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Research paper

Associated degeneration of ventral tegmental area dopaminergic neurons in the rat nigrostriatal lactacystin model of parkinsonism and their neuroprotection by valproate

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HIGHLIGHTS

- Intranigral lactacystin causes degeneration of adjacent VTA dopaminergic neurons.
- Valproate is protective to VTA neurons in the lactacystin rat model of Parkinson's.
- Valproate is a candidate for extra-nigral as well as intra-nigral neuroprotection.

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ABSTRACT

Parkinson's disease (PD) manifests clinically as bradykinesia, rigidity, and development of a resting tremor, primarily due to degeneration of dopaminergic nigrostriatal pathways in the brain. Intranigral administration of the irreversible ubiquitin proteasome system inhibitor, lactacystin, has been used extensively to model nigrostriatal degeneration in rats, and study the effects of candidate neuroprotective agents on the integrity of the dopaminergic nigrostriatal system. Recently however, adjacent extra-nigral brain regions such as the ventral tegmental area (VTA) have been noted to also become affected in this model, yet their integrity in studies of candidate neuroprotective agents in the model have largely been overlooked. Here we quantify the extent and distribution of dopaminergic degeneration in the VTA of rats intranigrally lesioned with lactacystin, and quantify the extent of VTA dopaminergic neuroprotection after systemic treatment with an epigenetic therapeutic agent, valproate, shown previously to protect dopaminergic SNpc neurons in this model. We found that unilateral intranigral administration of lactacystin resulted in a 53.81% and 31.72% interhemispheric loss of dopaminergic SNpc and VTA neurons, respectively. Daily systemic treatment of lactacystin lesioned rats with valproate however resulted in dose-dependant neuroprotection of VTA neurons. Our findings demonstrate that not only is the VTA also affected in the intranigral lactacystin rat model of PD, but that this extra-nigral brain region is substrate for neuroprotection by valproate, an agent shown previously to induce neuroprotection and neurorestoration of SNpc dopaminergic neurons in this model. Our results therefore suggest that valproate is a candidate for extra-nigral as well as intra-nigral neuroprotection.

1. Introduction

Parkinson's disease (PD) is a complex movement disorder classically characterised by clinical presentation of rigidity, tremor and bradykinesia, primarily resulting from degeneration of the dopaminergic nigrostriatal pathway [10]. Degenerating dopaminergic neurons within the Substantia Nigra pars compacta (SNpc) of PD patients display intracytoplasmic protein inclusions, known as Lewy bodies and Lewy neurites, composed predominantly of a misfolded synaptic protein called α-synuclein (αSyn) [30]. As PD

Abbreviations: PD, Parkinson's disease; SNpc, Substantia Nigra pars compacta; αSyn, α-synuclein; VTA, ventral tegmental area; UPS, ubiquitin proteasome systems; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDACI, histone deacetylase inhibitor; TH, tyrosine hydroxylase.

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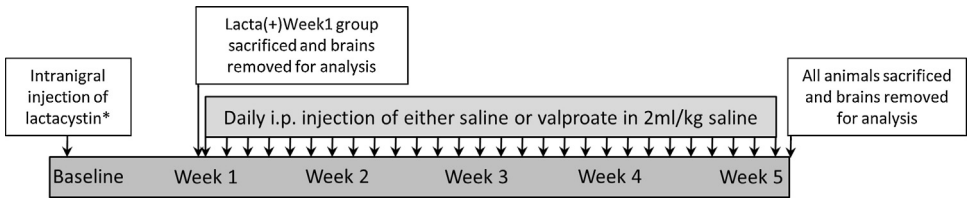

Fig. 1. Animal study design.
*Lacta(+) Week 1, Lacta(+)VPA(−), Lacta(+)VPA(+) and Lacta(+)VPA(++) groups were intranigrally injected with lactacystin. Control groups (Lacta(−)VPA(−) and Lacta(−)VPA(++)) remained surgically naïve.

develops this αSyn pathology is thought to progressively spread rostrally throughout the brain, gradually affecting higher order centres with disease progression, bringing about dementia and neuropsychiatric symptoms which are synonymous with late stage PD [2].

It has recently been suggested that the misfolding of αSyn is self-propagating, spreading from neuron to neuron *via* a 'prion-like' mechanism [16,18]. The concept of spreading αSyn pathology in PD, particularly by direct transfer of αSyn between neurons suggests that there would be neuronal cell death in regions spatially nearby the SNpc, where αSyn pathology in PD is most evident. The SNpc lies just lateral to another dopaminergic centre, the ventral tegmental area (VTA), which like the SNpc has reciprocal projections to caudal nuclei, such as mesocortical projections to the prefrontal, orbitofrontal and cingulate cortices [23]. Spread of αSyn pathology in the brain and the proximity of the SNpc to the VTA would suggest that this region is also affected in PD. Correspondingly, it has previously been shown that this is the case in PD: VTA neuron numbers being reduced in PD brains compared to control [9,28] thought, possibly to be related to duration or severity of depression in PD patients as these neurons normally function as part of the brain's positive affect system, in reward or reinforcement processes [6].

Toxin based models of PD tend to focus exclusively on nigrostriatal neurodegeneration and although the SNpc and striatum are obvious regions of interest, such study designs do not consider the progressive nature of PD in which several neuronal systems gradually become affected. A recent animal model of PD takes advantage of the irreversible ubiquitin proteasome system (UPS) inhibitor, lactacystin, to model parkinsonian nigrostriatal neurodegeneration by causing intracytoplasmic accumulation of altered proteins [19,20] and degeneration of dopaminergic and non-dopaminergic SNpc neurons [12]. Additionally however, when injected into the SNpc of rats, lactacystin has recently been observed to produce both intra- as well as extra-nigral pathology: affecting nearby regions such as the pedunculopontine nucleus (PPN) [26], the VTA [17] and also more rostral regions such as the primary motor (M1) and somatosensory cortices [32]. It remains unanswered however as to whether these neuronal populations susceptible to lactacystin induced cell death are also substrates for neuroprotective therapies known to successfully target nigrostriatal dopaminergic neurons in this model.

In healthy cells, histone acetylation is carefully controlled by the activity of two enzyme classes: histone acetyltransferases (HATs) which acetylate histone N-terminal tails, and histone deacetylases (HDACs) which deacetylase histone N-terminal tails. It was discovered recently that αSyn 'masks' histone proteins, preventing their acetylation, and as such the resulting histone hypoacetylation is thought to contribute to neurodegeneration in PD [14]. In addition it has been observed that a misbalance in the activities of HATs and HDACs exists in neurodegenerative scenarios, leading to an exacerbation of the histone hypoacetylation and associated apoptosis [29]. We therefore postulate that this deregulation of the balance of histone acetylation and deacetylation could be rectified with the use of HDAC inhibitors (HDACIs), to reduce the neurodegeneration observed as a result of histone hypoacetylation in PD [8]. Correspondingly, we have shown recently that systemic treatment with valproate, an inhibitor of HDAC classes I (HDACs 1–3 & 8) and IIa (HDACs 4, 5, 7 & 9), causes neuroprotection and neurorestoration of dopaminergic neurons within the SNpc of lactacystin lesioned rats [7]. The SNpc is known to express HDACs 2–5 and 11 most abundantly, as does the VTA [3]. Therefore, if valproate is to cause protection/restoration of neurons in the SNpc by inhibition of HDACs it would therefore be likely that valproate also protects against lactacystin induced neurodegeneration in the VTA.

In this study, we firstly aim to quantify the effect of a nigral proteasome inhibitor lesion on the integrity of the nearby VTA in the rat brain, with specific focus on the extent and distribution of dopaminergic neuronal toxicity within this region. Secondly we seek to determine whether or not dopaminergic VTA neurons, like SNpc neurons, are a substrate for valproate mediated neuroprotection/restoration in this proteasome inhibitor model of PD.

2. Materials and methods

2.1. Experimental animals

Animal procedures were carried out in accordance with the Home Office Animals (Scientific Procedures) Act, UK, 1986 and were previously approved by the Imperial College Animal Welfare and Ethical Review Board. Male Sprague-Dawley rats (260 ± 10 g, Charles River, UK) were housed in groups of two or three at 21 ± 1 °C on a 12 h light–dark cycle with the relative humidity maintained at 55 ± 10%. Standard rat chow and drinking water were available *ad libitum* throughout the duration of the study, and were supplemented with standard rat wet diet for seven days post-surgery.

2.2. Animal treatment groups

Six animal treatment groups were included in the study (Table 1): four groups which received intranigral injection of lactacystin and were either culled 7 days post-surgery, or subsequently treated for 28 days with either saline or valproate (200 or 400 mg/kg) administered i.p. starting 7 days post-surgery, and two groups which remained surgically naïve whilst receiving subsequent treatment with either saline or valproate (400 mg/kg). See Fig. 1 for study design.

2.3. Stereotaxic lesioning of the SNpc with lactacystin

The left SNpc was stereotaxically lesioned using the irreversible proteasome inhibitor lactacystin (Enzo Life Sciences, Exeter, UK) as previously described by us [7,26] and others [5,22,31–33]. Briefly, isoflurane (IsoFlo®, Abbot Laboratories, Maidenhead, UK) anaesthetised animals were positioned in a stereotaxic frame (Kopf Instruments, Tujunga, USA) in the horizontal skull position, and a midline incision made in the scalp to expose the skull, where