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Brain tyrosine increases after treating with prodrugs: comparison with tyrosine

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Abstract—After mice had been treated with L-tyrosine, *O*-phospho-L-tyrosine, L-tyrosine methyl ester or *N*-acetyl-L-tyrosine, tyrosine was assayed by HPLC coupled with fluorometric detection. *O*-Phospho-L-tyrosine behaved as a tyrosine prodrug after its hydrolysis by acid and alkaline phosphatases. After the intraperitoneal administration of *O*-phospho-L-tyrosine or the methyl ester, there was a substantial increase in bioavailability in terms of the effect of tyrosine. The two prodrugs were as powerful as tyrosine following oral administration. *N*-Acetyl-L-tyrosine was the least effective prodrug tested. The stability, solubility and bioavailability of *O*-phospho-L-tyrosine are consistent with proposing it for use as a tyrosine prodrug. In addition, it can be used parenterally. The use of a tyrosine aminotransferase inhibitor is necessary for limiting the hepatic breakdown of tyrosine and for increasing its bioavailability.

Tyrosine is the precursor of catecholamine (CA) synthesis. The quantities of phenylalanine and tyrosine required daily by an adult are about 16 mg kg^{-1}. The dose used in the clinic is commonly 100 mg kg^{-1} (Cuche et al 1985). It is rapidly metabolized in the liver: Noda & Ichihara (1976) reported that 20% of a tracer dose of [^{14}C]tyrosine (i.v.) was excreted as $^{14}CO_2$ in 1 h. After ligature of the hepatic vessels, that percentage dropped to 0·6%.

Tyrosine used at pharmacological doses has shown its value in both animal experimentation and human clinical trials, in pathophysiological situations combined with an increased turnover of catecholamines. In particular, these situations have included experimental haemorrhagic shock (Laborit et al 1969 a–d; Conlay et al 1981), septic shock (Simon et al 1987) and inescapable electric shock (Reinstein et al 1984). The activity of tyrosine has also been described in the treatment of experimental high blood pressure (Laborit et al 1970; Sved et al 1979; Yamori et al 1980; Bresnahan et al 1980) but its efficacy has been questioned in animals by Lockley et al (1985) and by Henley et al (1986), and in man by Sole et al (1985). Its clinical use has been proposed for depression and aggression, as well as in Parkinson's disease (Laborit 1969). Several clinical studies have confirmed its value in treating depression (Gelenberg et al 1980, 1983; Goldberg 1980) and particularly in dopamine-dependent depressions (Mouret et al 1987). Tyrosine increases the homovanillic acid level in the cerebrospinal fluid of parkinsonian patients (Growdon et al 1982). That clinical result suggests that tyrosine has a potential therapeutic use in treating this disease.

The clinical use of tyrosine would be facilitated by the use of precursors with higher solubility and better bioavailability.

We have thus investigated three molecules, of which two are known tyrosine precursors. L-Tyrosine methyl ester is widely used in experimental studies and *N*-acetyl-L-tyrosine has been used intravenously in rats (Im et al 1985). *O*-Phospho-L-tyrosine has apparently never been described as a tyrosine precursor, but its use appears feasible since it is a substrate for acid and alkaline phosphatases (Apostol et al 1985).

Materials and methods

Male OF1 SPF mice (Iffa Credo, France), 30 g, had free access to food (AO4, UAR, France) and water. The drugs were administered intraperitoneally (i.p.) or orally in a volume of 20 mL kg^{-1}. After treatment the mice were decapitated and the brain was rapidly removed and stored at −80°C until analysis when it was homogenized with an Ultra-Turrax apparatus in 1 mL of 0·4 M perchloric acid containing 0·1% each of EDTA and sodium metabisulphite. After centrifugation for 13 000 *g* for 10 min at 4°C, the supernatant was removed and pH was adjusted to 5·0 by adding 70 μL of 10 M potassium acetate. Following a second centrifugation, the supernatant was filtered and used for chromatographic analysis of tyrosine as described by Anderson et al (1979).

Chromatographic separation was on a reversed phase 300 mm long, 3·9 mm inner diameter column (C_{18} μBondapak (Waters)). A Waters 6000 A pump was used in conjunction with a U6K injector. The mobile phase was 0·01 M sodium acetate–methanol (85/15, v/v). The pH was adjusted to 4 with glacial acetic acid. The phase was filtered through 0·45 μm pore size membranes (Millipore) and degassed by ultrasound (Bransonic tank). The flow-rate was 1·5 mL min^{-1}.

An Aminco-Bowman SPF 500 fluorimeter, equipped with a 9 μL continuous flow cell was used for detection at wavelengths of 280 nm (excitation) and 330 nm (emission).

Tyrosine concentrations were determined with reference to the peak height of an external standard. No deviation from linearity was observed with standards or samples in the range of 75 to 750 ng. The detection limit was 100 pmol with a signal/noise ratio greater than 2.

Brain tyrosine concentrations were 60 to 80 nmol g^{-1}, in agreement with published values (Im et al 1985; Marcou et al 1987). The brain and plasma concentrations are similar and vary in parallel after i.p. administration of tyrosine (Topall & Laborit 1987). It is thus consistent to believe that the presence of blood in the brain homogenate would not affect the validity of the results.

The different doses of tyrosine, with *O*-phospho-L-tyrosine at 5·52 mmol kg^{-1}, were administered as a suspension (10% Tween 80 in isotonic saline). For the other treatments, the compounds were dissolved in isotonic saline alone. Control animals treated with NaCl or the aqueous emulsion of Tween 80 alone exhibited no changes in brain tyrosine concentrations.

All reagents were analytical grade and were obtained from Sigma, Fluka or Merck.

Data are expressed as mean ± standard error of the mean and were analysed with Student's *t*-test.

Results

Fig. 1 shows the dose–response curves obtained after the i.p. injection of the compounds 1 h before death at the doses of 0·55, 1·66 and 5·52 mmol kg^{-1} (100, 300 and 1000 mg kg^{-1} equivalent dose of free tyrosine).

Beginning at 0·55 mmol kg^{-1}, *O*-phospho-L-tyrosine and L-tyrosine methyl ester led to a greater increase in brain tyrosine than L-tyrosine itself. The difference between the two prodrugs and L-tyrosine increased and became significant at 1·66 and 5·52 mmol kg^{-1}. At the highest dose, the increase in brain tyrosine caused by *O*-phospho-L-tyrosine was 1112% ($P < 0·001$), equivalent to a 400% increase ($P < 0·001$) compared with the use of

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Table 1. Brain concentrations of tyrosine 1 and 4 h after oral administration of tyrosine or the three tyrosine prodrugs at the dose of 1·66 mmol kg^{-1} (300 mg kg^{-1} equivalent dose of free tyrosine). Results are the mean ± s.e.m. obtained with 6–7 mice, in nmol g^{-1} fresh weight. a: $P<0·01$; b: $P<0·001$ compared with the control group. c: $P<0·001$ compared with the effect of tyrosine.

Control	Tyrosine	Tyrosine Me ester	*N*-Acetyl tyrosine	*O*-Phospho tyrosine
1 hr after treatment				
89·1 ± 3·6	340·7 ± 16·8 b	374·8 ± 15·5 b	122·9 ± 8·5 a, c	368·5 ± 21·1 b
4 h after treatment				
81·4 ± 2·5	105 ± 7·8 a	86·8 ± 4·6	95.5 ± 3·5 b	97·3 ± 3·61 b

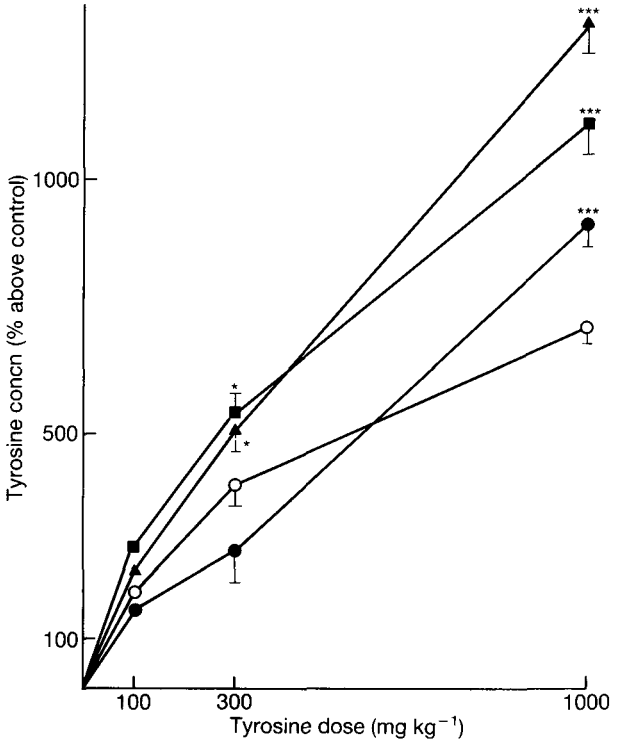

FIG. 1. Brain concentrations of tyrosine 1 h after intraperitoneal administration of L-tyrosine (○), *O*-phospho-L-tyrosine (▲), L-tyrosine methyl ester (■) or N-acetyl-L-tyrosine (●) at the doses of 0·55, 1·66 and 5·5 mmol kg^{-1} (100, 300 and 1000 mg kg^{-1} equivalent dose of free tyrosine). Results are the mean ± s.e.m. (bars) obtained with 12 (controls) or 5 mice (treated). All increases are significant compared with the control animals.*: $P<0·05$; ***: $P<0·001$ in comparison to the effect of tyrosine.

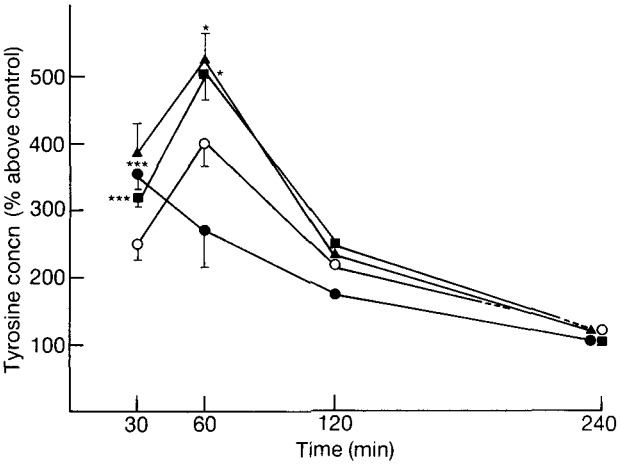

FIG. 2. Mean time course of percentage changes of brain concentrations of tyrosine after intraperitoneal administration of L-tyrosine (○), *O*-phospho-L-tyrosine (▲), L-tyrosine methyl ester (■) or *N*-acetyl-L-tyrosine (●) at the dose of 1·66 mmol kg^{-1} (300 mg kg^{-1} equivalent dose of free tyrosine). Control animals were also killed at each point. Results are the mean ± s.e.m. (bars) obtained with 5 mice. *: $P<0·05$, ***: $P<0·001$ compared with the effect of tyrosine.

tyrosine itself. In the same conditions, the methyl ester caused a 1300% increase ($P<0·001$) equivalent to a 590% increase compared with the use of tyrosine.

The curve of *N*-acetyl-L-tyrosine differs significantly from that of tyrosine only at the 5·52 mmol kg^{-1} dose, when there was an increase of 908% ($P<0·001$), which represents an increase of 196% ($P<0·001$) compared with the use of tyrosine.

Changes in brain tyrosine concentrations were analysed 0·5, 1, 2 and 4 h after administration of the different prodrugs i.p. at 1·66 mmol kg^{-1} (Fig. 2). *N*-acetyl-L-tyrosine exerted its maximal effect 30 min after injection. At that time, the molecule was significantly more active than tyrosine, although no further differences compared with tyrosine were recorded later. The maximal effects of L-tyrosine methyl ester and of *O*-phospho-L-tyrosine appeared at 1 h, as did that of tyrosine. The two prodrugs were more active than tyrosine during the first hour after treatment, but this effect disappeared at 2 and 4 h.

The oral administration of tyrosine and the prodrugs showed that the bioavailability of *O*-phospho-L-tyrosine and that of methyl ester were the same as that of tyrosine 1 h after treatment with 1·66 mmol kg^{-1} (Table 1). For all the treatments, the values returned to basal levels after 4 h (Table 1). After administration of *N*-acetyl-L-tyrosine at 1·66 mmol kg^{-1}, there was only a modest increase in the cerebral brain concentration of tyrosine 1 h later (Table 1). This increase was significantly lower than that obtained with tyrosine ($P<0·001$) and also lower than that obtained when the prodrug was administered i.p.

Discussion

This study has shown the quality of *O*-phospho-L-tyrosine as tyrosine precursor. The molecule is stable in aqueous solution over a broad pH range (Robert et al 1985). Its quality as prodrug undoubtedly stems from its dephosphorylation by acid and alkaline phosphatases (Apostol et al 1985). After i.p. administration, there is a clear increase in tyrosine availability, compared with that obtained with L-tyrosine methyl ester. After oral administration, however, the activity of the two molecules is no higher than that of L-tyrosine, probably as a result of their

hydrolysis in the gastrointestinal tract. The use of the methyl ester is limited to animal experimentation, since this releases methanol. Tyrosine ethyl ester, another tyrosine prodrug (Raveux 1970) also appears to be rapidly hydrolyzed after ingestion. *O*-Phospho-L-tyrosine, on the other hand, could be used clinically, primarily as a result of its water solubility at physiological pH. It is known that the acute administration of high doses of tyrosine (600 mg kg^{-1} orally) activates hepatic tyrosine aminotransferase (TAT) (Lin & Knox 1958), which reduces the bioavailability of tyrosine (Gibson 1988). Since the use of tyrosine for parenteral feeding remains limited because of its low solubility (Im et al 1985), those authors recommended using *N*-acetyl-L-tyrosine. In our experimental conditions, this substance was less interesting than *O*-phospho-L-tyrosine. The presence of acid and alkaline phosphatases in the bloodstream and in the liver is consistent with proposing the phospho derivative in the present framework. Its use may be limited, however, by the acidity resulting from the release of the phosphate group by hydrolysis.

Finally, the kinetics of action of the three tyrosine prodrugs enables limits of their interest to be set. It was found that their effects no longer differed from those of tyrosine 4 h after treatment, in spite of a substantial increase in bioavailability during the first hour. This is probably the result of increased hepatic catabolism. It is known that hepatic TAT has a broad capacity for adaptation (Lin & Knox 1958). The best tactic for increasing the bioavailability of exogenous tyrosine would thus be the inhibition of hepatic TAT, as we have shown (Topall & Laborit 1987).

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