METABOLIC CONSEQUENCES OF DRUG-INDUCED INHIBITION OF THE PENTOSE PHOSPHATE PATHWAY IN NEUROBLASTOMA AND GLIOMA CELLS

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SUMMARY: 6-Aminonicotinamide leads to a considerable accumulation of 6-phosphogluconate, which is 3 times higher in C-6 glial cells than it is in C-1300 neuroblastoma cells. Dephosphorylation of the accumulated 6-phosphogluconate causes a rise of intracellular gluconate, which can be released from the cells. The higher dephosphorylating capacity of neuroblastoma cells leads to an intracellular gluconate content which is 4 times that found in C-6 glial cells. Although 6-phosphogluconate is a potent competitive inhibitor of glucose phosphate isomerase, no reduction of glycolytic flux and ATP content in stationary phase neuroblastoma cells was found in contrast to observations in C-6 glial cells. Morphological changes are only found in C-6 glial cells during the experimental period.

The nucleotide 6-ANADP, synthesised in the cells of the central nervous system of rats after administration of the antimetabolite 6-AN, is a very potent inhibitor of the NADP dependent 6-phosphogluconate dehydrogenase (EC 1.1.1.44) (1, 2). In our studies on the molecular bases of the neurotoxicity of 6-AN we found an enormous accumulation of 6-phosphogluconate in the central nervous system, which influences the function of the glucosephosphate isomerase (EC 5.3.1.9) (3, 4). Furthermore, the accumulated 6-phosphogluconate is dephosphorylated to gluconate, which can be released from the cells (5). The most severe lesions were found in those regions of the central nervous system, in which the highest concentrations of 6-phosphogluconate were found (6).

Electron microscopic studies showed fundamental differences in the sensitivity of nerve and glial cells to the antimetabolite. The preferential lesion of the neuroglial cells was particularly conspicuous. Schneider

Abbreviations: 6-AN = 6-aminonicotinamide
6-ANADP = 6-aminonicotinamide adenine dinucleotide phosphate

and Cervos-Navarro (7) speak of an acute gliopathy. The nerve cells were affected only secondarily if at all. The different behaviour of the cells might be explained as follows:

1) The preferential lesion of the neuroglial cells is due to their characteristic topography. They seem to fulfill a barrier function, which protects the nerve cells from the direct action of the toxic compound.
2) The fundamental differences in the sensitivity of neuroglial and nerve cells in case of equal uptake of 6-AN are due to still unknown deviations in metabolism.

Our biochemical studies on rat brain and spinal cord did not enable us to decide, whether particularly high concentrations of 6-phosphogluconate occur within a certain cell type. The cellular complexity of the whole brain made us use two established cell lines of neural and glial origin, i. e. C-1300 neuroblastoma cells (clone N2A), and C-6 glial cells. Both cell types have differentiated traits grossly comparable to their normal counterparts in intact brain (8, 9). This study deals with metabolic differences between both cell types after inhibition of the hexose phosphate pathway and is aimed at establishing a relationship between biochemical and morphological changes in the central nervous system.

MATERIALS AND METHODS:

C-1300 Neuroblastoma cells (clone N2A) and C-6 glial cells were obtained from the American Type Culture Collection. Cultivation methods and growth characteristics of both cell types correspond to the description in the American Type Culture Collection manual (10). During the stationary phase with a final density of 1.5×10^5 cells/cm^2 for neuroblastoma cells and $4\text{-}5 \times 10^5$ cells/cm^2 for C-6 glial cells the growth medium was exchanged for either new medium or new medium + 0.01 mg/ml 6-AN. The growth medium was decanted 24 h later. After washing the monolayer twice the experimental medium (i. e. N_2-saturated Hanks balanced salt solution, 10 mg% glucose, 25 mM HEPES, pH 7.4) was poured onto the cells. After incubation for 1 h at 37°C in N_2-atmosphere, glucose consumption as well as gluconate and lactate production were determined in the experimental medium. The cells were fixed by means of perchloric acid and subsequently prepared for the determination of intracellular metabolite contents of 6-phosphogluconate, gluconate, Glc-6-P and ATP as described previously (11). To be able to compare our values with tissue concentrations, we chose the packed cell volume determined with an ordinary microhematocrit centrifuge as reference.

Abbreviation:
HEPES = 2- [4-(2-hydroxyethyl)-piperazinyl-(1)] -ethanesulfonic acid

Table 1:

Glucose consumption and production of lactate and gluconate by stationary phase C-1300 neuroblastoma (clone N2A) and C-6 glial cells under experimental conditions, i.e. 1 h incubation in Hanks balanced salt solution + 10 mg% glucose at 37°C under an atmosphere of nitrogen.

Metabolites in the medium	nmoles/min x ml cells									
	Neuroblastoma N2A					C-6 Glial cells+)				
	control	n	6-AN	n	p	control	n	6-AN	n	p
Glucose	936 ± 18	6	905 ± 55	6	n.s.	531 ± 18	4	< 250		s
Lactate	2025 ± 79	6	1954 ± 59	6	n.s.	1071 ± 54	6	456 ± 24	6	s
Gluconate	< 1		39 ± 4	11	s	< 1		24 ± 1.4	6	s

+)Values taken from Keller et al. (11)

s = significant ($p < 0.05$)
n.s. = not significant ($p > 0.05$)

Cells were counted in a Coulter Counter model ZF.

Microscopic observations were done with an inverse Zeiss Axiomat. Cultivation media, i.e. nutrient mixture (Ham's F 10) medium for C-6 glial cells and minimum essential medium with Hanks balanced salt solution for C-1300 neuroblastoma cells, were purchased from GIBCO-BIOCULT Ltd. and from FLOW Lab., materials for Hanks balanced salt solution from Merck, Darmstadt.

RESULTS AND DISCUSSION:

The first experiments (Table 1) show the differences in glucose metabolism between neuroblastoma and glioma cells at stationary phase of growth based on the production of lactate and gluconate and glucose consumption. During incubation with Hanks balanced salt solution plus glucose in N_2 atmosphere, the ratio of lactate production to glucose consumption was 2 : 1. This could be expected under conditions in which glucose is the only substrate available for energy supply. Neither glucose consumption nor glycolytic flux were affected by 6-AN in neuroblastoma cells. In contrast we were able to demonstrate that, compared to the controls, the glycolytic flux of glioma cells is reduced by more than 50%.

Table 2:

Intracellular contents and tissue contents of some intermediates in C-1300 neuroblastoma cells, C-6 glial cells and rat brain (steady state values from animals wholly frozen by submerging in Freon 13 at -190°C). Values for brain and C-6 glial cells from Keller et al. (4, 11).

Metabolite	nmoles/g w. wt.		nmoles/ml cells					
	Brain		C-6 Glial cells		C-1300 Neuroblastoma N2A			
	control	6-AN	control	6-AN	control	n	6-AN	n
6-PG	12 ± 2	1704 ± 37	< 150	20000 ± 900	< 150		6794 ± 409*	17
Gluconate	< 10	960 ± 46	< 150	15200 ± 900	< 150		53462 ± 4538*	13
Glc-6-P	58 ± 2.1	102.8 ± 2.4	83.8 ± 13.6	318 ± 21	49 ± 5	11	203 ± 10*	11
ATP	2019 ± 53	1706 ± 60	2550 ± 220	1920 ± 166	5047 ± 165	12	5465 ± 391**	12

* = significant ($p < 0.05$)
** = not significant ($p > 0.05$)

In all these experiments we could show that a considerable amount of gluconate is released into the incubation medium. The gluconate efflux which in neuroblastoma cells is about twice the value observed in glioma cells, points to the most conspicuous changes in the contents of hexose monophosphate pathway metabolites (Table 2). In both cell types, as in brain, the 6-AN effect leads to a considerable accumulation of 6-phosphogluconate, which is more pronounced in glioma cells. Conversely, in neuroblastoma cells with much lower 6-phosphogluconate values, gluconate amounts to 8 times the value of 6-phosphogluconate and 4 times the gluconate content observed in glioma cells. This could be explained by more effective dephosphorylation of 6-phosphogluconate in the neuroblastoma cells.

The reduced glycolytic flux and the diminished ATP content of the glioma cells as well as their lower dephosphorylating capacity indicate differences between both cell types as far as the inhibition of the hexose monophosphate pathway and its influence on glycolysis are concerned. In addition, morphological alterations were only observed in glioma cells during the experimental period. In phase contrast microscopy the glioma

cells showed edematous alterations and lost their sharply defined body contours and processes which can be seen in control cells (11).

Biochemical as well as morphological alterations point to the selective vulnerability of glial cells previously shown in brain (1, 6, 12). The fact that neuroglial cells constitute an exchange zone between neuron and extraneuronal fluid in the central nervous system might not be the only cause of the different behaviour of nerve and glial cells found by electron microscopy (12). In our study we were able to show differences between neuroblastoma and glioma cells in metabolizing glucose after 6-AN which correspond well with morphological findings in intact brain and spinal cord.

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