Role of Metabotropic Glutamate Receptors in the Mechanisms of Experimental Parkinsonism Development

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We studied the effects of metabotropic glutamate receptor 5 (mGluR5) antagonist 6-methyl-2-(phenylethynyl)-pyridine (MPEP) on the development of catalepsy and NO generation in the striatum of rats under conditions of long-term treatment with low doses of rotenone, a mitochondrial complex I inhibitor. In rats receiving single intraperitoneal injection of rotenone (1.5 mg/kg), NO concentration in the striatum did not differ from that in animals receiving sunflower oil. No signs of catalepsy were observed at these terms in both animal groups. It was demonstrated that long-term rotenone treatment induced catalepsy associated with enhanced NO production in the rat striatum. mGluR5 antagonist MPEP alleviated catalepsy caused by long-term rotenone treatment and prevented rotenone-induced stimulation of NO generation.

Key Words: *metabotropic glutamate receptors; rotenone; parkinsonism; nitric oxide; catalepsy*

The determinant role of dopaminergic neurotransmission disturbances in the pathogenesis of Parkinson's disease (PD) is now generally accepted. It was demonstrated that degeneration of dopaminergic neurons in this pathology results in reduced content of extracellular dopamine in the *substantia nigra*. This deficiency leads to considerable imbalance of neurotransmitter content in the basal ganglia [12,13] and development of parkinsonism symptoms [3]. There are published reports relating PD development to continuous contact with pesticides, in particular, with mitochondrial complex I inhibitor rotenone [11]. In 2000, a principally new experimental model of PD was developed based on supposed high selectivity of the nigrostriatal system of the brain to mitochondrial complex I inhibitor rotenone [5]. This hypothesis is now confirmed by both *in vivo* and *in vitro* experiments. At the same time, neurochemical mechanisms of rotenone-induced neuronal damage remain poorly studied. A large body of data suggests that other neurotransmitter systems (first of all glutamatergic system), also play a role in the mechanisms of parkinsonism [6]. The contribution of metabotropic glutamate receptors (mGluR) of various subtypes into the mechanisms of extrapyramidal disorders was hypothesized, but the available data on neurochemical peculiarities of these processes are scanty and fragmentary [8]. We previously have demonstrated a modulating effect of mGlu1/5R ligands on the development of audiogenic seizures and NO generation in the brain of DBA/2 mice [1].

Here we studied the effects of metabotropic glutamate receptor 5 (mGluR5) antagonist 6-methyl-2-(phenylethynyl)-pyridine (MPEP) on the development of catalepsy and NO generation in the striatum of rats under conditions of long-term treatment with low doses of rotenone, a mitochondrial complex I inhibitor.

MATERIALS AND METHODS

Experiments were carried out on male Sprague Dawley rats weighing 180-230 g (n=57, Nursery of Tübingen University). The experiments were performed with strict adherence to Order No. 267 of the Ministry of Health of the Russian Federation (19.06.2003) and German Animal Protection Law (01.09.1990). The animals were kept in cages (8 rats per cage) with free

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access to standard mixed feed and water. To avoid the influence of diurnal rhythms on behavioral parameters of animals, all experiments were performed during the period from 9:00 to 14:00 in a laboratory room at 22±1°C.

Catalepsy was determined by the time during which both forelimbs remained on a horizontal bar suspended 10 cm above the floor (lecturer posture). Catalepsy was scored using the Morelli scale: cataleptic posture maintenance for 15-29 sec (1), 30-59 sec (2), and >60 sec (3). Catalepsy was tested over 1 min.

NO content in the brain tissue was directly and quantitatively measured by EPR method based on the use of diethyldithiocarbamate (DETC) as a free-radical trap [2]. DETC interaction with endogenous NO and Fe^{2+} ions leads to the formation of mononitrosyl iron complexes recorded by EPR. All animals received an intraperitoneal injection of Na-DETC (500 mg/kg) and a subcutaneous injection of $FeSO_4$ (37.5 mg/kg) with sodium citrate (165 mg/kg) 30 min before behavioral experiments; after behavioral tests the rats were decapitated, the striatum was isolated and frozen in liquid nitrogen. NO content was measured on a Radiopan radiospectrometer.

Rotenone (Sigma) was emulsified in natural refined deodorized oil and injected intraperitoneally in a dose of 1.5 mg/kg in a volume of 1 ml/kg body weight once a day over 60 days. Control animals received 1 ml/kg deodorized sunflower oil according to the same scheme (once a day for 60 days). Selective mGlu5 receptor antagonist MPEP (Merz) was dissolved in 0.9% NaCl and intraperitoneally injected in a dose of 3 mg/kg every other day over 60 days (30 injections).

The data were processed statistically by ANOVA.

RESULTS

Single administration of mitochondrial complex I inhibitor rotenone in a dose of 1.5 mg/kg produced no appreciable effect on NO content in the rat striatum (Table 1). No catalepsy symptoms, a typical sign of parkinsonism development in rats, were noted. Long-term (60 days) administration of rotenone in the specified dose induced sustained catalepsy (Table 1). This was accompanied by pronounced enhancement of NO generation in the rat striatum. mGluR5 antagonist MPEP considerably prevented the rotenone-induced elevation of NO content and also partially reduced rotenone-induced catalepsy (Table 1).

These data on the absence of NO accumulation in the rat striatum after single administration of rotenone agree with previous reports on the absence of activation of free-radical processes. For instance, single administration of rotenone in a higher dose (15 mg/kg) had no effect on generation of hydroxyl radical [7]. The behavioral effects induced by long-term rotenone administration corresponded to our previous observations [4] and published data [11]. These findings suggest that rotenone produces a neurotoxic effect and induces enhanced generation of free radicals, including NO, only after long-term chronic administration. This in turn supports the hypothesis that the biochemical cascade inducing the development of neurotoxic processes can be triggered only after repeated rotenone administration [5,7].

A large body of data suggests that glutamatergic neurotransmission also plays a role in the mechanisms of dopaminergic brain disorders [9]. Special attention is attracted to ligands of different subtypes of metabo-

TABLE 1. Effects of Metabotropic Glutamate Receptor Ligands on Catalepsy Development in Animals under Conditions of Long-Term Rotenone Treatment

Parkinsonism markers, time of ligand administration	Group		
	control (oil)	rotenone, 1.5 mg/kg	MPEP, 3 mg/kg+ rotenone, 1.5 mg/kg
Catalepsy			
1 day (n=10)	0	0.10±0.02	0
60 days (n=9)	0.43±0.24^{+}	2.57±0.22**	1.53±0.19*$^{+a}$
NO content, nmol/g tissue over 30 min			
1 day (n=10)	1.64±0.35	2.28±0.32	2.05±0.51
60 days (n=9)	2.49±0.48^{+}	6.53±0.76**	2.71±0.43^{+a}

Note. n: number of rats in the group, a: MPEP was injected every other day and rotenone was administered daily. *p<0.05, **p<0.01 in comparison with the control; ^{+}p<0.05 in comparison with animals receiving daily injections of rotenone over 60 days.

tropic glutamate receptors as modulators of activity of other neurotransmitter systems of the brain. Modulation of mGluR is now considered as a more promising way of regulation of the brain glutamatergic system than direct influence on ionotropic NMDA and AMPA glutamate receptors [10]. MPEP produces an inhibitory effect on NO synthase, which probably indicates the relationship between the nitroergic, glutanatergic, and dopaminergic systems of the brain.

Thus, our results attest to a substantial contribution of mGluR5 into the mechanisms of the development of experimental parkinsonism. These findings suggest that the neurotoxic effect caused by long-term rotenone treatment manifests in considerable enhancement of NO generation in the striatum. Partial prevention of this effect observed in our experiment after administration of selective antagonist MPEP probably suggests that activation of dopaminergic neurotransmission induced by mitochondrial complex I inhibitor is NO-dependent and is mediated primarily by inducable NO synthase.

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