Br. J. clin. Pharmac. (1980), **10**, 611–615

METABOLISM OF AN ORAL TRYPTOPHAN LOAD. II: EFFECT OF PRETREATMENT WITH THE PUTATIVE TRYPTOPHAN PYRROLASE INHIBITORS NICOTINAMIDE OR ALLUPURINOL

A.R. GREEN & J.K. ARONSON

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

G. CURZON

Department of Neurochemistry, Institute of Neurology, Queen Square, London, WC1N 3BG

H.F. WOODS

Department of Clinical Pharmacology and Therapeutics, The Royal Hallamshire Hospital, Sheffield

1 The effect of seven days administration of either allopurinol (300 mg daily) or nicotinamide (500 mg twice daily) on the metabolism of an oral L-tryptophan load (50 mg/kg) has been investigated.

2 Administration of either drug failed to alter the plasma total or free tryptophan or plasma kynurenine curve. Nor was the urinary excretion of tryptophan, kynurenine, 5-hydroxyindoleacetic acid or indole acetic acid influenced.

3 Allopurinol pretreatment did increase the volume of distribution of tryptophan.

4 These data suggest that allopurinol and nicotinamide are unlikely to be of value as tryptophan pyrrolase inhibitors *in vivo* and therefore would not increase the therapeutic effect of L-tryptophan when it is given to treat depressive illness.

Introduction

In the previous communication (Green, Aronson, Curzon & Woods, 1980) the problems involved in administering tryptophan were discussed and it was pointed out that large amounts of tryptophan are metabolised by tryptophan pyrrolase to kynureine in the liver, so that relatively little is available for the synthesis of the central neurotransmitter 5-hydroxytryptamine (5-HT). Therefore inhibition of tryptophan pyrrolase may enhance 5-HT synthesis.

Two compounds, allopurinol and nicotinamide, have been suggested to have potential use as *in vivo* pyrrolase inhibitors. Allopurinol is an *in vitro* pyrrolase inhibitor and has some inhibitory action on tryptophan pyrrolase following adminstration to rats (Becking & Johnson, 1967; Green & Curzon, 1968). Both drugs have been used with tryptophan to treat depression (Shopsin, 1978; McSweeney, 1975).

We have therefore examined whether pretreatment with these drugs for 1 week affects the metabolism of an oral tryptophan load.

Methods

Subjects

The subjects investigated were healthy young male members of staff (mean age 28 years; range 23–35). None of the subjects was taking any drugs, other than those under investigation and none took alcohol during the week of the study.

Protocol

Control (drug-free) subjects were those reported in the previous investigation (Green *et al.*, 1980). Most of them were then used in this study of the effects of allopurinol and nicotinamide.

Allopurinol ('Zyloric' Wellcome) was taken at a dose of 300 mg/day, once daily for 7 days. On Day 8, 300 mg was taken just before the tryptophan load. Nicotinamide was taken at a dose of 1 g/day in two divided doses of 500 mg for 7 days. On Day 8, 500 mg was taken just before the load.

0306–5251/80/060611–05 $01.00

A 24 h urine collection was started at 10.00 h on Day 7 and continued until 10.00 h on Day 8 when a new 24 h collection was started, at the time of tryptophan administration. Oral tryptophan (50 mg/kg) was given and blood collected as described in the previous paper (Green *et al.*, 1980).

Analytical procedures and mathematical methods

These are described in the previous paper (Green *et al.*, 1980). In addition plasma uric acid was measured on Day 8 in the subjects taking allopurinol 1 h following the last dose of allopurinol using the method of Kageyama (1971).

Results

Effect of allopurinol and nicotinamide administration on plasma tryptophan and kynurenine concentrations

Seven days' administration of either allopurinol or nicotinamide did not significantly affect plasma total or free tryptophan concentration curves following oral tryptophan (Figure 1a and 1b). Nor was any change seen in the plasma kynurenine concentration curve (Figure 2).

Kinetic analysis of the plasma total tryptophan and plasma free tryptophan curves revealed that the only significant effect of either drug was that allopurinol increased the volume of distribution of total tryptophan (V_d) by 60%. The V_d value for free tryptophan was also increased but this failed to reach statistical significance (Table 1).

Allopurinol decreased plasma uric acid concentrations (control: 377 ± 31 μmol/l(4); experiment: 269 ± 81 μmol/1 (4): $P < 0.05$).

Urinary excretion of tryptophan and tryptophan metabolites following allopurinol and nicotinamide administration

Allopurinol or nicotinamide administration did not alter urinary excretion of tryptophan, kynurenine, 5-hydroxyindole acetic acid (5-HIAA) or indole acetic acid (IAA) during Day 7 (the day before the tryptophan load) compared to drug free controls (Table 2). The drugs also failed to alter the excretion of these compounds following the oral tryptophan load (Table 2).

Discussion

The suggestion that the therapeutic effects of L-tryptophan would be enhanced by concomitant administration of a pyrrolase inhibitor has been current for some time. The two inhibitors suggested have been allopurinol (Green & Curzon, 1968; Badawy & Evans, 1974) and nicotinamide (Young & Sourkes, 1974). Certainly Fernando, Joseph & Curzon (1975) found that allopurinol but not nicotinamide pretreatment modestly enhanced the rise of rat brain tryptophan following tryptophan injection.

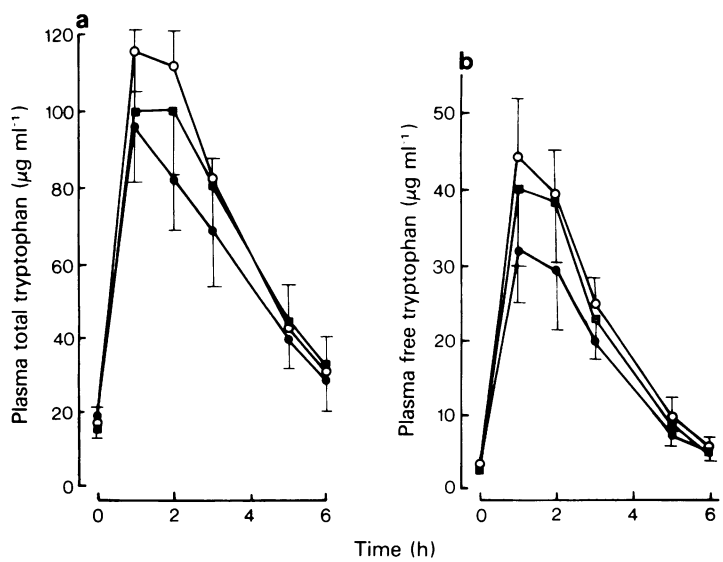

Figure 1 Plasma tryptophan concentration following an oral L-tryptophan load (50 mg/kg) in control subjects (■, n = 7) and in subjects who had taken either allopurinol (300 mg daily) (●, n = 6) or nicotinamide (500 mg twice daily) (○, n = 6) for 7 days. Results shown as mean ± s.d. of (a) total tryptophan concentrations or (b) free tryptophan concentrations in the 6 h following the load.

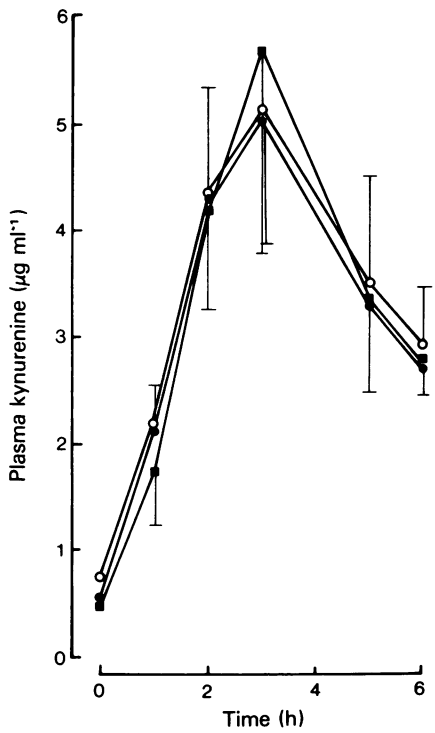

Figure 2 Plasma kynurenine concentration following an oral L-tryptophan load (50 mg/kg) in control subjects (■) and in subjects who had taken either allopurinol (300 mg daily) (●) or nicotinamide (500 mg twice daily) (○) for 7 days. Results shown as mean ± s.d. during the 6 h following the load, number of subjects the same as **Figure 1**.

Badawy & Evans (1975), however, found that nicotinamide was also effective in producing an enhanced tryptophan accumulation.

The greater increase in brain tryptophan seen in animals pretreated with allopurinol before a tryptophan load was, however, difficult to explain, since studies on the metabolism of a tryptophan load by a perfused liver preparation suggested that allopurinol did not, in fact, inhibit pyrrolase activity in the presence of high tryptophan concentrations (Green, Joseph & Woods, 1976). Furthermore, allopurinol did not inhibit tryptophan catabolism in the rat liver following a tryptophan load although the previously observed enhancement of the brain tryptophan concentration was confirmed (Joseph, Young & Curzon, 1976).

This present study may partly explain these seemingly contradictory findings. In agreement with the previous rat liver perfusion data (Green *et al.*, 1976) and rat *in vivo* data (Joseph *et al.*, 1976), allopurinol did not apparently alter the metabolism of a tryptophan load in human subjects. Nevertheless it did increase the volume of distribution of the amino acid. This indicates an increase in the tryptophan concentration in body compartments which is consistent with the enhanced increase of brain tryptophan following a load in allopurinol pretreated animals.

Another recent study has claimed that allopurinol did not alter human tryptophan metabolism (Moller & Kirk, 1978). However the data were both variable and inconclusive.

At present there seems to be no explanation for the altered volume of tryptophan distribution produced by allopurinol but not nicotinamide administration. A plot of free against total tryptophan values in con-

Table 1 Plasma half-life ($T_{½}$), plasma clearance, area under the curve of the plasma concentration versus time (AUC) and apparent volume of distribution (V_d) of tryptophan following an oral tryptophan load

	Drug pretreatment	n	$T_{½}$ *(h)*	*AUC* *(μg ml⁻¹ h)*	V_d *(l kg⁻¹)*	*Clearance* *(ml min⁻¹ kg⁻¹)*
Total						
	None	6	2.31±0.42	317±48	0.54±0.07	2.67±0.48
	Allopurinol	6	2.62±0.52	253±53	0.84±0.21**	3.86±1.48
	Nicotinamide	4	2.16±0.21	339±36	0.46±0.05	2.47±0.27
Free						
	None	6	1.40±0.14	113±29	0.90±0.25	7.80±2.27
	Allopurinol	6	1.52±0.13	94±21	1.21±0.38	9.20±2.31
	Nicotinamide	4	1.45±0.27	122±13	0.85±0.18	6.83±0.68

Values calculated from tryptophan measurements and free (non-albumin bound) tryptophan. All subjects received an oral dose of L-tryptophan (50 mg kg⁻¹) either after no pretreatment or 7 days administration of allopurinol (300 mg daily) or nicotinamide (500 mg twice daily). Different from no pretreatment ** $P < 0.01$. Details of mathematical methods given in previous paper (Green, *et al.*, 1980).

Table 2 The urinary excretion of tryptophan and some tryptophan metabolites following nicotinamide or allopurinol pretreatment

Pretreatment drug	*Day*	n	*Tryptophan*	*Kynurenine*	*5-HAA*	*IAA*
None	Control	6	7.6 ±2.2	1.55±0.39	4.9 ±2.1	4.88±0.99
	Load	6	12.3 ±3.1	13.6 ±6.2	6.3 ±4.5	15.09±2.19
Allopurinol	Control	6	7.8 ±3.1	1.35±0.54	3.31 ±1.0	6.23±3.62
	Load	6	11.54±4.2	14.7 ±8.8	3.5 ±2.1	10.05±7.32
Nicotinamide	Control	5	11.0 ±4.7	2.02±1.0	5.7 ±3.7	5.74±2.54
	Load	5	11.8 ±4.0	13.8 ±7.4	9.0 ±7.4	14.18±8.54

Subjects were given either allopurinol (300 mg daily) or nicotinamide (500 g twice daily) for 7 days. On day 7 a 24 h urine collection was made (control day). On day 8 an oral tryptophan load (50 mg kg^{-1}) was given and a further 24 h urine collection made (load day). Results show mean ± s.d. of the number of observations shown in column 3.5-HIAA:5-hydroxyindoleacetic acid, IAA: Indole acetic acid. All results reported as mg compound excreted/24 h.

trol subjects and those taking allopurinol does not reveal any effect of the drug on tryptophan binding. Conceivably allopurinol has some effect on membrane transport of amino acids. Furthermore whilst the ribonucleotide derivative of the drug is a protein synthesis inhibitor (McCollister, Gilbert, Ashton & Wyngaarden, 1964), the effect of protein synthesis inhibition would probably be to increase tissue amino acid concentrations before administration of the tryptophan load, thereby decreasing the apparent volume of distribution of this subsequent load.

Both nicotinamide (McSweeney, 1975) and allopurinol (Shopsin, 1978) have been reported to be useful in enhancing the therapeutic effect of L-tryptophan. However such suggestions were made following administration of both tryptophan plus the drug in open uncontrolled trials. Indeed the authors did not compare the efficacy of the combination with L-tryptophan administration alone, so no conclusions are justified.

As nicotinamide did not apparently alter tryptophan metabolism and as allopurinol had marginal effects, this study does not encourage the use of either drug to enhance the therapeutic effect of the amino acid although clearly we are not able to exclude the possibility that the increase in the volume of tryptophan distribution might be of value in increasing brain tryptophan content.

We gratefully acknowledge the excellent technical assistance of Mr M.R. Bloomfield and Mrs B.D. Krantameneni, the co-operation of our colleagues in taking part in this study and E. Merck Ltd, both for supplying the Optimax tablets and for providing a grant to help defray some of the costs of the investigations.

References

BADAWY, A.A-B. & EVANS, M. (1974). Tryptophan plus a pyrrolase inhibitor for depression. *Lancet*, **ii**, 1209.

BADAWY, A.A-B. & EVANS, M. (1975). Tryptophan plus a pyrrolase inhibitor for depression. *Lancet*, **ii**, 869.

BECKING, G.C. & JOHNSON, W.J. (1967). The inhibition of tryptophan pyrrolase by allopurinol, an inhibitor of xanthine oxidase. *Can. J. Biochem.*, **45**, 1667–1672.

FERNANDO, J.C., JOSEPH, M.H. & CURZON, G. (1975). Tryptophan plus a pyrrolase inhibitor for depression? *Lancet*, **i**, 171.

GREEN, A.R., ARONSON, J.K., CURZON, G. & WOODS, H.F. (1980). Metabolism of an oral tryptophan load. I. Effects of dose and pretreatment with tryptophan. *Br. J. clin. Pharmac.*, **10**, 603–610.

GREEN, A.R. & CURZON, G. (1968). Decrease of 5-hydroxytryptamine in the brain provoked by hydroxytryptamine and its prevention by allopurinol. *Nature, Lond.*, **220**, 1095–1097.

GREEN, A.R., JOSEPH, M.H. & WOODS, H.F. (1976). Tryptophan metabolism in the isolated perfused liver of the rat: effects of tryptophan concentration hydrocortisone and allopurinol on tryptophan pyrrolase activity and kynurenine formation. *Br. J. Pharmac.*, **57**, 103–114.

JOSEPH, M.H., YOUNG, S.N. & CURZON, G. (1976). The metabolism of a tryptophan load in rat brain and liver. The influence of hydrocortisone and allopurinol. *Biochem. Pharmac.*, **25**, 2599–2604.

KAGEYAMA, N. (1971). A direct colorimetric determination of uric acid in serum and urine with uricase/catalase system. *Clinica Chim. Acta*, **31**, 421–426.

McCOLLISTER, R.J., GILBERT, W.R., ASHTON, D.M. & WYNGAARDEN, J.B. (1964). Pseudofeedback inhibition of purine synthesis by 6-mercaptopurine ribonucleotide and other purine anlaogues. *J. biol. Chem.*, **239**, 1560–1563.

McSWEENEY, D.A. (1975). Treatment of unipolar depression. *Lancet*, **ii**, 510–511.

MOLLER, S.E. & KIRK, L. (1978). The effect of allopurinol on the kynurenine formation in humans following a tryptophan load. *Acta Vitamin Enzym.* **32**, 159–162.

SHOPSIN, B. (1978). Enhancement of the antidepressant response to L-tryptophan by a liver pyrrolase inhibitor: a rational treatment approach. *Neuropsychobiology*, **4**, 188–192.

YOUNG, S.N. & SOURKES, T.L. (1974). Antidepressant action of tryptophan. *Lancet*, **ii**, 897–898.

(*Received March 17, 1980*)