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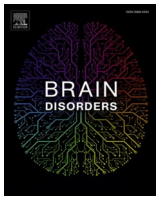

CAV2.3 expression is upregulated in the substantia nigra pars compacta of humans with Parkinson's disease

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Main

Selective degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) leads to the motor symptoms of Parkinson's disease [1]. The neighbouring dopaminergic neurons in the ventral tegmentum area (VTA) and other areas are relatively spared from degeneration in the early stages of the disease. The SNpc neurons are highly arborised axons with a persistent firing rate that impose a high cellular energetic demand [2,3]. These properties of the neurons stem from their differential gene expression profile [4,5]. The robust pacemaking activity of SNpc neurons, for example, is regulated by the voltage-gated Ca^{2+} channels, while that of the VTA is mediated by voltage-dependent sodium channels [6–8].

Pacemaker activity of SNpc neurons results in broad action potentials allowing Ca^{2+} influx through voltage-gated Ca^{2+} channels, possibly predisposing them to degeneration. These observations motivated a clinical trial of isradipine, the most potent of the clinically available dihydropyridine L-type calcium channel antagonist with excellent central nervous system penetration [9]. Amongst other reasons, the failure of this trial may have actually been due to the selectivity of the drug to the L-type channels, allowing currents through from other voltage-gated Ca^{2+} channels. For example, in SNpc neurons, Cav2.3 (R-type) channels account for 50% of Ca^{2+} flux into the soma [10,11]. Furthermore, Cav2.3 expression increases with age, unlike L-type calcium channels which decrease in an age-dependent manner [11–13]. Cav2.3 levels were also higher in rodent SNpc neurons compared with the VTA [11]. These spatio-temporal changes in Cav2.3 expression suggest a more significant role for R-type channels in the vulnerability of SNpc neurons than may have been recognised before the STEADY-PD III trial [9,14].

Here we examined the expression of CAV2.3 in the healthy and PD brains to motivate a more rational consideration of drugs targeting R-type channel contribution to neuronal degeneration. We quantitatively examined the distribution of Cav2.3 α1-subunit (CACNA1E) in tyrosine-hydroxylase-stained midbrain sections in 6 normal brains (2 males and 4 females; 79.7 ± 9.6 years) and 9 PD brains (5 males and 4 females; 75.0 ± 5.3 years; Supple. Table 1). In patients with Parkinson's disease, we found that Cav2.3 expression in SNpc neurons ($M = 26.20 \pm 1.22$ AFU arbitrary fluorescent units (AFU)) was significantly elevated compared with expression in the neighbouring VTA ($M = 16.20 \pm 0.75$ AFU). In control brains, however, there was no statistically significant difference in CAV2.3 expression amongst the dopaminergic neurons in either region (Fig. 1). Qualitatively, as noted in rodent studies [11,15], we confirmed that CAV2.3 is predominantly restricted to the soma and the proximal hillock region in human neurons.

Failure of the recent STEADY-PD III trial was predominantly attributed to a low dose of the dihydropyridine Isradipine (5.0 mg twice a day). This drug and the dose were selected based on tolerability (safety) and relative affinity for L-type calcium channels. However, drug underdosing as the primary cause of limited trial efficacy fails to consider the contribution of the other Ca^{2+} channel types to calcium overload and neurodegeneration. Recent studies in an animal model of PD suggest R-type channels as an important contributor to the selective vulnerability and degeneration of SNpc neurons [11]. Our results extend these observations to humans by showing that CAV2.3 expression is increased in the SNpc of patients with Parkinson's disease and seems to be located predominantly in the soma of the neurons, as seen in the animal studies. In light of these observations, one might argue that treatment of PD with a less selective calcium channel blocker, targeting both L- and R-type channels and aimed at maximal elimination of the calcium overload, would result in enhanced neuroprotection. For example, in large epidemiological studies, less selective dihydropyridines such as amlodipine and nifedipine have been shown to reduce the

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risk of Parkinson's disease by up to 30%. Of these, the one with broad coverage (for L- and R-type) and the highest known affinity for R-type channels is nicardipine (IC50 < 1.0 μM) [16,17]. This drug is particularly well-tolerated at high oral doses with an excellent safety profile [18]. In short, if the calcium channel neuroprotection hypothesis is correct in humans, then nicardipine seems the most likely of the existing dihydropyridines to prove it.

Methods

Subjects

Formalin-fixed, paraffin wax-embedded, slide-mounted brain sections from 6 control subjects (2 males and 4 females) and 9 PD patients (5 males and 4 females) were provided by the Parkinson's UK Tissue Bank at Imperial College (Supple Table 1). All subjects consented to donate their brains after death. The mean age of control individuals was years (range 66–88 years) and the mean age of PD patients was 79.7 ± 9.6 75.0 ± 5.3 years (range 69–83 years). Cases were selected based on pathology reports and were matched for age, disease duration (PD 10.5 ± 3.9 years) and postmortem interval of tissue (control: 37.3 ± 13.2 h, PD: 24.2 ± 11.4 h). Clinical diagnosis of PD was confirmed by neuropathological analysis. Control cases demonstrated no PD pathology, with clear pigmentation in the SNpc, and did not meet diagnostic criteria for PD or Alzheimer's disease (AD) apart from 1 control case (CO4) which was clinically diagnosed with AD. PD cases had prolonged PD, characterised by motor and cognitive impairments, such as depression and mood disturbances (Mallach et al., 2019). The SNpc was pale, indicating DA neuron loss, and Lewy Bodies were present. All of the PD cases had Lewy Body pathology confined to the brainstem. AD-like pathology was seen in 2 PD cases. During neuropathological analysis, the grade of α-synuclein pathology was rated and cases were chosen with an α-synuclein Braak stage of 3, representing early PD (Braak et al., 2003).

Immunohistochemistry

Immunohistochemistry, image acquisition and analysis were performed according to our published protocol [19]. Briefly, heat-induced epitope retrieval was performed on 10 μm thick formalin-fixed paraffin-embedded sections by bathing the slides in ethylenediaminetetraacetic acid buffer (1 mM EDTA, pH 8) and heated. The slides were then blocked at room temperature with blocking buffer (2% goat serum, 0.3% triton in PBS) for 1 h and incubated overnight at room temperature with chicken anti-tyrosine hydroxylase (1:100, RayBiotech, USA) and rabbit anti-CAV2.3 (1:50, Abbkine Scientific, USA) antibodies. Secondary antibodies 1:1000, (Santa Cruz Biotechnology, USA) were applied for 1 h at room temperature. The slides were blocked with Sudan Black (0.1% Sudan Black, 70% ethanol) for 10 min and washed for 10 min with water. The nuclei were stained by incubation in 4′,6-diamidino-2-phenylindole (DAPI, 0.5 μg/ml) solution for 10 min. The tissue was mounted using Vectashield (Vecta Laboratories, UK).

Image acquisition and analysis

Images were acquired by using a Leica DMi8 Microscope and Leica DMi8 and the Leica application suite X (Leica Micro-systems, Wetzlar, Germany) and a 40x oil-based immersion objective. *Z*-stacks of at least one randomly selected area from SNpc and VTA were acquired, with an interval of 0.27 μm along the *z*-axis. *Z*-stacks were averaged and quantitative analysis of fluorescence intensity was performed using the elliptical selection tool in ImageJ (National Institutes of Health, Maryland, USA). 10 randomly selected areas within each tyrosine hydroxylase-positive neuron were used for quantification. The parameters of selection tool were kept constant throughout. Average signal intensities of the fluorescent antibody in individual TH+ SNpc and VTA neurons were determined using ImageJ software and recorded in Excel. Values were expressed as the mean ± standard error of the mean (S.E.M.). The statistical analysis was performed using Graphpad Prism. Means were determined for each of the groups (CTL, PD) and the resulting values were subjected to one-way ANOVA followed by Tukey HSD post-hoc comparisons.

Author contributions

PS conducted the experiments, analysed the data, and wrote an initial draft of the manuscript. HB assisted with immunohistochemistry and microscopy and commented on the manuscript. DG and SG prepared the postmortem brains and patient reports. KNA and PS designed the study. KNA and TST wrote and revised the manuscript with critical input from PJSS. KNA provided oversight for the work and assurance of data integrity.

Ethical approval

Parkinson's UK Brain Bank brain donations and research protocols have been approved by the relevant Research Ethics Committee.

CRediT authorship contribution statement

Parnaz Sharifi: Investigation, Formal analysis, Writing – original

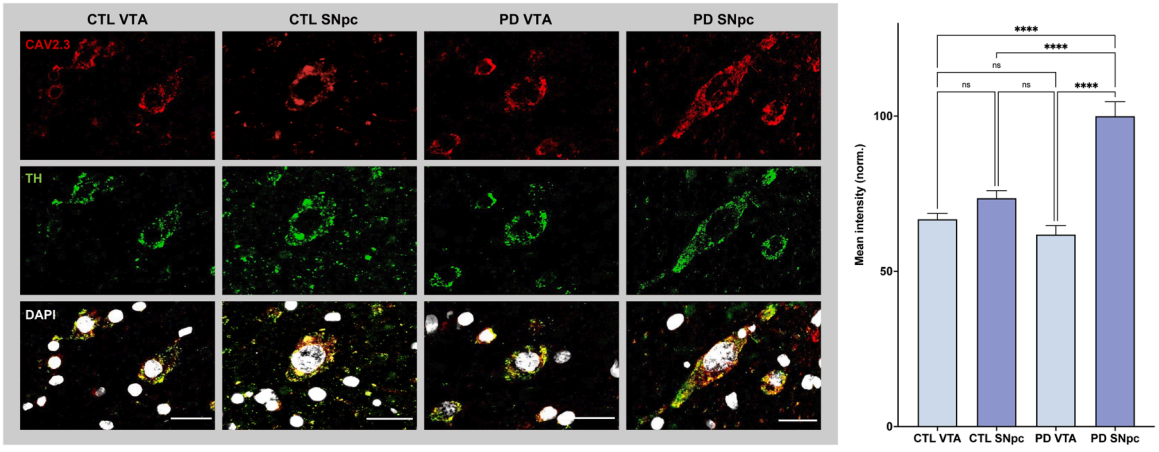

Fig. 1. Relative expression of CAV2.3 Parkinson's disease and control brains. Immunolabelling of CAV2.3 in tyrosine hydroxylase-positive neurons of the VTA and SNpc of control (A,B) and PD patient (C,D) sections. Relative mean intensity of CAV2.3 signal in ventral dopaminergic neurons of control (CTL) and PD human brains (E). All values have been normalised against the mean for PD SNpc group. Scale bars: 25 μm. NS, not significant. **** for $p < 0.0001$ (one-way ANOVA).

draft, Visualization. **Haesoo Bae: . Djordje Gveric:** Resources. **Steve M Gentleman:** Resources. **Peter JS Smith:** Conceptualization. **Travis S Tierney:** Writing – review & editing. **Kambiz N. Alavian:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. No author has any financial or in-kind conflicts of interest related to this work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dscb.2022.100031.

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