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Review

The R6 lines of transgenic mice: A model for screening new therapies for Huntington's disease

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ABSTRACT

Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an expanded CAG repeat in the *HD* gene that results in cortical and striatal degeneration, and mutant huntingtin aggregation. Current treatments are unsatisfactory. R6 transgenic mice replicate many features of the human condition, show early onset of symptoms and fast disease progression, being one of the most used models for therapy screening. Here we review the therapies that have been tested in these mice: environmental enrichment, inhibition of histone deacetylation and methylation, inhibition of misfolding and oligomerization, transglutaminase inhibition, rescue of metabolic impairment, amelioration of the diabetic phenotype, use of antioxidants, inhibition of excitotoxicity, caspase inhibition, transplantation, genetic manipulations, and restoration of neurogenesis. Although many of these treatments were beneficial in R6 mice, they may not be as effective in HD patients, and thus the search for a combination of therapies that will rescue the human condition continues.

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Contents

1. Introduction — The R6 mice

To address the question of CAG repeat stability in the mouse genome, Mangiarini et al. (1996) created transgenic mice with truncated fragments of the mutant huntingtin gene, referred to as the R6 lines. The authors were surprised to find that these fragments were sufficient to generate a neurological phenotype that was similar to Huntington's disease (HD) in humans (Mangiarini et al., 1996). Among the five original lines, the R6/1 and R6/2 have been the most studied. These mice express exon 1 of the human *HD* gene (which corresponds to 3% of the entire gene) with approximately 115 (R6/1) and 150 (R6/2) CAG repeats (Mangiarini et al., 1996). These mice, and in particular the R6/2 line, have an early onset of symptoms and a fast progression of the disease, showing a life expectancy of approximately 12 to 17 weeks, depending on the colony.

The phenotype and neuropathology observed in these mice replicate several features observed in humans, including a progressive motor (Carter et al., 1999; Luesse et al., 2001; Bolivar et al., 2003, 2004) and cognitive impairment (Lione et al., 1999; Murphy et al., 2000), weight loss, decreased striatal and brain size (Mangiarini et al., 1996), ubiquitinated nuclear and cytoplasmic inclusions of the mutant protein (Davies et al., 1997; Li et al., 1999), altered levels of neurotransmitters (for review see Cha, 2000) and their receptors (Cha et al., 1998), altered gene expression (Luthi-Carter et al., 2000, 2002, 2003; Zucker et al., 2005), diabetes (Hurlbert et al., 1999; Luesse et al., 2001; Andreassen et al., 2002; Björkqvist et al., 2005), which was previously reported in 10–25% of HD patients (Podolsky et al., 1972; Farrer, 1985), as well as several other neuroendocrine changes (Björkqvist et al., 2006; for review see Petersén and Björkqvist, 2006), and premature death (Mangiarini et al., 1996). Interestingly, both R6/2 (Gil et al., 2004, 2005) and R6/1 (Lazic et al., 2004, 2006) mice show a specific decrease in hippocampal cell proliferation and neurogenesis, which may account for some of their cognitive deficits (see below).

However, despite showing gross brain and striatal atrophy (Mangiarini et al., 1996), R6/2 mice show minimal neuronal loss in the striatum. To date, only one study reported a 25% decrease in the total number of striatal cells in 12-week-old transgenic mice (Stack et al., 2005), while another described a marked increase in the number of apoptotic cells in the striatum (Keene et al., 2002) of 13 week-old R6/2 mice. On the other hand, "dark cell degeneration" has also been described in a small number of neurons in 12- (Stack et al., 2005) and 14-week-old mice (i.e., at a very late stage in the development of the disease) (Iannicola et al., 2000; Turmaine et al., 2000; Yu et al., 2003). Moreover, a progressive loss of orexin neurons was found in the lateral hypothalamus of R6/2 mice, which was correlated with the occurrence of narcoleptic-like episodes (Petersén et al., 2005) and seemed to be related with a disintegration of the sleep–wake cycle and of the circadian rhythm in these mice (Morton et al., 2005b). Furthermore, a loss of retinal ganglion neurons was reported in these transgenic animals (Helmlinger et al., 2002).

2. Therapeutic strategies

Although clinically relevant, it is particularly challenging to perform therapeutic trials in HD patients for several reasons: (1) The low prevalence of the disease often makes it difficult to recruit a sufficient number of patients; (2) Due to the slow progression over many years, drugs that are hypothesized to affect disease progression may require very long follow up periods; (3) There is substantial variability in the rate of progression, even in patients with similar CAG repeats (Squitieri et al., 2002); (4) There are no well-established biomarkers (i.e., quantifiable measures that change predictably with disease progression) for HD, which means that for the most part, clinical trials have to rely on subjective clinical rating scales as outcome parameters; (5) There are no established methods to assess disease progression in preclinical gene carriers; (6) Therapies in mutation carriers early in the disease will probably have minor benefits and may cause adverse effects. Once a reliable set of biomarkers for HD progression becomes available, it will be possible to test the efficacy of many therapies in preclinical gene carriers in delaying disease onset. These biomarkers will allow the design of clinical trials with shorter durations and smaller groups of patients (Wild and Tabrizi, 2006). Thus, it is particularly important that all novel therapies for HD are extensively tested in cell cultures and animal models before studied in patients.

In this perspective, the R6 transgenic lines have served as a useful tool to evaluate new therapeutic strategies and are

probably the most used transgenic HD models for this purpose. However, the accelerated phenotype in R6 mice may not reflect the actual human condition and, in some circumstances, reduce the ability to detect subtle improvements due to a specific therapy. Moreover, the number of CAG repeats that are expressed in these mice (approximately 115 and 150 repeats in the R6/1 and R6/2 lines respectively) do not reflect the number of repeats (approximately 39) that cause the most common adult-onset form of the disease in humans. In fact, in humans longer repeats are normally associated with high allele instability during paternal transmission and result in juvenile and infantile cases that are characterized by a more severe phenotype and a faster progression of the disease (for review, see Gil and Rego, 2008). Thus, it is reasonable to suggest that the R6 transgenic lines reflect more accurately the infantile/juvenile HD cases than the adult-onset form of the disease.

It is also important to note that therapies that are predicted to function at the level of the full-length mutant huntingtin are unlikely to be optimal to test in this model, which expresses a truncated form of the mutant protein. Furthermore, both genetic (e.g., number of CAG repeats) and environmental factors (e.g., number of pups in a litter, maternal nutrition status, age at weaning, cage size, degree of environmental enrichment, diet, number of animals per cage, etc.) can contribute to the overall wellbeing and survival of the mice, and consequently to the observed results (Hockly et al., 2003b). Thus, these factors should be taken into consideration when comparing the effects of different therapeutic strategies. Moreover, the search for therapies is also dependent on a careful standardization of experimental protocols (namely on the use of appropriate behavioral tests) and a sophisticated statistical approach to analyze the outcome parameters. In this respect, a careful study by Hockly et al. (2003b) has shown that rotarod performance, grip strength and weight loss produce quantitative, reliable and reproducible data that can be easily analyzed by standard statistical methods and reveal the progressive nature of the disease. In a subsequent study (Hickey et al., 2005), R6/2 mice were shown to present behavioral deficits in tests of motor function, namely the running wheel activity and climbing behavior, as early as 4.5 weeks of age, a time when rotarod performance and grip strength were still normal. Power calculations further showed that the running wheel test was appropriate for efficient drug screening at this early stage (Hickey et al., 2005). On the other hand, one should keep in mind that the specific cause for the progressive behavioral deterioration of R6 mice, such as the decrease in rotarod performance, is not completely understood. Indeed, the peripheral features of the R6 pathology may also contribute for the progressive decline in the ability to perform certain behavioral tests including the rotarod. Finally, although it has been observed that R6 mice can die with epileptic seizures (Mangiarini et al., 1996; Gil et al., unpublished observations), the actual cause of death of these mice is still unknown and deserves further investigation.

So far, most of the experimental treatments that have been tested in R6 mice, have focused on drug administration. However, alternative therapeutic strategies, including genetic manipulations and transplantation, have also been studied (Table 1). Many of these treatment paradigms have been found to exert ameliorative effects on the behavior and neuropathology of these transgenic mice and to increase their survival by up to 40%. However, one should be careful when interpreting these results, since the actual beneficial effects of these various treatments in this and other animal models can only be properly inferred after performing controlled clinical trials in human HD patients. Nevertheless, the R6 mice have constituted an invaluable tool to screen potential therapies for the treatment of this devastating disorder. In the following sections we will review the therapeutic strategies (along with some molecular mechanisms related to HD pathogenesis) that have been evaluated to date in these transgenic mouse lines.

2.1. Environmental enrichment

Environmental enrichment can be beneficial in different experimental models of neurodegeneration (Johansson, 1996; Young et al., 1999). Two independent groups have analyzed the effect of an increased environmental stimulation on the phenotype and neuropathology of R6 mice (Carter et al., 2000; van Dellen et al., 2000; Hockly et al., 2002; Glass et al., 2004; Spires et al., 2004). These studies have demonstrated that the exposure of R6/1 (van Dellen et al., 2000; Glass et al., 2004; Spires et al., 2004) or R6/2 (Hockly et al., 2002) mice to an enriched environment (consisting of large standard cages with cardboard, paper and plastic objects, which were changed every two to three times per week) delayed the onset of motor symptoms, decreased the severity of the clasping phenotype, and reduced the loss of the peristriatal cerebral volume. Carter et al. (2000) also demonstrated that, improving the access to food and water had beneficial effects.

Although it is not clear how enrichment exerts its beneficial effects on disease progression, the structural changes in the brain, such as plasticity and neurogenesis that result from exposure to a stimulating environment (Kempermann et al., 1997; Gould et al., 1999; van Praag et al., 1999), could improve neuronal function and stimulate protective mechanisms in the brain, retarding the degenerative process. As stated above (Section 1), both R6/1 (Lazic et al., 2004, 2006) and R6/2 mice (Gil et al., 2004, 2005) show reduced hippocampal neurogenesis. Therefore, it is possible that the beneficial effects observed in an enriched environment may be due, at least in part, to a restoration of the neurogenic process. In agreement with this hypothesis, 25 week-old symptomatic R6/1 mice housed in an enriched environment (from 4 weeks of age), were shown to have an increase number of proliferating cells [bromodeoxyuridine (BrdU)-positive] and migrating neuroblasts (doublecortin-positive) in the dentate gyrus (DG) of the hippocampus as compared to control R6/1 mice housed in standard conditions (Lazic et al., 2006), showing that an enriched environment can indeed restore hippocampal neurogenesis in these transgenic mice.

On the other hand, it has been shown that an enriched environment can have beneficial effects at the level of gene transcription or protein transport, which in turn can contribute to an improvement in synaptic function and plasticity (for review, Hannan, 2004). In agreement with this hypothesis,

Table 1 – Therapeutic strategies for HD tested in R6 transgenic mice grouped according with their mode of action

Treatment	Mouse line	Effect on RR performance	Effect on survival	Reference	Clinical trials
Environmental enrichment					
Environmental enrichment	R6/1	n.d.	n.d.	van Dellen et al., 2000 Glass et al., 2004 Spires et al., 2004 Lazic et al., 2006	
Environmental enrichment	R6/2	↑ performance (accelerated RR)	n.d.	Hockly et al., 2002	
Environmental enrichment and enhanced diet	R6/2	n.d.	↓ early deaths	Carter et al., 2000	
Inhibition of histone deacetylation and methylation					
SAHA	R6/2	↑ performance (accelerated RR)	n.d.	Hockly et al., 2003a,b	
Sodium butyrate	R6/2	↑ performance	↑ 20.8%	Ferrante et al., 2003	
Chromomycin	R6/2	n.d.	↑ 13.1%	Stack et al., 2007b	
Mithramycin	R6/2	↑ performance	↑ 29.1%	Ferrante et al., 2004	
Mitramycin + cystamine	R6/2	↑ performance	↑ 40%	Ryu et al., 2006	
Inhibition of misfolding and oligomerization					
Overexpression of Hsp70	R6/2	n.d.	no effect	Hansson et al., 2003	
Overexpression of Hsp27	R6/2	n.d.	no effect	Zourlidou et al., 2007	
Congo red	R6/2	↑ performance no effect	↑ 14.2% no effect	Sánchez et al., 2003 Wood et al., 2007	
Trehalose	R6/2	↑ performance	↑ 10.1%	Tanaka et al., 2004	
C2-8	R6/2	↑ performance	no effect	Chopra et al., 2007	
Tiagabine	R6/2	↑ performance (10 and 12 weeks)	↑ 17.5%	Masuda et al., 2008	
Transglutaminase (Tgase) inhibition					
"Tissue" Tgase ablation	R6/1	↑ performance	↑ 12%	Mastroberardino et al., 2002	
Cystamine	R6/2	n.d. ↑ performance n.d.	↑ 10.7% ↑ 19.5% n.d.	Karpuj et al., 2002 Dedeoglu et al., 2002 Wang et al., 2005a,b,c	
Energy supplementation and rescue of metabolic impairment					
A2a agonist: CGS21680	R6/2	↑ performance	no effect	Chou et al., 2005	
A2a antagonist: SCH 58261	R6/2	no effect	n.d.	Domenici et al., 2007	
Creatine	R6/2	↑ performance ↑ performance	↑ 17.4% ↑14.4%	Ferrante et al., 2000 Dedeoglu et al., 2003	
Dichloroacetate	R6/2	↑ performance	↑ 5.8%	Andreassen et al., 2001b	
Pyrimidine nucleoside: PN401	R6/2	↑ performance	↑ 10%	Saydoff et al., 2006	
Pyrimidine nucleoside: KP544	R6/2	↑ performance	n.d.	Dey et al., 2007	
Antioxidants					
Essential fatty acids	R6/1	n.d.	n.d.	Clifford et al., 2002	
Lipoic acid	R6/2	n.d.	↑ 7.1%	Andreassen et al., 2001a	
Ascorbate	R6/2	n.d. n.d.	n.d. n.d.	Rebec et al., 2003 Rebec et al., 2006	
Co-enzyme Q10	R6/2	↑ performance	↑ 25.3%	Smith et al., 2006	No effect (Feigin et al., 1996; Huntington Study Group, 2001)
TUDCA	R6/2	↑ performance	n.d.	Keene et al., 2002	
BN82451	R6/2	↑ performance (10 weeks)	↑ 15.3%	Klivenyi et al., 2003	
Clioquinol	R6/2	↑ performance	↑ 20%	Nguyen et al., 2005	
Inhibition of excitotoxicity					
Remacemide + Co-enzyme Q10	R6/2	↑ performance	↑ 31.8%	Ferrante et al., 2002	No effect (Huntington Study Group, 2001)
Riluzole	R6/2	no effect (accelerated RR)	↑ 10.2%	Schiefer et al., 2002	↓ chorea (Huntington Study Group, 2003)

Table 1 (*continued*)

Treatment	Mouse line	Effect on RR performance	Effect on survival	Reference	Clinical trials
		no effect	n.d.	Hockly et al., 2006	No effect (Landwehrmeyer et al., 2007)
mGluR2 agonist: LY379268	R6/2	no effect	↑ 9.5%	Schiefer et al., 2004	
mGluR5 antagonist: MPEP		↑ performance	↑ 13.5%		
Ceftriaxone	R6/2	n.d.	n.d.	Miller et al., 2008	
Deafferentation of: corticostriatal pathway nigrostriatal pathway	R6/2	n.d. n.d.	↑ 13.4% ↑ 21.3%	Stack et al., 2007a	
Caspase inhibition					
Caspase-1 dominant negative	R6/2	↑ performance	↑ 20%	Ona et al., 1999	
Caspase inhibitor: zVAD-fmk		↑ performance	↑ 25%		
Inhibitors of Caspases 1 and 3 (YVAD-fmk+DEVD-fmk)	R6/2	↑ performance	↑ 17.3%	Chen et al., 2000	
Overexpression of Bcl-2	R6/2	no significant effect	↑ 10.3%	Zhang et al., 2003	
Minocycline	R6/2	↑ performance n.d. no effect	↑ 14% n.d. n.d.	Chen et al., 2000 Wang et al., 2003 Smith et al., 2003	↑ neuropsychological and motor function (Bonelli et al., 2004)
Minocycline+Co-enzyme Q_{10}	R6/2	↑ performance	↑ 18.2%	Stack et al., 2006	
Alternative pharmacological therapeutic strategies					
Lithium	R6/2	↑ performance (heavier mice)	no effect	Wood and Morton, 2003	
Anti-inflammatory drugs: acetylsalicylate and rofecoxib	R6/2	no effect	no effect	Norflus et al., 2004	
Anti-convulsants: gabapentin or gabapentin-lactam	R6/2	n.d.	no effect ↑14% n.s.	Zucker et al., 2004	
Combination: acetylcholinesterase inhibitor (tacrine) and antidepressant (moclobemide)+creatine	R6/2	no effect	minor effect	Morton et al., 2005a	
Sedative: chloral hydrate	R6/2	n.d.	↓ survival	Pallier et al., 2007	
Benzodiazepine: alprazolam		↑ performance	no effect		
Transplantation					
Striatal transplantation	R6/2	n.d.	n.d.	Dunnett et al., 1998	
Anterior cingulate cortical transplantation	R6/1	n.d.	n.d.	van Dellen et al., 2001	
Transplantation of C17.2 stem cells into the lateral ventricles + trehalose	R6/2	n.d.	Up to ↑ 26.3%	Yang and Yu, 2009	
Genetic manipulations					
Anti-huntingtin siRNA	R6/2	↑ performance	↑ 12.4%	Wang et al., 2005a,b,c	
Anti-huntingtin shRNA	R6/2	n.d.	n.d.	Huang et al., 2007	
Lentiviral GDNF	R6/2	n.d.	n.d.	Popovic et al., 2005	
Therapies aimed to restore neurogenesis					
asialoEPO	R6/2	no effect	n.d.	Gil et al., 2004	
FGF2	R6/2	↑ performance	↑ 20%	Jin et al., 2005	
Adenoviral (BDNF+Noggin)	R6/2	↑ performance	↑ 16.8%	Cho et al., 2007	
Voluntary exercise	R6/2	n.d.	n.d.	Kohl et al., 2007	
Antidepressant: fluoxetine	R6/2	n.d.	n.d.	Grote et al., 2005	
Antidepressant: sertraline	R6/2	↑ performance (10 weeks)	↑ 20%	Peng et al., 2008	

Note. n.d., not determined; RR, rotarod.

recent studies have shown that the delayed onset of HD in R6/1 mice maintained in an enriched environment is correlated with a delayed loss of cannabinoid CB1 receptors (Glass et al., 2004), and that an enriched environment entirely rescues the severe decrease of cortical DARPP-32 (dopamine and cyclic-adenosine monophosphate regulated phosphoprotein of a molecular weight of 32 kDa) and striatal brain-derived neurotrophic factor (BDNF) (in this case by possibly promoting the anterograde transport of this neurotrophin from the anterior cortex to the striatum) that are observed in 5 month-old R6/1 mice (Spires et al., 2004). Furthermore, in this last study, the enriched environment was also shown to

reverse the loss of hippocampal BDNF that is observed in these mice (Spires et al., 2004). Since BDNF has been shown to increase neurogenesis (Benraiss et al., 2001; Pencea et al., 2001), an appealing hypothesis is that the restoration of hippocampal BDNF levels by enriched environment may lead to an increase hippocampal neurogenesis, which can in turn contribute to the observed behavioral improvements.

Based on these studies, many groups are now using an enriched environment as the standard housing for these mice, and any given therapy would need to exert additional effects to be proven effective (Hockly et al., 2003b). The described findings also suggest that promoting environmental stimulation could have an effect on disease progression by improving the quality of life of patients afflicted with this disorder.

2.2. Inhibition of histone deacetylation and methylation

Post-translational modifications of histones (e.g. acetylation, methylation, phosphorylation) contribute to the overall chromatin structure and play an important role in the regulation of gene transcription. Transcriptional dysregulation might play a role in the pathogenesis of HD, and several transcription abnormalities have been reported in the R6/2 mouse model (for review see Cha, 2000). Furthermore, data from *Drosophila* models suggested that inhibitors of histone deacetylases (HDACs) might be used to ameliorate these transcriptional changes (Steffan et al., 2001). In line with this hypothesis, it was shown that suberoylanilide hydroxamic acid (SAHA), a potent HDAC inhibitor, crosses the blood-brain barrier and increases histone acetylation in R6/2 brains (Hockly et al., 2003a). Moreover, oral administration of this compound (starting at postnatal week 5) significantly improved rotarod performance and reduced the loss of striatal Nissl staining. However, this treatment had no ameliorative effect on body weight loss and higher SAHA doses were shown to be toxic (Hockly et al., 2003a), reflecting a narrow therapeutic window and indicating that HDAC inhibitors should be used with caution. Moreover, a closer scrutiny of the reported positive effects also suggests that they were mild. For example, SAHA treatment appeared to induce an analogous improvement in the rotarod performance of wild-type mice. Furthermore, the effects on the morphology of striatal neurons were only descriptive and no objective quantification was reported.

Sodium butyrate (SB) is another HDAC inhibitor that was tested in R6/2 mice (Ferrante et al., 2003). Intraperitoneal (i.p.) administration of this compound extended the survival of R6/2 mice in a dose-dependent manner (with the highest dose tested improving the survival of R6/2 mice by 20.8%), improved the rotarod performance and delayed the decrease in body weight observed in R6/2 mice. SB administration reduced brain weight loss and striatal neuronal atrophy, although no effect was observed on the number of striatal ubiquitinated neuronal intranuclear inclusions (NIIs) (Ferrante et al., 2003). Furthermore, treatment with SB increased the acetylation of histones H3 and H4 and of the transcription factor specific protein-1 (Sp1) in R6/2 brains. The consequent improvement of transcriptional regulation (as demonstrated by microarray analysis) resulted in an increased expression of proteins involved in transcription and metabolism, which, according to the authors, might contribute for the observed protective effects of SB (Ferrante et al., 2003).

Since DNA/RNA-binding anthracyclines may have therapeutic potential by correcting pathological nucleosome changes and realigning transcription, Stack et al. (2007b) have recently tested the effect of the anthracycline chromomycin in R6/2 mice. Chromomycin treatment (starting at postnatal day 28) realigned chromatin homeostasis, reduced histone H3 hypermethylation, and restored acetylation of histone H4 to wild-type levels. Chromomycin treatment also extended the survival of R6/2 mice by 13.1% and improved some motor abnormalities. Chromomycin-treated R6/2 mice also showed a decrease in gross brain atrophy and the average striatal cell area. Furthermore, chromonycin reduced the brain levels of 8-hydroxy-2-deoxyguanosine (8-OH2dG), a well-established marker of oxidative stress, in R6/2 mice. Importantly, similar results were obtained with a different HD transgenic mouse model (N171-82Q mice) (Stack et al., 2007b).

In another study, the inhibition of histone H3 methylation with the antitumor and antibiotic mithramycin (which binds to guanosine–cytosine-rich DNA sequences and interferes with several transcription factors of the Sp1 family) also proved to have beneficial effects in R6/2 mice (Ferrante et al., 2004). I.p. administration of mithramycin significantly improved the survival of R6/2 mice in a dose dependent manner, with the optimal dose extending the survival of R6/2 mice by 29.1%. Moreover, treatment of R6/2 mice with mithramycin significantly improved rotarod performance and had a positive effect on brain atrophy, ventricular enlargement and striatal neuronal atrophy (Ferrante et al., 2004). The potential beneficial effect of a combined pharmacological treatment with mithramycin and cystamine [a transglutaminase (Tgase) inhibitor; see Section 2.4] in R6/2 mice has also been recently evaluated (Ryu et al., 2006). This combined therapeutic strategy was shown to reduce hyper-trimethylation of histone H3 and to extend R6/2 mice survival over 40%, well beyond any other treatment that has been reported to date in R6/2 mice. In addition, the combined treatment significantly improved rotarod performance, delayed gross brain atrophy, ventricular hypertrophy, striatal neuronal atrophy, and the number of striatal NIIs (likely due to cystamine treatment, as mitramycin alone had no effect on the number of NIIs) (Ryu et al., 2006).

These results suggest that the improvement of gene transcription, namely by acting at the level of histones, may have beneficial effects in HD, especially when combined with other neuroprotective drugs.

2.3. Inhibition of misfolding and oligomerization

Molecular chaperones (including heat shock proteins, HSPs) prevent the misfolding and consequent aggregation of newly synthesized stress-denatured proteins and mutant proteins, thereby reducing the overload of the ubiquitin–proteasome system that is observed in several neurodegenerative diseases (for review see Ross and Poirier, 2004). Furthermore, these proteins co-localize with NIIs in several models of polyglutamine diseases (for references see Hansson et al., 2003), and mutations in HSP70 were shown to increase polyglutamine-

induced toxicity in *Drosophila* (Warrick et al., 1999). To determine whether overexpression of HSPs could have beneficial effects in an *in vivo* HD model, Hansson et al. (2003) crossed R6/2 mice with mice overexpressing HSP70. However, overexpression of this molecular chaperone had no effect on survival, development of paw clasping, and neuropathology (brain weight, striatal atrophy, striatal neuronal atrophy, and number and size of NIIs) of R6/2 mice. Although highly expressed in the hippocampus, cortex and striatum, the cytoplasmic levels of HSP70 seemed reduced in many neurons, with a clear sequestration into NIIs (Hansson et al., 2003). In agreement with this study, cross-breeding R6/2 mice with mice overexpressing a different HSP, HSP27, had no effect on the development of the R6/2 HD phenotype (Zourlidou et al., 2007). Therefore, despite their well-established role as modulators of protein misfolding and consequent aggregation, overexpression of HSPs may delay polyglutamine toxicity only up to a certain level, which may depend on the level of expression of the mutant protein, the size of the polyglutamine tract and the protein context in which it is expressed.

Another study also reported the beneficial effects of Congo red, an azo-dye that binds preferentially to β-sheets containing amyloid fibrils and can specifically inhibit oligomerization (Klunk et al., 1989; Heiser et al., 2000). Congo red administration, either i.p. or through an intracerebroventricular cannula (i.c.v.), promoted the clearance of expanded polyglutamine repeats, reducing the number of aggregates in the basal ganglia of R6/2 mice. Furthermore, this treatment also ameliorated the loss of body weight, inhibited the hind limb dyskinesia (clasping), improved the rotarod performance and prolonged the life span of these transgenic HD mice by 14.2%. Since this treatment was started well after the onset of symptoms (at postnatal week 9), these results strongly indicate that Congo red not only inhibits polyglutamine aggregation, but also promotes the disruption of preformed aggregates, suggesting that aggregation may be crucial for the toxicity of expanded polyglutamine (Sánchez et al., 2003). This is a controversial effect that is open for interpretation. It suggests that the ubiquitin proteasome system is likely to increase the clearance of soluble ubiquitinylated huntingtin peptides, ultimately promoting the slow dissociation of large inclusions and aggregates in the brains of R6/2 mice. In agreement, NIIs have also been found to disappear in another mouse model of HD once the transgene was turned off by administration of doxyciclin, a tetracycline analogue (Tet-Off regulatory system) (Yamamoto et al., 2000). Nevertheless, more recently, Wood et al. (2007) failed to replicate the Congo red data described by Sánchez et al. (2003). Although using a similar dose and administration regime, Congo red treatment had no overall effect on survival, motor and cognitive deficits, as well as on the number of striatal and hippocampal NIIs in R6/2 mice, and the authors suggest that this lack of effect is due to the inability of Congo red to cross the blood-brain barrier (Wood et al., 2007). The reason for this discrepancy is currently not understood, although differences in the degree of permeability of the blood brain barrier between the two mouse colonies may account, at least in part, for these results. However, until this mechanism is fully understood, the relevance of the results reported initially by Sánchez et al. (2003) will remain unknown.

Tanaka et al. (2004) have shown that various disaccharides can reduce polyglutamine aggregates and increase survival in an *in vitro* model of HD by binding to the expanded polyglutamine tails and stabilizing the partially unfolded polyglutamine-containing proteins. Importantly, oral administration of trehalose (the most effective of these disaccharides) starting at 21 days, was also proven to be beneficial in R6/2 mice by decreasing the number of polyglutamine aggregates in their brains, improving their motor dysfunction (as assessed by the rotarod and the footprint tests), and extending their lifespan by 10.14% (Tanaka et al., 2004). To date there has been no demonstration that trehalose crosses the blood brain barrier and therefore the exact mechanism of action of this disaccharide deserves further investigation. Nevertheless, given its lack of toxicity and high solubility, trehalose might be proven to be a good candidate for future clinical trials.

The small molecule C2-8 inhibits polyglutamine aggregation in cell culture and brain slices and rescues neurodegeneration in a *Drosophila* model of HD (Zhang et al., 2005). Therefore, the same group subsequently assessed its therapeutic potential in R6/2 mice (Chopra et al., 2007). C2-8 (administered orally starting at 3 weeks of age) penetrates the blood–brain barrier and is present in the brain at a high concentration. R6/2 mice treated with C2-8 showed improved motor performance (as assessed by the rotarod and the wire-hang endurance tests). Furthermore, C2-8 significantly reduced neuronal atrophy and the size of striatal NIIs. However, C2-8 treatment had no effect on body weight loss and survival of these transgenic mice (Chopra et al., 2007).

Nipecotic acid is a Food and Drug Administration (FDA)-approved compound that significantly reduces mutant huntingtin aggregation and blocks cell toxicity in an inducible cell model of HD (Wang et al., 2005a,b,c). However, since nipecotic acid does not cross the blood–brain barrier, the same group recently evaluated the effects of its analogue, tiagabine (an FDA-approved anti-convulsive drug, which crosses the blood brain barrier) in R6/2 mice (Masuda et al., 2008). Six week-old symptomatic R6/2 mice were treated daily with tiagabine. The highest dose of tiagabine tested significantly increased the survival of R6/2 mice by 17.5%, improved motor performance on the rotarod test at 10 and 12 weeks of age, but had no effect on the loss of body weight. At the neuropathological level, tiagabine treatment attenuated brain atrophy but had no effect on the number of NIIs. Similar beneficial effects were observed in N171-82Q mice. Importantly, the serum levels of tiagabine at effective doses in these transgenic models are comparable to the levels in human patients treated with tiagabine. Thus, tiagabine may be a promising candidate for future clinical trials for the treatment of HD (Masuda et al., 2008).

2.4. Transglutaminase inhibition

Tgase catalyses the formation of γ-glutamyl isopeptide bonds between polyglutamine tracts and lysine residues. It has been suggested that this enzyme is involved in NII formation in HD (Kahlem et al., 1998). However, the number of striatal and cortical aggregates are increased in a transgenic mouse model generated by crossbreeding R6/1 mice with "tissue" Tgase

knock-out mice [R6/1, TG2$^{(-/-)}$ mice], suggesting that this may not be the case (Mastroberardino et al., 2002). Despite an increase in the number of NIIs, the absence of Tgase did improve rotarod performance and extend the life span of R6/1 mice by 12% (Mastroberardino et al., 2002). The rather unexpected finding that an increased level of NIIs is associated with a general improvement of R6/1 symptoms and leads to an increased survival, favors the hypothesis that the formation of aggregates may be a protective mechanism of the cell against the more toxic effect of the soluble oligomeric intermediates of mutant protein, which are believed to be responsible for many of the intracellular disturbances observed in HD (for review, Ross and Poirier, 2004).

The use of cystamine (a classical Tgase inhibitor) as a therapeutic strategy for HD has also been evaluated in R6/2 mice (Dedeoglu et al., 2002; Karpuj et al., 2002). In one study, i.p. administration of cystamine (starting at 7 weeks of age) significantly reduced the abnormal movements (resting tremor, abnormal gait, and grooming), delayed the onset of clasping and the loss of body weight, and slightly increased the mean survival of R6/2 mice by 10.7% (Karpuj et al., 2002). However, no effect on the frequency of NIIs was detected with this treatment paradigm. In another report, Dedeoglu et al. (2002) evaluated the effects of cystamine treatment in R6/2 mice, either by i.p. administration (starting at 21 days of age), or via the drinking water (starting at the prenatal period through oral dosing of pregnant females and continuing post-natal). Cystamine treatment significantly extended the survival of R6/2 mice (the percentage of increased survival varied between 16.8% and 19.5%, depending on the dose and administration paradigm), increased their performance on the rotarod and improved their body weight, decreased the loss of brain weight and the gross brain atrophy and reduced the striatal neuronal atrophy. Furthermore, in this study, a significant reduction in the number of striatal and cortical aggregates was detected at 90 days of age (Dedeoglu et al., 2002). A study by Wang et al. (2005a,b,c) also showed that i.p. administration of cystamine (starting at 8 weeks) ameliorates cerebral glucose metabolism and dopamine (DA) D2 receptor function in the striatum of R6/2 mice, as assessed by micro positron emission tomography imaging. These authors also reported a trend towards an increased locomotor activity in the open field test, a non-significant decrease in the loss of body weight, and a dose-dependent decrease in the size of striatal NIIs in cystamine-treated R6/2 mice (Wang et al., 2005a,b,c). Although one can argue that the discrepancy between these studies regarding the effects of cystamine (i.e., Tgase inhibition) at the level of NIIs formation may reflect dosing differences or different starting times [prenatal/postnatal day 21 (Dedeoglu et al., 2002) versus 7 (Karpuj et al., 2002) or 8 weeks of age (Wang et al., 2005a,b,c)], one should bear in mind that the complete ablation of the *Tgase* gene resulted in an increased number of NIIs in the R6/1 model (Mastroberardino et al., 2002). Clearly, further research is needed to clarify the actual contribution of this enzyme for the formation of NIIs and its role in the pathology of HD. As suggested by Dedeoglu et al. and further corroborated by the study of Wang et al., it might be that the protective effects of cystamine are not directly linked with Tgase inhibition, but rather with its reported antioxidant and antiapoptotic properties (for references see Dedeoglu et al., 2002) and/or its ability to increase brain BDNF levels (Borrell-Pagès et al., 2006).

2.5. Energy supplementation and rescue of metabolic impairment

Mitochondrial dysfunction, oxidative stress and energy depletion have long been implicated in the pathogenesis of HD (for review see Gil and Rego, 2008). Therefore, a possible therapeutic approach consists in the administration of compounds that prevent the depletion of ATP or promote mitochondrial function. Dichloroacetate (DCA), a compound that stimulates the activity of the pyruvate dehydrogenase complex (PDHC) (therefore increasing oxidative phosphorylation), was shown to be therapeutically beneficial in R6/2 mice (Andreassen et al., 2001c). DCA (dosed orally, through the drinking water, from 4 weeks of age) significantly improved rotarod performance, decreased the loss of body weight, and slightly increased the survival of R6/2 mice by 5.8%. At the neuropathological level, DCA prevented striatal neuronal atrophy, but had no effect in the number of striatal and cortical NIIs. These beneficial effects are thought to be directly related with the maintenance of normal levels of active PDHC by DCA, and a resulting decrease in the R6/2 metabolic deficits (Andreassen et al., 2001c).

A decrease in the activities of mitochondrial complexes II–III has been implicated in HD (for review see Gil and Rego, 2008). Since the biosynthesis of uridine nucleotides is directly coupled to the mitochondrial respiratory chain, cells with impaired mitochondrial function become uridine auxotrophs and can be maintained with high micromolar concentration of uridine and pyruvate. The uridine pro-drug 2′,3′,5′-tri-O-acetyluridine (PN401) is a pyrimidine nucleoside that was previously shown to be protective in a 3-nitropropionic acid (3-NP) lesion model of HD (Saydoff et al., 2003). Recently, these findings were extended to the R6/2 mouse model (Saydoff et al., 2006). PN401 was administered orally and the treatment started between postnatal days 30 and 35. PN401 increased R6/2 mean survival by 10%, improved rotarod performance, significantly decreased neurodegeneration in both the cortex and striatum, and decreased the number of striatal NIIs. Importantly, this drug was also shown to have similar beneficial effects in the N171-82Q HD mice model (Saydoff et al., 2006). In another study, the potential therapeutic effect of KP544, another substituted pyrimidine that has been shown to amplify the effects of nerve growth factor (NGF), was also assessed in R6/2 mice (Dey et al., 2007). Six week-old female R6/2 mice received KP544 orally and were subsequently tested (from 8 to 12 weeks of age) on a battery of motor tasks (clasping assessment, rotarod performance, and spontaneous activity in the open field). KP544 treatment improved rotarod performance, reduced the hind limb clasping phenotype, and delayed the onset of hypoactivity characteristic of R6/2 mice. At the neuropathological level, KP544 treatment reduced the enlargement of the lateral ventricles in R6/2 mice (Dey et al., 2007). However, the effect of KP544 treatment on other neuropathological hallmarks of the disease (e.g., number of NIIs) as well as on R6/2 survival was not addressed in this study.

Adenosine is an important modulator that regulates various physiological functions through the activation of its receptors (A1, A2a, A2b, and A3). In early stages of HD (grade 0) there is a major loss of excitatory A2a receptor [abundant in striatal γ-aminobutyric acid (GABA)-containing neurons] binding in the caudate nucleus, putamen and globus pallidus externus (Glass et al., 2000). Thus, the potential benefits of CGS21680 (CGS; a selective agonist of adenosine A2a receptors) were evaluated in the R6/2 HD mouse model (Chou et al., 2005). Daily i.p. administration of CGS (starting at 7 weeks) improved rotarod performance, but had no effect on the life span and body weight of R6/2 mice. GCS-treated animals showed a decrease in the enlargement of the lateral ventricles and a correspondent increase in brain weight and a reduction in the size of NIIs. Functionally, CGS treatment reversed the elevated choline/creatine ratio (indicative of metabolic impairment and neurodegeneration) and reduced the activation of 5′AMP-activated protein kinase (AMPK), a major metabolic sensor and downstream mediator of stress-induced pathways, suggesting that CGS-induced activation of A2a receptors modulates abnormal metabolic pathways in R6/2 mice. In agreement with an improved energy metabolism, CGS administration also decreased the elevated blood glucose levels in diabetic R6/2 mice (Chou et al., 2005). Further experiments will be needed to evaluate the effects of an early administration of this compound on survival, body weight and frequency of NIIs in the R6/2 mouse model. In a more recent study, an opposite approach was tested, whereby therapeutic inhibition of the adenosine A2a receptors was achieved by chronic treatment with the selective antagonist SCH 58261 (Domenici et al., 2007). Starting from 5 weeks of age, R6/2 mice were treated daily with SCH 58261 for 7 days. This regime fully prevented the alterations in anxious responses displayed by R6/2 mice (assessed by the plus-maze test), but had no effect on the impairment in motor coordination (assessed by the rotarod test). At the electrophysiological level, SCH 58261 treatment abolished the increase in N-methyl-D-aspartate (NMDA)-induced toxicity in R6/2 corticostriatal slices (Domenici et al., 2007). Because the results obtained by either activating (Chou et al., 2005) or inhibiting (Domenici et al., 2007) adenosine A2a receptors at similar symptomatic phases of the disease (5 or 7 week-old) were found to trigger neuroprotective effects in R6/2 mice, one may deduce that A2a receptors can mediate different intracellular mechanisms and thus it is not clear whether therapeutic approaches aimed at targeting this receptor subtype will have beneficial outcomes in HD patients.

Creatine kinase and its substrates creatine and phosphocreatine form an energy-buffering and transport system between sites of energy production and energy consumption. As a result, creatine prevents the depletion of the cellular energy reserves. Moreover, it is believed that creatine can play a role in the maintenance of intracellular calcium homeostasis (for review see Hemmer and Wallimann, 1993). Two studies have demonstrated that dietary supplementation with creatine has beneficial effects in R6/2 mice, either when the treatment started at a pre-symptomatic stage (21 days) (Ferrante et al., 2000) or at a symptomatic stage (6 and 8 weeks) (Dedeoglu et al., 2003). In the first study, oral administration of creatine from 21 days onwards significantly increased the survival of R6/2 mice by 17.4%. This treatment paradigm also improved rotarod performance throughout the entire life span of R6/2 mice, increased the gain of body weight, delayed the loss of brain weight (from 6 to 10 weeks of age) and reduced gross brain and striatal atrophy, and the number of striatal NIIs. Interestingly, creatine supplementation also had an effect on the pancreatic pathology, by decreasing the number of huntingtin aggregates in the pancreas of 90 days-old R6/2 mice and delaying the onset of diabetes (assessed by a glucose tolerance test performed at 8.5 weeks of age) (Ferrante et al., 2000). The survival of R6/2 mice was still improved when the administration of creatine was initiated at later stages (Dedeoglu et al., 2003). Thus, initiation of the treatment at 6 weeks of age resulted in an increase in survival by 14.4%, while at 8 weeks of age the survival increased by 9.7%. Dietary creatine supplementation started either at 6 or 8 weeks of age also resulted in an improvement of the rotarod performance. However, effects on the body weight loss and neuropathology were only observed in mice treated with creatine from 6 weeks of age. In this group, creatine treatment resulted in an increase in brain weight and striatal area, and a decrease in gross brain atrophy, striatal neuronal atrophy and number of striatal NIIs (Dedeoglu et al., 2003). The beneficial effects of creatine seem to be directly related with an increase in striatal ATP levels (Dedeoglu et al., 2003), and an overall improvement of the metabolic state of R6/2 mice. Dietary creatine supplementation was also reported to have similar beneficial effects in another transgenic mouse model of HD (N171-82Q mice) (Andreassen et al., 2001a). However, the effects of creatine in these HD mouse models could not be reproduced in HD patients. Indeed, one year of creatine intake (5 g/day, at a concentration reported to improve muscle functional capacity in healthy subjects and patients with neuromuscular disease) did not improve functional, neuromuscular or cognitive status in stages I and III HD patients (Verbessem et al., 2003).

2.6. Therapies aimed to ameliorate the diabetic phenotype

As stated above (Section 1), 10–25% of HD patients have been suggested to display altered glucose metabolism (Podolsky et al., 1972; Farrer, 1985). However, these estimations were based on questionnaires and thus no robust clinical evidence supporting the relationship between HD and diabetes has been shown to date, making it difficult to interpret the actual relevance for HD of therapies aimed to treat the diabetic phenotype. Nevertheless, it is reasonable to speculate that while these treatments might not be effective in mitigating the neurological impairment, they might improve certain peripheral symptoms, and thus the general well-being and quality of life of patients who might suffer from HD-associated metabolic dysregulation. Interestingly, the diabetic phenotype is evident in the R6/2 mouse model (Hurlbert et al., 1999; Luesse et al., 2001; Andreassen et al., 2002; Björkqvist et al., 2005) and may be due to impaired gene expression (Andreassen et al., 2002) and exocytotic defects (Björkqvist et al., 2005). Thus, this model has also been used to evaluate the efficacy of various anti-diabetes treatments.

An interesting therapeutic approach based on immunization against mutant huntingtin was experimented by Miller et al. (2003). In this study, 5 and 7 week-old R6/2 mice were vaccinated with a plasmid encoding a huntingtin N-terminal

fragment with 17 amino acids and 103 glutamine residues fused to green-fluorescent protein (pHD103-GFP). Interestingly, DNA vaccination prevented the development of the R6/2 diabetic phenotype (as detected by an increased insulin immunoreactivity in β-cells and normal blood glucose levels after an overnight fast or a glucose challenge), although these beneficial effects were not correlated with a decrease in the number of aggregates in the pancreatic islets. However, DNA vaccination had no effect on body weight, progression of motor symptoms and neuropathology of R6/2 mice (Miller et al., 2003). Although the authors admit that the observed beneficial effects on the diabetic phenotype may be due, at least in part, to a nonspecific stimulation of the immune system (Miller et al., 2003), DNA vaccination may be an option for the treatment of this peripheral abnormality.

In another study, Hunt and Morton (2005) evaluated the effect of two oral hypoglycemic agents commonly used in the treatment of diabetic patients in the development of atypical diabetes in R6/2 mice. The mice responded acutely to glibenclamide (which induces exocytosis of insulin) but not to rosiglitazone (which induces sensitization to insulin), supporting the idea that in R6/2 mice, the diabetes is caused by impairment in insulin release rather than insulin insensitivity (Björkqvist et al., 2005). However, chronic treatment with these hypoglycemic agents (starting at 5.5 weeks of age) had no effect on survival, weight loss, rotarod performance, and the course of diabetes (as determined by onset of glycosuria and blood glucose levels) (Hunt and Morton, 2005). Factors such as intracellular availability of insulin vesicles ready to be released, and abnormalities in the exocytotic machinery (which have been already reported in the R6/2 model) may contribute to the inefficacy of this chronic treatment.

A recent study has also evaluated the effect of metformin, a widely used anti-diabetes drug, in R6/2 mice (Ma et al., 2007). The treatment started at 5 weeks of age, and was shown to prolong the survival of male HD mice by 20.1% and decrease hind limb clasping in 11-week-old mice. However, metformin was not effective in female HD mice (Ma et al., 2007), and the reasons for this gender discrepancy remain unknown.

2.7. Antioxidants

In line with mitochondrial dysfunction and oxidative stress playing a role in HD (for review see Gil and Rego, 2008), increasing the antioxidant defenses may also prove to be beneficial, and several antioxidant compounds have been tested in R6/1 or R6/2 mice. In one study, the diet of R6/1 mice was supplemented with several essential fatty acids (linoleic acid, γ-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, α-lipoic acid, d-α-tocopherol acetate) from conception and throughout adulthood (Clifford et al., 2002). The essential fatty acids linoleic acid and γ-linolenic acid are crucial for the regulation of membrane proteins and constitute central components in several neuronal signaling pathways, whereas α-lipoic acid (discussed below) and α-tocopherol (vitamin E) are antioxidant compounds. This dietary supplementation showed to be effective in ameliorating some of the behavioral abnormalities (such as clasping, the locomotor impairment, the reduced rearing and the enhanced grooming) that are characteristic of the R6 phenotype. Furthermore, this treat-

ment seemed to increase the survival of R6/1 mice (within the 43-week period of the study, the mortality appeared to decrease from 42% in the placebo-treated mice to 15% in the animals treated with essential fatty acids). However, the combination of essential fatty acids had no significant effect on the loss of body weight, and on the decreased expression of DA D1 and D2 receptors, suggesting that the fatty acids do not act at the level of the transcriptional abnormalities observed in these mice (Clifford et al., 2002). Systemic administration of α-lipoic acid (starting at 4 weeks of age) has also been shown to significantly improve the survival of R6/2 mice, although no effect on body weight was observed (Andreassen et al., 2001b). The beneficial effects of this treatment may be related with the antioxidant properties of lipoic acid, which has been shown to improve mitochondrial function, decrease oxidative damage and lipid peroxidation and increase several antioxidant defenses, such as α-tocopherol, ascorbate (vitamin C) and reduced glutathione (Hagen et al., 1999; Arivazhagan et al., 2001). However, it should be pointed out that the effect of α-lipoic acid administration was only modest, leading to an increase in the survival of R6/2 mice by 7.1% (Andreassen et al., 2001b). Therefore, one can speculate that the primary effect of α-lipoic acid may not necessarily be at the level of the CNS.

It has been shown that the levels of the antioxidant ascorbate are reduced in the striatal extracellular fluid of R6/2 mice during periods of behavioral activation (Rebec et al., 2002). I.p. administration of ascorbate to symptomatic R6/2 mice (from 6 to 10 weeks of age) prevented the decrease of this vitamin in the striatal extracellular fluid when the animals became behaviorally active (Rebec et al., 2003). According with a role of ascorbate in the behavioral phenotype of R6/2 mice, this treatment significantly reduced stereotypical hind limb grooming and increased the performance in a plus-maze test, although no effect was observed on the overall locomotion (evaluated in an open field apparatus) (Rebec et al., 2003). Furthermore, restoring the levels of striatal extracellular ascorbate to wild-type levels was enough to decrease striatal impulse activity (which was shown to be significantly elevated in these animals), suggesting a role for ascorbate in normalizing neuronal function in the striatum of R6/2 mice (Rebec et al., 2006).

Recently, Smith et al. (2006) have performed a dose ranging study administering co-enzyme Q_{10} (an essential cofactor of the mitochondrial electron transport chain that can act as a potent antioxidant) in R6/2 mice. The treatment was initiated at postnatal day 28 and high doses of co-enzyme Q_{10} significantly extended survival in R6/2 mice up to 25.3%. Co-enzyme Q_{10} treatment also resulted in a marked improvement in motor performance and grip strength, a reduction in weight loss, brain atrophy, and NIIs in R6/2 mice. Furthermore, this treatment elevated co-enzyme Q_{10} plasma levels and significantly increased brain levels of co-enzyme Q_9, co-enzyme Q_{10}, and ATP, while reducing the levels of the oxidative damage marker 8-OH2dG (Smith et al., 2006).

The administration of tauroursodeoxycholic acid (TUDCA), a bile acid with antioxidant and antiapoptotic properties, starting at an early symptomatic stage (6 weeks of age) was also shown to have beneficial effects in R6/2 mice (Keene et al., 2002). Surprisingly, in contrast to the studies reporting virtually no striatal cell death until a very late stage of the

R6/2 phenotype (Iannicola et al., 2000; Turmaine et al., 2000; Yu et al., 2003), these authors were able to observe a marked increase in the number of apoptotic cells [TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling)-positive cells] in the striatum of R6/2 mice at 13 weeks of age, a striking finding that was virtually abolished by TUDCA. Furthermore, TUDCA decreased cerebral atrophy, increased striatal volume and significantly reduced the number and size of striatal NIIs. At the behavioral level, TUDCA administration was shown to increase the activity in the open-field of 10 week-old R6/2 mice as well as the general rotarod performance of 10–12 week-old animals (Keene et al., 2002). However, it is difficult to discern the actual robustness of the effect of TUDCA on this behavioral parameter.

The potential beneficial effect of BN82451, a novel compound with antioxidant and anti-inflammatory properties, has also been evaluated in R6/2 mice (Klivenyi et al., 2003). Oral administration of BN82451 (starting at 4 weeks of age) significantly delayed the onset of the HD phenotype and increased R6/2 mean survival by 15.3%. Although no effect on the loss of body weight was observed, 10 week-old BN82451-treated R6/2 mice performed significantly better in the rotarod, and showed a decrease in gross brain atrophy, ventricular enlargement, striatal neuronal atrophy and in the number of striatal NIIs (Klivenyi et al., 2003).

Metals like iron, copper or zinc, which appear to be increased in brains of HD patients (Dexter et al., 1991), may participate in the generation of reactive oxygen species and therefore promote oxidative stress. Therefore, the use of metal-binding compounds can prove to be an efficient antioxidant strategy. In line with this idea, Nguyen et al. (2005) tested the efficiency of the metal-binding compound clioquinol (5-chloro-7-iodo-8-hydroxy-quinoline) in mitigating the development of the HD phenotype in R6/2 mice. Orally administration of clioquinol (starting at 3 weeks of age) significantly extended the life span of R6/2 mice by 20%, increased the body weight of 10 and 11 weeks-old mice, decreased the incidence of clasping and improved rotarod performance. At the neuropathological level, clioquinol treatment reduced the enlargement of the lateral ventricles (an indication of striatal atrophy) and significantly decreased the number of cortical and striatal NIIs. Furthermore, clioquinol-treated 11 week-old R6/2 mice showed normal fasting blood glucose and insulin levels comparable to the ones observed in wild-type controls, indicating that this treatment also ameliorated the diabetic phenotype of these mice (Nguyen et al., 2005).

Supporting the overall results that have been obtained when treating R6 mice with different antioxidants, recent *in vitro* studies have also shown that targeting mitochondrial defects induced by mutant huntingtin can rescue striatal neurons (Bae et al., 2005; Benchoua et al., 2006). Therefore, drugs directed against mitochondrial dysfunction and oxidative stress may also be efficacious in humans.

2.8.   Inhibition of excitotoxicity

Excitotoxicity has also been implicated in HD (for review see Gil and Rego, 2008), and the potential therapeutic effects of some antagonists of glutamate receptors have been tested in R6 mice. In one of these studies, R6/2 mice were treated either with remacemide (a low affinity NMDA receptor channel blocker), co-enzyme Q_{10} (a lower dose than the one used in the study by Smith et al., 2006, Section 2.7), or a combination of both compounds (Ferrante et al., 2002). Oral administration of remacemide and co-enzyme Q_{10} alone (starting at the early age of 21 days) improved the survival of R6/2 mice, by 15.5% and 14.5%, respectively. However, the combination of both drugs proved to be even more efficient, increasing the survival of R6/2 mice by 31.8%. Similarly, all three paradigms (remacemide, co-enzyme Q_{10}, and remacemide plus co-enzyme Q_{10}) significantly improved the performance of R6/2 mice on the rotarod and decreased the loss of body weight, with the greatest effect achieved when the two drugs were combined. Although all three treatments were shown to have beneficial effects at the neuropathological level (brain weight, gross brain atrophy, and striatal neuron atrophy), again the combination of both compounds produced the best results. Additionally, administration of remacemide plus co-enzyme Q_{10} reduced the loss of striatal volume and the number of striatal NIIs in 9 and 13 week-old R6/2 mice (no results were presented regarding the effect of each drug alone on these neuropathological parameters) (Ferrante et al., 2002). Identical results were observed with another HD transgenic mouse model (N171-82Q mice) (Ferrante et al., 2002). Interestingly, even before the publication of these studies, clinical trials using co-enzyme Q_{10} (Feigin et al., 1996; Huntington Study Group, 2001), remacemide and a combination of both compounds (Huntington Study Group, 2001) were conducted in HD patients with no positive outcome. Indeed, in a multicenter, parallel group, double-blind, randomized clinical trial performed in HD patients showing early symptoms of HD, the administration of remacemide, co-enzyme Q_{10} or a combination of both over a period of 30 months, produced no significant effect on the functional decline observed in early HD (Huntington Study Group, 2001). It might be possible that the doses given to patients (approximately 5 times less than the doses used in mice) could not produce sufficient pharmacological effects to be protective. Future clinical trials testing the eficacy of higher doses of these compounds in HD patients might help clarify this discrepancy.

A different study reported the beneficial effects of riluzole treatment in R6/2 mice (Schiefer et al., 2002). Riluzole was shown to reduce the release of glutamate through inactivation of voltage-dependent sodium channels on glutamatergic nerve terminals, and through the activation of a G-protein-dependent signal transduction pathway. Moreover, riluzole also blocks some of the postsynaptic effects of glutamate by noncompetitive blockage of NMDA receptors (for review see Doble, 1996) and is currently used in the clinic for the treatment of patients with amyotrophic lateral sclerosis (for review see Bensimon and Doble, 2004). Oral administration of riluzole (starting at 3 weeks of age) significantly increased the mean survival of R6/2 mice by 10.2% and delayed the onset of body weight loss. Analysis of the R6/2 locomotor activity in an open field apparatus revealed an attenuation of the initial hyperactive phase in the animals treated with riluzole. However, no effect on rotarod performance was observed upon riluzole treatment (Schiefer et al., 2002). Furthermore, this compound altered the pattern of striatal NIIs, which were

significantly smaller and appeared remarkably less ubiquitinated and surrounded by several ubiquitinated nuclear micro-aggregates. Based on these observations, these authors speculate that riluzole might induce an increase in the degradation of aggregates (Schiefer et al., 2002). However, one cannot rule out the possibility that riluzole may instead decrease the initial oligomerization of the mutant protein. Nevertheless, in a more recent study, oral administration of riluzole was inefficient at the level of aggregation and did not improve the R6/2 phenotype (body weight loss, rotarod performance, grip strength, and open field activity) (Hockly et al., 2006). Despite these discrepancies, clinical trials of riluzole in HD patients have been performed (Rosas et al., 1999; Seppi et al., 2001; Huntington Study Group, 2003; Landwehrmeyer et al., 2007). In 2003, the Huntington Study Group (2003) conducted an 8-week double-blind dose-ranging multicenter study involving 63 HD patients and found that riluzole treatment (200 mg/day) ameliorated chorea intensity without improving functional capacity or other clinical features of illness. However, in a more recent study involving a total of 379 adult HD patients that were treated with riluzole (50 mg twice daily) for 3 years, no differences in a combined score derived from the motor and total functional capacity subscores of the Unified HD Rating Scale were observed between the patients treated with riluzole and the ones that received placebo (Landwehrmeyer et al., 2007).

The effects of glutamate release inhibition through stimulation of the presynaptic metabotropic glutamate receptor (mGluR) 2 or blockage of the postsynaptic excitatory mGluR5 have also been evaluated in R6/2 mice (Schiefer et al., 2004). In this study, LY379268 (an mGluR2 agonist) or 2-methyl-6-(phenylethynyl)-pyridine (MPEP; an mGluR5 antagonist) were orally administered to R6/2 mice starting at the pre-symptomatic age of 3.5 weeks. Both compounds were found to significantly increase R6/2 survival (MPEP by 13.5%; LY379268 by 9.5%), and to attenuate the early hyperactivity (assessed by the open field test). Additionally, MPEP-treated mice were shown to perform better on the rotarod, but LY379268 had no effect on this test of motor coordination. However, none of the treatments had an effect on the number of NIIs (Schiefer et al., 2004).

Another factor that can contribute for striatal excitotoxicity in R6/2 mice is the down-regulation of the primary astroglial glutamate transporter GLT1, which in turn results in a deficient glutamate uptake by the glial cells. Thus, Miller et al. (2008) have recently tested the effects of ceftriaxone, a beta-lactam antibiotic known to elevate GLT1 expression. Ceftriaxone was administered to symptomatic 6 week-old R6/2 mice and the animals were submitted to behavioral testing for 7 days after receiving the last drug injection. Ceftriaxone attenuated paw clasping and improved motor flexibility (as measured in a plus maze test, and an open-field apparatus). As expected, ceftriaxone induced an increase in striatal GLT1 expression and consequently reversed the deficit in glutamate uptake characteristic of these mice (assessed by microdialysis) (Miller et al., 2008). Given these promising results, future studies should investigate whether ceftriaxone treatment (i.e., an increase in glutamate uptake by the glial cells) will also have a long-term effect on the behavior and neuropathology of R6/2 mice.

Since altered afferent neuronal projections may modulate the release of glutamate, contributing to striatal excitotoxicity (for review see Gil and Rego, 2008), Stack et al. (2007a) examined the effects of deafferentation of the corticostriatal and nigrostriatal pathways in R6/2 mice. Surgical decortication was performed either at 4 or 6 weeks of age through a unilateral cortical aspiration lesion targeting the primary motor cortex, whereas lesion of the nigrostriatal pathway was performed in 6-week-old R6/2 mice through infusion of 6-hydroxydopamine into the substantia nigra. Surprisingly, both cortical and substantia nigra lesions significantly extended the survival of R6/2 mice (decortication at 4 weeks: by 13.4%; decortication at 6 weeks: by 8.25%; nigrostriatal deafferentation at 6 weeks: by 21.29%). Furthermore, both types of lesions improved the hind limb clasping phenotype. At the neuropathological level, decortication reduced the decrease in striatal neuronal area and the number of striatal NIIs, but nigrostriatal deafferentation had no effect on these parameters. Importantly, cortical and substantia nigra lesions resulted in a significant decrease in the striatal levels of either glutamate or glutamate and DA respectively, a change that was accompanied by a reduction in the levels of 8-OH2dG and an increase in the N-acetyl aspartate/creatine ratio, which might reflect an improvement in neuronal survival (Stack et al., 2007a).

To summarize, some of the treatments that target excitotoxicity, as well as mitochondrial dysfunction and oxidative stress, and that have already been shown to be beneficial in transgenic mouse models (e.g., riluzole) may ameliorate some of the symptoms shown by HD patients. Moreover, the combined use of some of these strategies may produce cumulative beneficial effects. However, more clinical trials in HD patients are warranted in order to investigate the actual value of these combined therapies.

2.9.   Caspase inhibition

As discussed above (Section 1), in the R6 model striatal neuronal death is very limited and most of the studies reported so far were only able to detect the occurrence of "dark cell degeneration" in a limited number of striatal neurons in very late stages of the R6/2 phenotype (Iannicola et al., 2000; Turmaine et al., 2000; Yu et al., 2003). Nevertheless, the fact that caspase-1 can cleave both mutant and wild-type huntingtin in vitro (Wellington et al., 1998) raised the question whether inhibition of this caspase could interfere with the progression of the disease. Therefore, Ona et al. (1999) crossbred R6/2 mice with transgenic mice expressing a dominant-negative mutant of caspase-1 in the brain (Ona et al., 1999). Expression of the dominant-negative caspase-1 mutant extended the life span of R6/2 mice by 20% and significantly delayed the onset of the disease by 7.3 days. Moreover, this genetic manipulation improved rotarod performance, delayed the loss of body weight and the appearance of NIIs, and significantly inhibited the alterations in the expression of adenosine A2a, and DA D1 and D2 receptors (Ona et al., 1999). Furthermore, in the same study the authors showed that continuous i.c.v. administration of Val-Ala-Asp-fluoromethyl ketone (zVAD-fmk, a broad caspase inhibitor), starting at 7 weeks of age, also delayed mortality in the R6/2

mice by 25%, and increased their rotarod performance (Ona et al., 1999). These results supported the idea that caspase-1 was involved in HD. Besides its role in apoptosis, caspase-1 is also known to be a mediator of the inflammatory response. However, the mechanism by which this enzyme contributes for the neuropathology of R6/2 mice is still not clear, although caspase-1-mediated cleavage of mutant huntingtin is unlikely to play a role in this transgenic model (which expresses only 3% of the entire mutant protein).

An alternative strategy that can be applied to decrease apoptosis is the up-regulation of anti-apoptotic members of the Bcl-2 family (e.g., Bcl-2 and Bcl-X$_L$). Based on this assumption, Zhang et al. (2003) crossed R6/2 mice with mice over-expressing Bcl-2 in neurons. Double transgenic mice demonstrated a statistically significant delay in the onset of motor symptoms and an increase in survival by 10.3%. Behaviorally, over-expression of Bcl-2 only resulted in a non-significant trend towards a better rotarod performance, and the effects of this anti-apoptotic protein were not evaluated at the neuropathological level (Zhang et al., 2003). Although the results from this study point out for an involvement of the Bcl-2 family in the R6/2 phenotype (possibly through the regulation of mitochondrial permeability and caspases activation), no mechanistic results were presented in order to clearly understand the increased survival observed in the double transgenic mice.

Tetracyclines are broad-spectrum antibiotics that have been shown to inhibit caspases and prevent the mitochondrial release of pro-apoptotic factors (Scarabelli et al., 2004) and to inhibit aggregation in several models of amyloidoses (Forloni et al., 2001). Although tetracycline itself does not cross the blood–brain barrier, its derivatives, doxycycline and minocycline, can enter the brain. Based on these properties, several groups have studied the effects of tetracycline derivatives in R6/2 mice, reporting contradictory results (Chen et al., 2000; Smith et al., 2003; Wang et al., 2003). In the first published study, i.p. administration of minocycline (starting at 6 weeks of age) significantly extended the survival of R6/2 mice by 14% and delayed the decline in rotarod performance (Chen et al., 2000). However, unlike the expression of the dominant-negative caspase-1 transgene (Ona et al., 1999), minocycline-mediated inhibition of caspase-1 did not affect NIIs formation (Chen et al., 2000). Since NIIs can be first detected in the cortex and striatum as early as 3.5 weeks (Meade et al., 2002), the fact that minocycline treatment was started at 6 weeks of age (when NIIs are already widely distributed throughout the brain), may account for this lack of effect. Minocycline was efficient in reducing the activities of caspase-1 and the inducible isoform of nitric oxide synthase (iNOS) and decreasing the up-regulation of caspases 1 and 3 expression in R6/2 brains. On the other hand, the combined i.c.v. administration of Tyr-Val-Ala-Asp-chloromethyl ketone (YVAD-fmk, a caspase-1-like inhibitor) and Asp-Glu-Val-Asp-fluoromethyl ketone (DEVD-fmk, a caspase-3-like inhibitor) (starting at 7 weeks of age) significantly improved the rotarod performance and extended the survival of R6/2 mice by 17.3% (a higher increase than the one obtained with minocycline) (Chen et al., 2000). Thus, the authors speculated that the observed protective effects of minocycline were related with caspases inhibition (Chen et al., 2000). A follow-up study also

demonstrated that i.p. administration of minocycline (from 8.5 to 10.5 weeks of age) significantly inhibited the mitochondrial release of the apoptotic proteins AIF (apoptosis inducing factor), Smac/DIABLO [second mitochondria-derived activator of caspase/direct IAP (inhibitor of apoptosis protein) binding protein with low pI], and cytochrome c, and decreased the activation of caspases 1, 3 and 9 and the cleavage of the pro-apoptotic factor Bid in brains of symptomatic R6/2 mice (Wang et al., 2003). These results suggested that minocycline acts upstream of caspases (at the mitochondrial level) and can interfere with both caspase-independent (AIF) and -dependent (Smac/DIABLO and cytochrome c) mitochondrial cell death pathways (Wang et al., 2003). However, although mitochondrial dysfunction (and the consequent metabolic impairment and oxidative stress) and caspases activation (and the resulting cleavage of a wide variety of intracellular proteins, including normal and mutant huntingtin) can contribute to a generalized cellular dysfunction, one should take into account that apoptosis is a rare event in R6/2 brains. Thus, and as mentioned above, the effect that minocycline may have on caspases-mediated mutant huntingtin cleavage cannot be properly evaluated in the R6 models. On the other hand, Smith et al. (2003) failed to reproduce the protective effects of minocycline on the R6/2 phenotype. In this study, both tetracycline derivatives doxycycline and minocycline significantly reduced aggregation in R6/2 hippocampal slice cultures. However, oral administration of doxycycline or minocycline (in doses that achieve concentrations in the brain close to the ones reported to inhibit aggregation in vitro) had no effect on the loss of body weight, rotarod performance, grip strength and aggregate load in R6/2 mice. Interestingly, both tetracycline derivatives reduced the hyperglycemia seen in late stage R6/2 mice, although this protective effect was not due to a decrease in the number of aggregates in the islets of Langerhans (Smith et al., 2003).

A recent study has also evaluated the combined effects of minocycline and co-enzyme Q$_{10}$ in R6/2 mice (Stack et al., 2006). The combined treatment started when the animals were 26 day-old and was shown to provide enhanced beneficial effects when compared to both compounds administered alone. Thus, while minocycline and co-enzyme Q$_{10}$ alone extended the survival of R6/2 mice by 11.2% and 14.6%, respectively, the combination of minocycline and co-enzyme Q$_{10}$ extended R6/2 survival by 18.2%. Furthermore, the combined therapy also improved rotarod performance, attenuated gross brain atrophy, striatal neuron atrophy and huntingtin aggregation to a greater degree than either minocycline or co-enzyme Q$_{10}$ alone (Stack et al., 2006).

Despite the contradictory results that have been reported in the R6/2 mouse model, clinical trials have been performed to evaluate the potential beneficial effects of minocycline in HD patients (Bonelli et al., 2004; Huntington Study Group, 2004; Thomas et al., 2004). The first two studies only evaluated the tolerability of this drug in HD patients and both concluded that administration of minocycline over a period of 8 weeks (Huntington Study Group, 2004) and 6 months (Thomas et al., 2004) was well tolerated and no serious adverse events were noted. A follow-up two-year study conducted in 11 HD patients reported an improvement in the general motor and neuropsychological function of the patients, followed by

stabilization at the endpoint of the study. Minocycline-treated patients also showed a significant amelioration of their psychiatric symptoms as evaluated by several HD rating scales (Bonelli et al., 2004). Although these results look promising and indicate a neuroprotective effect of this agent in HD (possibly by inhibiting apoptotic neuronal death in the striatum), a long-term, double-blinded, placebo-controlled trial should be conducted to definitively establish the value of minocycline in HD.

2.10. Alternative pharmacological therapeutic strategies

Several other drugs have been tested in the R6 HD transgenic lines. Lithium has been commonly used as a treatment for bipolar disorder, showing beneficial effects during both phases of the disease (mania and depression). Furthermore, lithium was shown to increase the levels of Bcl-2 and to prolong survival and the growth and regeneration of axons in the brain [for references see (Wood and Morton, 2003)]. Based on these anti-apoptotic and neuroprotective properties and on the fact that HD patients also suffer from depression, Wood and Morton (2003 evaluated the effects of chronic administration of lithium chloride in R6/2 mice. Lithium treatment was initiated both at pre- and post-symptomatic stages, but no overall effect on survival was observed in either of the groups. Surprisingly, lithium treatment seemed to cause distinct effects in the post-symptomatic group, depending on the initial weight of the animals. In lighter mice, lithium treatment had adverse effects, whereas in heavier mice treatment with lithium showed a significant improvement on motor performance, although no effects were detected on animal survival and loss of body weight (Wood and Morton, 2003). These disparate results are not clearly understood, although the authors speculate that they may be due to differences in the ability of individual mice to clear lithium chloride from the plasma (Wood and Morton, 2003). Nevertheless, the fact that the deleterious effects were observed in lighter mice suggests that lithium chloride can interfere and aggravate the metabolic abnormalities observed both in R6/2 mice and HD patients.

Inflammation is thought to play a role in the pathogenesis of HD. Although no major signs of inflammation have been detected in R6 brains, the up-regulation of certain genes involved in inflammation (Luthi-Carter et al., 2000) and the activation of iNOS and caspase-1 (enzymes that are known to be involved in the inflammatory response) (Chen et al., 2000) have been reported to occur in R6/2 mice. Within this context, a recent study evaluated the potential therapeutic benefits of two commonly used anti-inflammatory drugs (acetylsalicylate and rofecoxib, inhibitors of cycloxygenases 1 and 2, respectively) in both N171-82Q and R6/2 transgenic mouse models of HD (Norflus et al., 2004). However, the administration of both drugs from weaning (at doses comparable to those tolerated by humans) had no significant effect on survival, behavioral changes, loss of body weight, or gross cerebral and striatal atrophy of both N171-82Q and R6/2 mice (Norflus et al., 2004), suggesting that inflammation is not a major contributor for the pathology of these mice.

Gabapentin (GBP), an anti-convulsant widely used in the treatment of epilepsy and neuropathic pain syndrome, and its derivative gabapentin-lactam (GBP-L) have been shown to have neuroprotective properties that are mediated by the GBP-L-induced opening of mitochondrial ATP-dependent K^+ channels (for references see Zucker et al., 2004). The efficacy of these compounds in ameliorating the R6/2 phenotype was tested through the subcutaneous implantation of GBP- or GBP-L-delivering pumps into 6 weeks-old R6/2 mice (Zucker et al., 2004). GBP-L treatment resulted in a non-significant trend towards an increased survival of about 14%, increased motor function and coordination, and a reduction in the size and density of cytoplasmic and nuclear aggregates in the striatum, neocortex and hippocampus of R6/2 mice. On the contrary, GBP had no effect on the R6/2 phenotype, further suggesting that conversion into GBP-L is required for neuroprotection (Zucker et al., 2004). The precise mechanism behind the observed beneficial effects of GBP-L was not addressed in this study. However, as the authors suggest, the GBP-L-induced opening of mitochondrial ATP-dependent K^+ channels may result in an improvement of neurotransmission, especially of glutamate release (which is known to be altered in R6/2 mice). Alternatively, modulation of these channels by GBP-L may also improve mitochondrial function (which, as mentioned above, is also disturbed in R6/2 mice). Since the occurrence of epileptic seizures have been reported to occur in R6/2 mice, especially during the late stage of the disease (Mangiarini et al., 1996; Gil et al., unpublished observations), this therapeutic approach is of particular relevance, and would be interesting to see if the incidence of epileptic episodes decrease in animals treated with this anticonvulsant compound.

Since deregulated gene expression, reduced neurotransmitter levels, and abnormal synaptic function contribute to the development of the HD-like phenotype in R6/2 mice, a recent study tested the effects of a combination of drugs aimed to enhance synaptic function, on the cognitive deficits of R6/2 mice (Morton et al., 2005a). The drugs used were tacrine (an acetylcholinesterase inhibitor that prevents the degradation of acetylcholine), and moclobemide (an antidepressant that reversibly inhibits monoamine oxidase A, preventing the breakdown of noradrenaline and 5-hydroxytryptamine), both reported to increase the levels of transmitters in the brain [for references see (Morton et al., 2005a)]. Furthermore, due to its reported beneficial effects in R6/2 mice (Section 2.5), creatine was also added to this drug combination (Morton et al., 2005a). The triple treatment (started at 5 weeks of age) significantly improved the performance of R6/2 mice on spatial tasks of the Morris water maze test, as well as on a T-maze test. In contrast to these cognitive improvements, the combined treatment had only a minor effect on the survival of R6/2 mice and no significant effects were detected on body weight, rotarod performance, and number of NIIs. However, the triple treatment had an effect on the progression of the diabetic phenotype, significantly delaying the onset of glycosuria. Mechanistically, the observed cognitive improvement is likely to be related with a significant effect of the triple treatment on global gene expression patterns. Indeed, many genes significantly up- or down-regulated in untreated transgenic animals returned to normal levels in the triple-treated R6/2 mice (Morton et al., 2005a).

As stated above (Section 1), there is a disintegration of the normal sleep–wake cycle (Petersén et al., 2005) and circadian

rhythms in R6/2 mice (Morton et al., 2005b), and this deregulation can contribute, at least in part, to the cognitive deficits observed in these transgenic mice. Thus, in a recent study R6/2 mice were treated with the sedative drug chloral hydrate or alprazolam (a short-acting benzodiazepine) to test whether circadian and cognitive disturbances could be reversed by imposing a daily cycle of sleep (Pallier et al., 2007). Daily treatment with chloral hydrate (from 9 to 16 weeks of age) or alprazolam (either from 4 to 16 weeks, from 9 to 16 weeks, or from 10 to 17 weeks of age) markedly improved cognitive performance of R6/2 mice in a two-choice visual discrimination task. Moreover, alprazolam treatment significantly improved the performance of R6/2 mice on the rotarod test. However, no differences in the overall survival of R6/2 mice were detected following alprazolan treatment, whereas chloral hydrate had a negative effect on body weight and survival of these mice (Pallier et al., 2007). Nevertheless, these results show, for the first time, that treatments aimed at restoring circadian rhythms may slow the cognitive decline characteristic of the HD phenotype.

2.11.　Transplantation

Transplantation of embryonic stem cells may be an alternative therapeutic approach for the treatment of HD. Neural transplantation studies have been performed in animal models that show marked neuronal death (for review, Lindvall et al., 2004). The R6 mice differ in this respect because they do not exhibit overt striatal cell loss. Therefore, the aim of neural grafting in R6 mice would be to replace or improve the function of dysfunctional neurons. So far, only two studies have analyzed the effects of neuronal transplantation in R6 mice (Dunnett et al., 1998; van Dellen et al., 2001). One of these studies evaluated the survival and differentiation of striatal grafts and their effects on the neurological deficits of R6/2 mice (Dunnett et al., 1998). Striatal tissue was harvested from embryonic day 14 wild-type mouse embryos and stereotactically injected into the striatum on both sides of the brain of 10 week-old R6/2 mice. Despite of good survival and apparent integration within the host brain, the grafts had no effect on the loss of body weight and the behavioral deficits were only minimally improved (as assessed by the open field test) (Dunnett et al., 1998). The lack of positive effects upon transplantation may be due to a late intervention, and the same procedure could be repeated in younger pre-symptomatic animals in order to clearly evaluate the effects of striatal grafts on disease progression. In a subsequent study, van Dellen et al. (2001) transplanted wild-type cortex from embryos at day 16 into the anterior cingulate cortex of neonatal R6/1 mice. This procedure had no effect on the performance of the mice on the horizontal rod test, but was able to delay the onset of paw clasping, suggesting that this cortical region (which is one of the sources of the corticostriatal fibers that innervate the striatum) is involved in the development of some of the motor deficit characteristics of this HD model (van Dellen et al., 2001). The effects of this repair strategy at the level of survival, loss of body weight and neuropathology were not evaluated on this study, and therefore limited conclusions can be inferred regarding the beneficial effects of this surgical procedure.

A dysfunction of the endosonal/lysosomal pathways might contribute to protein aggregation and neurodegeneration in HD (for review see Gil and Rego, 2008). C17.2 neural stem cells have the potential of differentiating into a neuronal phenotype and were shown to be effective in the treatment of a variety of lysosomal storage disorders (for references see Yang and Yu, 2009). Thus, in a very recent study, the efficacy of C17.2 neural stems cells was tested in R6/2 mice. Mice received a diet enriched with trehalose (which was previously shown to decrease aggregate formation; see Section 2.3) starting at 3 weeks of age and at 8 weeks C17.2 cells were transplanted into their lateral ventricles. This combined treatment decreased ubiquitin-positive aggregation in the striatum, reduced the enlargement of the lateral ventricles, delayed the onset of clasping, improved motor function (assessed by the footprint test), and extended R6/2 life span by up to 26.3% (Yang and Yu, 2009), providing a strong basis for the development of further transplantation protocols for the treatment of HD.

2.12.　Genetic manipulations

Gene therapy has been regarded as an attractive alternative therapeutic strategy for various neurodegenerative diseases. Moreover, since the primary cause of HD was identified as a mutation in the *huntingtin* gene (Huntington's Disease Collaborative Research Group, 1993), suppressing the neuronal expression of mutant *huntingtin* is expected to delay the onset and mitigate the severity of the disease. Therefore, a recent study reported the use of small interfering RNAs (siRNAs) directed against the *huntingtin* gene to repress the expression of the transgenic mutant protein in R6/2 mice (Wang et al., 2005a,b,c). I.c.v. administration of siRNAs at postnatal day 2 significantly extended the longevity of R6/2 mice by more than 2 weeks (12.4%), slowed down the characteristic loss of body weight as well as the onset of paw clasping, and improved the locomotor deficits (assessed by rotarod performance and open field activity). Furthermore, this approach significantly attenuated the enlargement of the lateral ventricles and decreased the number of striatal NIIs near the injection site (Wang et al., 2005a,b,c). Future research will be needed in order to develop safe delivery vectors to target the most affected neurons in the brain. Importantly, in agreement with this study DiFiglia et al. (2007) have demonstrated that a single administration into the adult striatum of an siRNA targeting mutant huntingtin can silence the mutant gene, attenuate neuronal pathology, and delay the abnormal behavioral phenotype observed in a rapid-onset viral transgenic HD mouse model (DiFiglia et al., 2007).

In addition, Huang and collaborators (2007) generated a high-capacity adenoviral (HC-Ad) vector expressing a short hairpin RNA (shRNA) targeted to exon 1 of the *huntingtin* gene. Stereotaxic injection of this construct into the striatum of 5 week-old R6/2 mice resulted in a significant reduction in the number of NIIs (Huang et al., 2007). Since this study only evaluated the effect of this genetic manipulation on the number of aggregates, further studies are warranted to test its effects on survival, behavioral deficits, and other neuropathological features of the R6/2 mouse model.

Several studies have shown that increased levels of glial cell line-derived neurotrophic factor (GDNF) can exert

neuroprotective effects in acute HD animal models generated by intrastriatal injections of excitotoxins (for references see Popovic et al., 2005), making GDNF a good candidate for gene therapy in HD. Therefore, in order to evaluate if this neurotrophic factor was also protective in a chronic mouse model of HD, Popovic et al. (2005) performed intrastriatal injections of lentiviral vectors containing the *gdnf* gene (LV-GDNF) in 5 week-old R6/2 mice. However, although a significant increase in the striatal expression of GDNF was detected in LV-GDNF-injected animals, no behavioral improvements were observed. Furthermore, at the neuropathological level, no significant differences were seen in the number of NIIs, striatal neuronal atrophy, and number or proliferating BrdU-positive cells in the DG of the hippocampus of these mice (Popovic et al., 2005). The fact that the treatment was initiated when the animals were 5 week-old (at an age when NIIs are already formed in the brain), may account for the lack of effect of GDNF. However, as discussed above, several other therapeutic strategies have been shown to be protective, even when started in older animals. Furthermore, grafting of neural stem cells stably expressing GDNF and the firefly luciferase gene prevented the degeneration of striatal neurons and reduced the amphetamine-induced rotational behavior in an excitotoxic HD mouse model (Pineda et al., 2007). Nevertheless, the study by Popovic et al. (2005) proves that overexpression of neurotrophic factors using lentiviral vectors is doable in the R6 mouse model, and therefore, the same techniques may be used to evaluate the effects of overexpression of other neurotrophic factors.

2.13. Therapies aimed to restore neurogenesis

The neuroprotective and neurogenic properties of many growth factors have been well documented in the literature. In particular, fibroblast growth factor 2 (FGF2) has been shown to protect striatal neurons in toxin-induced models of HD, exert trophic effects on striatal neurons, and stimulate proliferation of striatal neural stem cells (for references see Jin et al., 2005). Thus, Jin et al. (2005) evaluated the potential therapeutic effects of FGF2 in R6/2 mice. FGF2 was administered subcutaneously starting at 8 weeks of age. Surprisingly, FGF2 was shown to stimulate cell proliferation in the subventricular zone (SVZ) by 150%, as well as the migration of new neurons from this region into the cerebral cortex and striatum (where they differentiate into DARPP-32-expressing medium spiny neurons). Moreover, FGF2 treatment reduced the number of NIIs, improved rotarod performance, and increased the average survival of these animals by 20%, suggesting that potentiation of SVZ neurogenesis may be used for the treatment of HD (Jin et al., 2005). It remains to be shown whether FGF2 can also restore DG neurogenesis in these mice.

Ependymal overexpression of BDNF stimulates neurogenesis in the SVZ and integration of new neurons in the striatum, a process that can be potentiated by noggin-induced suppression of subependymal gliogenesis and increase in progenitor availability (for references see Cho et al., 2007). Thus, in a recent study 4- and 6-week-old R6/2 mice received intraventricular injections of adenoviral BDNF (AdBDNF) and adenoviral noggin (AdNoggin), which suppress astroglial

differentiation by subependymal zone progenitor cells, thereby promoting their neuronal differentiation. Recruitment of βIII-tubulin-positive and doublecortin-positive neurons, which developed as DARPP-32-positive and became GABAergic medium spiny neurons that expressed either enkephalin or substance P and extended fibers to the globus pallidus, was observed in AdBDNF/AdNoggin-treated R6/2 mice. Importantly, AdBDNF/AdNoggin treatment also improved rotarod performance and open-field activity and increased the survival of R6/2 mice by 16.8% (Cho et al., 2007). Given these promising results, it would be interesting to evaluate the effects of AdBDNF/AdNoggin treatment on the striatal neuropathology of R6/2 mice. Indeed, the importance of BDNF in HD neurodegeneration was previously demonstrated by disrupting the expression of the *bdnf* gene in R6/1 mice (Canals et al., 2004). Decreased levels of this neurotrophin were shown to advance the onset of motor dysfunction and cause the specific degeneration of enkephalinergic striatal projection neurons (the most affected neurons in HD). Importantly, this neuronal dysfunction was restored by administration of exogenous BDNF, suggesting that this neurotrophic factor might help delaying or stopping illness progression (Canals et al., 2004). More recently, CEP-1347, a mixed lineage kinase inhibitor with neuroprotective and neurotrophic effects, was shown to restore the expression of BDNF (Apostol et al., 2008; Conforti et al., 2008) and improve motor performance in R6/2 mice (Apostol et al., 2008).

Erythropoietin (EPO) is a cytokine that is produced in the kidneys and regulates haematopoiesis. EPO has been found to enter the brain after peripheral administration, where it can act through the activation of brain EPO receptors and several studies showed robust neuroprotective effects in various models of acute neurodegeneration (for references see Gil et al., 2004). The natural EPO metabolite asialoerythropoietin (asialoEPO) has the same neuroprotective potency as EPO, can equally cross the intact blood-brain barrier, and has the advantage of not affecting the hematocrit (Erbayraktar et al., 2003). Based on these properties and on the reported neurogenic effect of this cytokine, we evaluated the effects of chronic administration of asialoEPO in the locomotor deficits, neuropathology and reduced hippocampal cell proliferation in R6/2 mice (Gil et al., 2004). However, asialoEPO treatment (starting at 5 weeks of age) had no effects on rotarod performance, paw clasping, open field activity, and any of the neuropathological parameters evaluated (number of striatal NIIs, striatal atrophy and striatal neuronal atrophy). Furthermore, asialoEPO administration did not prevent the significant decrease in hippocampal cell proliferation observed in the DG of 12 week-old R6/2 mice (Gil et al., 2004). The lack of effects of asialoEPO in the R6/2 mice indicates that this drug is not as neuroprotective in chronic neurodegenerative models (with limited cell death) as in acute situations. Furthermore, one of the proposed EPO neuroprotective mechanisms involves the nuclear factor-κB (NF-κB)-mediated up-regulation of neuroprotective and anti-apoptotic genes (for references see Gil et al., 2004). Thus, transcriptional abnormalities, which are believed to play an important role in the pathology of R6/2 mice, may account for a lack of asialoEPO neuroprotection in this model.

Since physical activity is known to stimulate adult hippocampal neurogenesis, Kohl et al. (2007) examined

whether running was capable to rescue the impaired hippocampal neurogenesis in 5 week-old R6/2 mice, by housing them with free access to running wheels. However, a 4-week running period failed to stimulate proliferation and survival of newly generated neurons in the R6/2 mice. Although the authors suggest that the mechanisms responsible for upregulating neurogenesis following physical exercise might be compromised in R6/2 mice, a different explanation for these findings might be that the R6/2 mice simply just did not exercise enough due to their locomotor impairments.

Antidepressants that act as serotonin reuptake inhibitors are also known to stimulate neurogenesis. Thus, Grote et al. (2005) sought to investigate the effects of the antidepressant fluoxetine in R6/1 mice. Fluoxetine treatment (starting at 4 weeks of age) improved hippocampal-dependent cognitive function (assessed by a T-maze test) and reversed the depressive phenotype (evaluated with the Porsolt forced swim test), but had no effect on the locomotor deficits and the loss of body weight characteristic of these transgenic mice. Importantly, fluoxetine also rescued the neurogenic process in the R6/1 DG, strongly supporting the hypothesis that modulation of hippocampal neurogenesis might improve cognitive function in HD (Grote et al., 2005). In a more recent study, Peng et al. (2008) tested the potential beneficial effects of another serotonine reuptake inhibitor sertraline in R6/2 mice. Sertraline treatment was initiated when the mice were 6 weeks of age and was shown to prolong R6/2 survival in a dose-dependent manner (5 mg/kg by 13%; 10 mg/kg by 20%) but had no effect on the loss of body weight. Furthermore, sertraline-treated R6/2 mice performed significantly better on the rotarod test at 10 weeks of age. At the neuropathological level, sertraline treatment ameliorated striatal atrophy and the enlargement of the lateral ventricles, but had no effect on the number of NIIs. Interestingly, these beneficial effects are associated with an increase in brain BDNF levels and neurogenesis (cell proliferation and survival of the newly generated cells) in the DG of R6/2 mice (Peng et al., 2008). Similar results have also been obtained with a different HD transgenic model (the N171-82Q mouse model) (Duan et al., 2008). Importantly, the serum and brain levels of sertraline that were shown to be beneficial in these transgenic models are comparable to the levels achieved in humans treated with this antidepressant, making sertraline an interesting candidate for HD clinical trials. Nevertheless, one should keep in mind that the behavioral improvement observed upon treatment with trophic factors (e.g., BDNF) and serotoninergic agents might not be a direct consequence of increased cell proliferation. Furthermore, the actual implications of reduced hippocampal cell proliferation for the development of cognitive deficits in HD remais to be elucidated.

3.   Concluding remarks

There are today over 100 publications reporting the effects of a variety of therapeutic strategies in the pathology of R6 transgenic HD mice. The findings from all these studies suggest that the phenotype of these transgenic models may be due to several parallel pathological events, both within the nervous system and in peripheral tissues. Thus, to successfully treat these mice, and most likely human HD patients as well, it might be necessary to apply several different therapeutic strategies simultaneously. Although several of the studied therapies were proven to exert certain beneficial effects individually, the search continues for a combination of treatments that may also be of significant clinical relevance.

Naturally, as discussed above, there is concern that the R6 mice may not model critical aspects of the pathogenic process in HD and therefore therapies that are effective in R6 mice may not turn out to be relevant in patients (as in the case of creatine, co-enzyme Q_{10}, and remacemide). Nevertheless, the characterization of the R6 transgenic HD mouse has greatly increased our understanding of the pathogenic process in HD. Thus, by paralleling findings in mice and humans, important similarities and discrepancies will continue to be identified, which will further increase the understanding of the disease and ultimately the identification of more effective therapies.

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