STUDIES ON THE INHIBITION OF GLYCOGENOLYSIS BY D-GALACTOSAMINE IN RAT LIVER HEPATOCYTES.*

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SUMMARY

Effect of galactosamine on glycogenolysis was studied in isolated hepatocytes. It was found that addition of galactosamine strongly inhibited glycogenolysis in normal hepatocytes. Galactosamine-inhibited glycogenolysis was not stimulated by epinephrine or glucagon. This inhibition was specific as no such inhibition was observed with galactose, 2-deoxy-glucose or glucosamine. The glucagon-stimulated cyclic AMP formation in galactosamine-treated hepatocytes was the same as in normal cells; Glc-1-P and Glc-6-P did not accumulate nor was lactate formation enhanced. The glucose production by hepatocytes from regenerating liver was only slightly inhibited by galactosamine and glucagon addition stimulated glycogenolysis in the presence of the amino sugar.

D-Galactosamine administration to fed adult rats in vivo was shown to lead to a depletion of the liver glycogen stores (1). Since the amino sugar induces a rapid depletion of the hepatic UDPG content, the net loss of glycogen was assumed to be due to failure of synthesis (2). However, a stimulated breakdown of glycogen under these conditions could not be ruled out. As a consequence of decreased levels of UTP, UDPG and UDPGal, inhibition of macromolecular synthesis, e.g. of RNA (3), protein (4-6), and especially of glycoproteins (7) and glycolipids (8) occurs, leading to alterations of membrane structures and func-

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Abbreviations used: GalN, D-galactosamine; cAMP, cyclic 3',5'-adenosine monophosphate; cGMP, cyclic 3',5'-guanosine monophosphate; cUMP, cyclic 3',5'-uridine monophosphate

tions. In this communication data will be presented showing inhibition of glucagon-sensitive glycogenolysis after GalN administration to normal hepatocytes despite a normal production of cAMP. This GalN effect is almost absent in hepatocytes from regenerating liver.

MATERIALS AND METHODS

Female Wistar rats weighing 165-180 g (Ivanovas, Kissleg, West Germany) were maintained on Altromin R diet (Altrogge GmbH, Lage/Lippe, West Germany). Partial hepatectomies were performed by removing two-thirds of the livers while the animals were kept under light ether anaesthesia. Normal and partially hepatectomized animals were anaesthesized with pentobarbital, the liver removed and perfused with collagenase: hepatocytes were isolated as reported previously (9). An aliquot of the final cell suspension (1 ml, 50-60 mg of cell weight) was incubated in 2 ml of Umbreit-Ringer-25 mM $NaHCO_3$ buffer (10) in stoppered plastic vials with various substrates and hormones at 37°C and 90 oscillations/min in a metabolic incubator. Vials were gassed with 95% O_2/5% CO_2 for 2 min. At the end of incubation the vial contents were placed in iced centrifuge tubes and centrifuged at 3000 x g for 5 min. Aliquots of the supernatant were assayed for glucose by the glucose oxidase method (11). Glycogen (12), Glc-1-P (13), Glc-6-P (13) and lactate (14) were assayed enzymatically, cAMP by radio-immune assay (15). All experiments were conducted in duplicate.

RESULTS AND DISCUSSION

GalN inhibits glucose formation in isolated hepatocytes in a dose-dependent manner (Table I), almost complete inhibition being observed in the presence of 1 mM GalN. Under these conditions, neither glucagon nor epinephrine were able to stimulate glycogenolysis. The inhibiting effect of GalN on glucose production could be abolished if the cells were washed 5 min after exposure to the amino sugar; however, reversal of the inhibition could not be observed when the cells were exposed to GalN for 30 min or longer.

The effect of GalN on glycogenolysis appears to be rather specific as no inhibition of glucose release from hepatocytes could be found when comparable concentrations of galactose, 2-deoxyglucose and D-glucosamine were added to the medium instead of GalN (Table II). Furthermore, the GalN inhibition could not be reversed by the addition of cAMP, cGMP or cUMP to the isolated cells.

TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF GALACTOSAMINE ON GLYCOGENOLYSIS IN ISOLATED HEPATOCYTES

Galactosamine added, mM	Without hormone	glucagon (10^{-8}M)	epinephrine (10^{-6}M)
none	46.8 ± 6.0	80.2 ± 10	74.0 ± 7
0.05	44.0 ± 5.5	82.5 ± 9	73.2 ± 7
0.10	38.0 ± 6	76.5 ± 8	65.0 ± 8
0.15	30.0 ± 5	46.5 ± 6	40.2 ± 6
0.2	18.0 ± 2	22.6 ± 3	20.2 ± 3
0.4	13.1 ± 2	12.8 ± 3	-
1.0	6.5 ± 1	8.2 ± 2	-

50-60 mg of isolated cells were incubated in 3 ml of Ringer bicarbonate buffer (10) for 60 min at 37°C as described previously (16). Values are expressed as μmole glucose released into the medium per g of wet cells. They are the averages of five or more observations.

TABLE II

EFFECT OF D-GALACTOSE, 2-DEOXYGLUCOSE AND D-GLUCOSAMINE ON GLYCOGENOLYSIS IN ISOLATED HEPATOCYTES

Substrate added (0.2 mM)	Without glucagon	glucagon (10^{-8}M)
none	49.2 ± 8	76.8 ± 10
D-galactose	43.6 ± 7	69.5 ± 8
2-deoxyglucose	56.8 ± 8	88.0 ± 12
D-glucosamine	44.0 ± 9	62.5 ± 10
D-galactosamine	21.0 ± 4	25.8 ± 5

Experimental conditions are the same as in TABLE I.

TABLE III

EFFECTS OF D-GALACTOSAMINE AND GLUCAGON ON GLYCOGEN BREAKDOWN AND METABOLITE FORMATION IN ISOLATED RAT HEPATOCYTES

	Additions			
	none	GalN* (1 mM)	Glucagon (10^{-8}M)	GalN (1 mM)* Glucagon (10^{-8}M)
Glycogen breakdown (μmole glycosyl units/g wet wt/30 min)	64.5	27	137	34
glucose formed (μmole/g wet wt/30 min)	21	6	47	11
lactate formed (μmole/g wet wt/30 min)	30	4	11.5	-2
cAMP formed (nmole/g wet wt) after 2 min	0.97	1.0	3.83	3.97
G-1-P (nmole/g wet wt) after 30 min	<10	<10	12	<10
G-6-P (nmole/g wet wt) after 30 min	40	30	262	123

*GalN added 20 min prior to 0 time

Experimental conditions are the same as on TABLE I.

GalN-1-P was shown to accumulate to a considerable extent within liver cells after administration of GalN (1); if this compound would interfere with the metabolism of sugar phosphates and inhibit the release of glucose, an accumulation of Glc-1-P should occur. An impairment of Glc-6-phosphatase by GalN or one of its metabolites would result in an increase of the Glc-6-P level and/or a concomitant rise in lactate formation. The data in Table III do not support either possibility. Since UDPG (and UDPGal) contents of GalN-treated hepatocytes are very low (17) and both glycogen and UDP-glucuronate synthesis virtually absent in GalN-treated livers (2) one has to assume that GalN inhibits the production of Glc-1-P from glycogen rather than its further metabolism.

The secondary effects of GalN on protein and heteroglycan synthesis (18) may lead to disturbances of membrane functions including hormone receptor-adenyl cyclase systems. Therefore, the production of cyclic AMP was measured in the presence of GalN and glucagon (Table III). These experiments show a normal response of the hepatocytes to glucagon stimulation after 20 min of GalN pretreatment.

Studies on the effect of GalN on glycogenolysis in isolated cells from regenerating liver are summarized in Table IV. Cells obtained from regenerating liver, two days after partial hepatectomy, show some inhibition of glycogenolysis by GalN but a good response to glucagon. However, at 3, 4 and 5 days after hepatectomy, only a slight inhibition of glycogenolysis by GalN was observed and addition of glucagon almost fully stimulated glycogenolysis in the presence of GalN. Cells obtained from rats 10 days after partial hepatectomy showed again the strong inhibition in the presence of GalN; added glucagon had no effect.

These observations are in line with previous findings of Reutter et al (19) on the insensitivity of regenerating liver *in vivo* to the cytotoxic action of a single dose of GalN. This GalN-refractory state is the result of an alteration of the metabolite deficit period (20) required for irreversible cell damage to occur.

The data presented in this paper show that the inhibitory effect of GalN on the hormone-sensitive glucose formation from

TABLE IV

EFFECT OF D-GALACTOSAMINE ON GLYCOGENOLYSIS IN ISOLATED HEPATOCYTES FROM REGENERATING LIVER

Days after partial hepatectomy	Additions			
	none	glucagon (10^{-8}M)	GalN (0.2 mM)	GalN (0.2 mM) glucagon (10^{-8}M)
2	38.0 ± 6	68.0 ± 8	24.5 ± 4	55.0 ± 8
3	46.0 ± 6	84.0 ± 10	39.0 ± 5	78.0 ± 8
4	57.0 ± 7	118.0 ± 12	46.0 ± 6	106.0 ± 10
5	60.3 ± 5	121.8 ± 13	43.2 ± 7	110.0 ± 12
10	48.4 ± 6	83.8 ± 10	26.5 ± 6	31.2 ± 6

Experimental conditions are the same as in TABLE I

glycogen is neither due to an impaired cAMP production nor to an alteration of the metabolism of Glc-1-P. This inhibition is observed only to a minor extent in fast growing liver tissue, e.g. after partial hepatectomy. The use of GalN appears to be an interesting tool for studies on cAMP-mediated enzyme activations.

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