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RESEARCH ARTICLE

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Atypical diabetes associated with inclusion formation in the R6/2 mouse model of Huntington's disease is not improved by treatment with hypoglycaemic agents

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Abstract The R6/2 transgenic mouse model of Huntington's disease (HD) develops a progressive neurological phenotype that involves severe motor and cognitive dysfunctions. Although not a cardinal sign, diabetes has been described in R6/2 mice. It is not clear, however, whether the diabetes contributes to the HD-like phenotype of R6/2 mice. In our study we found that the severity of diabetes in R6/2 mice was associated with the progressive formation of ubiquinated inclusions in pancreatic beta cells. Diabetes is dissociated from early motor and cognitive dysfunctions and did not correlate with motor impairment and survival of R6/2 mice. However, chronic behavioural testing (at a level higher than that which is reported to improve several aspects of the R6/2 phenotype) exacerbated the onset of diabetes. Pharmacological treatment of the diabetes was attempted using two oral hypoglycaemic agents commonly used by diabetics. The mice responded acutely to glibenclamide (which induces exocytosis of insulin) but not to rosiglitazone (which induces sensitization to insulin). This supports the suggestion that the diabetes in R6/2 mice is caused by an impairment in insulin release rather than insulin insensitivity. However, chronic treatment with these hypoglycaemic agents had no effect on either the course of the diabetes or the disease in R6/2 mice.

Keywords Huntington's disease · Diabetes · Glycosuria · Glibenclamide · Rosiglitazone

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat within the coding region of the HD gene (Huntington's Disease Collaborative Research Group 1993). It is characterised by a triad of abnormal motor, cognitive and psychiatric symptoms (Squitieri et al. 2002). Although not one of the cardinal signs, abnormal glucose utilisation has been identified in some HD patients (Podolsky et al. 1972; Schubotz et al. 1976; Podolsky and Leopold 1977). Indeed, an extensive survey of 620 HD patients revealed the prevalence of diabetes mellitus to be 10.5% compared to the general occurrence of 1.9% in the neurologically normal population (Farrer 1985). Diabetes has also been associated with other neurodegenerative diseases, including Parkinson's and Alzheimer's disease, and may contribute to some of the neurological symptoms (Craft and Watson 2004). The co-morbidity of diabetes in neurodegenerative diseases is therefore of particular importance and the possible contribution of diabetes to the symptoms of HD a factor that needs to be investigated further.

The R6/2 transgenic mice carry exon 1 of the human HD gene with an expanded CAG repeat (Mangiarini et al. 1996). These mice develop progressively worsening motor and cognitive impairments from around 4 to 5 weeks (Carter et al. 1999; Lione et al. 1999) and die prematurely. The R6/2 mice have been shown to develop diabetes (Hurlbert et al. 1999) with progressive reductions in both pancreatic insulin levels and insulin gene expression (Andreassen et al. 2002). Although diabetes (assessed by glycosuria and glucose intolerance) is present in our colony, diabetes has not been reported consistently in other colonies of R6/2 mice—some groups have found severe diabetes (Hurlbert et al. 1999) whereas others report normal glucoregulation (Fain et al. 2001). A recent report has attempted to reconcile some of these differences (Luesse et al. 2001). These authors suggested that, although all R6/2 mice developed glucose intolerance, there are two distinct subpopulations of R6/2 mice, with overt of latent diabetes.

The objectives of this study were to examine;

1 The onset of diabetes and its possible contribution to the mortality and motor impairment of R6/2 mice;

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2 The impact of behavioural testing on the onset of diabetes; and
3 The effect of two orally active anti-diabetic drugs, glibenclamide and rosiglitazone, on the onset and severity of diabetes.

We also examined the effect of the drugs in combination, because addition of rosiglitazone to glibenclamide treatment has been shown to improve glycaemic control (Wolffenbuttel et al. 2000).

Materials and methods

Mice

A colony of R6/2 transgenic mice was established in the Department of Pharmacology, University of Cambridge, and maintained by backcrossing on to CBA×C57/BL6 F1 mice. Mice were housed in cages of mixed genotype with 12 h light and dark cycles in a temperature/humidity-controlled room. Genotyping was carried out using a PCR based on a modification of the method by Mangiarini et al. (1996). The DNA was extracted from tail tips as described previously (Carter et al. 1999) and the primers used were 31329 HD (5′ to 3′ ATG AAG GCC TTC GAG TCC CTC AAG TCC TTC) and 33934 HD (5′ to 3′ GGC GGC TGA GGA TGA GGA).

Onset of glycosuria

Mice were placed on a clean acetate sheet. Most mice urinated spontaneously, otherwise firm but gentle pressure was applied to the abdomen to induce micturition. Urine was tested twice weekly with Diastix reagent sticks, enabling semi-quantitative analysis of glycosuria. The presence of glycosuria was regarded as indicative of diabetic status. The correlation between glucose levels in blood and urine was examined by measuring samples taken within 2 min of each other in R6/2 mice ($N=92$).

Glucose challenge

Mice were fasted for 12 h (R6/2 mice $N=8$, WT mice $N=12$). After food deprivation the tip of each mouse-tail was anaesthetised (ethyl chloride, BP). A drop of blood from the tail was dropped on to a Glucotide stick (Bayer, UK). Blood glucose concentrations were analysed using a Glucometer 4 blood glucose calibrator (Bayer Diagnostics, UK). Mice were then dosed with an intraperitoneal (i.p.) glucose challenge (1.5 g kg^{-1} glucose (Sigma, UK). Blood glucose concentrations were measured again 1 and 2 h after the glucose challenge.

Effect of acute oral antidiabetic treatment

Drug-naïve male mice were given a glucose challenge 1.5 mg kg^{-1} (i.p.) at 6 and 10 weeks of age. This challenge was repeated using the same mice 2–3 days later; this time, however, the mice were divided into four groups and administered with either an oral dose of glibenclamide (5 mg kg^{-1}; Approved Prescription Services; R6/2 mice $N=10$, WT mice $N=12$), rosiglitazone (3 mg kg^{-1}; SmithKlineBeecham; R6/2 mice $N=9$, WT mice $N=7$), a combination of both drugs together at these doses (R6/2 mice $N=6$; WT mice $N=10$), or distilled water as the vehicle (R6/2 mice $N=8$; WT mice $N=11$ mice). These doses were chosen on the basis of results from studies in rats (Fuhlendorff et al. 1998; Jucker et al. 2002).

Effect of chronic oral antidiabetic treatment

The R6/2 mice were treated daily with 5 mg kg^{-1} glibenclamide ($N=11$), 3 mg kg^{-1} rosiglitazone ($N=10$), a combination of both doses ($N=8$), or vehicle ($N=9$) from 5.5 weeks of age until death. All mice were weighed daily and tested for glycosuria twice weekly. At weekly intervals from 6 to 11 weeks of age blood glucose levels of all mice were measured after fasting (12 h) and then 1 and 2 h after a glucose challenge (1.5 g kg^{-1}). Testing took place immediately after administration of oral hypoglycaemic agents.

Rotarod

The rotarod apparatus (Ugo Basile, Varese, Italy) was used to measure motor coordination and balance. Training consisted of placing mice on the rotarod at 24 revolutions per minute (rpm) for four trials over three consecutive days. Performance was measured by recording the latency to fall off the rotarod within a maximum of 60 s. The performance of mice was calculated by the mean latency to fall off the rotarod, over two trials at three different speeds (8, 24 and 33 rpm).

Repeated swimming trials

Mice were trained from to swim from one end of a water-filled tank to a visible escape platform at the opposite end. The glass tank was 100 cm long and 6 cm wide and filled to a depth of 30 cm. The water was maintained at 23°C. Mice received 10 trials per day, with an inter-trial interval of at least 15 min, for 6 days per week, between the ages of 5 and 12 weeks of age.

Immunohistochemistry

The pancreas from 17 R6/2 mice aged between 3 and 16 weeks were used in this study. Mice were killed in an increasing concentration of CO_2 and their pancreas was dissected, snap frozen rapidly in isopentane, and stored at −80°C until use. Tissue was cryosectioned (30 μm) and processed for histochemistry and immunocytochemistry for inclusions using rabbit polyclonal anti-ubiquitin

(1:1000, DAKO) antibody. A horse radish peroxidase-conjugated secondary antibody (1:1000, DAKO) was used, and staining was visualised using diaminobenzidine.

Islets were identified by haematoxylin and eosin staining, and corresponding islets on the sections stained with ubiquitin and Hoechst 33342 were identified. The total number of Hoechst 33342-positive nuclei were counted in each islet. The total number of ubiquitin-positive inclusions was also counted, and the percentage of the total nuclei containing inclusions was calculated.

Statistics

All behavioural data were analysed using repeated measures analysis of variance (ANOVA) with genotype and treatment as between subject factors. Onset of glycosuria and survival were analysed with the Log-rank test. A Student's unpaired *t*-test was used for comparing blood glucose measurements between groups of mice. A paired *t*-test was used for comparisons within the same group of mice. Correlation analysis was performed using the two-tailed Pearson test. A critical value of $P < 0.05$ was used throughout this study.

Results

General characteristics of diabetes in R6/2 mice

We found that most R6/2 mice developed glycosuria with the youngest age at which we observed glycosuria being 9.3 weeks. By 14 weeks of age 72% of surviving R6/2 mice tested positive for glycosuria (Fig. 1A), although some of the R6/2 mice (9/40) did not develop glycosuria before they died. The presence of glycosuria in R6/2 mice was always associated with blood glucose values exceeding 18 mmol L^{-1} (Fig. 1B) suggesting that glycosuria is a good non-invasive indicator of hyperglycaemia. In a separate study, between 6 and 13 weeks of age, diabetes was assessed by glucose challenge (1.5 g kg^{-1} i.p) after a 12 h overnight fast. There were no significant differences between the fasted blood glucose values of R6/2 mice and WT mice at any time-point ($F_{(1,18)} = 5.39$, $P > 0.05$). However, we found that R6/2 mice displayed progressively worsening glucose intolerance ($F_{(1,18)} = 7.8$, $P < 0.001$, Fig. 1C) that was significant from 9 weeks of age. Two hours after the glucose challenge, blood glucose levels in WT mice always returned to original baseline values whereas from 9 weeks of age R6/2 mice maintained significantly higher blood glucose concentrations ($F_{(1,18)} = 7.8$, $P < 0.001$).

The histopathological hallmark of diabetes in R6/2 mice is the presence of abnormal ubiquitin-positive inclusions in pancreatic islets cells (Hurlbert et al. 1999; Sathasivam et al.1999; Andreassen et al. 2002). Figure 1D shows the progressive nature of ubiquitin-positive inclusions in pancreatic tissue taken from R6/2 mice.

There was no correlation between the age of onset of glycosuria and the age at death of R6/2 mice ($r^2 = 0.0458$, $P = 0.315$). Further, there was no significant difference between the mean age at death of the R6/2 mice that developed glycosuria (15.9 ± 0.4 weeks) and those that died without developing glycosuria (15.2 ± 0.5 weeks). Similarly, in a separate study, the

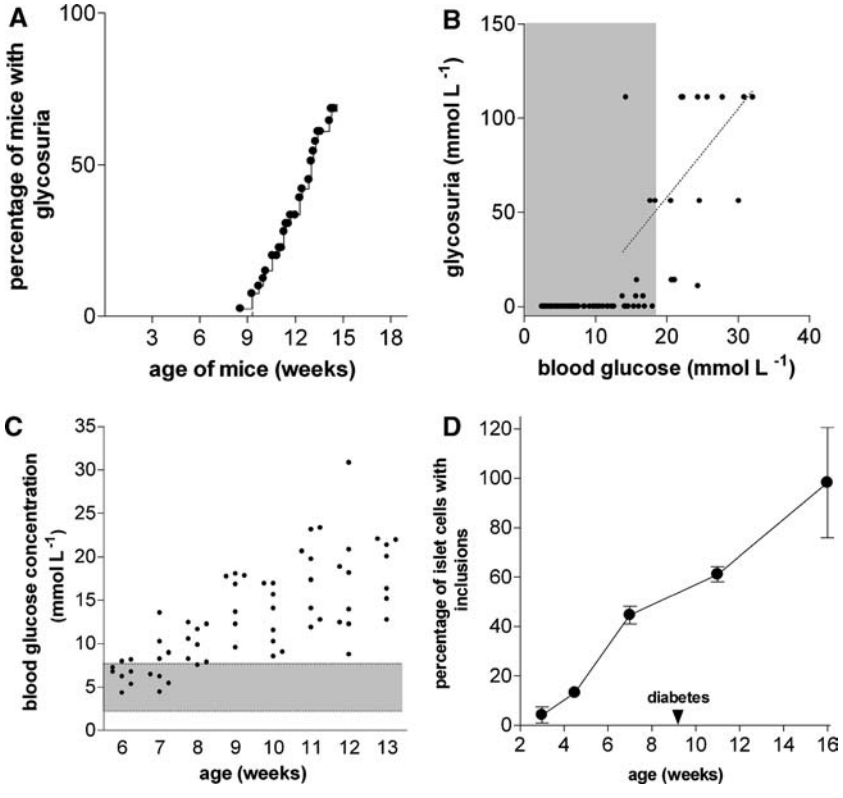

Fig. 1 General features of diabetes in R6/2 mice: **A**. The R6/2 mice progressively developed glycosuria from approximately 9 weeks of age. **B**. Glycosuria was associated with blood glucose values in excess of 18 mmol L^{-1}. **C**. Time course of post-glucose challenge blood glucose concentrations from R6/2 mice, superimposed against the range of WT values (*shaded area*). **D**. The number of ubiquitin-positive inclusion bodies in pancreatic beta cells from R6/2 mice increased with age. Note that inclusion formation occurs well before the onset diabetes

severity of glucose intolerance at 13 weeks of age did not correlate with age at death ($r^2 = 0.012$).

Behavioural testing accelerates onset of diabetes

We, and others, have shown that behavioural testing can increase the survival of R6/2 mice (Carter et al. 2000; Hockly et al. 2002; Glass et al. 2004). In this study we tested the effect of behavioural testing (repeated swimming), between 5 and 13 weeks of age, on the onset of diabetes. Surprisingly, we found that the mice that were tested in the swim tank 6 out of 7 days a week developed glycosuria significantly earlier ($P < 0.05$) than mice that were tested only once a week. Further, the first 50% of the chronically tested group of mice died significantly earlier than those tested only once a week ($P < 0.01$; Figs. 2A and 2B). Although no correlation was seen between glycosuria and death in mice that had only moderate handling, a positive correlation between the age of glycosuria onset and age at death in the mice receiving chronic behavioural testing was found ($r^2 = 0.4029$, $P < 0.05$, Fig. 2C). This suggests that although behavioural testing can be beneficial to R6/2 mice, it can produce deleterious effects when testing continues into the advanced stages of phenotype.

Effect of acute treatment with oral antidiabetic drugs (6 weeks)

We examined the acute effect of oral antidiabetic treatments on the diabetes in R6/2 mice. The onset and severity of diabetes varied between animals. Thus, to avoid inter-subject variability, paired studies were performed. Figure 3 shows results from two consecutive blood glucose challenges (1.5 g kg^{-1}) conducted 2–3 days apart, with each mouse acting as its own control. The first challenge was administered with vehicle, to determine baseline glucose sensitivity, and the second challenge was administered with vehicle, glibenclamide and/or rosiglitazone. Although administration of vehicle at the same time as the challenge did not affect blood glucose values (Fig. 3A), the presence of glibenclamide in the second challenge reduced blood glucose concentrations in R6/2 mice at both 1 and 2 h post-challenge ($P < 0.01$ for all, Fig. 3B). Rosiglitazone alone did not modify blood glucose concentrations (Fig. 3C), although the combination of glibenclamide and rosiglitazone reduced blood glucose concentrations 1 and 2 h post-challenge in both R6/2 mice ($P < 0.05$ and 0.01, respectively) and in WT mice ($P < 0.001$, for both, Fig. 3D).

Acute treatment with oral antidiabetic drugs (10 weeks)

At 10 weeks of age, R6/2 mice were hyperglycaemic after glucose challenge (Fig. 4A). Compared with baseline levels glibenclamide significantly reduced blood glucose concentrations in R6/2 mice and WT mice 1 h after the challenge ($P < 0.05$), although blood glucose

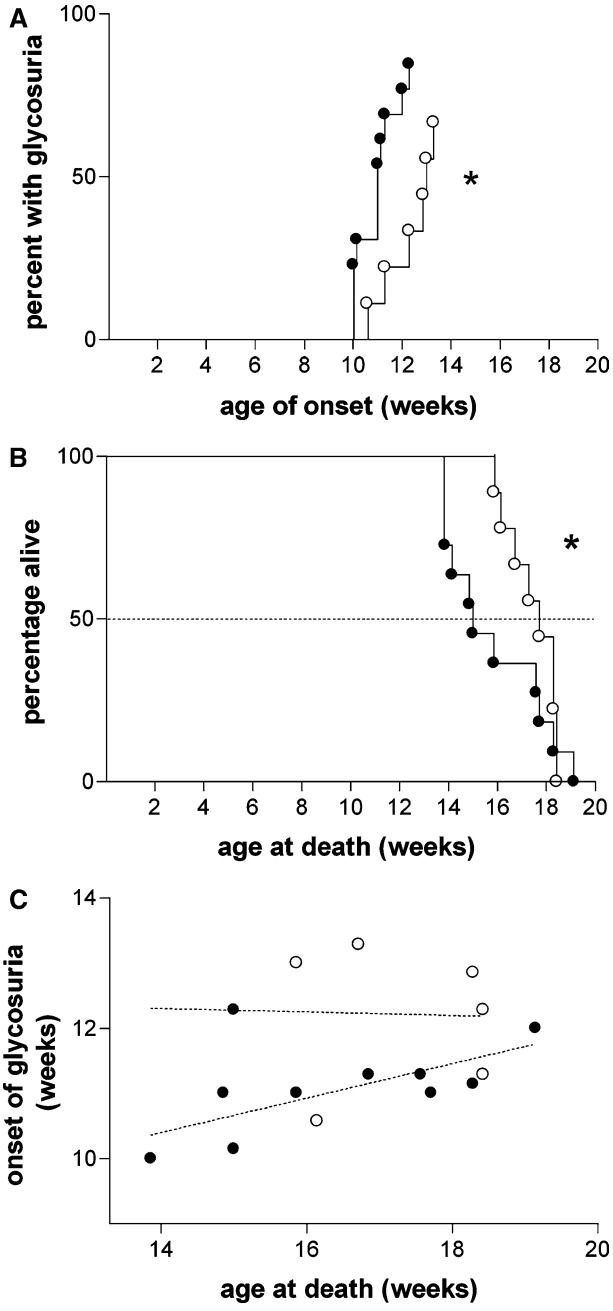

Fig. 2 Behavioural testing exacerbates the onset of glycosuria. The effects of chronic behavioural testing were examined in R6/2 mice within the same generation. In (**A**) mice tested chronically between 5 and 12 weeks of age (*filled circles*, $N = 13$) developed glycosuria significantly earlier than those subjected to acute testing (*filled circles*, $N = 9$). The first 50% of those mice tested chronically died significantly earlier (**B**). A significant correlation between age of onset and age at death was found in mice tested chronically ($r^2 = 0.4029$, $P < 0.05$, *lower line*) but not in mice tested acutely ($r^2 = 0.215$, $P > 0.05$, *upper line*) (**C**). Onset of glycosuria and survival were analysed by the Log-rank test. *Asterisks* indicate significant differences between acute and chronic behavioural testing (*$P < 0.05$)

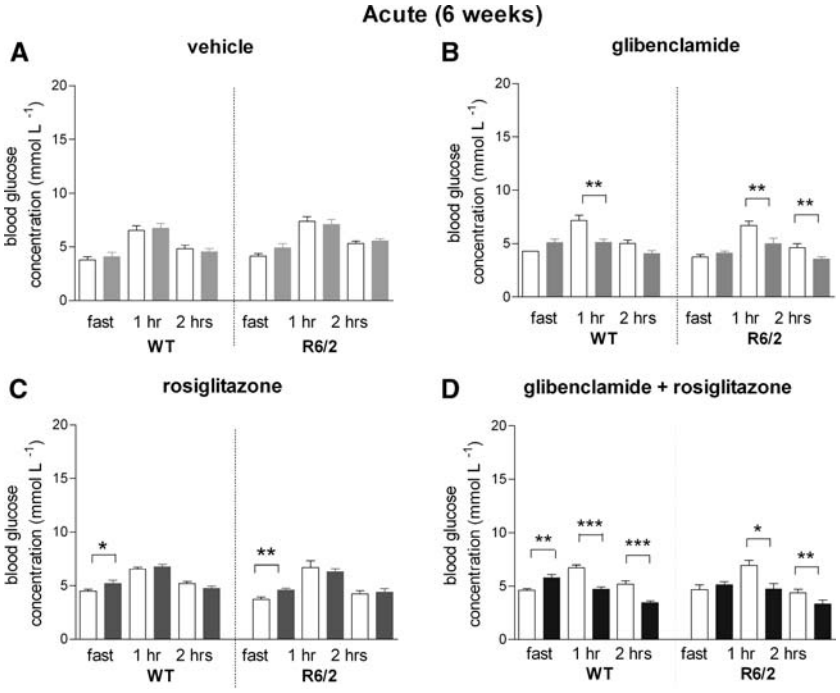

Fig. 3 The acute effect of glibenclamide and rosiglitazone used alone or in combination at 6 weeks of age. Blood glucose concentrations are shown after the first glucose challenge (*open bar*) at fasting, 1, and 2 h post-glucose challenge (1.5 g kg^{-1}) (**A**). The second challenge (*closed bar*) was co-administered with a vehicle (WT mice $N=11$; R6/2 $N=8$), glibenclamide (WT mice $N=12$; R6/2 $N=10$), rosiglitazone (WT mice $N=7$; R6/2 $N=9$), or glibenclamide plus rosiglitazone (WT mice $N=10$; R6/2 $N=6$). Glibenclamide administered alone (**B**) or in combination with rosiglitazone (**D**) resulted in a significant reduction in blood glucose levels in both WT and R6/2 mice. Rosiglitazone administered alone failed to change blood glucose values in both WT and transgenic mice (**C**). Data are mean ± SEM. *Asterisks* indicate significant differences between the first (*open bar*) and second (*closed bar*) glucose challenges. Data were compared using paired Student's *t*-test (*$P<0.05$; **$P<0.01$; ***$P<0.001$)

levels in R6/2 mice remained significantly higher than in WT mice (Fig. 4B). Consistent with our findings at 6 weeks of age, rosiglitazone did not alter post-challenge blood glucose values in older R6/2 or WT mice (Fig. 4C). However, when both drugs were administered together a reduction in blood glucose concentrations was detectable post-challenge in both R6/2 and WT mice ($P<0.05$, Fig. 4D).

Effect of chronic treatment with oral antidiabetic drugs

We examined the effect of rosiglitazone in a chronic study, because chronic treatment with rosiglitazone has been shown to improve glycaemic control in mouse models of diabetes (Young et al. 1995; Connor et al. 1997). Chronic daily treatment with a combination of rosiglitazone and glibenclamide significantly reduced the fasting blood glucose concentrations in all mice at 10 weeks of age ($F_{(3,34)}=26.44$, $P<0.05$). However, at 9 weeks of age R6/2 mice still displayed significantly elevated blood glucose concentrations at 1 h ($F_{(3,34)}=54.36$, $P<0.001$) and 2 h ($F_{(3,34)}=36.99$, $P<0.001$) post-challenge. Post hoc analysis revealed no significant effects after chronic antidiabetic treatment post-glucose challenge. It is possible that long-term treatment may have induced temporary depletion of the insulin releasable pools, resulting in a failure to respond in the glucose tolerance test. However, together these data show that there was no effect of chronic treatment on survival, onset of glycosuria, and weight loss (all $P>0.05$, Figs. 5A–C).

The effect of chronic treatment on motor performance was assessed using the rotarod. As expected, R6/2 mice became progressively less able to maintain balance on the rotarod compared with WT mice ($F_{(3,34)}=2.94$, $P=0.001$, Fig. 5D), but hypoglycaemic treatment had no effect—either beneficial or deleterious—on motor performance the mice. Notably, at no age did hyperglycaemia correlate with motor impairment on the rotarod.

Discussion

In this study we have shown that from 9 weeks of age R6/2 mice developed glycosuria and glucose intolerance that progressively worsened with age, until by 14 weeks of age more than 70% of the mice had developed diabetes. The onset of glycosuria and glucose intolerance was variable and a small proportion of R6/2 mice never developed diabetes. Onset of diabetes followed the formation of ubiquitin-positive inclusions in pancreatic beta cells and was exacerbated by chronic behavioural testing. When we attempted to

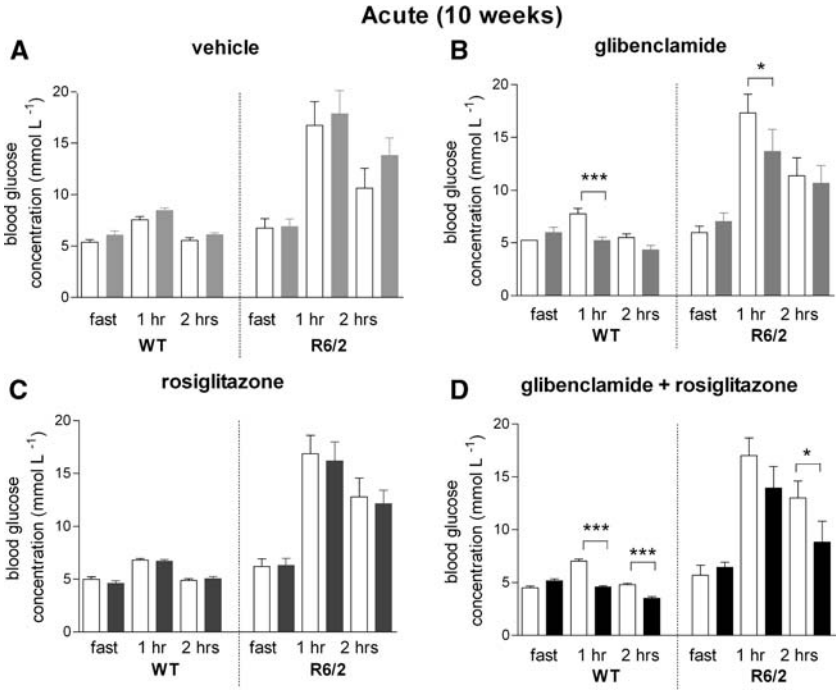

Fig. 4 The acute effect of glibenclamide and rosiglitazone used alone or in combination at 10 weeks of age. Blood glucose concentrations are shown after the first glucose challenge (*open bar*) at fasting, 1, and 2 h post glucose challenge (1.5 g kg^{-1}). The second challenge (*closed bar*) was co-administered with a vehicle (WT mice $N=11$; R6/2 $N=8$), glibenclamide (WT mice $N=12$; R6/2 $N=10$), rosiglitazone (WT mice $N=7$; R6/2 $N=9$), or glibenclamide plus rosiglitazone (WT mice $N=10$; R6/2 $N=6$). By 10 weeks of age, glibenclamide administered alone (**B**) or in combination with rosiglitazone (**D**) reduced blood glucose values, but rosiglitazone alone did not alter blood glucose values (**C**). In all cases, post-challenge values were significantly higher in R6/2 mice than in WT mice. Data are mean ± SEM. *Asterisks* indicate significant differences between the first (*open bar*) and second (*closed bar*) glucose challenges. Data were compared using paired Student's *t*-test (*$P<0.05$; ***$P<0.001$)

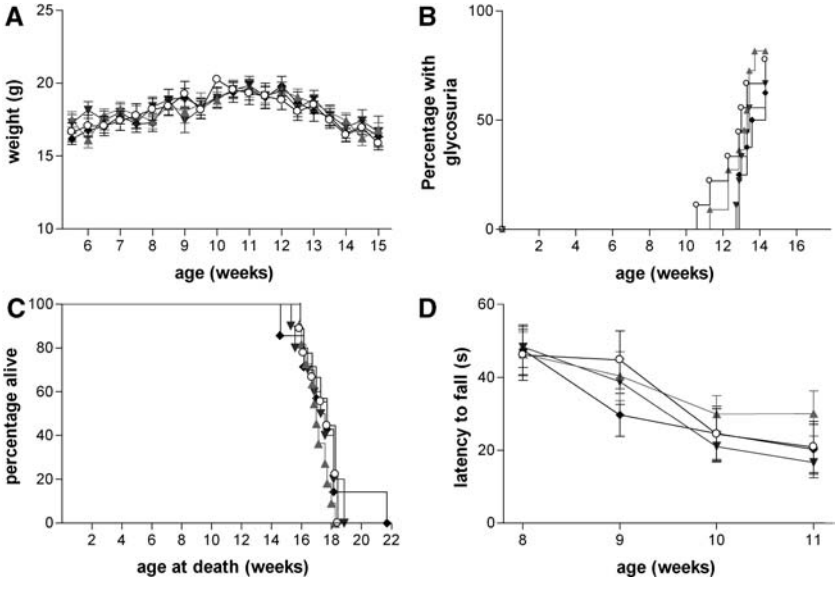

Fig. 5 The effect of chronic treatment with glibenclamide and/or rosiglitazone from 5.5 weeks of age. Chronic treatment did not alter the weight (**A**), onset of glycosuria (**B**), survival (**C**), or rotarod performance (**D**). Symbols represent R6/2 vehicle-treated (*filled circles*, $N=9$), R6/2-glibenclamide-treated (*filled circles*, $N=11$), R6/2-rosiglitazone-treated (*filled circles*, $N=10$), and R6/2-glibenclamide and rosiglitazone-treated (*filled diamonds*, $N=8$)

treat the diabetes, using oral hypoglycaemic agents, R6/2 mice were found to be sensitive to glibenclamide but not rosiglitazone treatment. However, chronic treatment did not ultimately change the course of the diabetes or the R6/2 phenotype.

Diabetes in R6/2 mice

In most of the R6/2 mice glycosuria and glucose intolerance became detectable at the age (9–10 weeks) when the overt phenotypic changes first became discernible

(increased dyskinetic movements, reduced locomotor activity, and weight loss). However, in contrast with the motor symptoms (that all mice displayed), not all R6/2 mice developed diabetes. With increasing age, a broad scatter of post-challenge blood glucose values was measured in R6/2 mice, with some mice maintaining near-control values even into the terminal stage of the phenotype. This is in agreement with other reports (Jenkins et al. 2000; Luesse et al. 2001; Miller et al. 2003; Bjorkqvist et al. 2005) and supports the notion that diabetes occurring in R6/2 mice is heterogeneous in onset and severity. Despite variable onset and severity of glucose intolerance, post-challenge values did not correlate with motor impairments on the rotarod or with the age at death of R6/2 mice. These findings are in accordance with those of Luesse et al (2001) and with reports showing that motor and cognitive impairments (Carter et al. 1999; Lione et al. 1999) and neurotransmitter changes (Cha et al. 1998) occur well before the age at which R6/2 mice become glucose intolerant. Recently, plasmid vaccination against mutant huntingtin dramatically improved the diabetic phenotype, without producing any overt changes in the behavioural phenotype (Miller et al. 2003). Therefore, although diabetes develops in most R6/2 mice in parallel with the overt phenotype, it is unlikely to be the cause of either the neurological phenotype or the rapid decline in general function of R6/2 mice.

In this study, we used glycosuria as a marker of diabetes occurring in R6/2 mice. We found glycosuria was always present when blood glucose values were ≥18 mmol L^{-1}. This indicates that the presence of glycosuria is a good non-invasive marker of glucose intolerance. Assessment of glycosuria as a quantitative test of diabetes has distinct methodological advantages over conventional blood glucose tests. Because it is a non-invasive test, it is less stressful to perform, unlike blood glucose sampling that involves restraint, application of a local anaesthetic, and blood withdrawal. Because in some colonies R6/2 mice develop handling-induced seizures which can be fatal (Mangiarini et al. 1996) stressful procedures should be avoided wherever possible. Further, stress responses can confound the measurement of blood glucose concentrations (Armario et al. 1990; DeBoer et al. 1990). Indeed, in this study we show that physical stress can exacerbate diabetes in R6/2 mice (see below). The test for glycosuria seems to be an accurate and markedly less stressful test for the presence of diabetes and we recommend that this non-invasive method should be routinely employed in drug trials.

The cause of diabetes in R6/2 mice remains unclear. Peripheral pathology in R6/2 mice is not surprising because mutant huntingtin is expressed ubiquitously (Huntington's Disease Collaborative Research Group 1993). The histopathological hallmark of HD, intranuclear inclusions, is present in pancreatic cells (Sathasivam et al. 1999; Ferrante et al. 2000; Andreassen et al. 2002). Here, we show that inclusions can be identified in the pancreas from 3 weeks of age, 5 weeks earlier than previously shown. This is approximately the same age when inclusions are detected in neurons (Morton et al. 2000) and well before the onset of diabetes. This suggests that peripheral and neuronal pathologies are likely to occur in parallel. However, as in the brain, the role of inclusions in the pathophysiology of the beta islet cells remains unclear. In R6/2 mice at 6 weeks of age at least 50 % of pancreatic islet cells contain nuclear inclusions, although pancreatic function as assessed by glucose tolerance testing and sensitivity to glibenclamide seems to be normal. In contrast, at 10 weeks of age, when an abnormal overt phenotype can be detected, inclusions are present in almost all pancreatic islet cells and most R6/2 mice are intolerant to a glucose bolus. A temporal association between the presence of inclusions and insulin mRNA expression has been reported (Andreassen et al. 2002). Indeed our findings suggests that R6/2 mice can maintain normal blood glucose levels up to a certain threshold of inclusion pathology, but above this level diabetes occurs. This is consistent with our previous suggestion (Morton et al. 2000), that initial inclusion formation may be protective (or at least benign) but when inclusion load increases it has a deleterious effect on cell function.

Chronic behavioural testing exacerbates diabetes

Between 5 and 12 weeks of age R6/2 mice were exposed to a behavioural testing regime that incorporated daily exercise. Exercise has been found to reduce the severity of diabetes, particularly type-2 diabetes, and to increase glucose uptake and metabolism in diabetic patients and rodent models of diabetes (Henriksen 2002; Nakai et al. 2002; Goodyear and Kahn 1998). We found that chronic behavioural testing induced an earlier age of onset of diabetes and age of death in R6/2 mice. It seems that daily repeated exercise rather than behavioural testing per se was the major contributory factor to these changes, because moderate behavioural testing actually improves survival (Carter et al. 2000). Stress is known to exacerbate hyperglycaemia both in diabetic patients and in rodent models of diabetes (Surwit et al. 1992). Indeed, the C57BL/6 *ob*/*ob* mouse model is not consistently hyperglycaemic except when exposed to environmental stress (Surwit et al. 1985). In these mice it is likely that stress induced the release of glucocorticoids, which elevate blood glucose levels (Surwit et al. 1986; Durant et al. 1998). In R6/2 mice glucose may not be adequately metabolised by skeletal muscle, in which progressive atrophy is observed from 8 weeks of age in R6/2 mice (Sathasivam et al. 1999, Ribchester et al. 2004). Another effect of stress is activation of the stress-activated protein kinase (SAPK) pathway, which can be stimulated by a variety of endogenous and exogenous stress-inducing stimuli, including hyperglycaemia-induced oxidative stress (Ho et al. 2000). This activation

has been associated with impaired insulin secretion and insulin resistance (Evans et al. 2002). Interestingly, both mild and moderate occupational stress have been associated with an earlier age of onset in HD patients (Brackenridge 1979). Furthermore, the expression of mutated huntingtin has been shown to stimulate SAPK activity (Liu 1998) and nuclear inclusions from post mortem HD brains co-aggregate with SEK-1, an activator of SAPK (Yasuda et al. 1999). The role of SAPK in the phenotypic changes occurring in R6/2 mice is unknown. However, activation of the SAPK pathway, leading to impaired secretion of insulin, may represent a putative mechanism whereby exposure of R6/2 mice to chronic stressful behavioural testing may exacerbate the diabetes. A similar mechanism, if occurring in neuronal tissue, may have also accelerated the neurological phenotype, leading to an earlier age at death.

R6/2 mice are sensitive to glibenclamide but not rosiglitazone

Glibenclamide is a sulfonylurea that depolarises pancreatic beta cells by blocking ATP-sensitive potassium channels causing exocytosis of insulin (Trube et al. 1986; Ashcroft and Ashcroft 1992). At both 6 and 10 weeks of age glibenclamide co-administered with a glucose challenge induced a significant fall in the blood glucose levels of R6/2 mice. The sensitivity of younger R6/2 mice to glibenclamide suggests that insulin is present and that secretion can be induced by glibenclamide. This supports the previous findings of Bjorkqvist et al. (2005) that diabetes occurs in R6/2 mice as a result of impairment of the release of insulin. Glibenclamide induced a small but significant reduction in post-challenge blood glucose concentrations in older mice, consistent with reports of progressive depletion of pancreatic insulin and insulin gene expression with age (Hurlbert et al. 1999; Andreassen et al. 2002; Bjorkqvist et al. 2005). Thus, the efficacy of glibenclamide in R6/2 mice seems to depend on the availability of insulin-releasable pools. It has recently been shown that at 12 weeks of age exocytosis is virtually abolished in beta cells, which has been attributed to a marked decline in the number of insulin-containing secretory vesicles (Bjorkqvist et al. 2005). Other factors may modify the release of insulin. For example, a progressive reduction in the synaptic protein, complexin II has been identified in the brains of R6/2 mice (Morton and Edwardson 2001) and a similar reduction in the pancreas may contribute to the progressive failure of R6/2 mice to maintain normal glucoregulation. Indeed, the related peptide complexin I regulates glucose-induced secretion in pancreatic beta-cells (Abderrahmani et al. 2004). When R6/2 mice were treated chronically with glibenclamide there was no change in the onset of the disease as assessed by weight loss, motor impairment, and survival. Thus, although glibenclamide produced short-term effects, chronic treatment did not change the progression of the disease. Although it is possible that optimum therapeutic levels of glibenclamide were not maintained for 24 h (because we dosed the mice only once a day, and clearance of the drug has not been measured in mice), a decrease in the amount of insulin available for release seems to be the most likely explanation of our results.

To examine whether insulin resistance develops in R6/2 mice we treated the mice with rosiglitazone. Although we did not observe an effect after acute administration, chronic treatment with rosiglitazone is reported to induce insulin-sensitising effects in *db*/*db* and *ob*/*ob* models of diabetes (Young et al. 1995; Connor et al. 1997). In our study chronic treatment did not modify blood glucose levels in R6/2 mice. Again, although it is possible that effective therapeutic levels of rosiglitazone were not sustained, it seems more likely that insulin resistance is not a primary factor in the diabetes occurring in R6/2 mice. In support of this, R6/2 mice and adipose tissue taken from R6/2 mice respond normally to exogenous insulin (Hurlbert et al. 1999; Fain et al. 2001). Together, our findings are consistent with reports of progressive depletion of pancreatic insulin and an impaired insulin-release mechanism. The R6/2 mice are sensitive to agents that induce release of insulin, but this ultimately depends on the availability of insulin releasable pools and normally functioning mechanisms of release. Chronic treatment with oral hypoglycaemic agents does not, however, alter the course of the HD phenotype in R6/2 mice.

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