Effect of Azaserine on the Fine Structure of the Liver and Pancreatic Acinar Cells

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SUMMARY

Intraperitoneal administration of azaserine to adult rats resulted in characteristic alterations of rough endoplasmic reticulum, large areas of focal degradation and formation of osmiophilic plaques in pancreatic acinar cells. The hepatocytes showed a marked enlargement of the crystalloid of microbodies, disorganization of endoplasmic reticulum and focal degradation. The lesions were similar to those produced by ethionine. Oral administration of azaserine produced a similar but less drastic change.

Cellular injury is manifested at the ultrastructural level by a limited series of nonspecific alterations (17, 19, 20—23). These include focal degradation, mitochondrial swelling and the appearance of inclusions, disruption of the organized endoplasmic reticulum, and the abnormal elaboration of Golgi membranes. Although these changes are recognizable in the majority of injured cells, they occur in different degrees with different toxic agents. Thus the sum of these nonspecific changes can provide characteristic patterns for individual types of injury.

The cellular reaction to injury has been investigated after administration of analogs of phenylalanine (β-3-thienylalanine, β-3-furylalanine), of cholesterol synthesis (triparanol) and of choline incorporation (diethanolamine) (17, 19, 20—23). The acute effects of azaserine on the fine structure of hepatocytes and pancreatic acinar cells were investigated in the present study.

Azaserine (o-diazoacetyl-L-serine) is an anti-metabolite isolated from cultures of Streptomyces (2) known to be an inhibitor of de novo synthesis of purine (13, 26). It has been widely studied as a possible anti-neoplastic agent (11, 14, 27, 28, 31, 35). Administration of azaserine to animals produces light microscopic changes similar to those resulting from ethionine (34). In the present study we have found that the ultrastructural alterations produced by azaserine differ markedly from the changes produced by certain other inhibitors of protein synthesis (19, 22, 23). The lesions are more severe in pancreatic acinar cells than in hepatocytes, and strikingly resemble the alterations produced by ethionine (15, 16, 32). Both compounds produce a marked alteration of rough endoplasmic reticulum, formation of large areas of focal degradation, and the appearance of dense membrane-bound lamellar inclusions, which have been called osmiophilic plaques (16).

MATERIALS AND METHODS

Adult male Sprague-Dawley rats were used. The animals weighed on the average 415 gm in the 1st, 300 gm in the 2nd, 240 gm in the 3rd and 4th experiments, 130 gm in the 5th, and 280 gm in the 6th experiment. The rats in Group 5 were fed chow diet ad libitum. The other groups were force-fed semi-synthetic diets similar to those previously described (39) in 3 divided doses at 6-hr intervals. The protein was supplied as enzymatic casein hydrolysate.

Azaserine tablets (Parke, Davis & Company), supplied by Dr. J. H. Burchenal, were used for oral administration, powdered and mixed with the diet. The rats in Experiments 1—4 received 15, 30, and 50 mg azaserine/kg body weight/day. Azaserine (NSC-742) for injections was supplied by Cancer Chemotherapy National Service Center. It was dissolved in saline before each injection and given i.p., in a single daily injection in Experiment 5 and in 2 daily injections in Experiment 6. The animals were treated as indicated in Table 1. The rats in Group 4 were given also Strep-Dicyristein fortis (Squibb), 0.2 ml/day i.m. in an attempt to control diarrhea.

Samples of pancreas and liver for electron microscopic studies were taken from rats surviving treatment under light ether anesthesia, fixed for 1 hr at 5°C in 1% osmium tetroxide buffered with s-collidine buffer (5) at pH 7.4, dehydrated in graded alcohols, and embedded in methacylate monomers containing 2% benzoyl peroxide as catalyst. Thin sections were stained with lead hydroxyde Karnofsky method A (24). Selected areas were photographed in an RCA type EMU 3 c electron microscope.

Following the sampling for electron microscopic studies, the rats were exsanguinated and samples of liver, pancreas, kidneys, and salivary glands were fixed in formalin and Carnoy’s fixative for light microscopic studies.

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TABLE I

SCHEDULE OF EXPERIMENTS

<table>
<thead>
<tr>
<th>Group and animal</th>
<th>Azaserine (mg/kg/day)</th>
<th>Days of treatment</th>
<th>Last feeding</th>
<th>Time of killing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>15, p.o.</td>
<td>1</td>
<td>Fasted overnight</td>
<td>2nd day</td>
</tr>
<tr>
<td>1B</td>
<td>15, p.o.</td>
<td>3</td>
<td>Fasted overnight</td>
<td>4th day</td>
</tr>
<tr>
<td>2</td>
<td>30, p.o.</td>
<td>3</td>
<td>Fasted overnight</td>
<td>4th day</td>
</tr>
<tr>
<td>3A</td>
<td>50, p.o.</td>
<td>2 hr</td>
<td></td>
<td>2 hr</td>
</tr>
<tr>
<td>3B</td>
<td>50, p.o.</td>
<td>5 hr</td>
<td></td>
<td>5 hr</td>
</tr>
<tr>
<td>3C</td>
<td>50, p.o.</td>
<td>1</td>
<td>Fasted overnight</td>
<td>24 hr</td>
</tr>
<tr>
<td>3D</td>
<td>50, p.o.</td>
<td>2</td>
<td>5 hr before killing</td>
<td>3rd day</td>
</tr>
<tr>
<td>3E</td>
<td>50, p.o.</td>
<td>3</td>
<td>Fasted overnight</td>
<td>4th day</td>
</tr>
<tr>
<td>4</td>
<td>50, p.o.</td>
<td>3</td>
<td>6 hr before killing</td>
<td>4th day</td>
</tr>
<tr>
<td>5A, B</td>
<td>50, i.p.</td>
<td>2</td>
<td>Not fasted</td>
<td>4 hr after 2nd injection, 2nd day</td>
</tr>
<tr>
<td>5C, D</td>
<td>50, i.p.</td>
<td>2</td>
<td>Not fasted</td>
<td>3rd day</td>
</tr>
<tr>
<td>5E, F</td>
<td>50, i.p.</td>
<td>4</td>
<td>Not fasted</td>
<td>4th day, 3 hr after 4th injection</td>
</tr>
<tr>
<td>5G</td>
<td>50, i.p.</td>
<td>4</td>
<td>Not fasted</td>
<td>5th day</td>
</tr>
<tr>
<td>5H</td>
<td>50, i.p.</td>
<td>5</td>
<td>Not fasted</td>
<td>6th day</td>
</tr>
<tr>
<td>6A</td>
<td>100, i.p. in 2 divided doses</td>
<td>2</td>
<td>2 hr before killing</td>
<td>3rd day, 2 hr after 5th injection</td>
</tr>
<tr>
<td>6B</td>
<td>100, i.p. in 2 divided doses</td>
<td>2</td>
<td>5 hr before killing</td>
<td>3rd day, 2 hr after 5th injection</td>
</tr>
</tbody>
</table>

Tissues from rats which died during the experiments were used for light microscopic studies only. Of 38 rats, force-fed 15, 30, and 50 mg azaserine/kg body weight/day, 29 died on the 3rd and 4th days. Of 17 rats receiving 50 and 100 mg azaserine/kg body weight/day i.p., 7 died between the 3rd and 7th days.

RESULTS

LIGHT MICROSCOPIC STUDIES

Animals receiving i.p. injections of azaserine showed alterations similar to those previously described (34). Occasional necrotic cells were seen in pancreas on the 2nd day. At 4 to 6 days moderate to marked patchy necrosis was seen in pancreas (Figs. 15, 17, insets), but some intact pancreatic acini with zymogen granules were still apparent around the islets of Langerhans.

Moderate to marked necrosis was seen also in the parotid gland; the sublingual gland was less affected. The external lacrimal gland showed mild atrophy and decrease of nuclear size. The sections of livers (Figs. 1, 2, insets) showed, from the 4th day on, necrosis with fragmentation of nuclei of many hepatocytes; this lesion was most pronounced in rats receiving the highest i.p. dose. In the kidney vacuolization and necrosis of convoluted tubules, particularly in the inner cortex, was seen from the 4th day on.

Section of liver, pancreas, kidneys, and salivary glands from rats receiving azaserine p.o. showed only minor changes. The sections of intestine from rats receiving azaserine p.o. or i.p. showed loss of epithelia from the intestinal villi. This alteration was considered to be the probable cause of death in the nonsurviving animals.

ELECTRON MICROSCOPIC STUDIES

The ultrastructural changes found in pancreatic acinar cells were more marked than those in hepatocytes. The alterations seen after i.p. administration of azaserine were more severe than those resulting from p.o. administration.

The hepatocytes showed disruption of the parallel arrangement of rough cisternae, slight to moderate increase of smooth endoplasmic reticulum, increase in the size of the crystalloid in microbodies, and an increase of cytoplasmic fat droplets. The pancreatic acinar cells showed dilatation of rough cisternae, appearance of short round cisternae, separation of cisternae, free ribosomes, focal degradation including large ergastoplasmic lesions (16), and appearance of osmiophilic plaques. These changes are discussed in detail below.

Liver.—The alterations in hepatocytes of rats fed azaserine p.o. were very slight. With large doses of azaserine and prolonged feeding (Rat 3E), the stacks of cisternae were replaced by cisternae wrapped closely around mitochondria and microbodies. The amount of smooth endoplasmic reticulum was slightly increased. The crystalloid of the microbodies was larger than normal and focal degradation was observed uncommonly.

The hepatocytes from rats receiving 50 mg azaserine/kg/day i.p. showed an early disorganization of rough endoplasmic reticulum, although a few stacks of cisternae were observed even after prolonged treatment (Rats 5C and 5D). The smooth endoplasmic reticulum was moderately abundant from the 4th day on. The Golgi complex was hyperplastic in some cells and extended occasionally to the nucleus (Fig. 1). Appearance of a small number of fine neutral lipid droplets was an early change. The nucleoid in many microbodies was strikingly enlarged (Figs. 3, 4). Multivesiculate bodies were seen occasionally (Fig. 1). Focal degradation (37) was moderately pronounced from the 4th day on. Occasional cells were necrotic. Amorphous dense inclusions were seen in the mitochondria in degenerating cells (Fig. 7).

The livers from rats receiving 2 daily injections of aza-
serine (Group 6) showed severe alterations. Many cells showed early necrotic changes (Fig. 2). The cisternae were short and round. Vacuoles with clumps of dense material were often seen (Fig. 2). Mitochondria were swollen and in a very close mutual contact (Figs. 2, 6) resembling mitochondria observed in starved rats (36). Focal degradation was marked. The nucleoli showed separation of their particulate and amorphous components (Fig. 5).

Pancreas.—Force-feeding of azaserine produced only slight alterations in pancreatic acinar cells. The rough cisternae were often arranged in concentric whorls and showed localized dilatations (Fig. 14). Annulate lamellae were seen rarely (Fig. 8). Mature zymogen granules were present and varied markedly in size. Prozymogen granules were rare. Focal degradation was mild consisting of areas of sequestration and of heterogeneous dense bodies of the size of zymogen granules. Large, dense, membrane-bound lamellar inclusions (osmiophilic plaques) were seen in some cells. Several acini from a rat fed azaserine for 3 days (3E) were filled by heterogeneous prozymogen granules which occasionally showed direct contact with rough cisternae and often were fused with each other (Fig. 9).

Animals injected with 50 mg azaserine/kg/day showed on the 2nd and 3rd day focal dilatations of rough cisternae (Fig. 16) and arrangement of cisternae in whorls. Marked dilatation of cisternae was seen from the 4th day on. The Golgi complex was not hyperplastic. The zymogen granules varied markedly in size and their number was reduced. Prozymogen granules were rare. Small areas of focal degradation were seen occasionally. Large areas of focal degradation similar to the ergastoplasmic lesions described from ethionine-treated rats (12), were seen at all days of treatment (Figs. 12, 18–21). Many of the sequestered portions of rough endoplasmic reticulum were surrounded by 2 smooth limiting membranes (Figs. 12, 18). Osmiophilic plaques were found from the 3rd day on (Figs. 23, 25) and some appeared to form by condensation of vacuoles containing loosely arranged membranous material (Figs. 22–24). Multivesiculate bodies were seen in some cells (Fig. 13). Portions of some mitochondria were free of cristae and were covered by a single membrane (Figs. 12, 15).

Marked alteration of endoplasmic reticulum and signs of necrosis were characteristic of prolonged treatment with large doses of azaserine. Many cells from animals treated with 50 mg azaserine/kg/day for 5 and 6 days and from rats treated with 100 mg azaserine/kg/day for 3 days showed necrosis. The endoplasmic reticulum in some cells was greatly distended and contained cytoplasmic vesicles and ribosomes (Fig. 15). In other cells endoplasmic reticulum was reduced to a mass of small spherical vesicles (Figs. 11, 17); zymogen granules were rarely seen in such cells. Portions of the cytoplasm also contained smooth vesicles (Fig. 10) or dense granular material probably of necrotic cells. Dissolution of the nuclear envelope, fragmentation of nuclei (Fig. 15) and shrinkage of cytoplasm (Fig. 13) was seen in severely damaged cells, as stages in necrotic breakdown.

**DISCUSSION**

The cellular injury produced by i.p. administration of azaserine is quite characteristic, although largely unspecific. The alterations in pancreatic acinar cells are strikingly similar to changes produced by ethionine (16, 32). The hepatocytes of azaserine-treated rats show less marked and less characteristic changes, which also resemble those produced by ethionine (1, 3, 9, 10). “Glycogen bodies,” described in liver cells after prolonged treatment with ethionine (33), were not observed in our acute experiments. Only minor changes are seen in the liver and pancreas of rats which were fed azaserine. The mortality of rats receiving azaserine p.o. and i.p. is however similar. Azaserine administered p.o. is incompletely absorbed (8, 34) and detoxified in the liver (30); the mortality appears to be related to damage of the intestinal mucosa.

The most striking effect of azaserine is the alteration of rough endoplasmic reticulum in pancreatic acinar cells. This involves either certain configurational changes in cisternae or degeneration of rough membranes through sequestration, focal degradation, and “lysosome” formation (37).

The configurational changes may be divided into 3 types; whorl formation (Fig. 14), fragmentation of cisternae to form numerous small round vesicles (Fig. 17), and dilatation of cisternae, so that adjacent vesicles fuse, often capturing small portions of cytoplasm as round ribosome-containing inclusions (Fig. 15). Fragmentation of cisternae is of common occurrence in normal cell processes. It has been considered a concomitant of cell division by Porter (29). The dilatation of cisternae occurs normally in some cell types, when protein is accumulated in the endoplasmic reticulum, for example in plasma cells, and coagulating gland (7). In our material, however, it is probably associated with osmotic imbalance and approaching cell death. Similar changes occur in polio-infected HeLa cells, associated with nuclear shrinkage, and the extrusion of nuclear sap into the cytoplasm (6).

The alterations of rough reticulum in hepatocytes are less distinct. The rough cisternae are frequently seen wrapped around mitochondria and microbodies; the portion of cisternae which are in close contact with these organelles are often free of ribosomes. The smooth endoplasmic reticulum is moderately abundant. A characteristic change in hepatocytes is the enlargement of the crystallloid of microbodies. The crystallloid represents crystalline uricase (18), and its enlargement may be related to the effect of azaserine on the synthesis of uric acid (25). The mitochondrial alterations are in general less severe than alterations in ergastoplasm.

The pancreatic damage produced by azaserine has been observed in light microscopic studies and the similarities with the effects of ethionine have been discussed (34). In contrast to ethionine, azaserine intoxication is characterized by a marked necrosis of hepatocytes, slight fatty change of liver and sparing of acini adjacent to islets (34). Comparison of the ultrastructural alterations in pancreatic acinar cells produced by azaserine with those produced by ethionine (16, 32) shows that both compounds affect the
rough endoplasmic reticulum in a similar characteristic fashion. Azaserine and ethionine also show similarities in their biochemical effects. Azaserine is an analog of glutamine and inhibits the de novo synthesis of purine (26). Ethionine produces a rapid decrease in the concentration of hepatic adenosine triphosphate and thus probably inhibits the synthesis of messenger ribonucleic acid (RNA) (38). The morphologic alterations produced by azaserine are indistinguishable from those of ethionine in either liver or pancreas. With both compounds, however, the pancreas is much more seriously affected than the liver. The mechanism of action of these 2 compounds may thus be different on the liver and on pancreatic acinar cells.

Application of biochemical methods, previously used to study the mechanism of action of ethionine in liver (4, 38), certainly need to be extended to the pancreas, and also to a comparison with the biochemical lesions produced by azaserine. While both compounds could be expected to produce primary changes in RNA synthesis, a major morphologic expression of the cellular injury by azaserine and ethionine is the alteration of the lipoprotein part of the rough cytomembranes. It thus may be speculated that active protein renewal is necessary to maintain the organization of the endoplasmic reticulum.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1.—Portion of a hepatocyte from rat 5G. Four centrioles in the center, multivesiculate bodies (V), Golgi complex (G) and portion of a nucleus (N) are seen. × 34,000. Hepatocytes from the same rat as seen in light microscope. Inset, H and E, × 810.

Fig. 2.—Portions of three hepatocytes from rat 6B. The endoplasmic reticulum is disorganized. Some mitochondria are swollen, others are in a close contact (arrows). A bile canaliculus is seen in the center. The small dense bodies are microbodies. The nucleus (N) contains a small nucleolus. × 11,000. Small vacuoles are seen in the hepatocytes of the same rat in light microscope. Inset, H and E, × 880.
FIGS. 3 AND 4.—Microbodies from hepatocytes of rat 5E. Two crystalloids are in cross-section showing a characteristic structure of small and large cylinders. Fig. 3, × 110,000; Fig. 4, × 120,000.
FIG. 5.—Nucleolus of a hepatocyte from rat 6B shows separation of its components. × 32,000.
FIG. 6.—Mitochondria from a hepatocyte of rat 6B show close contact. × 30,000.
FIG. 7.—Portion of a severely damaged hepatocyte of rat 5A. Mitochondria contain dense amorphous material. × 32,000.
FIG. 8.—Annulate lamellae from an acinar cell of rat 4. × 38,000.
FIG. 9.—Continuity between proxymogen granules and rough cisterna (arrow). Acinar cell of rat 3D. × 42,000.
FIG. 10.—Portion of two acinar cells from rat 6B. Dense granular material in cytoplasm is seen on the right. Vesicular rough reticulum and smooth vesicles are seen on the left. × 32,000.
Fig. 11.—Portion of two acinar cells from rat 6B. The endoplasmic reticulum is vesicular, zymogen granules are absent, lipid droplets are seen in right lower corner. Two nuclei are present. X 8,000.

Fig. 12.—Portion of an acinar cell of rat 5H shows a large area of sequestered endoplasmic reticulum limited by a double smooth membrane. Two proxymogen granules, mitochondria partially covered by a single membrane and a small lipid droplet are seen. X 29,000.

Fig. 13.—Portion of four acinar cells of rat 5H. The dark cell shows severe damage. Several bodies probably representing stages in formation of osmiophilic plaques (O), small area of sequestration (S) and multivesiculate bodies (M) are seen. X 29,500.
FIG. 14.—Endoplasmic reticulum from an acinar cell of rat 3E. The reticulum forms two whorls; a lipid droplet is seen in the center of the lower one. The cisternae show communications between their lumina. Separation of smooth wall vesicles is seen at the arrow. X 67,000.

FIG. 15.—Portion of an acinar cell of rat 5H. The endoplasmic reticulum is dilated. The mitochondria show localized swelling.

FIG. 16.—Endoplasmic reticulum from an acinar cell of rat 5B. The cisternae are separated and show localized dilatation. X 86,000.

FIG. 17.—Endoplasmic reticulum from an acinar cell of rat 6B. Cisternae are replaced by vesicles covered by ribosomes. Many free ribosomes are present. The mitochondrion is partially covered by a single membrane. X 45,000. Pancreatic acinar cells of the same rat seen in light microscope; acinar organization is lost. Many nuclei are pyknotic. Inset, H and E X 810.
Fig. 16.—Endoplasmic reticulum from an acinar cell of rat 5B. The cisternae are separated and show localized dilatation. $\times$ 86,000.

Fig. 17.—Endoplasmic reticulum from an acinar cell of rat 6B. Cisternae are replaced by vesicles covered by ribosomes. Many free ribosomes are present. The mitochondrion is partially covered by a single membrane. $\times$ 45,000. Pancreatic acinar cells of the same rat seen in light microscope; acinar organization is lost. Many nuclei are pyknotic. Inset, H and E $\times$ 810.
Figs. 18 to 21.—Large areas of focal degradation from acinar cells of rats, Group 5.

Fig. 18.—A portion of dense endoplasmic reticulum is limited by a double smooth membrane except a short segment on the left formed by a single smooth membrane. X 27,000. Portion of the wall is enlarged in the inset. The outer smooth membrane (o) is straight, the inner smooth membrane (i) is wrinkled. X 130,000.

Fig. 19.—Large area of sequestration contains ribosomes, a mitochondrion and smooth membranes. X 30,000.

Fig. 20.—An area of focal degradation with packed smooth membranes and a zymogen granule. X 25,400.

Fig. 21.—Advanced focal degradation with dense granular and membranous material. X 27,000.

Figs. 22 to 25.—Osmiophilic plaques from acinar cells of rats, Group 5.

Fig. 22.—A body limited by a single membrane contains moderately dense material, smooth and myeloid membranes and ribosomes (arrow). X 34,000.

Fig. 23.—The body on right probably represents a stage in formation of the osmiophilic body on the left. X 44,000.

Fig. 24.—Body with osmiophilic lamellar material and smooth membranes is limited by a single membrane. X 36,000.

Fig. 25.—Group of osmiophilic plaques. X 35,000.
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