J. Anat. (2000) **196**, pp. 629–633, with 4 figures Printed in the United Kingdom

Review

The AS/AGU rat: a spontaneous model of disruption and degeneration in the nigrostriatal dopaminergic system

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(*Accepted 26 October 1999*)

ABSTRACT

The AS/AGU rat provides an alternative to experimentally produced laboratory models of basal ganglia disorders. This mutant is characterised by disturbances of movement including clumsy gait, whole body tremor, rigidity and difficulty in initiating movement. From an early age, there is a profound depletion of extracellular dopamine in the dorsal caudate-putamen as measured via in vivo microdialysis; levels are only 10–20% of those found in the parent Albino Swiss (AS) strain. Subsequently a depletion of whole tissue dopamine levels occurs and, later still, loss of dopaminergic cells in the substantia nigra pars compacta. The dysfunction in movement and the nigrostriatal dopaminergic system are clearly linked, since movement can be ameliorated by L-DOPA administration. Furthermore, there are depletions in glucose utilisation in several regions of the basal ganglia circuitry, including the substantia nigra pars compacta, the subthalamic nucleus and the ventrolateral thalamus. The AS/AGU rat represents a unique opportunity to investigate the intrinsic factors controlling the integrity of dopaminergic systems and the recent successful positional cloning of the *agu* gene will allow the molecular mechanisms underlying this interesting phenotype to be analysed.

Key words: AS/AGU rat; nigrostriatal dopaminergic system.

INTRODUCTION

Many attempts have been made to devise suitable laboratory models of neurodegeneration which combine motor abnormalities with deficits in the basal ganglia and/or aminergic systems, and thus give insight into human disorders such as Parkinson's disease, multiple system atrophy or progressive supranuclear palsy. These models have tended to involve electrolytic or pharmacological lesions to selected brain areas (reviewed by Jenner & Marsden, 1993; Bezard et al. 1998). They thus possess a number of limitations, one being that they cannot readily mimic the progressive nature of change, nor can they give insight into genetic predispositions to dysfunction which are of increasing interest in, for example, Parkinson's disease (Bandmann et al. 1998; Gasser, 1998).

A spontaneous animal model in which the disorder exhibits progressive characteristics would therefore be of considerable use alongside existing models artificially induced by 6-hydroxydopamine (6-OH-DA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This short review will argue that the AS/AGU rat represents such a model.

The AS/AGU rat arose as a spontaneous mutation within a closed inbred colony of Albino Swiss (AS) rats. The mutation is recessive (Campbell et al. 1996) and AS/AGU rats have been successfully isolated as a true breeding substrain. The parent AS strain has little detectable heterozygosity (Shiels et al. 1995) and phenotypic differences between the lines are therefore the result of the single *agu* mutation; there should thus be no effect due to variation in the genetic background. The AS/AGU mutant is characterised by serious movement impairments including rigidity, a staggering gait and a tendency for the hind quarters to slip over every few steps, a slight whole body

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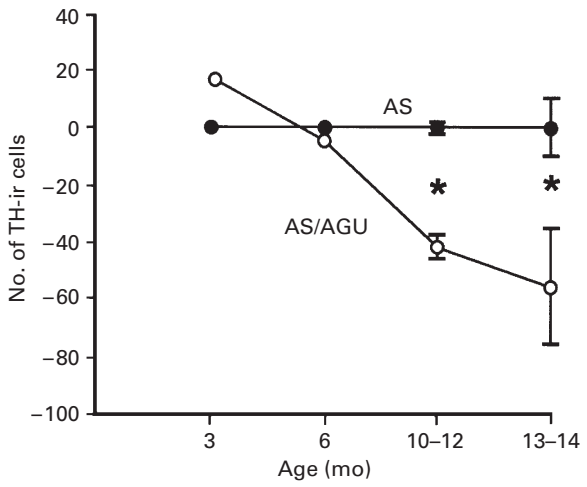

Fig. 1. Graph showing the percentage difference in the numbers of tyrosine hydroxylase immunoreactive (TH-ir) cells in the substantia nigra pars compacta of Albino Swiss (AS) and mutant AS/AGU male rats at different ages. The results are presented as percentages rather than absolute numbers because of differing protocols used over a 5 y period of investigation. (From Clarke & Payne, 1994, and D. J. Clarke, R. Branton and J. M. Campbell, personal communication). * Differs at $P < 0.05$.

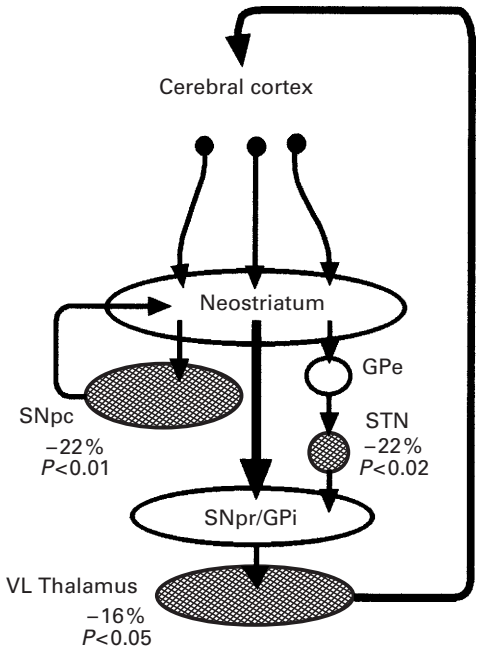

Fig. 2. Simplified diagram of basal ganglia circuitry showing areas of markedly reduced 2-deoxy-glucose utilisation in the AS/AGU mutant compared with the AS control. It is unclear whether the reduced utilisation in any particular area is related to its intrinsic cells or to the synaptic terminals of extrinsic afferent cells. (From Lam et al. 1998). GPe, globus pallidus externus; Gpi, globus pallidus internus; SNpc, substantia nigra pars compacta; SNpr, substantia nigra pars reticulata; STN, subthalamic nucleus; VL, ventrolateral thalamus.

tremor and difficulty in initiating movement (Clarke & Payne, 1994; Payne et al. 1998).

There are no obvious gross morphological differences between the brains of normal and mutant AS/AGU rats: neocortical and cerebellar histology appears normal. Initial immunocytochemical studies revealed deficits in the number of tyrosine hydroxylase-immunoreactive (dopaminergic) cells in the substantia nigra pars compacta of mutant animals aged 12–14 mo (Clarke & Payne, 1994). This reduction in cell numbers was of sufficient magnitude (−60%) to suggest that it could readily underlie the observed locomotor disorders. However, subsequent unpublished studies showed that there was no significant dopaminergic cell loss at earlier ages when movement itself was already clearly impaired (D. J. Clarke, R. Branton, J. M. Campbell, personal communication). These data are summarised in Figure 1. Conversely, data quite clearly confirming an impairment of basal ganglia function in much younger animals came from studies by Lam et al. (1998) who showed significant depletion of 2-deoxy-glucose uptake in the substantia nigra pars compacta, subthalamic nucleus and ventrolateral thalamus of AS/AGU rats compared with AS rats (Fig. 2).

If the AS/AGU mutant is to be of use in investigating basal ganglia-related and/or dopamine-related degenerative disorders, several questions must be addressed. (1) At what age do motor disturbances appear; are they progressive and can they be reversed by appropriate pharmacological treatment? (2) If dopaminergic cell numbers themselves are not depleted when movement disorders initially occur, do other measures of dopaminergic function show deficits? (3) Are there possible links between these deficits and eventual cell loss?

QUANTITATIVE LOCOMOTOR DIFFERENCES BETWEEN AS AND AS/AGU RATS AND THE EFFECTS OF L-DOPA ON PERFORMANCE

A number of very simple tests have proved useful in distinguishing motor performance between adult AS controls and AS/AGU mutants (Payne et al. 1998). Two representative tests are as follows.

Mid-air righting. Animals are held upside down 50 cm above a soft substrate and dropped. Rats can normally turn around within a vertical drop of 20 cm to land on all fours and thus the success rate of control AS animals is virtually 100% until 1 y of age when success declines. By contrast, AS/AGU mutants have

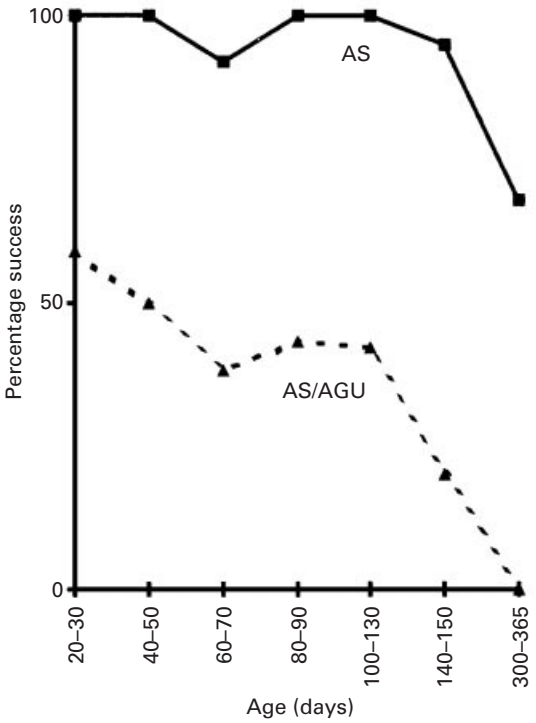

Fig. 3. Percentage of AS (solid line) and AS/AGU mutant (dotted line) male rats capable of mid-air righting at different ages up to 1 y. AS/AGU rats perform poorly compared with the parent strain.

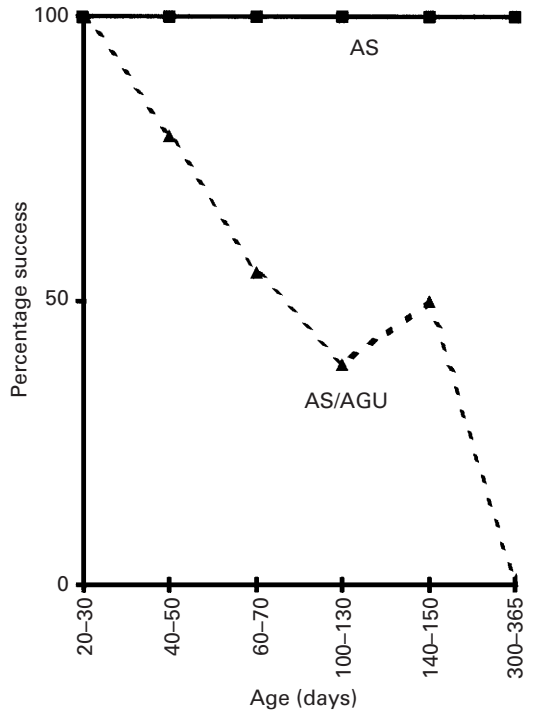

Fig. 4. Percentage of AS (solid line) and AS/AGU mutant (dotted line) male rats capable of walking down an inclined 70 mm ramp without falling off at different ages up to 1 y. AS/AGU rats perform poorly compared with the parent strain.

an initial success rate of only 60% and this shows a steep decline over the period of a year after birth (Fig. 3).

Table. *Percentage of mutant AS/AGU rats capable of* (*a*) *mid-air righting and* (*b*) *walking down an inclined 70 mm wooden ramp without falling off, when given daily injections of* L-*DOPA* (*25 mg/kg/day i.p. in 0.9% saline*) + *benzeraside* (*2.5 mg/kg/day i.p. in 0.1% sodium bisulphite*) *or saline control* (*from Russell et al. 1998*)

	Week			
Task group	1	2	3	4
Mid-air righting				
AS/AGU + saline	30	20	0	0
AS/AGU + L-DOPA	20	50	80	90
Inclined ramp				
AS/AGU + saline	40	10	0	0
AS/AGU + L-DOPA	20	0	50	80

* Animals were 3-mo old at the beginning of the test period, received treatment for 1 mo and were tested at weekly intervals.

Inclined ramp test. Animals are placed at the top of an inclined wooden ramp 85 cm long and of varying width, angled downwards at 14° into a large container filled with wood chips. Very wide or very narrow ramps are poor discriminators of performance between control and mutant animals. However, whilst AS controls are readily able to walk down a ramp of 70 mm width with no decrease in performance over a year, AS/AGU rats are increasingly unable to negotiate the plank without falling off (Fig. 4).

These 2 tests are simple, provide robust differences between mutants and controls, and also demonstrate that deficits are both evident from an early age, and progressive with age. Importantly, performance by mutants in these tests can be improved by the daily administration of L-DOPA (25 mg/kg/day i.p. in 0.9% saline) plus the peripheral DOPA decarboxylase inhibitor benserazide (2.5 mg/kg/day i.p. in 0.1% sodium bisulphite). Indeed, success, rates in both mid-air righting and negotiating an inclined ramp reach 80–90% by 3–4 wk of treatment (Campbell et al. 1997; Russell et al. 1998; Table). The restorative effects of L-DOPA occur in animals as young as 3 mo of age. Thus treatment is effective long before there is any detectable loss of dopaminergic neurons, suggesting that loss of functional capacity precedes cell death.

STRIATAL DOPAMINE IN THE AS/AGU RAT

If locomotor defects are reversible by L-DOPA administration, this argues strongly that dopamine is involved in the overall phenotype, even though nigral cell loss may not occur until later. When whole tissue levels of dopamine are measured in striatal micro-punches using high performance liquid chromato-

graphy with electrochemical detection, AS/AGU mutants do indeed show a reduction of some 20–30% compared with the parent AS strain. However, this difference does not appear until about 6 mo of age (Campbell et al. 1996, 1997), again well after the time of onset of locomotor abnormalities. However, if dopamine is measured by in vivo microdialysis using a probe placed in the dorsal caudate-putamen of conscious, freely-moving rats, a massive deficit in extracellular dopamine can be detected at 3 mo of age and beyond (Campbell et al. 1998*b*). Three months is about the earliest age that stereotactic surgery can be carried out with confidence using standard rat brain atlases, but the massive scale of the decrement at this time (an 80–90% decrease compared with AS controls) makes it likely that biologically significant deficits occur at an early age. Not only is dopamine substantially reduced in striatal extracellular fluid, but dopamine metabolites such as DOPAC and HVA are considerably elevated, by a factor of 4–5 times (Campbell et al. 1998*b*).

DOPAMINE RELEASE AND METABOLISM IN THE AS/AGU RAT

From the above, it will be appreciated that extracellular dopamine is decreased, but whole tissue levels are normal and extracellular dopamine metabolites are raised in these mutant rats from at least 3 mo after birth. Perhaps the simplest explanation is to envisage that an abnormally high proportion of dopamine in the synaptic terminals occurs in the cytoplasm (rather than in vesicles) where it can be metabolised by mitochondrial enzymes such as monoamine oxidase A (MaO-A) and the breakdown products eliminated. Quite why that should occur (possibly failure of packaging/release mechanisms or 'leaky' vesicles) is not clear at present; nevertheless, a series of recent investigations (Campbell et al. 1998*a*) suggest that this hypothesis may be correct. (1) Release of dopamine can be evoked by a variety of stimuli including systemically administered reserpine, caffeine and amphetamine or probe infusion of potassium. However, evoked extracellular levels in AS/AGU rats never exceed basal levels in AS animals, while evoked levels in the latter are increased even further. (2) Systemic administration of the dopamine reuptake blocker nomifensine leads to a large rise in extracellular dopamine in AS animals but a much smaller rise in AS/AGU mutants. This suggests that reuptake rates cannot underlie the observed differences in extracellular dopamine. (3) Systemic administration of the MaO-A inhibitor clorgyline leads to a marked reduction in extracellular DOPAC and HVA and is the only treatment found to date which results in comparable extracellular levels of dopamine both in the AS parent strain and the AS/AGU mutant.

THE SEQUENCE OF COMPROMISE IN THE NIGROSTRIATAL DOPAMINERGIC SYSTEM

The nigrostriatal dopamine system is clearly defective in the AS/AGU rat from an early age; moreover, this deficiency is behaviourally significant since locomotor impairment is reversed by L-DOPA administration. However, the nature of the deficiency follows a chronological sequence with dopamine release being impaired first, followed later by a decrease in whole tissue dopamine levels and, later still, an actual reduction in dopaminergic cells. Two features of this require discussion.

First, do these events form an interconnected sequence? It might be that a single point mutation could cause multiple cellular effects such as a failure of dopamine handling and cell death with each, in turn, depending separately on the mutation. Alternatively, it may be that the mutation causes the first deficiency (i.e. in the packaging/release of dopamine) and that subsequent changes follow from this. Dopamine is itself cytotoxic and, if present in above average amounts in the cytosolic compartment of the terminal, could cause an inhibition of the dopamine transporter system (Berman et al. 1996) or neurodegeneration through the formation of quinones (Graham, 1978; Hastings et al. 1996; Stokes et al. 1999) or reactive oxygen species (Cohen et al. 1997).

Secondly, in the sequence of events outlined here, locomotor symptoms are already well established some time before cell death. By contrast, in human neurodegenerative conditions involving the basal ganglia, the assumption is often made that cell loss is the precipitating factor for symptoms to occur. Nevertheless, it has been pointed out that, in Huntington's disease, metabolic changes often precede neuronal degeneration (Grafton et al. 1992). In Parkinson's disease, cellular inclusions have been considered a hallmark of degeneration 'even when cell loss is slight' (Gibb, 1993), while some PET studies also suggest that a substantial part of the dopaminergic nigrostriatal system is still intact in living parkinsonian patients, even though dopamine release may be impaired (Leenders et al. 1990). This suggests that cell malfunction can produce symptoms during an (as yet undetermined) period prior to actual cell loss.

The AS/AGU rat represents a unique opportunity to investigate the intrinsic factors controlling the integrity of dopaminergic systems and may provide a valuable model for the investigation of dopamine deficiency syndromes in man. Work is in progress on the mode of onset and progression of this dopamine deficiency, and the recent successful positional cloning of the *agu* gene will allow the molecular mechanisms underlying this interesting phenotype to be analysed.

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