Vinblastine-induced autophagocytosis: effects on liver glycogen

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The possible similarities of the mechanism by which vinblastine induces autophagocytosis in liver were compared with the known effects of glucagon in glucagon-induced autophagocytosis. A single intraperitoneal injection of vinblastine produced a wave of autophagocytosis in <0.5 h in mouse hepatocytes. Liver glycogen content decreases simultaneously and blood glucose first increased and then decreased below control values. Both liver cAMP concentration and the activity of glycogen phosphorylase remained unchanged. These findings provide evidence that the induction of autophagocytosis after vinblastine injection is not mediated by cAMP. The increased degradation of glycogen may occur in the lysosomal system by means of increased autophagocytosis.

Vinoblastine *Autophagocytosis* *Liver* *Glycogen* *cAMP*

1. INTRODUCTION

In autophagocytosis cells digest their own organelles in membrane-limited vacuoles by means of lysosomal enzymes. Recently, it has been found that this mechanism is also the main system for protein degradation in the liver [1,2]. During studies of cellular autophagocytosis several experimental methods have been used [3–5] in order to increase the formation of autophagic vacuoles (AV), which occur rather infrequently in normal cells [9]. The classical method of inducing autophagocytosis is intraperitoneal injection of glucagon, which increases autophagocytosis within 30 min in rat hepatocytes [3,6]. Some evidence has been presented that the true inducer might be the elevated liver cyclic AMP (cAMP) concentration caused by glucagon injection, since dibutyryl cAMP alone has been implicated in the induction of autophagocytosis [7].

The microtubule inhibitor vinblastine has also proved to be a powerful inducer of autophagocytosis [8,9]. The mechanism by which vinblastine induces autophagocytosis is not clear. It has been shown that vinblastine increased the pituitary cAMP concentration by inhibition of cAMP phosphodiesterase. Although vinblastine usually inhibits secretory processes, it actually stimulates the release of glucagon from the pancreas, at least in vitro [11].

This study was undertaken in order to elucidate any possible similarities in the mechanisms by which vinblastine and glucagon induce autophagocytosis. For this purpose the effects of an intraperitoneal injection of vinblastine on mouse liver cAMP and glycogen as well as on blood glucose levels were studied. The activity of liver glycogen phosphorylase was also estimated. The ultrastructural changes of hepatocytes were monitored by electron microscopy.

2. MATERIALS AND METHODS

2.1. *Experimental animals*

150 male NMRI-mice (2–3 months old, 27–34 g body wt) were used in the experiments. Animal care was conducted as in [4]. Vinblastine sulphate (E. Lilly, Indianapolis IN) was dissolved in 0.9% saline, and injected as a single intraperitoneal dose of 10 mg/kg body wt. Control animals received a corresponding volume of saline. The animals were divided into 3 groups which were fed in different

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ways during the experiments. One group starved overnight (12 h), the second group starved after the administration of vinblastine or saline, and the third group was given food ad libitum. Liver glycogen estimations were made from all 3 groups, blood glucose from the first and third groups, and estimations of cAMP concentration and glycogen phosphorylase *a* activity, as well as specimens for electron microscopy, were made from the third group.

2.2. *Assay procedures*

Specimens for electron microscopy were prepared as in [4] and examined in a JEM 100 U electron microscope. Cyclic AMP concentration was assayed as in [12] with the reagents and instructions of Boehringer Mannheim GmbH. Radioactivity was determined using an LKB (Wallac) liquid scintillation counter. Blood glucose was estimated by the *o*-toluidine method as in [13] and liver glycogen level was estimated using anthrone reagent [14]. Estimation of liver glycogen phosphorylase *a* activity was carried out by measurement of the rate of liberation of inorganic phosphate from glucose 1-phosphate in the presence of glycogen; i.e., in the direction of synthesis of glycogen [15,16] as follows: a 10%-homogenate (w/v) was made in ice-cold 0.1 M glycylglycine buffer (pH 7.4) containing 0.15 M NaF in order to inhibit phosphorylase activity. Caffeine (final concentration 0.5 mM: inhibits phosphorylase *b* activity) was also added to the homogenate. The substrate solution contained 1% glycogen, 50 mM glucose 1-phosphate and 0.15 M NaF. The incubation was performed at 37°C for 10 min and the reaction was stopped by adding 10% TCA. The mixture was centrifuged and the supernatant was used for the determination of inorganic phosphate [17].

Satatistical analysis was performed using Student's *t*-test.

3. RESULTS

3.1. *Vinblastine-induced autophagocytosis*

As early as 30 min after vinblastine injection several newly formed, double membrane-limited

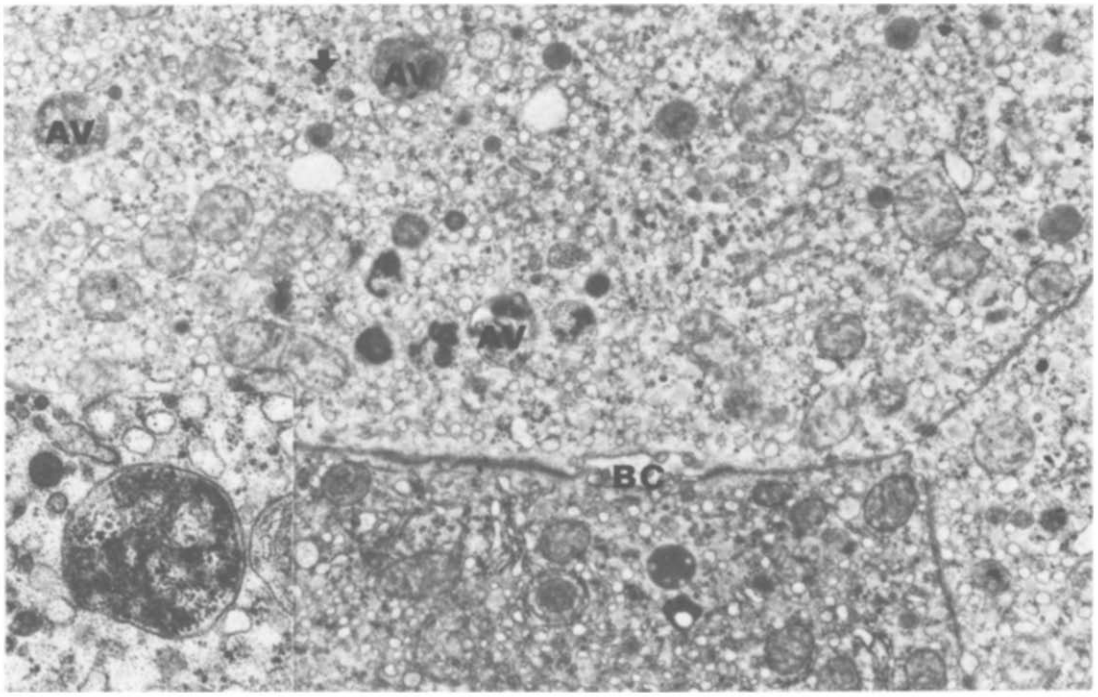

Fig.1. Mouse hepatocytes 1 h after vinblastine injection (10 mg/kg). Several autophagosomes (AV) with variable contents can be seen in the cytoplasm. Arrow: Cytoplasmic glycogen, BC: Bile canalisulus. M: ×12000. Inset: An autophagosome containing glycogen granules. M: ×30000.

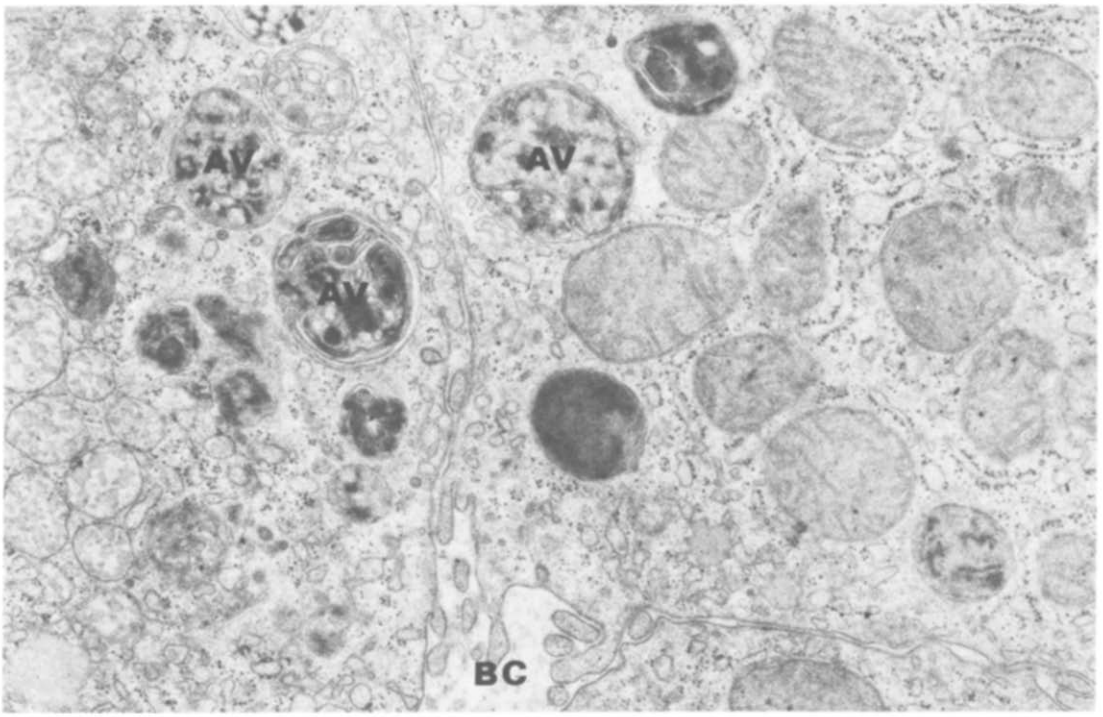

Fig.2. Mouse hepatocytes 12 h after vinblastine injection (10 mg/kg). Autophagosomes (AV) with partially degraded contents can be seen around the bile canaliculus (BC). No glycogen can be seen in the cytoplasm. M: ×15000.

AV were observed in the cytoplasm of the hepatocytes. The contents of the vacuoles were variable including glycogen and various cell organelles (fig.1,2) as in [4]. Glycogen was observed in the hepatocytes of the freely feeding animals, but disappeared completely during the 4 h after injection (fig.2). Other ultra-structural changes also occurred in the hepatocytes after vinblastine administration. These changes have been described in [4].

3.2. *Biochemical assays*

Blood glucose level was significantly elevated 1 h after vinblastine administration. This elevation was reversed after 2 h in the group which had free access to food (fig.3). The results expressed in fig.4 show that the liver glycogen content decreased significantly 1 or 2 h after injection in the group of animals which was starved after injection as well as in the freely feeding group, respectively. The cAMP concentrations in the livers of the animals receiving vinblastine did not differ significantly from the control values (table 1). The glycogen phosphorylase *a* activity also showed no significant changes after vinblastine injection (table 2).

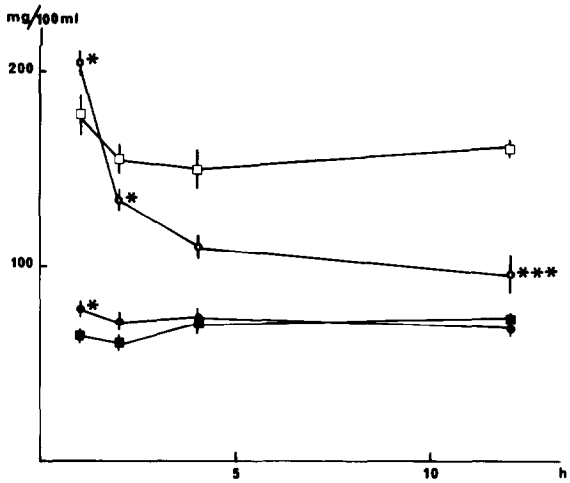

Fig.3. Blood glucose levels in mice after vinblastine (10 mg/kg; circles) or saline (squares) injection. Open symbols denote that the animals had free access to food throughout the experiment. Filled symbols denote that the animals were starved overnight before killing. Each symbol is the mean value of 4 animals. The vertical bars indicate the standard error. Levels of significance as compared with controls: +, $p < 0.050$; ++, $p < 0.010$; +++, $p < 0.001$.

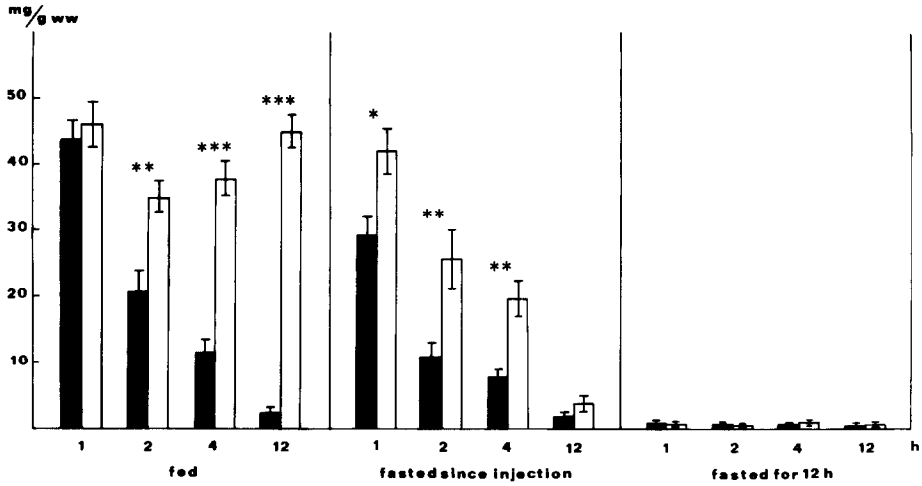

Fig.4. Mouse liver glycogen levels 1, 2, 4 and 12 h after vinblastine (10 mg/kg, black columns) or saline (white columns) injection. Each column represents the mean value from 4 animals. The vertical bars indicate the standard error. Levels of significance as compared with controls: +, $p < 0.050$; ++, $p < 0.010$; +++, $p < 0.001$.

Table 1

Cyclic AMP content of mouse liver 15, 30 and 60 min after VBL injection (10 mg/kg) as compared with controls

Treatment	Saline	Time after VBL injection (min)		
		15	30	60
pmol/mg	0.423 ± 0.007	0.386 ± 0.039	0.408 ± 0.020	0.416 ± 0.039
n	6	6	6	6

Results are expressed as pmol cAMP/mg wet wt of liver tissue. Values given are means ± standard error

Table 2

Glycogen phosphorylase *a* activity in mouse liver 15, 30 and 60 min after VBL injection (10 mg/kg) as compared with controls

Treatment	Saline	Time after VBL injection (min)		
		15	30	60
μmol.min^{-1}.g protein^{-1}	122 ± 6.56	113 ± 6.39	119 ± 3.91	120 ± 5.72
n	8	8	8	8

Results are expressed as μmol liberated P_i.min^{-1}.g protein^{-1}. Values given are means ± standard error

4. DISCUSSION

Injection of vinblastine to the test animals was followed by a loss of glycogen from the liver. Blood glucose values were elevated 1 h after vinblastine injection, resembling the sharp rise in blood glucose typical for glucagon-stimulated gly-

cogenolysis [18]. The hypoglycemic activity of vinblastine later observed in the group of animals feeding freely has been described in [19].

There are two principal enzymatic pathways by which glycogen can be degraded to glucose in the liver: by glycogen phosphorylase in the cytoplasm and by α-glucosidase in the lysosomal system. Phosphorolysis is involved in the rapid breakdown of glycogen when glucose or glucose phosphates are required for the energy needs of the body. The lysosomal α-glucosidase is necessary when glycogen is engulfed in AV [20]. According to our results increased glycogen degradation due to glycogen phosphorylase activation appeared to be improbable, because this enzyme is activated by elevated cAMP levels in the liver. Glucagon inreases the cAMP content in isolated rat liver within few min [21,22]. Here, neither the cAMP level nor the glycogen phosphorylase *a* activity changed after vinblasting injection. Furthermore, it has been found [23] that vinblastine and colchicine inhibit glucagon-stimulated adenylate cyclase and cAMP phosphodiesterase in rat liver plasma membrane. Inhibition of adenylate cyclase activity could thus explain the unchanged cAMP concentrations after vinblastine injection in his study despite the possible inhibition of cAMP phosphodiesterase activity, which could otherwise lead to increased cAMP concentrations as in the pituitary [10]. Unchanged cAMP levels have also been observed in autophagocytosis induced by amino acid deprivation in perfused rat liver, when glycogen was simultaneously frequently found in AV [5].

It has been shown that the uptake of cytoplasmic proteins by autolysosomes apears to be an obligatory step in proteolysis at all levels of regulation. As much as 99% of the intracellular proteins of the hepatocytes may be degraded in autolysosomes [1,2]. According to our results the increased degradation of glycogen after vinblastine injection may occur in the lysosomal system by means of increased autophagocytosis. The results also show that vinblastine induces autophagocytosis independently of alterations in the level of liver cAMP.

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