GLYCOLYSIS IN RAT PERITONEAL MAST CELLS

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ABSTRACT

Glycolytic activity of rat peritoneal mast cells has been measured by the Cartesian ampulla diver technique. The rates of anaerobic glycolysis, expressed as CO_2 expelled from a bicarbonate medium, are 1.70×10^{-6} μl and 1.43×10^{-6} μl per cell per hour with and without glucose, respectively. The aerobic glycolysis rate in the presence of glucose, assuming the respiratory quotient to be 1, is 0.93×10^{-6} μl CO_2 per cell per hour. It is pointed out that the anaerobic and non-respiratory aerobic carbon dioxide production by mast cells is much higher than the respiratory oxygen uptake reported previously. These values have been interpreted in terms of glucose utilization.

INTRODUCTION

We have reported previously the respiration rate of rat peritoneal mast cells (1). The present paper deals with the glycolytic activity of the same cells. Anaerobic glycolysis has been measured by the rate of evolution of carbon dioxide from a bicarbonate buffer using the ampulla diver technique (5). The same principle has been used to study aerobic glycolysis, assuming the respiratory quotient to be 1; the rate of gas evolution then reflects the non-respiratory carbon dioxide production or aerobic glycolysis.

MATERIAL AND METHODS

Mast Cells

Mast cells were obtained from male Sprague-Dawley rats weighing 450 to 570 gm, as described previously (1), with slight modification: 30 and 40 per cent albumin solutions were prepared from sterile human albumin powder (without preservative) obtained from KABI, Stockholm, Sweden. The cells were kept throughout at 0–4°C till shortly before filling the diver. The speed of centrifugation used for the isolation of cells was 700 RPM (110 *g*) in the International Refrigerated Centrifuge for 5 minutes. The subsequent steps in the isolation of the cells were the same as described previously (1), but the washing procedure was slightly different. The mast cells obtained in the concentrated albumin solution were first washed with solution A (see Table I) in air and next with solution B with or without substrate under the same gas mixture used for that experiment. The composition of the solutions (*cf.* reference 3) is shown in Table I. The cells were collected in a small siliconed test tube, which was then closed with a rubber stopper provided with a gas inlet and a wider outlet needle so arranged that the tip of the inlet needle reached just above the surface of the medium. The cell suspension was thus exposed to the gas mixture with gentle shaking at 37°C for 10 minutes, and kept stoppered in the same bath until introduced into the diver. The filling operation closely followed the equilibration of the cell suspension with the gas mixture. When an inhibitor was used it was either introduced into the solution for the second washing or added to the cell suspension after this washing. When sodium fluoride was used as an inhibitor, calcium was omitted from solution B, in which it was dissolved.

TABLE I

Composition of Solutions A, B, and C

Component	Solution A	Solution B	Solution C
NaCl, 0.154 M	116 ml	100 ml	109 ml
KCl, 0.154 M	4 "	4 "	
$CaCl_2$, 0.11 M	3 "	3 "	
$MgSO_4$, 0.154 M	1 "	1 "	
KH_2PO_4, 0.154 M		1 "	
Phosphate buffer ($Na_2HPO_4 + KH_2PO_4$, pH 7.4), 0.067 M	6 "		
$NaHCO_3$, 0.154 M		21 ml	21 ml
Human albumin		130 mg	
Final pH	7.25	7.35*	7.35*

* Read after 5 per cent CO_2 has been bubbled through the medium.

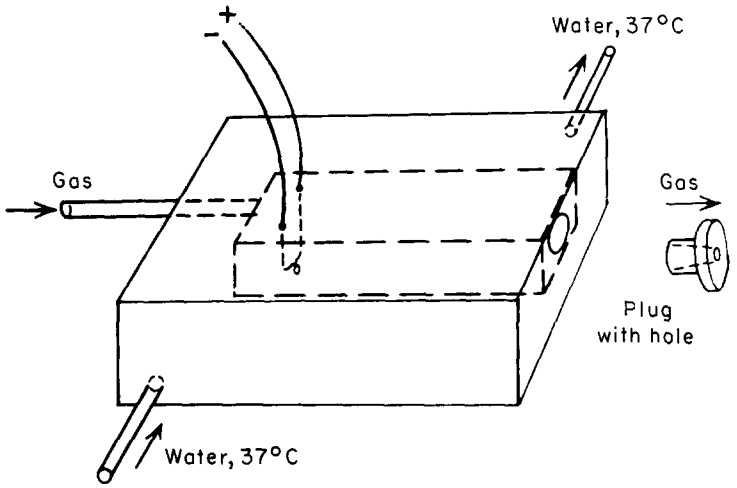

FIGURE 1 Chamber for filling diver at 37°C for glycolysis experiments. The chamber is made of double walls of Plexiglas on four sides, the outer and inner chambers measuring 110 × 80 × 42 mm and 85 × 30 × 30 mm, respectively. The walls on the upper and right surface are, however, common to the two chambers, thus providing for a clear view of the floor of the inner chamber where cells and solutions are placed, and allowing manipulations through the hole in the plug which closes the opening of the inner chamber during operation. The loop is electrically heated and contains a droplet of beeswax for sealing divers. The solutions and the droplet of cell suspension are placed in small cups and on a siliconed glass square, respectively, immediately in front of the loop close to the side walls.

Experiments in the absence of inhibitors have been carried out both in complete and in calcium-free solution; the results were the same.

The solutions were filtered through Seitz or sintered glass filters, and general aseptic precautions were taken to keep the number of bacteria in the diver well below a level where it might interfere with the result. The gas mixture was bubbled through solutions B and C (see Table I) for 30 minutes at 37°C and kept in well stoppered test tubes in the same bath.

Microgasometry

The amount of carbon dioxide evolved from solution B containing 25 mM bicarbonate was used to measure the glycolytic activity of the cells. The CO_2

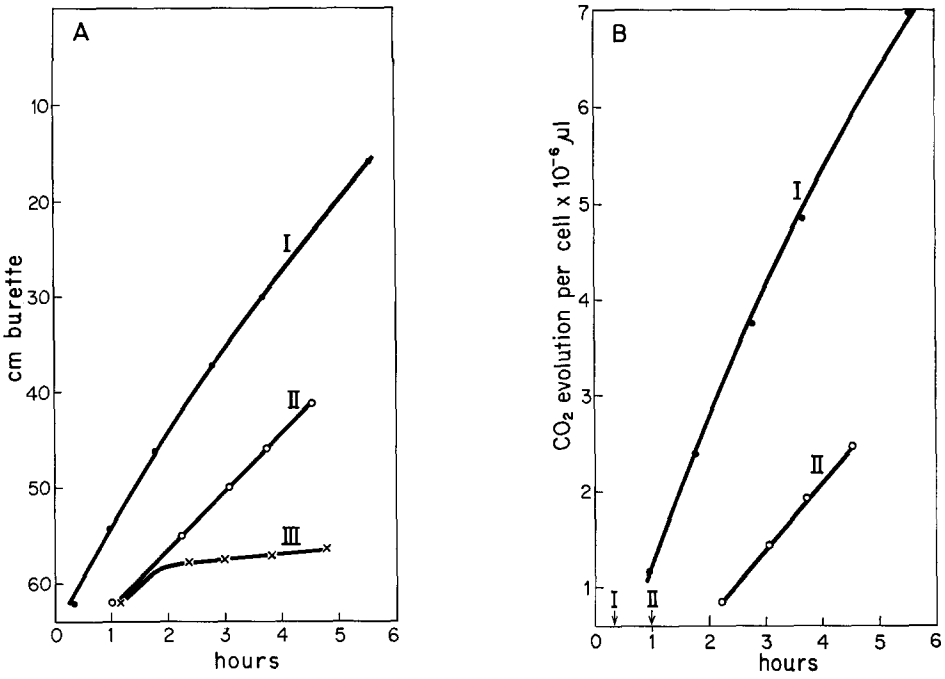

FIGURE 2 I, a typical mast cell glycolysis experiment; II, partial inhibition by iodoacetate; III, a control slope in the absence of cells.

I. 388 cells; V_D (gas volume of the diver), 0.32 µl; V_F (liquid charge in the diver), 0.24 µl; medium, solution B + 5 mM glucose; 1 cm burette $\sim 1.65 \times 10^{-4}$ atm.

II. 415 cells; V_D, 0.30 µl; V_F, 0.13 µl; medium, solution B + 5 mM glucose + 2 mM iodoacetate; 1 cm burette $\sim 1.54 \times 10^{-4}$ atm.

III. No cells; V_D, 0.23 µl; V_F, 0.10 µl; medium, solution B + 5 mM glucose; 1 cm burette $\sim 1.13 \times 10^{-4}$ atm.

A, actual experiment. B, calculated CO_2 evolution per cell for I and II. Arrows indicate times of closing the burettes.

system described by Zajicek and Zeuthen (4, 5) for measuring cholinesterase activity was used with modifications to permit measurements on a few hundred cells. Pyrex test tubes of inside/outside diameter ratio 0.85 were used to pull the capillaries (diameter, 0.3 to 0.4 mm) from which the divers were made. The gas volume (V_D) of the divers ranged from 0.2 to 0.8 µl. The liquid charge (V_F) was 0.08 to 0.5 µl. The tail, ranging in length from 10 to 25 mm, was sufficiently narrow to prevent leakage, and gave a "brake" value (see reference 1) of 3 to 10 mm/min. The procedure for filling mast cells in the divers was essentially the same as already described (1), but all the operations had to be done in an atmosphere of 5 per cent CO_2 + 95 per cent nitrogen[1] or 5 per cent CO_2 + 95 per cent air. A heating lamp was focused on the microscope stage so that the field of operation was heated to about 36–37°C. A double-walled chamber maintained at 37°C, as shown in Fig. 1, was placed on the stage of a dissecting microscope under low magnification. Small cups and silicone-coated glass squares meant for the solutions and the cell suspension, respectively, were then placed inside the chamber. The plug was inserted tightly into the wall of the chamber, thus permitting gassing and pipetting through the narrow opening in the plug itself. Five per cent CO_2 in air or in nitrogen, preheated and saturated with water vapor at 37°C, was passed through the chamber at the rate of about 100 ml/min. for 10 minutes. The rate of flow was then reduced to 40 to 50 ml/min., and this rate was maintained throughout the filling procedure. The solutions were introduced into the small cups with Pasteur pipettes, and a droplet of the cell suspension was placed on the glass square with a braking pipette. The diver was then charged essentially as illustrated previously (see Fig. 3 in reference

[1] Commercially available gas mixtures were used. Oxygen contamination of nitrogen was approximately 0.05 per cent.

TABLE II

Anaerobic CO_2 Production by Mast Cells in Bicarbonate Medium

Gas phase: 5 per cent CO_2 + 95 per cent N_2

Group	*a* Wt. of rat	*b* Av. diam. of cells	*c* No. cells in diver	*d* Substrate	*e* Total CO_2 evolution per hr. $\times 10^{-5}$ µl	*f* CO_2 evolution per cell per hr. $\times 10^{-6}$ µl	*g* Mean value ± SE $\times 10^{-6}$ µl
	gm	µ					
A	470	12.7	419	0	65.6	1.50	1.43 ± 0.17 (6.38 × 10^{-8} µmole)
	470	12.7	554	0	64.3	1.11	
	565	13.3	152	0	28.6	1.69	
B	530	13.4	388	Glucose, 5 mM	51.6	1.26	1.70 ± 0.15 (7.60 × 10^{-8} µmole)
	448	13.5	235	"	31.7	1.23	
	504	—	211	"	45.0	2.00	
	504	—	443	"	83.1	1.81	
	518	—	632	"	132.8	2.06	
	518	—	652	"	124.5	1.86	

Ten control divers all run simultaneously with the experimental divers were filled as follows: solution B (4 control divers), solution B with 5 mM glucose (3), solution B with 5 mM glucose + 2 mM iodoacetate (1), solution B + 20 mM NaF (2). As in experimental divers, the tail end and the flotation vessel contained solution C in all cases. There was no indication that the different solutions caused any difference in the control values. The CO_2 evolution in the control divers ranged from 0 to 5 × 10^{-5} µl per hour. The mean value 2.9 × 10^{-5} µl has been deducted from the CO_2 evolution shown in column *e*. The corrected CO_2 evolution thus obtained divided by the number of cells gives the values shown in column *f*.

1). However, steps I to VIII were performed in a flow of 5 per cent CO_2 in air, or 5 per cent CO_2 in N_2, operating through the narrow opening in the plug shown in Fig. 1. Other differences were: (*a*) in step I, the ampulla was flushed with a considerable volume of gas; (*b*) in step II, solution C (Table I) was introduced; (*c*) in steps IV to VI, solution B (Table I) was used; (*d*) steps X and XI were combined into one. In this operation the diver was equilibrated by the stepwise removal of gas (as separate bubbles) from the diver's interior. For this purpose suction was applied to the whole system. It was essential to re-saturate the flotation medium frequently with 5 per cent CO_2. Solution C was used in all steps after IX, and thus also served as a flotation medium in the manometric measurements. The procedure for gassing of the manometers was the same as described previously (4, 5).

Usually two divers were filled with cells and a third (the control) with only the solutions. It takes about 3 to 5 minutes to charge a diver. In practice the time that elapsed between the placing of the cells and the solution inside the chamber and the filling of the last diver varied from 12 to 25 minutes. The interval between the killing of the animal and the introduction of the isolated mast cells into the diver was 4 to 8 hours. After the closing of the flotation vessel an initial period of ½ to 1 hour was allowed, and readings were then taken every 20 to 40 minutes for 2 to 4 hours.

The question of CO_2 retention by the solutions used in the diver was tested using Warburg's apparatus. A standard solution of citric acid (0.05 ml, 40 mM) was tipped in from the side arm of the flask to solution B or C (2.95 ml) in the main chamber under 5 per cent CO_2 + 95 per cent N_2. Manometer readings showed 95 to 97 per cent recovery of the expected amount of CO_2 evolution. The CO_2 retention thus being negligible, no correction factor for retention was introduced in the calculation of CO_2 production in the diver experiments.

RESULTS

Fig. 2 shows a typical experiment, the actual burette readings being given in Fig. 2 A. The medium used was solution B plus glucose. Curve I represents the CO_2 evolution with time by 388 cells, curve II shows the CO_2 evolution by 415 cells in the presence of 2 mM iodoacetate, and curve III gives the control value for a diver filled with the medium without cells or inhibitor. The total amount of CO_2 evolved per hour for about 400 cells—as calculated from the V_D and calibration values for the burettes (see legend for Fig. 2)—was 48.7 and 29.7 × 10^{-5} µl, respectively, for I and II; in comparison, the control value represented by III was only 1.6 × 10^{-5} µl per hour. The amount of carbon dioxide evolved

TABLE III

Aerobic Balance of Gaseous Exchange. CO_2 Produced Minus O_2 Taken Up (= Nonrespiratory CO_2 Evolution) by Mast Cells in Bicarbonate Medium Containing 5 mM Glucose

Gas phase: 5 per cent CO_2 + 95 per cent air

a Wt. of rat	*b* No. cells in diver	*c* Total measured gas evolution (non-respiratory CO_2) $\times 10^{-5}$ µl	*d* Measured gas evolution (non-respiratory CO_2) per cell per hr. $\times 10^{-6}$ µl	*e* Mean value ± SE $\times 10^{-6}$ µl
gm				
509	615	48.1	0.81	0.93 ± 0.08 (4.15 × 10^{-8} µmole)
509	360	26.6	0.80	
518	284	27.6	1.04	
572	321	36.3	1.19	
572	490	36.6	0.79	

Three control divers, all run simultaneously with the experiments and filled with solution B + 5 mM glucose (solution C in tail end and flotation vessel) showed the following values: 2.53 (gas uptake), 3.86 (gas uptake), and 0.33 (gas evolution) × 10^{-5} µl. The mean value 2.0 × 10^{-5} µl (gas uptake) has been added to the total CO_2 evolution shown in column *c* to obtain the corrected value, which divided by the number of cells gives the figures of column *d*.

TABLE IV

Inhibition of Anaerobic CO_2 Production by Mast Cells in Bicarbonate Medium

Gas phase: 5 per cent CO_2 + 95 per cent N_2

Inhibitor	Substrate	No. experiments	CO_2 evolution per cell per hr. $\times 10^{-6}$ µl. Mean values ± SE
0	0	3	1.43 ± 0.17
Sodium fluoride, 15–20 mM	0	3	0.61 ± 0.08
0	Glucose, 5 mM	6	1.70 ± 0.15
Iodoacetate (sodium salt), 2 mM	"	3	0.56 ± 0.04

per cell is shown in Fig. 2 B without deduction of the small control value.

Table II gives the anaerobic CO_2 evolution in all the experiments. It may be seen that there is no significant difference between the two groups, namely, A, without substrate, and B, with glucose. Aerobic, but non-respiratory, CO_2 production (in solution B with glucose), shown in Table III, was calculated on the assumption that the R.Q. of the cells incubated in the medium is 1. The gas evolution calculated from the slopes is, like those demonstrated in Fig. 2, a measure of the non-respiratory CO_2 production caused by the cells in the presence of oxygen. The aerobic glycolytic activity with glucose is thus 55 per cent of the anaerobic value (see Table II, group B, and Table III).

The experiments with inhibitors are shown in Table IV. Sodium fluoride (15 to 20 mM) and iodoacetate (2 mM) caused 50 to 70 per cent inhibition of anaerobic glycolysis. The control experiments for both aerobic and anaerobic glycolysis with or without inhibitor are described in the notes to Tables II and III.

DISCUSSION

The carbon dioxide evolution caused by mast cells incubated anaerobically in bicarbonate solution seems to be largely due to glycolysis, since 50 to 70 per cent of the gas evolution could be blocked by sodium fluoride (15 to 20 mM) or iodoacetate (2 mM). However, it seems possible that there may be some other source of CO_2 pro-

duction apart from glycolytic acid formation. This question is being further studied.

It may be pointed out that the CO_2 production by mast cells in both anaerobic and aerobic media is much higher than respiratory oxygen uptake. In this respect the metabolic property of mast cells resembles that of leukocytes. The aerobic glycolytic activity of human polymorphonuclear leukocytes has been estimated by Martin *et al.* (2) to be 1.66 and 0.58 $\times$ 10^{-6} μl per cell per hour with and without glucose, respectively, the corresponding respiration values being 0.21 $\times$ 10^{-6} μl with glucose and 0.34 $\times$ 10^{-6} μl without glucose.

The oxygen uptake of mast cells (in the presence of glucose) expressed in micromoles O_2 consumed per cell per hour equals 2.1 $\times$ 10^{-8} (1). This amounts to only one-half the non-respiratory aerobic CO_2 production (4.15 $\times$ 10^{-8} μmole per cell per hour) and to one-third to one-fourth the value (7.6 $\times$ 10^{-8} μmole per cell per hour) for anaerobic CO_2 production (see Table III and Table II, B, respectively). In terms of glucose utilization, the oxygen uptake (1) would represent 0.35 $\times$ 10^{-8} μmole of glucose oxidized per cell per hour. The non-respiratory aerobic CO_2 production would correspond to 2.08 $\times$ 10^{-8} μmole glucose converted to lactic acid. The total aerobic glucose consumption would thus be 2.43 $\times$ 10^{-8} μmole per cell per hour. The CO_2 liberated under anaerobic conditions would correspond to the consumption of 3.8 $\times$ 10^{-8} μmole glucose per cell per hour, all transformed into lactic acid. All calculations refer to measurements in the presence of 5 mM glucose. The lower rate of substrate utilization in the presence than in the absence of oxygen suggests a mild Pasteur effect.

The studies on the respiration (1) and glycolysis of mast cells were inspired by a desire to understand the metabolic functions of mast cells, because in this way a direct evaluation of metabolic function in relation to histamine release would be possible. It may not be revealing to attempt an evaluation of the existing data on histamine release from minced or sliced tissues with relation to the present findings. The relation of the metabolic aspect to the histamine release phenomenon has to be studied in the same or a similar preparation of isolated cells.

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