The inhibition of protein synthesis and myocardial hypertrophy by oxythiamine may be caused by disturbance of the synthesis of nucleic acids as a result of the inhibition of the transketolase and pentose-phosphate pathway, as well as by inhibition of the energy production due to the depression of ferments associated with vitamin B_1, e.g. pyruvate- and ketoglutarate dehydrogenase which catalyse the oxidating decarboxylation of keto acids. However, this latter explanation seems to be less plausible since, in disturbance of utilization of the carbohydrates, the myocardium may largely use, as a source of energy, fatty acids, the oxidation of which avoids the reaction of the oxidative decarboxylation of the keto acids[12,13].

On the whole the present results support the suggestion of the important role of the pentose-phosphate pathway in activation of nucleic acids and protein synthesis which underlies the hypertrophy and the adjustment of the heart to a sustained load.

Выводы. У кроликов с гиперфункцией сердца, вызванной экспериментальным стенозом аорты, активация синтеза нуклеиновых кислот и ьелков в миокарде сопровождается резким повышением активности транскетолазы - фермента пентозо-фосфатного пути превращения глюкозы - в миокарде. Подавление транскетолазы специфическим ингиьитором окситиамином полностью снимает активацию синтеза нуклеиновых кислот и тормозит развитие гипертрофии миокарда.

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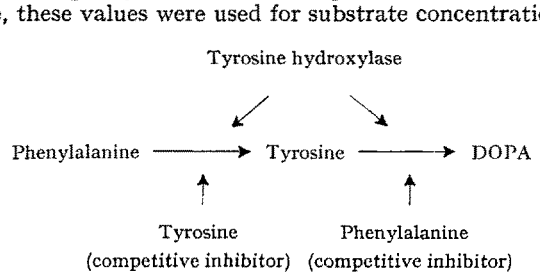
The Effect of High Phenylalanine Concentration on the Formation of DOPA from Phenylalanine and Tyrosine by Tyrosine Hydroxylase

IKEDA, LEVITT and UDENFRIEND[1] reported that the hydroxylation of phenylalanine to tyrosine is also catalysed by tyrosine hydroxylase[2], which catalyses the conversion of tyrosine to DOPA, the initial step of biosynthesis of norepinephrine, in brain and sympathetically innervated tissues. They found that phenylalanine and tyrosine are competitive inhibitors of the enzyme[1]. It has been suggested that in phenylketonuria huge amounts of phenylalanine may inhibit norepinephrine formation by competing with tyrosine on tyrosine hydroxylase[2]. However, it is not clear since phenylalanine also produces DOPA[1]. A theoretical and experimental study as to the effect of large amounts of phenylalanine on the formation of DOPA from both phenylalanine and tyrosine by tyrosine hydroxylase is reported in this communication.

In the theoretical kinetic treatment, the following values were used from the report of IKEDA, LEVITT and UDENFRIEND[1]: Km for phenylalanine, $3 \cdot 10^{-4} M$; Km for tyrosine, $5 \cdot 10^{-5} M$; V_{max} for tyrosine/V_{max} for phenylalanine, 20/1. Blood concentration of tyrosine and phenylalanine in health and that of tyrosine in phenylketonuria is about $1 \cdot 10^{-4} M$, whereas that of phenylalanine in phenylketonuria is as high as $3 \cdot 10^{-3} M$. Therefore, these values were used for substrate concentrations.



To calculate the amount of DOPA formed through the above-mentioned process[3], following symbols are used: P, concentration of phenylalanine; T, concentration of tyrosine; D, concentration of DOPA; K_P, Michaelis constant for phenylalanine; K_T, Michaelis constant for tyrosine; V_P, V_{max} for phenylalanine; V_T, V_{max} for tyrosine; t, time.

$$x = \frac{P}{K_P}, \qquad y = \frac{T}{K_T}, \qquad z = \frac{D}{K_T},$$

$$x' = \frac{dx}{dt}, \qquad y' = \frac{dy}{dt}, \qquad z' = \frac{dz}{dt},$$

$$a = -\frac{V_P}{K_P}, \qquad b = \frac{V_P}{K_T}, \qquad c = \frac{V_T}{K_T}.$$

The decrease of phenylalanine is expressed by LINEWEAVER-BURK equation[4] in the presence of a competitive inhibitor, tyrosine

$$\frac{dP}{dt} = -\frac{V_P}{1 + \frac{K_P}{P} + \frac{K_P}{P} \cdot \frac{T}{K_T}}$$

and is rewritten as

$$x' = \frac{a}{1 + \frac{1}{x} + \frac{y}{x}} = \frac{a\,x}{1 + x + y}.$$

The variation of tyrosine concentration dT/dt is expressed as the difference of 2 LINEWEAVER-BURK equations in the presence of competitive inhibitors

$$\frac{dT}{dt} = \frac{V_P}{1 + \frac{K_P}{P} + \frac{K_P}{P} \cdot \frac{T}{K_T}} - \frac{V_T}{1 + \frac{K_T}{T} + \frac{K_T}{T} \cdot \frac{P}{K_P}}$$

The first term in the right side is the one for tyrosine formation from phenylalanine and the second term is the

one for tyrosine oxidation to DOPA. The equation is rewritten as

$$y' = \frac{b}{1 + \frac{1}{x} + \frac{y}{x}} - \frac{c}{1 + \frac{1}{y} + \frac{x}{y}} = \frac{b\,x - c\,y}{1 + x + y}.$$

The LINEWEAVER-BURK equation from tyrosine to DOPA is

$$\frac{dD}{dt} = \frac{V_T}{1 + \frac{K_T}{T} + \frac{K_T}{T} \cdot \frac{P}{K_P}}$$

and is rewritten as

$$z' = \frac{c}{1 + \frac{1}{y} + \frac{x}{y}} = \frac{c\,y}{1 + x + y}.$$

The MACLAURIN's series of $z(t)$ for small t is

$$z(t) = z(0) + z'(0)\,t + \frac{z''(0)}{2}\,t^2 + \cdots$$

$$= z(0) + \frac{c\,y(0)}{1 + x(0) + y(0)}\,t + \frac{c}{2}\,\frac{y'(0)\,(1 + x(0)) - y(0)\,x'(0)}{(1 + x(0) + y(0))^2}\,t^2 + \cdots.$$

When t is very small, the terms where the index of t is larger than 2 are negligible. The amount of DOPA ($K_T\,z(t)$) formed in short time t in case of $z(0) = 0$ is calculated as follows:

in the normal state

$$x(0) = \frac{P(0)}{K_P} = \frac{1 \cdot 10^{-4} M}{3 \cdot 10^{-4} M} = \frac{1}{3},$$

$$y(0) = \frac{T(0)}{K_T} = \frac{1 \cdot 10^{-4} M}{5 \cdot 10^{-5} M} = 2,$$

$$K_T\,z(t) = \frac{y(0)}{1 + x(0) + y(0)}\,K_T\,c\,t = \frac{2}{1 + 1/3 + 2}\,K_T\,c\,t \fallingdotseq 0.6\,K_T\,c\,t$$

in phenylketonuric state

$$x(0) = \frac{P(0)}{K_P} = \frac{3 \cdot 10^{-3} M}{3 \cdot 10^{-4} M} = 10,$$

$$y(0) = \frac{T(0)}{K_P} = \frac{1 \cdot 10^{-4} M}{5 \cdot 10^{-5} M} = 2,$$

$$K_T\,z(t) = \frac{y(0)}{1 + x(0) + y(0)}\,K_T\,c\,t = \frac{2}{1 + 10 + 2}\,K_T\,c\,t \fallingdotseq 0.15\,K_T\,c\,t.$$

The ratio

$$\frac{\text{the amount of DOPA formed in phenylketonuric state}}{\text{the amount of DOPA formed in normal state}} = \frac{0.15\,K_T\,c\,t}{0.6\,K_T\,c\,t} = \frac{1}{4}.$$

Therefore, kinetic analysis showed that when phenylalanine concentration increases from $1 \cdot 10^{-4} M$ to $3 \cdot 10^{-3} M$, the overall conversion of phenylalanine and tyrosine to DOPA should decrease from 100% to about 25%.

This theoretical kinetic result was further examined experimentally. Tyrosine hydroxylase was partially purified from bovine adrenal medulla[2]. Incubation mixture contained: 200 μmoles acetate buffer (pH 6.0), L-phenylalanine and L-tyrosine containing L-tyrosine-C^{14} and L-phenylalanine-C^{14} (uniformly labelled) to give a specific activity of 800 cpm/mμmole, 100 μmoles mercaptoethanol, 1 μmole of 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydro-pteridine, enzyme in 0.2 ml volume (2 mg protein), and water to 1.0 ml. The incubation was carried out at 37 °C for 15 min. DOPA-C^{14} was isolated by alumina and counted by a scintillation spectrometer[2]. Boiled enzyme was used for the blank incubation.

As shown in the Table, the presence of same concentration ($1 \cdot 10^{-4} M$) of phenylalanine as that of tyrosine ($1 \cdot 10^{-4} M$) did not affect the formation of DOPA. However, when increasing phenylalanine concentration to $3 \cdot 10^{-3} M$ (30 times higher than tyrosine concentration), formation of DOPA decreased to about 30%.

These results suggest that an enormous preponderance of phenylalanine over tyrosine in blood, and therefore in brain in phenylketonuria, can inhibit the formation of DOPA by tyrosine hydroxylase in vivo, causing the decrease of catecholamines[5].

Effect of high concentration of phenylalanine on the formation of DOPA from phenylalanine and tyrosine by tyrosine hydroxylase

Substrate concentration in M		Formation of DOPA in mμmoles
Tyrosine	Phenylalanine	
$1 \cdot 10^{-4}$	0	5.16
$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	5.06
$1 \cdot 10^{-4}$	$3 \cdot 10^{-3}$	1.53

Zusammenfassung. Auf der Basis gegenseitiger Hemmung von Phenylalanin konnte die enzymatische Bildung des DOPA über Phenylalanin und Tyrosin mittels Tyrosinhydroxylase untersucht werden. Eine 70%ige Hemmung der enzymatischen DOPA-Bildung ergab sich im Fall der dreissigfachen Konzentration des Phenylalanin. Die Möglichkeit einer Hemmung der Norepinephrin-Bildung in Phenylketonurie wird diskutiert.

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[3] Product inhibition by DOPA is negligible, when small percentage of the substrates is converted to DOPA. It is assumed that the enzyme-tyrosine complex dissociates before conversion to DOPA as reported by IKEDA, LEVITT and UDENFRIEND[1].

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