Protection but maintained dysfunction of nigral dopaminergic nerve cell bodies and striatal dopaminergic terminals in MPTP-lesioned mice after acute treatment with the mGluR5 antagonist MPEP

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Abstract

The mGluR5 antagonist MPEP was used to study the role of mGluR5 in MPTP-induced injury of the nigrostriatal DA neurons. The findings indicate that acute blockade of mGluR5 may result in neuroprotective actions against MPTP neurotoxicity on nigral DA cell bodies and striatal DA terminals using stereological analysis of TH immunoreactivity and microdensitometry. Biochemical analysis showed no restoration of DA levels and metabolism indicating a maintained reduction of DA transmission.

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In a previous paper [2] it was reported that icv injections of the group I metabotropic glutamate receptor (mGluR) antagonist (RS)-1-aminoindan-1,5-dicarboxilic acid (AIDA) counteracted the nigral dopamine (DA) nerve cell loss in the C57bl/6 mouse produced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment. It was therefore of interest to examine whether mGluR5 could mediate these neuroprotective actions using the mGluR5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) [6]. Previously methamphetamine neurotoxicity on DA terminals was partially counteracted by mGluR5 antagonists [4]. Recently mGluR5 knockout mice have been shown to be less sensitive to MPTP-induced neurotoxicity than wild type mice [3]. In the present paper both icv and ip treatment with MPEP has been tested for neuroprotective actions against the MPTP-induced loss of the nigral tyrosine hydroxylase-immunoreactive (TH-IR) nerve cell bodies and of striatal TH-IR nerve terminals, as well as against MPTP-induced DA depletion in the substantia nigra (SN) and the striatum.

Sixty male C57bl/6 mice were used. MPTP hydrochloride (Research Biochemical, USA) in saline was given sc (40 mg/kg). MPEP (Tocris-Cookson, UK, and Glax-
oSmithKline, UK), in 0.1 M PBS pH 7.4, was administered icv (10 nmol per animal in 2 μl) 15 min before MPTP administration, using a chronic cannula in the brain right lateral ventricle. In the icv-injected mice the brains were analyzed 10 days after MPTP with TH immunohistochemistry [2] (for stereological analysis [7] and microdensitometry [1]). MPEP (20 mg/kg) was also administered acutely ip alone or 15 min before the MPTP treatment [6]. The brains of these mice were analyzed both by TH immunohistochemistry and by biochemistry (HPLC with electrochemical detection) 10 days after MPTP treatment. The mice were divided into four groups: (1) saline; (2) MPEP; (3) saline plus MPTP; (4) MPEP plus MPTP. The total number of three different types of cell populations of the entire SN (TH-IR nerve cells, non-TH-IR nerve cells with cresyl violet (CV)-stained Nissl substance, and non-neuronal nuclei) and volume fraction estimations for TH-IR cell bodies were estimated by means of the optical fractionator [7]. The experiment was approved by the local ethical committee (Stockholm Norra Försöksdjurs Etiska Kommittee).

Acute ip and icv treatment with MPEP fully counteracted the MPTP-induced loss of TH-IR and CV counterstained nigral nerve cell bodies without influencing by itself the

![Graph](image)

**Fig. 1.** Effects of MPEP ip (20 mg/kg) (A) or MPEP icv (10 nmol in 2 μl per animal) (B) on MPTP (40 mg/kg sc)-induced reduction of the total number of TH-IR nerve cells on one side of the mouse substantia nigra as evaluated on day 10 after MPTP. In all sections the TH-IR nerve cells were counterstained with cresyl violet (CV). Average total number of TH-IR nerve cells in each experiment group is shown. One-way ANOVA and the Newman–Keuls post hoc test (mean ± SEM, n = 6–8; *P < 0.05; **P < 0.01; ***P < 0.001), CE group value = coefficient of error for the estimated total cell number in each group. (C–F) Photomicrographs from the substantia nigra showing the effects of ip treatment with MPTP (D), MPEP (E), and MPTP+MPEP (F) on TH-IR in DA nerve cells compared with the saline group (C). Crossed arrows indicate dorsal and medial direction in the four panels. Scale bar is 250 μm.
total number of TH-IR and CV-positive nerve cell bodies as evaluated 10 days after the MPTP treatment (Fig. 1A–F). None of the treatments had any influence on the volume of remaining nigral TH-IR nerve cell bodies or the total number of other cell types or nuclear profiles (data not shown).

Acute ip treatment with MPEP partially counteracted the marked MPTP-induced disappearance of total TH-IR in the dorsal striatum as evaluated by microdensitometry (Fig. 2A). MPTP-induced disappearance of total TH-IR in the dorsal striatum was in contrast unaffected by the prior acute icv treatment with MPEP. However, the icv treatment with MPEP alone produced a significant loss of striatal TH-IR by 34% (Fig. 2B).

MPTP produced only a small non-significant depletion by 14% of nigral DA levels that were further significantly reduced by the acute MPEP treatment (Fig. 3A) and MPTP induced only a small non-significant nigral DOPAC and HVA disappearance that became significant after MPEP pretreatment (Fig 3B). The significant decrease in nigral DA turnover [(DOPAC + HVA) / DA] produced by MPTP treatment alone (\( P < 0.001 \), ANOVA) was not influenced by the MPEP pretreatment. MPEP treatment alone also resulted in a highly significant reduction of nigral DA turnover (Fig. 3C).

The acute MPEP treatment had no significant effect on the MPTP-induced striatal DA, DOPAC, and HVA depletion (Fig. 3D). MPEP alone caused a significant reduction of the striatal DOPAC and HVA levels and of striatal DA turnover (Fig 3E–F) with no effects of MPEP on striatal DA turnover in the MPTP-treated group.

The major finding in the present study was that acute icv and ip treatment with the mGluR5 antagonist MPEP resulted in a full neuroprotective effect on the total number of TH-IR nerve cell bodies in the SN and a partial maintenance of TH-IR in the DA nerve terminals in the dorsal striatum of the MPTP-treated mice after systemic MPEP treatment. With the stereological analysis it was clearly shown that a normal number (25,000 to 30,000) of DA cells was found in the entire SN after the combined MPEP + MPTP treatment (full neuroprotection) in contrast to previous biased cell counts from certain nigral sections showing only a weak neuroprotection with MPEP [3]. A demonstration of survival of cells is only possible when a method is based on a solid and unbiased collection of numerical data on cell bodies as made in the stereological analysis.

The biochemical analysis in contrast shows that the neuroprotective action of acute ip MPEP treatment on MPTP-lesioned nigral DA nerve cell bodies is associated with a further unexpected reduction of DA levels as well as of nigral DOPAC and HVA levels with no change of nigral DA turnover. This may be explained in part by assuming an MPEP-induced reduction of phosphorylation of TH at Ser 31 and Ser 40 by extracellular regulated kinase (ERK1/2) and PKA-dependent mechanisms, leading only to a weak activation of TH and reduced DA synthesis [8].

One important feature of the neuroprotective action of MPEP treatment is that MPTP-induced neurodegeneration of nigrostriatal DA neurons is fully counteracted in the SN but was only partially but significantly effective in the dorsal striatum with ip MPEP as seen from the microdensitometric determination of striatal TH-IR. However, the MPTP-induced reduction of striatal DA levels was not counteracted by acute ip MPEP treatment nor the MPTP-induced disappearance of striatal DOPAC and HVA levels. The present results indicate that like the DA cell bodies the striatal DA terminal systems are protected but still dysfunctional after MPEP and MPTP cotreatment with reduced DA synthesis and metabolism probably due in part to reduced TH activity (see above and Ref. [8]). The significant and long lasting reduction of the striatal DOPAC and HVA levels and DA turnover by MPEP alone would be in agreement with this view. It may also be that the remaining dysfunction in the striatal

Fig. 2. Semiquantitative analysis of total TH immunoreactivity (TH-IR) in the dorsal striatum. Effects of MPEP ip (20 mg/kg) (A) or icv (10 nmol in 2 μl per animal) (B) pretreatments on MPTP (40 mg/kg sc)-induced reduction of total striatal TH-IR as evaluated on day 10 after MPTP. Total TH-IR was calculated as OD × FA and is given in arbitrary units. The sampled field area was the entire dorsal striatum at the 0.74 mm bregma level. OD: optical density; FA: field area; sampled area: 2 ± 0.27 mm². (**\( P < 0.001 \); *\( P < 0.05 \); one-way ANOVA with intergroup differences analyzed by Fisher’s Protected Least square Difference (PLSD) test, corrected by Bonferroni’s procedure, \( n = 6–8 \)).
DA terminals after MPEP and MPTP cotreatment is related to a mGluR5-mediated reduction in the activity of the DAT carrier [9], and thus the blockade of mGluR5 by MPEP may increase the DAT activity enhancing MPP⁺ uptake and thus excitotoxicity. Furthermore, the mGluR5 located on the astroglia [5] may activate the astroglial trophism, which is essential for the survival of DA terminals after MPTP injury. It may therefore be difficult to develop mGluR5 antagonists for the neuroprotective treatment of Parkinson’s disease.

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References


