GLUCOSE METABOLISM IN MIXED GLIOBLASTOMA AND NEUROBLASTOMA CULTURES[1]

R. W. Newburgh[2] and Roger N. Rosenberg, Dept. of Neurosciences, Univ. of Calif., San Diego, La Jolla, CA 92037

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Summary: When glioblastoma and neuroblastoma cells are mixed, an inhibition of $^{14}CO_2$ evolution from [1-^{14}C]-glucose occurs. This does not occur when Hela and Glioblastoma cells or Hela and neuroblastoma cells are mixed. Mixing the cells has no effect on the incorporation of [1-^{14}C] glucose.

The addition of norepinephrine to cultured glioblastoma cells results in an inhibition of [^{14}C]-glucose uptake.(1) In addition, if the glioblastoma cells are pre-labeled with [^{14}C]-glucose, norepinephrine causes an increase in the release of radioactivity. These effects were not observed with cultured neuroblastoma cells.

Of interest is the possibility that a release of a neurotransmitter, from a neuron could alter glucose metabolism in glial cells. Such a possibility is suggested by the studies reported here.

Methods

The procedure for obtaining and culturing the glioblastoma and neuroblastoma cells were the same as previously reported.(1)

Approximately 3 x 10^6 glioblastoma (clone C-6) or neuroblastoma (clone 46) cells were added to 25 ml. Erlenmeyer flasks containing a center well. The total volume of medium (Dulbeccos Modified Eagles Medium(2) plus 10% fetal calf serum) plus cells was 5 ml. In addition, 5 x 10^6 glioblastoma or

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2 On leave from: Department of Biochemistry and Biophysics, Oregon State University, Corvallis OR 97331

neuroblastoma cells were added to petri dishes containing glass beads and the total volume of medium plus cells was 10 ml. After incubation at 37°C in 95% air and 5% CO_2 for 48 hours, the cells were washed with Eagles Balanced Salt Solution (EBSS)[3] or 0.9% NaCl. The glass beads on which cells were growing, as observed by microscopic examination, were then added to erlenmeyer flasks containing growing cells. A control consisted of adding glass beads alone and this had a minor effect on the evolution of $^{14}CO_2$ from [1-^{14}C]-glucose.

Prior to adding the radioactive medium ([1-^{14}C]-glucose (1.08×10^6 DPM) in 2 ml of EBSS or 0.9% NaCl), 0.2 ml of 2N KOH and a piece of flutted filter paper were added to the center well. After addition of the radioactive medium the flasks were sealed with a rubber serum stopper and incubated for 30 minutes at 37°C. At this time 1 ml of 2N HCl was added to the flask by injection through the stopper and the flasks incubated an additional 60 min. The filter paper and the KOH were removed and placed in a scintillation vial. After addition of a scintillation fluid (1) the vials were counted 24 hours later.

Sufficient KOH was then added to the erlenmeyer flasks to make the solution alkaline. The purpose of this was to remove the cells adhering to the flask and the glass beads. The solution was then removed, homogenized, and an aliquot removed for protein determination (4).

For the experiments on the incorporation of radioactivity from [1-^{14}C]-glucose the procedure was the same as reported previously (1).

Table I. $^{14}CO_2$ evolution from [1-^{14}C]- glucose in the Presence of Glioblastoma or Neuroblastoma Cells. The experimental procedure is in text. Experiments 1, 3 were done in EBSS. Experiments 2, 4, 5, 6, 7 & 8 were done in 0.9% NaCl.

Substrate - Glucose-1-^{14}C

Cell used in inoculation		Protein (mg)	DPM in $^{14}CO_2$	R	Specific Activity (DPM in $^{14}CO_2$ mg Protein)
Experiment 1					
Glioblastoma in Flask	Neuroblastoma on Beads				
+	-	1.5	14,200		9,500
-	+	1.1	1,095		1,140
+	+	2.4	7,505	0.54	3,200
Experiment 2					
Glioblastoma in Flask	Neuroblastoma on Beads				
+	-	1.29	27,000		20,900
-	+	0.08	206		2,600
+	+	1.32	5,400	0.2	4,100
Experiment 3					
Neuroblastoma in Flask	Glioblastoma on Beads				
+	-	0.56	2,700		4,850
-	+	1.7	14,650		8,700
+	+	1.9	8,270	0.56	4,300
Experiment 4					
Neuroblastoma in Flask	Glioblastoma on Beads				
-	+	1.4	53,000		37,000
+	-	0.18	5,700		31,500
+	+	1.5	17,000	0.29	11,400

Cells used in inoculation	Protein (mg)	DPM in $^{14}CO_2$	R	Specific (Activity DPM in $^{14}CO_2$ mg Protein)

Table I. (continued)

Experiment 5

Glioblastoma in Flask	Glioblastoma on Beads				
+	-	1.5	11,900		9,150
-	+	0.54	1,960		3,650
+	+	1.8	8,100	0.65	4,450

Experiment 6

Neuroblastoma in Flask	Neuroblastoma on Beads				
-	+	0.92	11,600		12,800
+	-	0.45	880		1,950
+	+	1.3	7,100	0.78	5,500

Experiment 7

Hela in Flask	Neuroblastoma on Beads				
+	-	1.5	11,800		7,800
-	+	0.42	1,530		3,650
+	+	1.9	13,200	1.04	7,000

Experiment 8

Hela in Flask	Glioblastoma on Beads				
+	-	1.9	13,200		6,700
-	+	0.34	1,900		5,600
+	+	2.2	13,100	0.9	6,000

Results and Discussion

[1-^{14}C]-glucose was added to flasks containing either glioblastoma, neuroblastoma, Hela cells or a mixture of two different cell types. The results of these experiments are shown in Table I. A calculated specific activity is obtained by dividing the total DPM's in $^{14}CO_2$ from the two different cell types when grown alone by the total protein. $\left(\frac{\text{DPM in } C^{14}O_2 \text{ glia} + \text{DPM in } ^{14}CO_2 \text{ neuroblastoma}}{\text{protein}_{\text{glia}} + \text{protein}_{\text{neuroblastoma}}}\right)$

This value which is the expected specific activity if there

Table II. Incorporation of Glucose- 1-[^{14}C] into Neuroblastoma, Glioblastoma and Hela Cells. Procedures are given in the text.

			Glucose-1-^{14}C Incorporated	
Experiment 1 (Cells indicated inoculated into a petri dish)				
Glioblastoma	Neuroblastoma	Hela	Experimental	Calculated
+	-	-	1,400	
-	+	-	2,270	
+	+	-	3,750	3,670
+	-	-	1,850	
-	-	+	1,420	
+	-	+	3,150	3,270
Experiment 2 (Cells indicated inoculated into a petri dish containing glass beads)				
Glioblastoma	Neuroblastoma			
+	-		760	
-	+		7,000	
+	+		7,200	7,760

is no effect of one cell type on the other is used to calculate R. R is the experimental specific activity obtained when the cells are grown together divided by the expected specific activity as described above. A value of one suggests no effect of mixing. When glass beads on which neuroblastoma cells were grown are added to a flask containing glioblastoma cells (Experiment 1, 2), an inhibition of $^{14}CO_2$ evolution occurs. Similar results are obtained if glass beads with glioblastoma cells are added to a flask containing neuroblastoma cells (Experiment 3, 4). Some inhibition occurs if glioblastoma cells on glass beads are added to a flask containing glioblastoma cells (Experiment 5) or neuroblastoma cells on glass beads are added to a flask containing

neuroblastoma cells (Experiment 6). In the experiments with the same cell types on the beads and in the flask the inhibition is slightly less. All of the experiments were repeated at least twice in each of the media and similar results obtained. These results indicate that an interaction occurs between glioblastoma and neuroblastoma cells that results in an inhibition of $^{14}CO_2$ evolution from [1-^{14}C]-glucose.

In contrast to this if Hela cells are grown in erlenmeyer flasks and either glioblastoma or neuroblastoma cells on beads added no inhibition occurs (Experiments 7, 8). This is indicative of a rather specific interaction between neuroblastoma and glioblastoma cells.

In another experiment the affect of mixed cells on the incorporation of [1-^{14}C]-glucose was examined. The results are shown in Table II. From these results it is apparent that mixing the cells have no affect on the incorporation.

These results suggest that an interaction occurs between neuroblastoma or glioblastoma cells that results in a change in the metabolism of [1-^{14}C]-glucose but not on its incorporation.

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