Contribution of the mGluR7 receptor to antiparkinsonian-like effects in rats: a behavioral study with the selective agonist AMN082

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Abstract:

Background: Metabotropic glutamate receptors (mGluRs) have been shown to be potential targets for numerous neurological diseases, including Parkinson’s disease (PD). We previously reported that ACPT-1, a non-selective group III mGluRs agonist, injected locally into the globus pallidus, striatum or substantia nigra pars reticulata (SNr), significantly attenuated the haloperidol-induced catalepsy in rats. N,N’-dibenzhydryl-ethane-1,2-diamine dihydrochloride (AMN082) is a potent, brain penetrating mGluR7 agonist, selective over other mGluRs.

Methods: The aim of the present study was to determine whether (1) activation of mGluR7 by systemic administration of AMN082 may produce antiparkinsonian-like effects in the haloperidol-induced catalepsy and reserpine-induced akinesia models in rats; (2) striatal and nigral mGluR7 is likely to contribute to such an effect.

Results: We found that AMN082 (1 and 3 mg/kg) decreased the haloperidol (0.25 mg/kg)-induced catalepsy, but was not efficient in attenuating the reserpine (2.5 mg/kg)-induced akinesia. When given locally, AMN082 also significantly diminished catalepsy in rats; however, its effective striatal doses were 10-fold lower than those used in the SNr (2.5 and 7.5 pmol/0.5 µl/side vs. 25 and 75 pmol/0.5 µl/side, respectively).

Conclusion: The above findings support the idea that the activation of mGluR7 can produce antiparkinsonian-like effects in rats. Furthermore, our results indicate contribution of both striatal and nigral mGluR7 to the anticaeleptic effects of AMN082.

Key words:
Parkinson’s disease, metabotropic glutamate receptor, AMN082, basal ganglia, catalepsy, akinesia

Abbreviations: AMN082 – N,N’-dibenzhydryl-ethane-1,2-diamine dihydrochloride, BG – basal ganglia, DA – dopamine, DAT – dopamine transporter, Met-1 – N-benzhydrylethane-1,2-diamine, mGluRs – metabotropic glutamate receptors, α-MT – α-methyl-p-tyrosine, NET – norepinephrine transporter, 6-OHDA – 6-hydroxydopamine, PD – Parkinson’s disease, SERT – serotonin transporter, SNr – substantia nigra pars reticulata

Introduction

Glutamate is the main excitatory neurotransmitter in the central nervous system, its action being mediated by both ionotropic and metabotropic glutamate receptors (mGluRs). To date, eight subtypes of mGluRs
(mGluR1–8) have been cloned and subdivided into three groups (I–III) on the basis of their sequence homology, pharmacological profile and signal transduction mechanism [38]. Group III mGluRs (mGluR4, 6, 7, 8) are localized mainly presynaptically at glutamatergic and GABAergic terminals [4–6, 14, 18] where they regulate the release of glutamate and GABA [23, 35, 40, 43]. A growing body of evidence suggests that group III mGluRs may be potential targets for the treatment of Parkinson’s disease (PD). In fact, three members of group III mGluRs (mGluR4, 7, 8) are widely expressed in the basal ganglia (BG), a group of brain nuclei which are involved in the control of motor functions and are critical to the motor deficits observed in PD.

Like other group III mGluRs, mGluR7 is located mainly presynaptically at the active zone of glutamatergic and GABAergic terminals [4, 14, 28, 38]. Because of the low affinity of mGluR7 for glutamate, this receptor needs a high concentration of glutamate for its activation [14, 37]. The density of mGluR7 within the BG is the highest in the striatum and substantia nigra pars reticulata (SNr) [18, 24], i.e., structures which receive glutamatergic inputs from the cortex and subthalamic nucleus, respectively. Since glutamatergic corticostriatal and subthalamonoigral pathways are thought to be overactive in parkinsonian condition, the reduction of glutamate release by activation of mGluR7 at the terminals of these pathways may be of significant importance in attenuating PD symptoms. So far, however, this receptor has been the least frequently studied target for potential antiparkinsonian drugs because of the lack of highly selective ligands.

N,N’-dibenzhydrylethylene-1,2-diamine dihydrochloride (AMN082) is a very potent and brain penetrating mGluR7 agonist. This compound (< 10 µM) fails to show appreciable effects at other mGluRs [25]. Thanks to its binding site within the transmembrane domain of mGluR7, AMN082 acts like an allosteric agonist. A previous study showed some antiparkinsonian-like effects of the systemically and intrastriatally administered AMN082 [10]. However, there are still some controversies regarding the involvement of nigral mGluR7 in the therapeutic effects of mGluR7 agonists. Using high doses of non-selective group III agonists, most of the studies have indirectly demonstrated either anti-parkinsonian [2, 22] or pro-parkinsonian-like effects [21] of mGluR7 activation in the SNr. Only one study showed an antiakinetic effect of intranigral administration of AMN082 in a reserpine model [7]; however, it has not yet been ascertained whether beneficial effects are observed after peripheral administration of AMN082. Therefore, the aim of the present study was to compare (1) potential antiparkinsonian-like effects of systemic administration of AMN082 in two animal models of PD, i.e., the haloperidol-induced catalepsy and reserpine-induced akinesia in rats; (2) the effects of mGluR7 activation in the striatum and SNr in the haloperidol-induced model of catalepsy in rats. Part of these data were previously presented in the form of an abstract [15].

Materials and Methods

Animals

The experiment was performed on male Wistar rats (250–320 g). The animals were kept in a well-ventilated room on an artificial 12-h light/dark cycle (the light on from 7 a.m. to 7 p.m.) at a room temperature of 21–22°C, with free access to food and water. All experiments were carried out in compliance with the Animal Protection Act of August 21, 1997 (published in Poland’s Current Legislation Gazette [Dziennik Ustaw] no. 111/197, item 724), and according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institute’s Local Bioethics Commission.

Drugs

Haloperidol (Polfa, Warszawa, Poland; ampoules of 5 mg/1 ml) was diluted to a concentration of 0.25 mg/kg with distilled water. Reserpine (Polfa, Warszawa, Poland) was dissolved in a solution containing a 0.25% citric acid, a 2% benzyl alcohol and a 10% Tween 80; α-methyl-p-tyrosine (α-MT; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water. AMN082 (Ascent Scientific, Bristol, UK) was dispersed in a suspension of a 0.5% methylcellulose for systemic administration, or was dissolved in a 50% DMSO for intracerebral injection.
Surgery and intracranial injection

The animals were operated under pentobarbital anesthesia (Vetbutal; 30 mg/kg ip; Biowet, Pulawy, Poland). Two guide cannulae (11.9 mm long; 0.4 mm o.d.) were bilaterally implanted in the striatum or SNr. Intracerebral injections of the drug were made to conscious animals using Hamilton microsyringes connected, via polyethylene tubing, to two inner cannulae (0.3 mm o.d.) which protruded by 0.7 mm (striatum) or 2.1 mm (SNr) from the guide cannulae. Cannulae tips were placed in the rostral striatum (A/P = +1.6; L = ± 2.4 mm; D/V = –6.0 from the bregma) or SNr (A/P = –5.6; L = ± 2.0; D/V = –8.4 from the bregma) (Figs. 1A, B) [32]. The injections (0.5 µl/side) lasted 60 s, and the cannulae were left in place for another 60 s to allow diffusion. Localization of all the cannula tips was checked histologically on frozen coronal slices of the brain. All the rats used for the data analyses had injection cannula tips located bilaterally in the striatum or SNr.

Behavioral experiments

Catalepsy

A total of 177 rats were included in this study. Catalepsy was induced with haloperidol (0.25 mg/kg ip) in all the animals tested. The dose of haloperidol was selected on the basis of our earlier findings, because it produced catalepsy below the cut-off time (300 s). Higher doses of the drug induced the maximal catalepsy in our test, which made it impossible to evaluate the conceivable procataleptic effect of AMN082.

The animals were divided into three groups. The first group (66 animals) was given AMN082 (1–10 mg/kg ip) or solvent systemically, and the second (47 animals) and third groups (64 animals) were locally injected with AMN082 or solvent into the striatum (0.5–2.5 nmol/0.5 µl/side) or SNr (2.5–75 pmol/0.5 µl/side), respectively, 60 min after haloperidol administration. Each animal received only one injection and was tested only once. Since the experiment with systemic administration of the highest dose of AMN082 (10 mg/kg) was performed at a different time than that with the other doses, we used a separate haloperidol-treated group for that study.

Catalepsy was determined using a 9-cm cork test. Both forepaws of a rat were put on a cork and descent latency was measured 3 times at most, for up to 300 s each time. The longest time of keeping at least one forepaw on the cork was accepted as a catalepsy score. The test was evaluated twice: at 30 and 60 min after systemic administration of AMN082 (90 and 120 min after haloperidol), or at 15 and 30 min after intracerebral injection of that compound (75 and 90 min after haloperidol).

Locomotor activity

A total of 34 rats were included in this study. To induce akinesia, the rats were treated systemically with...
Antiparkinsonian-like effects of AMN082 in rats

Fig. 2. The influence of systemic administration of AMN082 in doses of 1 and 3 mg/kg (A) or 10 mg/kg (B) on the haloperidol-induced catalepsy in rats. AMN082 (1–10 mg/kg, ip) was administered 60 min after haloperidol (0.25 mg/kg, ip). Catalepsy was estimated twice: at 30 and 60 min after AMN082. The values are the mean ± SEM. Statistics: the Kruskal-Wallis and the Mann-Whitney U test. Symbols indicate the significance of differences in the Mann-Whitney U test: * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control; ^ p < 0.05, ^^ p < 0.01 vs. haloperidol. The number of animals per group is given in parentheses.

Fig. 3. The influence of local administration of AMN082 on the haloperidol-induced catalepsy. AMN082 was administered bilaterally into (A) the striatum (0.5 pmol to 25 pmol/0.5 µl/side) or (B) SNr (2.5 to 75 pmol/0.5 µl/side) 60 min after haloperidol (0.25 mg/kg, ip). Catalepsy was estimated twice: at 15 and 30 min after AMN082 (doses higher than 75 pmol/0.5 µl/side are not shown in the figure). The values are the mean ± SEM. Statistics: the Kruskal-Wallis and the Mann-Whitney U test. Symbols indicate the significance of differences in the Mann-Whitney U test: * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control (group which locally received solvent instead of AMN082); ^ p < 0.05 vs. haloperidol 0.25 mg/kg (group which locally received solvent instead of AMN082); * p < 0.05 vs. haloperidol + AMN082 2.5 pmol/0.5 µl/side; ** p < 0.05 vs. haloperidol + AMN082 2.5 pmol/0.5 µl/side. The number of animals per group is given in parentheses.
reserpine (2.5 mg/kg 

\textit{ip}), and 16 h later with \(\alpha\text{-MT} \)

(125 mg/kg \textit{ip}). AMN082 (1 and 3 mg/kg \textit{ip}) was
given 19.5 h after reserpine (3.5 h after \(\alpha\text{-MT}\)). Control
rats received solvents instead of reserpine or AMN082.

Akinesia was recorded in Opto-Varimex cages (Col-
lumbus Instruments, Columbus, OH), linked on-line
to a compatible IBM computer. Each cage (43 × 44 × 25 cm) was surrounded with a 15 × 15 array of photo-
cell beams located 3 cm above the floor surface.
Three parameters were estimated: the distance trav-
elled, which expressed horizontal locomotor activity
(in cm); the ambulation time, and the resting time (in s).
The rats were injected with AMN082 in their home
cages. Thirty min later, the animals were individually
placed in locomotor activity chambers. Immediately
afterwards, the locomotor activity was recorded every
15 min and measured for 60 min. The obtained data
were analyzed using Auto-Track software (Columbus
Instruments).

\section*{Statistical analysis}

The statistical analysis of catalepsy was carried out by
the Kruskal-Wallis and the Mann-Whitney U tests.

For the statistical evaluation of locomotor activity,
a one-way ANOVA was used, followed – when the
F-ratio was significant (\(p < 0.05\)) – by the Tukey
\textit{post-hoc} test.

\section*{Results}

\subsection*{The effect of systemic administration of AMN082 on the haloperidol-induced catalepsy}

Haloperidol (0.25 mg/kg) injected \textit{ip} to rats induced
moderate catalepsy, measured 90 and 120 min after its
administration (Fig. 2A, B). AMN082 (3 mg/kg), ad-
ministered systemically, markedly decreased the
haloperidol-induced catalepsy at the time points of 30
and 60 min (Fig. 2A). The effect of its lower dose
(1 mg/kg) was weaker and disappeared after 60 min.
In contrast, the highest dose of AMN082 (10 mg/kg)
produced no effect at all (Fig. 2B). AMN082 alone
(10 mg/kg) did not induce catalepsy in rats (Fig. 2B).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{The influence of systemic administration of AMN082 on the reserpine-induced akinesia. The rats were treated systemically with reserpine (2.5 mg/kg \textit{ip}) and 16 h later with \(\alpha\text{-MT} \)
(125 mg/kg \textit{ip}). AMN082 (1 and 3 mg/ kg \textit{ip}) was given 19.5 h after reserpine. Measurements started 30 min after AMN082 administration and locomotor activity was recorded for 60 min. The values are the mean ± SEM. Statistics: a one-way ANOVA, followed, when the F-ratio was significant (\(p < 0.05\)), by the Tukey \textit{post-hoc} test. Symbols indicate the significance of differences in the Tukey \textit{post-hoc} test: * \(p < 0.05\) vs. control. The number of animals per group is given in parentheses.}
\end{figure}
The effect of local administration of AMN082 on the haloperidol-induced catalepsy in rats

Catalepsy was measured in two groups of rats 75 and 90 min after haloperidol injection. Interestingly, the intensity of catalepsy differed between the groups: the group with cannulae implanted in the striatum showed catalepsy twice as strong as that when cannulae were implanted in the SNr (Figs. 3A, B). However, the latter results were not surprising, since a similar effect was observed in our earlier study [18]. AMN082 (2.5 and 7.5 pmol/0.5 µl/side) injected into the striatum markedly attenuated the haloperidol-induced catalepsy (Fig. 3A). A potent effect of AMN082 was observed only 15 min after its administration, but a tendency to reduce catalepsy was still present at 30 min after injection. The lowest (0.5 pmol/0.5 µl/side) and higher doses (25 pmol, 0.25 nmol and 2.5 pmol/0.5 µl/side) of AMN082 did not wholly abolish catalepsy in animals (the data on doses higher than 25 pmol/0.5 µl/side are not shown). Similarly, local administration of AMN082 into the SNr decreased the haloperidol-induced catalepsy in rats (Fig. 3B); however, 10-fold higher doses of AMN082 (25 and 75 pmol/0.5 µl/side) than those effective in the striatum were necessary to evoke an anticaeteleptic effect. AMN082 administered alone into the striatum (2.5 pmol/0.5 µl/side) or the SNr (2.5 and 25 pmol/0.5 µl/side) did not evoke catalepsy in rats (Figs. 3A, B).

The effect of systemic administration of AMN082 on the reserpine-induced akinesia in rats

Reserpine (2.5 mg/kg) administered in combination with α-MT (125 mg/kg) induced strong akinesia in rats, measured as a marked decrease in the distance travelled and the ambulation time, and as a distinct increase in the resting time (Fig. 4). AMN082 given in doses that effectively attenuated the haloperidol-induced catalepsy (1 and 3 mg/kg) did not change any parameters measured. AMN082 (3 mg/kg) given alone had no influence on locomotor activity in comparison with control rats. Since AMN082 given systemically did not affect any akinesia symptoms, the effects of local administration were not examined in the reserpine model.

Discussion

In the present study, we used rat haloperidol and reserpine models of PD to investigate potential antiparkinsonian effects of the AMN082-induced selective mGluR7 activation. It was found that AMN082 attenuated the haloperidol-induced catalepsy after both systemic and local administration. In contrast, AMN082 was not effective in reducing the reserpine-induced akinesia following systemic administration. These data are consistent with our earlier findings showing anticaeteleptic effects of the non-subtype-selective group III mGluR agonist ACPT-1 given into rat striatum, GP or SNr [17], and with other authors studies demonstrating antiparkinsonian-like effects of group III agonists, given systemically or intracerebrally, in various animal PD models [2, 3, 22, 40].

The potent anticaeteleptic effect of AMN082 (1 and 3 mg/kg) observed in our study is in line with the observations of Greco et al. [10], who found a similar effect after oral administration of AMN082. Although we did not confirm our findings using knock-out models, it was shown that the anticaeteleptic effect of AMN082 was absent in mGluR7 knockout mice [10], which clearly demonstrates involvement of the receptor in these effects. In contrast, the higher dose of AMN082 (10 mg/kg) used in our study was not effective in attenuating catalepsy and it decreased locomotor activity in both wild-type [31] and knock-out mice [30]; hence other mechanisms, unrelated to mGluR7, may actually participate in these effects. Direct inhibition of dopamine (DA) nigrostriatal transmission can be excluded, since AMN082 at doses up to 20 mg/kg failed to alter extracellular DA levels in the striatum or nucleus accumbens [19, 20]. On the other hand, AMN082 is rapidly metabolized to a major metabolite, N-benzhydrylethane-1,2-diamine (Met-1), in rat liver [40]. Although Met-1 shows appreciable affinity for the serotonin transporter (SERT), DA transporter (DAT) and norepinephrine transporter (NET) (323, 3020 and 3410 nM, respectively) [40], involvement of SERT and NET blockade in the beneficial effect of AMN082 seems doubtful, given that selective SERT and NET inhibitors fail to reverse locomotor deficits in MPTP-treated marmosets [11]. In contrast, selective DAT inhibitors abolish akinesia in this model [11, 33]; however, since the affinity of Met-1 for DAT is ca. 10 times lower than for SERT, a potential benefit of DAT inhibition may be masked by the effects induced by SERT blockade. Furthermore, DAT inhibitors are known to elevate extracellular DA levels in the striatum and to potentiate locomotion [13, 26]. Since AMN082 is unable to increase either striatal DA release [20] or locomotion (our study), its effect
related to DAT inhibition also seems questionable. Moreover, it should be stressed that peak concentrations of AMN082 in the brain were observed at 30 min after treatment [39], i.e., when the anticaudaleptic effect was the strongest. Given that the brain levels of AMN082 and Met-1 were similar at that time [39], and that the agonistic potency of AMN082 at mGluR7 was about 100-fold higher than that of Met-1 (EC50 of 66–81 nM and 5936 nM, respectively) [25, 39], these findings strongly suggest that AMN082 is responsible for the above-described effect. However, it cannot be excluded that the decrease in locomotion after doses of AMN082 higher than those effective in attenuating catalepsy, or the antidepressant-like effects visible a few hours after AMN082 administration [31] may be off-target effects (e.g., they may be due to the action of Met-1 on SERT or NET).

The anticaudaleptic effects of AMN082, observed after local administration of the compound, strengthen the hypothesis that striatal and nigral mGluR7 contribute to the effect of the systemically administered AMN082. In fact, mGluR7 is the most abundant in the striatum [18, 24], being located mostly on terminals of the glutamatergic corticostriatal pathway where its activation may inhibit glutamate release. Interestingly, the U-shaped dose-dependence and the inefficiency of higher doses of AMN082 to attenuate catalepsy have been observed after both systemic and intrastriatal injection of the compound, which is generally attributed to the receptor desensitization due to prolonged agonist exposure. In fact, several studies with orthosteric agonists of group III mGluR have shown similar effects [2, 40, 41]. Hence, our findings confirm a previous in vitro study [34] that AMN082 may produce potent mGluR7 internalization. This may be related to the ability of AMN082 to fully activate mGluR7 through an allosteric site [25] in contrast to most allosteric modulators which do not directly activate the receptor but rather enhance the effects of endogenous agonists. These results may be of basic clinical significance, since they suggest that long-term treatment with mGluR7 agonists, even those acting via an allosteric site, may decrease their effectiveness. Surprisingly, the effective anticaudaleptic doses of the intrastriatally administered AMN082 (2.5 and 7.5 pmol/0.5 µl/side) were much lower than its effective doses (0.1 and 0.5 nmol/0.5 µl/side), as reported by Greco et al. [10]. Such a discrepancy may be due to the higher dose of haloperidol used by the latter authors. Furthermore, some differences were observed in the application site of AMN082, i.e., the rostral part of the ventrolateral striatum in our study vs. the more caudal dorsolateral area in the experiment of Greco et al. It is noteworthy that 6-hydroxydopamine (6-OHDA) administered into the ventrolateral striatum dramatically increased the response initiation time in a lever pressing paradigm even in animals with mild DA depletion [9], which may suggest that the examined region is particularly responsible for such deficits.

Outside the striatum, immunocytochemical studies gathered evidence for the presence of mGluR7 on terminals of GABA-ergic striatopallidal or glutamatergic subthalamonic projections [5, 18] which seem to be hyperactive in the parkinsonian condition. Hence, activation of pallidal and nigral mGluR7 should decrease parkinsonian-like symptoms by reducing GABA or glutamate release, respectively. On the other hand, activation of mGluR7 localized on terminals of the GABAergic striatonicral pathways [18], whose activity seems to be decreased in PD, may counteract antiparkinsonian effects by stimulating the SNr [43]. Participation of the pallidal mGluR7 in antiparkinsonian-like effects seems rather doubtful, since AMN082 administered directly into the GP does not decrease the haloperidol-induced catalepsy in rats [10]. On the other hand, the existing studies with high doses of non-selective group III agonists suggest either anti-parkinsonian [2, 22] or pro-parkinsonian-like effects [21] of mGluR7 activation in the SNr. Hence, it is of vital importance to verify the results concerning participation of nigral mGluR7 in the beneficial effects of group III mGluR agonists in view of such conflicting reports.

It is likely that the SNr is very sensitive to any pharmacological manipulations, since even application of physiological saline to this structure may induce stereotyped behavior in rats [8]. In fact, the intensity of catalepsy in rats injected with the solvent into the SNr was twice as weak compared to the animals injected with the solvent into the striatum. On the other hand, the intranigral doses of AMN082 and ACPT-1 [17] effective in decreasing catalepsy were much higher than those efficient in the striatum. It is likely that the pro-parkinsonian-like effects of low doses (compared to the intrastriatal ones) of the intranigraly administered AP4 and ACPT-1, regarded as potentiation of motor impairment in the reaction-time task in 6-OHDA-treated rats [21], may be caused by the dominant influence on the striatonigral pathway.
In line with the above assumption, an electrophysiological study with high concentrations of L-AP4 showed that inhibition of glutamatergic transmission in the SNr required a higher concentration of L-AP4 than did reduction of GABAergic transmission in that structure [43]. Another cause of the discrepancy between the present and the above-mentioned study may lie in different PD models used in the two experiments, i.e., a haloperidol vs. a 6-OHDA model. It has been demonstrated that nigral tonic DA levels may modulate the function of presynaptic group III mGluRs in the SNr [44]. Importantly, DA depletion by reserpine or the blockade of DA receptors by haloperidol decreases the ability of group III mGluR agonists to attenuate the inhibitory GABA-ergic transmission, but not the glutamatergic one, in the SNr, which, in turn, may enhance the therapeutic effect of group III agonists in DA deficiency conditions. However, the 6-OHDA model used by Lopez et al. represents an early stage of PD, evoking only partial (50–60%) depletion of striatal DA [1]. In such conditions, the extracellular DA content in the SNr remains at a normal level [36]. In line with the above findings, potentiation of the motor impairment induced by intranigral administration of group III agonists was observed in both 6-OHDA-treated and sham-operated animals [21].

In contrast to the anticataleptic effect of the systemically administered AMN082, the compound was completely ineffective in reversing the reserpine-induced akinnesia. The main difference between these two models lies in the fact that reserpine (given in combination with α-MT) evokes strong muscle rigidity [16] and produces an almost complete DA depletion [45], while a moderate dose of haloperidol induces catalepsy related to deficits in limb movement initiation [12] rather than to muscle rigidity [29, 42]. In accordance with the above results, systematically administered AMN082 was found to reverse deficits in movement initiation in 6-OHDA-lesioned rats with a partial depletion of striatal DA in a reaction-time task [1]. Since parkinsonian symptoms appear when 80% of the striatal DA is lost, it is unlikely that muscle rigidity should be produced at such a moderate level of DA depletion. Although we did not study the impact of local administration of AMN082 on the reserpine-induced akinnesia because of the lack of effects of this compound after systemic administration, a most recent study showed an antiakinetic effect of the intranigrally administered AMN082 in that model, reversed by the nonselective group III antagonist CPPG [7]. The above results also confirm the hypothesis that under conditions of DA deficiency, group III agonists may preferentially act on receptors localized on subthalamonomigral terminals. However, the extremely high effective doses of AMN082 used in the study (100–300 nmol) can activate other group III mGluRs, e.g., mGluR4. Indeed, in contrast to mGluR7, mGluR4 is fairly sparse at the striatonigral synapse [4, 18], which indicates that mGluR4 can more selectively modulate the subthalamonomigral pathway. Accordingly, studies with some more selective positive allosteric modulators of mGluR4 given systemically, icv or into the SNr demonstrated a potent antiakinetic effect in reserpine-treated rats [7, 23, 27]. However, it cannot be excluded that the lack of efficacy of AMN082 in the present study results from an almost complete DA depletion, since – in contrast to Broadstock et al. – we administered reserpine together with α-MT which further inhibits de novo DA synthesis. Besides, unlike haloperidol which inhibits fairly selectively DA receptors, reserpine also depletes other biogenic amines; hence, this model may reflect a late phase of the disease with more severe symptoms, when AMN082 can no longer be effective.

Summing up, our study demonstrates that AMN082 has a beneficial effect on the haloperidol-induced catalepsy, but not on the reserpine-induced akinnesia. It seems that the anticataleptic effect of AMN082 depends on the reversal of the impaired initiation of a movement rather than on the decrease in muscle rigidity. Our data imply that the effects of the systemically administered AMN082 are related to activation of both striatal and nigral mGluR7; however, intranigral injections require much higher doses of the compound to obtain a beneficial effect. It is also proposed that the off-target effects of the systemically administered AMN082 may be connected with its doses higher than anticataleptic ones, and with a longer period of time elapsing after its administration. On the other hand, since AMN082 can easily produce mGluR7 desensitization, long-term treatment with mGluR7 agonists may reduce their efficacy.

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