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CHANGES IN PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITIES IN MOUSE BRAIN, LIVER AND MUSCLE WITH AGE

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SUMMARY

Hypoxanthine (or guanine) phosphoribosyltransferase (EC 2.4.2.8) and adenine phosphoribosyltransferase (EC 2.4.2.7) activities have been evaluated in mouse brain, liver and muscle as a function of age. In these tissues adenine phosphoribosyltransferase activity (per g of tissue) decreased from birth until about 30 days of age, at which time it attained a plateau in the brain and increased slightly in liver and muscle. The pattern of hypoxanthine (or guanine) phosphoribosyltransferase activity differed more with the tissue. In muscle it decreased continuously; in liver it decreased until 30 days and then increased significantly; in brain it increased considerably and attained a plateau at 30 days. Until 20 days of age the total phosphoribosyltransferase activity per organ was greatest in the brain; subsequently the total liver activity became largest. Simultaneous determinations of xanthine oxidase and guanine deaminase indicated that, shortly after birth, hypoxanthine (or guanine) phosphoribosyltransferase in liver and muscle must compete for its substrates with high levels of these two enzymes. In brain from birth, the guanine deaminase activity was superior to the phosphoribosyltransferase activity with guanine; however, phosphoribosyltransferase activity with hypoxanthine as substrate was always considerably greater than the xanthine oxidase activity.

INTRODUCTION

During recent years considerable interest has awakened in the purine phosphoribosyltransferases. These enzymes, once thought of only as a means to reutilize the purine ring, apparently play important roles in the regulation of purine metabolism in general in the cell. In the Lesch-Nyhan syndrome the absence of hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) leads to severe neurological malfunction in young males [1]. Partial deficiences of this enzyme seem to be the basis for certain forms of gout [2]. Although some information is available on the distribution in hypoxanthine (or guanine) phosphoribosyltransferase and adenine phosphoribosyltransferase (EC 2.4.2.7) in mammalian tissues, it is limited [3–7]. As a part of a study of the regulation of mammalian purine metabolism, the activities of these two enzymes were evaluated in several tissues as a function of age, in order to determine

their importance relative to other enzymes or pathways utilizing the same substrates or forming the same products and to later correlate these results with investigations of the availability of one of the substrates, phosphoribosyl 5′-pyrophosphate.

EXPERIMENTAL PROCEDURE

Materials

[8-^{14}C]Adenine, [8-^{14}C]guanine and [8-^{14}C]hypoxanthine were obtained from Amersham/Searle. [8-^{14}C]Guanine was purified on Dowex AG 50W-X4, 200–400 mesh (Bio-Rad Laboratories), before use. Phosphoribosyl 5′-pyrophosphate, adenine, guanine and hypoxanthine were obtained from Sigma Chemical Co. Other chemical compounds were from the Fisher Scientific Co. All products used were of the highest available quality.

Methods

For each assay 10–20 male Swiss mice (CBL-HAM/ICR, Canadian Breeding Farm and Laboratories, Ltd, St. Constant, Quebec) were decapitated and the brains, livers and hind leg muscles rapidly removed and placed on ice. The tissues were rinsed rapidly with ice-cold 100 mM Tris–HCl buffer, pH 7.5, and then homogenized in a Virtis homogenizer with 10 vol. of ice-cold 100 mM Tris–HCl, pH 7.5. Care was taken not to heat the homogenate. The homogenates were centrifuged at 30 000 $\times$ g for 1 h at 4 °C, and the supernatants were removed with a Pasteur pipette and assayed immediately.

Purine phosphoribosyltransferase activity was determined as follows: the final volume of the incubations was 250 μl and consisted of 100 μl of supernatant in a medium containing 100 mM Tris–HCl (pH 7.5)–5 mM $MgCl_2$–0.5 mM phosphoribosyl 5′-pyrophosphate–0.4 mM purine base (spec. act. between 2500 and 10 000 dpm/nmole). The supernatants were incubated with buffer–$MgCl_2$–phosphoribosyl 5′-pyrophosphate for 5 min at 37 °C. Purine base was added to start the reaction, and the tubes were incubated for 5 min at 37 °C (the reaction was linear during this time). To stop the incubations, the contents of the tubes were frozen rapidly in acetone–solid CO_2 and they were stocked in a freezer at −20 °C until evaluation. Assays were carried out in triplicate.

Nucleotide formation was measured by adsorption onto DEAE-cellulose disks using a modification of the method of Atkinson and Murray [8]. Three disks of Whatman DEAE-cellulose paper were placed in a millipore filtration apparatus, and the contents of an incubation filtered. The incubation tube was rinsed onto the filters with a total of 10 ml of water, and then the filters were rinsed with 10 ml of 2 mM $(NH_4)_2CO_3$, 30 ml of water and 5 ml of 95% ethanol. The filters were placed in a scintillation vial, 1 ml of ethanol and 14 ml of toluene–PPO–POPOP scintillation fluid added, and the samples were counted in a Nuclear-Chicago Mark II liquid scintillation apparatus. The counting efficiency of this system, as measured with [8-^{14}C]AMP applied to the disks as described, was 60%. With this method care must be taken to avoid having large quantities of anions in the incubations which can displace the labelled nucleotide.

Products of the incubations were also separated by column chromatography on Dowex AG 50W-X4, 200–400 mesh, using the method of Sweetman and Nyhan [9],

by descending paper chromatography on Whatman 3 MM paper with 5% Na_2HPO_4 or butanol–water–88% formic acid (77:11.5:11.5, v/v/v) as solvent, or by high-voltage electrophoresis on Whatman 3 MM paper using 50 mM sodium borate–50 mM EDTA, pH 9.0, at 1800 V for 75 min. For Dowex column chromatography triplicate incubations were combined, 1.25 ml of 0.4 M perchloric acid, 0.7 ml of carriers, and 0.2 ml of 5 M HCl were added, and one-third of this was placed on the column. The column was monitored with an ISCO UV monitor, and 1-ml fractions were collected and counted with 15 ml of Bray's scintillation fluid [10]. For paper chromatography 50 μl of 50 mM EDTA were added to the incubations and the tubes were heated for 1 min at 100 °C and then centrifuged. The supernatant was removed, and to it was added sufficient carriers to detect the various nucleotides, nucleosides, and purine bases after separation. The compounds were detected by ultraviolet light, and the spots were cut out, placed in scintillation vials, and counted in 15 ml of toluene–PPO–POPOP. The separation of various purine compounds obtained using each of these methods is shown in Table I.

Protein was measured by the method of Lowry et al. [11].

TABLE I

SEPARATION OF PURINE COMPOUNDS BY VARIOUS METHODS

For paper chromatography R_F values were calculated using the solvent front, except where indicated. For Dowex chromatography the values represent the elution position in ml. Butanol–water–88% formic acid (77:11.5:11.5, v/v/v); Whatman 3 MM; 12 h migration, solvent front 33 cm; 5% Na_2HPO_4; Whatman 3MM; 6 h migration, solvent front 48 cm; high-voltage electrophoresis in 50 mM sodium borate–50 mM EDTA, pH 9; Whatman 3MM, 30 cm × 56 cm; 75 min at 1800 V, 320 mA; migration of IMP taken as reference; Dowex AG 50W-X4, 200–400 mesh, 0.9 cm × 44 cm, 1 ml/min; 300 ml of 0.5 M HCl were passed, followed by 300 ml of a linear gradient 0.5–5 M HCl.

Compound	Butanol–water–formic acid	Na_2HPO_4	High-voltage electrophoresis	Dowex AG 50W-X4	
				0.5 M HCl	0.5–5 M HCl
Adenine	0.28	0.35	0.07		185
Adenosine	0.20	0.47	0.30		42
AMP	0.02	0.69	0.88	182	
Hypoxanthine	0.32	0.52	0.45	232	
Inosine	0.15	0.67	0.63	164	
IMP	0.02	0.81	1.00	73	
Xanthine	0.22	0.42	0.60	164	
Guanine	0.12	0.38	0.20		95
Guanosine	0.12	0.60	0.50	220	
GMP	0.02	0.77	0.98	94	

RESULTS AND DISCUSSION

The activities of the purine phosphoribosyltransferases, as determined by fixation of the nucleotides on DEAE-cellulose disks, were compared with the values obtained by chromatography of the reaction products on Dowex AG 50W-X4, a method which is considerably more discriminating. At four different ages of mice and with all substrates, similar values were obtained for the appropriate nucleotide product using both methods. Thus, as dephosphorylation was not significant under these reac-

tion conditions, DEAE-cellulose fixation was used for subsequent studies of enzyme activity as a function of age.

As presumably the needs for purine reutilization in various tissues can vary during the life of an animal, we have investigated the changes in the activities of purine phosphoribosyltransferases in mouse brain, liver and muscle as a function of age. In Fig. 1 the results are expressed per g of tissue (wet weight) and in Fig. 2 per total organ. It is evident that these values do not necessarily represent the actual physiological activities, however, they are indicative of the potentials present. Initially, the activities (per g of tissue) were greatest in liver, and for both liver and muscle there

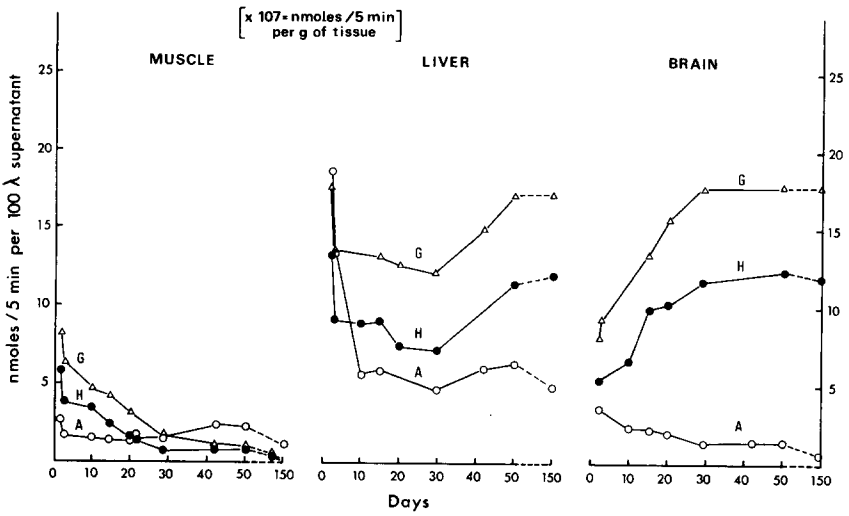

Fig. 1. Activity of purine phosphoribosyltransferases, expressed per 100 μl of supernatant (or per g of tissue), as a function of age. Supernatants were incubated with [8-^{14}C]adenine (A), [8-^{14}C]hypoxanthine (H), or [8-^{14}C]guanine (G), and the nucleotides formed were evaluated by fixation on DEAE-cellulose disks as described in the Experimental Procedure.

was a very marked decrease in activity during the first few days of life. The level then continued to decrease slowly, stabilized, and, in the liver the level of hypoxanthine (or guanine) phosphoribosyltransferase rose again considerably after 30 days. Adenine phosphoribosyltransferase in brain followed a pattern qualitatively and quantitatively similar to that in muscle, however, the hypoxanthine (or guanine) phosphoribosyltransferase activity was quite different. It rose steadily during the first 30 days of life and attained a stable plateau. Somewhat similar results have been reported for mouse liver by Murray [12] and for rat brain by Gutensohn and Guroff [7]. Only in the liver of very young mice or in the muscle of adult mice was the adenine phosphoribosyltransferase activity greater than that of hypoxanthine (or guanine) phosphoribosyltransferase. The total purine phosphoribosyltransferase activity per organ was greatest in brain from birth until about 20 days of age, at which time the total liver activity became more important. This was due to the continuing growth of the liver as compared with the termination of the weight increase of the brain.

Muscles were not separated into slow or fast types, nor were brains divided into regions. This latter is probably of greater importance. It has been previously

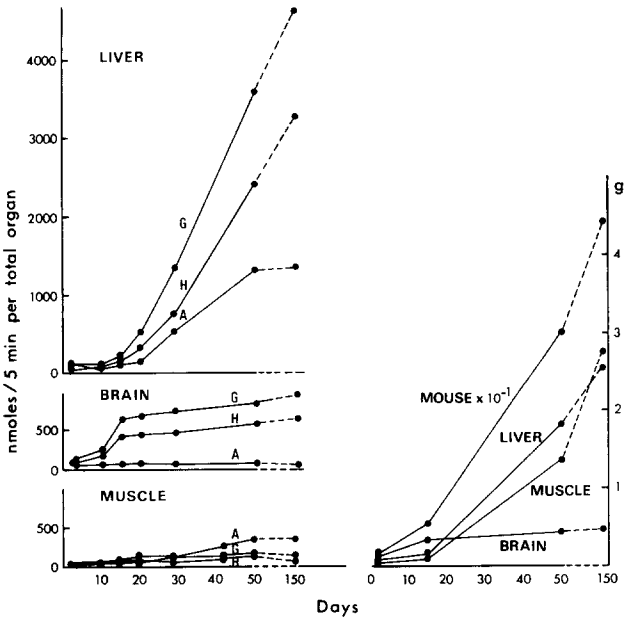

Fig. 2. Total purine phosphoribosyltransferase activities per organ as a function of age.

shown by other workers that there is a significant difference in the hypoxanthine (or guanine) phosphoribosyltransferase activity in different regions of the brain [4, 6, 7]. These values were obtained, however, using only young adult or adult tissues. In a subsequent study purine phosphoribosyltransferases should be evaluated in various regions of the brain as a function of age, as their patterns of change could be quite different from one region to another.

Evaluation of the incubations by chromatography permitted a concomitant determination of certain other enzymatic activities, i.e. xanthine oxidase and guanine deaminase, which utilize common substrates with the purine phosphoribosyltransferases. These results are presented in Table II. As one sort of chromatography permitted the separation of compounds not adequately separated by another (Table I, *Methods*), the values given are the means of different evaluations or the value obtained by the discriminating method. Xanthine oxidase was measurable and remained constant in brain and muscle, whereas it increased significantly in liver with age. Guanine deaminase remained fairly constant in muscle, whereas in brain and liver it increased considerably with age. From at least day 22 these two enzyme activities were superior to hypoxanthine (or guanine) phosphoribosyltransferase activity in liver and muscle and presumably could limit hypoxanthine and guanine reutilization. In brain from birth the guanine deaminase activity was greater than the phosphoribosyltransferase activity with guanine, and the difference increased with age. However, there also exists a regional distribution of guanine deaminase in the brain [6], and thus the possibility for guanine reutilization in vivo would depend upon the relative activities of the two enzymes in a given region. Phosphoribosyltransferase activity with hypoxanthine was always considerably greater than xanthine oxidase activity and thus hypoxanthine reutilization in brain in vivo is presumably quite important.

TABLE II

REPARTITION OF [^{14}C]PURINE AFTER INCUBATION WITH SUPERNATANTS FROM MOUSE BRAIN, LIVER AND MUSCLE

Supernatants for this experiment were prepared from tissues homogenized using a Potter–Elvehjem teflon–glass tissue grinder for brain and liver and a Potter–Elvehjem glass–glass tissue grinder for muscle. Activities obtained with these supernatants were somewhat higher than those obtained from homogenates prepared with a Virtis homogenizer.

Supernatants were incubated with [8-^{14}C]adenine, [8-^{14}C]hypoxanthine, or [8-^{14}C]guanine as described in Experimental Procedure. The values given are nmoles of product formed/5 min per 100 μl of supernatant. Adenine phosphoribosyltransferase activity was evaluated from the formation of AMP; Hypoxanthine phosphoribosyltransferase activity was evaluated from the formation of IMP; Xanthine oxidase activity was evaluated from the formation of xanthine; Guanine phosphoribosyltransferase activity was evaluated from the formation of GMP; Guanine deaminase was evaluated from the formation of xanthine.

	2-day old mice			22-day old mice			58-day old mice		
	Brain	Liver	Muscle	Brain	Liver	Muscle	Brain	Liver	Muscle
Adenine phosphoribosyltransferase	4.7	29.4	2.7	2.5	6.2	1.7	1.4	6.3	2.0
Hypoxanthine phosphoribosyltransferase	5.6	17.2	5.7	14.8	11.7	1.4	13.1	13.6	0.5
Xanthine oxidase	2.5	4.3	3.0	1.9	14.2	2.5	2.8	25.5	1.7
Guanine phosphoribosyltransferase	11.0	25.1	10.5	22.0	18.9	3.4	20.1	22.2	0.8
Guanine deaminase	17.9	13.0	2.2	67.0	46.0	7.1	72.0	44.0	4.1

Little is known about the relative importance of the pathways of purine metabolism in various tissues. Certain tissues, e.g. bone marrow, erythrocytes and leukocytes, are unable to synthesize purines de novo [13–15] and thus presumably depend upon the purine phosphoribosyltransferases to supply the purine nucleotides necessary. Liver, on the other hand is known to have a very active purine biosynthesis [16], while other tissues, notably brain, synthesize purines de novo to a small extent [17]. In addition, in all tissues the manner by which the pathways of de novo synthesis and reutilization are regulated and coordinated remains little known. There are indications that the supply of phosphoribosyl 5′-pyrophosphate, a common substrate for de novo synthesis and base reutilization, has a great influence [16]. Studies are currently underway to evaluate the amount of phosphoribosyl 5′-pyrophosphate in mouse brain, liver, and muscle and to determine the influence that this can have on the regulation of their purine phosphoribosyltransferases and of de novo purine synthesis.

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REFERENCES

1 Seegmiller, J. E., Rosenbloom, F. M. and Kelley, W. N. (1967) Science 155, 1682–1684
2 Kelley, W. N., Greene, M. L., Rosenbloom, F. M., Henderson, J. F. and Seegmiller, J. E. (1969) Ann. Intern. Med. 70, 155–205
3 Murray, A. W. (1966) Biochem. J. 100, 664–670
4 Rosenbloom, F. M., Kelley, W. N., Miller, J., Henderson, J. F. and Seegmiller, J. E. (1967) J. Am. Med. Assoc. 202, 103–105
5 Krenitsky, T. A. (1969) Biochim. Biophys. Acta 179, 506–509
6 Dawson, D. M. (1971) Neurology 21, 621–626
7 Gutensohn, W. and Guroff, G. (1972) J. Neurochem. 19, 2139–2150
8 Atkinson, M. R. and Murray, A. W. (1965) Biochem. J. 94, 64–70
9 Sweetman, L. and Nyhan, W. L. (1969) Anal. Biochem. 31, 358–365
10 Bray, G. A. (1960) Anal. Biochem. 1, 279–285
11 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
12 Murray, A. W. (1967) Biochem. J. 140, 675–578
13 Nicol, T. and Bilbey, D. L. J. (1958) Nature 182, 191–192
14 Lowy, B. A., Ramot, B. and London, I. M. (1960) J. Biol. Chem. 235, 2920–2923
15 Scott, J. L. (1962) J. Clin. Invest. 41, 67–79
16 Henderson, J. F. (1972) Regulation of Purine Biosynthesis, American Chemical Society, Washington, D.C.
17 Wong, P. C. L. and Henderson, J. F. (1972) Biochem. J. 129, 1085–1094