Biochemical Pharmacology, Vol. 17, pp. 643–645. Pergamon Press. 1968. Printed in Great Britain

Uptake and oxidative utilization of glucose, fructose and galactose by rat mast cells

(*Received* 4 *May* 1967; *accepted* 11 *October* 1967)

ANAPHYLACTIC histamine release is inhibited by lack of oxygen,[1–5] but the inhibition is less effective or ineffective in the presence of glucose.[6] The histamine release in the presence of oxygen is blocked by dinitrophenol[7] suggesting that oxidative energy metabolism is linked with the process. The restoration of histamine release by glucose in an anoxic milieu led to the view that high-energy compounds formed by the glycolytic pathway may be utilized in the anaerobic state.[8] The inhibitors of glycolysis, iodoacetate[9] and 2-deoxyglucose,[10] were indeed found to counteract the restoring effect of glucose. In contrast to the glucose effect, however, histamine release inhibited by anoxia could not be restored by fructose and galactose.[8] In mammalian tissue, fructose[11, 12] and galactose[13, 14] utilization are generally thought to follow the Embden–Meyerhof pathway. The difference between the effect of glucose on the one hand and of fructose and galactose on the other hand on histamine release raises the question of whether fructose and galactose are metabolized differently by the mast cells. A comparative study of the metabolism of these monosaccharides could thus be a tool to test the hypothesis that glycolytic energy production is the basis of glucose effect on histamine release under anaerobic conditions. The experiments reported here were undertaken to explore the uptake and and oxidative utilization of glucose, fructose, and galactose, and to study whether the rate of glucose oxidation is influenced during histamine release induced by compound 48/80.

EXPERIMENTAL AND RESULTS

Mast cells were incubated with ^{14}C-labeled glucose, fructose, and galactose with sufficient carrier to give a final concentration of 5 mM, and the oxidative metabolism of the sugars was followed by measuring the radioactivity of the $^{14}CO_2$ produced. Radioactive sugars were obtained from New England Nuclear Corp.; glucose and fructose were uniformly labeled and galactose was labeled at C_1.

Peritoneal mast cells were isolated from Wistar rats (male or female 300–500 g) in concentrated human albumin solution as described previously.[15, 16] The mast cells (98–100 per cent pure) were then suspended in Krebs-Ringer solution containing phosphate buffer and 1 mg/ml human serum albumin.[15] Mast cells, 10^5–5×10^5, in a final volume of 1 ml were incubated at 37° in a 10-ml glass tube under gentle shaking, and the carbon dioxide produced was trapped in hyamine hydroxide by a modification of the method of Cuppy and Crevasse.[17] The incubation time was 20 or 30 min. The shorter incubation time was used to study the effect of compound 48/80 (Wellcome Laboratories), which was added after 4 min. At the end of incubation the reaction was terminated and CO_2 in the solution was released by adding 0·5 ml of 6 N H_2SO_4. $NaHCO_3$ (1 μM) was also added as carrier CO_2. After equilibration for 2 hr at room temperature under shaking, the tube containing hyamine hydroxide solution was transferred to 10 ml liquid scintillator[17] and its radioactivity was measured in Beckman or Packard Tri-Carb liquid scintillation spectrometer. Appropriate standards and blanks were taken and quenching was corrected by external or internal standardization. With $NaH^{14}CO_3$, it was found that after 2 hr of equilibration trapping of $^{14}CO_2$ was essentially complete.

The number of cells incubated was determined by counting a sample in the hemocytomer. The cells were kept cold (0–4°) throughout after their withdrawal from the peritoneal cavity, until incubation at 37°. The glass surfaces which came in contact with the cells were coated with desicote (Beckman). When the effect of compound 48/80 on glucose oxidation was studied, the amount of histamine released by the compound was determined in separate samples of the same cell suspension as described earlier.[18] Histamine was assayed by the fluorometric method.[19]

Table 1 shows the oxidative utilization of glucose added to the medium: 0·24–0·49 $\times$ 10^{-8} μmole glucose was converted to CO_2/cell/hr. The variation between the experiments is mainly due to the error involved in determining the number of mast cells incubated. To judge the effect of compound 48/80, also shown in Table 1, equal volumes of the same cell suspension were used for the untreated and compound 48/80-treated samples; the error due to a difference in the size of the sample was thus practically eliminated. To ensure that the variation between duplicate untreated samples was low, the counts for $^{14}CO_2$ were determined for duplicate samples in three experiments; the counts

of the second sample varied from the first by −2, +7 and +10 per cent. As seen in Table 1, the amount of glucose converted to CO_2 by the mast cells was not materially altered by incubation with compound 48/80. The difference from untreated cells was −2 to +13 per cent. Thirteen to 49% histamine was released by the same concentrations of compound 48/80 (0·3–1·0 μg/ml).

TABLE 1. THE EFFECT OF COMPOUND 48/80 ON OXIDATIVE UTILIZATION OF EXOGENOUS GLUCOSE BY MAST CELLS*

Expt. No.	Glucose (μmoles × 10^{-8}) converted to CO_2/cell/hr		Difference (%)	Concn of compd 48/80† (μg/ml)	Histamine release (% of total content)	
	Untreated	With compd 48/80			Spontaneous	With compd 48/80
1	0·41	0·39	−5	0·3	Not detectable	13
2	0·37	0·35	−5	0·8	Not detectable	20
3	0·31	0·35	+13	0·5	Not detectable	16
4	0·24	0·24	0	0·5	3	13
5	0·49	0·52	+6	1·0	5	49
6	0·38					
Mean	0·37		+2			22

* Glucose: uniformly labeled; concentration in medium 5 mM; sp. act., 0·4 to 1 μc/μmole.

† The concentration of compound 48/80 shown for each experiment was used for its effect both on the metabolic activity and on histamine release.

The oxidative utilization of fructose and galactose is compared with that of glucose in Table 2. Expressed as the amount of sugar converted to CO_2 ,the fructose values were 0·19 × 10^{-8}, 0·17 × 10^{-8}, and 0·13 × 10^{-8} μmole/cell/hr. The mean value, 0·16 × 10^{-8} μmole 43 per cent of the mean rate of glucose oxidation to CO_2 (Table 1). When fructose and glucose were used in different samples of the same cell suspension, CO_2 production from fructose was about one-half that from glucose. Glucose in the medium is thus oxidized about twice as fast as fructose. The rate of production of CO_2 from galactose supplied to the medium was quite high (Table 2). The galactose values, however, cannot be directly compared with the results obtained with the other two sugars because of the difference in the site of labeling galactose.

TABLE 2. COMPARISON OF THE OXIDATIVE UTILIZATION BY MAST CELLS OF GLUCOSE, FRUCTOSE, AND GALACTOSE ADDED TO THE MEDIUM IN 5 mM CONCENTRATION*

	$^{14}CO_2$ production (cpm)†		
	Glucose	Fructose	Galactose
	777	443	1150
	1053	373	836
	505	329	1194
	644		
Mean	745	382	1060

* Glucose and fructose were uniformly labeled and galactose was labeled at C_1. The sp. act. of the sugars was 1 μc/μmole for all except the third experiment with fructose in which the sp. act. was 0·91 μc/μmole.

† cpm = Counts per min after background deduction and quenching correction for $^{14}CO_2$ produced by 10^5 cells in 1 hr.

DISCUSSION

As judged by the production of $^{14}CO_2$, all three sugars (glucose, fructose, and galactose) were taken up from the medium by the mast cells and metabolized by the oxidative pathway. Compound 48/80, which released 13–49 per cent histamine from the mast cells, had no appreciable effect on the

conversion of oxegenous glucose to CO_2. It is of interest to compare this finding with mast cell respiration during histamine release. The oxygen uptake of mast cells has been reported to be unaffected during compound 48/80-induced histamine realease,[20, 21] but shows a short-lived stimulation when histamine is released by antigen–antibody reaction.[18]

The utilization of fructose and galactose is of particular interest for the histamine release problem, since these sugars do not share the glucose effect on histamine release in an anaerobic medium.[8] The oxidative utilization of fructose and galactose indicates the presence of the respective kinases in the mast cells so that the sugars are phosphorylated and transported into the cells. The experiments do not give a clue, however, to the pathways of fructose and galactose oxidation. The possibility remains that the hexose monophosphate shunt may oxidize the sugars, bypassing the glycolytic system, since fructose and galactose may be utilized by the hexose monophosphate pathway by other mammalian cells.[22, 23] The present finding that fructose and galactose are taken up and oxidized to CO_2 by the mast cells thus calls for further study to determine whether the difference in their effect on histamine release under anaerobic conditions (as compared to that of glucose) stems from a difference in their metabolic fate in the mast cells.

Peritoneal mast cells isolated from Wistar rats were incubated with labeled glucose, fructose, and galactose to determine their uptake and oxidative utilization. With a 5 mM total glucose concentration, the amount converted to CO_2 was $0{\cdot}37 \times 10^{-8}$ μmole/cell/hr. This rate was not significantly influenced by incubation of the cells with compound 48/80, which caused 13–49 per cent histamine release. Fructose and galactose were taken up by the mast cells and metabolized to CO_2.

Acknowledgements—I wish to express my appreciation and thanks sto Dr. Andres Goth for providing all the facilities to carry out this work, and for his kind hospitality and for valuable discussions. My thanks are also due to Miss Mary Knoohuizen for her assistance with the histamine assay.

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