SUBSTANTIA NIGRA PARS COMPACTA DOPAMINERGIC NEURONS SURVIVOR AFTER SYSTEMIC ADMINISTRATION OF PROTEASOME INHIBITOR MG-132 – MORPHOLOGICAL AND NEUROCHEMICAL CHARACTERISTIC

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Located in the midbrain, immediately dorsal to the cerebral crus, the substantia nigra belongs to the basal ganglia and is considered an important motor centre. Majority of the cells from substantia nigra pars compacta (SNC) produce dopamine. This neurotransmitter passes via axoplasmic flow to the nerve terminals in the caudate nucleus and putamen, two other components of the basal ganglia, which together form the dorsal striatum. Projections of dopaminergic cells from SNC regulate the activity of striatal cells and thus have a significant impact on locomotor activity. These nigrostriatal connection are thought to be the lesion site in Parkinson's disease. Our previous studies have shown that in the rats the number of SNC dopaminergic cells is significantly reduced after intraperitoneal administration of proteasome inhibitor MG-132.

The aim of this study were: (1) to describe morphological features of rat SNC neurons, that did not undergo degeneration after systemic, intraperitoneal administration of MG-132, and (2) to determine the expression of dopamine (DA) and tyrosine hydroxylase (TH) - enzyme necessary for DA synthesis (Fig.1), in those neurons in comparison to control.

10 adult male Wistar rats (initial weight between 230 and 270 g) were used in the study. All efforts were made to reduce the number of animals and to minimize their suffering.
The animals were randomly divided into two groups: control (n=5), which received intraperitoneal injection of 10% DMSO solution and (n=5) group of rats that received reversible proteasome inhibitor - MG-132 (0.5 μg/g body weight per dose, total of 7 doses) dissolved in 10% DMSO. Eight weeks after first MG-132 administration the rats were deeply anaesthetized, brains were perfused with 4% solution of paraformaldehyde and postfixed. Then the brains were cut into coronal sections. Immunohistochemical stainings with primary antibodies against: TH (MAB318, Millipore, USA; 1:500) and DA (Millipore, USA; 1:500) followed by appropriate secondary antibodies coupled with fluorescent dye were performed.
The high-magnification images were taken using a confocal laser scanning microscopy (CLSM) system (Radiance 2100, Bio-Rad UK) The CLSM images were analysed (including measured of the intensity fluorescent signal emitted by the SNC cells) with LaserSharp 2000 and LaserPix v. 2.0 software (both Bio-Rad, UK).
Data are expressed as mean ± standard deviation (SD). Differences were considered significant when $p<0.05$.

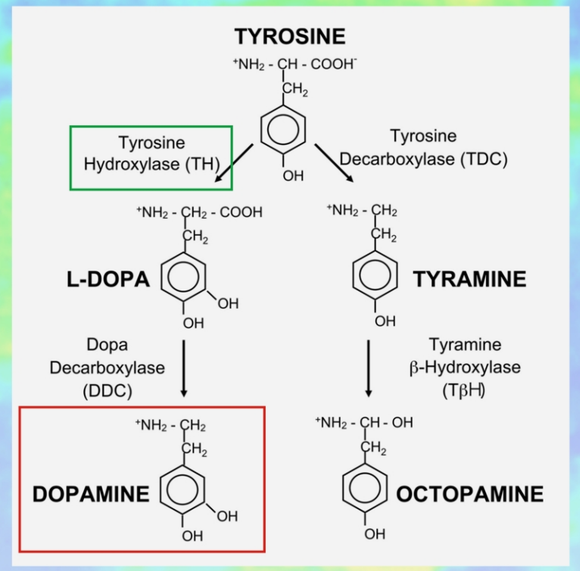

Fig.1.Chemical relationships and biosynthetic pathways linking derivatives of tyrosine: dopamine, tyramine, and octopamine. In order to synthesize dopamine, tyrosine is first converted to DOPA by tyrosine-hydroxylase, which is then decarboxylated by DOPA-decarboxylase to yield dopamine. This figure summarizes the most common synthesis pathways.

R E S U L T S

Results base on analysis of TH-ir SNC cells (control n=952 and MG-132 n=839) as well as DA-ir SNC cells (control n=218 and MG-132 n=222). Within the population of SNC TH-ir and DA-ir neurons almost identical qualitative changes were observed, namely significant differences in distribution of TH-ir and DA-ir cells profiles in control and MG-132 administered rats. In MG-132 rats the average area of TH-ir neurons was 16% ($p<0.05$)while DA-ir neurons was 18% ($p<0.01$) lower as compare to control (Fig. 2A,C; Fig.3A; Fig. 4A,C; Fig.5A). Detailed analysis showed that within the SNc of MG-132 administered rats there was a marked decrease of large (bigger than 200 μm2) TH-ir and DA-ir neurons.

Expression of DA and TH were measured on the basis of the intensity fluorescent signal emitted by the SNC cells. In rats treated with MG-132 cytoplasmatic intensity of TH was significantly lower (20%, $p<0.05$) as compare to the intensity of this enzyme in untreated control. Also the intensity of DA within cytoplasm of SNC neurons was significantly different between studied groups – but it was higher (19%, $p<0.05$) in MG-132 treated rats (Fig. 2B,D; Fig.3B; Fig. 4B,D; Fig.5B).

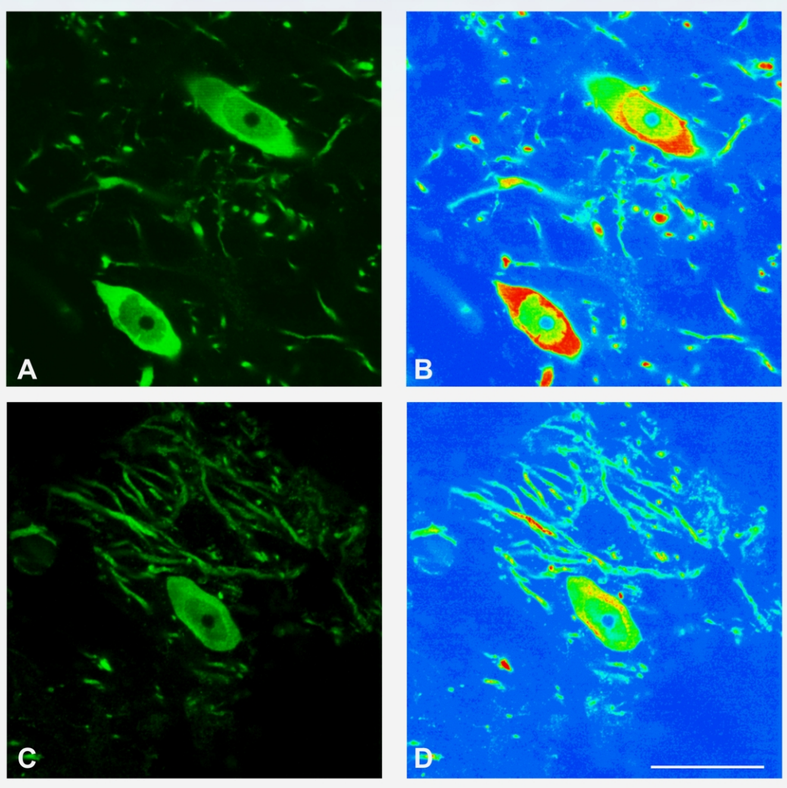

Fig. 2. CLSM images of SNC TH-ir cells in Control (A,B) and MG-132 (C,D). Intensity mask (B,D) – blue: very week signal/red: very strong signal) used to measure intracellular TH-ir intensity. Scale bar 25μm.

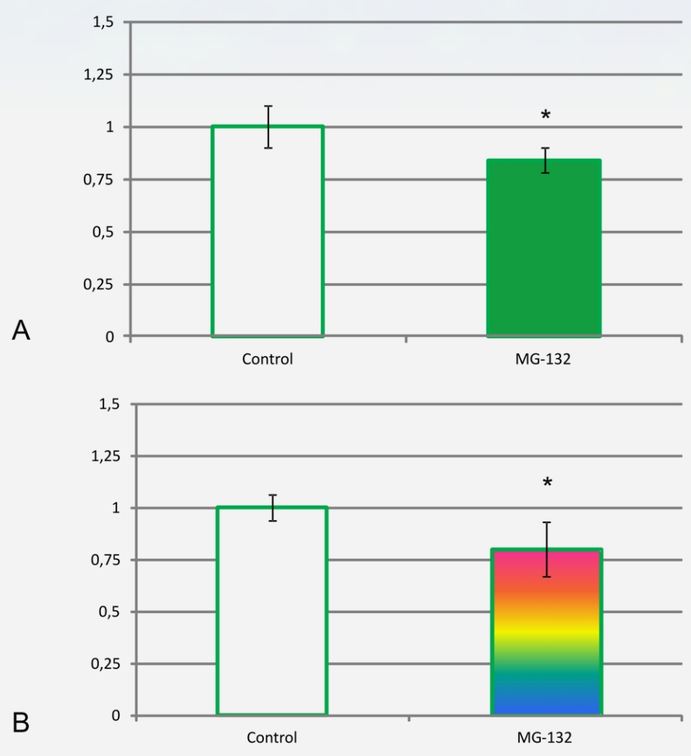

Fig. 3. Graphic illustration of comparison between Control and MG-132: (A) average area of TH-ir cell profile and (B) average intracellular intensity of TH-ir within SNC neurons.

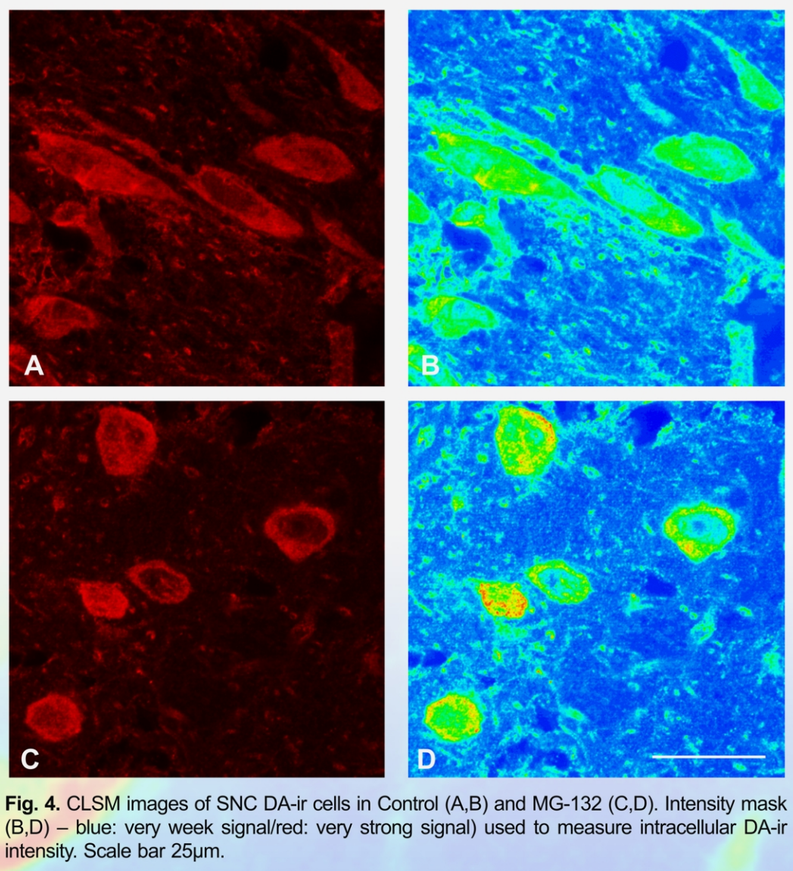

Fig. 4. CLSM images of SNC DA-ir cells in Control (A,B) and MG-132 (C,D). Intensity mask (B,D) – blue: very week signal/red: very strong signal) used to measure intracellular DA-ir intensity. Scale bar 25μm.

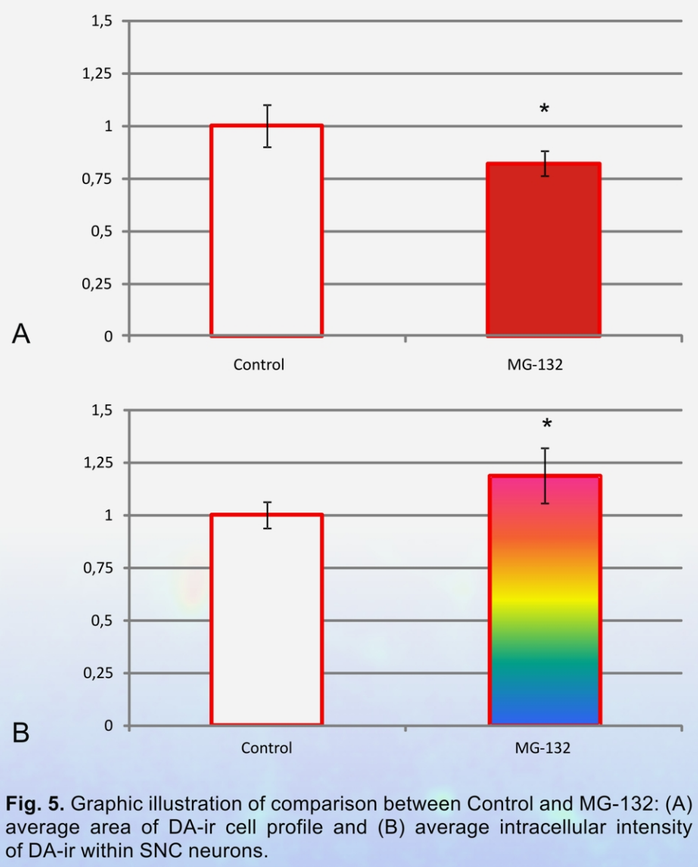

Fig. 5. Graphic illustration of comparison between Control and MG-132: (A) average area of DA-ir cell profile and (B) average intracellular intensity of DA-ir within SNC neurons.

C O N C L U S I O N S

Our results indicate that the decrease amounts of DA neurons in SNC, what mainly concern larger neurons, is accompanied by a decrease of TH intensity but increase of DA. Taking into the consideration previous results from other experimental models, indicating that the impairment of ubiquitin-proteasome system produces cell death selectively for DA-containing neurons that depend on the occurrence of endogenous DA, observed by us decrease of TH intensity in surviving SNC neurons may be a manifestation of DA synthesis limitations, designed to protect the SNC cell.