Tryptophan metabolism in rheumatoid neuropathies

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Peripheral neuropathy is an established complication of rheumatoid arthritis (Pallis and Scott, 1965). The clinical, electrophysiological, and histological features have been described previously (Chamberlain and Bruckner, 1970; Weller, Bruckner, and Chamberlain, 1970). The underlying aetiology of the neuropathy is usually ascribable to a vascular lesion, but an occasional case is reported in which no vasculitis is found in spite of extensive *post mortem* histological search (Hart and Golding, 1960). It is tempting to speculate that other factors, such as metabolic disturbances, might contribute to the pathogenesis of the neuropathy.

Abnormalities of tryptophan metabolism have been found to occur in patients with rheumatoid arthritis (Bett, 1962; McMillan, 1960; Spiera, 1966), and to cause an increased urinary excretion of kynurenine, xanthurenic acid, and 3-hydroxyanthranilic acid after L-tryptophan loading. These results have been interpreted as indicating a functional pyridoxine deficiency, one of the features of which is a peripheral neuropathy (McKusick, Sherwin, Jones, and Hsu, 1964). Thus it is possible that a peripheral neuritis in rheumatoid arthritis may result from this functional pyridoxine deficiency; alternatively the metabolite 3-hydroxyanthranilic acid may have an effect on the neuropathy, as it has been shown to inhibit mitochondrial respiration (Quagliariello, Papa, Saccone, and Alifano, 1964).

We therefore set out to investigate tryptophan metabolism in patients with rheumatoid neuropathies, and to see if the metabolic abnormality and clinical neuropathy were affected by a 6-month course of oral pyridoxine.

Patients and methods

Of 32 patients with rheumatoid neuropathies who had been previously evaluated clinically, electrophysiologically, and histologically (Chamberlain and Bruckner, 1970; Weller and others, 1970), ten were available for further study. There were two males and eight females

and the mean age was 54 years (range 35 to 75). The mean duration of the arthritis at the time of study was 13.6 years (range 3 to 25), and the mean duration of the neuropathy was 3.3 years (range 4 mths to 9 yrs). Only one patient (Case 4) had the severe sensorimotor type of neuropathy, and she was on long-term therapy with azathioprine 2.5 mg./kg. body weight as well as prednisolone. The remaining nine patients had the predominantly sensory type of neuropathy. Of these, eight were on oral corticosteroid therapy, six were having aspirin, two were on indomethacin, and one was on phenylbutazone.

The extent and severity of the neuropathy was estimated clinically and electrophysiologically. At each visit sensation was tested to light touch and pin-prick, and vibration sense and sensory loss were charted. Tendon reflexes were also tested and their absence recorded. Electromyographic studies and motor and sensory nerve conduction velocity measurements were performed by the methods described previously (Chamberlain and Bruckner, 1970).

After a baseline clinical assessment and L-tryptophan loading test, four patients were given a 6-month course of oral pyridoxine 100 mg. daily. The clinical assessment and L-tryptophan loading test were repeated 2 weeks, and again 6 months, after starting pyridoxine treatment. The other six patients acted as controls, having clinical assessments and L-tryptophan load tests 6 months after base-line studies, but without being given pyridoxine. Of these six control patients, two were subsequently given a 6-month course of pyridoxine 100 mg. daily, and the clinical assessment of the severity of the neuropathy and the L-tryptophan load test were repeated at the end of the course of pyridoxine therapy. Thus, at the end of the study, there were six patients with a rheumatoid neuropathy who were assessed after a 6-month course of pyridoxine therapy and six who were assessed after 6 months without having pyridoxine.

In addition, L-tryptophan loading tests were performed on a woman aged 63 years (Case 5) with classical rheumatoid arthritis, who did not have a neuropathy and was not on corticosteroid therapy. After a baseline test she was given a 6-month course of pyridoxine 100 mg. daily and the test was repeated. Similar L-tryptophan loading tests were performed on six healthy volunteers, four males and two females with a mean age of 22 years (range 21 to 23).

Material

3-Hydroxy-DL-kynurenine and xanthurenic acid were obtained from Koch-Light Laboratories Ltd., Colnbrook.

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3-Hydroxyanthranilic acid and Dowex-50W $\times$ 12 (200–400 mesh) ion exchange resin were supplied by Sigma Chemical Co., St. Louis. All other chemicals were of analytical grade, and distilled water was used throughout.

Tryptophan loading

5 g. L-tryptophan was administered orally as a suspension in yoghurt at 10 a.m. to patients and control subjects. Urine was collected for 6 hrs after ingestion of the tryptophan load, acidified with hydrochloric acid, and stored at -20°C . Patients already on oral corticosteroid therapy were given hydrocortisone hemisuccinate 100 mg. intravenously just before the test to eliminate the effects of fluctuation of corticosteroid intake.

Determination of tryptophan metabolites in urine

Xanthurenic acid was determined according to the method

of Satoh and Price (1958). Separation of the tryptophan metabolites, 3-hydroxykynurenine and 3-hydroxyanthranilic acid, was carried out by ion-exchange chromatography (Smith and Lakatos, 1971), using modifications of the methods of Brown and Price (1956) and Heeley (1965). All urine samples were hydrolysed before column chromatography (Smith and Lakatos, 1971).

Results

The results of the L-tryptophan loading test in patients with rheumatoid arthritis are shown in Table I, and the results in normal control subjects in Table II (opposite). Sex and age did not appear to affect the results.

Table I *Excretion of tryptophan metabolites in patients with rheumatoid arthritis*

3OH-Kynurenine, 3OH-anthranilic acid, and xanthurenic acid were measured in urine collected for 6 hrs after oral administration of 5 g. L-tryptophan

Patient no.	Sex	Age (yrs)	Steroids	Pyridoxine	3OHK ($\mu\text{M.}/6$ hrs)	3OHA ($\mu\text{M.}/6$ hrs)	XA ($\mu\text{M.}/6$ hrs)	3OHK: 3OHA	3OHK: XA	3OHA: XA
1	M	72	—	—	578	119	119	4.86	4.86	1.00
			—	—	460	253	56	1.82	8.21	4.52
			—	—	148	52	50	2.85	2.96	1.04
2	F	47	+†	—	276	142	79	1.94	3.49	1.79
			+	—	378	153	102	2.47	3.71	1.50
			+	—	658	128	165	5.14	3.99	0.78
3	F	58	+	—	295	227	152	1.30	1.94	1.49
			+	—	620	409	274	1.52	2.26	1.49
4	F	40	+	—	3223	619	553	5.20	5.83	1.12
			+	—	2580	379	404	6.82	6.39	0.94
			+	—	3313	653	274	5.07	12.09	2.38
			+	6 mths	922	599	109	1.54	8.46	5.49
5*	F	60	—	—	476	205	142	2.33	3.35	1.44
			—	—	691	251	176	2.75	3.93	1.43
			—	6 mths	311	276	37	1.13	8.41	7.46
6	F	71	—	—	168	47	52	3.57	3.23	0.90
			—	2 wks	72	45	17	1.60	4.24	2.65
			—	6 mths	13	29	8	0.45	1.63	3.63
7	F	56	+	—	987	266	90	3.71	10.97	2.96
			+	2 wks	82	174	7	0.47	11.71	24.86
			+	6 mths	131	174	12	0.75	10.92	14.50
8	F	70	+	—	—	—	207	—	—	—
			+	2 wks	—	—	24	—	—	—
9	F	36	+	—	813	103	529	7.89	1.54	0.19
			+	—	374	54	173	7.93	2.16	0.31
			+	6 mths	43	91	53	0.47	0.81	1.72
10	F	53	+	—	806	182	235	9.83	3.43	0.77
			+	2 wks	882	206	155	4.28	5.69	1.33
			+	6 mths	117	110	21	1.06	5.57	5.24
11	M	41	+†	—	152	99	74	1.54	2.05	1.34
			+	2 wks	136	150	36	0.91	3.78	4.17
			+	6 mths	350	117	83	2.99	4.22	1.41

* Rheumatoid arthritis without neuropathy.

† Intravenous hydrocortisone not given before tryptophan loading.

Table II Excretion of tryptophan metabolites in control subjects

3OH-Kynurenine, 3OH-anthranilic acid, and xanthurenic acid were measured in urine collected for 6 hrs from six normal subjects after oral administration of 5 g. L-tryptophan. Each value represents the mean ($\mu\text{M}/6 \text{ hrs}$) $\pm$ standard deviation

Metabolite	3OHK	3OHA	XA	3OHK: 3OHA	3OHK: XA	3OHA: XA
mm $\pm$ s.d.	176 $\pm$ 60	97 $\pm$ 32	50 $\pm$ 22	1.81	3.52	1.94

Steroid therapy was associated with increased excretion of all the metabolites, and this was most prominently seen in Case 4, who was also having azathioprine. During pyridoxine therapy, there was a tendency for the level of urinary 3-hydroxykynurenine and 3-hydroxyanthranilic acid to fall, but this was not statistically significant. However, the fall in xanthurenic acid excretion, the fall in the ratios 3-hydroxykynurenine:3-hydroxyanthranilic acid, and the rise in the ratios 3-hydroxyanthranilic acid:xanthurenic acid on pyridoxine therapy, were all highly significant ($P \leq 0.05$; $P \leq 0.025$; $P \leq 0.025$ respectively). The results were analysed by the paired T-test. The urinary levels of metabolites in the patient without a neuropathy (Case 5) were similar to those in the patients with neuropathies. However, the ratio of 3-hydroxykynurenine:xanthurenic acid rose markedly on pyridoxine in Case 5, in contrast to the group of rheumatoid patients with neuropathies who showed no significant change in this ratio.

The extent of clinical sensory loss varied considerably from visit to visit in the same patient. There was no significant improvement in the sensory loss in the six patients who had been given a 6-month course of pyridoxine treatment, and there was no change in their tendon reflexes.

Of the four patients in this study who had originally had electrophysiological tests, repeat electromyography and nerve conduction studies were available

in two (Cases 4 and 6). No significant change was found after the 6-month course of pyridoxine.

Discussion

Increased urinary excretion of tryptophan metabolites has been described in patients with a wide spectrum of conditions, such as congenital hypoplastic anaemia (Price, Brown, Pfaffenbach, and Smith, 1970), carcinoma of the bladder (Bryan, 1969), epilepsy (Hansson, 1968), and rheumatoid arthritis (McMillan, 1960).

In rheumatoid arthritis, increased urinary excretion of kynurenine, 3-hydroxyanthranilic acid, and xanthurenic acid have been found (Spiera, 1966; Bett, 1962; McMillan, 1960), and our present study has confirmed these findings. We were unable to show any significant difference between our rheumatoid patients with neuropathies and the one without a neuropathy in respect of level of the urinary metabolites after tryptophan loading. Nor was there any difference between these two groups after treatment with pyridoxine.

There was a trend in our patients for pyridoxine treatment to reduce the excretion of urinary 3-hydroxykynurenine (3HK), 3-hydroxyanthranilic acid (3HA), and xanthurenic acid (XA), as found by previous workers (Bett, 1963; Jaffe and Altman, 1964). However, only in respect of XA did this reach statistical significance. Perhaps of more interest than

FIGURE Metabolic pathway of 3-hydroxykynurenine. PLP = Pyridoxal phosphate

the absolute values of these metabolites are the ratios. There is a significant fall in our ratios 3HK:3HA after pyridoxine therapy, and a significant rise in 3HA:XA. These results suggest that pyridoxine (or pyridoxal phosphate) augments the conversion of 3HK to 3HA down the main niacin pathway, at the expense of the XA pathway (see Figure). As the

enzymes concerned (kynureninase and transaminase) are pyridoxal phosphate dependent, it is likely that this is a quantitative rather than a qualitative effect.

Heeley (1965) found a similar rise in the 3HA:XA ratio after pyridoxine administration in two normal children, and Hughes and Raine (1966) found that the urinary excretion of XA fell in the only normal adult tested after administration of pyridoxine. These findings suggest that our observations of the effect of pyridoxine on tryptophan metabolism are not specific for patients suffering from rheumatoid arthritis, with or without a neuropathy, and should not be interpreted as confirming a functional pyridoxine deficiency in this condition.

Corticosteroid therapy is known to increase the urinary excretion of 3HK, 3HA, and XA, probably by inducing tryptophan pyrrolase (oxygenase) (Altman and Greengard, 1966; Rose and McGinty, 1968). Other drugs commonly used in the treatment of patients with rheumatoid arthritis may profoundly affect the metabolism of L-tryptophan (Smith and Lakatos, 1971). They reduce protein-binding of tryptophan (Smith, 1971a; McArthur, Dawkins, Smith, and Hamilton, 1971), and inhibit kynureninase and the transaminase which converts 3-hydroxy-kynurenine to xanthurenic acid (Smith, 1971b) (Fig. 1). Thus, the effects of drug therapy might be masking any quantitative differences in urinary tryptophan metabolites between those rheumatoid patients with and those without neuropathies. On the other hand, it is quite possible that all the abnormalities of tryptophan metabolism shown in our patients and those of other authors could be due to drug therapy alone. This possibility needs urgent elucidation in rheumatoid patients seen before therapy of any kind has been instituted.

The course of the neuropathy in our patients fluctuated spontaneously, and there was no significant improvement on pyridoxine therapy. The only reliable clinical parameter which could be quantitated to show changes in the neuropathy was the area of sensory diminution. Many factors other than neurogenic weakness give rise to loss of muscle power in patients with rheumatoid arthritis, e.g. pain, stiffness or deformity of joints, tendon involvement or rupture, and primary muscle disease. This renders conventional testing for muscle power of relatively little value in the follow-up of these patients.

Unfortunately, we were unable to obtain sufficient repeat studies of nerve conduction in the patients treated with pyridoxine to arrive at any conclusions, but since this is the most sensitive test of nerve

function currently available to us further studies are being carried out.

In our patients it is likely that the long-standing changes were irreversible. Investigation and treatment of possible metabolic abnormalities might yet yield fruitful results in patients with a predominantly sensory neuropathy of recent onset.

Summary

- (1) Ten patients with rheumatoid neuropathies were studied. Nine had a mild predominantly sensory neuropathy and one a severe sensorimotor neuropathy. The extent of the neuropathy was estimated clinically and electrophysiologically.
- (2) Tryptophan metabolism was studied in these ten patients and in another patient with rheumatoid arthritis without neurological complications. After a 5-g. load of L-tryptophan, the metabolites 3-hydroxykynurenine, 3-hydroxyanthranilic acid and xanthurenic acid were measured in a 6-hour urine collection.
- (3) Six patients were given a 6-month course of oral pyridoxine 100 mg. daily. Two patients acted as their own controls, so that in effect there were also six control subjects.
- (4) During pyridoxine therapy, there was a statistically significant fall in the urinary excretion of XA ($P \leq 0.05$) and in the ratio 3HK:3HA ($P \leq 0.025$) and a significant rise in the ratio 3HA:XA ($P \leq 0.025$). This suggests that pyridoxal phosphate augments the conversion of 3HK to 3HA down the main niacin pathway at the expense of the XA pathway.
- (5) The raised levels of urinary tryptophan metabolites seen in patients with rheumatoid arthritis, with or without neuropathies, can be attributed at least partially to drug therapy. Drugs such as corticosteroids and aspirin may have more effect on the levels of these metabolites than the disease itself.
- (6) The 6-month course of pyridoxine had no demonstrable effect on the course of the neuropathy, probably because the neuropathic changes were irreversible.

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