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Glutamate as a hippocampal neuron survival factor: An inherited defect in the trisomy 16 mouse

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ABSTRACT **The survival of cultured mouse hippocampal neurons was found to be greatly enhanced by micromolar concentrations of the excitatory neurotransmitter glutamate. Blockade of kainate/AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) glutamate receptors increased the rate of neuron death, suggesting that endogenous glutamate in the cultures promotes survival. Addition of glutamate (0.5–1 μM) further increased neuron survival, whereas glutamate in excess of 20 μM resulted in increased death. Thus, the survival vs. glutamate dose–response relation is bell-shaped with an optimal glutamate concentration near 1 μM. We found that hippocampal neurons from mice with the genetic defect trisomy 16 (Ts16) died 2–3 times faster than normal (euploid) neurons. Moreover, glutamate, at all concentrations tested, failed to increase survival of Ts16 neurons. In contrast, the neurotrophic polypeptide basic fibroblast growth factor did increase the survival of Ts16 and euploid neurons. Ts16 is a naturally occurring mouse genetic abnormality, the human analog of which (Down syndrome) leads to altered brain development and Alzheimer disease. These results demonstrate that the Ts16 genotype confers a defect in the glutamate-mediated survival response of hippocampal neurons and that this defect can contribute to their accelerated death.**

The virtual absence of neurogenesis in the adult mammalian brain underscores the necessity of ensuring the survival of neurons over many years. Since it has been suggested that all cells require a constant trophic signal to block cell death (1), the loss of neurons associated with normal aging and the accelerated neuronal death characteristic of neurodegenerative disorders could result from the failure of one or more intrinsic survival mechanisms.

Many agents have been shown to promote the survival of neurons. Polypeptide neurotrophic factors (2–5) include basic fibroblast growth factor (bFGF), glial cell line-derived neurotrophic factor, and the neurotrophins—e.g., nerve growth factor and brain-derived neurotrophic factor. Not all types of neurons respond to every neurotrophic factor, but many neurons respond to multiple factors, providing a redundancy of trophic inputs to ensure long-term neuron survival.

Activation of excitatory amino acid receptors has also been shown to elicit a trophic response in neurons. Although very high concentrations of glutamate are toxic to most neurons (for review, see ref. 6), lower concentrations of glutamate or glutamate agonists have been reported to increase survival of postnatal cerebellar granule cells *in vitro* (7–9), while glutamate receptor (GluR) antagonists can decrease their survival (7). Similarly, metabotropic GluR activation has been found to promote the *in vitro* survival of cerebellar Purkinje and granule cells (10, 11). It has also been shown that *in vivo* treatment with *N*-methyl-D-aspartate (NMDA) GluR antagonists decreases the numbers of surviving granule cells in the developing dentate gyrus of the hippocampus (12), suggesting that endogenous glutamate promotes the survival of these cells. The trophic actions of excitatory amino acid receptor stimulation may be mediated by increases in cytoplasmic calcium concentration ($[Ca^{2+}]_{cyt}$), as has been reported for the survival-promoting effects of depolarization (13, 14).

Many neurodegenerative disorders, including Alzheimer disease (AD) and amyotrophic lateral sclerosis, result in the premature loss of specific classes of neurons in the central nervous system. Down syndrome (DS or trisomy 21) is of interest for the study of neurodegeneration because, in addition to a variety of neurological and systemic disorders, all DS individuals develop dementia and the neuropathological symptoms of AD (15, 16). A substantial part of human chromosome 21, including genes for β-amyloid precursor protein, the free radical scavenging enzyme superoxide dismutase, and the type 5 ionotropic GluR (GluR5), and most of the region associated with DS, can be found on mouse chromosome 16 and the genes in this region are triplicated in the trisomy 16 (Ts16) mouse (17, 18), suggesting that the Ts16 mouse may be an animal model for DS. Although Ts16 mice do not survive to birth, Ts16 fetuses have been shown to exhibit abnormal brain development and other abnormalities that parallel the symptoms of DS (19–23). Furthermore, since Ts16 cholinergic basal forebrain neurons appear less likely to survive when grown *in vitro* (24) or transplanted into normal mouse brain (25), neuron survival and development may be affected in Ts16.

Since neurodegeneration in the hippocampus is a characteristic finding in AD, we focused our studies of Ts16 on the hippocampus and found that Ts16 hippocampal neurons *in vitro* die at an accelerated rate. The following experiments were undertaken to elucidate the mechanism of this accelerated neuron death. We found that micromolar concentrations of glutamate promote the survival of euploid neurons but that this response is lacking in Ts16 cells. A preliminary report of these findings has appeared (26).

EXPERIMENTAL PROCEDURES

Generation of Trisomic Mice and Karyotyping. Male mice doubly heterozygous for appropriate Robertsonian chromosome translocations [Rb(6,16)24LuBXRb(16,17)7BNRFI] were mated with C57BL/6J female mice. The next day was designated as day 1 of gestation (embryonic day 1). Normal and trisomic fetuses were easily distinguished; however, karyotypes were routinely generated from livers to confirm the genotype. Mice were obtained from The Jackson Laboratory.

Neuron Cell Culture. Cultures of embryonic hippocampus were prepared from euploid and Ts16 mouse fetuses at embryonic day 16. For each Ts16 fetus, a euploid fetus from the same litter was used. The hippocampi were freed of

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Abbreviations: GluR, glutamate receptor; K/A, kainate/AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) subtype of glutamate receptor; Ts16, trisomy 16; NMDA, *N*-methyl-D-aspartate; bFGF, basic fibroblast growth factor; DS, Down syndrome; AD, Alzheimer disease; $[Ca^{2+}]_{cyt}$, cytoplasmic Ca^{2+} concentration; APV, D-2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DIV, day(s) *in vitro*.

meninges, digested with trypsin, and dissociated by trituration in MEM 10/10 [minimum essential medium (MEM) with Earle's salts/2 mM glutamine/10% (vol/vol) fetal bovine serum/10% (vol/vol) horse serum/penicillin (100 units/ml)/streptomycin (100 units/ml)]. Cells were plated at 50,000 cells per cm^2 on 12-mm glass coverslips photoetched with a lettered grid of 175 μm × 175 μm squares (Eppendorf). The coverslips were pretreated with poly(L-lysine) (Sigma). At 1 day *in vitro* (DIV), the MEM 10/10 was replaced with MEM supplemented with B27 (ref. 27; obtained from GIBCO/BRL) and 10 μM cytosine β-D-arabinofuranoside (Sigma) to kill proliferating cells, including astrocytes and neuroblasts. The B27 supplement contains optimized concentrations of neuron survival factors including triiodothyronine, cortisol, transferrin, glutathione, DL-α-tocopherol, and insulin. At 2 DIV, the medium was changed to MEM with B27. The cultures were maintained at 37°C in 95% air/5% CO_2. Each coverslip was kept in a separate well; two to four coverslips were used for each condition in each experiment.

Neuron Survival. At 2 DIV, all the live neurons in each of four 175 μm × 175 μm squares were counted and photographed by using a Nikon Diaphot inverted microscope. Neurons that were in the process of degenerating (i.e., that displayed nuclear condensation, membrane blebbing, and extensive neurite fragmentation) were excluded. On each subsequent day, the same areas were located and recounted. All treatments began at 2 DIV and the number of live neurons remaining at each day was expressed as a percentage of the number at 2 DIV. Similar live neuron counts were obtained whether the cultures were counted daily or every third day. Thus, daily removal of the cultures from the incubator for counting does not significantly affect neuron survival.

Materials and Reagents. Glutamate and D-2-amino-5-phosphonovaleric acid (APV) were obtained from Sigma. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) was obtained from Research Biochemicals (Natick, MA). CNQX, APV, and glutamate were prepared as stock solutions in MEM. Unless otherwise stated, all tissue culture media and supplements were obtained from GIBCO/BRL.

RESULTS

Cultures of hippocampal neurons were prepared from euploid and Ts16 mouse fetuses at embryonic day 16, plated on coverslips etched with a lettered grid, and maintained in serum-free medium containing B27 supplement (27). Astrocytes made up <10% of the cells throughout these experiments. Fig. 1 *A* and *B* shows phase-contrast micrographs of typical fields in euploid and Ts16 cultures, respectively, at 2 DIV. Euploid and Ts16 cultures were similar in appearance and cell density. Two days later (4 DIV) most of the euploid neurons were still present (Fig. 1*C*), while a substantial number of the Ts16 cells had died (Fig. 1*D*) leaving only debris.

Ts16 and euploid cell body size and neuron shape were not obviously different at 2 DIV. Both Ts16 and euploid neurons had an average of three or four neurites per cell with similar distributions around the mean value (data not shown). Thus, despite the increased death of Ts16 neurons, there was no evidence for morphological differences between euploid and Ts16 neurons at 2 DIV.

To quantitatively analyze the accelerated neuron death, the total number of cells identifiable as live neurons by phase-contrast microscopy in each grid square was counted daily for 6 days. Live neurons were characterized by their small (10 μm) phase-dark cell bodies and fine processes. The neuronal identity of cells with this morphology was confirmed by immunocytochemistry with anti-neurofilament antibodies. Cell counts at each day are expressed as a percentage of the starting number of cells at 2 DIV. The results of five experiments are summarized in Fig. 2. About half of the euploid neurons

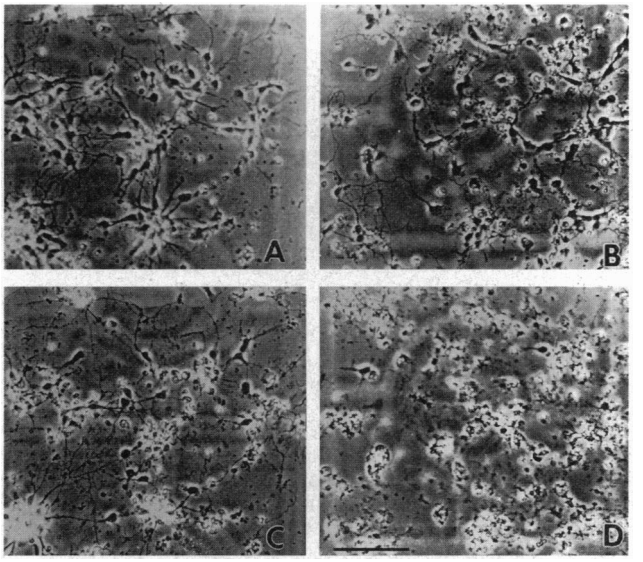

FIG. 1. Phase-contrast photomicrographs showing euploid (*A* and *C*) and Ts16 (*B* and *D*) mouse hippocampal cultures at 2 DIV (*A* and *B*) and 4 DIV (*C* and *D*). *A* and *C* show the same field; *B* and *D* show the same field. The etched grid used to define the area counted is visible but out of focus. (Bar = 50 μm.)

survived to 5 DIV. This degree of survival of mouse hippocampal neurons is similar to that reported for embryonic rat hippocampal neurons grown in serum-free B27-supplemented DMEM in the absence of glia (27). In contrast, <10% of the Ts16 neurons remained at 5 DIV. The decrease in survival for both euploid and Ts16 neurons was linear on a semilogarithmic plot, indicating that neuron death is an exponential process—i.e., the probability of any one neuron dying is constant throughout the experiment. The lines through the data points are linear regressions, indicating death rates for euploid and Ts16 neurons of 26% per day and 58% per day, respectively. Thus, the Ts16 neurons die more than twice as fast as euploid neurons. To characterize the possible mechanisms that could

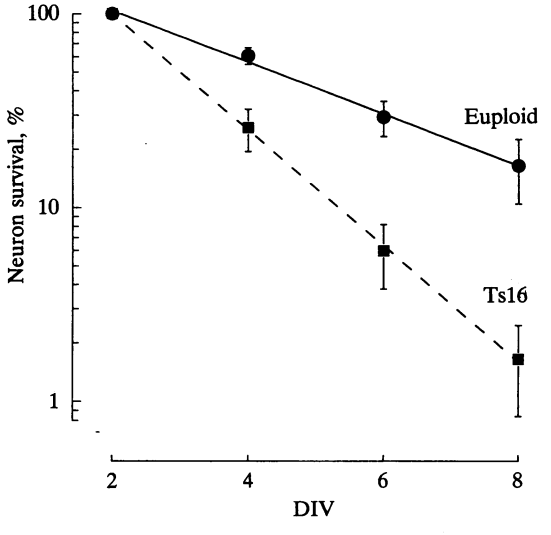

FIG. 2. Survival of euploid (●) and Ts16 (■) hippocampal neurons maintained in B27-supplemented MEM. Live neurons were counted daily by using phase-contrast microscopy in identified regions of the cultures (see Fig. 1). For each experiment, survival was calculated as the percent of cells at 2 DIV that remain at each subsequent day. Data are the mean ± SEM of five experiments. Lines are linear regressions through the means. The slopes of the lines are significantly different at $P < 0.01$.

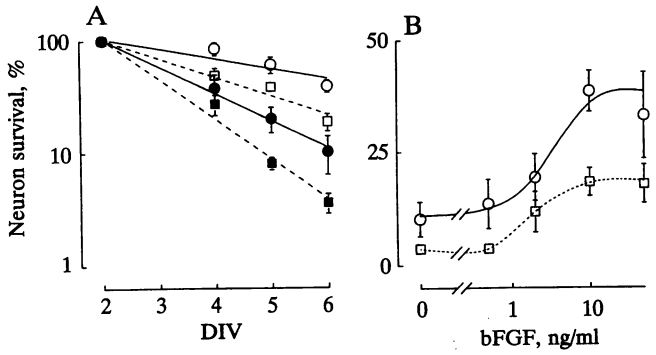

FIG. 3. Effect of bFGF on euploid (circles) and Ts16 (squares) hippocampal neuron survival. Medium was changed daily and survival was determined as in Fig. 2. Data are the mean ± SEM of four or five experiments. (*A*) Survival (logarithmic scale) in the absence (solid symbols) and presence (open symbols) of bFGF (10 ng/ml). Lines are linear regressions. bFGF significantly increased the survival ($P < 0.01$) of both euploid and Ts16 neurons. ○, Euploid plus bFGF; □, Ts16 plus bFGF; ●, euploid; ■, Ts16. (*B*) bFGF dose–response curves for euploid (○) and Ts16 (□) hippocampal neurons. Survival was determined at 6 DIV after 4 days of treatment with bFGF. The lines have no theoretical significance. Neuron survival in bFGF at 10 ng/ml was significantly greater ($P < 0.01$) for euploid than for Ts16 cells.

contribute to the observed impaired survival of Ts16 neurons, we carried out the following experiments.

When the B27 supplement was removed from the medium, both euploid and Ts16 neurons died substantially faster; but Ts16 neurons still died several times faster than euploid neurons (data not shown). Doubling the amount of B27 in the medium did not increase survival of either euploid or Ts16 neurons (data not shown). Thus, the accelerated death of Ts16 neurons was not due to an altered response to one or more components of the B27 supplement.

Survival of cultured neurons is increased by a variety of polypeptide growth factors, only one of which (insulin) is present in the B27-supplemented medium. Rodent hippocampal neuron survival is promoted by bFGF, which is normally produced by astrocytes (28). To determine whether the accelerated death of Ts16 neurons was due to lower levels of, or a diminished response to, polypeptide growth factors, survival of euploid and Ts16 hippocampal neurons was studied in the absence and presence of bFGF at 10 ng/ml (Fig. 3*A*). In these experiments, the medium was changed daily (in contrast to every 4 days for Fig. 2) to maintain constant bFGF levels [bFGF has been reported to have a half-life of <24 h in culture (29)]. Untreated Ts16 neuron survival was still compromised relative to euploid survival under these conditions, although, as would be expected, survival of both euploid and Ts16 neurons was reduced by the daily medium changes (compare survival in Figs. 2 and 3*A*). Addition of bFGF enhanced the survival of both euploid and Ts16 neurons (Fig. 3). The bFGF dose–response curves (Fig. 3*B*) reveal that euploid and Ts16 neurons are equally sensitive to bFGF ($K_{bFGF} \approx 3$ ng/ml). Therefore, the Ts16 neurons have a normal response to bFGF. Further, the ability of bFGF to rescue Ts16 cells shows that Ts16 neurons are not irrevocably destined to die at the time of bFGF addition (2 DIV). Indeed, in the presence of bFGF at 3 ng/ml, the survival of Ts16 neurons is indistinguishable from the survival of euploid neurons in the absence of bFGF. However, bFGF does not cure the Ts16 deficit since Ts16 neurons are still less viable than similarly treated euploid neurons even at maximally effective concentrations of bFGF. This suggests that the Ts16 defect is unlikely to be due to insufficient levels of, or an abnormal response to, bFGF or a similarly acting substance.

Since both excitotoxic death and neuron survival can be mediated through GluRs (6–9), the presence of the gene coding for a kainate-preferring GluR (GluR5) on murine chromosome 16 (18) raised the possibility that the Ts16 neurons might have an altered response to glutamate that could lead to their accelerated death, possibly an increased sensitivity to endogenous glutamate in the cultures, which could cause neuron death by an excitotoxic mechanism. To test this hypothesis, euploid and Ts16 hippocampal neurons were treated with a mixture of the NMDA-type GluR blocker APV and the kainate/AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) (K/A) ionotropic GluR blocker CNQX. This mixture of GluR blockers did not improve the survival of Ts16 neurons (Fig. 4*B*), indicating that the increased rate of Ts16 neuron death was not due to increased excitotoxicity. However, the GluR blockers decreased the survival of euploid neurons (Fig. 4*A*), suggesting that activation of GluRs by endogenous glutamate contributes to euploid neuron survival. In four experiments, treatment with GluR blockers reduced euploid neuron survival to Ts16 levels (Fig. 4*C*). When tested individually, CNQX, but not APV, significantly decreased the survival of euploid neurons. Neither a combination of APV and CNQX (Fig. 4) nor each separately (data not shown) affected survival of Ts16 neurons. These results show that endogenous glutamate acting at K/A GluRs promotes the survival of euploid hippocampal neurons and that either this response is lacking in Ts16 neurons or insufficient glutamate is present in Ts16 cultures to promote neuron survival.

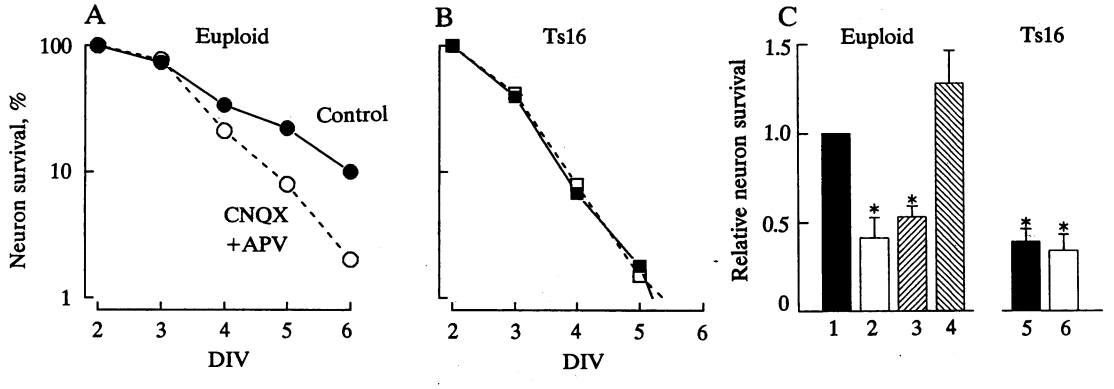

FIG. 4. Effect of GluR blockers on euploid and Ts16 hippocampal neuron survival. Survival was determined as described in Fig. 2. Blockers used were 50 μM CNQX and 100 μM APV. (*A* and *B*) Time course of euploid (*A*) and Ts16 (*B*) survival was determined in the absence (solid lines) and presence (dashed lines) of a mixture of CNQX and APV. Data are the means of quadruplicate measurements from one experiment; similar results were obtained in three other experiments. (*C*) Summary of the effects of a mixture of CNQX and APV on euploid and Ts16 neurons (bars 2 and 6) and of CNQX (bar 3) and APV (bar 4) individually on euploid neurons at 5 DIV, 3 days after addition of GluR antagonists. Controls (bars 1 and 5) are the values for untreated cultures. For each experiment, survival was normalized to that of untreated euploid neurons (bar 1). Data are the mean ± SEM of four experiments. *, Significantly different from 1.0 at $P < 0.02$.

The effects of GluR activation on euploid and Ts16 neuron survival were further investigated by determining survival in the presence of various concentrations of glutamate. The medium was changed daily to compensate for variations due to glutamate uptake or release by cells in the cultures. As described above, in the absence of added glutamate, euploid neuron survival was greater than Ts16 neuron survival. CNQX (50 μM) had no effect on Ts16 survival but reduced euploid neuron survival to the Ts16 level (Fig. 5). The addition of micromolar concentrations of glutamate enhanced euploid neuron survival with a maximally effective glutamate concentration of 0.5–1 μM. In contrast, glutamate concentrations in the same range had no effect on Ts16 survival. Concentrations of glutamate >20 μM decreased the survival of both euploid and Ts16 neurons to levels below those observed in the absence of added glutamate (data not shown), probably reflecting the well-established excitotoxic effects of glutamate on neurons (6). In five similar experiments where the effects of glutamate concentration on hippocampal neuron survival were examined, the maximal effect of glutamate on euploid neurons was a 2.5 ± 0.5 times increase in survival compared to the survival in the absence of added glutamate and presence of CNQX. The maximally effective concentration of added glutamate varied from 0.2 to 1 μM, possibly reflecting culture-to-culture variation in endogenous glutamate levels. Nevertheless, in every case, added glutamate (0.2–1 μM) increased survival of euploid neurons, while glutamate failed to rescue Ts16 neurons at any concentration tested. Therefore, the lack of effect of CNQX on Ts16 neurons resulted from their inability to respond to glutamate rather that from a lack of glutamate in the Ts16 culture medium.

There are several possible sources of the endogenous glutamate active in our cultures. (*i*) Many of the hippocampal neurons are glutamatergic and may release glutamate in the vicinity of neuronal GluRs. (*ii*) Although we have attempted to reduce the number of astrocytes in these cultures, the remaining astrocytes may release glutamate, as demonstrated in rat hippocampal cultures (30). (*iii*) We cannot rule out the possibility that while the culture medium is nominally glutamate-free, a low level of contamination of the glutamine (2 mM) in the medium could lead to the presence of micromolar glutamate.

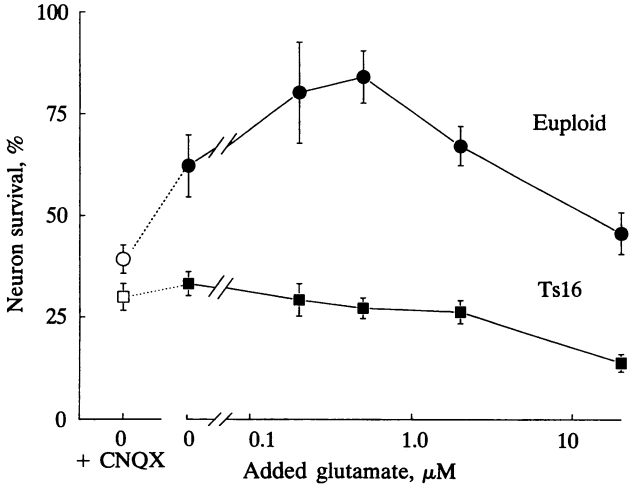

FIG. 5. Dependence of euploid (circles) and Ts16 (squares) hippocampal neuron survival on glutamate. The ordinate shows the concentration of added glutamate, which acts together with the endogenous glutamate present in the cultures to affect neuron survival. Survival was determined as in Fig. 2 at 5 DIV after 3 days of glutamate treatment. Open symbols show data obtained in the absence of added glutamate and the presence of 50 μM CNQX. Solid symbols show data in the absence of CNQX. Data are the mean ± SEM determined from four fields per coverslip on three coverslips in a single experiment. The experiment was repeated four additional times with similar results: added glutamate always increased survival of euploid neurons and never affected survival of Ts16 neurons.

DISCUSSION

These experiments demonstrate that micromolar concentrations of glutamate act through K/A receptors to promote mouse hippocampal neuron survival *in vitro*. Cells from Ts16 hippocampus were found to have an accelerated rate of death and to lack this trophic response to glutamate. The defective response of Ts16 neurons to glutamate is sufficient to explain most of the increase in their rate of death.

Glutamate Is Required for Optimal Survival of Normal Hippocampal Neurons. The decreased survival of euploid neurons in the presence of CNQX (Fig. 4 *A* and *C*) shows that endogenous glutamate acting through K/A GluRs promotes the survival of hippocampal neurons. Neuron survival was further increased by the addition of 0.5–1 μM glutamate (Fig. 5). If neuron survival in the presence of CNQX is taken as the true basal level of cell survival in the absence of K/A GluR activation, it is clear that optimal levels of glutamate can cause a 2- to 3-fold enhancement of neuron survival.

There is considerable evidence demonstrating that survival of cerebellar granule cell neurons *in vitro* is decreased by blocking NMDA or non-NMDA GluRs while NMDA and glutamate themselves promote granule cell survival (7–9). *In vivo*, NMDA receptor antagonists also decrease neuron survival in the dentate gyrus (12). Studies have also shown a trophic role for metabotropic GluR activation in cerebellar Purkinje and granule cell survival (10, 11). These results and our finding of a trophic role for K/A GluR activation in the hippocampus suggest that glutamate may act throughout the brain as a trophic factor and that its trophic actions may be mediated by distinct receptor subtypes in different neuronal populations. Moreover, since micromolar glutamate is known to be present in the adult brain (31), glutamate may contribute to neuron survival throughout life.

A "Glutamate Window" for Optimal Neuron Survival. Exposure to very high concentrations of glutamate is toxic to neurons. This excitotoxic neuron death is an important component in neuron loss in brain injuries and stroke (6). Chronic exposure to 10–50 μM glutamate also compromises cell survival (32). However, while elevated glutamate is lethal, previous studies in the cerebellum (7–9) and our results in the hippocampus imply that glutamate levels that are too low are also detrimental to neuron survival, suggesting the existence of a glutamate window—a range of glutamate concentrations optimal for neuronal survival. While we have not directly measured the concentration of endogenous glutamate in our cultures, the substantial increase in euploid neuron survival observed with 0.2–1 μM added glutamate (Fig. 5) indicates that endogenous levels are in a similar range. Thus we estimate that the concentration of glutamate optimal for neuron survival is 1–3 μM. Interestingly, it has been reported that in the adult hippocampus, extracellular levels of glutamate *in vivo* are ≈3 μM (31) and, thus, would be expected to provide maximal trophic support based on our *in vitro* experiments.

Johnson and colleagues (33, 34) have suggested that $[Ca^{2+}]_{cyt}$ must be maintained near the Ca^{2+} set point (between 150 and 250 nM) for optimal neuron survival. Excitotoxic concentrations of glutamate lead to cell death by raising $[Ca^{2+}]_{cyt}$ above the set point (35). In contrast, both depolarization (with elevated K^+) and low concentrations of glutamate cause smaller increases in $[Ca^{2+}]_{cyt}$ and are thought to enhance cell survival by increasing $[Ca^{2+}]_{cyt}$ from suboptimal levels toward the Ca^{2+} set point, an interpretation supported by the finding that inhibition of Ca^{2+} influx blocks the trophic effects of depolarization (7, 13, 14). The glutamate window for neuron survival may thus correspond to the concentration of

glutamate required to maintain neuronal $[Ca^{2+}]_{cyt}$ within this optimal range.

Maintaining glutamate concentrations within this trophic window may be an important *in vivo* role for astrocytes. Astrocytes are known to accumulate glutamate and convert it to the inactive amino acid glutamine, thus protecting neurons from the excitotoxic effects of high glutamate (36, 37). Recently, cultured rat hippocampal astrocytes have also been shown to be capable of releasing glutamate by a Ca^{2+}-dependent mechanism (30). Thus, our findings and this observation suggest that astrocytes may play a dual role in ensuring neuron survival not only by protecting neurons from high excitotoxic levels of glutamate but also by supplying glutamate when endogenous levels fall below those that are optimal for neuron survival.

Hippocampal Neurons from the Ts16 Mouse Die Faster than Euploid Neurons. When cultured under identical conditions in serum-free chemically defined medium, Ts16 hippocampal neurons died at least twice as fast as did neurons from euploid littermates. The Ts16 neurons were not, however, irrevocably destined to die faster, since they, as well as euploid neurons, could be partially rescued by bFGF. Indeed, with bFGF at 3 ng/ml, the survival of Ts16 neurons was virtually indistinguishable from that of untreated euploid neurons (Fig. 3*B*). The decreased survival of Ts16 neurons could not be attributed to an altered sensitivity to bFGF, because half-maximal rescue occurred at ≈3 ng/ml in both euploid and Ts16 neurons, and, when euploid and Ts16 cultures were compared at maximally effective bFGF concentrations, the Ts16 neurons still died faster (Fig. 3*B*).

Glutamate-Promoted Hippocampal Neuron Survival Is Absent in the Ts16 Mouse. The failure of CNQX or added glutamate to affect Ts16 neuron survival (Figs. 4 and 5) suggested that the accelerated Ts16 neuron death was due to the inability of the Ts16 neurons to respond to endogenous glutamate. Since the survival of euploid neurons in the presence of K/A GluR blockers was not significantly different from that of Ts16 neurons (Fig. 4*C*), this single defect may be sufficient to account for the accelerated death of Ts16 neurons. Further, the normal response of Ts16 cells to bFGF suggests that the Ts16 defect is specific to some part of the glutamate signaling pathway and does not simply reflect an inability to respond to trophic stimuli. Although several other studies have suggested that neurons from the Ts16 basal forebrain may be reduced in number (24, 25), herein we report a cellular mechanism that could underlie the observed neuropathology of Ts16.

The failure of glutamate to increase Ts16 neuron survival may be due to one or more causes. Ts16 neurons may lack the appropriate GluRs. It is possible that the increased gene dosage of GluR5 may alter the glutamate response in Ts16. A second possibility is that the signal transduction mechanism for glutamate-promoted survival may be defective in Ts16 neurons leading to an impaired ability of GluR activation to elevate cytoplasmic Ca^{2+} or to the failure of increases in intracellular Ca^{2+} to elicit a normal survival response.

This defect is not necessarily fatal, as the accelerated neuron death can be overcome, at least in part, by addition of another survival factor (bFGF). The presence of such multiple trophic inputs may help to explain one of the features of many neurodegenerative diseases including AD, which is that neurodegeneration ensues only after several decades of normal function, even in individuals genetically fated to develop the disease. Neurons with a defect in one survival mechanism may live, provided adequate amounts of other survival factors are available. The decreased production of neurotrophic factors with aging (5) could unmask such a defect, leading to neuron death. Our experiments in the Ts16 mouse suggest the hypothesis that a defect in the glutamate survival pathway could contribute to the pathophysiology of neurodegenerative diseases such as AD.

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