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Noradrenergic loss enhances MDMA toxicity and induces ubiquitin-positive striatal whorls

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Abstract Movement disorders involve a number of neurodegenerative conditions, mostly affecting basal ganglia. Parkinson's disease (PD) is classically defined by the selective loss of dopaminergic neurons in the substantia nigra pars compacta. Administration of specific neurotoxins represents a common tool to reproduce this lesion. Among these, amphetamine derivatives act as powerful monoamine neurotoxins, impairing striatal dopamine (DA) axons in mice. Despite the well-investigated effects on striatal DA terminals, only sporadic studies have focused on the potential toxicity of amphetamines towards post-synaptic neurons within the striatum. In the present work we found that 3,4-methylenedioxymethamphetamine (MDMA) produces ultrastructural alterations in striatal cells, featuring as membraneous whorls, positive for ubiquitin and heat shock protein 70. These morphological alterations were enhanced in locus coeruleus-lesioned mice.

Most degenerative disorders involve the basal ganglia. Parkinson's disease (PD) is characterized by a selective loss of dopaminergic (DA) neuronal cells belonging to the nigrostriatal pathway [1].

Damage to striatal DA fibers can be produced either by microinfusing monoamine neurotoxins into the substantia nigra pars compacta (SNpc), or systemically administering specific DA neurotoxins like amphetamine derivatives [2]. These compounds are, unfortunately, largely abused by humans and produce a robust effect on the DA system, increasing at first striatal DA release [3], so leading to the damage of the nigrostriatal DA pathway, possibly due to the production of free radicals and reactive oxygen species.

Recently, a number of experimental and clinical studies documented the importance of the locus coeruleus (LC) in modulating the progression of neurodegenerative disorders; concomitantly, reduction of noradrenergic (NA) neurotransmission in various brain areas innervated by the LC is found as a constant element of the pathobiochemistry of PD. Altogether, these findings strongly suggest that NA loss could have a specific role in sustaining the disease [4]. Among amphetamine derivatives, 3,4-methylenedioxymethamphetamine (MDMA) is well known for its neurotoxic effects against monoamine axons, while the effects in striatal GABA cells are less investigated.

In this study we evaluated, at the ultrastructural level, the effects of MDMA on striatal neurons of the mouse. In addition, we investigated the role of LC in modulating the occurrence of subcellular alterations in striatal perikarya.

Male C57 black mice (C57BL/6J) 9–10 weeks old were divided into five groups of 10 mice each. To one group we administered intraperitoneally the NA neurotoxin DSP-4 (50 mg/kg). Three days later, a subgroup of DSP-4 treated mice and a group of controls received MDMA hydrochloride (three administrations of 30 mg/kg at 2-h intervals). One week after MDMA administration, one group was sacrificed and the striatum was processed in order to measure catecholamines and their metabolites by HPLC as previously described [5].

Another group of mice was anaesthetized with chloral hydrate (4 ml/kg), perfused and processed for electron microscopy and immunocytochemistry. Thin sections of 50 nm were deosmicated in a saturated solution of Na-metaperiodate for 30 min, washed in PBS and incubated in the primary antibody (Ab-I) for 24 h at 4° C. We used Ab-I anti-ubiquitin and anti-heat shock protein 70 (HSP-70), diluted 1:100; secondary antibodies were conjugated with gold particles of 10 nm diameter. Measurement of striatal whorls was carried out by counting the number of nuclear or cytosolic whorls out of the total number of neurons under observation. The same procedure was followed for each animal from each different group of treatment. Data are given as percentage of whorls and they were compared using ANOVA with Sheffè's post-hoc analysis. Null hypothesis (H_o) was rejected when $p<0.05$.

Administration of MDMA to intact mice caused a significant decrease in DA level compared with controls. In contrast, striatal DA was not affected by DSP-4 treatment, whereas in LC-lesioned mice MDMA produced significantly more severe damage.

Ultrastructural observation revealed the occurrence of morphological changes within striatal cells of MDMA-treated mice. These alterations consisted in some multilamellar bodies (Fig. 1) and they were found both in the cytosol (12.13%±1.69%) and in the nucleus of striatal neurons (29.02%±2.04%). These whorls, which were only exceptional in the striatum of control animals, constantly appeared as very electron dense inclusions, composed by multiple, concentric membranes (Fig. 1) and were found more abundantly within the nucleus of striatal cells of DSP-4- and MDMA-treated mice (41.22%±2.45%). Immunocytochemical investigation showed that whorls were positive for ubiquitin and HSP-70.

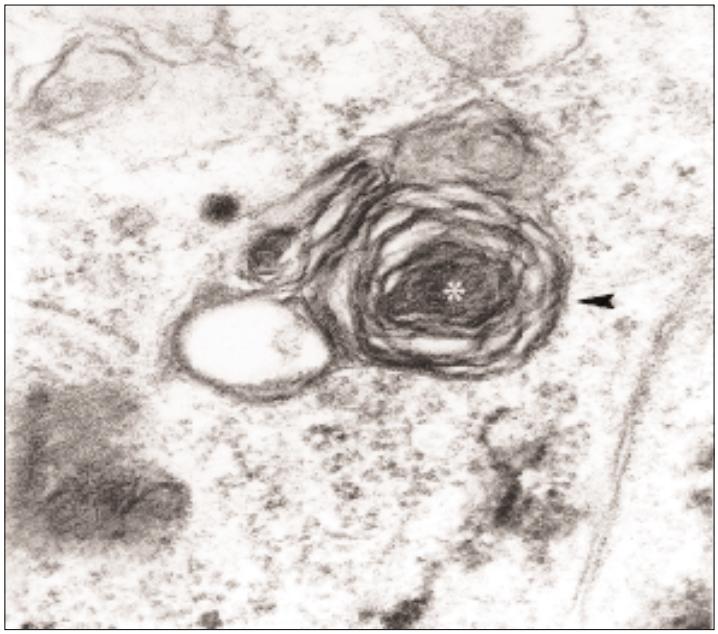

Fig. 1 Electron microscope micrograph of a cytosolic whorl within a striatal cell from MDMA-treated mouse. Multiple concentric membranes (*arrowhead*) and a more electron-dense core (∗) are evident

Discussion

This work points out the occurrence of ultrastructural alterations into striatal post-synaptic neurons following MDMA treatment, consisting of whorls of concentric membranes. Ubiquitin immunostaining suggests these formations to be functionally related to the ubiquitin-proteasome pathway, a cellular system which plays a pivotal role in proteolysis and which is involved in the regulation of cell cycle [6]. Our findings of ubiquitin-positive whorls in association with the smooth endoplasmic reticulum, as well as their positive staining for the stress-induced chaperone-like protein HSP-70, strongly support the hypothesis that they represent the morphological profile of an alteration of the ubiquitin-proteasome pathway.

Membranous whorls were described within neurons of specific brain areas in several pathological conditions; in PD typical neuronal inclusions, Lewy bodies (LB), consist of eosinophilic material found in the cytoplasm. Immunostaining shows ubiquitin as a prominent and constant component of LB. Lewy bodies are also present in other neurodegenerative disorders, such as diffuse LB disease and Alzheimer's disease.

Recently, mutant TorsinA, a novel protein identified as responsible for the early-onset torsion dystonia, has been found to stain LB [7]. TorsinA in human brain is mainly expressed in DA neurons of the SNpc; this protein, which contains an ATP-binding site, is thought to perform chaperone functions, involved in the identification of misfolded proteins and their refolding or degradation [7]. In this respect, our finding of HSP-70 immunostaining in striatal whorls might represent an example of a chaperone molecule recruited following oxidative damage induced by MDMA treatment.

In conclusion, we hypothesize that whorls represent, at the morphological level, an enhancement of a more general, physiological response of the cell to the production of altered proteins. In particular, the marked striatal DA release observed after MDMA administration, constantly associated with the production of highly reactive species, can oxidize large amounts of intracellular proteins. Moreover, a recent study [8] demonstrated that DA inhibits the proteasome pathway, making more critical the oxidative effects of DA on "proteasome-impaired" striatal cells.

References

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