Vol. 89, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 12, 1979 Pages 279-285

MANNOSE-6-P AND MANNOSE-1-P IN RAT BRAIN, KIDNEY AND LIVER

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Received June 4,1979

Summary

The approximate concentrations of mannose-6-phosphate and mannose-1-phosphate in female rat brain, kidney and liver are respectively 51, 29 and 99 nmole/g (Man-6-P), and 13, 12, 15 nmol/g (Man-1-P). Intraperitoneal injection of mannose (20 mmol/kg body weight, 15, 30 or 60 min before sacrifice) raises the liver Man-6-P to 0.4 to 4.3 µmol/g and Man-1-P to 100 to 186 nmol/g.

Mannose injection has been shown to relieve the symptoms of hypoglycemia resulting from hepatectomy (1) and to restore to normal the electrical activity of brains of rabbits made hypoglycemic by hepatectomy or insulin injection (2). These results were interpreted as evidence that mannose is converted to glucose by other tissues prior to utilization by the brain. However, in 1972, Sloviter and colleagues (3) reported that mannose can replace glucose as an energy substrate for perfused rat brain. These authors also determined the concentration of Man-6-P in perfused and normal rat brain (3) and described its isolation from this tissue (4). Man-1,6-P_2 has been found in erythrocytes of humans and various mammals (5).

Although it is presumed that Man-6-P, formed from Fru-6-P, is converted to GDP-Man via Man-1-P, a synthetic pathway established in certain microorganisms, evidence for this sequence in mammals is limited. Man-1-P has not been reported in animal

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Journal Article No. J-3656 of the Oklahoma Agricultural Experiment Station.

0006-291X/79/130279-07$01.00/0

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tissues, PMI[+] of animal origin has not been extensively studied and mammalian PMM has not been detected. Although PGM can catalyze the interconversion of Man-6-P and Man-1-P (6), its physiological role in this reaction remains in doubt. Herein we report the concentrations of Man-6-P and Man-1-P in several rat tissues, with and without mannose loading.

Methods

Materials - Glucose, fructose and perchloric acid were from J. T. Baker; ATP, NADP, hexose-phosphates, mannose and triethanolamine base from Sigma; PMI from Boehringer, the other enzymes from either Boehringer or Sigma; charcoal (Norit A) from Matheson. All solutions used in fluorometric assays were filtered through a 0.45 μ Millipore filter.

Preparation of Tissue Extracts - Female Holtzmann rats were used throughout; the sucklings' sex was not determined. Animals were in the laboratory with access to food and water and acclimated to handling for at least two days before being killed; usually at 10-12 A.M. Under mild ether anesthesia, skin above the forehead was removed and the abdominal cavity exposed. The rat was then immersed in liquid nitrogen and desired tissues chiseled from the frozen carcass. When brain was not required, tissues were excised from the anesthetized animal and pressed with a precooled pestle in a mortar filled with liquid nitrogen; a flattened tissue was obtained within 5 sec after excision.

Mannose was injected intraperitoneally under ether anesthesia, at a dose of 20 mmol/kg body weight given as a 25% solution. The animal was allowed to recover and after an appropriate interval was reanesthetized and killed.

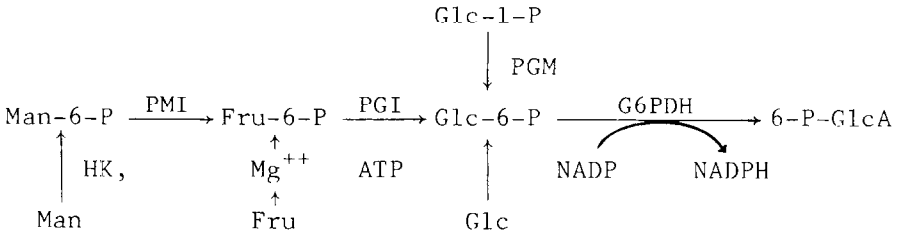
After removal of fat and excess blood (without thawing), tissues were put in a steel mortar surrounded by dry ice, pulverized, transferred to a glass homogenizing tube cooled in dry ice and weighed.

Protein-free tissue extracts were prepared according to Williamson and Corkey (7). When Man-1-P was to be determined, neutralization was done with 5M KOH; the use of buffer is a disadvantage here because of the requirement for alkaline and acid hydrolysis. Since some extracts of liver and kidney were yellowish-brown and highly fluorescent, they were decolorized with charcoal (8).

Assay of Metabolites - The increase in absorbance or fluoresence of the NADPH formed (7) was measured. The validity of

[+]Enzyme abbreviations: HK, hexokinase; PGI, phosphoglucose isomerase; PMI, phosphomannose isomerase; PMM, phosphomannomutase; PGM, phosphoglucomutase; G6PDH, glucose-6-phosphate dehydrogenase.

assay was checked with internal standards and recoveries determined by addition of metabolites to some tissue samples. The reactions were as follows:



All assays were done at 30° in 2 ml of 0.1 N triethanolamine buffer, pH 7.6, containing the following as appropriate: $MgCl_2$, 3 mM; ATP, 500 µM; NADP, 400 µM; PMI (yeast), 1.5 U; HK (yeast), 1.5 U; PGI (yeast), 1 U; PGM (rabbit muscle), 1 U; G6PDH (yeast), 1 U.

Some batches of PMI contain PGM activity, therefore PGM was added before PMI to remove any Glc-1-P present which might give an overestimate of Man-6-P. In this case EDTA, an activator of PGM, was added at a final concentration of 0.1 mM.

Glucose was measured by the addition of HK after measurement of Glc-6-P. Addition of PGI gave fructose and Fru-6-P and addition of PMI, mannose and Man-6-P. Free fructose and mannose were then obtained by subtracting the Fru-6-P and Man-6-P readings in the absence of HK, from those in its presence, respectively.

Determination of Man-1-P - Without PMM, mannose-1-P cannot be assayed enzymatically using the system described above. Therefore three other methods were tried; they gave similar results. Two of them involved enzymatic determination of mannose released from Man-1-P by enzymatic or acid hydrolysis, after removal of hexoses and their 6-phosphates by 1) enzymatic conversion to 6-P-gluconate or 2) reduction to the corresponding polyols with $NaBH_4$.

In the method of choice, advantage was taken of the differences in stability towards acid and alkali of hexose-1-phosphates and reducing sugars (free hexoses and their 6-phosphates. Hexose-1-phosphates are stable in alkali while acid hydrolysis releases an equivalent amount of phosphate and the respective free sugar. Reducing sugars on the other hand are relatively stable in dilute acid whereas treatment with alkali results in degradation products, among others, phosphate, saccharinic acids and lactate.

The reducing sugars were first destroyed by boiling in a water bath for 30 min in 0.05 N KOH. Acid was added, HCl or H_2SO_4, to 0.1 N, and hydrolysis was achieved by heating in boiling water for 20 min. The acid hydrolysate was decolorized with charcoal before neutralization and assay.

Neither free hexoses nor hexose-6-phosphates were detected after treatment with alkali. After acid hydrolysis, Glc-6-P

and Man-6-P were detected, presumably due to the presence of the respective 1,6-bisphosphates. Free glucose and mannose, detected after acid hydrolysis (above), originated from the respective 1-phosphates. In standard runs known Man-1-P was quantitatively recovered as mannose.

The sequence of events is:

$$\left[\begin{array}{l}\text{Hexose}\\ \text{Hexose-1-P}\\ \text{Hexose-6-P}\\ \text{Hexose-(1,6)-P}_2\\ \text{UDP-Glc}\end{array}\right] \xrightarrow[\Delta]{OH^-} \text{Hexose-1-P} \quad \text{Hexose-(1,6)-P}_2 \xrightarrow[\Delta]{H^+} \begin{array}{l}\text{Hexose}\\ \text{Hexose-6-P}\end{array}$$

Addition of amino acids to standard runs did not cause interference. GDP-Man, if present, might contribute to the mannose measured after acid hydrolysis. However, no mannose was detected when a solution of GDP-Man was put through the procedure. Similar treatment of UDP-Glc, which differs from GDP-Man at the hydroxyl group on C_2 of the hexose, yielded glucose in the acid hydrolysate.

That hexose-1-P is present in an alkaline hydrolysate of a liver extract was verified by separating the sugar phosphates from other intermediates by anion exchange chromatography. Enzymatic analysis showed hexose-6-P to be absent. Hydrolysis of the hexose-P peak gave a ratio of glucose + mannose:P_i of 0.8.

Results and Discussion

The results are presented in Table I. Considerable data concerning the concentration of Glc-6-P and Fru-6-P in several tissues are not presented. The values we obtain from normal animals are in good agreement with those reported by several other laboratories (9); those obtained from freeze blown brain‡ are slightly lower than, but comparable to, those of others (10). This fact indicates that our methodology of tissue freezing, extraction and assay is satisfactory. The size of animal used varied from 60 to 215 g. However, metabolite concentrations did not vary substantially with animal size; hence we have combined the data.

In normal female rats the average concentration of Man-6-P ranges from about 23 nmol/g in kidney to 100 nmol/g in liver.

‡The authors are indebted to Drs. Richard Veech and Todd King for supplying the freeze blown brains.

Table 1

Concentration (nmol/g) of Mannose, Man-6-P and Man-1-P in Rat Tissue

Treatment	Man-6-P	Man-1-P	Mannose
Brain			
suckling	41,52		
freeze blown	51 ± 3 (3)*		
normal	21 ± 4 (5)	11,14,15	0
+ mannose			
15 min	85-162 (4)	15,28	2300-3900 (3)
30 min	112,195		4500
60 min	58	14	1800
Kidney			
suckling	32		
normal	29 ± 4 (6)	12,12	0
+ mannose			
15 min	23,24,44	14,28	35100,35000
30 min	53,82,107		
60 min	32		15100
Liver			
suckling	48,54		
normal	99 ± 6 (9)	15 ± 3 (5)	0
+ mannose			
15 min	410-2620 (5)	100,134	11000-27500 (4)
30 min	790-4310 (4)		10600
60 min	1010,1560	186	6900,24700
Blood			
control			0
+ mannose			
15 min			5600,12000
60 min			7350

*In parentheses are the number of observations in a range of values or used in calculation of S.E.M.

The brain Man-6-P values need comment. When the animals were killed in this laboratory (see Methods) Man-6-P values for brain are approximately 21 nmol/g. However, with freeze blown brains‡ the value is approximately 51 nmol/g (Table I). In our hands the latter figure seems to be the more nearly correct value for Man-6-P in rat brain. We should note that Sloviter and colleagues (3) found considerably more Man-6-P in rat brains perfused with either glucose or mannose. The reason for

the difference between their values and ours is not immediately apparent. It may be noted that we found Fru-6-P concentrations in brain (19 nmol/g) close to the 12-15 nmol/g reported by Sloviter et al. (3). Our data indicate that in rat brain the PMI reaction (Man-6-P $\rightleftharpoons$ Fru-6-P) is near equilibrium (Keq $\cong$ 1; see 11) whereas the results of Sloviter et al. suggest that this reaction is in the direction of Man-6-P.

Although the data are more limited, we report for the first time the presence of Man-1-P in three rat tissues at concentrations of 10-15 nmol/g (Table I). Whereas in liver the PMM reaction (Man-1-P $\rightleftharpoons$ M-6-P) seems to be near equilibrium (Keq $\cong$ 10, see 6), in kidney and brain this reaction is somewhat removed from equilibrium in the direction of Man-1-P. However, because the Man-1-P data are limited, this apparent shift from equilibrium should not be overemphasized.

Some animals were injected with glucose or fructose in a manner similar to that described (Methods) for mannose loading. Treatment with glucose or fructose prior to sacrifice had little effect on the Man-6-P or Man-1-P concentrations of liver, kidney or brain (data not shown), though fructose administration did increase the Man-1-P content of liver. Loading with mannose caused a striking increase in the concentration of Man-1-P and Man-6-P in liver and to a lesser extent in brain (Table I). In one animal, 30 min after mannose administration, the Man-6-P concentration exceeded 4 μmol/g. In another, 60 min after mannose administration, liver Man-6-P concentration was about 1.5 μmol/g and mannose about 24.7 μmol/g. Although fructose loading resulted in fructose concentrations in liver, brain and blood of several mM, it did not cause order of magnitude changes in concentrations of Fru-6-P (data not

shown). We conclude that when exposed to large concentrations of mannose, rat liver, and to a lesser extent rat brain, rapidly forms Man-6-P which accumulates to very high concentrations because of a less rapid removal. Since the concentrations of Man-6-P we observe in liver following mannose administration are several fold the Ki for Man-6-P as an inhibitor of PGI (Ki = 0.46 mM; 12), it may be inhibiting this step in glycolysis, although Fru-6-P concentrations were not elevated (data not shown).

Of note is the large increase in Man-1-P in liver following mannose loading (Table I). At present this must be assumed to be due to the presence of an as yet undescribed PMM in rat liver which interconverts Man-6-P and Man-1-P. To date we have been unable to detect PMM in rat tissues.

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