The Pitx3-deficient aphakia mouse: a naturally occurring mouse model of dopamine deficiency

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Chapter 8

Summary
Introduction

The neurotransmitter dopamine (DA) is an important modulator of many central nervous system (CNS) functions including motor control, neuroendocrine hormone release, cognition, emotional behaviour and reward-related behaviour. In mammals, DA neurons are mainly located in the ventral midbrain/mesencephalon to form the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). DA neurons from the SNc project to the neostriatum (caudate-putamen), the main structure of the basal ganglia which receives inputs from virtually all cortical areas and in turn affects the frontal cortex. Degeneration of SNc DA neurons in humans causes Parkinson’s disease (PD), whereas disturbances of VTA DA neuronal projections to the nucleus accumbens and prefrontal cortex are associated with schizophrenia, addictive behavioural disorders, obsessive-compulsive disorder, Gilles de la Tourette’s syndrome and attention-deficit hyperactivity disorder. Since the physiological role and clinical relevance of DA neurons are well recognized, mechanisms underlying their embryonic development have been the subject of intense investigation.

Thesis

Chapter 1 provides an introduction about the early developmental genetic events that precede the appearance of the first mesencephalic DA (MesDA) neurons at embryonic day (E) 11.5 of the 21 days of mouse development. Anterior-posterior patterning genes like the homeobox transcription factors Otx2, Gbx2, En1, En2, Pax2, Pax5 and Lmx1b, and signalling molecules Wnt1 and FGF8 determine proper formation of the future midbrain and hindbrain. The signalling molecule Shh induces dorsal-ventral patterning of cell types in the future midbrain. The first specific signs of MesDA neurons shortly follow induction of the orphan nuclear hormone receptor Nurr1 (E10.5) and homeobox transcription factor Pitx3 (E11), when expression of tyrosine hydroxylase (TH), the key enzyme in DA synthesis, is initiated. The Pitx3 gene maps to the region of the aphakia (ak) mutation, a spontaneously occurred recessive mutation that causes microphthalmia with an absence of lens, iris and pupil. The aim of this thesis is to test the hypothesis that the ak mouse is a knockout for Pitx3 at the CNS level, and that absence of Pitx3 expression during MesDA neuron development results in SNc/VTA DA neuron depletion and subsequent downstream loss of DA innervation in the neostriatum.

In Chapter 2 we show that in wild-type (wt) mice only a subset of MesDA neurons, those in the ventral tier of the SNc (vSNc) and half of the VTA, express Pitx3. Ak mice have no detectable midbrain Pitx3 expression and markedly reduced MesDA neurons, with 71%
loss of SNc DA neurons during early development and 52% loss of VTA DA neurons during postnatal day (P) 21 and 100. Within the ak SNc, vSNc is almost depleted of DA neurons, whereas the dorsal tier of the SNc (dSNC) is relatively spared. The ak MesDA neuronal depletion causes a dramatic reduction of DA innervation in the striatum, with 93% reduced DA levels in the neostriatum and 69% reduced levels in the nucleus accumbens, associated with marked hypokinesia. The preferential loss of vSNc DA neurons in ak mice together with the severe reduction of neostriatal DA levels and associated movement disorder are very similar to PD.

**Chapter 3** examines in more detail the expression pattern of Pitx3 in the wt MesDA system and selective loss of MesDA neurons in ak mice. We show that the expression of Pitx3 in vSNc and half of VTA DA neurons is largely complementary with the expression of the calcium-binding protein calbindin D$_{28K}$ (CB), a marker of resistant MesDA neurons in PD and neurotoxin-induced animal models of PD, in dSNC and the other half of VTA DA neurons. In ak mice, nearly all surviving MesDA neurons express CB. In wt mice, exposure to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes preferential loss of Pitx3-expressing neurons. The strong resemblance between the pattern of MesDA neuron loss in ak mice, in neurotoxin-induced MesDA neurodegeneration, and in PD suggests that the Pitx3 pathway underlies a common vulnerability of this MesDA neuronal subpopulation.

In **Chapter 4** we examine the effects of the loss of MesDA neurons in ak mice on the neostriatum and nucleus accumbens. We report that the onset of the ak locomotor deficit is established by P40, when only the SNc but not the VTA is affected by neuronal loss. The ak hypokinesia can be rescued by injection of the DA precursor 3,4-dihydroxyphenylalanine (L-DOPA), the most effective drug for symptomatic treatment of PD. We further show that downstream striatal correlates of MesDA neuronal loss in ak mice, as assessed by expression levels of DA receptors, dynorphin, enkephalin and neurotensin, are highly similar to the neuroadaptive phenomena as observed with neurotoxin-induced rapid neurodegeneration in animals models of PD, or following slowly progressive neurodegenerative processes as seen in PD patients.

Neurons of the neostriatum are organized in anatomically and chemically distinct patch and matrix compartments. **Chapter 5** explores the effects of early developmental DA depletion in the ak mouse on the development of the neostriatum. We show scant or absent DA fibers in the ak dorsolateral and medial neostriatum, altering the chemical anatomy of µ-opioid receptor stained patches. Furthermore, neostriatal neuron numbers are comparable in ak and wt animals, but the volume of the ak neostriatum and its
neurons is significantly reduced. We propose that early nigrostriatal DA loss in ak mice results in loss of trophic support to the developing neostriatum.

Animal models have provided tremendous insight in the pathogenesis of various diseases, and are a valuable tool for testing new treatment strategies. In the last four decades, several toxin and genetic animals models of DA deficiency have been generated to study disease pathways of PD. Chapter 6 presents the Pitx3-deficient ak mouse as a naturally occurring mouse model of DA deficiency, and compares it with other available mouse models of DA deficiency. Ak mice faithfully model the selective parkinsonian pattern of vSNc DA neuron loss, associated striatal pathology, and hypokinetic locomotor deficits which can be reversed by L-DOPA. However, their SNc DA neuron loss is complete at birth. ATM knockout, En1 heterozygote/En2 double knockout, Girk2 knockout/weaver, MitoPark, MPTP/rotenone-treated, Nurr1 heterozygote knockout, Parkin-Q311X overexpressing, and VMAT2 low expressor mice exhibit SNc DA neuron loss during adult life, and are therefore better models to study the cellular mechanisms that occur during SNc DA neuron loss.

Chapter 7 discusses the results of our studies against the recent developments. Our finding that the wt MesDA system is composed of two previously unrecognized DA neuronal subpopulations, that are differentiated by the expression of Pitx3, is at variance with reports from other research groups. Whether our immunohistochemical protocols or the characteristics of our anti-Pitx3 antibodies are too stringent, or whether the results from the other groups are too unspecific, remains to be verified. Our findings of absent Pitx3 immunostaining and dramatic reduction of MesDA neurons in ak mice, together with reduced DA levels in the neostriatum and associated locomotor deficits, were confirmed by several groups. A sequentially orchestrated genetic cascade involving FoxP1, Lmx1b, Pitx3 and Ahd2 is crucial for survival of Pitx3-expressing MesDA neurons. Although the neuropathological findings in PD are more extensive than in ak mice, Pitx3 seems essential for the MesDA neuronal subpopulation most susceptible to degeneration in PD. Interestingly, polymorphisms of the human PITX3 gene put people throughout the world at risk for developing PD.

Conclusion

Pitx3-deficiency in the ak MesDA system causes >70% loss of mainly vSNc DA neurons during early development and loss of half of VTA DA neurons during adult life. This loss causes >90% reduction of DA levels in the neostriatum, neostriatal DA-receptors/neuropeptides adaptations, 20% neostriatal atrophy, and hypokinetic locomotor deficits which can be reversed by L-DOPA. Ak mice faithfully model the pattern
of vSNc DA neuron loss and associated neostriatal pathology as observed in PD patients. Notwithstanding the neuropathological differences between the highly selective midbrain phenotype in ak mice and the extensive pathology in PD patients, Pitx3 seems necessary for survival of the MesDA neuronal subpopulation most susceptible to degeneration in PD. Future research is needed to further elucidate how this homeobox transcription factor regulates vulnerability to neurodegeneration.