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EFFECT OF MERCAPTOPICOLINIC ACID AND OF TRANSAMINASE INHIBITORS ON GLYCOGEN SYNTHESIS BY RAT HEPATOCYTES[1]

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SUMMARY: To explore the mechanism of the stimulation of glycogen synthesis by amino acids (1) we have studied the effects of transaminase inhibitors and of mercaptopicolinic acid, (MPA) an inhibitor of phosphoenol pyruvate carboxykinase. Mercaptopicolinic acid enhanced glycogen synthesis from fructose, dihydroxyacetone and xylitol. Stimulation of glycogen synthesis with hepatocytes from fasted rats by 0.5 mM mercaptopicolinic acid was 50-70% as effective as 10 mM glutamine. With hepatocytes from fed rats, the stimulation of glycogen synthesis by mercaptopicolinic acid was more pronounced, and stimulation by mercaptopicolinic acid and amino acids was additive. Glycogen synthesis as high as 1% in wet weight per hour was attained in hepatocytes with a high initial glycogen content. Over 80% of glycogen synthase was in the active (a) form. Amino oxyacetic acid greatly depressed or abolished the stimulatory effect of glutamine and asparagine and of mercaptopicolinic acid, and induced extensive glycogen breakdown in hepatocytes of fed rats.

We have previously shown that efficient glycogen synthesis by isolated rat hepatocytes occurs from gluconeogenic precursors in the presence of glucose and the amino acids, glutamine, asparagine or alanine (1, 2). Glucose and glycogen synthesis proceed concurrently. The amino acids, at a concentration of 5-10 mM, are essential for efficient net glycogen synthesis. The mechanism for the stimulation by the amino acids was obscure. Uptake of lactate or other gluconeogenic precursors approximately equalled carbohydrate (glucose+glycogen) synthesis, and the disappearance of glutamate was nearly balanced by alanine formation (1). This indicated that glutamine did not serve as net carbon source for carbohydrate synthesis. We suggested that the amino acids served as precursors for some unidentified compound which somehow activated glycogen synthesis. To test this hypothesis we have in the present study used mercaptopicolinic acid (MPA)[3], an inhibitor of PEP carboxykinase (3, 4), and the transaminase inhibitors aminooxyacetic acid (AOA) 5, 6), and cycloserine (7).

METHODS: Male rats of the Wistar strain, fed ad libitum, 180-250g in body weight, were used. The preparation of hepatocytes, incubation of cells and methods of analysis were as previously described (1, 2). In short, 0.12-0.15 ml packed cells volume, (20-25 mg cell protein) were incubated for

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3. Abbreviations: AOA, aminooxyacetic acid; CS, cycloserine; MPA, mercaptopicolinic acid, PEP, P-enolpyruvate, GTMN, glutamine.

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TABLE I. EFFECT OF AMINOOXYACETIC ACID (AOA) AND CYCLOSERINE (CS) ON CARBOHYDRATE SYNTHESIS FROM LACTATE AND DIHYDROXYACETONE BY HEPATOCYTES FROM FASTED RATS.

	Amino Acid	Inhibitor	Δ Glucose	Δ Glycogen
			µmol/100 mg/hr	
Glucose Plus	-	-	33	3.7
Lactate-Pyruvate	-	AOA	8.2	- 0.2
	Glutamine	-	25	11
	Glutamine	AOA	13	3.0
	Asparagine	-	32	21
	Asparagine	AOA	7.5	3.5
Glucose Plus	-	-	64	11
Dihydroxyacetone	-	AOA	60	3.3
	Glutamine	-	53	25
	Glutamine	AOA	55	16
	Asparagine	-	53	26
	Asparagine	AOA	55	6.0
	Alanine	-	59	26
	Alanine	CS	53	12

Cells, 20-25 mg protein were incubated for one hour in 3 ml Krebs-bicarbonate buffer at 38°, in an atmosphere of 95% O_2-5% CO_2. The concentration of glucose was 10 mM, and of lactate-pyruvate (9:1) 20 mM, of dihydroxyacetone 20 mM, of amino acids 10 mM. Aminooxyacetic acid concentration was 1 mM, and cycloserine 5 mM. Averages from 3 to 6 rats.

one hour at 38° in 3 ml Krebs bicarbonate buffer in an atmosphere of 95% O_2-5% CO_2. The medium contained glucose, a gluconeogenic precursor, and an amino acid, either glutamine, asparagine or alanine. Glycogen synthase was extracted and assayed as previously described (2, 8) except that 10 mM UDPG (rather than 1 mM) was used. 10 mM sulfate was present for the assay of the a form, and 10 mM glucose-6P, but no sulfate, for total (a+b) synthase. Results are expressed as µmol of glucose or glucose equivalents per 100 mg cell protein per hour, and for synthase assays, as µmol UDPG converted per minute per ml packed cells. 1 gram liver is equivalent to about 200 mg protein or 0.7 ml packed cells.

RESULTS:

Hepatocytes from Fasted Rats. Aminooxyacetic acid, a competitive inhibitor of glutamic oxalacetic transaminase, markedly inhibited glucose synthesis from lactate and from glutamine (Table 1). There was no inhibition from fructose or dihydroxyacetone. Gluconeogenesis from xylitol was depressed by 15-25% (not shown). Similar findings have been reported for perfused liver (9) and hepatocytes . In the absence of amino acids, aminooxyacetic acid abolished glycogen formation. Wet glycogen synthesis in the presence of glutamine was inhibited by 60-80% (Table 1), and depression was even more pronounced with asparagine (see below).

Mercaptopicolinic acid is a noncompetitive inhibitor of PEP carboxykinase (4). In perfused rat liver it inhibited gluconeogenesis from pyruvate, but not from fructose (3, 4). In rat hepatocytes 0.5mM MPA nearly

TABLE 2. EFFECT OF MERCAPTOPICOLINIC ACID (MPA) ON CARBOHYDRATE SYNTHESIS BY HEPATOCYTES FROM FASTED RATS.

Additions	Glutamine mM	MPA mM	Δ Glucose μmol/100mg/hr	Δ Glycogen	^{14}C from Glutamine in CO_2	Glucose % Dose in	Glycogen
Glucose	0	0	33	3.7	-	-	-
Plus	10	0	25	16	17	13	3.1
Lactate	0	0.5	0.1	0.2	-	-	-
Pyruvate 9:1	10	0.5	0.1	3.6	10	2.6	0.4
Glucose	0	0	64	10	-	-	-
Plus	10	0	53	25	20	6.3	1.3
Dihydroxy-	0	0.5	53	21	-	-	-
acetone	10	0.5	45	28	13	0.9	0.4
Glucose	0	0	59	5.8	-	-	-
Plus	10	0	56	16	15	3.3	0.6
Fructose	0	0.5	58	11	-	-	-
	10	0.5	49	22	11	0.2	0.1

Conditions as in Table 1. Analytical data from 7 experiments (lactate and dihydroxyacetone) and 4 experiments with fructose. [U-^{14}C] Glutamine was present in 2 experiments.

abolished carbohydrate synthesis from lactate and glutamine, but not from fructose, dihydroxyacetone and xylitol[4]. (Table 2) In the presence of glutamine and these compounds MPA nearly abolished incorporation of ^{14}C from glutamine into carbohydrate, but the production of glucose and glycogen from gluconeogenic precursors was not impaired. To our surprise MPA augmented the effect of amino acids on glycogen synthesis and was found to be by itself an efficient activator. Glycogen formation with MPA at 0.5 mM was 70 to 80% that with 10 mM glutamine (Table 2). Total carbohydrate synthesis was not greatly altered, but there was a diversion of gluconeogenic flux from glucose to glycogen synthesis.

In Table 3 the effects of the three amino acids and MPA on carbohydrate synthesis from xylitol are compared. MPA was somewhat less effective than the amino acids in stimulating glycogen synthesis. When both MPA and amino acids were present glycogen formation was further enhanced, and approached the rate of glucose synthesis. Aminooxyacetic acid markedly depressed the stimulatory effect of the amino acids and MPA. Cycloserine, a competitive inhibitor of alanine-pyruvate transaminase (7) also inhibited glycogen syn-

4. There was 20-25% inhibition of gluconeogenesis from glycerol, but no inhibition of total carbohydrate synthesis (glucose + glycogen). It was reported (4) that MPA unexpectedly inhibited by 20-30% gluconeogenesis from glycerol in perfused rat liver. We suggest that the depression was due to a diversion of synthesis from glucose to glycogen. We find glycerol inferior to dihydroxyacetone and xylitol as a precursor for both glucose and glycogen synthesis.

TABLE 3. EFFECT OF THE MERCAPTOPICOLINIC ACID (MPA), AMINOOXYACETIC ACID (AOA) OR CYCLOSERINE (CS) ON GLYCOGEN SYNTHESIS FROM XYLITOL IN HEPATOCYTES FROM FASTED RATS.

Additions			Δ	Δ
Amino Acids	MPA	AOA	Glucose µmol/100 mg/hr	Glycogen µmol/100 mg/hr
None	-	-	50	3.7
	+	-	43	17
	-	+	42	- 0.4
	+	+	37	1.1
Glutamine	-	-	42	20
	+	-	37	23
	-	+	47	7.3
	+	+	40	6.1
Asparagine	-	-	38	21
	+	-	31	27
	-	+	44	0.7
	+	+	42	2.4
		CS		
Alanine	-	-	44	21
	+	-	33	25
	-	+	44	4.4
	+	+	39	11

Xylitol (10 mM) and glucose (10 mM) was present in all flasks. Amino acids were at a concentration of 10 mM, mercaptopicolinic acid (MPA) 0.5 mM, aminooxyacetic acid (AOA) 1 mM and cycloserine (in the presence of alanine) 1 mM. Other conditions as in Table 1. Average of 3 experiments.

thesis induced by alanine. Thus the activation of glycogen synthesis by both MPA and the amino acids depends on transamination.

Stimulation of glycogen synthesis by concentrations of 0.1 MPA was somewhat less than with 0.5 mM and there was no further increase at 1 mM. A number of SH compounds, (glutathione, mercaptoethanol, dithioerythrytol) were tested and were without effect.

Hepatocytes from Fed Rats. Cell preparations from such animals have a high glycogen content, from 2 to 4% wet weight. When incubated with glucose and gluconeogenic precursors glycogenolysis usually occurs. With glutamine, there is significant glycogen synthesis from dihydroxyacetone, fructose or xylitol but not from lactate (Table 4). MPA was an effective inducer of glycogen synthesis in these cells. The response differed with the gluconeogenic substrate. For synthesis from xylitol and fructose 0.5 mM MPA and 10 mM glutamine were about equally effective. With dihydroxyacetone MPA was a better activator than glutamine. Highest rates were obtained when glutamine and MPA were both present, and the stimulation was often additive. (Table 4). Net deposition of glycogen from dihydroxyacetone in the presence of MPA and glutamine ranged from 20 to 40 µmol glucose equivalents per

100 µg cell protein per hour, or maximal rates of over 1% net weight per hour, in cells containing initially 2-3% glycogen. As far as we are aware, net glycogen deposition has not previously been reported in perfused liver or cells containing high levels of glycogen. In Fig. 1, the synthesis of glycogen from dihydroxyacetone in the presence of glutamine and MPA is shown as a function of time. When MPA and glutamine were present, the glycogen content increased in 80 minutes from 90 to 130 µmol of glucose equivalents per 100 mg protein, or from 2.9 to 4.2% wet weight. Aminoacetic acid inhibited the stimulatory effects of glutamine or MPA (Table 4 and Fig. 1.) and induced extensive glycogenolysis.

We have previously shown the activation of glycogen synthase by glutamine (2). In Fig. 2 the activation of synthase during glycogen synthesis from dihydroxyacetone in the presence of glutamine and MPA is shown. There is a close correlation between the activity of active synthase and the rates of glycogen synthesis. Active synthase was higher in the presence of MPA than of glutamine, and highest when MPA and glutamine were both present, when about 80% of the total synthase was in the a form.

DISCUSSION: Our findings that MPA does not block the activation of glycogen synthesis by amino acids show that the effect is not due to a supply of carbon from these acids. Since the transaminase inhibitors depress or abolish their effect, either transformation of the amino acids or their action as amino group donors is in some way essential for activation of glycogen synthesis. The amino acids themselves thus do not seem to activate, but serve as precursors for an activator.

The activation of glycogen synthesis by MPA was unexpected. Since AOA abolished this effect it appears that transamination is also required for the action of MPA. MPA has been reported (3, 4) to be a rather specific inhibitor of PEP carboxykinase, and is unlikely to have a direct effect on the enzymes of glycogen synthesis. The response to aminooxyacetic acid suggest a common mechanism of action for MPA and the amino acids.

The effect of MPA was most pronounced in cells of fed rats. The degree of glycogen stimulation varied with the nature of the gluconeogenic substrate, and was most pronounced with dihydroxyacetone. With this substrate, in the presence of both amino acids and MPA the content of glycogen was increased by over 1% wet weight per hour, a rate as high as maximal rates of glycogen deposition observed in vivo. This is to our knowledge the first demonstration in vitro of rapid hepatic glycogen deposition in the presence of initial high glycogen content.

Glutamine induces glycogen synthesis by stimulating the conversion of synthase b to the active a form, but it has little effect on phosphorylase (2).

TABLE 4. EFFECT OF GLUTAMINE (GTMN), MERCAPTOPICOLINIC ACID (MPA) AND AMINOOXYACETIC ACID (AOA) ON GLYCOGEN SYNTHESIS BY HEPATOCYTES FROM FED RATS.

Rat No.:				1		2		1-7
Initial Cell Glycogen µmol/100mg				95		106		102±8
	Addition			Δ Glycogen				
Substrates	GTMN	MPA	AOA	Net	Above Control	Net	Above Control	Above Control
				µmol/100 mg/hr				
Glucose Plus	-	-	-	-17	0	- 7.5	-	-
Dihydroxy-	+	-	-	1.5	18	7.5	15	18±4
acetone	-	+	-	10	27	25	32	30±6
	+	+	-	24	41	32	40	42±9
	-	+	+	42	-25	-16	- 8	- 8±8
Glucose Plus	-	-	-	6.2	0	-22	0	-
Xylitol	+	-	-	7.2	13	- 0.5	21	19±6
	-	+	-	3.5	10	2.5	25	21±7
	+	+	-	9.5	16	13	35	21±9
	-	+	+	-20	-14	-44	-22	-17±5
Glucose Plus	-	-	-	- 4.9	0	0.7	0	-
Fructose	+	-	-	8.9	14	11	10	-
	-	+	-	9.1	14	16	15	-
	+	+	-	18	23	22	21	-
	-	+	+	- 0.7	4	6.1	5	
Glucose Plus	-	-	-	-19		-23		
Lactate	+	-	-	- 6.2		- 7.1		

Concentrations of compounds and conditions as in Table 3. Means and standard errors are given for 7 rats.

Conversion of synthase to the a form was higher with MPA than with glutamine, and when both glutamine and MPA were present, over 80% of synthase was converted to the a form.

The mechanism of action of amino acids and MPA is unknown. The concentration of amino acids in our studies, 10 mM, is well above normal levels. Although the activators of glycogen synthesis in hepatocytes in our system are unphysiological, it is most likely that MPA and the amino acids affect a normal regulatory mechanism for hepatic glycogen synthesis. We postulate that efficient glycogen synthesis requires the presence of a yet unknown activator, which is largely lost in the preparation of hepatocytes. This compound is formed in the presence of excess amino acids. It also accumulates when the conversion of oxalacetate to PEP is blocked, and its formation requires also a supply of amino groups from endogenous sources. The need to accumulate this compound would account for the lag in the onset of glycogen synthesis. In cells of fed rats the presence of both MPA and amino acids leads to a maximal accumulation of this stimulator.

This hypothesis provides the best rationale for the interpretation of

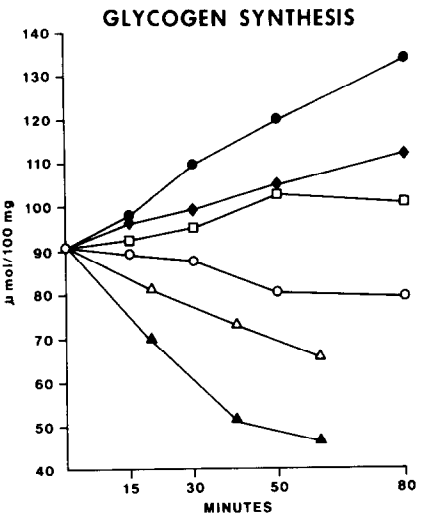

Fig. 1. GLYCOGEN SYNTHESIS FROM DIHYDROXYACETONE BY HEPATOCYTES FROM ONE FED RAT.
Cells were incubated with 10 mM glucose and 20 mM dihydroxyacetone. The additions when present, were glutamine 10 mM, mercaptopicolinic acid (MPA) 0.5 mM, and aminooxyacetic acid (OAA) 5 mM. Results are expressed as μmole glucose equivalents per 100 mg cell protein. From top to bottom: -●- MPA plus glutamine; -◆- MPA; -□- glutamine; -○- control (no addition); -△- MPA + OAA; -▲- no substrate.

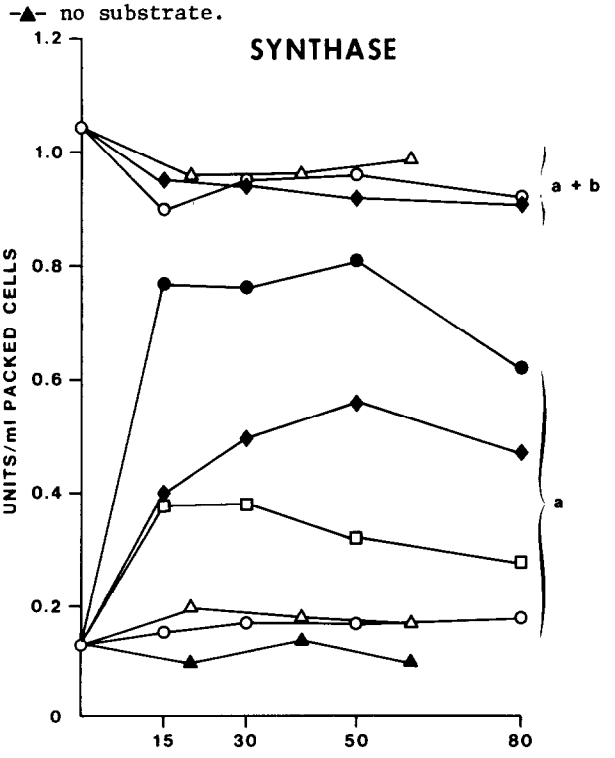

Fig. 2. Active (a) and total (a + b) glycogen synthase in hepatocytes from one fed rat. Conditions and symbols as in Fig. 1.

our experiments. The nature of the activator, and how it would interact with the enzymes of glycogen synthesis is speculative but our hypothesis lends itself to experimental testing.

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