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The role of metabotropic glutamate receptor (mGluR) ligands in parkinsonian muscle rigidity

S. Wolfarth[1], **J. Konieczny**[1], **E. Lorenc-Koci**[1], **K. Ossowska**[1], and **A. Pilc**[2]

[1] Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland,
[2] Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

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Summary. It has been shown that the primary striatal dopaminergic hypofunction which is at the origin of Parkinson's disease, results in a secondary hyperactivity of glutamatergic neurotransmission. In the search for a therapy of Parkinson's disease, ionotropic, mainly NMDA, receptor antagonists were found to have moderately beneficial, yet also some undesirable side-effects. Therefore the present study was aimed at determining whether some metabotropic glutamate receptor (mGluR) ligands may have antiparkinsonian effects in the haloperidol-induced muscle rigidity. To this end three mGluR ligands were used: the potent and selective mGluR I antagonist (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA), the mixed group II agonist/group I antagonist (S)-4-carboxy-3-hydroxyphenyl-glycine ((S)-4-C3HPG), and the potent group II agonist (+)-2-aminobicyclo[3.1.0.]hexane-2,6,-dicarboxylic acid (LY354740). Only LY354740 penetrated the brain from the periphery; for this reason other drugs were injected bilaterally into the rostral striatum or nucleus accumbens. The muscle tone was recorded by a mechanomyographic/electromyographic (MMG/EMG) method which measured the resistance of a rat's hind foot and the EMG reflex response of its muscles to passive movements. (S)-4C3HPG (5 and 15 μg/0.5 μl) and LY354740 (5 and 10 mg/kg i.p.) diminished the muscle rigidity induced by haloperidol (1 mg/kg i.p.). AIDA (0.5–15 μg/0.5 μl) injected into the striatum was only slightly effective in the highest dose used. However, when injected into the nucleus accumbens AIDA (15 μg/0.5 μl) significantly and strongly counteracted the haloperidol-induced muscle rigidity. Our results suggest that stimulation of group II striatal mGluRs seems to play a major role in diminution of parkinsonian-like muscle rigidity. However, it seems that the antagonism of group I mGluRs located in the nucleus accumbens may also be of importance to the antiparkinsonian effect.

Keywords: Amino acids – Parkinsonian-like muscle rigidity – Ionotropic glutamate receptors – Metabotropic glutamate receptors – Striatum

Introduction

It is widely accepted that main symptoms of Parkinson's disease (akinesia, muscle rigidity) result from degeneration of dopaminergic nigrostriatal neurons, which leads to loss of dopamine in the striatum (c.f. Lee, 1989). On the other hand, parkinsonian symptoms occurring in the course of a neuroleptic therapy have been shown to be caused by a blockade of striatal dopaminergic receptors. Furthermore, it has been demonstrated that the primary striatal dopaminergic hypofunction results in a secondary hyperactivity of glutamatergic neurotransmission (Klockgether and Turski, 1990). As some uncompetitive antagonists of the ionotropic glutamate NMDA receptor, e.g. amantadine and memantine, have been shown to slightly alleviate parkinsonian symptoms in humans (for ref. see Danysz et al., 1994), a number of studies were carried out to find antagonists of the NMDA receptors which more effectively counteract symptoms of this disease (for ref. see Ossowska, 1994).

The NMDA receptor is a very complex heterooligomeric structure which has a number of binding sites. Some uncompetitive antagonists which bind within the ion channel of the receptor complex, such as memantine, amantadine (Danysz et al., 1994) or dizocilpine (MK-801) (Lorenc-Koci et al., 1995, 1996), are effective in animal models of Parkinson's disease, catalepsy (akinesia) and muscle rigidity (Ossowska et al., 1994, 1996). However, when used in higher doses, they are known to produce potent psychotomimetic effects in humans (phencyclidine and ketamine) (for ref. see Ossowska, 1994). Hence, some ligands of other binding sites, e.g. competitive antagonists of the NMDA receptor, such as CGP37849, CPP and AP-5, have been regarded as possible antiparkinsonian drugs. However, these drugs either (1) poorly penetrate the blood/brain barrier (for ref. see Ossowska, 1994), or (2) act simultaneously in an opposite direction on NMDA receptors in various brain areas, either causing muscle rigidity or counteracting it (Ossowska and Konieczny, 1996), or (3) act weakly in the reserpine model, this effect suggesting that they would rather be efficacious against neuroleptic-induced parkinsonian symptoms than against Parkinson's disease itself. Therefore, competitive and uncompetitive antagonists of the NMDA receptor are not expected to create a virtually new avenue for the therapy of Parkinson's disease.

Consequently, studies on a possible antiparkinsonian action of other antagonists of the NMDA receptors, i.e. antagonists of the glycine site of the NMDA receptor, have been initiated. Since, in contrast to akinesia, the influence of NMDA receptor antagonists on muscle rigidity has not been thoroughly examined, we focused our attention especially on the effect of antagonists of the glycine site on that very important symptom of Parkinson's disease (for ref. see Ossowska, 1994; Lorenc-Koci et al., 1996, 1995). Unfortunately, the firstly synthetized powerful and selective antagonists of the glycine site, kynurenic acid derivatives (5-chlorokynurenic acid and 5,7-dichlorokynurenic acid), only poorly penetrated the brain from the periphery (Danysz et al., 1989). However, when injected directly into the striatum they

potently antagonized both catalepsy and muscle rigidity (Danysz et al., 1994; Maj et al., 1994; Lorenc-Koci et al., 1998). Effects of L-701,324, another antagonist of the glycine site which is also powerful and selective but crosses readily the blood/brain barrier, were controversial (Konieczny et al., 1999). The drug under study counteracted muscle rigidity, but was without effect on the haloperidol catalepsy even when injected in extremely high doses (20 mg/kg ip)(Konieczny et al., 1999). Some similar results, i.e. the lack of an anticataleptic effect of L-701,324 (Danysz et al., 1996) and ACEA 1021, glycine B site antagonists have already been reported (Kretschmer et al., 1995). Moreover, close observation of the behaviour and results of the rotarod test indicated that L-701,324 in doses approximately equal to those counteracting the muscle rigidity, induced a marked ataxia, disturbed rats' balance and the control over their hind limbs (Konieczny et al., 1999). Hence, it seems that also compounds of this group can hardly be expected to be effective against Parkinson's disease.

Glutamate receptors are divided in two large groups of ionotropic and metabotropic receptors. To the ionotropic receptors there belong NMDA, AMPA and kainate receptors (for ref. see Ossowska, 1994). Since AMPA and kainate receptors do not seem to play an important role in Parkinson's disease, and since NMDA receptor antagonists seem to possess a number of dangerous side-effects (for ref. see Ossowska, 1994), our attention was focused on the other group of glutamate receptors, i.e. metabotropic ones.

Like the former, the metabotropic glutamate receptors are protein complexes, with numerous splice variants, though. Furthermore, they do not form ion channels but – when activated – generate, via G proteins, a cascade of second messengers within a cell. So far, 8 different types of metabotropic receptors have been cloned (Nicoletti et al., 1996). Regarding their molecular form, the kind of the activated second messenger and the pharmacological profile, these 8 kinds of metabotropic receptors are divided into 3 groups. Activation of the group I proceeds via stimulation of phospholipase C and hydrolysis of polyphosphoinositides, yielding inositol triphosphate and diacylglicerol which, in turn, induce further metabolic processess within a cell. Group I receptors are known to be negatively coupled to K^+ channels (for ref. see Nicoletti et al., 1996). Activation of group II and III receptors inhibits the activity of adenylcyclase and diminishes cAMP formation.

One of the most interesting features of mGluRs is that they are promising drug targets in the experimental therapy of acute or chronic neurodegenerative diseases (Nicoletti et al., 1996).

In contrast to the ionotropic receptors which are mainly localized on the postsynaptic membrane, the metabotropic ones are localized on both sides of the synapse. They can influence postsynaptically the impulse flow, or can lower or raise the glutamate level via a receptor (autoreceptor) on the presynaptic side. It has been shown that group I postsynaptic receptors are activated only by excessive amounts of glutamate during the synaptic hyperactivity, whereas activation of presynaptic receptors which probably belong to this group enhances the glutamate release (for ref. see Nicoletti et al., 1996). It is generally assumed that activation of group I mGlu receptors increases neuronal excita-

tion and excitability, whereas activation of group II or group III mGlu receptors reduces synaptic excitation (Nicoletti et al., 1996). Moreover, activation of presynaptic group II receptors (autoreceptors) inhibits glutamate release. Apart from a few exceptions, it is expected that excitotoxicity should be prevented by group I antagonists, whereas group II and III agonists are likely to be neuroprotective. Since the glutamatergic hyperactivity seems to underlie neurodegeneration and the occurrence of symptoms of Parkinson's disease, new potential antiparkinsonian compounds should be searched among agonists of group II mGluRs and antagonists of group I ones.

Methods

As previously, substances which fairly selectively stimulate or block the respective metabotropic receptors were injected bilaterally (with one exception) into either the rostral striatum or the nucleus accumbens of male Wistar rats through the previously implanted guide cannulae. About 1 hour before administration to a brain structure, haloperidol (1 mg/kg) was injected intraperitoneally to induce parkinsonian-like muscle rigidity (Lorenc-Koci et al., 1996).

Mechanomyogram

Muscle tone was assessed as resistance developed by a rat's hind foot during its passive flexion and extension by 25 deg in the ankle joint with a velocity of 100 deg/s. The resistance was recorded using a force sensor (torque) connected – via an analog/digital converter – to a PC computer; the experiment consisted of 60 or 90 up-and-down movements (1–1.5 hours) of the foot (30 s apart). Mean maximum resistance of hind limb muscles (MMGmax), developed in response to flexions and extensions of the foot, was estimated. For further information, see Lorenc-Koci et al. (1996) and Ossowska et al. (1994).

Electromyogram

To test the reflex response of muscles to passive movements, an electromyogram (EMG) was recorded using two pairs of flexible, stainless-steel wire electrodes, inserted percutaneously into two antagonistic muscles of the ankle joint: the gastrocnemius and tibialis anterior. For quantification, the EMG activity was rectified and averaged with a time constant of 20 ms. For further information, see Lorenc-Koci et al. (1996) and Ossowska et al. (1996).

Ligands of metabotropic receptors, used in the present study

1. (RS)-1-aminoindan-1,5dicarboxylic acid (AIDA) – a potent and selective mGluRs group I antagonist,
2. (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4-C3HPG) – a mixed mGluRs group II agonist/group I antagonist,
3. (+)-2-aminobicyclo[3.1.0.]hexane-2,6-dicarboxylic acid (LY354740) – a potent mGluRs group II agonist.

Only LY354740 penetrated the brain from the periphery, and was therefore injected intraperitoneally. All the other metabotropic ligands were injected bilaterally in a volume of 0.5 μl/site through the previously implanted guide cannulae, directly into the brain.

Results

(S)-4-C3HPG (5 and 15 μg/site), injected into the rostral striatum, and LY354740 injected intraperitoneally (5 and 10 mg/kg), diminished in a dose-dependent manner the muscle rigidity and the EMG reflex response increased by haloperidol (1 mg/kg) whereas AIDA (0.5, 2, 7.5 and 15 μg/site) injected into the rostral striatum had only, at the highest dose used, a moderate diminishing effect on the muscle rigidity. However, AIDA (15 μg/0.5 μl) injected into the nucleus accumbens diminished clearly the muscle rigidity induced by haloperidol (1 mg/kg) as well as the EMG reflex response to it. LY354740 per se neither increased nor decreased the muscle tension.

Discussion

It has been shown that LY354740, a selective and potent agonist of group II mGluRs, counteracts both the increased mechanic muscle resistance (MMG) – muscle rigidity (Konieczny et al., 1998) and the augmented EMG reflex activity induced by haloperidol in the respective muscles of the rat's hind limb. This antiparkinsonian action of LY354740 on group II mGluRs has been confirmed by similar results obtained after intrastriatal injection of (S)-4C3HPG, a mixed group II agonist/group I antagonist. The action of (S)-4C3HPG on striatal group I mGluRs seems to be rather weak as AIDA, a potent and selective mGluRs I antagonist was only slightly effective, in the highest dose used, in counteracting the haloperidol muscle rigidity when injected into the rostral striatum. However, AIDA injected into the neighboring nucleus accumbens strongly counteracted this symptom.

It was shown previously that the nucleus accumbens transmits impulses connected with some parkinsonian symptoms, mainly akinesia and catalepsy. Its connections with neuronal circuits which convey impulses transferring muscle tension are not quite clear, although it has already been demonstrated that baclofen (1 and 2.5 mg/0.5 μl), injected into this stucture, counteracts the reserpine (10 mg/kg)-induced parkinsonian muscle rigidity (Stefański et al., 1990; Lorenc-Koci et al., 1994). Moreover, it cannot be excluded that AIDA action counteracting the rigidity after its injection into the striatum was realized via the action of the drug on mGluR1 receptors located in the nucleus accumbens since an equal dose of AIDA to that administered to the striatum has been more effective when injected to the nucleus accumbens.

Summing up, it seems that stimulation of group II mGluRs is the most important factor in diminishing the parkinsonian-like, haloperidol-induced muscle rigidity. Moreover, stimulation of group II mGluRs seems to be directed mainly towards receptors located in the rostral striatum, as the injection of (S)-4-C3HPG, a substance which also counteracts the haloperidol-induced muscle rigidity, was guided to this brain region. Nevertheless, additional contribution of the blockade of Group I mGlu receptors of the nucleus accumbens to its antiparkinsonian action against the haloperidol-induced muscle rigidity cannot be excluded.

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Authors' address: Dr. Stanisław Wolfarth, Department of Neuro-Psychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna St. PL-31-3434 Kraków, Poland.

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