METABOLISM OF AN ORAL TRYPTOPHAN LOAD III: EFFECT OF A PYRIDOXINE SUPPLEMENT

In a previous communication it was shown that when oral L-tryptophan was administered in a dose of 50 mg kg⁻¹ twice daily for 7 days plasma kynurenine concentrations 12 h after the last dose were increased in comparison with the plasma concentration in subjects who had not previously taken L-tryptophan (i.e. without pretreatment). In addition the peak plasma kynurenine concentration following a subsequent single dose of 50 mg kg⁻¹ was at least twice that seen following a single 50 mg kg⁻¹ dose without pretreatment (Green, Aronson, Curzon & Woods, 1980). Urinary kynurenine excretion following the final single L-tryptophan dose was also markedly increased following 7 days L-tryptophan pretreatment. In contrast, the urinary excretion of indole acetic acid (IAA),

which normally increases following a single dose (Green, Bloomfield, Woods & Seed, 1978; see Table 1) did not increase in subjects who had taken L-tryptophan pretreatment during the previous week (Green *et al.*, 1980). It was suggested that these changes might indicate that L-tryptophan administration for 7 days led to a relative pyridoxine deficiency, since some of the enzymes degrading kynurenine are pyridoxal dependent, as is the decarboxylase enzyme which metabolizes tryptophan to tryptamine (the precursor of indole acetic acid). Since kynurenine can compete with tryptophan for entry into the brain (see later) it was suggested that a pyridoxine supplement might be of advantage when L-tryptophan is being given chronically (Green *et al.*, 1980).

Table 1 Effect of chronic L-tryptophan administration with and without vitamin supplement on the metabolism of a subsequent tryptophan load.

Pretreatment	The concentration of plasma tryptophan and kynurenine and the urinary excretion of various tryptophan metabolites following a single L-tryptophan load		
	None	L-tryptophan (50 mg kg ⁻¹ twice daily) for 7 days	L-tryptophan (50 mg kg ⁻¹ twice daily) + pyridoxine HCl (0.5 mg kg ⁻¹) for 7 days
Compound measured:	(n=6)	(n=4)	(n=4)
Plasma tryptophan (just before load)	16 ± 4	44 ± 14 ¹	29 ± 7 ¹
Plasma tryptophan (peak concentration following load)	100 ± 18	101 ± 7	102 ± 11
Plasma kynurenine (just before load)	0.53 ± 0.16	3.53 ± 1.58 ⁶	1.51 ± 0.27 ⁵
Plasma kynurenine (peak concentration following load)	5.7 ± 2.4	12.5 ± 5.8 ¹	6.1 ± 1.1
Urinary kynurenine excretion (24 h)	13.6 ± 6.2	55 ± 26 ³	13.4 ± 9.4
Urinary indole acetic acid excretion (24 h)	15.1 ± 2.2	4.3 ± 0.9 ³	22.4 ± 5.6
Urinary 5-hydroxyindole acetic acid excretion (24 h)	6.3 ± 4.5	3.6 ± 2.2	11.4 ± 3.5 ²
Urinary tryptophan excretion (24 h)	12.3 ± 3.1	13.7 ± 7.6	15.7 ± 3.5

¹ Different from no pretreatment $P < 0.01$, no statistical difference between the two pretreatment groups.

² Different from chronic L-tryptophan group $P < 0.01$, not statistically different from no pretreatment group, no statistical differences between chronic L-tryptophan group and no pretreatment group.

³ Different from the other two groups $P < 0.001$.

⁴ Different from the other two groups $P < 0.01$.

⁵ Different from chronic L-tryptophan group $P < 0.05$ and from no pretreatment group $P < 0.01$.

⁶ Different from no pretreatment group $P < 0.01$.

Data for columns 1 and 2 taken from Green *et al.* (1980). Plasma tryptophan and kynurenine values are reported in $\mu\text{g ml}^{-1}$. Urinary kynurenine, IAA, 5-HIAA and tryptophan reported as mg compound excreted in 24 h following the load. Excretion of these compounds in a control 24 h period (no load given) are as follows: kynurenine 4.7 ± 3.2 mg/24 h; IAA 7.5 ± 0.8 mg/24 h; 5-HIAA 6.5 ± 1.6 mg/24 h; tryptophan 6.9 ± 0.5 mg/24 h ($n=10$ for all observations: data taken from Green *et al.*, 1978). The values reported above show the plasma tryptophan and kynurenine concentrations at 10.00 h following no pretreatment or following 7 days pretreatment with L-tryptophan (50 mg kg⁻¹ twice daily) or L-tryptophan (50 mg kg⁻¹ twice daily) with vitamin supplement. Peak plasma tryptophan and kynurenine values are those seen following a subsequent L-tryptophan 50 mg kg⁻¹ load and the urinary values are the total excretion for the 24 h following the load.

We have now examined the metabolism of an oral tryptophan load in subjects who have taken L-tryptophan (50 mg kg⁻¹) with a pyridoxine supplement for 7 days. The results have been compared with the previous data on the metabolism of a L-tryptophan load in subjects who have taken L-tryptophan at the same dose for 7 days but without the vitamin supplement.

Four healthy young male subjects who had taken part in the previous study were investigated. They were given tablets of Optimax with vitamins (E. Merck, Alton, Hants) twice daily (at 10.00 h and 22.00 h) for 7 days. Each tablet contains L-tryptophan 500 mg, pyridoxine HCl 5 mg and ascorbic acid 10 mg. Each dose contained L-tryptophan 50 mg kg⁻¹, pyridoxine HCl 0.5 mg kg⁻¹ and ascorbic acid 1 mg kg⁻¹.

On day 8, a 10 ml blood sample was taken at 10.00 h immediately before the L-tryptophan load. The load was given as powdered Optimax tablets, the powder being suspended in an orange drink as in the previous study. A 24 h urine collection was started and further blood samples taken at 11.00 h, 12.00 h, 13.00 h, 15.00 h and 16.00 h. All analytical procedures were exactly as used previously (Green *et al.*, 1980).

The results of this study are shown in the accompanying table. The mean basal plasma tryptophan concentration on day 8 just before the load was comparable to that seen in subjects who had taken L-tryptophan only pretreatment and higher than in subjects who had not taken L-tryptophan pretreatment. The peak plasma tryptophan concentration was identical to that seen after a load both in subjects pretreated for a week with L-tryptophan alone and in subjects who took no pretreatment.

When L-tryptophan pretreatment had been taken with a pyridoxine supplement, the basal plasma kynurenine concentration on day 8 was higher than that found in untreated subjects, but only half that found in subjects who had taken L-tryptophan pretreatment alone. Furthermore the peak plasma kynurenine concentration was the same as that found in subjects who had not taken pretreatment but only about half that attained in subjects taking L-tryptophan alone for a week.

Whilst the urinary kynurenine excretion in the L-tryptophan plus pyridoxine pretreatment group was the same as that seen after no pretreatment, it was only 25% of that seen in the L-tryptophan alone group.

Indole acetic acid excretion increased after a single L-tryptophan load and also following a load in the subjects given L-tryptophan plus pyridoxine pretreatment for a week, in contrast to the subjects pretreated with L-tryptophan alone in whom no increase was seen. Urinary 5-HIAA excretion did increase in the L-tryptophan plus pyridoxine group compared with those subjects who had taken L-tryptophan pretreatment alone. However, the excretion was not increased significantly compared with the no pretreatment

group. Again, a pyridoxal phosphate dependent enzyme (decarboxylase) is involved in the pathway of 5-HIAA and IAA production.

Urinary tryptophan excretion following a load was unaltered by pretreatment with either L-tryptophan or L-tryptophan plus pyridoxine.

It appears therefore that when L-tryptophan is administered alone for 7 days at a dose of 50 mg kg⁻¹ twice daily there are marked alterations in the metabolism of a subsequent oral L-tryptophan load compared with the metabolism of a load without pretreatment. In contrast if the L-tryptophan is administered for 7 days with a pyridoxine supplement these metabolic changes are avoided.

The most important finding is that when L-tryptophan has been taken with a pyridoxine supplement neither the peak plasma kynurenine concentration nor the urinary kynurenine excretion is increased above that seen in the absence of L-tryptophan pretreatment, in contrast to the increases seen when L-tryptophan is given without pyridoxine. This probably indicates that when L-tryptophan is given with the pyridoxine supplement the postulated relative pyridoxine deficiency caused by the repeated administration of L-tryptophan is avoided and kynurenine and several other products on this pathway are metabolized at a normal rate by the pyridoxal phosphate dependent enzymes (see Green *et al.*, 1978). This view is supported by the observation that without the pyridoxine supplement, IAA excretion does not increase following a load in subjects who have taken L-tryptophan alone for the previous week, since the decarboxylase enzyme forming tryptamine is also pyridoxal phosphate dependent.

Whilst the tablets Optimax plus vitamins also contain ascorbic acid, there is little evidence to suggest that ascorbate will alter tryptophan metabolism *in vivo* and we think it more likely that the pyridoxine hydrochloride is implicated in the changes observed.

Green & Curzon (1970) and Kiely & Sourkes (1972) found that kynurenine interferes with tryptophan transport into rat brain. The data of Gal, Young & Sherman (1978) suggest that in subjects taking L-tryptophan alone the plasma kynurenine concentrations attained after a load may be associated with diminished tryptophan transport into the brain. Singleton & Marsden (1979) showed that when L-tryptophan was given to rats tryptophan transport into the brain decreased with increasing time of administration. Furthermore, Chadwick, Trimble, Jenner, Driver & Reynolds (1978) found that when L-tryptophan (Optimax without vitamin supplement) was given in a dose of 8 g daily for 90 days there was only a small rise in CSF tryptophan and no increase in CSF 5-HIAA in man.

Thus it is possible that the increased plasma kynurenine concentrations which result from chronic L-tryptophan administration may cause diminished

transport of peripherally administered tryptophan into the brain and this in turn may explain why low doses of tryptophan (around 6 g daily) have an anti-depressant effect whilst higher doses are ineffective (see review of Young & Sourkes, 1977). We would, therefore, suggest during long term L-tryptophan therapy a pyridoxine hydrochloride supplement should be given.

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A.R. GREEN & J.K. ARONSON

MRC Unit & University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, OX2 6HE

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THE EFFECT OF β -ADRENOCEPTOR AGONISTS ON URINARY INCONTINENCE IN THE ELDERLY

Stimulation of the body of the bladder by the sympathetic nervous system in man is predominantly via β -adrenoceptors (Benson, McConnell & Wood, 1979), and agonists at these sites inhibit bladder contraction during the filling phase in cats (Nergardh & Boreus, 1972). However the physiological importance of these receptors in man is uncertain, and yet drugs such as salbutamol and terbutaline are advocated for treating urinary incontinence due to unstable detrusor contractions in man (Stanton, 1978). Since there has been no formal investigation of their role in this condition the present study was undertaken.

Salbutamol 4 μ g/kg was given intravenously to six elderly patients with urinary incontinence associated with unstable detrusor contractions. The mean age of the patients was 80.8 years (range 73–93 years), and four of them were female. All underwent an initial cystometry for routine investigation of their incontinence. After giving informed consent they were then enrolled in the study which had local Ethical Committee permission. Fifteen to thirty minutes after salbutamol, cystometry was repeated,

and the mean $\pm$ s.e. mean results of both are shown in Table 1. Salbutamol produced no benefit in any patient, but significantly reduced the volume at which the first spasm occurred ($P < 0.05$). The volume at

Table 1 Mean $\pm$ s.e. mean cystometric results of six elderly patients before and after salbutamol 4 μ g/kg i.v.

	Baseline	After salbutamol	Significance
Volume at first spasm (ml)	183 (± 37)	136 (± 23)*	$P < 0.05$
Pressure at first spasm (cm H ₂ O)	44 (± 14)	42 (± 14)	NS
Volume at capacity (ml)	192 (± 38)	142 (± 23)	NS
Pressure at capacity (cm H ₂ O)	61 (± 18)	62 (± 20)	NS

* $P < 0.05$ ($t = 2.714$, Student's paired t -test)