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Autonomic impairment in a transgenic mouse model of amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of motor neurons, however it is increasingly recognized that nonmotor manifestations may occur, including autonomic nervous system dysfunction. To better understand the autonomic involvement in ALS we measured autonomic functions in transgenic (TG) mice carrying an SOD1 (G93A) mutation and wild-type (WT) control mice. TG mice had a higher heart rate at rest and following stress than WT mice at all ages except for the advanced stages of the disease (19–20 weeks of age). The mean pupil diameter at rest was similar in WT and TG mice; however, TG mice had decreased mydriasis following administration of morphine. The rectal temperature did not differ between TG and WT mice at rest, during exposure to cold stress and following administration of morphine (30 mg/kg) except for the advanced stages of the disease in which TG mice had significantly lower temperatures than WT mice during cold stress and following morphine administration. The results suggest autonomic nervous system impairment in this ALS model, consistent with clinical data in humans.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of motor neurons in the cerebral cortex, brain stem and spinal cord, eventually leading to paralysis and ultimately death (Swash, 2000). Accumulating evidence exists for subclinical dysfunction of cardiovascular, gastrointestinal, salivary and lacrimal regulation, even in early stages of the disease (Baltadzhieva et al., 2005). Increased resting heart rate, blood pressure and muscle sympathetic nerve activity (MSNA) and decreased heart rate variability (HRV) have been reported in ALS patients (Sachs et al., 1985; Pisano et al., 1995; Linden et al., 1998; Oey et al., 2002; Shindo et al., 1995). Autonomic disturbances may lead to circulatory collapse or sudden death, particularly in respirator-dependent patients (Shimizu et al., 1994). Awareness of the presence of autonomic impairment allows for more precise evaluation of some of the complaints of ALS patients as well as for therapeutic considerations (Baltadzhieva et al., 2005).

Due to the inherent variability in characteristics of disease and the severe motor handicap, especially in the more advanced stages, it is difficult to examine extensively the autonomic nervous system function in patients, and we therefore studied autonomic involvement in transgenic (TG) mice carrying the G93A mutant form of the human SOD1 transgene (Gurney et al., 1994), a universally recognized animal model of ALS, including heart rate (HR) regulation, thermoregulation and pupillary reactions. To the best of our knowledge the ANS involvement in the ALS mouse model has not been examined previously.

2. Materials and methods

The mice used in this study were inbred C57BL/6 mice obtained from our animal breeding facility and TG mice of strain B6SJL-TgN(SOD1-G93A)1Gur/J (Jackson Laboratories, Bar Harbor, ME). Progeny for experimental analysis was obtained from breeding between male SOD1-G93A and female C57BL/6 wild-type (WT) mice. Offspring were genotyped by PCR of tail clips removed after completion of the experiments. The PCR assay recommended by Jackson Laboratories used a mixture of four sets of primers, two for mutant human SOD1 (5′-CAT CAG CCC TAA TCC ATC TGA-3′ and 5′-CGC GAC TAA CAA TCA AAG TGA-3′), which amplify a 236 bp fragment from mice carrying the human G93A SOD1 TG construct. The other two primers were for interleukin-2 (IL-2, 5′-CTA GGC CAC AGA ATT GAA AGA TCT-3′ and 5′-GTA GGT GGA AAT TCT AGC ATC ATC C-3′), which amplify a 324 bp fragment from WT mice. The IL-2 primers were run in the same reaction as a control for DNA amplification. The transgene carriers showed amplification of both SOD1 and IL-2 products, whereas the WT gave only the IL-2 product. The PCR protocol consisted of 1 cycle of 3 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 60 °C, and 45 s at 72 °C, and a final extension for 2 min at 72 °C. The PCR products were

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electrophoresed on 1.5% agarose gels, and size comparisons were done with reference to a 100-bp ladder.

Mice were maintained at the Tel-Aviv University Medical School animal facility and cared for in accordance with the guidelines published by the National Institutes of Health (Clark, 1996). They were housed in groups of 5 or less in 26×15×12 cm polycarbonate cages, or up to 7 in 20×30×12 cm cages with food (standard pellets) and water freely available, in thermostable rooms (21±2 °C) and humidity of 50–70%. A light–dark schedule of 14:10 h was maintained. Body weight and motor function measurements began at age 5 weeks and were repeated weekly. Testing of HR, thermoregulation and pupillary reactions started at 7–8 weeks of age and were repeated monthly (e.g. at age 11–12, 15–16 and 19–20 weeks) in order to assess possible correlations with the severity of disease. Experiments lasted up to the point when TG mice became paralyzed. All experiments were performed blinded to the animals' genotype.

3. Evaluation of motor function

Motor function was evaluated using a rotarod 3.0 cm in diameter with a knurled surface, suspended at a height of 15 cm. Mice were given 3 days of practice to become acquainted with the rotarod and human handling beginning at 40 days of age. Then, the mice were evaluated on the rotarod on a weekly basis at three consecutive 1 min rounds of 11 rpm with 15 s breaks between rounds. The time they remained on the rotarod was registered automatically. Mice that fell off at any time during the test session received a score that corresponded to the time at which they fell. Mice able to stay on the rod for the entire test session received a score of 60. Progression of disease was measured by the decrement in the ability of mice to remain on the rotarod.

3.1. Thermoregulation

The mice were left unrestrained in individual cages, moving freely. To investigate thermoregulation, rectal temperature was measured in an ambient temperature of 21±2 °C and during exposure to cold stress in a "walk-in" cold room (5±1 °C) using a rectal probe inserted to a depth of 1.5 cm. All experiments began at 10.00 AM in order to avoid circadian rhythm effects. During cold stress, the rectal temperature was measured at half-hour intervals for 4 h. The mice were then returned to the animal facility, where the ambient temperature was 21±2 °C, and the rectal temperature continued to be monitored until recovery. Changes of body temperature were also measured for 5 h after subcutaneous injection of (−)-morphine hydrochloride (30 mg/kg), in an attempt to cause central, rather than environmental, hypothermia.

3.2. Pupillary size changes

Pupillary diameters were measured using an Olympus binocular microscope with a magnification of ×20, as described before (Korczyn and Maor, 1982). One of the oculars was fitted with a divided 0.1 mm ruler. All the measurements were made while the animals were non-sedated and held gently under the microscope. Handling may cause stress to the mouse and may alter autonomic function, demanding a total handling of less than 5 s. Both pupils of each animal were measured, and the average value was recorded. (−)-Morphine was injected subcutaneously at a dose of 30 mg/kg. The pupil size was measured at baseline and at 30 min intervals for 5 h after injection.

3.3. Regulation of heart rate

To assess cardiac autonomic function, the ECG was recorded using stainless steel needle electrodes inserted subcutaneously on the two front limbs and left rear limb in freely moving animals. The HR was recorded by an ECG machine (Grass 7P6B) with a paper speed of 30 mm/s, as previously described (Wang et al., 2003). The HR was identified by visually inspecting R-waves. During the test, mice were kept individually in cages placed in a stable temperature (21±2 °C) and noise free environment.

The HR was measured three times and the mean HR was recorded as resting HR. HR following stressful stimulation was measured immediately after strongly shaking the cage for 1 min.

3.4. Statistical analysis

Repeated measures (for time points) analysis of variance (ANOVA) using the mixed model was used in the analysis of weight change, rotarod performance, thermoregulation and pupil diameter to compare between TG and WT mice, gender and the interaction between these variables for each age group separately. Comparisons between time points were performed adjusted for multiple comparisons using the Hochberg method (Benjamini and Hochberg, 1995). Data for the analysis of HR regulation comparing HR at rest and HR after stress between TG and WT mice were examined by Student's two-tailed unpaired *t*-test. Statistical analyses were performed using SAS statistical software 9.13 (SAS institute Inc, Cary, NC) and GraphPad Prism for Windows (version 5.0). The study was approved by the Tel Aviv University Animal Research Ethics Committee.

4. Results

The TG group comprised of 8 males and 12 females, whereas the control WT group consisted of 7 males and 12 females. The number of TG mice at the advanced stages of the disease (19–20 weeks) was reduced due to death, as a natural result of the course of the disease. The mean age at death of TG mice was 20.5 weeks (SD, 1 week). Control and TG mice were similar in body weight from the onset of testing until a decline began in the affected mice at approximately 12 weeks of age (Fig. 1). A statistically significant difference in body weight between TG and WT mice appeared at the age of 16 weeks ($p<.0001$, repeated measures ANOVA).

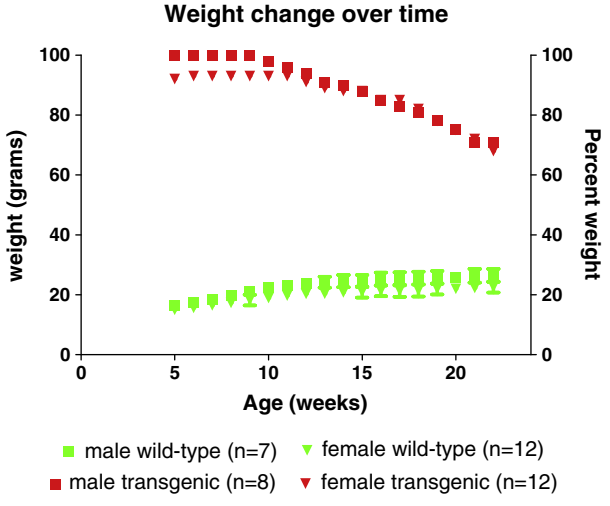

Fig. 1. Body weight change over time in male wild-type (WT) and female WT mice (lower figure) and the percentage change in weight of male transgenic (TG) and female TG mice relative to that of the WT mice (upper figure). A statistically significant change in weight between TG and WT mice was first seen at 16 weeks of age for both males and females ($p=0.047$ and $p=0.042$ respectively, repeated measures ANOVA). TG mice had normal weight until 10 weeks of age, after which they gradually lost weight. The difference in body weight between TG and WT mice was statistically significant ($p<.0001$, repeated measures ANOVA). The vertical bars indicate standard error of mean.