The Inhibition of Intestinal Absorption of Phenylalanine in the Rhesus Monkey (35159)

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Phenylketonuria is the commonest of the genetic aminoacidopathies and is an important disease in which mental retardation is the most prominent feature. The administration of a diet low in phenylalanine is generally accepted as useful (1–3) although its efficacy has been questioned (4, 5). This diet is very restrictive and may be a major factor in the emotional climate of the home (6) and any method that could make the dietary intake more liberal and varied without unduly increasing the phenylalanine content would be useful. One approach that might allow this would be to feed synthetic amino acids which compete with phenylalanine for active transport in the ileum.

We have previously reported that β-2-thienylalanine inhibits renal tubular reabsorption of phenylalanine (7) and because ileal and renal absorptive mechanisms for amino acids share many common characteristics, it was decided to try the effect of this phenylalanine antagonist upon the absorptive phenomenon. In addition, parachlorophenylalanine, a well known inhibitor of phenylalanine metabolism (8) and cycloleucine, a substance known to produce a generalized aminoaciduria in man (9) were used. Cycloleucine is currently under trial (Phase II of Clinical Drug Evaluation Program of Cancer Chemotherapy National Cancer Institute) in patients with leiomyosarcoma in whom side-effects are mild and reversible.

Materials and Methods. Four healthy, adult male rhesus monkeys (*Macaca mulatta*), which weighed 6.25 to 8.75 kg, were used. They were usually fed Purina monkey chow, fruits and vitamins, but were fasted for 8 hr before the experiment during which they were offered water *ad libitum*.

Each monkey had a control blood sample drawn, nasogastric tube passed, and urine collector attached to the external genitalia, and the animal was placed in a special metabolic chair (10). The dose of phenylalanine administered orally to each monkey with or without the antagonist was 750 mg/kg of body weight. This dose, and the timing of blood samples was chosen after 5 preliminary administrations were shown to produce maximum effect and no emesis. Blood samples were drawn at 1, 2, 3, 4, and 6 hr.

The dose of the inhibitors was as follows: β-2-thienylalanine, 187.5 mg/kg; cycloleucine, 225 mg/kg; parachlorophenylalanine, 225 mg/kg. These doses were chosen on the basis of solubility characteristics and previous known effects on phenylalanine metabolism. Both the phenylalanine and antagonists were dissolved in 300 ml of tap water.

Urine was collected in a plastic bottle containing a few crystals of thymol and kept in ice. At the end of the 6-hr period suprapubic pressure was applied to empty the bladder.

Acidified urine and all sera samples (11) were analyzed on a Beckman-Spinco amino acid analyzer by the method of Spackman *et al.* (12) for neutral and acidic amino acids. β-2-thienylalanine and parachlorophenylalanine are clearly separated by this method, each appearing as a peak before tyrosine.

Results. Phenylalanine absorption. A typical experiment is shown in Fig. 1: the blood level of phenylalanine (mg/100 ml) is shown at zero time, 1, 2, 3, 4, and 6 hr. In each case the addition of the inhibitor to the 750 mg/kg of phenylalanine administered reduces the absorption of phenylalanine. To quantitate this reduction the area under each curve above base line was calculated and phenylalanine absorbed expressed in arbi-

TABLE I. Total Phenylalanine Absorption Expressed as Area Under Curve After 750 mg/kg of Phenylalanine Load by Naso-Gastric Tube With and Without Synthetic Amino Acids.[a]

	Load	B56	E27	D43	E65	Mean
1.	Phenylalanine	339.8	428.0	363.65	80.25	302.9
2.	Phenylalanine + β-2-th.	356.0	286.0	303.9	50.3	249.0 (NS)
3.	Phenylalanine + cycloleucine	139.7	182.4	124.4	67.2	128.4 (p <.100)
4.	Phenylalanine + Parachloro	178.1	268.0	142.8	29.9	154.7 (p <.200)
	Ratio					
5.	line 2/line 1	1.05	0.67	0.84	0.63	0.80 (p <.10)
6.	line 3/line 1	0.41	0.43	0.34	0.84	0.51 (p <.05)
7.	line 4/line 1	0.52	0.63	0.39	0.37	0.48 (p <<.001)

[a] Each monkey received 4 loads: in each he received 750 mg of phenylalanine/kg; in load 2 he also received β-2-thienylalanine, 187.5 mg/kg; in load 3 cycloleucine, 225 mg/kg; in load 4 parachlorophenylalanine, 225 mg/kg. Each analog decreased the amount of phenylalanine absorbed when given the analog (with the exception of B56 given β-2-thienylalanine). When results expressed as a ratio for each individual monkey (thereby reducing individual variance) each analog significantly reduced absorption.

trary units.

These calculated areas are shown in Table I where the mean absorption of phenylalanine in monkeys fed phenylalanine is compared with the mean absorption when given phenylalanine plus each synthetic amino acid. The phenylalanine absorption in Table I is the mean of two experiments on each animal with a 750 mg/kg dose. All three phenylalanine antagonists reduce phenylalanine absorption. When cycloleucine is added, the mean absorption is significantly lower and when parachlorophenylalanine is added it only approaches significance ($p <$ 0.2). The variation in intestinal absorption of phenylalanine by individual monkeys is shown by line 1. The absorption of phenylalanine when both phenylalanine and inhibitor are given is compared in lines 5, 6, and 7 for each individual monkey. In each case, except monkey B56 given β-2-thienylalanine, the value of this ratio is less than unity, indicating that the amino acid analogue reduces intestinal absorption. Expressed this way the reduction of absorption is significant and especially so in the case of parachlorophenylalanine ($p <.001$).

Serum tryptophan. As previously reported (13) the phenylalanine load lowered serum tryptophan levels. After 4 hr, the tryptophan levels started to rise again as shown in Fig. 2. When the synthetic amino acid was administered in addition, the tryptophan fell again with the same recovery after 4 hr. In Table II the fall of serum tryptophan (expressed as the area between the curve and base line) for the first 4 hr is shown. β-2-Thienylalanine failed to produce a significantly different fall in serum tryptophan compared with phenylalanine alone but both cycloleucine and parachlorophenylalanine did. When expressed as a ratio to allow for individual variation, parachlorophenylalanine produced

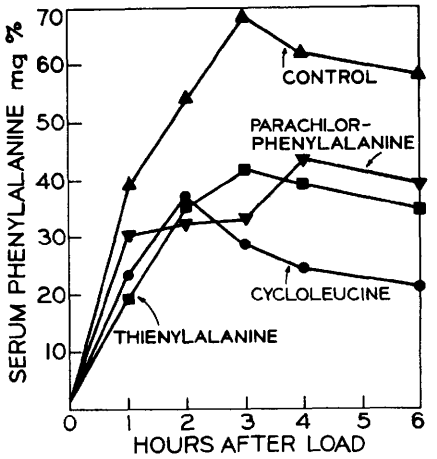

FIG. 1. Rise in serum phenylalanine in monkey E27 following four oral loads of phenylalanine ± antagonist: Control load was phenylalanine, 750 mg/kilo. Each of the 3 other loads contained phenylalanine, 750 mg/kg plus either β-thienylalanine, 187.5 mg/kg; cycloleucine, 225 mg/kg; or parachlorphenylalanine, 225 mg/kg.

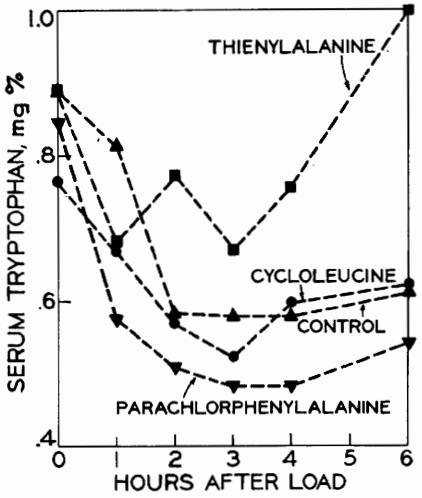

FIG. 2. Fall in serum tryptophan in monkey E27 following four oral loads of phenylalanine ± antagonist as described under Fig. 1.

a significantly greater fall in tryptophan than phenylalanine alone. Also in the three monkeys not shown in Fig. 1 the plasma level of tryptophan failed to rise by the usual 6 hr when parachlorophenylalanine was administered.

Serum tryosine. This was measured either by the method of Wong *et al.* (14) or by amino acid analyzer. The rise in serum tyrosine over 6 hr was small but consistent. Although the synthetic amino acids produced a trend for slower rise in tyrosine this was not significant with any analogue. These data are not presented but are available from the authors.

Urinary amino acids. The urinary excretion of phenylalanine during the 6 hr of oral load is shown in Table III. In all cases, the excretion is less when the synthetic amino acid is concurrently administered with one exception: β-2-thienylalanine given to Monkey B56 promotes greater excretion but this was the only instance in which intestinal absorption of phenylalanine was greater when synthetic amino acid was given (see Table I). This confirms the impression that the reason for the lower urinary excretion of phenylalanine in the presence of analogues is the lower absorption from the intestine.

Discussion. The results shown indicate that both cycloleucine and parachlorophenylalanine are potent inhibitors of intestinal absorption of phenylalanine, as is β-2-thienylalanine but to a lesser extent. The finding that this compound also inhibits the kidney transport system again demonstrates the similarity of transport systems in these two tissues.

Parachlorophenylalanine caused a marked rise in plasma phenylalanine in two out of five patients who received it on a chronic basis (15). There was an initial fall in plasma phenylalanine in two of the five patients, but no acute studies on intestinal absorption were performed. Presumably the elevation of plasma phenylalanine after chronic dosage

TABLE II. Fall in Serum Tryptophan Level Expressed as Area Above Curve After 750 mg/kg of L-Phenylalanine by Naso-Gastric Tube With and Without Synthetic Amino Acids.

Load	B56	E27	D43	E65	Mean and significance
1. Phenylalanine only	0.571	0.595	0.755	0.415	0.588
2. Phenylalanine + β-2-thienylalanine	0.581	1.100	0.730	0.730	0.820 ($p < .200$)
3. Phenylalanine + cycloleucine	1.16	1.18	0.85	0.920	1.04 ($p < .05$)
4. Phenylalanine + parachlorophenylalanine	1.45	2.28	1.84	1.37	1.235 ($p < .05$)
Ratio					
line 2/line 1	1.018	1.849	0.967	1.759	1.148 (NS)
line 3/line 1	2.035	1.983	1.126	2.217	1.840 ($p < .05$)
line 4/line 1	2.544	3.832	2.437	3.301	3.028 ($p <<< .001$)

[a] Experiment as described under Table I; β-2-thienylalanine failed to produce a significantly greater fall in serum tryptophan than phenylalanine alone. When cycloleucine or parachlorophenylalanine was added to the oral load the fall in serum tryptophan was significantly greater than when the same dose of phenylalanine was administered alone.

TABLE III. Urinary Excretion of Phenylalanine for 6 hr After 750 mg/kg Load of Phenylalanine by Naso-Gastric Tube With and Without Synthetic Amino Acids.[a]

Load	B56	D43	E27	E65	Mean
Phenylalanine only	13.4	148.5	26.1	73.3	65.3
Phenylalanine					
+ β-2-thienylalanine	22.9	4.6	4.9	1.2	8.4
+ parachlorophenylalanine	2.1	0.8	5.8	0.8	2.4
+ cycloleucine	2.5	19.2	6.4	0.5	7.2

[a] Each monkey excretes less phenylalanine when the synthetic amino acid is concurrently administered with the exception of B56 when given β-2-thienylalanine.

was due to inhibition of phenylalanine hydroxylase. Parachlorophenylalanine has been found to be a potent inhibitor of rat phenylalanine hydroxylase *in vivo* (8, 14) although a poor inhibitor *in vitro* (16).

Weitzman *et al.* (17) also administered parachlorophenylalanine intraperitoneally to monkeys (*Macaca mulatta*) but only measured sleep patterns and brain serotonin. Mouret *et al.* (18) performed a similar study in the rat. Unfortunately neither paper cites data from which absorption characteristics can be derived.

Cycloleucine has been shown to be an inhibitor of amino transport into the Ehrlich cell by Oxender and Christensen (19) affecting both their postulated systems for neutral amino acid transport. Brown (9) showed that cycloleucine administration markedly increased human urinary excretion of cysteine, ornithine, lysine, arginine, taurine, threonine, serine, valine, tyrosine, and histidine. Brown did not specifically mention phenylalanine but the figure in the text indicates that its excretion is increased. Goyer (20) found a similar aminoaciduria in rats injected with cycloleucine intraperitoneally in which urinary phenylalanine excretion was definitely elevated. Thus there is additional evidence that cycloleucine inhibits transport of phenylalanine in both intestinal and renal tissues.

Elevated plasma phenylalanine is known to be associated with lowered plasma tryptophan levels (13). Phenylalanine inhibits transport of tryptophan in the intestine (21) and at the blood brain barrier (13). The more severe and prolonged depression of plasma tryptophan by parachlorophenylalanine is of interest as this compound inhibits tryptophan hydroxylase (8, 22, 23) and this action in the brain has been considered to be the cause of the lowered brain serotonin (18, 23–26). These low levels of tryptophan cause real concern when considering the use of such synthetic amino acids as a mode of treatment in phenylketonuria. Although this study shows that the analogues decrease the absorption of phenylalanine, Aoki and Siegel (27), have shown that cerebral tryptophan deficiency may be the mechanism whereby brain damage occurs with elevated blood phenylalanine. Further reduction of plasma tryptophan by the administratiion of these synthetic amino acids may even further deplete the brain of tryptophan. Such a lowering of cerebral tryptophan seems to be a nonspecific phenomenon in that a number of amino acids when elevated in plasma reduce the brain free tryptophan (28). Such lowering by branched chain amino acids in brain can occur without lowering of plasma levels (13). Thus the possible detrimental effects of these synthetic amino acids can only be assessed by their prolonged oral administration —this is currently being undertaken in our laboratory.

Summary. β-2-Thienylalanine, cycloleucine, and parachlorophenylalanine have been shown to acutely reduce intestinal absorption of an oral phenylalanine load. Combined with phenylalanine, they reduce plasma tryptophan levels more than phenylalanine alone.

Whether their oral administration will be of benefit in allowing a more liberal diet in phenylketonuric children requires more investigation.

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