Vol. 105, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1982 Pages 75-81

DIRECT INHIBITION OF BRAIN SEPIAPTERIN REDUCTASE BY A CATECHOLAMINE AND AN INDOLEAMINE

Setsuko Katoh, Terumi Sueoka and Shozo Yamada

Department of Biochemistry, Josai Dental University, Sakado, Saitama 350-02, JAPAN

Received January 18, 1982

SUMMARY: Rat brain sepiapterin reductase, which is involved in the biosynthesis of tetrahydrobiopterin, was directly inhibited by a catecholamine and an indoleamine which require tetrahydrobiopterin as a cofactor in the biosyntheses of themselves. A competitive inhibition appeared for sepiapterin reductase with respect to pterin substrate by l-norepinephrine (catecholamine) and serotonin, N-acetyl-serotonin and melatonin (indoleamines): K_i values of them were estimated as 3.4 mM, 2.3 mM, 0.20 μM and 30 μM, respectively with partially purified sepiapterin reductase from whole rat brain. Some possible co-regulatory mechanisms to control the formation of catecholamines or indoleamines and of tetrahydrobiopterin are discussed.

INTRODUCTION

In the biosyntheses of catecholamines and indoleamines, tetrahydrobiopterin is an essential component as a cofactor of tyrosine 3-monooxygenase (1,2) and tryptophan 5-monooxygenase (3), the first and rate limiting enzymes of these active monoamine biosyntheses. We have found, in this study, that rat brain sepiapterin reductase (EC 1.1.1.153), which catalyzes the formation of dihydrobiopterin from sepiapterin with NADPH (4,5) and is considered to be involved in the biosynthesis of tetrahydrobiopterin from GTP in brain (5,6,7), is directly inhibited by a catecholamine or an indoleamine *in vitro*. Since brain level of pteridine cofactor is relatively low and may be limiting, rapid modulation of tyrosine or tryptophan monooxygenase activity could occur *in vivo* by small change of an available pool of tetrahydrobiopterin to control the regulation of the biosyntheses of cate-

0006-291X/82/050076-07$01.00/0

cholamines and serotonin in brain (8). This finding in this study of interesting to suggest a possible co-reguratory mechanism between the formation of catecholamines and indoleamines and of pteridine cofactor in vivo. Accordingly, we have investigated the mechanism of inhibition of sepiapterin reductase by these active monoamines in vitro using a sepiapterin reductase purified from rat erythrocytes besides the enzyme preparation partially purified from whole rat brain.

EXPERIMENTAL PROCEDURES

Materials: Sepiapterin was extracted and crystalized from adult flies of sepia mutant of Drosophila melanogaster (9). NADPH and N-acetyl-serotonin were obtained from Sigma; L-Phenylalanine, L-tyrosine, L-tryptophan, serotonin from Wako Chemical Co.; L-Dopa, dopamine, l-norepinephrine, l-epinephrine from Tokyo Kasei; 5-Hydroxytryptophan, melatonin from Aldrich.

Enzyme preparation: Male Sprague-Dawley rats (150-200g) were used for enzyme source. Brain sepiapterin reductase was partially purified from whole brain through ammonium sulfate fractionation and hydroxylapatite column chromatography according to the method for liver sepiapterin reductase (5). Erythrocyte sepiapterin reductase was purified to apparent homogenity from the hemolysate by an affinity chromatography on Mātrex Red A and isoelectric focusing (10) after the same processes for brain enzyme preparation.

Sepiapterin reductase assay: Sepiapterin reductase was assayed photometrically at 420 nm (5) in the assay mixture contained 50 μM sepiapterin, 100 μM NADPH, 100 mM potassium phosphate buffer and a suitable amount of sepiapterin reductase in a final volume of 2.0 ml at pH 6.4. The reaction was performed at 25°C for 4 min starting by the addition of enzyme. One unit of sepiapterin reductase is defined as the amount which catalyzes the reduction of 1 μmole of sepiapterin per min at 25°C.

Inhibition of sepiapterin reductase: Inhibition of sepiapterin reductase was examined in the standard reaction mixture containing a compound to test for 4 min at final pH 6.4. The reaction was started by the addition of enzyme and measured photometrically at 420 nm. The reaction ommited the enzyme was performed, for each compound, as the control. Each compound was observed to be unchanged at least 4 min in the presence of sepiapterin and NADPH at pH 6.4. Amino acids except 5-hydroxytryptophan, and serotonin were dissolved in water. Catecholamines and 5-hydroxytryptophan were dissolved in 0.1N HCl. N-Acetyl-serotonin and melatonin were dissolved in absolute ethanol and were diluted with water just before adding to the reaction mixture. The reaction containing the same amount of ethanol ommited inhibitor was performed as the control.

Protein determination: Protein concentrations were estimated by the method of Lowry et al. (11) with bovine serum albumin as a standard.

TABLE 1

Effect of Catecholamines, Indoleamines and their Precursors on Sepiapterin Reductase Activity

Compound added*	Activity %		K_i (versus sepiapterin)	
	Brain enzyme	Erythrocyte enzyme	Brain enzyme	Erythrocyte enzyme
L-Phenylalanine**	100	100		
L-Tyrosine**	100	100		
L-Dopa	96	100		
Dopamine	88	92		
l-Norepinephrine	74	73	3.4 mM	3.1 mM
l-Epinephrine	100	100		
L-Tryptophan**	97	100		
L-5-Hydroxytryptophan	100	100		
Serotonin	79	73	2.3 mM	2.5 mM
N-Acetyl-serotonin	0	0	0.20 μM	0.17 μM
Melatonin	7	4	30 μM	25 μM

K_i's were determined by the methods of Dixon plots for l-norepinephrine and melatonin, and of Lineweaver-Burk plots for serotonin and N-acetyl-serotonin. The apparent K_m values of sepiapterin reductase of sepiapterin with NADPH were 14.3 μM with both erythrocyte and brain enzymes ((10) and this time estimation, respectively). *3mM added. **1 mM added.

REFERENCE

In an series of metabolites of catecholamine, 3 mM l-norepinephrine inhibited 30 % of sepiapterin reductase activity in the presence of 50 μM sepiapterin (Table 1). Dopamine has slite effect but dopa and epinephrine had no effect for the enzyme activity. As for indoleamine series, serotonin, N-acetyl-serotonin and melatonin inhibited brain sepiapterin reductase (Table 1). Almost all of the activity of sepiapterin reductase was inhibited by 3 mM of N-acetyl-serotonin and melatonin. As sepiapterin reductase is localized in all parts of rat brain, it was partially purified from whole brain for the experiment. Pure sepiapterin reductase from erythrocytes, which was prepared by the method developed recently for blood sepiapterin reductase in our laboratory (10), was also inhibited by these compounds with the same rate as observed in brain sepiapterin reductase (Table 1). Either L-phenylalanine, L-tyrosine, L-dopa, the precursors of

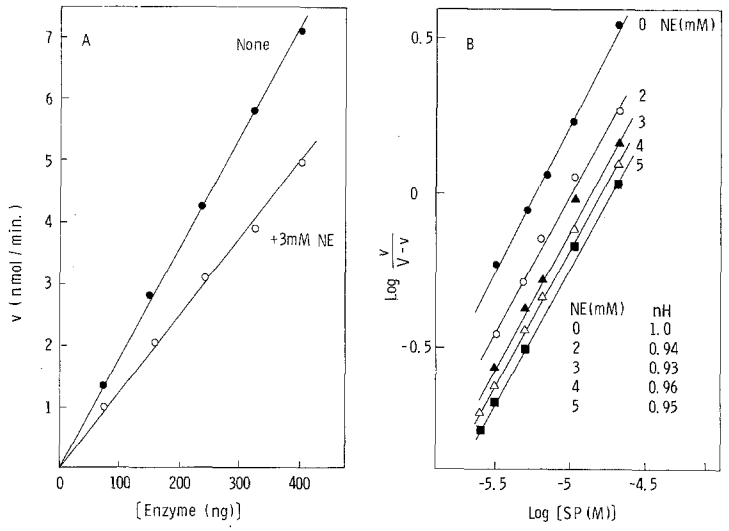

Fig.1 Inhibition on Sepiapterin Reductase by l-Norepinephrine and Melatonin analized with purified enzyme preparation from erythrocytes. (A)Effect of enzyme concentration on the rate of inhibition by l-norepinephrine. (B)Hill plots of enzyme inhibition by l-norepinephrine versus sepiapterin concentration. The apparent maximal velocity (V) for sepiapterin with NADPH was obtained by Lineweaver-Burk plots. (C and D)Lineweaver-Burk plots of enzyme inhibition by l-norepinephrine(C) and melatonin(D) as a function of pteridine substrate concentration. SP; sepiapterin, NE; l-norepinephrine, MT; melatonin.

catecholamine, nor L-tryptophan, L-5-hydroxytryptophan, the precursors of indoleamine, had any effect on the activity of sepiapterin reductase both from brain and erythrocytes. Inhibition mechanism of sepiapterin reductase by l-norepinephrine or by melatonin was analized as shown in Fig.1 using a pure preparation of sepiapterin reductase from erythrocytes. As the rate of inhibition by 3 mM l-norepinephrine on the different amount of the enzyme was observed as constant (A), this compound might inhibit the enzyme reversibly. By Hill plots of the enzyme inhibition by l-norepinephrine versus sepiapterin (B), Hill coefficient, nH was approximately 1.0 for sepiapterin in the presence or absence of l-norepinephrine. This is indicative of noncooperativity among the sites. Lineweaver-Burk plots of the enzyme inhibition by l-norepinephrine and melatonin appeared to be competitive with respect to the pterin substrate shown in (C) and

(D), respectively. Serotonin and N-acetyl-serotonin were also competitive inhibitors of the enzyme by Lineweaver-Burk plots with respect to sepiapterin. K_i value of each inhibitor was estimated and summarized in Table 1. N-Acetyl-serotonin and melatonin were far more effective inhibtors than l-norepinephrine and serotonin : K_i's of N-acetyl-serotonin and melatonin were about 1/20000 and 1/100 of that of l-norepinephrine, respectively (Table 1).

DISCUSSION

Using either a partially purified sepiapterin reductase from whole brain or a purified enzyme preparation from erythrocytes, a catecholamine and indoleamines proved to be a competitive inhibition against pterin substrate. These compounds appear to be the first pterin substrate-competitive inhibitors of sepiapterin reductase among many inhibitors including substrate analogue pteridines reported previously (5). Since sepiapterin reductase shows a strict specificity for sepiapterin as the substrate (5), catecholamines or indoleamines are not suitable for binding at the pterin substrate site of the enzyme. The similar kinetics were observed in catecholamines for pteridine cofactor-competitive inhibition in tyrosine 3-monooxygenase as feed back inhibition by end product (1) (Fig.2) showing IC_{50} versus tetrahydrodimethyl pterin and K_i versus tetrahydrobiopterin with l-norepinephrine of 1 mM and 0.1 mM, respectively (12,13). Though K_i's of N-acetyl-serotonin and melatonin of brain sepiapterin reductase were much smaller than the values of l-norepinephrine and serotonin (Table 1), the former two compounds are formed only in pineal gland and end product melatonin moves into brain while the latter two are formed in brain. Then, there might be a regulatory system in the formation of norepinephrine or serotonin, in brain, by the amount of melatonin besides the co-regulatory mechanism of the for-

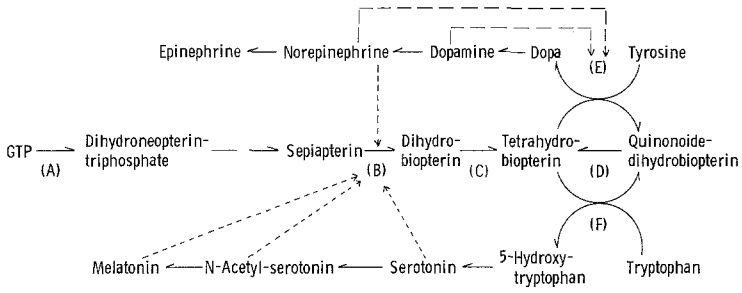

Fig.2 Relationship between the Biosynthetic Pathways of Tetrahydrobiopterin, Catecholamines and Indoleamines. Inhibitions (in vitro) were observed in this study (-----) or previous works (— — —)(1). (A);GTP cyclohydrolase, (B);Sepiapterin reductase, (C);Dihydrofolate reductase, (D);Dihydropteridine reductase, (E);Tyrosine 3-monooxygenase, (F);Tryptophan 5-monooxygenase.

mation of norepinephrine and serotonin via the regulation of tetrahydrobiopterin biosynthesis through the direct inhibition of sepiapterin reductase by catecholamines and indoleamines themsleves (Fig.2). It is recently observed (14) that intracellular biopterin content of rat pineal glands in organ culture was reduced 50 percent by treatment with l-norepinephrine or cyclic adenosine monophosphate presumably by an adrenergic cyclic adenosine monophosphate-dependent mechanism via inhibition of biopterin synthesis. This decline of biopterin content might be, in part, caused by a direct inhibition of sepiapterin reductase by norepinephrine in the process of biopterin biosynthesis, but further in vivo animal studies are required in relation to the metabolic regulation between pteridine cofactor and catecholamines and indoleamines in brain.

REFERENCES

1. Nagatsu, T., Levitt, M. and Udenfriend, S. (1964) J. Biol. Chem. 239, 2910-2917.
2. Brenneman, A.R. and Kaufman, S. (1964) Biochem. Biophys. Res. Commun. 17, 177-183.
3. Nakamura, S., Ichiyama, A. and Hayaishi, O. (1965) Fed. Proc. 24, 604.
4. Nagai, M. (1968) Arch Biochem. Biophys. 126, 426-435.
5. Katoh, S. (1971) Arch Biochem. Biophys. 146, 202-214.
6. Lee, C.L., Fukushima, T. and Nixon J.C. (1979) Chemistry and Biology of Pteridines (Kislik, R.K. and Brown, G.M. eds) ppl25-128, Elsevier North-Holland, New York.

7. Kapatos, G., Katoh, S. and Kaufman, S. (1982) Function and Regulation of Monoamine Enzymes - Basic and Clinical Aspects (Usdin, E., Neiner, N. and Youdim, M. eds), Macmillan Publishers Ltd. New York, (in press)
8. Kaufman, S. (1975) Neurobiological Mechanisms of Adaptation and Behavior (Mandell, A.J. ed) ppl27-136, Reven Press, New York.
9. Tsusue, M. and Akino, M. (1965) Zool. Mag. 74, 91-94.
10. Sueoka, T. and Katoh, S. (Manuscript submitted for publication).
11. Lowry, O.H., Rosebrough, N,J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
12. Udenfriend, S., Zaltzman-Nierenberg, P. and Nagatsu, T. (1965) Biochem. Pharmacol. 14, 837-845.
13. Nagatsu, T., Matsuura, S. and Sugimoto, T. (1972) Biochem. Pharmacol. 21, 1945-1953.
14. Kapatos, G., Kaufman, S., Leller, J.L. and Kleen D.C. (1981) Science, 213, 1129-1131.