Electron Microscopic Autoradiography of the Pancreas in the Hamster Treated with Tritiated N-Nitroso-2,6-dimethylmorpholine

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ABSTRACT

Syrian golden hamsters were given a single dose of [3H]-N-nitroso-2,6-dimethylmorpholine and killed 8 hr later. The pancreas was processed for electron microscopic autoradiography to detect binding of radioactivity to cellular constituents. The pancreatic acinar cells and duct epithelia were found to be labeled, while islet cells, centroacinar cells, and all nonepithelial elements were not. Acinar cells active in secreting zymogen had a high concentration of grains over the zymogen granules and the rough endoplasmic reticulum. Their nonsecreting counterparts contained abundant bound material in the nuclei and rough endoplasmic reticulum. Labeling was lower in the duct epithelia than in acinar cells, with the majority of grains associated with the heterochromatin. Our findings suggest that the acinar cells are the principle site of metabolic activation in this organ.

INTRODUCTION

2,6-DMNM2 is a powerful pancreatic carcinogen in the Syrian golden hamster, inducing a high incidence of duct carcinomas within 30 weeks of treatment (10, 14). 2,6-DMNM induces tumors which are composed of cells with the morphology of duct cells (10, 14, 15), but the cell from which the tumor originates has not yet been identified. Duct cells (10, 15), islet cells (14), and pancreatic acinar cells (15) have all been suggested as precursors of the pancreatic tumors induced by this compound. Pancreatic duct carcinomas induced in Syrian hamsters by N-nitrosobis(2-hydroxypropyl)amine (13) and N-nitrosobis(2-oxo-cyclopropyl)amine (11, 12) [which are both converted to some extent to the same metabolites as is 2,6-DMNM] when given to hamsters (6, 7) seem to arise from duct epithelia. However, while one of these studies (8) emphasized the involvement of pancreatic acinar cells in the neoplastic process, in the other study (11) islet cells were claimed to be an important component of ductal neoplasms. The organotropy of the pancreatic carcinogens listed above and their effects on the various cell types may be closely linked to their requirement for metabolic activation. It would be helpful to detect binding of radioactivity to cellular constituents.

MATERIALS AND METHODS

Five 4-month-old male Syrian golden hamsters were each given a single intragastric dose of 1 mCi [3H]-2,6-DMNM (labeled in the 3,5-position) in 1.93 mg of the nitrosamine (purity > 99% by chromatography) in ethanol. Eight hr later, the animals were fixed in situ by vascular perfusion with 2% cacodylate-buffered glutaraldehyde (pH 7.4) under sodium pentobarbital anesthesia (Diabital; Diamond Laboratories Inc., Des Moines, Iowa). Twenty tissue samples per organ were excised at random from the pancreas and heart. (The heart was chosen as an intraindividual background control; heart muscle is well vascularized but most unlikely to metabolize the compound. If labeled material due to insufficient exsanguination had remained in the tissues, this would have been detected in autoradiograms from heart tissue.) They were immersed for an additional 2 hr in the same fixative and then postfixed for 1 hr in 1% cacodylate-buffered osmium tetroxide. The samples were dehydrated in a graded series of ethanol and embedded in Epon 812 (Ladd Research Industries, Inc., Burlington, Vt.). During the dehydration steps, the level of activity was monitored by liquid scintillation counting to ensure that all unbound radioactivity had been removed. Ultrathin sections were cut on an LKB Ultratome IV (LKB, Bromma, Sweden) using diamond knives. The sections were mounted on copper grids and coated with a monolayer of Ilford L4 emulsion by the loop technique. They were exposed in the dark for 5 months and then developed with Kodak D-19 developer. The autoradiograms were stained with uranyl acetate in 50% methanol and lead citrate. Electron micrographs were taken at random with a Philips 201C transmission electron microscope. Quantitative measurements of the autoradiograms were made on the prints by the method of Williams (16). Twenty-five cells of each type per animal were examined. To ensure that different cells and not merely serial sections of the same cell were examined, only one section per sample was evaluated. To obtain background levels, control autoradiograms from heart tissue of the same animals and pancreas tissue from untreated control animals were evaluated. The background was less than 2 grains/100 sq μm of cell. To check the validity of the autoradiographic findings, grain counts were compared with the random distribution of circles (on transparent overlays which were placed on the prints) the diameters of which equalled the resolution of the system (16). To establish an objective basis with which to compare the grain concentrations over cell organelles and to avoid invalidation of the results arising from differences in size and shape of the cells and their constituents, all grain counts were carried out with a Leitz A. S. M. image analysis system (E. Leitz, Inc., Rockleigh, N. J.). The results were tested

1 To whom requests for reprints should be addressed.
2 The abbreviation used is: 2,6-DMNM, N-nitroso-2,6-dimethylmorpholine.
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for significant differences by the paired t test and analysis of variance.

RESULTS

The distribution of autoradiographic grains over cellular constituents differed significantly (p < 0.001) from that of the random circles. In the pancreas, autoradiographic grains were associated only with epithelial cells. All other tissue elements were unlabeled. Among the epithelial cells, only the acinar cells and duct cells contained bound radioactivity, whereas the centroacinar cells and the various cell types constituting the endocrine pancreas were unlabeled. Two types of acinar cells with different functions were distinguished: one active in the production of zymogen (Fig. 1), and the other which contained no zymogen granules (Fig. 2). The pattern of grain distribution (Table 1) was different in these 2 cell types. While in the actively secreting acinar cells the concentration of grains was highest over the cytoplasm, in the nonsecreting cells the majority of the grains per unit area was seen over nuclear constituents. Further classification of grain distributions at the subcellular level revealed that the high cytoplasmic labeling in secreting acinar cells was caused by an exceptionally high grain concentration (43/100 sq μm) over zymogen granules. The rough endoplasmic reticulum, which occupied the majority of the acinar cell cytoplasm, also contained considerable quantities of bound radioactivity in both cell types (Table 1). Grain counts on mitochondria, Golgi apparatus, and lysosomes were no higher than background levels. Therefore, these organelles were not considered to have been labeled.

The duct epithelial cells did not generally contain as much bound radioactivity per cell as did the acinar cells (Table 1; Figs. 1 to 3). Their cytoplasm, which possesses only sparse organelles, contained a fairly low concentration of grains. The sparse mitochondria and rough endoplasmic reticulum were unlabeled. The highest grain concentration (39/100 sq μm) in this cell type was associated with the marginally condensed heterochromatin of the nuclei. There were fewer grains (p < 0.001) per unit area (4/100 sq μm) associated with the noncondensed euchromatin, which constituted the majority of the nucleus in each of these cells (Table 1). The location of the ducts within the pancreas (e.g., intralobular ductule, perinuclear ductule, interlobular ducts) did not seem to be related to the level of epithelial labeling.

DISCUSSION

In autoradiograms made under the conditions used here, grains are generally regarded as indicators of labeled material bound to cellular constituents (1), although it is not possible to determine whether the activity is directly bound nitrosamine or material incorporated after metabolism.

Our findings suggest that neither the centroacinar cells nor any of the islet cells are important sites of activation of 2,6-DMNM in hamsters. These cells are therefore unlikely to be the precursors of the pancreatic tumors induced by this chemical. Only 2 cell types remain as likely precursors of the tumor cells formed, the acinar cells and the duct cells, both of which contain considerable levels of bound radioactivity after the administration of [3H]-2,6-DMNM.

The distribution of autoradiographic grains over the cytoplasmic organelles of the acinar cells indicates that these cells are the most likely to play a key role in the activation of this nitrosamine. These cells, which exhibit a high concentration of grains over their abundant rough endoplasmic reticulum, could provide the enzymes necessary for the metabolic activation of nitrosamines. In contrast, the duct cells with their sparse cytoplasmic organelles contained few grains in their cytoplasm, which corresponds with their low level of metabolic competence. The unusually high concentration of autoradiographic grains over the zymogen granules of the acinar cells suggests that there is extensive binding of 2,6-DMNM (or its metabolites) at this site. Recent studies have shown that the pancreatic juice does not contain measurable amounts of 2,6-DMNM or its metabolites (5).

This high proliferative capacity of pancreatic acinar cells after injury is well known (4), and studies of the pathogenesis of pancreatic duct carcinomas induced by implantation of 7,12-dimethylbenz(a)anthracene have identified them as precursors of the neoplastic duct cells (2). Moreover, Levitt et al. (8) noted

### Table 1

<table>
<thead>
<tr>
<th>Grains counts and areas of organelles in labeled pancreatic cells</th>
<th>Acinar cells with zymogen</th>
<th>Acinar cells without zymogen</th>
<th>Duct cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (sq μm)</td>
<td>Grains/100 sq μm</td>
<td>Area (sq μm)</td>
<td>Grains/100 sq μm</td>
</tr>
<tr>
<td>----------------</td>
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<td>----------------</td>
</tr>
<tr>
<td>Heterochromatin</td>
<td>12.1 ± 12.0*</td>
<td>20.4 ± 7.8</td>
<td>23.2 ± 7.7</td>
</tr>
<tr>
<td>Euchromatin</td>
<td>25.9 ± 10.4</td>
<td>8.3 ± 19.0</td>
<td>47.2 ± 11.0</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>25.6 ± 8.4</td>
<td>0.6 ± 1.4</td>
<td>21.0 ± 10.1</td>
</tr>
<tr>
<td>Rough endoplasmic reticulum</td>
<td>177.4 ± 26.4</td>
<td>20.3 ± 1.9</td>
<td>170.5 ± 16.0</td>
</tr>
<tr>
<td>Rough endoplasmic reticulum-mitochondria*</td>
<td>49.5 ± 70.9</td>
<td>0.5 ± 0.9</td>
<td>39.9 ± 11.1</td>
</tr>
<tr>
<td>Zymogen granules</td>
<td>26.8 ± 10.6</td>
<td>42.7 ± 12.4</td>
<td>---</td>
</tr>
<tr>
<td>Cytoplasmic filaments</td>
<td>---</td>
<td>---</td>
<td>1.8 ± 3.9</td>
</tr>
<tr>
<td>Golgi</td>
<td>4.2 ± 5.3</td>
<td>0</td>
<td>4.3 ± 5.9</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>2.2 ± 1.0</td>
<td>0</td>
<td>1.6 ± 1.5</td>
</tr>
<tr>
<td>Matrix*</td>
<td>24.6 ± 10.2</td>
<td>0</td>
<td>13.9 ± 12.9</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

a, 0, no grains on this organelle.

* A shared item (grains partly on rough endoplasmic reticulum and partly on mitochondria).

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a pronounced increase in the \([^{3}H]\)thymidine labeling in acinar and duct cells after treatment of hamsters with N-nitrosobis(2-hydroxypropyl)amine; in an accompanying experiment, they observed that both of these cell types proliferated after treatment. A plausible interpretation of our data would be that acinar as well as the duct cells are both susceptible to the carcinogenic effects of 2,6-DMNM, and later both produce duct carcinomas. The acinar cells are capable of activating 2,6-DMNM and perhaps can transform themselves into neoplastic cells and/or induce the malignant transformation of the duct cells. A serial sacrifice study with 2,6-DMNM in Syrian hamsters should clarify the pathogenesis of duct carcinomas induced by this compound. In addition, efforts will be made to isolate the individual cell types of the pancreas for study of the metabolism of 2,6-DMNM in these defined cell populations.

ACKNOWLEDGMENTS

We thank Dr. J. E. Tomaszewski for the preparation of \([^{3}H]\)-2,6-DMNM and Lee Ann Franklin for embedding and sectioning the tissues.

REFERENCES

Fig. 1. Electron microscopic autoradiogram of an acinar cell with zymogen granules. Numerous grains are associated with zymogen granules and rough endoplasmic reticulum. × 11,600.
Fig. 2. Electron microscopic autoradiogram of acinar cell without zymogen granules. Most grains are located over heterochromatin and rough endoplasmic reticulum. × 17,500.
Fig. 3. Electron microscopical autoradiogram of duct epithelial cells. The majority of grains are located over the nuclei, while the cytoplasm with its sparse organelles has a low grain concentration. × 27,000.
Fig. 4. Electron microscopic autoradiogram of islet cells. In the islets of Langerhans, the grain concentration is in the range of background levels (less than 2 grains/100 sq μm). These cells are hence considered unlabeled. × 15,700.

Fig. 5. Electron microscopic autoradiogram exemplifying an unlabeled centroacinar cell. In the upper part of the figure, labeled zymogen granules of an adjacent acinar cell are visible. × 22,400.
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