The toxic effect of L-cysteine on the rat retina
A morphological and biochemical study

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Subcutaneous injection of L-cysteine into 4-day-old rats (1.2 mg/gm body weight) leads to permanent retinal dystrophy. When the eyes were examined 4 to 6 weeks later, there was a reduction of ganglion cells to less than 50% of the controls. The inner plexiform layer was reduced to about 60%, and the inner nuclear layer was reduced with approximately one cell layer. The photoreceptors were unaffected. The same layers were reduced in retinas from L-glutamate-treated animals which were examined for comparison. Electron microscopy showed that no single class of neurons was entirely eliminated by the cysteine treatment, and no specific changes were observed in the remaining neurons. The retinal lesion induced by L-cysteine was not accompanied by any reduction in glutamate decarboxylase activity, choline acetyltransferase activity, or in the high-affinity uptake of glutamate, which are markers for some of the retinal neurotransmitters. This is in striking contrast to the retinal lesion induced by L-glutamate, which is followed by a severe reduction in these biochemical parameters. Eyes enucleated 4 hr after injection of L-cysteine showed widespread necrosis of neurons in the inner nuclear layer and of ganglion cells. In conclusion, L-cysteine, like L-glutamate, given systemically to newborn rats, affects neurons in the inner layers of retina. L-Cysteine, however, apparently affects other populations of cells than L-glutamate.

Key words: retina, toxicology, L-cysteine, neurotransmitters, electron microscopy, central nervous system, rats

The neurotoxic properties of the acidic amino acids, especially glutamate, on retinal neurons have aroused considerable interest.1, 2 Neurotransmitter enzymes and high-affinity uptake of putative neurotransmitters have been investigated in retinas from glutamate-treated animals.3, 4 The results obtained in these studies were in correspondence with previous results suggesting that gamma aminobutyric acid (GABA), glycine, acetylcholine (ACh), and dopamine are transmitters of populations of amacrine cells (for reviews see refs. 5 and 6). Similar results were also observed after intraocular injection of the glutamate analogue, kainic acid, which destroys retinal neurons.7 On the other hand, intraocular injection of DL-α-aminoadipic acid, previously suggested to be gliotoxic,8 led to destruction of Müller cells9 as well as to reduction in high-affinity uptake mainly of β-alanine,9 which is suggested to be a marker for glial cells.10 DL-α-Aminoadipic acid does not significantly affect the neuronal markers.4

In the present investigation, the long-term effects on the rat retina of the neutral amino acid L-cysteine, given subcutaneously to
newborn rats, were studied by light and electron microscopy and by measurements of some biochemical parameters. L-Cysteine has previously been reported to induce widespread brain damage and also to affect retinal neurons. It has been suggested that the neurotoxic properties of this compound are analogous to the effects of L-glutamate by conversion in vivo of L-cysteine to acidic metabolites. In the present work, the lesion induced by L-cysteine was therefore compared to the lesion induced by L-glutamate.

Materials and methods

White Wistar 4-day-old rats were given a single subcutaneous injection of L-cysteine (1.2 mg/gm body weight). A few animals were killed 4 hr after the injection, and the eyes were processed for histological study at the light microscopic level. The other animals were killed at the age of 4 to 6 weeks, and the retinas and optic nerves of these eyes were examined. Some animals were also injected subcutaneously with sodium glutamate (2 mg/gm body weight) during the first week of life. These eyes were examined when the animals were between 4 and 6 weeks old. The eyes of uninjected controls from the same litters were examined as well.

Morphology. The animals for morphology studies were anesthetized with a combination of Hypnorm (Janssen Pharmaceuticals, Belgium) and diazepam, and they were killed during perfusion fixation with 2.5% glutaraldehyde in 0.1M sodium phosphate buffer, pH 7.4. The eyes were immediately enucleated, the cornea and lens were removed, and the rest of the eyes were immersed in the above-mentioned fixative. The fixation was continued for 1 to 3 days. The tissues were then rinsed shortly in 0.1M phosphate buffer, pH 7.4, with 5% sucrose. Some eyes were at this stage divided in four parts, whereas others were postfixed and embedded with no further dissection. The optic nerves were cut off from the globes and processed separately. The tissues were postfixed for 3 hr in 1% OsO₄ in Millonig’s phosphate buffer, dehydrated in graded series of ethanol, treated with propylene oxide, and embedded in Epon 812. Semithin sections (1 to 2 μm thick) were cut with glass knives and stained with toluidine blue. Some eyes from each group were cut horizontally through the optic nerve head to the ora on both sides to ensure exact localization within the retina. Ultrathin sections were cut with diamond knives on a Reichert Ultracut ultramicrotome. The sections were stained with uranyl acetate and alkaline lead and examined in a Siemens Elmiskop la electron microscope.

Biochemistry. The animals for biochemical studies were rapidly killed by cervical dislocation, and the eyes were enucleated. Retina was isolated as a floating disc on 0.32M ice-cold sucrose.

High affinity uptake. A 2% homogenate (w/v) in 0.32M sucrose was prepared in a Teflon glass homogenizer in a way to preserve synaptosomes. Aliquots containing 10 to 60 μg of protein were used for uptake studies. For the measurements of high-affinity glutamate uptake, the samples were incubated for 3 min at 25° C. Final substrate concentration was 10⁻⁷M. The method has been described in detail previously. Choline acetyltransferase and glutamate decarboxylase were analyzed as described. Protein was determined according to the method of Lowry et al. Statistical analysis was done by the Student’s t test.

Results

Morphology. The retinas from 4- to 6-week-old animals treated with L-cysteine were markedly affected, as revealed by light microscopy. The changes affected the inner layers of retina, whereas the photoreceptors and pigment epithelium were unaffected. There was a marked reduction of the thickness of the ganglion and nerve fiber layers, the inner plexiform layer, and the inner nuclear layer, as compared to the untreated controls (Fig. 1). The number of ganglion cells was reduced to less than 50% in the cysteine-treated animals. The thickness of the inner plexiform layer was reduced to about 60%, and the reduction of the inner nuclear layer averaged one cell layer. The same retinal layers were affected by the glutamate treatment, but to a larger extent. The number of ganglion cells was reduced to less than 10% in these eyes, the inner plexiform layer to about 30%, and the thickness of the inner nuclear layer averaged one cell layer. The same retinal layers were affected by the glutamate treatment, but to a larger extent. The number of ganglion cells was reduced to less than 10% in these eyes, the inner plexiform layer to about 30%, and the thickness of the inner nuclear layer by nearly 50% (Fig. 1).

The areas of the transversally sectioned optic nerves were reduced to about 40% in the L-cysteine–treated animals and to less
Fig. 1. For legend see facing page.
than 10% in the glutamate lesion (Fig. 2, a to e). By electron microscopy of the optic nerves, the individual nerve fibers were found to be preserved, as judged from the presence of myelin sheaths and axoplasmic organelles. However, there were increased amounts of glial tissue in the cysteine-treated as well as in the glutamate-treated animals (Fig. 2, f to h).

In the cysteine-treated animals, electron microscopy did not reveal any specific changes in the neurons of the retina or their synaptic contacts. We could not decide which types of neurons had disappeared from the inner nuclear layer. Counting of different cell types to make statistical data was hampered by the rather numerous unidentified cells.

The ultrastructure of the photoreceptors, the pigment epithelium, and the Müller cells was also unaffected by the cysteine as well as the glutamate treatment.

In the eyes from 4-day-old rats, extensive necrosis of neurons in the inner nuclear layer and of ganglion cells was evident 4 hr after injection of L-cysteine (Fig. 3).

Biochemistry. In six separate experiments within each group, the high-affinity uptake of L-glutamate was 1.6 ± 0.2 pmol/3 min/mg of protein in the control retinas and 1.5 ± 0.2 in retinas from cysteine-treated animals. The activity of choline acetyltransferase was 96.3 ± 13.1 pmol/hr/mg of protein and 97.4 ± 10.5 in retinas from controls and L-cysteine–treated animals, respectively, and the activity of glutamate decarboxylase was 155.5 ± 15.0 and 153.2 ± 16.1. Thus no significant reduction of the neuronal markers included in the study was found in the animals treated with L-cysteine.

Discussion

The L-cysteine treatment was accompanied by an initial mortality of about 50%. Approximately 10% of the survivors developed a most severe brain damage with almost complete disappearance of the cortex, whereas the remaining animals developed a more moderate cortical atrophy. The retinal lesions were, however, not significantly different in the two brain-lesion types. (In the present paper, Fig. 2, e and g, are from animals with the most severe brain damage.)

The morphological changes observed in the retinas of the L-cysteine–treated animals affected the inner layers. The same layers were affected by the glutamate treatment, but to a greater extent. Apart from the ganglion cells, we could not determine with certainty which neurons were reduced in number in the L-cysteine–treated animals. No single class of neurons was entirely eliminated. Since the inner plexiform layer is mainly built up by the axons and dendrites of the bipolar and amacrine cells, evidently subpopulations of one or both of these cell types were affected.

The biochemical observations in the present study were rather unexpected. The retinal lesion induced by L-cysteine was not accompanied by any reduction in the neuronal markers included in the study. It has previously been shown that the retinal lesion induced by L-glutamate is accompanied by severe reductions in choline acetyltransferase activity (99% reduction) and glutamate decarboxylase activity (90% reduction). These marker enzymes are associated with certain populations of amacrine cells using ACh or GABA as their transmitters. Since these neuronal markers apparently were unaffected by cysteine treatment, the present observations suggest that differing populations of cells are destroyed by L-cysteine than by L-glutamate. In addition, the retinal lesion induced by L-glutamate is accompanied by a reduction in the high-affinity uptake of L-glutamate, suggesting uptake into retinal neurons of this compound. In the case of the...
Fig. 2. a to e, Cross-sections of optic nerves from 5-week-old rats. a and c, Untreated control animals. d and e, Animals treated with L-cysteine. b, Animal treated with L-glutamate. Note the marked reduction in the thickness of the optic nerves from animals treated with L-cysteine and L-glutamate. (Light microscopy; toluidine blue.) f to h, Electron micrographs from control animal (F), and from L-cysteine—(g) and L-glutamate—(h) treated animals. Note increased amount of glial tissue in the optic nerves from experimental animals.
L-cysteine lesion, there was no reduction in the high-affinity uptake of L-glutamate, again suggesting that a different population of neurons was destroyed by L-cysteine. These cells could be other populations of amacrine cells (not using GABA or ACh) or a subpopulation of bipolar cells.

The basal neurotoxic mechanism of L-cysteine is unknown. It has been suggested that acidic metabolites (cysteic acid or cysteine sulfonic acid) are responsible for the neurotoxic properties. L-Glutamate is thought to act as a neurotoxicological agent through overstimulation of glutamate receptors, and the transformation of L-cysteine to acidic excitatory amino acids could perhaps fit in with a similar mode of action of this compound. However, since widespread necrosis of neurons was demonstrable already 4 hr after the injection of L-cysteine, this may suggest that it is the compound itself and not a metabolite which acts as a neurotoxic agent. It has previously been shown that L-cysteine is a very potent inhibitor of the high-affinity uptake of L-glutamate. This observation may suggest that L-cysteine acts as a neurotoxic agent by increasing the extracellular concentration of L-glutamate, thereby causing overstimulation of glutamate receptors.

We have found that the retinal lesion induced by the neutral amino acid L-cysteine is interesting because it apparently affects mainly other cell types than the toxicological retinal lesions previously reported. This may provide an opening for new research concerning the relation and function of the different retinal cells. However, more research is required to characterize further the retinal lesion induced by this compound.

REFERENCES


