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DECREASE IN TISSUE-SPECIFIC RESISTANCE OF THE GASTRIC EPITHELIUM TO ACUTE CELL DEATH INDUCED BY INHIBITION OF DNA SYNTHESIS

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Administration of hydroxyurea to mice caused acute death of a few cells synthesizing DNA in the epithelium of the glandular stomach. If actinomycin D was given 8 h before the hydroxyurea, cell death was sharply intensified and about 80% of cells synthesizing DNA died during administration of the hydroxyurea. Administration of actinomycin D simultaneously with hydroxyurea had no potentiating effect on cell death. It is postulated that actinomycin D stimulates protein synthesis in stomach cells (the "superinduction" effect), thus increasing their sensitivity to inhibition of DNA synthesis.

KEY WORDS: *stomach; DNA synthesis; hydroxyurea; actinomycin D; cell death.*

Inhibition of DNA synthesis by hydroxyurea causes acute death of nearly all cells in the S phase in the epithelium of the small intestine but of only solitary cells in the epithelium of the stomach [1]. The ability of gastric cells to withstand inhibition of DNA synthesis without dying reflects the tissue-specific features of the organization of these cells which, since they persist after malignant degeneration, determine the resistance of tumors of the stomach to chemotherapy [2].

The object of this investigation was to study the action of substances which reduce the resistance of the gastric epithelium to hydroxyurea, an inhibitor of DNA synthesis, which is a matter of considerable interest.

EXPERIMENTAL METHOD

To determine acute death of cells synthesizing DNA, thymidine-^{3}H was injected intraperitoneally in a dose of 1 μCi/g body weight into noninbred mice, and 30 min later hydroxyurea was injected in a dose of 500 mg/kg body weight. At 8 h before injection of hydroxyurea or simultaneously with it, actinomycin D was administred in a dose of 0.25-0.5 mg/kg body weight. In some experiments hydroxyurea was injected twice at an interval of 4 h. The vari-

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TABLE 1. Acute Cell Death in Gastric Epithelium Following Inhibition of DNA Synthesis by Hydroxyurea

Expt.	Preparation	Interval between injections, h	Dose of preparation, mg/kg	Fraction of labeled cells which degenerated
1	Hydroxyurea	—	500	12±1,3
2	Actinomycin D	—	0,5	0
3	Actinomycin D	0	0,5	14,3±2,7
	Hydroxyurea		500	
4	Actinomycin D	8	0,25	84,7±2,9
	Hydroxyurea		500	
5	Actinomycin D	8	0,5	79±5,5
	Hydroxyurea		500	
6	Actinomycin D	0	0,5	85,5±2,5
	Hydroxyurea	4	500	
	"		500	
7	Actinomycin D	0	0,25	56,8±4,1
	Hydroxyurea	4	500	
	"		500	
8	Actinomycin D	0	0,5	67,5±10,6
	Hydroxyurea	4	500	
	"		500	
9	Actinomycin D	8	0,5	29,3±4,5
	Hydroxyurea		500	

Legend. In experiments Nos. 1-6 thymidine-^{3}H was given 30 min before the last preparation, and in experiments Nos. 7-9 it was given 30 min before the first preparation.

ous parts of the gastrointestinal tract were fixed in Carnoy's fluid 4 h after the injection of hydroxyurea. Section 5 μ thick were coated with type M emulsion and exposed for 2 weeks. The number of degenerating labeled cells was counted on autoradiographs.

EXPERIMENTAL RESULTS

Injection of hydroxyurea caused acute death of a very few cells synthesizing DNA in the epithelium of the glandular stomach (Table 1). No acute cell death was observed after injection of actinomycin D. After simultaneous injection of hydroxyurea and actinomycin D, approximately the same number of cells died as after injection of the hydroxyurea alone. When actinomycin D was injected 8 h before hydroxyurea, there was a sharp increase in cell deaths, for about 80% of DNA-synthesizing cells died during the injection of hydroxyurea (Table 1, experiments Nos. 4 and 5). In experiment No. 6, hydroxyurea was injected twice; the first injection was given simultaneously with actinomycin D. Thymidine-^{3}H was injected 4 h after the first injection of hydroxyurea, when DNA synthesis was resumed. Consequently, in this experiment the inhibition of DNA synthesis was more prolonged than in experiments Nos. 4 and 5, and virtually all the cells in the S phase during the injection of actinomycin D failed to emerge from this phase before the injection of thymidine-^{3}H. Cell death was equal in degree in experiments Nos. 5 and 6. This is evidence against the role of changes in the composition of the cell population in the S phase by actinomycin D in the increase in their sensitivity to hydroxyurea. In experiments Nos. 7-9, thymidine-^{3}H was injected before actinomycin D. If under these circumstances hydroxyurea was injected twice, thus blocking the departure of the cells from phase S, many cells died. If, however, hydroxyurea was injected only once and most of the labeled cells had left the S phase before it was given, far fewer of the labeled cells died.

The absence of acute cell death in the glandular part of the stomach following inhibition of DNA synthesis is evidently an indicator of tissue-specific differences between the responses of these cells to chemotherapy. The mechanism of acute cell death following inhibition of synthesis of DNA and RNA and alkylation of DNA is still unexplained. All that is known is that inhibition of protein synthesis prevents this death [4] and also prevents loss of clonogenic activity of cells in culture during exposure to hydroxyurea [3]. Possibly, therefore, the special features of protein synthesis in the cells of the glandular stomach are responsible for their resistance to the inhibitor of DNA synthesis and factors

acting on protein synthesis, especially stimulating it, may reduce this resistance. Actinomycin D, which causes "superinduction," i.e., an increase in the concentration and activity of specific proteins after inhibition of RNA synthesis [5], was used for this purpose. Actinomycin D in fact sharply reduced the resistance of the stomach cells to hydroxyurea. Only preliminary injection of actinomycin D, and not its simultaneous injection with hydroxyurea, proved to be effective. This confirms indirectly that "superinduction," and not the direct action of actinomycin D, reduces the resistance of cells, although of course this interpretation is highly hypothetical.

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ULTRASTRUCTURAL ANALYSIS OF THE ADRENAL CORTEX IN RATS AFTER BILATERAL SUBDIAPHRAGMATIC VAGOTOMY

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Electron-microscopic investigation of the zona fasciculata and zona reticularis of the adrenal cortex in rats showed that 7 days after bilateral subdiaphragmatic vagotomy the perinuclear space of the adrenocorticocytes and endothelial cells is widened, the tubules of the smooth cytoplasmic reticulum are dilated, the mitochondria edematous, their cristae reduced, and the number and size of the lipid droplets diminished. After 45 days some mitochondria were starting to undergo myelinization, lipid droplets were aggregating, and electron-translucent vacuoles appeared in them. Vagotomy depresses the function of the adrenocorticocytes of the zona fasciculata and zona reticularis of the rat adrenal cortex.

KEY WORDS: *vagotomy; lipid droplets; mitochondria; adrenocorticocytes.*

The function of the adrenal cortex when its innervation is disturbed has been inadequately studied [1]. One problem, in particular, that is still not settled is the role of the vagus nerve in the innervation and regulation of activity of the adrenal cortex, even though the wide use of vagotomy for the surgical treatment of duodenal ulcer necessitates a study of changes in the structure and function of the secretory cells of the adrenal cortex after disturbance of its vagal innervation.

The object of this investigation was to study the ultrastructure of the adrenal cortex after bilateral subdiaphragmatic vagotomy.

EXPERIMENTAL METHOD

Male Wistar rats (54) weighing 120-140 g were used. Bilateral subdiaphragmatic vagotomy was performed under ether anesthesia. The animals were killed 7 and 45 days after the

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