BRAIN DISORDERS INDUCED BY PHARMACOLOGICAL BLOCKADE OF THE PENTOSE PHOSPHATE PATHWAY

by

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Received May 23, 1969

SUMMARY

6-ANADP synthesized by the endoplasmic glycohydrolase in the brain cells of rats after application of 6-AN is an inhibitor of NADP-dependent dehydrogenases. Under conditions in vivo, 6-phosphogluconate dehydrogenase seems to be the most sensitive enzyme. In the brain, a high accumulation of 6-phosphogluconate and, to a smaller extent, of glucose 6-phosphate was found.

The determination of the ratio glucose 6-phosphate : fructose 6-phosphate in the brains of animals pre-treated with 6-AN showed that the accumulation of 6-phosphogluconate also seems to inhibit the PGI in vivo.

After application of ^{14}C D-glucose the specific activity of the RNA from cell nuclei of the brain is considerably decreased in animals pre-treated with 6-AN. This is supposed to be caused by the inhibition of the oxidative and the non-oxidative part of the pentose phosphate cycle.

Studies on the relations between the pentose phosphate cycle (review see Horecker, 1968) and the glycolytic pathway showed that some intermediates of the pentose phosphate cycle, such as 6-phosphogluconate, erythrose 4-phosphate, and seduheptulose 7-phosphate are potent inhibitors of phosphoglucose isomerase (PGI) (Parr, 1957; Grazi, de Flora, and Pontremoli, 1960; Venkataraman and Racker, 1961).

Racker (1965) pointed out that these intermediates are providing a negative feedback mechanism for the control of the glycolytic pathway. It is not yet known, whether disturbances of such regulations are possible in vivo, and which consequences they would have for the cell metabolism.

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

Assays with the neurotoxic antimetabolite 6-AN opened a possibility to study this problem. Application of 6-AN induces the biosynthesis of derivatives of NAD and NADP containing 6-AN by the glycohydrolase (EC 3.2.2.6) of the endoplasmic reticulum of different cells of albino rats (Kaplan and Ciotti, 1954, 1956). The derivatives of 6-AN are pharmacologically particularly active in the brain (Coper and Herken, 1963). The symptoms of disorders in the central nervous system which can be observed after administration of 6-AN mainly consist of paralysis (Johnson and McColl, 1956; Sternberg and Philips, 1958; Wolf, Cowen, and Geller, 1959). A continuous drop of body temperature and a prolongation of sleeping time after application of hexobarbital not due to a delayed metabolism of the drug are found to be further central nervous symptoms caused by the biosynthesis of pyridine nucleotides containing 6-AN in the brain (Coper, Hadass, and Lison, 1966; Herken, 1968). These abnormally structured nucleotides are unable to act as hydrogen carriers in oxidoreductase systems (Dietrich, Friedland, and Kaplan, 1958). Furthermore, 6-ANADP is a competitive inhibitor for several NADP-dependent enzymes (Coper and Neubert, 1964). It is, however, not yet known which enzymic reactions are preferentially inhibited in the brain in vivo after application of 6-AN.

The blood-brain-barrier being easily penetrated by glucose, the brain tissue has a potential capacity for metabolizing glucose. Preliminary experiments carried out with [1-^{14}C] and [6-^{14}C] D-glucose on tissue slices of brains of rats which had received 35 or 70 mg/kg 6-AN 6 hours previous to being killed by decapitation, and on a hyaloplasmic fraction obtained from the brain by centrifugation (150.000 x g; temp. 2° C) showed a considerable decrease of CO_2-production from [1-^{14}C] D-glucose as compared with the untreated control animals. This led to the conclusion that the two initial NADP-dependent steps of the oxidative part of the pentose phosphate pathway are also particularly affected in vivo.

METHODS

Male albino rats (Wistar) weighing from 120 to 150 g were given 35 and 70 mg/kg 6-AN by i.p. injection. After different lapses of time (see Fig. 1) the animals were killed by decapitation. The brains were quickly extracted, weighed (wet weight) and, within 30 seconds after decapitation,

homogenized in 5% perchloric acid, and centrifugated. The residue was washed. After precipitation of the perchloric acid as potassium perchlorate at pH 6 and centrifugation, to the clear supernatant aqua dest. to a total of 10 ml was added.

Measurement of substrate concentrations

The determination of glucose 6-phosphate, fructose 6-phosphate, and 6-phosphogluconate in the solutions obtained by the above mentioned procedure was carried out with D-glucose 6-phosphate : NADP oxidoreductase (EC 1.1.1.49), D-glucose 6-phosphate ketol isomerase (EC 5.3.1.9), and 6-phospho D-gluconate : NADP oxidoreductase (decarboxylating) (EC 1.1.1.44) in the optical test by measuring the produced $NADPH_2$ at 340 nm in the Double-Beam Recording Spectrophotometer DMR 21 (Zeiss). All measurements were performed at pH 7.6.

Labelling of the RNA from cell nuclei of rat brains after injection of D-glucose ^{14}C-(U) into animals treated with 6-AN and control animals

Four animals each out of the test group (35 mg/kg 6-AN i.p.) and the control group were injected 1.300 μCi/kg D-glucose ^{14}C-(U) (Boehringer, specific activity 200 mCi/mmol), solved in physiological saline solution, into the tail vein 6 hours after the test animals had received 6-AN. One hour later, the animals were killed by decapitation, and the brains prepared at 4° C. Pure nuclei were prepared following the method of Widnell and Tata (1964) as reported by Bass (1967): homogenization of the brains in fourfold (v/v) quantity 0.32 M saccharose with a plexiglass homogenizer Type Potter-Elvehjem. The homogenate was centrifugated (700 x g) for 10 minutes. The crude nuclear fraction thus obtained was once more homogenized, filtered through eight layers of gauze and washed several times in 0.32 M saccharose + 0.02 M tris + 3 mM Mg^{++}. The washed crude nuclear fraction was suspended in a 2.4 M saccharose, so that a final concentration of 1.9 M was obtained. Centrifugation at 50.000 x g for 60 minutes, isolation of the RNA from the pure nuclei following the method of Ogur and Rosen (1950) as modified by Neubert, Helge, and Bass (1965): extraction of the acid-soluble fraction with 0.2 M perchloric acid in the cold, washing 3 to 4 times, extraction of lipids by 4 x ether alcohol (1 : 3), benzene, and ether. Afterwards extraction of the RNA by 1.0 M perchloric acid in the

cold for 16 hours. Determination of the RNA by the orcin reaction, measuring of radioactivity in a Packard Liquid Scintillation Spectrometer Type 3375.

RESULTS AND DISCUSSION

The results reproduced in Fig. 1 show the considerable accumulation of 6-phosphogluconate in the brains of the animals treated with 35 or 70 mg/kg 6-AN. It was also possible to demonstrate an increase of glucose 6-phosphate. Following up the course of time of these concentrations showed a distinct difference. After 10 hours the quantity of 6-phosphogluconate reached a peak value in the brain cells with about 2 $\mu g/g$. The largest quantity of glucose 6-phosphate was found after 6 hours; it diminished slightly in the further course of the experiments. With the control animals which

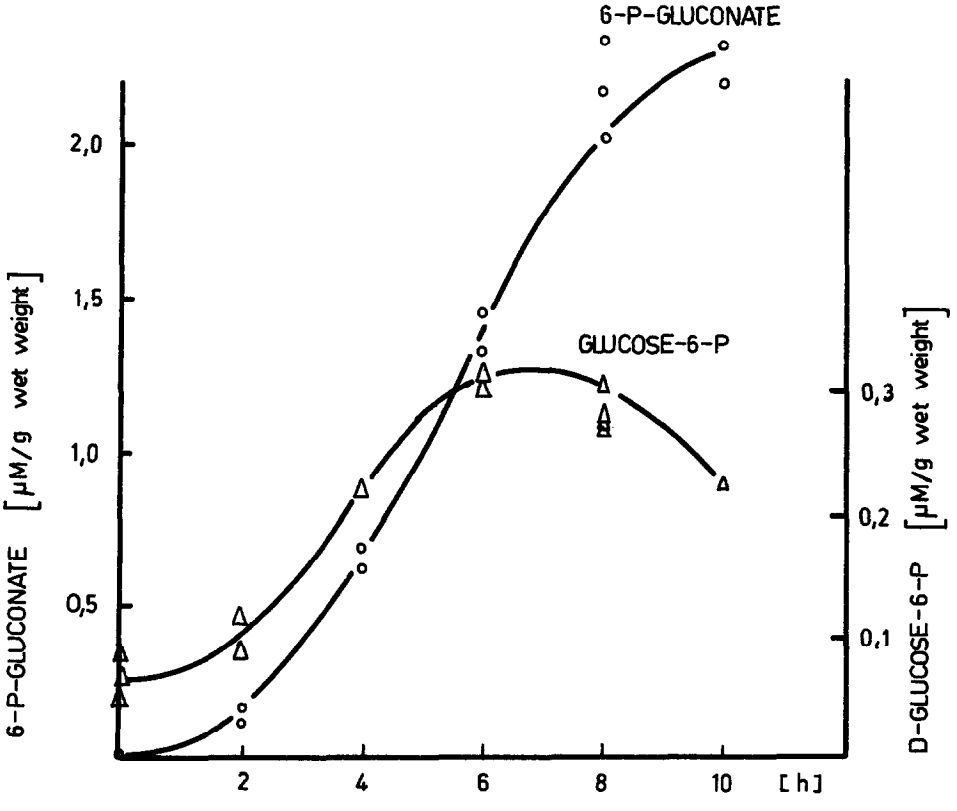

Fig. 1: Concentration of 6-phosphogluconate and glucose 6-phosphate in the brain of the rat after application of 35 or 70 mg/kg 6-AN by enzymic methods. Each point represents the concentration of the metabolite from one rat brain at different times.

had not received 6-AN, no measurable quantities of 6-phosphogluconate could be found under the present conditions. The results prove that the function of the 6-phospho D-gluconate : NADP oxidoreductase (EC 1.1.1.44) is preferentially inhibited by 6-ANADP. The conditions for a functional disorder of the NADP-dependent enzyme are favourable, because of the competitive antagonism of 6-ANADP to NADP. Coper, Hadass, and Lison (1966) have reported that, after application of 20 or 50 mg/kg 6-AN, considerably greater amounts of 6-ANADP than of NADP were found in the brains of rats. The ratio of 6-ANADP : NADP concentrations was about 2 : 1 resp. 3 : 1 with the higher dose. The inhibition of the NAD-dependent enzymes should be negligible, because there is a considerable excess of NAD, and because only about 1/10 of this coenzyme was converted into the derivative containing 6-AN.

The marked differences in the accumulation of the metabolites seem to indicate that the inhibition of the 6-phospho D-gluconate : NADP

Table I

Average values of the substrate concentrations in μmol/g fresh weight measured in the brain 6 hrs after i.p. injection of 35 mg 6-AN/kg rat.

	6-Phospho-gluconate (6-PG)	Glucose 6-phosphate (G 6-P)	Fructose 6-phosphate (F 6-P)	$\frac{\text{6-PG}}{\text{G 6-P}}$	$\frac{\text{G 6-P}}{\text{F 6-P}}$
Controls (I)	0.012 $\pm$ 0.0026	0.0607 $\pm$ 0.0014	0.0156 $\pm$ 0.0011	0.198	3.9
(n)	(5)	(5)	(5)		
6-AN (35 mg/kg rat)	1.37 $\pm$ 0.12	0.232 $\pm$ 0.014	0.0149 $\pm$ 0.0025	5.9	16.3
(n)	(5)	(5)	(5)		
p	< 0.0002	< 0.0002	0.54		

oxidoreductase (EC 1.1.1.44) is the decisive reaction leading to disturbances of the brain metabolism. Up to now, no drug is known to cause such an effective inhibition of 6-phosphogluconate : NADP oxidoreductase in the brain. The enzymic determination of fructose 6-phosphate in the brain showed that the normal relation of glucose 6-phosphate to fructose 6-phosphate which, according to results published amounts to 3 : 1 (Kahana, Lowry, Schulz, Passonneau, and Crawford, 1960), is being shifted in favour of glucose 6-phosphate by the increase of the concentration of glucose 6-phosphate. This disturbance of the balance indicates an inhibition of the PGI by 6-phosphogluconate in vivo and on principle confirms the results published by Parr (1956, 1957); Noltmann and Bruns (1959), and Salas, Vinuela, and Sols (1965) (Table I).

An inhibition of the PGI which occupies a central position within the carbohydrate metabolism might explain the severe damage to different cells of the central nervous system by impairment of the glycolytic path-

Table II

Incorporation of ^{14}C into the RNA of isolated nuclei of brain cells.

Assay	Control animals (dpm/μg RNA)	Animals treated with 6-AN (dpm/μg RNA)
I	15.0	7.85
II	19.95	6.35
III	13.70	5.90

Isolation of cell nuclei from 4 pooled rat brains per assay.
Controls: decapitation 1 hour after 1.300 μCi/kg D-glucose ^{14}C-(U) specific activity 200 mCi/mmol
Animals pre-treated with 6-AN: injection of 1.300 μCi/kg D-glucose ^{14}C-(U) 6 hours after application of 6-AN. Decapitation 1 hour later.

way. The following findings seem to indicate a further restriction of the function of the pentose phosphate cycle probably in its oxidative and non oxidative parts by formation of 6-phosphogluconate: After application of D-glucose ^{14}C-(U) a well measurable activity is found in the RNA of the isolated cell nuclei of the brain which is probably due to the incorporation of ribose. After application of 6-AN, the specific activity is reduced to about 1/2 of the control values (Table II).

Studies on the activity of the glucose 6-phosphate dehydrogenase and of the 6-phosphogluconate dehydrogenase in the brain have resulted in showing remarkable differences in the efficiency. In contrast to other organs in which the ratio of activity of both enzymes amounted to 1 : 1 in most cases, several authors have found that the activity of the phosphogluconate dehydrogenase in the brain is mostly considerably lower than that of the glucose 6-phosphate dehydrogenase (Robins, 1960; Brunnemann and Coper, 1964; Novello and McLean, 1968). Therefore, the activity of the gene controlling the synthesis of the 6-phosphogluconate dehydrogenase can physiologically be of importance for the regulation of the carbohydrate metabolism in the different brain cells, and the inhibition of the enzyme by a drug with its consequences can serve to explain the selective vulnerability of some brain regions.

References

Bass, R.: Inaug. Diss. FU Berlin (1967)

Brunnemann, A. and H. Coper: Naunyn-Schmiedebergs Arch. exp. Path. Pharmakol. 246, 493 (1964)

Coper, H., H. Hadass, and H. Lison: Naunyn-Schmiedebergs Arch. Pharmakol. exp. Path. 255, 97 (1966)

Coper, H. and H. Herken: Dtsch. med. Wschr. 88, 2025 (1963)

Coper, H. and D. Neubert: Biochim. biophys. Acta (Amst.) 89, 23 (1964)

Dietrich, L. S., I. M. Friedland, and L. A. Kaplan: J. biol. Chem. 233, 964 (1958)

Grazi, E., A. De Flora, and S. Pontremoli: Biochem. biophys. Res. Comm. 2, 121 (1960)

Herken, H.: In: Proc. 3rd Intern. Pharmacological Meeting, Vol. 4, p. 3, Oxford-New York, Pergamon Press (1968)

Horecker, B. L.: In: Carbohydrate metabolism and its disorders, Vol. I, p. 139, London-New York: Academic Press (1968)

Johnson, W. J. and J. D. McColl: Fed. Proc. 15, 284 (1956)

Kahana, S. E., O. H. Lowry, D. W. Schulz, J. V. Passonneau, and E. J. Crawford: J. biol. Chem. 235, 2178 (1960)

Kaplan, N. O. and M. M. Ciotti: J. Amer. Chem. Soc. 76, 1713 (1954)
Kaplan, N. O., M. M. Ciotti, and F. E. Stolzenbach: J. biol. Chem. 221, 833 (1956)
Neubert, D., H. Helge, and R. Bass: Naunyn-Schmiedebergs Arch. exp. Path. Pharmakol. 252, 258 (1965)
Noltmann, E. and F. H. Bruns: Biochem. Z. 331, 436 (1959)
Novello, F. and P. McLean: Biochem. J. 107, 775 (1968)
Ogur, M. and G. Rosen: Arch. Biochem. 25, 262 (1950)
Parr, C. W.: Nature (Lond.) 178, 1401 (1956)
Parr, C. W.: Biochem. J. 65, 34 P (1957)
Racker, E.: Mechanisms in bioenergetics, p. 207. New York-London: Academic Press (1965)
Robins, E.: J. Histochem. Cytochem. 8, 431 (1960)
Salas, M., E. Vinuela, and A. Sols: J. biol. Chem. 240, 561 (1965)
Sternberg, S. S. and F. S. Philips: Science 127, 644 (1958)
Venkataraman, R. and E. Racker: J. biol. Chem. 236, 1876 (1961)
Widnell, C. C. and J. R. Tata: Biochem. J. 92, 313 (1964)
Wolf, A., D. Cowen, and L. M. Geller: Transact. Amer. neurol. Ass. 1959, 140