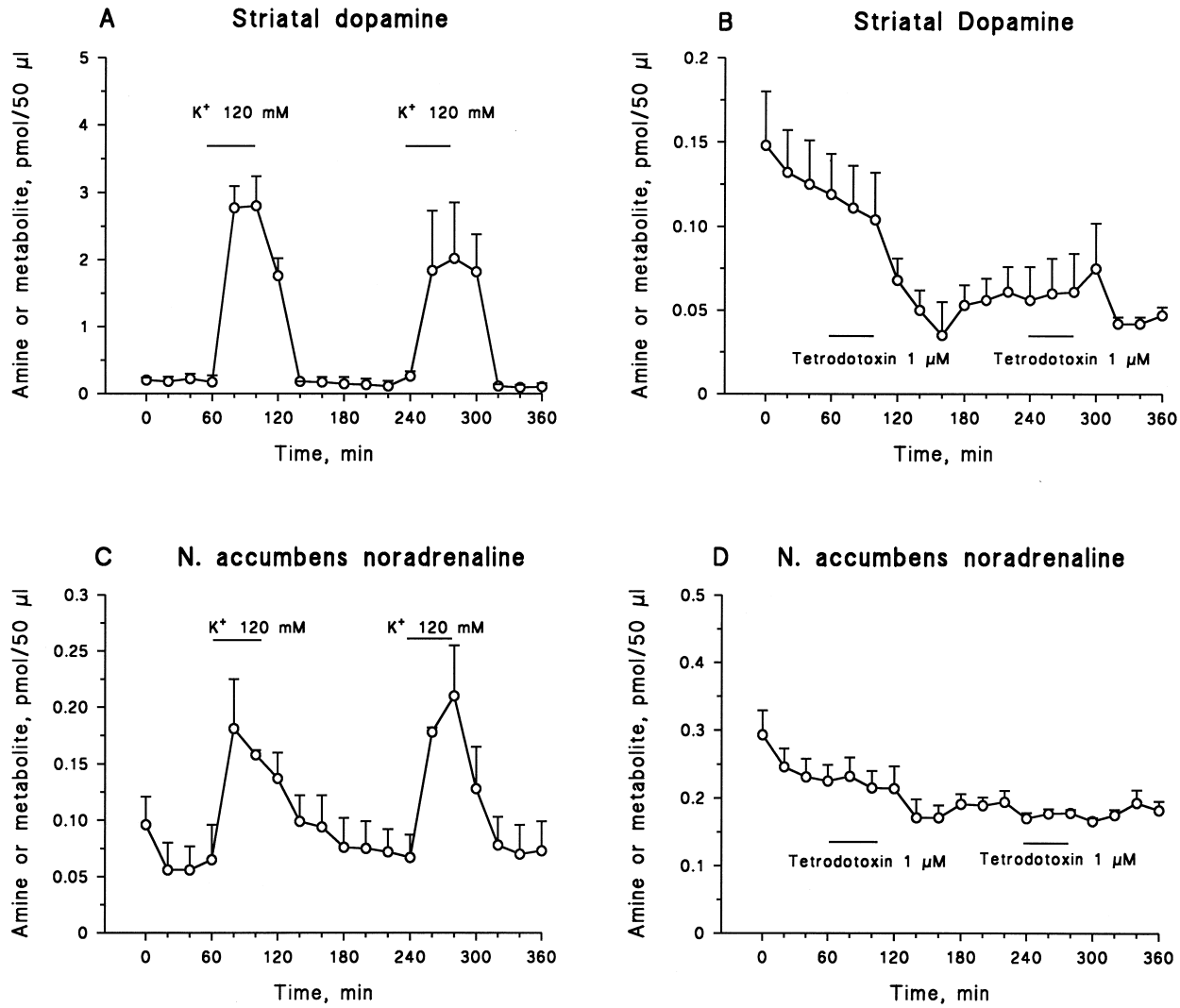



Fig. 1. Effect of $K^+$-depolarization (A) and 1 µM of tetrodotoxin (B) on striatal extracellular dopamine, and nucleus accumbens noradrenaline (C,D). Mean ± S.E.M. $n = 5–8$.

0.16% for dopamine, 11.8 ± 2.0% for DOPAC and 14.0 ± 0.6% for HVA ($n = 5$). The results were not corrected for recovery.

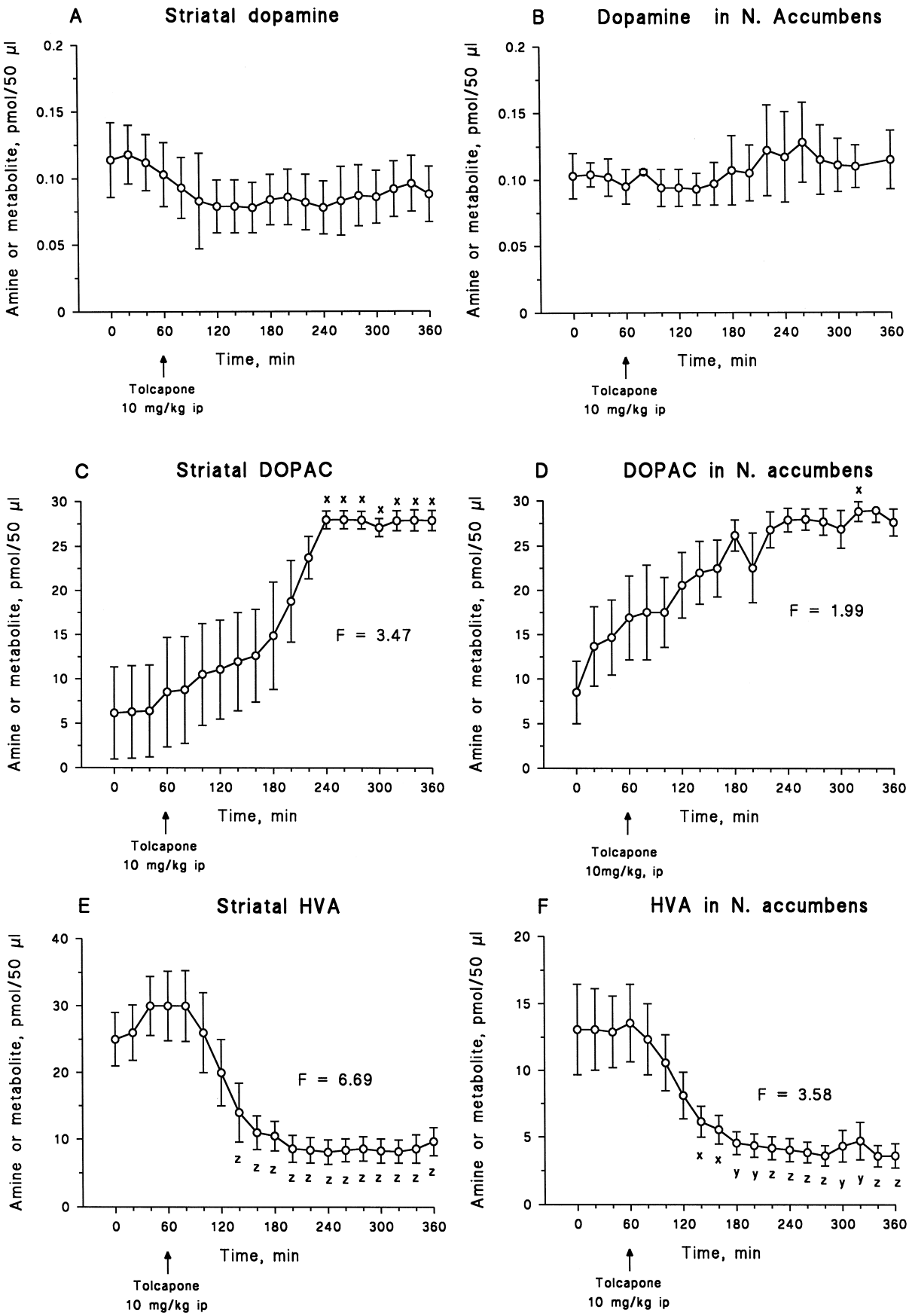
Tolcapone (10 or occasionally 30 mg/kg i.p.) was injected just after the 60-min baseline period. These doses of tolcapone are effective in blocking the brain catechol-*O*-methyltransferase activity for several hours (Männistö et al., 1992a). The collection of 20-min samples started from the tolcapone injection and lasted for 280 min. There were 5–8 rats in each group.

### 2.3. Turnover studies

An approximation of the turnover rate of catecholamine was obtained by examining whether the catechol-*O*-methyltransferase inhibitors were able to alter the rate of depletion of dopamine and noradrenaline in distinct brain regions induced by treatment with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine administered as the methyl ester, 250 mg/kg, i.p. 20 min after the catechol-*O*-methyltransferase inhibitors (doses 30 mg/kg, i.p.). Compounds which accelerate turnover potentiate the depletion in dopamine and noradrenaline after α-methyl-*p*-tyrosine and conversely, compounds which inhibit release of catecholamine transmitters decrease the rate at which the stores are depleted. The rats were killed 2 h after α-methyl-*p*-tyrosine or its saline control, this period being during the phase of linear decline in catecholamine stores (Scheinin et al., 1986). The rats were sacrificed by decapitation and the following brain regions dissected on ice; striatum, hypothalamus, frontal cortex. These tissues were stored at −80°C until determination of biogenic amines and metabolites.

### 2.4. Chemical assays

Dialysate levels of dopamine, DOPAC, HVA, noradrenaline and 3-methoxy-4-hydroxy phenylethylene glycol (MHPG) levels were analyzed using a high-perfor-


A
Striatal dopamine
B
Dopamine in N. Accumbens
C
Striatal DOPAC
D
DOPAC in N. accumbens
E
Striatal HVA
F
HVA in N. accumbens
Amine or metabolite, pmol/50 µl
Time, min
Tolcapone
10 mg/kg ip
Tolcapone
10mg/kg, ip
F = 3.47
F = 1.99
F = 6.69
F = 3.58

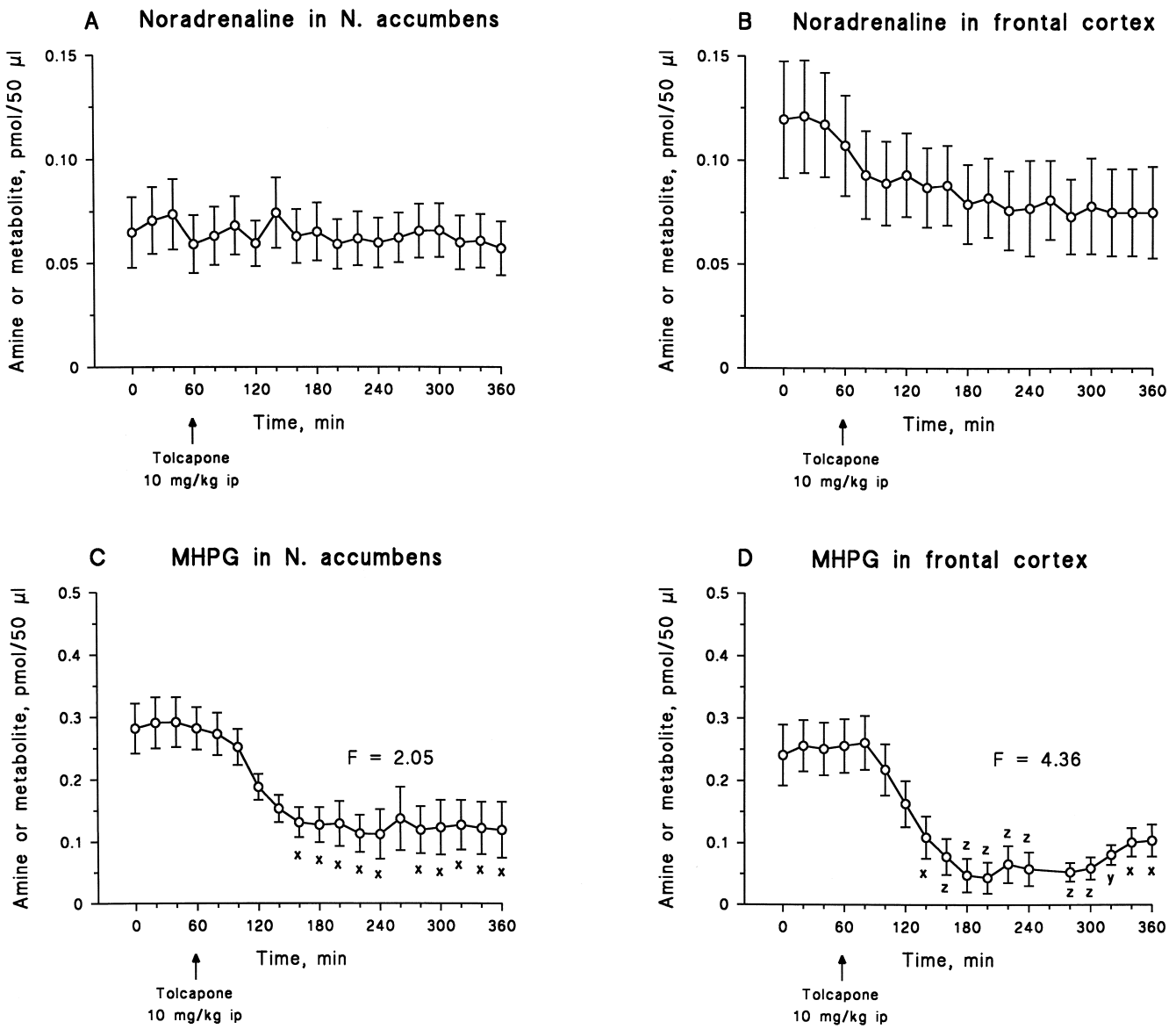



Fig. 3. Effect of 10 mg/kg i.p. of tolcapone on noradrenaline and MHPG concentrations in microdialysis perfusion fluid in nucleus accumbens (A,C) and in frontal cortex (B,D). Mean ± S.E.M. $n = 5–7$. If significant, $F$-values of ANOVA are given in the figure. The detailed effect of tolcapone (Dunnett's test) is shown as follows: $^{x}P < 0.05$, $^{y}P < 0.01$ and $^{z}P < 0.001$.

mance liquid chromatography (HPLC) with electrochemical detection as earlier described (Tuomainen et al., 1996). The HPLC system consisted of an isocratic Waters model 6000A pump with dual SSI suppressors in series, a Waters 712 WISP autoinjector (Waters Association, Milford, MA, USA) and a Hewlett Packard 3396 series II recording integrator (Palo Alto, CA, USA). An analytical cell 5011 of an ESA 5100A coulometric detector (ESA, Bedford, MA, USA) set at +0.10 V/−0.30 V with a conditioning cell 5021 set +0.50 V were used. The column was LiChrospher 100 RP-18 (5 μm) (Merck, Darmstadt, Germany). The mobile phase contained 10% methanol in 0.1 M phosphate buffer, 20 mM citric acid, 0.15 mM EDTA and 2.2 mM octane sulphonic acid, pH 2.7. The solution was filtered through a FP Vericel™ membrane filter with 0.45-μm pores (Gelman Sciences, Ann Arbor, MI, USA). The flow rate of the mobile phase was 0.9 ml/min and injection volume 20 μl. The detection limits of the compounds (pmol/ 20 μl) were as follows: dopamine 0.01, DOPAC 0.02, HVA 0.02.

In turnover studies, the brains regions were homogenized in 9 volumes of 0.2 M $HClO_4$ which contained the

Fig. 2. Effect of 10 mg/kg i.p. of tolcapone on dopamine, DOPAC and HVA concentrations in microdialysis perfusion fluid in striatum (A,C,E) and in nucleus accumbens (B,D,F). Mean ± S.E.M. $n = 5–7$. If significant, $F$-values of ANOVA are given in the figure. The detailed effect of tolcapone (Dunnett's test) is shown as follows: $^{x}P < 0.05$, $^{y}P < 0.01$ and $^{z}P < 0.001$.

internal standards for the HPLC analysis done according to Mefford (1981) with minor modifications. Separation was achieved with a Ultrasphere ODS column (5 μm, 25 × 0.6 cm) and detection with an ESA coulometric detector working only with oxidation (electrode 0.45 V). The mobile phase consisted of 0.1 M sodium acetate, 0.1 M citric acid, 75 mg/l sodium octyl sulphate (ion pairing agent) and methanol 15% (v/v) with a flow rate of 1.3 ml/min. Injection volumes ranged from 8–15 μl. The analysis was performed in two stages. First the homogenates were centrifuged (16 500 × *g*; 15 min) and the supernatant injected directly into the HPLC for assay of serotonin, 5-hydroxyindoleacetic acid (neither reported here) and the metabolites of dopamine, DOPAC and HVA. A second aliquot of supernatant was extracted through activated alumina for purification of catecholamines which elute during the elution front in unextracted samples. At the sensitivity settings used, minimum detection levels were 0.01 nmol/g for dopamine and noradrenaline and 0.05 nmol/g for DOPAC and HVA.

### 2.5. Drugs and chemicals

Tolcapone (Ro 40-7592; OR-1404; 3,4-dihydroxy-4′-methyl-5-nitrobenzophenone) and entacapone (OR-611; (*E*)-2-cyano-*N*,*N*-diethyl-3(3,4-dihydroxy-5-nitro-phenyl)-propenamide) were synthesized at Orion Pharmaceutica (Espoo, Finland) by Ms Aino Pippuri, M.Sci. Their purity

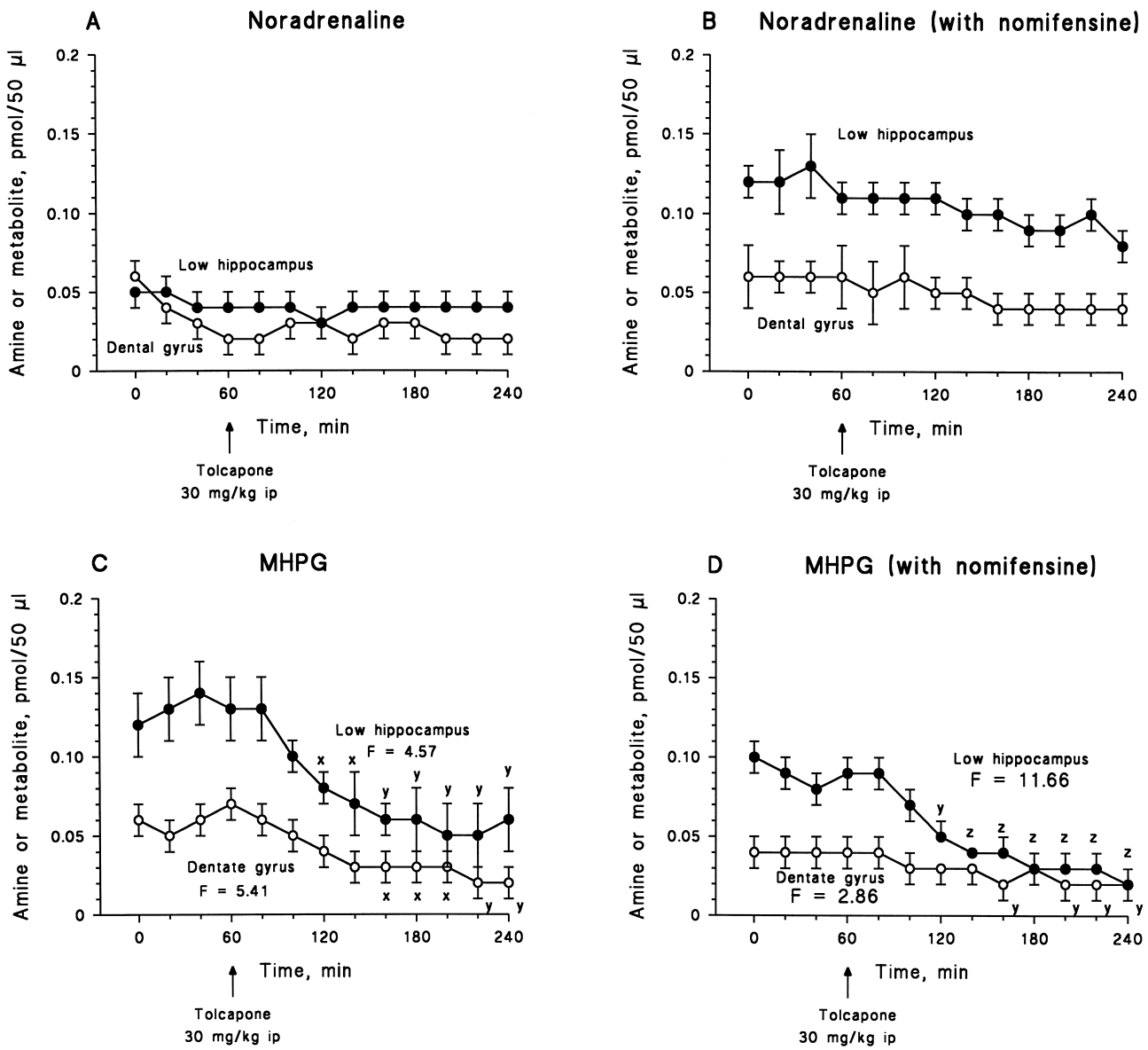



Fig. 4. Effect of 30 mg/kg i.p. of tolcapone on noradrenaline and MHPG concentrations in microdialysis perfusion fluid in two parts of hippocampus, dentate gyrus of CA3 area and the low part of CA3 (A,C). Experiments shown on the right were performed with 10 μM of nomifensine in the dialysis fluid during the whole procedure (B,D). Mean ± S.E.M. $n = 5–7$. If significant, *F*-values of ANOVA are given in the figure. The detailed effect of tolcapone (Dunnett's test) is shown as follows: $^{x}P < 0.05$, $^{y}P < 0.01$ and $^{z}P < 0.001$.

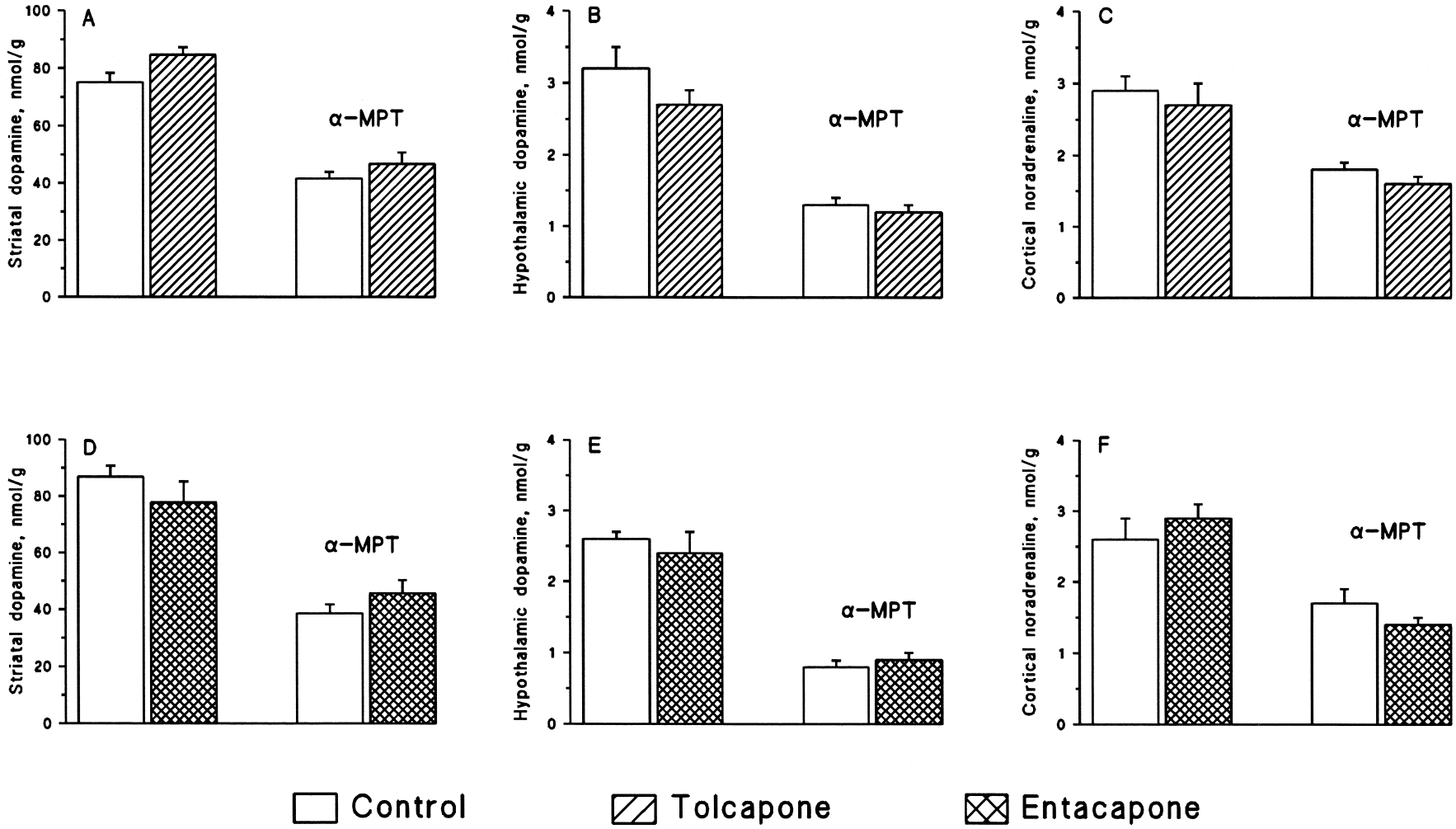



Fig. 5. Effect of tolcapone (30 mg/kg i.p., 2 h 20 min before sampling) on striatal (A) and hypothalamic (B) dopamine and cortical noradrenaline (C) with and without α-methyl-*p*-tyrosine (α-MPT; 250 mg/kg i.p., 2 h prior sampling). The effect of entacapone (30 mg/kg i.p.) in a similar experiment is shown in D–F. Mean ± S.E.M. $n = 5$. There were no significant differences between controls and catechol-*O*-methyltransferase-inhibitor treated animals with or without α-methyl-*p*-tyrosine treatment.

was higher than 99% according to thin layer chromatography and nuclear magnetic resonance. Catechol-*O*-methyltransferase inhibitors were suspended in 0.9% NaCl containing few drops of Tween 80 (European Pharmacopoeia, Vol. 3) and diluted with 0.9% NaCl in water for i.p. injections. α-Methyl-*p*-tyrosine methyl ester HCl and 1-octane sulphonic acid were from Sigma (St. Louis, MO, USA), tetrodotoxin citrate from RBI (Natick, MA, USA) and HPLC grade methanol from Rathburn (Walkenburg, UK). Other chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany).

### 2.6. Statistics

In the microdialysis studies, absolute concentrations (without any correction for the probe recovery) in each fraction were analyzed and the arithmetic mean, S.D. and S.E.M. of each group were then calculated. The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test comparing to the values before tolcapone injection (Tallarida and Murray, 1987).

For brain samples, analysis was ANOVA followed by Scheffe's test. Changes in turnover were estimated comparing the decrease in dopamine and noradrenaline levels in α-methyl-*p*-tyrosine rats without catechol-*O*-methyltransferase inhibition to those treated with entacapone or tolcapone and α-methyl-*p*-tyrosine.

## 3. Results

### 3.1. Microdialysis studies

#### 3.1.1. Functionality of dopamine release in the striatum and noradrenaline release in nucleus accumbens

$K^+$-depolarization (120 nM of KCl in the dialysis fluid for 40 min) caused regular secretion peaks of dopamine and noradrenaline levels. It appeared that the peak levels of dopamine decreased faster than those of noradrenaline (Fig. 1A and C). In striatum, DOPAC levels were also increased by $K^+$-depolarization (not shown). Tetrodotoxin (1 μM in the dialysis fluid) reduced clearly dopamine release in the striatum but affected less noradrenaline release in the nucleus accumbens (Fig. 1B,D).

#### 3.1.2. Dopamine, DOPAC and HVA levels in striatum and nucleus accumbens

Dopamine was not increased by tolcapone although DOPAC levels were tripled (striatum: $F = 3.47$, $P < 0.001$; nucleus accumbens: $F = 1.99$, $P = 0.028$) and HVA levels decreased to about 30% (striatum: $F = 6.69$, $P < 0.001$; nucleus accumbens: $F = 3.58$, $P < 0.001$). Detailed statistics are given in Fig. 2.

#### 3.1.3. Noradrenaline and MHPG levels in frontal cortex and nucleus accumbens, and in two parts of hippocampus

Noradrenaline levels remained stable in all brain regions after tolcapone although MHPG levels were significantly suppressed (nucleus accumbens: $F = 2.05$; frontal cortex: $F = 4.36$; low hippocampus: $F = 4.57$; dentate gyrus: $F = 5.41$; low hippocampus (with nomifensine): $F = 11.66$ and dentate gyrus (with nomifensine): $F = 2.86$. In all cases, $P$-values were $< 0.05$. The detailed statistics are given in Figs. 3 and 4.

### 3.2. Turnover studies

There was clear evidence for catechol-*O*-methyltransferase inhibition in brain 2 h after tolcapone. For example, in striatum, the concentration of DOPAC had doubled (control = $9.3 \pm 1.3$ nmol/g; tolcapone = $21.3 \pm 1.6$ nmol/g; $P < 0.01$ Scheffe's test) whereas HVA levels had diminished by over 90% (control = $5.8 \pm 1.0$ nmol/g; tolcapone = $0.5 \pm 0.06$ nmol/g; $P < 0.01$). This pattern was repeated in other brain areas. In contrast, entacapone, which does not penetrate into the brain, caused no significant change in striatal DOPAC (control = $11.3 \pm 0.6$ nmol/g; entacapone = $11.9 \pm 1.4$ nmol/g) or HVA (control = $5.1 \pm 0.3$ nmol/g; entacapone = $4.7 \pm 0.8$ nmol/g). As expected, α-methyl-*p*-tyrosine decreased levels of DOPAC and HVA in all brain regions (not shown).

Neither drug induced any change in the concentrations of dopamine or noradrenaline in striatum, hypothalamus or frontal cortex (Fig. 5A–F). Furthermore, the ability of α-methyl-*p*-tyrosine to deplete stores of catecholamines in these brain regions which was seen as significant decreases of dopamine (Fig. 5A,B,D,E) and noradrenaline (Fig. 5C,F) was also unaffected, indicating that the turnover rate of dopamine and noradrenaline was unaffected by catechol-*O*-methyltransferase inhibition in brain and/or periphery.

## 4. Discussion

We have demonstrated that 10 or 30 mg/kg of tolcapone did not increase extracellular dopamine or noradrenaline levels in any of the brain regions studied even though these doses inhibited the formation of the catechol-*O*-methyltransferase-dependent end metabolites HVA and MHPG. Previous studies have shown that brain catechol-*O*-methyltransferase activity remains depressed for several hours after 30 mg/kg of tolcapone (Männistö et al., 1992a). Therefore, the negative results are not due to an insufficient dosage of tolcapone.

Our results support the consensus about the fate of the catecholamines in the synaptic cleft; the high affinity neuronal reuptake ($uptake_1$) is an efficient elimination system for the released catecholamines, being responsible of 90% or more of the elimination of the amine transmitters (Kopin, 1985; Männistö et al., 1992b). When dopamine re-uptake or monoamine oxidase-A is blocked, the increase in extracellular dopamine levels can be further elevated by tolcapone (Kaakkola and Wurtman, 1992).

Our earlier microdialysis studies (Törnwall et al., 1994; Tuomainen et al., 1996) have indicated that the intact striatum is very resistant to even marginal elevation of extracellular dopamine. This is in contrast to the situation in the lesioned striatum, where catechol-*O*-methyltransferase inhibitors potentiate the effect of exogenous levodopa on contralateral turning behaviour (Törnwall and Männistö, 1993). When levodopa and dopa decarboxylase inhibitors are given together, 3-OMD becomes the major metabolite (Da Prada et al., 1984; Fahn, 1974; Reilly et al., 1980; Rivera-Calimlim et al., 1977), and then catechol-*O*-methyltransferase inhibition becomes of major importance.

According to Nissbrandt and Carlsson (1987) the importance of *O*-methylation can vary between different dopamine containing brain areas. Furthermore, amphetamine increases dopamine release and 3-MT formation more effectively in the nucleus accumbens than in the striatum (Carboni et al., 1989; Karoum et al., 1994). Maj et al. (1990) have also shown that striatal catechol-*O*-methyltransferase is inhibited by doses of tolcapone lower than those needed to cause the same degree of inhibition in the nucleus accumbens. Despite the failure of tolcapone to modify amphetamine-induced turning, tolcapone has potentiated the effect of amphetamine to increase the locomotor activity of intact rats (Maj et al., 1990). Locomotor activity is dependent on the dopaminergic tone in the nucleus accumbens, whereas circling behaviour is based on the dopaminergic imbalance in the striatum (Ungerstedt and Arbuthnott, 1970). It seems that the dopamine-rich striatum possesses a remarkable capacity to maintain physiological dopamine levels even while subjected to different pharmacological insults. It is noteworthy that in our recent microdialysis studies also noradrenaline release in the frontal cortex was very well maintained even after treatment with noradrenergic toxin *N*-(chloroethyl)-*N*-ethyl-2-bromobenzylamine, DSP-4 (Kask et al., 1997).

Our simple turnover studies, where only the levels of dopamine or noradrenaline were followed after the blockade of catecholamine synthesis by α-methyl-*p*-tyrosine, did not show any change in amine turnover rate in the striatum or hypothalamus. In contrast, in monkeys, a recent positron emission tomography study utilizing 6-[$^{18}$F] L-dopa marker, showed a 55–65% decrease in dopamine turnover after tolcapone but no effect by monoamine oxidase inhibitors (Doudet et al., 1997). We think that this result is a reflection of the L-dopa marker used since its primary metabolism is strongly dependent on catechol-*O*-methyltransferase and not at all on monoamine oxidase.

The use of the α-methyl-*p*-tyrosine method to measure turnover of catecholamines, though widely used, is subject to certain limitations. For example, if catechol-*O*-methyltransferase inhibition decreased the elimination of α-methyl-*p*-tyrosine, then one could interpret a kinetic interaction as a change in turnover. However, by restricting the experiment to 2 h after treatment, when brain tyrosine hydroxylase is maximally inhibited by α-methyl-*p*-tyrosine, we hoped to circumscribe any such problem. Two hours was sufficient time for α-methyl-*p*-tyrosine to deplete stores of dopamine and noradrenaline in the brain and compounds which alter release (and thus turnover) through presynaptic $\alpha_2$-adrenoceptors have been shown to modify significantly the rate of α-methyl-*p*-tyrosine-induced depletion (MacDonald et al., 1988). Nonetheless, catechol-*O*-methyltransferase inhibition, whether in both brain and periphery as with tolcapone or only in the periphery with entacapone, did not alter the decline in brain regional dopamine or noradrenaline concentrations after α-methyl-*p*-tyrosine. Thus, even when synthesis of catecholamines is acutely inhibited and vesicle stores are being rapidly emptied, catechol-*O*-methyltransferase inhibition does not channel more catecholamines back to storage after their re-uptake from the synapse.

What then is the mechanism of action of catechol-*O*-methyltransferase inhibitor causing positive effects on mood and cognition? At least two possibilities still remain. First, there is good evidence that tolcapone (Zürcher et al., 1991) and CGP 28014, another centrally acting inhibitor of catechol *O*-methylation (Waldmeier et al., 1990) preserve brain *S*-adenosyl-L-methionine levels when methyl groups of *S*-adenosyl-L-methionine are not consumed. *S*-adenosyl-L-methionine has been proposed to act as an endogenous antidepressant (Bottiglieri et al., 1994; Friedel et al., 1989). Second, nitrocatechols are quite strong antioxidants (Suzuki et al., 1992) and therefore they may be able to protect brain tissue from oxidative damage.

In conclusion, the effect of catechol-*O*-methyltransferase inhibition on extracellular dopamine and noradrenaline levels remained negligible in all brain regions studied, and cannot explain the antidepressive or memory improving effects of tolcapone. Evidently, the role of metabolizing enzymes in elimination of synaptic dopamine and noradrenaline is minor when compared to the dominance of the reuptake process.

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