Enhancement of Tyrosine Transamination in vivo by Catecholamines

Several findings indicate that catecholamines may influence the activity of L-tyrosine: 2-oxyglutarate-aminotransferase EC 2.6.1.5 (TAT), but the results have been rather controversial. In adrenalectomized rats, the depletion of catecholamines by α-methyl-*p*-tyrosine or by reserpine increased, whereas repletion of catecholamines after administration of L-3,4-dihydroxyphenylalanine (L-dopa) decreased TAT activity in the supernatant of liver homogenates[1]; norepinephrine, however, had no effect[2]. Other authors found no definite correlation between adrenergic blockade and catecholamine depletion on the one hand and enhanced activity of TAT on the other hand. The increase of TAT activity induced by adrenergic blocking or catecholamine depleting agents was partly attributed to their stimulating effect on corticosterone secretion[3]. In cultures of fetal rat liver, epinephrine even caused induction of TAT[4]. In addition, it has recently been shown that the above mentioned catecholamine depletors decreased TAT activity in the central nervous system and that this effect was antagonized by L-dopa[5].

The present paper shows that in normal rats acute elevation of catecholamines lowers the level of endogenous plasma tyrosine and enhances the urinary excretion of *p*-hydroxyphenylpyruvic acid (PHPPA). Concomitantly, the activity of TAT in liver is enhanced.

Materials and methods. Male albino rats of Wistar origin (Füllinsdorf), weighing 80–120 g, kept on chow and water (ad libitum except during the period of the experiment), were injected i.p. with saline (controls), L-dopa, dopamine, L-norepinephrine and L-epinephrine respectively (see Tables). PHPPA was determined by the spectrophotometric method of HOLCOMB et al.[6] in urine collected for 2 h (dopa, dopamine, norepinephrine) or 4 h (epinephrine) after injection. Paper chromatography of the final butylacetate extracts (Whatman No. 1; butanol, glacial acetic acid, water 5:1:4; development with 2,4-dinitrophenylhydrazine) revealed the presence of only one spot with an Rf (0.9) and an UV-spectrum identical with those of authentic PHPPA.

In a parallel series of rats treated as indicated above and killed at different time intervals after the injection, the tyrosine content of blood plasma was determined colorimetrically with 1-nitroso-2-naphthol[7]. In addition, the TAT activity was measured in the supernatant of liver homogenates by a method similar to that of WEINSTEIN et al.[8]. The supernatant of liver homogenate (9 vol 0.1 *M* K-phosphate buffer, pH 7.4, 40,000 × *g*/30 min) was incubated at 37.5 °C for 10 min in the presence of $6 \times 10^{-3} M$ α-ketoglutarate and $5 \times 10^{-3} M$ L-2-^{14}C-tyrosine. After deproteinization (with $HClO_4$), the amino derivatives were removed by Dowex 50 × 4 (pH 2.5) and the radioactivity of the effluent containing the products of ^{14}C-tyrosine transamination was measured in a liquid scintillation counter.

A possible interference of L-dopa, catecholamines, pyruvic acid or hydroxypyruvic acid with the assay methods was excluded by addition of these substances to the blood plasma or urine prior to the extraction procedures. All rats were sacrificed at the same time of the day (11.00–12.00 h) in order to avoid variations in tyrosine concentration and PHPPA excretion due to the diurnal rhythm of TAT.

Results. After administration of single doses of L-dopa and dopamine or repeated injections of L-norepinephrine and L-epinephrine respectively, the levels of tyrosine in blood plasma show a significant decrease to a minimum of 60–70% of untreated controls. This diminution is rather rapid and transient. The tyrosine level begins to decrease $^1/_2$ h following injection, reaches its minimum within 1–$1^1/_2$ h and is partly recovered after 2 h (Table I). Concomitantly, the urinary elimination of PHPPA during 2 or 4 h is enhanced reaching values of 150–190% compared to untreated controls (100%) (Table II). Moreover, 1–2 h after injection of the catecholamines, the TAT activity in the supernatant of liver homogenates shows an increase of 150–200% over controls (100%) (Table III).

Discussion. PHPPA represents the first metabolite of tyrosine transamination, the major pathway of tyrosine metabolism, and no other major metabolic source for this keto acid has been described. After administration of

[1] I. B. BLACK and J. AXELROD, Proc. natn. Acad. Sci. *59*, 1231 (1968).
[2] I. B. BLACK and J. AXELROD, J. biol. Chem. *244*, 6124 (1969).
[3] W. C. GOVIER, W. LOVENBERG and A. SJOERDSMA, Biochem. Pharmac. *18*, 2661 (1969).
[4] W. D. WICKS, Science *160*, 997 (1968).
[5] J. W. GIBB and J. G. WEBB, Pharmacologist *11*, 275 (1969).
[6] I. J. HOLCOMB, D. S. MCCANN and A. J. BOYLE, Analyt. Chem. *37*, 1657 (1965).
[7] T. P. WAALKES and S. UDENFRIEND, J. Lab. clin. Med. *50*, 733 (1957).
[8] A. WEINSTEIN, G. MEDES and G. LITWACK, Analyt. Biochem. *21*, 86 (1967).

Table I. Effect of L-dopa and catecholamines i.p. on the concentration of tyrosine in blood plasma of rats

Time (min)	Controls	L-Dopa		Dopamine		L-norepinephrine		L-epinephrine	
	μg/ml	μg/ml	% of controls	μg/ml	% of controls	μg/ml	% of controls	μg/ml	% of controls
	12.05 ± 0.45								
30		9.67 ± 1.83	85.9 ± 11.1			11.48 ± 0.15	86.1 ± 1.6	10.08 ± 0.12	75.2 ± 0.4[a]
60		7.68 ± 2.00	64.8 ± 8.2[a]	7.31 ± 0.72	60.2 ± 1.8[a]	10.46 ± 0.57	84.6 ± 6.9		
90						9.76 ± 0.70	73.3 ± 5.9[a]	9.37 ± 0.25	70.3 ± 2.5[a]
120		10.89 ± 2.39	92.2 ± 7.8	10.36 ± 0.88	86.0 ± 5.9	10.16 ± 0.72	82.3 ± 7.7		

L-Dopa (200 mg/kg) or dopamine (100 mg/kg) were injected in single doses, whereas 100 μg/kg of L-norepinephrine or L-epinephrine were administered twice, i.e. at the beginning of the experiment and 60 min later. The values represent averages ± S.E. of 3 duplicate experiments each with a pool of plasma from 2 rats. [a] $p < 0.01$.

Table II. Increase of urinary elimination of *p*-hydroxyphenylpyruvic acid in rats treated with L-dopa or catecholamines i.p.

Time (min)	Controls	L-Dopa		Dopamine		L-norepinephrine		L-epinephrine	
	µg/rat	µg/rat	% of controls	µg/rat	% of controls	µg/rat	% of controls	µg/rat	% of controls
120	4.22 ± 0.65	8.74 ± 3.51	193.4 ± 6.7[a]	8.11 ± 2.34	172.8 ± 7.6[a]	8.88 ± 1.86	181.9 ± 24.8[a]		
240	13.91 ± 1.37							19.46 ± 0.40	153.9 ± 9.6[a]

L-Dopa (200 mg/kg) or dopamine (100 mg/kg) were administered in single doses before the start of the urine collection. 100 µg/kg L-norepinephrine or 50 µg/kg L-epinephrine were injected at the beginning of the experiment and 60 min later. The values represent means ± S.E. of 3–5 duplicate experiments each with a pool of urine from 6 rats. [a] $p < 0.01$.

Table III. Increase of metabolites of ^{14}C-tyrosine transamination calculated as µmoles of hydroxyphenylpyruvic acid in the supernatant of liver homogenates of rats pretreated with catecholamines i.p.

Controls	Dopamine		L-norepinephrine		L-epinephrine	
µmoles/g/h	µmoles/g/h	% of controls	µmoles/g/h	% of controls	µmoles/g/h	% of controls
12.96 ± 0.85	25.87 ± 2.36	203.8 ± 33.4[a]	18.94 ± 0.95	146.8 ± 7.7[a]	24.57 ± 1.70	192.2 ± 23.4[a]

Dopamine (100 mg/kg) was injected 120 min, L-norepinephrine (100 µg/kg) or L-epinephrine (100 µg/kg) 90 and 30 min before decapitation. The values represent averages ± S.E. of 3 experiments each with a pool of 3 livers. [a] $p < 0.01$.

catecholamines or L-dopa, not only the excretion of PHPPA is enhanced, but in addition there is a decrease in blood tyrosine as well as an increased activity of hepatic TAT. These findings clearly demonstrate that in normal rats an acute elevation of catecholamines or of their precursor L-dopa enhances tyrosine transamination. Accordingly, a decrease of plasma tyrosine has been recently observed in patients with Parkinson's syndrom treated with L-dopa[9].

Three pathways are known to be of importance for the metabolism of tyrosine, i.e. transamination[10,11], incorporation into proteins[12] and meta-hydroxylation to dopa[13]. The absolute level of tyrosine in blood and tissues is of similar order to the K_m value for the tyrosine hydroxylase ($5 \times 10^{-5} M$)[14]. Consequently, a decreased availability of tyrosine due to an enhanced transamination presumably diminishes the synthesis of catecholamines. Therefore, as suggested for the brain on the basis of experiments with catecholamine depleting drugs[5], tyrosine transamination may be involved in the regulation of the biosynthesis of catecholamines in the various tissues.

The mechanisms by which catecholamines enhance the activity of TAT are not yet known. An activation mediated by cyclic 3′,5′-adenosine monophosphate[4] and by corticosteroids[15] has to be considered. Some discrepancies in the literature (see above) might partly be attributed to the use of drugs which interfere not only with catecholamines but also with other hormones (e.g. corticosteroids)[3]. Furthermore, adrenalectomized animals[1] seem to react differently from normal animals with regard to TAT activation by catecholamines.

In conclusion, catecholamines markedly enhance the transamination of tyrosine in normal rats. Consequently, the biosynthesis of the catecholamines may not only be regulated by the hydroxylation[13], but also by the transamination of tyrosine.

Zusammenfassung. Bei normalen Ratten bewirken L-Dihydroxyphenylalanin, Dopamin, Noradrenalin und Adrenalin eine Herabsetzung des Plasma-Tyrosins, eine erhöhte Ausscheidung von *p*-Phenylbrenztraubensäure im Urin und eine Zunahme der Aktivität der Tyrosin-Aminotransferase der Leber. Es wird daraus geschlossen, dass Catecholamine die extrazerebrale Transaminierung von Tyrosin steigern und dass die Tyrosin-Transaminierung möglicherweise an der Regulation der Catecholamin-Biosynthese beteiligt ist.

G. Bartholini, K. F. Gey and A. Pletscher

Research Department,
F. Hoffmann-La Roche & Co. Ltd.,
CH-4002 Basle (Switzerland), 28 May 1970.

[9] M. H. Van Woert and M. B. Bowers jr., Experientia *26*, 161 (1970).
[10] W. E. Knox and L. M. Knox, Biochem. J. *49*, 686 (1951).
[11] B. N. La Du and D. M. Greenberg, J. biol. Chem. *190*, 245 (1951).
[12] A. Meister, *Biochemistry of the Amino Acids* (Academic Press, N.Y., London 1965).
[13] T. Nagatsu, M. Levitt and S. Udenfriend, J. biol. Chem. *239*, 2910 (1964).
[14] S. Udenfriend, Pharmac. Rev. *18*, 43 (1966).
[15] A. B. King, Proc. Soc. exp. Biol. Med. *130*, 445 (1969).