Effect of Subchronic Administration of Tolcapone on L-DOPA- and Carbidopa-Induced Release of Striatal Dopamine and Its Metabolites

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Effect of tolcapone (30 mg/kg), a catechol-O-methyltransferase (COMT) inhibitor, on the release of striatal dopamine and its metabolites induced by L-DOPA and carbidopa is studied in freely moving rats by brain microdialysis. It is shown that inhibition of COMT induced by single injection of tolcapone leads to pronounced shifts in the concentration of metabolites, the concentration of dopamine being unchanged. The preparation stimulated the release of the neurotransmitter induced by combined administration of L-DOPA (50 mg/kg) and carbidopa (50 mg/kg). Maximum efficiency of this combination is attained upon subchronic administration.

Key Words: *tolcapone; catechol-O-methyltransferase; dopamine release; striatum*

The use of catechol-O-methyltransferase (COMT) inhibitors in complex therapy of Parkinson's disease is now extensively studied [1,3-7]. Clinical studies have shown that these drugs potentiate the effect of conventional therapy by prolonging and enhancing the effect of L-DOPA [3,4,6]. It was assumed that this potentiation of the antiparkinson effect results from inhibition of peripheral L-DOPA transformation into 3-O-methyl-DOPA, which increases bioavailability of the dopamine precursor for the brain [1,3,7]. Effect of agents readily crossing the blood-brain barrier can be increased via inhibition of central methylation [3]. Tolcapone is a prospective COMT inhibitor possessing both peripheral and central activities [1,3,5,7].

Effects of single administration of tolcapone alone and in combinations with L-DOPA and inhibitors of peripheral decarboxylation have been studied in experiments [1,5,7]. However, clinical practice usually requires long-term administration of drugs.

Our objective was a neurochemical study of the effect of tolcapone administered in a combination with L-DOPA (50 mg/kg) and carbidopa (50 mg/kg) on the release of striate dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA).

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 220-240 g. The release of dopamine and its metabolites was assessed by the method of intracerebral striatal microdialysis in freely moving rats. Concentric microdialysis probes (CMC) were stereotactically implanted into the striatum (AP 0.7; L -3.5; DV -7.5) and fixed to the scull under chloral hydrate anesthesia. Perfusion with Ringer's solution was conducted at a rate of 2 μl/min 24 h postoperation, and samples were collected every 20 min. After collection of 3-4 basal samples, the test agents were intraperitoneally injected according to the following scheme: L-DOPA (Sigma) in a dose of 50 mg/kg 40 min after injection of tolcapone (30 mg/kg) and carbidopa (50 mg/kg, Orion Pharmaceutica). In the study of subchronic effect tolcapone was injected daily at 9:00 and 21:00 for one week prior to surgery. The duration of each microdialysis procedure was

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6 h. The samples were analyzed by high-performance liquid chromatography with electrochemical detection [8].

The data were processed statistically using the Student *t* and Wilcoxon-Mann-Whitney *U* tests.

RESULTS

Blockade of COMT by single injection of tolcapone (30 mg/kg) induced long-term shifts in the level of metabolites without causing marked changes in dopamine content (Fig. 1): the level of DOPAC significantly increased to 150%, while the content of HVA decreased to 53% of the baseline levels 40 min after administration of the drug. Maximum elevation of DOPAC (to 230-260%) was observed 3-4 h after injection of tolcapone. The concentration of HVA gradually decreased and 2 h postinjection attained 17% of the initial value. These findings agree with published data [1,3,5,7] and are very important for understanding the interrelationship of neurochemical processes underlying this pharmacological effect.

Tolcapone inhibits peripheral L-DOPA transformation into 3-O-methyl derivative, thus improving brain supply with L-DOPA [1,3,7]. In the brain, the dopamine precursor undergoes decarboxylation and, being transformed into dopamine, probably elevates only the intraneuronal concentration of the transmitter, which is then deaminated to DOPAC by monoaminoxidase.

The excess of DOPAC enters synaptic gap by diffusion, undergoes O-methylation, and is converted into HVA. However, under conditions of tolcapone-induced COMT inhibition this process becomes less intensive, which is assumed [7] to be responsible for the elevated content of DOPAC and decreased level

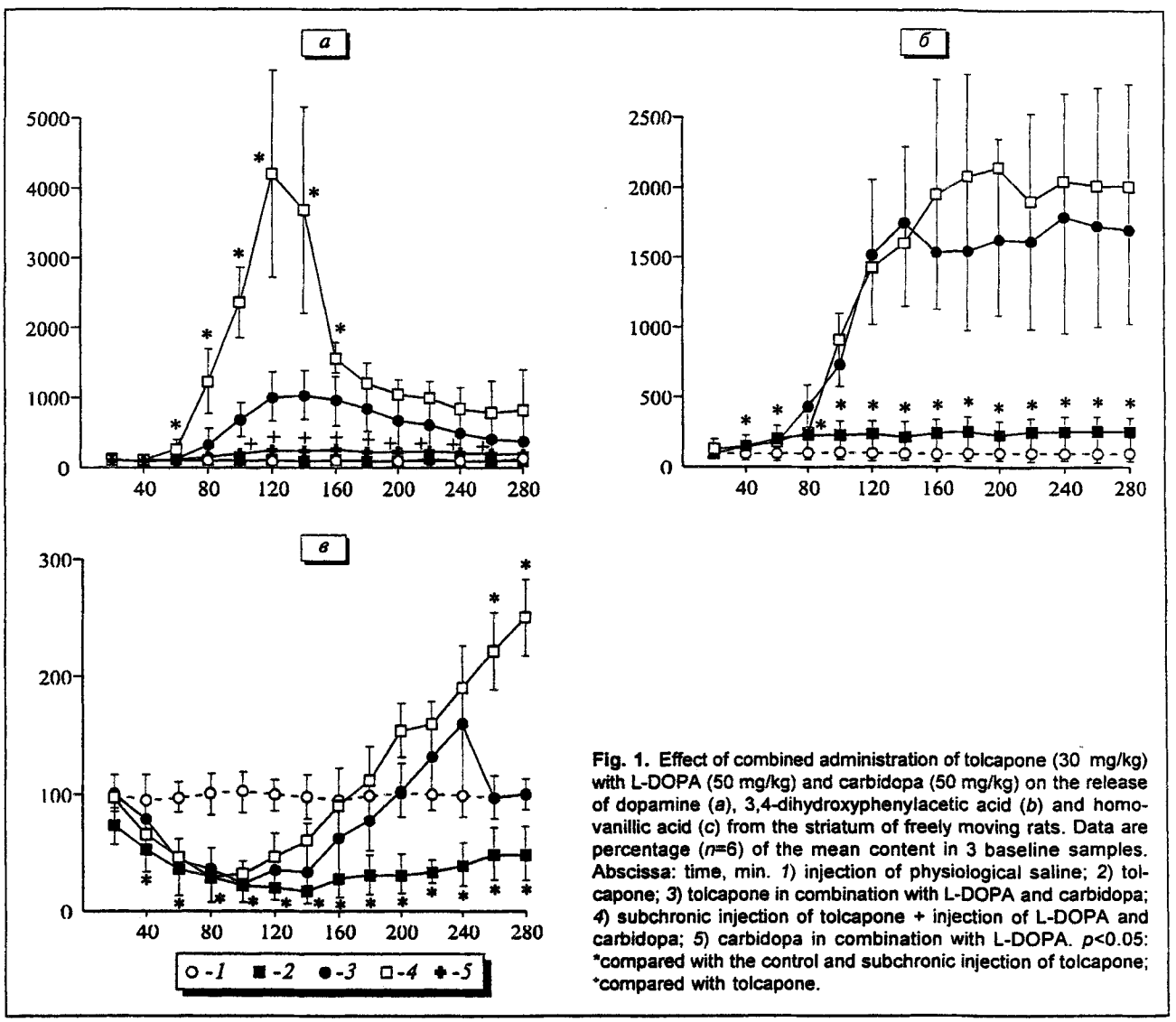

Fig. 1. Effect of combined administration of tolcapone (30 mg/kg) with L-DOPA (50 mg/kg) and carbidopa (50 mg/kg) on the release of dopamine (*a*), 3,4-dihydroxyphenylacetic acid (*b*) and homovanillic acid (*c*) from the striatum of freely moving rats. Data are percentage (n=6) of the mean content in 3 baseline samples. Abscissa: time, min. *1*) injection of physiological saline; *2*) tolcapone; *3*) tolcapone in combination with L-DOPA and carbidopa; *4*) subchronic injection of tolcapone + injection of L-DOPA and carbidopa; *5*) carbidopa in combination with L-DOPA. $p<0.05$: *compared with the control and subchronic injection of tolcapone; *compared with tolcapone.

of HVA in dialysate observed in our experiments and reported by others (Fig. 1, *b*, *c*).

The unchanged extraneuronal concentration of dopamine is probably a result of its enhanced intraneuronal deamination.

It can be assumed that minor elevation of extraneuronal dopamine concentration is difficult to detect because of neuronal reuptake. This assumption is confirmed by the fact that tolcapone potentiates the rise of dopamine content induced by the neuronal reuptake blocker nomifensine [5].

The second experimental series showed that although tolcapone had no effect on dopamine concentration, it markedly enhanced dopamine release induced by combined injection of 50 mg/kg L-DOPA and 50 mg/kg carbidopa (Fig. 1, *a*); the maximum effect was produced by subchronic administration of tolcapone. For instance, after injection of the combination L-DOPA and carbidopa, maximum concentration of dopamine did not exceed 260% of the baseline level, while single and subchronic injections of tolcapone potentiated this effect to 1030 and 4200%, respectively.

Based on the proposed mechanism of action of tolcapone, the potent dopamine-stimulating effect of L-DOPA in combination with carbidopa against the background of subchronic administration of tolcapone can be interpreted as follows.

Bioavailability of exogenous L-DOPA considerably increases due to inhibition of peripheral methylation and decarboxylation induced by tolcapone and carbidopa, respectively. The relatively high concentrations of L-DOPA are actively decarboxylated in the brain and replenish intraneuronal dopamine pool. It can be hypothesized that under these conditions the rise of newly synthesized dopamine cannot be completely compensated by deamination catalyzed by monoaminoxidase. Thus, the enhanced synthesis manifests itself in enhanced release of dopamine from presynaptic neuron endings. Subchronic inhibition of COMT suppresses dopamine metabolism in synaptic gap and therefore, far potentiates this effect.

An alternative explanation is that dopamine concentration in the extracellular space exceeds the compensatory capacity of neuronal reuptake.

In the analysis of the time-concentration curves for dopamine metabolites of special interest is an increase in the content of HVA 3 h after injection of L-DOPA against the background of subchronic administration of tolcapone (Fig. 1, *c*). Bearing in mind that this coincides with maximum accumulation of DOPAC (Fig. 1, *b*), it is difficult to explain the surprising inversion of this effect by deinhibition of central COMT followed by enhanced methylation, since the concentration of DOPAC in this case should decrease. However, HVA is formed not only due to demethylation of DOPAC, but also from 3-methoxytyramine in the reaction catalyzed by monoaminoxidase, hence changes in this metabolic chain can contribute to the observed effect. In this context, a possibility of compensatory activation of monoaminoxidase cannot be excluded.

Thus, the data of our neurochemical studies confirm rationality of combined administration of COMT inhibitors with preparation containing L-DOPA and L-aromatic amino acid decarboxylase inhibitor for more efficient therapy of Parkinson's disease.

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