Group I mGluR antagonist AIDA protects nigral DA cells from MPTP-induced injury

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The effects of i.c.v. injection of AIDA, a group I mGluR antagonist, were studied on the nigral DA cells after MPTP-induced injury in the black mouse, using TH immunocytochemistry and unbiased stereology. MPTP reduced the total number of TH-IR neurons by 55.2% and non-TH-IR neurons by 27.5%. A 15 min AIDA pre-treatment (10 nmol) selectively counteracted the loss of TH-IR cells caused by MPTP as evaluated 10 days after the insult without changing the total number of non-neuronal cell nuclei. The results suggest that group I mGluR antagonists may have a neuroprotective role against MPTP-induced degeneration of DA neurons and thus probably also against neurodegenerative processes occurring in Parkinson’s disease. NeuroReport 12:2615–2617 © 2001 Lippincott Williams & Wilkins.

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INTRODUCTION

A number of publications indicate that group I metabotropic glutamate receptors (mGluR1 and mGluR5) can have a role favoring both neurotoxicity and neuroprotection [1]. It is therefore of substantial interest to study the trophic role of the group I mGluR demonstrated in the nigral dopamine (DA) neurons [2–4]. In vitro studies have previously shown that mGluR group I mediates slow postsynaptic depolarization in rat midbrain DA neurons [5] and an inward current independent of calcium mobilization [6]. Therefore, in the present study the effects of the group I mGluR antagonist (RS)-1-aminoindan-1,5-dicarboxylic acid/UPF 523 (AIDA) [7,8] have been studied on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced nigral DA nerve cell degeneration [9,10]. This compound has previously been shown to protect against ischemic neuronal death in vitro and in vivo [11–13].

MATERIALS AND METHODS

Animals: Twenty-seven specific-pathogen-free male C57bl/6 mice (10 weeks old, body weight 25g) were purchased from B and K Universal (Sollentuna, Sweden). The mice were kept under standardized housing conditions (relative humidity 65–75%, temperature 21–24 °C) with a 12:12 h light-dark cycle (lights on at 06:00h) and free access to tap water and food pellets. MPTP-HCl (Research Biochemical, Natick, MA USA) dissolved in 0.1 M phosphate buffer (PB) pH 7.4, was administered intracerebroventricularly (i.c.v.; 10 nmol/animal in a volume of 2 μl) [7] 15 min before MPTP administration, using a chronic cannula (bregma +0.5 mm; lateral to the midline 0.5 mm; dorso-ventral −2.0 mm from the cortex). The mice were divided into four groups: (1) solvent (n=6); (2) AIDA (n=7); (3) solvent plus MPTP (n=7); (4) AIDA plus MPTP (n=7). All MPTP-treated animals were protected from hypothermia and received i.p. a solution of isotonic saline and 5% glucose (1 ml, twice daily).

Immunocytochemistry and unbiased stereology: After 10 days all animals were anaesthetised with sodium pentobarbital (100 mg/kg, i.p.) and intracardially perfused with 25 ml ice-cold 0.9% NaCl followed by 150 ml fixation fluid (4°C) for 6 min. The fixative consisted of 4% paraformaldehyde (w/v) and 0.2% picric acid (w/v) solution in 0.1 M sodium PB (PBS; pH 7.4). The brains were dissected out, postfixed for 2 h in the same fixative and cryoprotected in sucrose (10% for 24 h followed by 30% for 1 week, at 4°C). The midbrain was cut into coronal, serial 40 μm sections on a cryostat (Micron, HM 500 M, Walldorf, Germany). With a random start, every sixth section (f=6: the numerical fraction of the sections used for the stereological analysis) through the entire substantia nigra (SN; levels: bregma −2.54 to −4.04 mm) was sampled into three series of sections. The sections were processed free-floating. Endogenous peroxidase activity was removed by incubating the
sections with 0.3% H₂O₂ for 10 min. After blocking with 1% normal goat serum (Sigma, St. Louis, MO; 40 min, room temperature) the sections were incubated with a mouse monoclonal IgG1 antibody to rat PC 12 tyrosine hydroxylase (TH; Incstar, Stillwater, MI) diluted 1:1500 (4°C, overnight) in 0.1M PBS containing 0.3% Triton X-100 (Sigma, St. Louis, MO). The immunostaining was performed according to the ABC method using the Vectastain kit (Vector, Burlingame, CA). The chromogen used was 0.03% 3′,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) and 0.03% fresh H₂O₂ in 0.05M Tris–HCl (pH 7.6). Sections from all animals were processed simultaneously. After mounting on gelatin–chromium coated slides, the sections were counterstained with cresyl violet (CV; Sigma, St. Louis, MO), dehydrated and coverslipped with Mountex (Histolab, Gothenburg, Sweden). Nucleoli and Nissl substance were stained with CV without interfering with the brownish TH immunostaining. Every mounted section (from one series) was numbered following the rostrocaudal level corresponding to the mouse brain atlas [14] and was used for mean volume and cell number estimations. The total number of three cell populations of the SN–TH-immunoreactive (IR) nerve cells, non-TH-IR nerve cells with stained Nissl substance, and non-neuronal nuclei labeled by CV–were estimated by means of the optical fractionator, which combines the optical dissector [15] with a fractionator sampling scheme [16]; volume fraction estimations for TH-IR neurons were computed by applying the Cavalieri method based on point counts obtained during the application of the optical fractionator. An Olympus BH2 microscope (Olympus, Denmark) was interfaced with a computer (DGC systems, Stockholm, Sweden) and a color video camera (CCD-iris, Sony, Japan). The CAST-Grid software package (Olympus, Glostrup, Denmark) generated sampling frames with a known area and directed the motorised X-Y stage (Lang, Huttenberg, Germany), and a microcator (MT12, Heidenheim, Germany), which monitored the movements in the Z-axis with a resolution of 0.5 μm. One side of the entire SN (zona compacta, zona lateralis and zona reticulata) was defined using a ×4 objective. After having counted the objects (Σ Q−), the total number of TH-IR, non-TH-IR, and non-neuronal cells in the nigral region was then estimated as: N = ΣQ− × fs × fa × fb [16], where fs is the numerical fraction of the section used, fa is the areal fraction and fb is the linear fraction of section thickness [17]. The coefficient of error (CE) for each estimation and animal ranged from 0.05 to 0.1. The total CE of each group [16] ranged from 0.05 to 0.1. More complete details on these methods can be found elsewhere [10,16,18,19].

**Mean cell volume estimation:** The sampling grids that were laid systematically by the computer over the sectional profiles of the SN for the cell counting created a virtual lattice of points that was used for obtaining unbiased estimates of volume, applying the principle of Cavalieri [16]. This procedure provided individual point counts intercepting TH IR neurons that were used for estimating volume. The mean CE ranged between 0.05 and 0.10.

A one-way ANOVA with Newman-Keuls post-hoc test was applied for statistical significance between groups using GraphPad 2.01 Software.

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**RESULTS**

**Neuron and non-neuron number estimation:** Staining with CV allowed the visualisation of neuronal nucleoli, and the nuclei of non-neuronal cells. These varied in shape and staining, ranging from round and blue pale to irregular and pyknotic nuclei. The location of nucleoli (nerve cells) or nuclei (non-neuronal cells) within the height of the dissector and into the counting frame was used as criteria for counting [20].

**TH-IR and CV counterstained nigral nerve cells:** The main results from the stereological analysis are presented in Fig. 1. A marked reduction of the total number of TH-IR nerve cells was produced by the MPTP treatment 10 days after the injury. This action was significantly and strongly counteracted by the i.c.v. AIDA (10 nmol in 2 μl) treatment 15 min prior to the MPTP exposure. I.c.v. AIDA pre-treatment alone had no effect on the total number of nigral TH-IR nerve cells. As seen in Table 1, the MPTP-induced injury did not change the volume of the surviving DA cell bodies, nor did AIDA alone or in combination with MPTP.

**Non-TH-IR and CV counterstained nigral nerve cells:** As seen in Table 1, the total number of CV-only-stained nigral nerve cells was reduced by MPTP treatment, but not significantly. The pre-treatment with AIDA (10 nmol in 2 μl, i.c.v.) failed to modulate this decrease in the total number of non-TH-IR nigral nerve cells.

**Non-neuronal cell nuclei stained with CV:** As seen in Table 1, the MPTP treatment did not affect the total number of non-neuronal cell nuclei with or without prior i.c.v. AIDA treatment.

**Tissue shrinkage:** Although the fractionator does not need any absolute measurements (or reference space, stage movement, etc.), and therefore is unaffected by dimensional changes of the tissue during processing, the last...
linear fraction of section thickness requires fair estimates of the final section thickness. In our study the mean observed thickness was 51% of the microtome setting when mounted (i.e. 20.6 µm for the section cut at 40 µm).

DISCUSSION

The current stereological methods are founded on rigorous designs of sampling protocols and measuring tools with strong mathematical and statistical support [16,19]. When correctly applied, these methods guarantee the practical elimination of all sources of systematic error (bias) from the counting or measuring design, permit evaluation of the precision of the estimates obtained from each individual case, and exponentially increase the overall efficiency in the quantitative procedure [17].

The present results give evidence based on the unbiased stereological analysis that the group I mGlur antagonist AIDA given i.c.v. can protect the nigral DA nerve cells against the MPTP-induced injury in line with a possible involvement of glutamate in MPTP-induced toxicity. It is likely that these findings represent a protection against cell death but it is possible that some of the nigral DA neurons lacking TH-IR after MPTP treatment may only loose their capacity to express TH-IR [20,21], a property that may be restored by the AIDA treatment.

The neuroprotective action of AIDA appeared to be preferential for the nigral DA nerve cells, since the non-significant reduction of non-TH-IR nerve cells was not altered by the AIDA pre-treatment. In a previous study [10], this action of MPTP on non-TH-IR nerve cells was significant. It seems possible that the neuroprotective action of AIDA is related to the blockade of mGlur1 and mGlur5 isoforms demonstrated in the nigral DA nerve cells [2,4] operating mainly via increases of intracellular Ca²⁺ and protein kinase C activation, but also via regulation of voltage-sensitive Ca²⁺ channels and inducing slow depolarization of neurons. It has also been indicated that reactive astrocytes, as occurs after MPTP treatment, can express mGlur5 receptors enhancing the release of a neurotoxic factor, possibly nitric oxide contributing to neuronal degeneration [1]. It is of substantial interest that in vitro studies have shown recently that group I mGlur, when activated, increases necrotic cell death but reduces neuronal apoptosis [22]. If this is also true in vivo it opens up the possibility that MPTP-induced injury involves, to a major degree, necrotic DA cell death in the DA nerve cells that undergo degeneration.

CONCLUSION

Taken together, the present paper provides indications that in the MPTP mouse model of Parkinson’s disease, group I mGlur located in nigral DA nerve cells may be involved in MPTP-induced DA nerve cell death. Antagonists of group I mGlur, like AIDA, may therefore have a neuroprotective potential in Parkinson’s disease by counteracting DA nerve cell neurodegeneration.

REFERENCES


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