Inhibition of glycogenolysis by 2,5-anhydro-D-mannitol in isolated rat hepatocytes

Hope C. Stevens and William L. Dills, jr[+]*

Division of Nutritional Sciences and [+]Section of Biochemistry, Cell and Molecular Biology, Division of Biological Sciences, Cornell University, Ithaca, NY, 14853 and **Department of Chemistry, Southeastern Massachusetts University, N. Dartmouth, MA 02747, USA*

Received 21 November 1983

2,5-Anhydro-D-mannitol, an analog of D-fructofuranose, inhibited basal and glucagon-stimulated glycogenolysis and glucose production in hepatocytes isolated from fed rats. Glucose formation from galactose was unaffected by the inhibitor. 2,5-Anhydro-D-mannitol-1-phosphate inhibits phosphorylase *a* with a K_i value of 2.4 mM. This same phosphorylated metabolite accumulates to the extent of 9.2 μmol/g wet wt in treated hepatocytes suggesting that phosphorolysis is the locus of the inhibition of glucose production from glycogen. Our results suggest that 2,5-anhydro-D-mannitol can be used to produce a model of hereditary fructose intolerance and that it merits further study as a hypoglycemic agent.

2,5-Anhydro-D-mannitol *Glycogenolysis* *Glucose synthesis* *Glycogen phosphorylase* *Hereditary fructose intolerance* *Hepatocyte*

1. INTRODUCTION

As part of a program to evaluate the potential of fructose analogs as hypoglycemic agents, we are studying the effects of AM on glucose homeostasis in laboratory animals. The objective of our study was to determine the effects of AM on carbohydrate metabolism in the liver. AM has been shown to be a substrate for fructokinase (EC 2.7.1.4) and hexokinase (EC 2.7.1.1) [1,2]. The resulting AM-1-P can be phosphorylated by phosphofructokinase (EC 2.7.1.11) [3]. Neither the mono- nor the diphosphate can be cleaved by aldolase (EC 4.1.2.13) [4]. On the basis of these in vitro enzyme studies, we hypothesized that the administration of AM to isolated hepatocytes would result in the intracellular accumulation of mono- and diphosphate esters of AM that could have marked effects on hepatic glucose output. Data here show that AM inhibits glucose production from glycogen.

* To whom reprint requests should be sent

Abbreviations: AM, 2,5-anhydro-D-mannitol; AM-1-P, 2,5-anhydro-D-mannitol-1-phosphate; AMBP, 2,5-anhydro-D-mannitol-1,6-biphosphate

2. MATERIALS AND METHODS

Bovine serum albumin (Cohn Frac V), enzymes, sugars, substrates, and glucagon were purchased from Sigma (St.Louis, MO). AM was synthesized as in [5]. This synthesis was adapted for the synthesis of labelled AM [6]. Rat liver phosphorylase *a* (EC 2.4.1.1) was the generous gift of Dr Ted Chrisman of Vanderbilt University.

All experiments were done with 200–300 g male Sprague-Dawley rats obtained from Blue Spruce (Altamont, NY) and fed the stock diet (Charles River Rat, Mouse and Hamster Formula, Syracuse, NY) ad libitum.

Isolated hepatocytes were prepared from fed rats according to [7] as modified in [6,8].

Hepatocytes (40–60 mg) were incubated in 25-ml polycarbonate Erlenmeyer flasks with various substrates with Krebs-Henseleit buffer in a final volume of 2.5 ml. The flasks were gassed with 95%

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O_2:5% CO_2, sealed with parafilm and incubated at 37°C and 90 oscillations/min in a shaking water bath. At the end of the incubation period suspensions were deproteinized with 0.5 ml of 3 N perchloric acid, the precipitated protein was pelleted by centrifugation and the supernatant was assayed for glycogen [9,10], glucose [11], galactose [12], AM phosphates were determined by incubations with [^{3}H]AM followed by ion exchange chromatography [6,13].

Glycogen phosphorylase assays were performed as in [14]; inhibitor constants were determined as in [15].

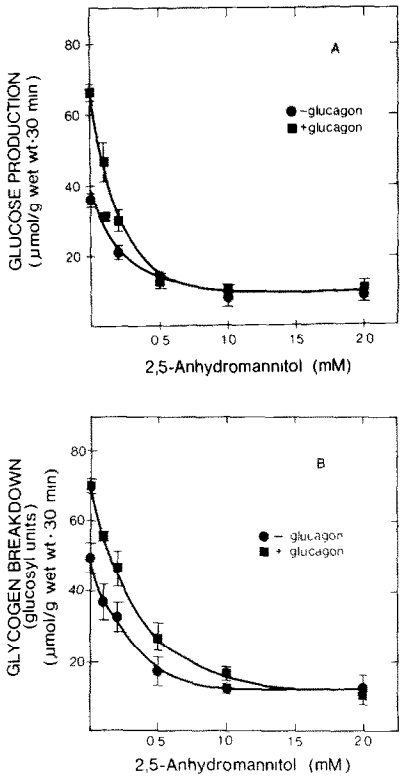

Fig. 1. Effects of AM and glucagon on glucose production (A) and glycogenolysis (B) in hepatocytes isolated from a fed rat. Hepatocyte suspensions were incubated for 10 min with the indicated concentrations of AM. Glucagon (0.1 ml of 2.5 × 10^{-7} M glucagon in 0.9% saline to give a final concentration of 10^{-8} M glucagon) or saline (0.1 ml of 0.9% saline) was added and incubation was continued for 20 min. Rates are expressed as μmol glucose formed/g wet wt hepatocytes (A) and μmol glycogen broken down/g wet wt hepatocytes (B) during the 30-min incubation period. Rates are means ± SD for triplicate incubations.

3. RESULTS

In hepatocytes prepared from fed rats, AM inhibited glucose production (fig.1A) and glycogen breakdown (fig.1B) in a dose-dependent manner. Substantial inhibition (70%) was observed at 1.0 mM AM. Stimulation of glucose production (fig.1A) and glycogen breakdown (fig.1B) by glucagon in control hepatocytes were essentially abolished by additions of 1.0 mM AM.

The ability of hepatocytes prepared from fed rats to metabolize galactose was not affected by AM. Glucose formation attributable to galactose was not inhibited by AM (fig.2A). In addition, galactose did not affect the glycogen content of

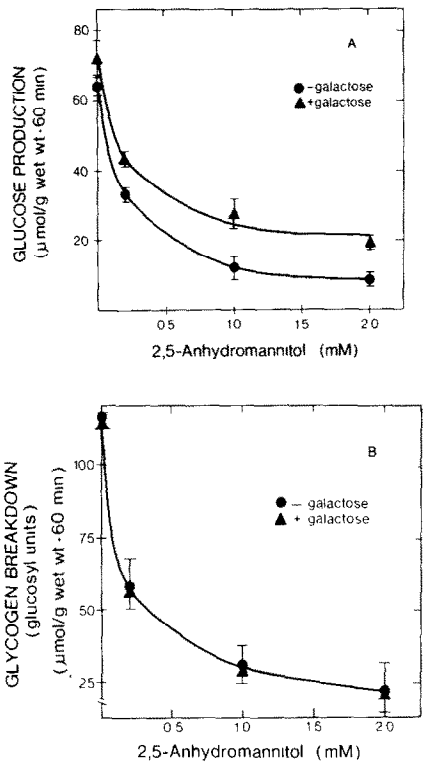

Fig. 2. Effects of AM and galactose on glucose production (A) and glycogenolysis (B) in hepatocytes isolated from a fed rat. Hepatocyte suspensions were incubated for 60 min with the indicated concentrations of AM and with or without 1.0 mM galactose. Rates are expressed as μmol glucose formed/g wet wt hepatocytes (A) and μmol glycogen broken down/g wet wt hepatocytes (B) during the 60-min incubation period. Rates are means ± SD for triplicate incubations.

hepatocytes incubated with or without AM (fig.2B).

Hepatocytes from fed aminals treated for 30 min with 2.0 mM tritiated AM accumulated phosphorylated metabolites of AM; AM-1-P accumulated to levels of 9.2 ± 2.2 μmol/g wet wt and AMBP to levels of 0.43 ± 0.23 μmol/g wet wt. These levels were not affected by 10^{-8} M glucagon.

In vitro enzyme studies with isolated rat liver phosphorylase *a* showed that AM-1-P and AMBP inhibited phosphorylase *a* activity. The K_m for inorganic phosphate was 3.0 mM. Inhibition by AM-1-P was competitive with phosphate and a K_i value of 2.4 mM was obtained. The inhibition by AMBP was slight.

4. DISCUSSION

Metabolism of AM in hepatocytes appears to be similar to the metabolism of fructose in the livers of patients with hereditary fructose intolerance [16–20]. The analog accumulates in the form of its mono- and biphosphate esters, glucose synthesis and glycogenolysis are inhibited, glucagon does not stimulate glucose production or glycogenolysis in AM-treated cells, and the conversion of galactose into glucose is not inhibited by AM treatment. Glucose formation from galactose indicates that phosphoglucomutase and glucose-6-phosphatase, two enzymes essential for glucose production from either galactose or glycogen, are not inhibited in hepatocytes incubated with AM. We therefore conclude that phosphorolsis is the locus of inhibition of glucose production from glycogen. This conclusion is further supported by the fact that AM-1-P accumulates to levels of 9.2 mM in treated hepatocytes, a value well in excess of its K_i value for liver phosphorylase *a*.

This data, taken together with reports of the inhibition of gluconeogenesis by AM [6,21–23] and hypoglycemia in AM-treated animals [24] support the suggestions that AM can be used to produce a model of hereditary fructose intolerance and that AM merits further study as a hypoglycemic agent.

ACKNOWLEDGEMENTS

We wisk to thank Geraldine McDonough for assistance in the preparation of isolated hepatocytes, Dr Ted Chrisman of Vanderbilt University for the gift of rat liver phosphorylase *a*, and Thomas Covey for the synthesis of AM. The technical assistance of Dale Loh, Barbara Montour and Leah Jenney is gratefully acknowledged. Supported, in part, by grants HD-10967, AM-20882 and CA-26755 from the National Institute of Health, US Public Health Service, Bethesda, MD 20014, by funds from the Lester G. Wood Foundation, and by grant 14-83 from the Southeastern Massachusetts University Foundation. H.C.S. is a predoctoral fellow supported in part by the Charles Quillman Fellowship Fund, Cornell University and in part by NIH Training grant T32-AM07158 to the Division of Nutritional Sciences, Cornell University.

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