Enhancement of rat brain catecholamine synthesis by administration of small doses of tyrosine and evidence for substrate inhibition of tyrosine hydroxylase activity by large doses of the amino acid

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Rat brain catecholamine synthesis is enhanced by small doses of tyrosine, but not by doses of 50 mg/kg body wt. and above. It is suggested that these latter doses overcome the above enhancement by causing a substrate inhibition of tyrosine hydroxylase activity.

There is considerable evidence suggesting that cerebral catecholamine synthesis is influenced by brain tyrosine concentration. Thus catecholamine synthesis is known to be decreased when brain tyrosine concentration is lowered by administration of tryptophan and other amino acids that compete with circulating tyrosine for the same cerebral uptake mechanism (Wurtman *et al.*, 1974; Wurtman & Fernstrom, 1975; Fernstrom, 1976; Carlsson & Lindqvist, 1978) and when transamination of brain tyrosine is enhanced at 2 or 12 h after administration of cortisol or ethanol respectively (Laboritt & Thuret, 1977; Badawy *et al.*, 1982). Conversely, cerebral catecholamine synthesis is enhanced when brain tyrosine concentration is increased by tyrosine administration. The results of these latter studies are, however, conflicting, because some of them have shown that catecholamine synthesis is unaltered by doses of tyrosine of 20–100 mg/kg body wt., whereas others have demonstrated an enhanced synthesis by doses of the amino acid of 50–1000 mg/kg body wt. (Wurtman *et al.*, 1974; Wurtman & Fernstrom, 1975, 1976; Fernstrom, 1976; Gibson & Wurtman, 1978; Carlsson & Lindqvist, 1978), and it was suggested by these groups of workers that the failure of tyrosine to enhance catecholamine synthesis in some of these experiments may involve feedback inhibition of tyrosine hydroxylase (L-tyrosine, tetrahydropteridine:oxygen oxidoreductase; EC 1.14.16.2) activity (see also the Results and discussion section). It was therefore considered important to perform a

detailed investigation of the effects of administration of various doses of tyrosine on rat brain catecholamine synthesis, the results of which form the subject of the present paper.

Materials and methods

Male Wistar rats (150–170 g) were locally bred and were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water. The animals were killed by decapitation between 12:45 and 13:45 h and the brains were rapidly removed (within 10 s of death), immersed in liquid N₂ for 3 min and stored at –20°C overnight before extraction.

Tetrahydropteridine was a gift from Hoffmann–La Roche (4002 Basle, Switzerland), and the aromatic L-amino acid decarboxylase inhibitor compound NSD-1015 (3-hydroxybenzylhydrazine hydrochloride) was purchased from Sandev (Gilston Park, Harlow, Essex, U.K.). All other chemicals (of the purest commercially available grades) were from standard suppliers. For intraperitoneal injection, compound NSD-1015 (100 mg/kg body wt.) and tyrosine (5–500 mg/kg body wt.) were dissolved in 0.9% (w/v) NaCl and were given in a volume of 1 ml/kg or 20 ml/kg respectively. Control rats received an appropriate volume of 0.9% NaCl. The tyrosine solution was prepared by dissolving the required amount in 1 ml of 1 M-NaOH, adding most of the 0.9% NaCl needed, neutralizing with 1 M-HCl and making up the final (20 ml) volume with 0.9% NaCl.

Serum and brain tyrosine concentrations were determined by the method of Waalkes & Udenfriend (1957). Brain tyrosine was present in the eluate containing (when compound NSD-1015 was ad-

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ministered) 3,4-dihydroxyphenylalanine. This and the eluates containing dopamine (3,4-dihydroxyphenethylamine) and noradrenaline were obtained by the ion-exchange column-chromatographic procedures of Bertler *et al.* (1958), Attack & Magnusson (1970) and Kehr *et al.* (1972). 3,4-Dihydroxyphenylacetic acid (and also homovanillic acid, which was not studied in the present work because of interference by column material in its fluorimetric determination) has been separated for the first time, by the above procedures, in the present work by utilizing the ability of aq. 60% (v/v) methanol to effect the early elution of biogenic acids from such columns. 3,4-Dihydroxyphenylacetic acid was present in the first 3 ml of the eluate obtained after application of the 20 ml sample of aqueous methanol immediately after the passage of the 0.4 M-HClO₄ extract of brain, which in our experiments contained 1.15 g wet wt. of tissue in every 5 ml applied. Concentrations of noradrenaline, 3,4-dihydroxyphenylacetate, dopamine and, when appropriate, 3,4-dihydroxyphenylalanine were determined in suitable portions of eluates by the methods described by Bertler *et al.* (1958), Spano & Neff (1971), Kehr *et al.* (1972) and Attack (1973) respectively. Recoveries of the above four compounds and of tyrosine from brain extracts after chromatography were 71, 74, 50, 57 and 86% respectively. The results have been corrected for full recovery.

Brain tyrosine hydroxylase activity was determined by the fluorimetric procedure of Yamauchi & Fujisawa (1978) in the presence of a 1.6 mM final concentration of tetrahydropteridine. Each 0.5 ml of the total incubation mixture contained 50 mg wet wt. of tissue in the form of a 0.2 ml portion of a 25% (w/v) homogenate from which catechols have previously been removed by rapid adsorption on aluminium oxide (300 mg of which were shaken with 4–7 ml of homogenate) followed by a brief centrifugation in the cold (at 300 g for 30 s).

Statistical analysis of results was performed by using Student's *t* test.

Results and discussion

Cerebral catecholamine synthesis can be examined *in vivo* by assessment of the rate of tyrosine hydroxylation (which is the rate-limiting step of the pathway) by determination of concentration of the normally undetectable 3,4-dihydroxyphenylalanine after administration of a suitable decarboxylase inhibitor. By using this method, we found (Fig. 1) that, although serum and brain tyrosine concentrations were increased in a dose-related fashion after administration of doses of tyrosine of 5–500 mg/kg body wt., that of brain 3,4-dihydroxy-

Fig. 1. Effects of administration of various doses of tyrosine on the concentrations of serum and brain tyrosine and brain 3,4-dihydroxyphenylalanine in rats treated with compound NSD-1015

Rats received an intraperitoneal injection of tyrosine (5–500 mg/kg body wt.) or an equal volume (20 ml/kg body wt.) of 0.9% NaCl and were killed 1.5 h later. The animals also received a similar injection of compound NSD-1015 (100 mg/kg body wt.) at 45 min after that of tyrosine or 0.9% NaCl. Concentrations of serum and brain tyrosine and brain 3,4-dihydroxyphenylalanine were determined as described in the Materials and methods section. Several experiments were performed, in each of which the effects of two doses of tyrosine were compared with those of 0.9% NaCl. Each single experiment was performed with five rats per group. The results are expressed as the percentage increase in concentrations of the above three parameters as a function of the tyrosine dose. Control values for serum tyrosine (means $\pm$ S.E.M. for each group of five rats, in μ g/ml) varied between 14.75 ± 0.75 and 21.32 ± 1.66 , whereas those of brain tyrosine (in μ g/g wet wt., expressed as above) varied between 18.22 ± 0.29 and 23.90 ± 0.48 . The corresponding values for brain 3,4-dihydroxyphenylalanine (expressed as for brain tyrosine) varied between 0.152 ± 0.005 and 0.206 ± 0.006 . Symbols used: $\circ$, serum tyrosine; $\bullet$, brain tyrosine; $\blacktriangle$, brain 3,4-dihydroxyphenylalanine.

phenylalanine was significantly increased ($P < 0.001$) only by doses of the precursor of 5, 7.5, 10, 20, 30 and 40 mg/kg body wt. (by 10, 22, 24, 31, 20 and 11% respectively). Although doses of tyrosine of 50 mg/kg body wt. and above did not cause a

Table 1. *Effects of administration of various doses of tyrosine on the concentration of rat serum and brain tyrosine and brain catecholamines*

Rats received, at 1.5 h before death, an intraperitoneal injection of tyrosine (20–500 mg/kg body wt.) or an equal volume (20 ml/kg body wt.) of 0.9% NaCl. The concentrations of the above compounds were determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for each group of five to six rats. The values observed in tyrosine-treated rats have been compared with those obtained in control (0.9% NaCl-treated) animals, and the significance of the differences is indicated as follows: * $P < 0.001$. Abbreviation used: dopac, 3,4-dihydroxyphenylacetic acid.

Tyrosine dose (mg/kg body wt.)	Concentration (μ g/ml of serum or per g wet wt. of brain)				
	Serum tyrosine	Brain tyrosine	Brain dopamine	Brain dopac	Brain noradrenaline
0	15.68 $\pm$ 0.46	18.90 $\pm$ 0.56	0.91 $\pm$ 0.007	0.24 $\pm$ 0.009	0.38 $\pm$ 0.005
20	25.75 $\pm$ 0.54*	29.75 $\pm$ 0.42*	1.14 $\pm$ 0.016*	0.43 $\pm$ 0.037*	0.46 $\pm$ 0.005*
0	17.47 $\pm$ 0.32	13.38 $\pm$ 0.36	0.75 $\pm$ 0.006	0.38 $\pm$ 0.015	0.45 $\pm$ 0.010
100	35.56 $\pm$ 2.71*	33.46 $\pm$ 0.60*	0.74 $\pm$ 0.012	0.35 $\pm$ 0.036	0.45 $\pm$ 0.020
500	102.00 $\pm$ 5.14*	116.58 $\pm$ 2.76*	0.77 $\pm$ 0.016	0.48 $\pm$ 0.042	0.48 $\pm$ 0.020

stimulation of 3,4-dihydroxyphenylalanine synthesis, they failed to decrease its concentration below the values observed in control rats (those given 0.9% NaCl plus compound NSD-1015).

To find out if the above changes in 3,4-dihydroxyphenylalanine synthesis are associated with parallel changes in that of catecholamines, the experiments whose results are shown in Table 1 were performed. Although all three doses of administered tyrosine increased serum and brain tyrosine concentrations, only the 20 mg/kg body wt. dose increased significantly those of brain dopamine, 3,4-dihydroxyphenylacetic acid and noradrenaline (by 25, 79 and 21% respectively).

The results described so far therefore demonstrate the ability of small doses of tyrosine to enhance cerebral catecholamine synthesis by increasing the availability to tyrosine hydroxylase of its amino acid substrate; a finding consistent with the concept of this enzyme existing normally unsaturated with tyrosine (see Wurtman & Fernstrom, 1976, and references cited therein; Carlsson & Lindqvist, 1978). The present results also show that the above enhancement is gradually overcome by increasing the dose of tyrosine such that it is totally abolished by doses of 50 mg/kg body wt. and above. The failure of large doses of tyrosine to enhance catecholamine synthesis has previously been suggested, on the basis of studies with the dopamine-receptor-blocking agent haloperidol to involve feedback inhibition of tyrosine hydroxylase activity by the newly synthesized catecholamines (Wurtman & Fernstrom, 1976; Carlsson & Lindqvist, 1978). This proposed feedback mechanism, however, requires assessment by techniques other than that using haloperidol; a drug that may exert as yet unrecognized effects on tyrosine metabolites, such as increasing of 3,4-dihydroxyphenylalanine concentration (Carlsson & Lindqvist, 1978).

Another possible explanation of the failure of large doses of tyrosine to enhance catecholamine synthesis may be that of substrate inhibition of tyrosine hydroxylase activity *in vivo*. The latter possibility is supported by the following findings: (1) 3,4-dihydroxyphenylalanine synthesis determined in the experiments in Fig. 1 is decreased from the enhanced level observed after administration of the 20 mg/kg body wt. dose of tyrosine when the amino acid concentration is increased in the brain by larger doses to values above 200 μ M; (2) tyrosine hydroxylase activity *in vitro* (determined in the presence of the natural tetrahydropteridine cofactor) is known (Watanabe *et al.*, 1981) to be inhibited by the addition to 10000 g supernatants of large concentrations of tyrosine, with a 50% inhibition being produced by a concentration of approx. 250 μ M; (3) such an inhibition has been confirmed in the present work in whole-brain homogenates in the presence of tetrahydropteridine. Thus hydroxylase activities (in nmol of 3,4-dihydroxyphenylalanine formed/min per g wet wt.; means $\pm$ S.E.M. for each group of four separate experiments) in the presence of concentrations of added tyrosine of 100, 150, 200, 300 and 500 μ M were 0.15 $\pm$ 0.011, 0.20 $\pm$ 0.006, 0.20 $\pm$ 0.013, 0.13 $\pm$ 0.012 and 0.13 $\pm$ 0.003 respectively. Further work on possible substrate inhibition of tyrosine hydroxylase activity *in vivo* may be of interest in relation to the regulation of cerebral catecholamine synthesis. The failure of large doses of tyrosine to decrease the 3,4-dihydroxyphenylalanine concentration to values below those observed in control rats treated with 0.9% NaCl plus compound NSD-1015 (Fig. 1) cannot be explained at present, except, perhaps by the possibility that tyrosine hydroxylase activity cannot be completely inhibited by a large excess of tyrosine, as the results of experiments *in vitro* described above and reported by Watanabe *et al.* (1981) suggest. Further work is

therefore required to examine such resistance of tyrosine hydroxylase to inhibition by its substrate.

The present findings (Fig. 1 and Table 1) may also be useful in relation to tyrosine loading in clinical practice in conditions requiring enhancement of cerebral catecholamine synthesis. It should, however, be noted that, although 20 mg/kg body wt. was the optimum dose, joint administration of compound NSD-1015 caused additional increases in serum and brain tyrosine concentrations (of 48 and 64% respectively; results not shown), probably by inhibiting liver tyrosine aminotransferase activity (see also Carlsson & Lindqvist, 1978). In view of these latter findings and of the strong possibility that the above type of patients may have a deficiency in circulating tyrosine, the clinician may wish to consider administration of the moderate, rather than the large, doses of tyrosine used in the present work.

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