dues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

19. S. R. Stone and J. Hofsteenge, *Biochemistry* **25**, 4622 (1986).
20. H. Schagger and G. von Jagow, *Anal. Biochem.* **166**, 368 (1987).
21. Clotting assays were done according to the manufacturer's directions with kits supplied by Sigma Chemical Co. and normal human plasma (George King Biomedical). Plasma was preincubated with TAP for 15 min before the addition of the activator, and the clotting time was measured with a fibrometer (BBL Fibrosystem).

22. We thank M. Sardana, J. Rodkey, and L. Wassel for amino acid and sequence analyses, C. Bennett for helpful discussions, and C. Hannan for helping to prepare this manuscript. R. Endris provided the identification of the species of tick used in this study.

31 October 1989; accepted 6 March 1990

L-Cysteine, a Bicarbonate-Sensitive Endogenous Excitotoxin

JOHN W. OLNEY, CHARLES ZORUMSKI, MADELON T. PRICE, JOANN LABRUYERE

After systemic administration to immature rodents, L-cysteine destroys neurons in the cerebral cortex, hippocampus, thalamus, and striatum, but the underlying mechanism has never been clarified. This neurotoxicity of L-cysteine, in vitro or in vivo, has now been shown to be mediated primarily through the *N*-methyl-D-aspartate subtype of glutamate receptor (with quisqualate receptor participation at higher concentrations). In addition, the excitotoxic potency of L-cysteine was substantially increased in the presence of physiological concentrations of bicarbonate ion. L-Cysteine is naturally present in the human brain and in the environment, and is much more powerful than β-*N*-methylamino-L-alanine, a bicarbonate-dependent excitotoxin, which has been implicated in an adult neurodegenerative disorder endemic to Guam. Thus, the potential involvement of this common sulfur-containing amino acid in neurodegenerative processes affecting the central nervous system warrants consideration.

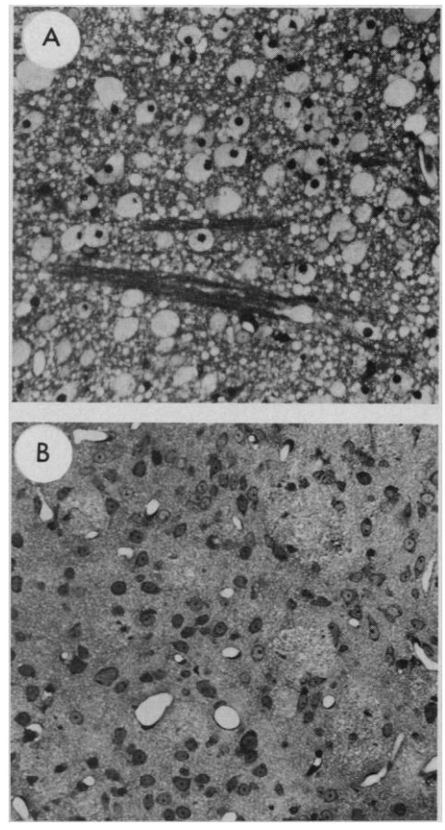

Fig. 1. (**A**) The caudate nucleus of a 4-day-old rat containing many acutely necrotic neurons ("bull's eye" profiles) 6 hours after L-Cys (1 g/kg) was administered subcutaneously. (**B**) In contrast, the caudate nucleus appears entirely normal in a 4-day-old rat 6 hours after a treatment consisting of the same dose of L-Cys accompanied by MK-801 (1 mg/kg). Magnification, ×180.

WHEN ADMINISTERED ORALLY IN high doses to infant mice, L-cysteine (L-Cys) reproduces the type of brain damage that is caused by glutamate (Glu) (*1*). This type of damage is typically restricted to circumventricular organ brain regions that lack blood-brain barriers and evolves very rapidly to reach end-stage neuronal necrosis within 2 to 3 hours. Paradoxically, lower doses of L-Cys resulted in a more devastating neurotoxic syndrome, which developed more slowly (4 to 6 hours) but damaged many more regions of the brain, including the cerebral cortex, hippocampus, caudate, and thalamus (*2*). L-Cys also causes a similarly widespread pattern of damage in the fetal rodent brain when administered orally or subcutaneously to the pregnant dam in late gestation (*2*). Curiously, neurons undergoing L-Cys–induced degeneration have an identical appearance by light or electron microscopy to those undergoing degeneration after exposure to Glu or various excitatory neurotoxic (excitotoxic) analogs of Glu. However, L-Cys has not been considered an excitotoxin because it lacks the Ω acidic group shared by all other excitotoxic analogs of Glu and it was not found to mimic the neuroexcitatory properties of Glu when administered microelectrophoretically onto spinal neurons (*3, 4*). The present experiments were undertaken to reexamine the neurotoxic properties of L-Cys in light of new information pertaining to excitotoxic mechanisms.

To determine whether a glutamate antagonist might protect against L-Cys neurotoxicity in vivo, we assigned 4-day-old Sprague-Dawley rat pups to one of two groups (ten per group) and treated one group subcutaneously (sc) with MK-801 (1 mg/kg), an antagonist of the *N*-methyl-D-aspartate subtype of glutamate receptor, and the other group with saline, then 30 min later we administered L-Cys (1 g/kg, sc) to both groups. The pups were returned to their mothers and observed for 6 hours, then anesthetized with halothane, and perfused with an aldehyde fixative solution; their brains were processed for histopathological evaluation (*4, 5*). All ten of the control pups had acute lesions in several brain regions; typically the most severely affected were the frontoparietal neocortex, hippocampus, thalamus, and caudate nucleus (Fig. 1A). None of the ten experimental pups sustained damage in any brain region (Fig. 1B). These findings implicate the NMDA receptor–ionophore complex in the in vivo neurotoxicity of L-Cys.

To evaluate mechanisms underlying L-Cys neurotoxicity in greater detail, we used the in vitro chick embryo retina, a preparation that is valuable for studying excitotoxic phenomena (*6*). Because the excitotoxic Glu agonists, NMDA, quisqualate (Quis), and kainic acid (KA), interact with different Glu-receptor subtypes that are distributed differently among retinal neurons, each agonist induces an acute cytopathological reaction in the chick retina that has its own distinctive cellular pattern. The typical pattern of neuronal degeneration induced by NMDA (80 μ*M*) in the chick retina is illustrated in Fig. 2A. Although L-Cys was nontoxic at low concentrations, it consistently induced a lesion pattern indistinguishable from the NMDA pattern at a concentration of 2 m*M* (Fig. 2B). The competitive NMDA antagonist, D-2-amino-5-phosphonopentanoate (D-AP5), at a concentration (50 μ*M*) known to block the neurotoxic action of NMDA (80 μ*M*) (*6*), completely prevented

Washington University School of Medicine, Department of Psychiatry, St. Louis, MO 63110.

the neurotoxic action of L-Cys (2 m*M*) (Fig. 2C).

Several agents known to prevent excitotoxicity were compared for potency in blocking the neurotoxicity of L-Cys (2 m*M*), NMDA (80 μ*M*), Quis (15 μ*M*), or KA (25 μ*M*) (Table 1). We chose D-AP5, 7-chlorokynurenic acid (7-CK), and MK-801 because each antagonizes the NMDA receptor–ionophore complex by a different mechanism; D-AP5 acts at the NMDA binding site (*7*), 7-CK at the glycine site (*8*), and MK-801 at the phencyclidine (PCP) site (*9*). CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) was chosen because it blocks the excitotoxic action of non-NMDA agonists more potently than that of NMDA (*10*). Each of the three NMDA antagonists totally blocked the excitotoxic actions of L-Cys in direct proportion to its potency in blocking NMDA toxicity, while displaying little or no blocking activity against Quis or KA at the concentrations tested. CNQX totally blocked the excitotoxicity of Quis and KA but displayed either equivocal or no protection against NMDA or L-Cys excitotoxicity. These in vitro experiments confirm the impression from the in vivo study that L-Cys neurotoxicity is mediated through the NMDA receptor–ionophore complex.

The divalent cation Zn^{2+} effectively blocks NMDA neurotoxicity in the chick embryo retina when introduced at a concentration of 250 μ*M*, but does not antagonize Quis or KA at substantially higher concentrations (*6*). Further corroborating the specificity of L-Cys neurotoxicity for the NMDA receptor complex, we found that Zn^{2+} also antagonizes L-Cys neurotoxicity at 250 μ*M*. Although the mechanism by which Zn^{2+} antagonized NMDA neurotoxicity was noncompetitive (with respect to NMDA and the NMDA receptor), its antagonist action with respect to L-Cys was competitive; that is, serial increases in the concentration of L-Cys overcame the Zn^{2+} blockade, but proportionate increases in Zn^{2+} restored the blockade (Fig. 3). Similar competition studies were conducted to evaluate the effects of D-AP5, 7-CK, and MK-801 on L-Cys excitotoxicity with the puzzling findings that 7-CK displayed a competitive action and that the ability of D-AP5 and MK-801 to block L-Cys neurotoxicity was completely abolished when the concentration of L-Cys was doubled and could not be restored by disproportionately large increases in the concentrations of either antagonist.

Further experiments revealed that D-AP5 and MK-801 lose their blocking activity against L-Cys when the concentration of L-Cys is increased because, although L-Cys is a pure NMDA agonist at a 2 m*M* concentration, it begins to express excitotoxicity through Quis receptors at higher concentrations, and NMDA antagonists such as D-AP5 and MK-801 are impotent against toxic reactions mediated through Quis receptors. We made this interpretation because the cellular degeneration associated with increased concentrations of L-Cys had a Quis pattern (very different from the KA pattern and similar but not identical to the NMDA pattern), and it could be blocked by a low concentration of MK-801 (200 n*M*), if accompanied by CNQX in appropriate stepwise concentration increases, or by D-AP5 when both D-AP5 and CNQX were applied in appropriate stepwise concentration increases. These findings support the interpretation that L-Cys toxicity is mediated by a direct action at the NMDA site (because it is blocked competitively by D-AP5) and that at higher concentrations it exerts toxic activity through the Quis receptor (because CNQX competitively blocks this action).

The competitive interaction between L-Cys and Zn^{2+} can be explained best in terms of the well-recognized ability of L-Cys to chelate Zn^{2+} ions (*11*). We propose that Zn^{2+} and L-Cys form a complex that sequesters and inactivates both molecules with respect to participation in NMDA receptor function (*12*). The competitive interaction between 7-CK and L-Cys can be explained on a different basis. 7-CK is a relatively potent, broad spectrum anti-excitotoxin, which blocks NMDA toxicity noncompetitively (because it acts primarily at the glycine rather than NMDA site) (*8, 13*), and Quis toxicity competitively (*13*). Therefore, in competition studies, when the concentration of L-Cys is increased enough for it to exert toxic activity through Quis as well as NMDA receptors, the blocking action of 7-CK assumes a competitive profile that reflects its competitive action against L-Cys toxicity at the Quis receptor.

BMAA (β-*N*-methylamino-L-alanine), which like L-Cys lacks the Ω acidic terminal com-

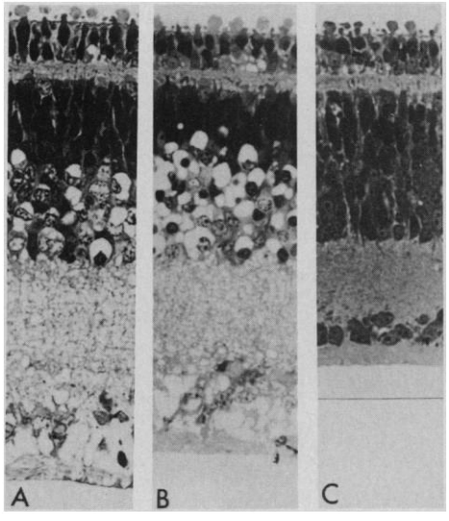

Fig. 2. Fifteen-day chick embryo retinas incubated for 30 min in balanced salt solution containing (**A**) 80 μ*M* NMDA, (**B**) 2 m*M* L-Cys, and (**C**) 2 m*M* L-Cys plus 50 μ*M* D-AP5. The lesions induced by (A) NMDA and (B) L-Cys have a similar appearance, each being characterized by edematous degeneration of neural elements in the inner half of the retina. The retina in (C) appears normal because D-AP5 totally protected it against L-Cys toxicity. Magnification, ×160.

Table 1. Potencies of antagonists in blocking NMDA, KA, Quis, or L-Cys toxicity. Compounds were rated according to the minimal concentration (μ*M*) required to provide total protection against the neurotoxic effects of NMDA (80 μ*M*), KA (25 μ*M*), Quis (15 μ*M*), or L-Cys (2000 μ*M*). Antagonists were tested over a range of concentrations from 100 μ*M* downward until a minimal effective concentration was established. If total protection was not obtained at 100 μ*M* this is indicated by a dash (–).

Antagonist	Versus			
	NMDA	KA	Quis	L-Cys
D-AP5	50	–	–	50
7-CK	50	–	–	50
MK-801	0.2	–	–	0.2
CNQX	–	50	15	–

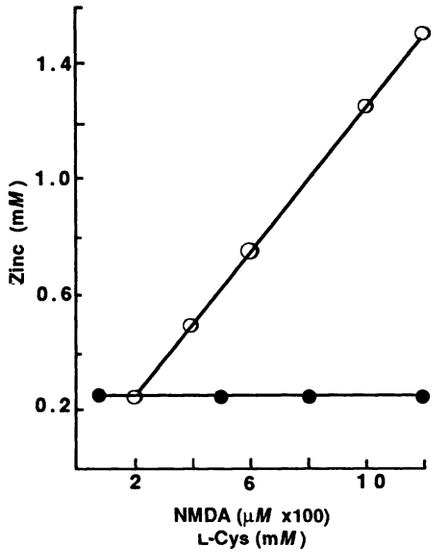

Fig. 3. Segments of chick embryo retina were incubated with NMDA or L-Cys in graduated concentrations. At each concentration of NMDA (●) or L-Cys (○), a range of Zn^{2+} concentrations was tested to determine the threshold concentration for totally blocking NMDA or L-Cys toxicity. A fixed low concentration of Zn^{2+} (250 μ*M*) was sufficient to block all concentrations of NMDA from 80 μ*M* to 1.2 m*M*. In contrast, each increase in the concentration of L-Cys overcame the blockade requiring a proportionate increase in Zn^{2+} to restore the blockade.

mon to all other excitotoxic straight-chain analogs of Glu, expresses neuroexcitatory and neurotoxic activity only in the presence of physiological concentrations of bicarbonate ion (*14*). Therefore, we tested whether bicarbonate might play a role in L-Cys excitotoxicity. Using cultured hippocampal neurons, which exhibit depolarizing responses to excitatory amino acids (*15*), we applied L-Cys (either in medium containing 20 m*M* bicarbonate or in bicarbonate-free medium) by pressure ejection. L-Cys elicited a small inward current in the absence of bicarbonate, and a substantially larger current in the presence of bicarbonate (Fig. 4).

The standard incubation medium used in the chick embryo retina assay contains bicarbonate ion at 4 m*M* concentration (*6*), and it requires >1 m*M* L-Cys to induce an excitotoxic reaction consistently in this medium. Increasing the bicarbonate in increments from 4 to 24 m*M* (physiological range) resulted in a stepwise increase in the excitotoxic potency of L-Cys such that, at 24 m*M* bicarbonate, it only required 0.2 to 0.3 m*M* L-Cys to induce an excitotoxic reaction. However, medium containing 24 m*M* bicarbonate underwent a change in *p*H (from 7.4 to 7.6) during the 30-min incubation period. In additional experiments, we varied *p*H from 7.0 to 7.6 and bicarbonate from 0 to 24 m*M* and found that, at either 0 or 24 m*M* bicarbonate, raising the *p*H from 7.0 to 7.6 caused an approximately twofold increase in the excitotoxic potency of L-Cys, whereas, at either *p*H 7.0 or 7.6, raising the bicarbonate concentration from 0 to 24 m*M* resulted in a six- to eightfold increase in excitotoxic potency (Fig. 4). Thus, although increases either in *p*H or bicarbonate concentration can increase L-Cys neurotoxicity, the bicarbonate influence is much more pronounced and seems to operate independently of *p*H changes.

Our findings identify L-Cys as an endogenous excitotoxin that can severely damage the immature central nervous system (CNS) by an action mediated primarily by the NMDA receptor–ionophore complex. L-Cys is present in significant concentrations in both the environment and brain, is transported from the blood into the brain, and also is synthesized within the brain (*16*). L-Cys requires only a simple cofactor (bicarbonate), which is also endogenously present in brain, to assume enhanced excitotoxic potency. L-Cys, in the presence of bicarbonate, is more powerful than L-Glu in destroying neurons in the chick retina. It is possible that L-Cys, under hypoxic-ischemic or other oxidative stress conditions, might be released along with L-Glu and L-Asp (*17*), and could act in concert with these endogenous excitotoxins to destroy neurons. The immature brain may be at particularly high risk in that neurons in the developing rat brain are hypersensitive to excitotoxic processes mediated through NMDA receptors (*18*). In conditions such as perinatal asphyxia, not only is the brain rendered hypoxic, but there is an accompanying respiratory acidosis resulting in an increased amount of bicarbonate ion reaching the extracellular compartment of brain. Thus, there is ample basis for postulating that L-Cys may contribute to developmental neuropathological processes.

An interesting parallel exists between L-Cys and BMAA, an environmental excitotoxin implicated in an adult neurodegenerative disease (amyotrophic lateral sclerosis–parkinsonism–dementia complex) endemic to Guam (*19*). Both molecules lack the acidic Ω terminal that other straight-chain excitotoxins possess, and this may facilitate their penetration of blood-brain barriers. Both are capable of assuming increased excitotoxic potency by combining with bicarbonate within the brain. Moreover, there is evidence (*20*) documenting a metabolic disturbance causing elevated blood levels of L-Cys in each of the three neurological disorders—motor neuron disease, parkinsonism, and Alzheimer's disease—that comprise components of the BMAA-linked Guamanian syndrome. Candidacy of L-Cys as a pathogen in such diseases is strengthened by evidence that it is a much more potent excitotoxin than BMAA (*21*), is more ubiquitously present in the environment, and is naturally present in the brain. Moreover, L-Cys may promote excitotoxicity both by receptor activation, which opens NMDA ion channels, and by Zn^{2+} chelation, which facilitates (disinhibits) ion flow through such channels.

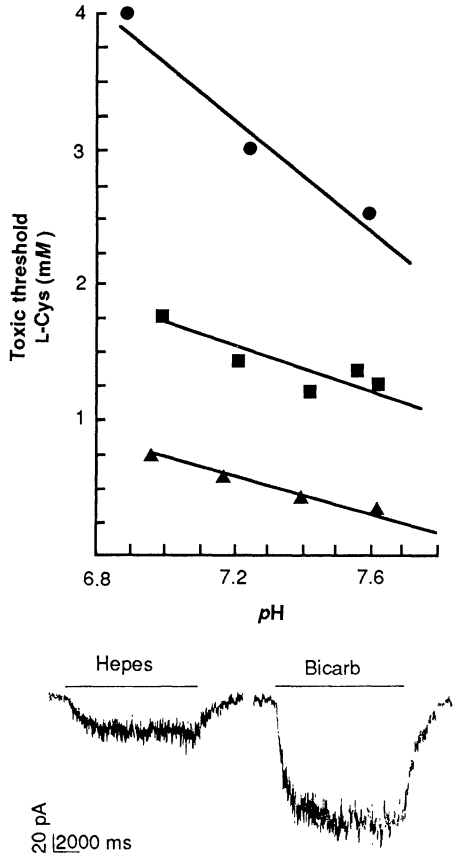

Fig. 4. The concentration of L-Cys required to induce a fully developed lesion in the 15-day chick embryo retina was influenced by changes in *p*H or in bicarbonate concentration. In medium containing no bicarbonate (●), MOPS buffer (5 m*M*) was used and *p*H was adjusted to various levels with NaOH or HCl. In medium containing bicarbonate, 4 m*M* (■) or 24 m*M* (▲) bicarbonate was used. The *p*H was regulated at various levels by addition of HCl with or without gassing with 95% O_2/5% CO_2. The *p*H was adjusted to a specific level before each retinal incubation and was measured in each specimen vial before and after incubation; a mean *p*H (±SEM) was then calculated for that experimental condition ($n > 30$ for each condition since multiple incubations were used for each Cys concentration, and multiple Cys concentrations were tested for each *p*H condition). Error bars are not shown as the SEM was never greater than 0.02 of a *p*H unit. Regardless of *p*H, adjusting the bicarbonate concentration from zero to a physiological range markedly reduced the threshold for L-Cys neurotoxicity. Varying the *p*H from 7.0 to 7.6 shifted the neurotoxic threshold in the same direction but only slightly. The electrographic recording depicts the currents induced in cultured hippocampal neurons by L-Cys when applied in Hepes buffer (no bicarbonate) or in the presence of 20 m*M* bicarbonate. In a series of 14 neurons, the response to L-Cys in bicarbonate was 3.2 ± 0.4 (±SEM) times that in the absence of bicarbonate, and this response was completely blocked by D-AP5 (200 μ*M*).

REFERENCES AND NOTES

1. J. W. Olney and O. L. Ho, *Nature* **227**, 609 (1970).
2. ———, V. Rhee, B. Schainker, *Brain Res.* **45**, 309 (1972); R. L. Karlsen, I. Grofova, D. Malthe-Sorenssen, F. Fonnum, *ibid.* **208**, 167 (1981).
3. D. R. Curtis and J. C. Watkins, *J. Physiol. (London)* 166, 1 (1963).
4. J. W. Olney, O. L. Ho, V. Rhee, *Exp. Brain Res.* **14**, 61 (1971).
5. J. W. Olney, *J. Neuropathol. Exp. Neurol.* **30**, 75 (1971).
6. ———, M. T. Price, L. Samson, J. Labruyere, *Neurosci. Lett.* **65**, 65 (1986); J. W. Olney, *Biol. Psychiatr.* **26**, 505 (1989). Fifteen-day chick embryos were decapitated, and their eyes were removed and cut into thirds after excising the cornea and removing the lens, vitreous, and iris. The retinal thirds were then gently separated from the pigment epithelium and incubated for 30 min at 37°C in a standard balanced salt solution (BSS) to which excitatory amino acid agonists and antagonists were added. The BSS contained 148 m*M* Na^+, 5.0 m*M* K^+, 1.2 m*M* Ca^{2+}, 0.9 m*M* Mg^{2+}, 150 m*M* Cl^-, 4 m*M* bicarbonate, 1.1 m*M* phosphate, and 5.6 m*M* glucose. After incubation, the retinal thirds were fixed by immersion in a phosphate-buffered solution containing 1.5% glutaraldehyde and 1% paraformaldehyde, then additionally fixed in 1% osmium tetroxide and embedded in araldite. Sections were

cut 1 μm thick, stained with methylene blue and azure II, and evaluated by light microscopy. To establish whether a neuroprotective effect was achieved, the tissue was evaluated by a rater who was blind to the experimental conditions. At least six retinal segments were examined for each experimental condition and these were matched with at least six concurrent controls. In the standard assay, an agonist was used at a fixed concentration (previously established in concentration-response studies as a threshold concentration that reproducibly causes a fully developed retinal lesion in 30 min: Glu, 1 m*M*; NMDA, 80 μ*M*; Quis, 15 μ*M*; or KA, 25 μ*M*), and excitatory amino acid antagonists were added to assess their ability to block the excitotoxic action of a given agonist. Although agents that are effective in antagonizing excitotoxic phenomena usually induce a partial blockade at concentrations lower than the threshold for total blockade, we used the concentration that totally prevented toxic activity for purposes of rating antagonist potency. To determine whether a given antagonist blocks an agonist toxic action by a competitive mechanism, we increased the concentration of agonist (for example, NMDA or L-Cys) in graduated steps and determined what concentration of antagonist was required to maintain a full blockade at each step.

7. J. C. Watkins and R. H. Evans, *Annu. Rev. Pharmacol. Toxicol.* **21**, 165 (1981).
8. J. W. Johnson and P. Ascher, *Nature* **325**, 529 (1987); J. A. Kemp *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6547 (1988).
9. D. Lodge and N. A. Anis, *Eur. J. Pharmacol.* *77*, 203 (1982); J. A. Kemp, A. C. Foster, E. H. F. Wong, *Trends Neurosci.* **10**, 294 (1987).
10. T. Honoré *et al.*, *Science* **241**, 701 (1988); M. T. Price *et al.*, *Soc. Neurosci. Abstr.* **14**, 418 (1988).
11. M. Friedman, *The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins* (Pergamon Press, New York, 1973), p. 27.
12. The ability of increasing concentrations of Zn^{2+} to block toxicity produced by increasing concentrations of L-Cys supports this interpretation since Zn^{2+} does not block toxicity mediated through Quis receptors. Therefore, the ability of Zn^{2+} to prevent increasing concentrations of L-Cys from inducing a Quis-type lesion implies that it formed complexes with L-Cys, thereby inactivating the L-Cys molecule.
13. J. W. Olney, M. T. Price, J. Labruyere, unpublished data. In the chick embryo retina, kynurenic acid is a broad spectrum antagonist that effectively blocks the toxic action of NMDA, Quis, or KA but is significantly more potent against NMDA (*6*). We have observed that 7-CK is approximately two times more potent than kynurenic acid in protecting retinal neurons against either NMDA, KA, or Quis neurotoxicity. Consistent with electrophysiological evidence (*8*) suggesting that 7-CK blocks NMDA responses by an action at the glycine site, we have found that the antagonism of NMDA neurotoxicity by 7-CK is reversible by adding glycine, and that 7-CK also blocks L-Cys neurotoxicity by a glycine-reversible mechanism.
14. J. H. Weiss and D. W. Choi, *Science* **241**, 973 (1988).
15. L. O. Trussell, L. L. Thio, C. F. Zorumski, G. D. Fischbach, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2834 (1988); C. E. Jahr and C. F. Stevens, *Nature* **325**, 522 (1987).
16. S. I. Rapoport, *Blood-Brain Barrier in Physiology and Medicine* (Raven Press, New York, 1976).
17. H. Benveniste, J. Drejer, A. Schousboe, N. M. Diemer, *J. Neurochem.* **43**, 1369 (1984).
18. J. W. McDonald, F. S. Silverstein, M. V. Johnston, *Brain Res.* **459**, 200 (1988); C. Ikonomidou *et al.*, *J. Neurosci.* **9**, 1693 (1989); C. Ikonomidou *et al.*, *ibid.*, p. 2809; J. W. Olney, C. Ikonomidou, J. L. Mosinger, G. Frierdich, *ibid.*, p. 1701.
19. P. S. Spencer *et al.*, *Science* **237**, 517 (1987).
20. M. T. Heafield *et al.*, *Neurosci Lett.* **110**, 216 (1990).
21. In the chick embryo retina, BMAA is not toxic in the absence of bicarbonate but becomes a weak toxin in the presence of physiologic concentrations of bicarbonate. The thresholds of BMAA and L-Cys for expressing excitotoxicity in the chick retina in the presence of physiologic concentrations of bicarbonate are approximately 3 m*M* and 300 μ*M*, respectively (G. R. Stewart, J. W. Olney, M. T. Price, unpublished data). Thus, L-Cys is ten times more potent than BMAA in this preparation.
22. Supported in part by HD 24237, DA 05072, and NIMH Research Scientist Award MH 38894 (J.W.O.), Physician Scientist Award MH 00630 (C.F.Z.) and Klingenstein Fellowship (C.F.Z.).

5 October 1989; accepted 8 March 1990

K^+ Current Diversity Is Produced by an Extended Gene Family Conserved in *Drosophila* and Mouse

AGUAN WEI, MANUEL COVARRUBIAS, ALICE BUTLER, KEITH BAKER, MICHAEL PAK, LAWRENCE SALKOFF

The *Drosophila Shaker* gene on the X chromosome has three sister genes, *Shal*, *Shab*, and *Shaw*, which map to the second and third chromosomes. This extended gene family encodes voltage-gated potassium channels with widely varying kinetics (rate of macroscopic current activation and inactivation) and voltage sensitivity of steady-state inactivation. The differences in the currents of the various gene products are greater than the differences produced by alternative splicing of the *Shaker* gene. In *Drosophila*, the transient (A current) subtype of the potassium channel (*Shaker* and *Shal*) and the delayed-rectifier subtype (*Shab* and *Shaw*) are encoded by homologous genes, and there is more than one gene for each subtype of channel. Homologs of *Shaker*, *Shal*, *Shab*, and *Shaw* are present in mammals; each *Drosophila* potassium-channel gene may be represented as a multigene subfamily in mammals.

POTASSIUM CHANNEL DIVERSITY IN *Drosophila* may result from an extended gene family coding for homologous proteins since the *Drosophila Shaker* gene (*1*) has three sister genes (*2*). The peptides encoded by the extended gene family *Shaker*, *Shal*, *Shab*, and *Shaw* share a conserved organization suggestive of common function (*2*). We now report that, like *Shaker*, the *Shal*, *Shab*, and *Shaw* gene products each expresses a K^+ current in the *Xenopus* oocyte system. Although the currents share general features (voltage-gating and K^+ selectivity), they differ greatly in their kinetic and voltage-sensitive properties.

Because of its resemblance to one of the four homologous domains of the Na^+ (*3*) and Ca^{2+} channels (*4*), the *Shaker* holoprotein is presumed to be a homotetramer (*5*). Like the peptide encoded by *Shaker*, the peptides encoded by *Shal*, *Shab*, and *Shaw* each form functional K^+ channels in *Xenopus* oocytes, all potentially function as homomultimers in vivo.

We have isolated cDNAs for two alternatively spliced mRNAs transcribed from *Shal* that encode the protein products Shal1 and Shal2 (*2*). The deduced amino acid sequence of Shal2 is compared to that of the conserved portions of *Shaker*, *Shab*, and *Shaw* cDNAs (Fig. 1A). Shal1 has a splice junction at residue 489 of Shal2 (Fig. 1A). In Shal1 the COOH-terminal residue from Shal2 (Met^{490}) is absent and 116 amino acids are added (*6*). The highly conserved regions of the deduced peptides include the hydrophobic domains, S1 through S6, which may be membrane-spanning structures (*1, 3*). One or more possible N-linked glycosylation sites, indicated by the consensus sequence Asn-X-Ser/Thr (*7*), are present between S1 and S2 in Shaker, Shab, and Shaw, but not in Shal. The deduced peptides for Shal2 of 490 amino acids (56 kD) and Shaw2 of 498 amino acids (56.5 kD) are somewhat smaller than Shaker, which average 71 kD. The deduced Shab peptide is the largest with 924 amino acids (100 kD). This size difference results from length variation in the domains at the NH_2- and COOH-termini, both of which are presumed to be cytoplasmic. The conserved core of Shaker, Shal, Shab, and Shaw encompassing the membrane-spanning portions S1 through S6 averages about 240 residues and varies little among the subfamily members. A pairwise comparison of homology for the entire conserved region (Shal residues 45 to 409) shows a degree of identity ranging from 38 to 42% among all four predicted peptide sequences (Fig. 1A).

Each member of the *Drosophila* gene family, *Shaker*, *Shal*, *Shab*, and *Shaw*, has one or more mammalian homologs, and thus, defines a subfamily for each member. A comparison of each fly K^+-channel protein to a homolog from mouse brain is shown in Fig. 1B. The mammalian homologs of the *Shaker* protein (*8*) are more closely related to the *Drosophila Shaker* protein (about 76% iden-

A. Wei, M. Covarrubias, A. Butler, K. Baker, M. Pak, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.
L. Salkoff, Department of Anatomy and Neurobiology and Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110.