Inhibition of Caspases Protects Cerebellar Granule Cells of the Weaver Mouse from Apoptosis and Improves Behavioral Phenotype*

Jun Peng‡, Zhijin Wu§, Yongqin Wu‡, Mike Hsu§, Fang Feng Stevenson‡, Rapee Boonplueang§, Suzanne K. Roffler-Tarlovy*, and Julie K. Andersen‡§

From the ‡Buck Institute for Age Research, Novato, California 94945, §Program in Molecular Biology, Department of Biologcal Sciences, University of Southern California, Los Angeles, California 90089, and the ¶Departments of Neuroscience and Anatomy and Cell Biology, Tufts University School of Medicine, Boston, Massachusetts 02111

The homozygous mouse mutant weaver exhibits a massive loss of cerebellar granule neurons postnatally. The death of these cells is associated with a single amino acid mutation in the G protein-activated inwardly rectifying potassium channel, Girk2. Evidence suggests that both the mutated Girk2 channel and the calcium channel-associated N-methyl-D-aspartate receptor play important roles in the apoptotic death of weaver cerebellar granule cells, but the downstream events associated with this process are unknown. In this study, we demonstrate that the consequences of the mutation result in caspase activation. In addition, our results show that caspase inhibition in vivo decreases caspase activation and granule cell apoptosis and significantly improves behavioral deficits associated with the weaver’s phenotype.

The homozygous murine mutant weaver (gene symbol wv) is characterized by ataxia, hyperactivity, and tremor (for a review, see Ref. 1). These neurological defects are associated with the large scale death of neurons in the cerebellum and midbrain during the first month of postnatal development. Homozygous weaver mice exhibit death of cerebellar granule cells (2–4), dopaminergic neurons in the substantia nigra (5–9), Purkinje cells in the cerebellum (10–12), and neurons in the deep cerebellar nuclei (13). The wv defect has been identified as a point mutation in the protein-activated inwardly rectifying potassium channel gene, Girk2 (14). Girk channels are activated by direct interaction with G proteins (15) and play an important role in controlling cell membrane excitability by maintaining the potassium equilibrium potential (16). It has been observed that the weaver’s cerebellar granule cells die by an apoptotic mechanism (17–19). However, the precise nature of the process underlying granule cell death is unclear.

Neuronal apoptosis often involves a family of proteases known as caspases. Caspases are synthesized as precursors that are activated after cleavage. Three categories of caspases have been characterized by the specificity of their substrate cleavage site: caspases generating mature proinflammatory cytokines (caspase-1, -4, and -5) and caspases that traditionally act as either initiators (caspase-6, -8, and -9) or downstream (caspase-2, -3, and -7) as executioners in the apoptotic pathway (20, 21). During development, cell death is essential for regulation of neuronal cell numbers as well as for protection against the propagation of aberrant cells (22). The evidence suggests that caspase-3 participates in neuronal cell death during development (23), after traumatic neuronal injury (24), and ischemia (25), suggesting that caspase-3 may play a critical role in the terminal stage of the apoptotic pathway in neurons.

Death of granule cells caused by the Girk2wv mutation can be rescued, at least for a time, both in vivo and in vitro by elimination of the NR1 subunit of the calcium channel-associated N-methyl-D-aspartate receptor, suggesting that this receptor may also be involved in cerebellar granule cell death (19). The studies reported here were carried out to attempt to determine the downstream events in the death of granule cells that carry two copies of the faulty GIRK2 channel gene. Here we supply direct evidence for the involvement of caspases in the death of the weaver’s granule cells. Furthermore, we demonstrate that caspase inhibition attenuates apoptosis in the weaver’s granule cells both in vitro and in vivo and also significantly attenuates behavioral deficits associated with this genetic lesion.

EXPERIMENTAL PROCEDURES

Reagents—(+)-5-Methyl-10,11-di hydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801),1 QX-314, verapamil, and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma. PCR reagents, DNA polymerase, and the digoxigenin DNA labeling and detection kit were from Roche Molecular Biochemicals. Rabbit polyclonal cleaved caspase-8 antibody was purchased from Smith-Kline Beecham Pharmaceuticals (King of Prussia, PA). Rabbit polyclonal cleaved caspase-9 and rabbit polyclonal cleaved caspase-3 antibodies were from New England Biolabs (Beverly, MA). Z-DEVD-FMK (caspase-3 inhibitor), Z-IETD-FMK (caspase-8 inhibitor), and Z-LEHD-FMK (caspase-9 inhibitor) were purchased from BD Biosciences (San Diego, CA). Anti-TAG-1 (4D7) antibody was from the Developmental Hybridoma Studies Bank, University of Iowa (Iowa City, IA). The FluorAce™ apopain assay kit and caspase substrates were purchased from Bio-Rad.

Animals—Weaver heterozygous mating pairs (B6CBA/C-AcK/J) were purchased from the Jackson Laboratory (Bar Harbor, ME). Generation of homozygous p35 transgenic mice has been described elsewhere (26). All animals used in this study were generated from matings between female wv/wv, p35+/− or we/wv, p35+/− mice and male wv/wv, p35+/− mice. They were maintained in the vivarium on a

1 The abbreviations used are: MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeled; ECL, enhanced chemiluminescence; IGL, internal granular layer; Z, zonulae occludentes; P, P5, P7, and P21, postnatal day 5, 7, and 21, respectively; FMK, fluoromethylketone; AFC, 7-amino-4-trifluoromethyl coumarin.

Received for publication, July 23, 2002, and in revised form, September 4, 2002

The Journal of Biological Chemistry
© 2002 by The American Society for Biochemistry and Molecular Biology, Inc.

Downloaded from http://www.jbc.org/ on November 7, 2016

This paper is available on line at http://www.jbc.org

44285
12-h (light/dark) cycle at 22 °C. All procedures were approved by the Institute Animal Care and Use Committee at the Buck Institute.

**Determination of Genotype**—Genomic tail DNA was isolated using a kit (Qiagen). uv genotypes were determined by a PCR protocol. This protocol uses a common reverse primer (5′-CAC GGA CTG GAT TAA GAG GAG AAT AAT-3′) in combination with a wild-type sequence forward primer (5′-GAG ACA GAA ACC ACC ATC G-3′) or a uv sequence forward primer containing the point mutation at the 3′-end (5′-GAG ACA GAA ACC ACC ATC A-3′). PCRs were performed in a total volume of 25 μl and included an initial denaturation at 94 °C/5 min followed by 30 cycles each consisting of denaturation at 94 °C/30 s, annealing at 47 °C/45 s, and extension at 72 °C/60 s, and a final extension of 10 min at 72 °C. Subsequent PCR products were subjected to electrophoresis, and the bands were visualized with ethidium bromide. Each genomic DNA sample was tested with both pairs of primers. Wild-type (+/+ ) DNA yielded an 87-bp band with wild-type primers but not with uv primers and vice versa for homozygous (uv/uv) DNA. Heterozygous (uv/+ ) DNA yielded bands with both primer pairs. p35-positive genotypes were identified by slot blot analysis of genomic DNA prepared from tails as described previously (26). Briefly, 5 μg of total DNA was slot-blotted onto positively charged membranes and UV-cross-linked, membranes were hybridized with digoxigenin-labeled RNA probes transcribed from p35 cDNA, and DNA was detected by chemiluminescence.

**Preparation of Cerebellar Granular Cells**—Primary cerebellar granule cell cultures were isolated from 5–7-day-old pups as described previously (27). Cells were seeded onto tissue culture plates coated with poly-L-lysine (Sigma) and B2COAT™ culture slides (Becton Dickinson) in minimum essential medium (Invitrogen) supplemented with 10% fetal calf serum, 33 μM glucose, 2 μM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 20 μM supplementary KCl.

**Immunocytochemistry**—Cultured cerebellar granule cells were fixed with 4% paraformaldehyde, washed in phosphate-buffered saline, and then incubated in phosphate-buffered saline containing 10% goat serum and 0.3% Triton-X for 1 h at room temperature. The cells were then incubated with primary antibodies (caspase-9, 1:100; caspase-8, 1:500; caspase-3, 1:50 and TAG-1, 1:50) in blocking solution overnight. The cells were washed with phosphate-buffered saline and incubated with fluorescein isothiocyanate-conjugated secondary antibodies (1:200; Molecular Probes, Inc., Eugene, OR) for 1 h at room temperature. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (Vector). Control experiments were performed in which one or the other of the primary antibodies was omitted. No staining was observed under these conditions.

**Caspase Activity Assay**—The enzymatic activity of individual caspases was determined using kits from Bio-Rad. Control experiments were performed in which one or the other of the caspase substrates was omitted. Wild-type (+/+ ) DNA yielded minimal activity of caspase-9, -3, and -8, whereas weaver cerebellar granule cells exhibited a significant 2–6-fold increase in levels of activated caspase-9, -3, and -8 as measured by immunocytochemistry. Caspase-9 induction as monitored by immunofluorescence in uv/uv granule cells occurred first at 18 h, followed by caspase-3 induction at 24 h, which in turn preceded caspase-8 induction at 36 h. In cultures from uv/uv mice, activated caspase-8 and -9 were demonstrated to be primarily in the cytoplasm, whereas caspase-3 showed both nuclear and cytoplasmic localization (Fig. 1A). Negligible caspase-positive activity was noted in +/+ cultures.

**Cell Viability by MTT Assay**—MTT tetrazolium salt (5 mg/ml) was added to cells grown in 96-well plates and incubated for 4 h at 37 °C. After incubation, the cells were washed and the formazan dye was dissolved, absorbance at 560 nm was measured using a spectrophotometer.

**Histology**—Postnatal day 7 and 21 pups were anesthetized with Nembutal and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and immersed in 4% paraformaldehyde for the same fixative overnight at room temperature. Brains were dehydrated in graded ethanol, cleared in xylene, and paraffin-embedded (18). 10-μm-thick serial sagittal sections were cut and mounted on glass slides, which were dried overnight at 42 °C. Sections were deparaffinized, rehydrated through a graded series of ethanol, and washed in water. Nuclei were counterstained with hematoxylin. In all cases, sections examined were those near the vermis of the cerebellum. Granule cell counts were performed as previously described (17).

**Behavioral Testing**—Spontaneous locomotor activity, rest time, and climbing ability were measured in an automated Tru Scan™ photobeam activity system (Coulbourn Instruments, Allentown, PA) under illumination. Animals were habituated to the apparatus for 15 min prior to running the test experiment. Behavioral data were collected in the apparatus over a 10-min period and then analyzed using Tru Scan 99 software (Coulbourn Instruments, Allentown, PA). Depth of holes for climbing experiments was 23 mm deep at a diameter of 22 mm. Rest times were considered any period of 2 s or longer of inactivity.

**Statistical Analysis**—Results shown represent the mean ± S.E. for the number (n) of independent experiments performed. Statistical analysis of the data was performed using an analysis of variance software package (Statview).

**RESULTS**

**Involvement of Caspases in Weaver Cerebellar Granule Cell Death**—Apoptosis is initiated by activation of specific proteases of the caspase family (21). To determine whether the major initiator caspase-8 and/or -9 or the executioner caspase, caspase-3, are involved in weaver granule cell death and to delineate their sequence of activation, proteolytic activities associated with these caspases were measured by immunocytochemistry and enzymatic assays. Cerebellar granule cells were purified from postnatal day 5 (P5) homozygous (uv/uv) and wild-type (+/+ ) mice and cultured for different times before assessing the processing of cleaved caspase-9, -8, and -3 via immunocytochemistry. Caspase-9 induction as monitored by immunofluorescence in uv/uv granule cells occurred first at 18 h, followed by caspase-3 induction at 24 h, which in turn preceded caspase-8 induction at 36 h. In cultures from uv/uv mice, activated caspase-8 and -9 were demonstrated to be primarily in the cytoplasm, whereas caspase-3 showed both nuclear and cytoplasmic localization (Fig. 1A). Negligible caspase-positive activity was noted in +/+ cultures.

To assess temporal activation of the individual caspases, we examined the activities of each using specific fluorometric caspase substrates. Wild-type cerebellar granule cells exhibited minimal activity of caspase-9, -3, and -8, whereas weaver cerebellar granule cells exhibited a significant 2–6-fold increase in levels of activated caspase-9, -3, and -8 at 18, 24, and 36 h in vitro, respectively (Fig. 1B). A selective caspase-9 inhibitor, Z-LEHD-FMK, significantly attenuated the increase in caspase-9, -3, and -8 activities, whereas neither the caspase-8 inhibitor (Z-IETD-FMK) nor the caspase-3 inhibitor (Z-DEVD-FMK) inhibited caspase-9 activity (Fig. 1B). These data corroborate our immunocytochemical data demonstrating the activation of these caspases in the weaver mouse and furthermore indicate that caspase-9 is activated upstream of both caspase-3 and -8.

**Rescue of Weaver Cerebellar Granule Cells by Caspase Inhibition**—Granule neurons purified from P5–7 cerebella were cultured, and cell viability was measured by the MTT assay. As shown in Fig. 2A, there was a 25–50% greater incidence of death among uv/uv cells compared with +/+ neurons at 2 and 3 days in vitro. Granule neurons isolated from uv/uv cerebella were cultured in the presence of 20 μM MK-801, 20 μM verapamil, and 100 μM QX-314. Our data corroborate earlier findings (28) demonstrating that these cationic channel blockers markedly enhanced uv/uv granule cell viability (Fig. 2A), supporting the hypothesis that the mutant channel is nonselective, fluxing Na⁺. Our data show that uv/uv granule cell...
Caspase-9, -8, and -3 are activated in wv/wv. Activation is involved in the granule cell death associated with the caspase-9-specific caspase inhibitor. This suggests that caspase-9 activation is restricted to the period of axonogenesis (34) but not by weaver granule cells, which normally fail to differentiate (28). Surviving untreated weaver granule cells were TAG-1-negative as expected but did express the antigen if treated with the cationic channel inhibitor QX-314 as previously shown (28). As illustrated in Fig. 3, wlvew, p35 granule cells also express TAG-1, demonstrating that caspase inhibition allows wlvew granule cell differentiation to proceed through axonogenesis at least in vitro.

**Caspase involvement in wlvew granule cell death.** P5 cerebellar granule cells were isolated from +/+ and wlvew mice. A, caspase-9-, -8, and -3 are activated in +/+ and wlvew cerebellar granule cells. Cells were fixed and immunostained for active caspase-9, -8, and -3 using antibodies specific for these proteins. 4',6-Diamidino-2-phenylindole staining was used to identify cell nuclei (blue). Original magnification, ×40. B, time course of caspase-9, -8, and -3 activities. Cytosolic protein extracts were from +/+ and wlvew cerebellar granule cells grown in the absence or presence of 25 μM caspase-9 inhibitor, 25 μM caspase-8 inhibitor, and 25 μM caspase-3 inhibitor as described under "Experimental Procedures" (n = 4). *, p < 0.01; **, p < 0.001, significantly different from +/+, #, p < 0.01, significantly different from wlvew.

viability was equally protected by either a general or a caspase-9-specific caspase inhibitor. This suggests that caspase-9 activation is involved in the granule cell death associated with the wlvew mutation (Fig. 2A).

Baculoviral p35 is a general caspase inhibitory protein similar to CrmA that acts to suppress host defense mechanisms that otherwise would eliminate virus-infected bacteria by apoptosis (29, 30). p35 is known to bind and to inhibit multiple vertebrate (26) and invertebrate caspases (31). It has been shown to protect against apoptosis induced by a variety of stimuli in a variety of different model systems (32). To assess the effect of the presence of GIRK2ew on caspase activation levels, time courses of activation of caspase-9, -3, and -8 in wlvew cells in the absence or presence of p35 expression via crossing weaver mice with a transgenic mouse model previously generated in our laboratory (26) or pharmacological cationic channel blockers were performed. As shown in Fig. 2B, the intracellular activity levels of caspase-9, -3, and -8 were significantly increased in wlvew granule neurons up to 48 h in vitro as compared with +/+ neurons. p35 expression had a profound effect on caspase activities, resulting in a significant decrease in caspase activation in granule cells from wlvew with p35 mice (Fig. 2B). Significantly, the channel blockers MK-801, verapamil, and QX-314 largely prevented caspase-9, -3, and -8 activation in wlvew granule neurons (Fig. 2B).

Developmental differentiation of cerebellar granule neurons can be monitored via the transient expression of TAG-1, a glycoprotein localized in the plasma membrane whose expression in cerebellar granule cells is restricted to the period of axonal elongation during the first two postnatal weeks in mice (28, 33). To test whether rescued wlvew granule cells are able to proceed with differentiation, cultured cells were assayed for TAG-1 expression. As previously shown (18), in situ end-labeling reactions demonstrated that caspase inhibition allows wlvew granule cell differentiation to proceed through axonogenesis at least in vitro.

**Rescue of weaver granule cells.** A, cell viability in cerebellar granule cells in vitro as measured by MTT assay in +/+ and wlvew cells grown in the absence or presence of 20 μM MK-801, 20 μM verapamil, 100 μM QX-314, wlvew cells expressing p35, or wlvew cells grown in the presence of 25 μM caspase-9 inhibitor, n = 4. *, p < 0.001, significantly different from +/+, #, p < 0.01, significantly different from wlvew. B, time course of caspase-9, caspase-3, and caspase-8 activities in presence of either cationic channel blockers or p35 (n = 4). *, p < 0.01; **, p < 0.001, significantly different from +/+, #, p < 0.01, significantly different from wlvew.
Effects of Caspase Inhibition on Weaver

Effects of Caspase Inhibition on Weaver

Effects of Caspase Inhibition on Weaver

Effects of Caspase Inhibition on Weaver

Effects of Caspase Inhibition on Weaver
weaver’s motor deficiencies. In the present experiments, the performance of P21+/+, wv+, and wv/wv mice both expressing p35 and not expressing p35 was compared on a battery of behavioral tests in an open field environment. The tests included spontaneous locomotion, mean velocity, rest time, and climbing ability. We observed no significant difference in these four parameters between +/+ and wv/+ mice, whether or not they expressed p35, consistent with past reports. In contrast, wv/wv mice had decreased locomotor behavior (i.e. movement was slower), and they moved shorter distances per move and increased rest time compared with +/+ or wv/+ over a 10-min trial period. During the rest periods, defined as lack of detectable activity by the apparatus for a period of 2 s or longer, we observed that wv/wv mice remained fairly stationary. In contrast, the +/+ mice were engaged in grooming behavior during the rest period. In addition, the wv/wv mice were ~10 times slower to crawl onto a platform from a hole 23 mm deep × 22 mm in diameter at the bottom of the apparatus. The behavioral deficits in the wv/wv were partially rescued in the presence of p35 expression (Fig. 6). These data point to an improvement in coordination in the weaver mice that express the p35 transgene.

**DISCUSSION**

Cell death in the weaver mouse has up until now been primarily described morphologically (3, 4, 18) with limited biochemical evidence suggesting by what mechanism this occurs (36, 37). Although a few studies have implicated apoptosis in weaver cerebellar granule cell death (18, 36), the exact cause of apoptotic cell death remains unknown. Apoptosis has been shown to occur by both caspase-dependent and -independent means. We show here that the mutation that leads to the weaver phenotype, Girk2wv, elicits apoptosis in granule cells by caspase activation. Furthermore, results from our pharmacological and immunocytochemical experiments establish that caspase-9 is the apical upstream caspase involved in cerebellar granule cell death in the weaver mouse. Caspase-9 is normally involved in propagating intracellular apoptotic stimuli. Caspase-9 can in turn cleave and activate downstream executioner caspases such as caspase-3. This leads to cleavage of additional cellular substrates, resulting in morphological changes associated with apoptosis including DNA fragmentation and cytoskeletal disruption (38, 39). Recent evidence from cell-free and in vitro expression systems have suggested that in addition to being a final effector in neuronal apoptosis, caspase-9 is also capable of eliciting cleavage and activation of the initiator caspase, caspase-8 (40, 41). Although caspase-8 activation is generally thought to occur upstream of caspase-9, we have recently demonstrated that caspase-8 activation in dopaminergic neurons in the MPTP mouse model of Parkinson’s disease occurs downstream of activation of both caspase-9 and caspase-3 (42). Neuronal cell death in the weaver’s cerebellum may involve a similar pathway of caspase activation. This is to our knowledge the first identification of a molecular cell death pathway acting downstream of the altered ion channel function responsible for cell loss in the weaver mutant.

Intriguingly, caspase-3 was found in our studies to be expressed throughout the EGL in P7 weaver mice including in the external-most subdivision where the proliferating granule cells reside. The Girk2 channel protein has also been found to be expressed in mitotic cells of the EGL (28, 43–45). In contrast, TUNEL staining (a marker of DNA fragmentation, a late event in the apoptotic cell death pathway) was absent (Fig. 4) (18). Taken together, these data suggest that caspase-induced apoptosis triggered by the Girk2 mutation is initiated during the mitotic phase in cerebellar granule neurons, although cell death be-
comes morphologically evident only later at the time of postmitotic differentiation.

These studies also show that neuronal expression of the baculoviral protein p35 significantly attenuates caspase activation both in vitro and in vivo, resulting in a reduction in numbers of apoptotic cerebellar granule cells in p35/wave mice and an increase in IGL cell numbers (Fig. 4), although not all granule cells containing the GIRK2 mutation undergo cell death but primarily those at the vermis (13, 19). This suggests decreased apoptosis and increased migration of cells from the EGL to the IGL in p35 mice in the presence of the p35 transgene. In previous studies of this line of p35 transgene, we observed the highest level of expression of the transgene in the cerebellum. In addition, neuronal expression of p35 in these animals was found to significantly lower caspase activation induced by either staurosporine or lowered extracellular K+ levels in primary cerebellar granule cells cultured in vitro (26).

Our present data demonstrate that caspase inhibition via p35 allows cerebellar granule cell differentiation to proceed in vitro as exemplified by their expression of the late neuronal differentiation marker TAG-1. Weaver cerebellar granule cells without p35 fail to extend axonal processes and to express TAG-1 (28, 46). This suggests that caspase inhibition and subsequent attenuation of granule cell death allows some cells to differentiate beyond the stage of migration and axonogenesis, which could explain the observed improvement in coordination in these animals in vivo. These data are further corroborated by in vivo increases in the number of cells in the P21 IGL of the p35-expressing weaver mice, suggesting that the presence of the transgene results in a delay in apoptotic cell death. Delayed cell death could allow some cells to undergo normal migration from the EGL to the IGL and to functionally differentiate. The presence of TUNEL cells in the IGL further suggests that apoptosis may be delayed in these cells. Whereas p35 expression does not fully reverse cerebellar granule cell death in the weaver, the rescue is sufficient to allow diminished cell loss and a partial attenuation of behavioral effects in the presence of the transgene.

The results of this study are consistent with the view that Na+ influx is responsible for subsequent caspase activation and apoptotic cell death in weaver cerebellar granule cells. Previous electrophysiological experiments using heterologous systems have suggested that GIRK2/ev results in Na+ influx through a nonselective channel (28, 43, 47, 48). Electrophysiological studies of the weaver’s granule neurons have proven more controversial. Some groups have reported that the mutated channel appears to be nonselective, leading to increased Na+ permeability (28, 43, 47, 48), whereas others have reported that the mutation results in loss in channel function (49, 50). Our in vitro data agree with earlier reports that neurons can be rescued from apoptotic cell death by the addition of cationic channel blockers (e.g. MK-801, verapamil, and QX-314). We found here that these same channel blockers also prevent caspase-9,-8,-7 activation. This suggests that cationic influx is required for caspase activation, triggering this otherwise irreversible program of cell death (Fig. 2).

In conclusion, we have demonstrated that the mutant channel in granule cells results in subsequent caspase activation in vitro and in vivo. The activation of caspases is initiated prior to differentiation in mitotic cells in the EGL. Furthermore, based on immunocytochemistry and enzymatic assays, caspase-9 is the apical caspase involved in the subsequent neuronal cell death process, which also involves caspase-3 and -8 (Fig. 7). Caspase inhibition by p35 decreases caspase activation and allows neuronal differentiation to proceed in the weaver’s granule cells, resulting in an attenuation in cerebellar granule apoptosis both in vitro and in vivo and at least some degree of normal migration and differentiation in vivo. Cerebellar granule cell viability in association with an improvement in weaver behavior has been previously reported by another group (19). Interestingly, in this case granule cells were reversed when NRI N-methyl-d-aspartate subunits were knocked out in weaver NR1 double mutants. The differences in behavioral deficits were described, but quantitative data were not presented. We also found that the partial block or delay in cell death has functional consequences. Homozygous weaver mice that also carried p35 were far better coordinated and less ataxic that homozygous weavers without p35. p35 reversed the motor deficits seen in homozygous weavers in all four tests of motor behavior administered. This was particularly striking in the hole climbing task. Weaver mice without p35 were dramatically impaired in terms of their ability to climb out of a 23-cm hole. However, in the presence of the p35 transgene, this was completely reversed to wild-type levels.

Understanding the molecular events underlying neuronal cell loss in the weaver’s mouse and how to reverse them may not only aid us in understanding this specific process but also may lend insight into treatment of related human disorders in which neurodegeneration plays a major role.

Acknowledgment—We thank Dr. Joan Schein for helpful suggestions on the PCR.

REFERENCES

35. Wullner, U., Weller, M., Scholz, J. B., Krajeski, S., Reed, J. C., and
Inhibition of Caspases Protects Cerebellar Granule Cells of the Weaver Mouse from Apoptosis and Improves Behavioral Phenotype
Jun Peng, Zhijin Wu, Yongqin Wu, Mike Hsu, Fang Feng Stevenson, Rapee Boonplueang, Suzanne K. Roffler-Tarlov and Julie K. Andersen

doi: 10.1074/jbc.M207407200 originally published online September 6, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M207407200

Alerts:
     • When this article is cited
     • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 49 references, 20 of which can be accessed free at http://www.jbc.org/content/277/46/44285.full.html#ref-list-1