TYROSINE HYDROXYLASE IN BLOOD LEUKOCYTES

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Tyrosine hydroxylase (TH), which catalyzes the oxidative conversion of L-tyrosine into L-dihydroxyphenylalanine (dopa), the initial stage of catecholamine biosynthesis, is recognized as a neurospecific enzyme [14]. The immediate product of the TH reaction, namely dopa, evidently does not participate in plastic processes but performs the role of catecholamine precursor. In animals at a lower level of evolutionary development, in the Coelenterata, for example, dopa performs the function of a neurotransmitter [12].

The blood dopa level depends on the physiological conditions and it rises sharply during emotional stress [2, 6]. It is considered that catecholamines and dopa, found in the blood, are produced in the adrenals, the chromaffin tissue of which is related to nerve tissue in its embryonic origin. However, the possibility cannot be ruled out that dopa biosynthesis may in fact take place in the blood also. The basis for this view is provided by the well known facts of the presence of nearly all enzymes of catecholamine biosynthesis in the blood: phenylalanine hydroxylase, dopa-decarboxylase, and dopamine β-hydroxylase [1, 9]. Phenylalanine-hydroxylase and tyrosine-hydroxylase activity may belong to the same enzyme with two active centers. Besides this, tuftsin, a fragment of IgG formed in leukocytes, exhibits affinity for TH, as has been shown for preparations of brain TH [3].

EXPERIMENTAL METHOD

Heparinized blood plasma containing cells was solubilized with Triton X-100, by adding a 1% solution of Triton X-100 in 0.05 M Tris-maleate, pH 6.0, up to a final concentration of 0.1%, after which it was centrifuged during cooling for 10 min at 15,000g to sediment the undissolved particles. The resulting supernatant was used for determination of TH activity. Blood plasma was fractionated in a Verografin–Ficoll density gradient [11], yielding fractions of leukocytes, lymphocytes, and platelets. The velocity of the tyrosine-hydroxylase reaction was determined spectrophotometrically [7]. Protein was determined by Lowry's method [15].

To compare activity in the blood plasma and brain structures of the rats, the striatum and hypothalamus were isolated from the animals. The tissue was homogenized in 10 volumes of 0.05 M Tris-maleate, pH 6.0, containing 0.1% of Triton X-100. The sample was centrifuged at 15,000g and the supernatant used to determine activity of the enzyme. The substance 6,7-dimethyl-5,6,7,8-tetrahydropterine ($DMPH_4$, from Calbiochem, USA), recrystallized from 65% ethanol, was used as the pterine coenzyme.

EXPERIMENTAL RESULTS

Assuming that the blood TH activity may be connected with the blood cells, they were destroyed by a nonpolar detergent. By varying the concentrations of tyrosine, $DMPH_4$, catalase, and dithiothreitol, and also the volume of plasma, the pH of the medium, and the temperature, conditions were found under which TH activity was discovered in blood plasma. The following conditions were optimal for determination of TH activity in the blood (37°C): 0.05 M Tris-maleate buffer (pH 6.0-6.2), 0.03-0.05 ml of blood plasma, 1-36 μM tyrosine, 35 units of catalase, 65 μM dithiothreitol, and 150-260 μM of pterine coenzyme.

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TABLE 1. TH Activity in Blood Plasma and Brain Structures (M ± m)

Test object	Reaction velocity, moles · min^{-1}/mg protein		
	blood plasma	hypothalamus	striatum
Rat (n = 18)	24,0±3,0	34,0±4,0	35,0±4,3
Man (n = 12)	19,4±2,6	—	—

Legend. Solubilized preparations of blood plasma, hypothalamus, and striatum. Tyrosine concentrations: 36 μM for plasma, 96 μM for homogenates of rat brain structures. $DMPH_4$ concentration 183 μM. Protein 30 μg/ml sample. n) Number of investigations.

TABLE 2. Velocity of Tyrosine Hydroxylase Reaction in Preparations of Rat Blood Plasma and Cells (M ± m, n = 5)

Test object	Reaction velocity, nmoles · min^{-1}/mg protein
Native plasma	1,0
Solubilized plasma	18,0±0,8
Solubilized leukocytes	24,0±0,8
Native leukocytes	3,0±0,5
Solubilized platelets	0
Solubilized lymphocytes	1,0

Legend. Here and in Table 4: 0.05 M Tris-maleate buffer (pH 6.0); tyrosine 36 μM, $DMPH_4$ 146 μM, protein 25 μg/ml of sample.

TABLE 3. Velocity of Tyrosine-Hydroxylase Reaction in Leukocytes Determined from Increase in $DMPH_2$ and Dopa

Expt. No.	$DMPH_2$	dopa	$DMPH_2$/dopa
1	21,0	20,5	1,02
2	22,0	27,5	1,3
3	23,1	22,4	1,03
4	22,8	23,2	0,98

Legend. 0.05 M Tris-maleate buffer (pH 6.0). Tyrosine 36 μM, catalase 33 units, dithiothreitol 65 mM, $DMPH_4$ 147 μM.

It will be clear from Table 1 that the velocity of the tyrosine-hydroxylase reaction in preparations of rat blood plasma is comparable with the reaction velocity in preparations of brain structures, if values obtained under optimal conditions for each preparation are compared; for solubilized blood plasma the tyrosine concentration in the medium was 36 μM, for solubilized brain homogenates it was 96 μM. TH activity in human blood was similar to that in rat blood (Table 1). TH activity in rat blood cells was concentrated mainly in the leukocyte fraction, and could be detected only after destruction of the cells, evidence that the location of the enzyme is intracellular (Table 2). To prove that the leukocytes did in fact possess TH activity, the reaction velocity was measured in the same samples on the basis of the increase not only in the oxidized coenzyme of the reaction ($DMPH_2$), but also of

TABLE 4. Effect of Dopamine, α-Methyl-p-tyrosine, and Flufenazine on Velocity of Tyrosine-Hydroxylase Reaction in Preparations of Peripheral Blood Leukocytes (M ± m)

Variant of experiment	Reaction velocity, nmoles·min^{-1}/mg protein
Control 1 (tyrosine 36 μM)	25,0±2,5
Dopamine (10^{-6} M)	19,2±1,9
Dopamine (10^{-5} M)	15,6±1,7
Dopamine ($2 \cdot 10^{-5}$ M)	4,8±0,5
α-Methyl-p-tyrosine ($5 \cdot 10^{-4}$ M)	17,0±1,5
α-Methyl-p-tyrosine ($5 \cdot 10^{-4}$ M)	5,8±0,5
Control 2 (tyrosine 72 μM)	12,8±1,2
Flufenazin (tyrosine 72 μM)	25,6±2,5

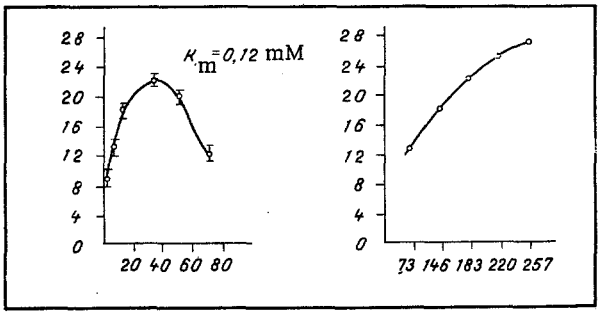

Fig. 1 Fig. 2

Fig. 1. Dependence of velocity of tyrosine-hydroxylase reaction on L-tyrosine concentration for preparations of peripheral blood leukocytes. Abscissa, concentration of L-tyrosine (in μM); ordinate, reaction velocity (in nmoles·min^{-1}/mg protein). Concentration of $DMPH_4$ 146 μM.

Fig. 2. Dependence of velocity of tyrosine-hydroxylase reaction on $DMPH_4$ concentration for preparations of peripheral blood leukocytes. Abscissa, concentration of $DMPH_4$ (in μM); ordinate, reaction velocity (in nmoles·min^{-1}/mg protein).

the specific reaction product — dopa, using the method described previously [7] for this purpose. As Table 3 shows, the $DMPH_2$/dopa ratio was close to 1, similar to what was found for preparations of brain tissue [7]. Meanwhile the presence of a hydroperoxide of the superoxide radical in the leukocytes necessitated the addition of catalase and dithiothreitol to the assay medium, which had to be added after tyrosine.

Data characterizing the kinetic properties of TH of the leukocytes and blood plasma relative to the reaction substrate, tyrosine, and the pterine coenzyme, $DMPH_4$, are given in Figs. 1 and 2. Leukocytic TH is characterized by substrate inhibition, manifested by the presence of a pterine coenzyme of synthetic origin ($DMPH_4$), and in this respect it is similar to the TH of the hypothalamus (Fig. 1). The Michaelis constants (K_m), calculated by the method of Eisenthal and Cornish-Bowden [5], were 0.01 and 0.12 mM respectively. K_m for tyrosine and leukocytic TH, as is clear from the above account, was much lower than K_m for TH from brain structures, which is about 0.05 mM [8]. The value of K_m for $DMPH_4$ and leukocytic TH was close to that for brain TH [8]. Like brain TH, leukocytic TH is inhibited by the tyrosine analog α-methyl-p-tyrosine, and undergoes retrograde inhibition by dopamine (Table 4).

The results are evidence that leukocytes and blood plasma possess TH activity, and that the properties of the enzyme from the blood and from brain structures are similar. The discovery of TH in the peripheral blood enables the regulation of leukocyte function to be regarded from a new aspect and, in particular, under stress conditions, when blood levels

of dopa and catecholamines, which are known to participate in the regulation of phagocytosis [4], are raised [10]. The outer membrane of the leukocytes (neutrophils) possesses α- and β-adrenoreceptors [13, 16]. These facts as a whole show that leukocytes have the essential components of the catecholaminergic system. The possibility cannot be ruled out that TH plays a definite role in the regulation of adrenoreceptor activity on the cell membrane of the leukocytes, while at the same time, it is under the regulatory influence of these receptors, just as takes place in the brain. It is very probable that TH in the leukocytes performs functions as yet unknown.

The qualitative similarity of the kinetic properties of TH in the blood and brain makes it possible to study the properties of the limiting enzyme of catecholamine biosynthesis in man, and it may prove a useful tool for the solution of problems connected with neurology and psychiatry.

This investigation thus showed for the first time that TH activity is present in leukocytes in mammalian peripheral blood.

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