Clinical Endocrinology (1997) 47, 667–677

Relationships between methoxyindole and kynurenine pathway metabolites in plasma and urine in children suffering from febrile and epileptic seizures

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(Received 8 March 1996; returned for revision 6 September 1996; finally revised 7 June 1997, accepted 1 July 1997)

Summary

OBJECTIVE The methoxyindole pathway metabolite, melatonin (aMT), and the kynurenine pathway metabolites, kynurenic acid (KYNA), xanturenic acid (XA) and 3-hydroxyantranilic acid (3HANA) are anticonvulsants, whereas the kynurenine pathway metabolites, L-kynurenine (KYN) and 3-hydroxykynurenine (3HK), are proconvulsants. It is thought that alterations in the concentrations of these compounds may be responsible for the excitotoxic aspect of human seizures. The aim of this study was to determine whether alterations in tryptophan metabolism might be related to the occurrence and type (febrile or non-febrile) of seizures in children.

DESIGN One hundred and eighteen children from the University of Granada Hospital were studied. They were divided into two main groups (febrile or epileptic convulsive) depending upon their clinical diagnosis. An age-, weight- and gender-matched control group was also studied. Each group was then divided into two subgroups of patients sampled between 0900 h and 2100 h (diurnal groups) and patients sampled between 2100 h and 0900 h (nocturnal groups).

MEASUREMENTS Plasma melatonin was measured in samples obtained from both the diurnal and nocturnal groups. Urinary excretion of melatonin and kynurenine metabolites were measured in an aliquot of 12-h urine samples collected from both the diurnal and nocturnal groups.

RESULTS Besides the typical circadian rhythm of melatonin we also found diurnal/nocturnal differences in the concentrations of all the kynurenines, which reached significantly higher levels during the day. In normal humans the production of methoxyindoles is lower during the day and rises at night, whereas the production of kynurenines is higher during the day and decreases at night. In patients suffering from febrile and epileptic convulsions, however, there was a significant increase in the nocturnal production of KYN, 3HK, KYNA and XA. Thus we found the circadian rhythm of kynurenines to be altered in convulsive patients. Furthermore, while the various kynurenine metabolites increased by the same amount during the night in febrile convulsive children, in epileptic children the increase in KYN and 3HK was significantly lower than the increase in KYNA and XA. During the day the proconvulsant KYN decreased significantly and the anticonvulsant XA increased in both convulsive groups. Moreover, plasma aMT increased during the day in febrile convulsive group and also during the night in both febrile and epileptic groups although showing no significant change in their urinary excretion levels.

CONCLUSIONS Our results point to the existence of an imbalance in the tryptophan metabolite pathways during convulsions, blunting the normal diurnal–nocturnal rhythm of kynurenines. They also support the idea of a difference in the production of tryptophan metabolites between febrile and epileptic patients, suggesting that the tryptophan pathways follow different routes depending upon the type and duration of the convulsion.

Several authors have proposed that excitatory amino acids are involved in the triggering and maintenance of seizures in human convulsive disorders (Anderson *et al.*, 1987; Meldrum, 1987; Meldrum *et al.*, 1988; Bleck & Klawans, 1992). Experimental data have focused on the possible role of glutamate and aspartate in seizure development and the potential therapeutic benefit of antagonists of excitatory amino-acid receptors (Stone & Javid, 1983; Anderson *et al.*, 1987; Meldrum, 1987; Meldrum *et al.*, 1988; Mody *et al.*, 1988). The fact that some metabolites of the

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kynurenine pathway are ligands for excitatory amino-acid receptors raises the possibility that tryptophan metabolites may be involved in the pathogenesis of seizure disorders (Lapin, 1982; Perkins & Stone, 1982, 1983; Vezzani *et al.*, 1988).

Tryptophan metabolism, including its main pathways, such as the methoxyindole and hydroxyindole routes, has been studied extensively in children (Aurichio *et al.*, 1960; Snyderman *et al.*, 1961; Gholson *et al.*, 1962; Narbona *et al.*, 1994). Nevertheless, the relationships between some of these metabolites and their participation in infantile pathology are as yet not completely understood. Quinolinic acid is an agonist of the NMDA receptor in the brain (Meldrum, 1987), and its administration induces an epileptiform discharge (Lapin, 1982; Perkins & Stone, 1982; Vezzani *et al.*, 1988). L-kynurenine (KYN) and 3-hydroxykynurenine (3HK) have also been reported to be convulsants (Snyderman *et al.*, 1961; Gholson *et al.*, 1962; Lapin, 1980, 1982). Kynurenic acid (KYNA), on the other hand, is an antagonist of the NMDA receptor in the brain and may protect it against excitotoxic compounds (Perkins & Stone, 1982; Foster *et al.*, 1984; Beninger *et al.*, 1986).

There is evidence to support the idea that the main tryptophan methoxyindole pathway metabolite, N-acetyl-5-methoxytryptamine (melatonin, aMT), plays a role in neuromodulation and in this way aMT is able to develop a neuroprotective effect in humans (Antón-Tay, 1974; Champney & Peterson, 1993; Molina-Carballo *et al.*, 1994a,b; Fauteck *et al.*, 1996) and in experimental animals (Reiter *et al.*, 1972, 1973; Reiter & Morgan, 1972; Albertson *et al.*, 1981; Sudgen, 1983). Melatonin depresses the brain excitability regulating Na^+,K^+-ATPase (Acuña-Castrovicjo *et al.*, 1992) and GABA-benzodiazepine receptor-complex activities (Acuña-Castroviejo *et al.*, 1986a,b). Melatonin also potentiates the capacity of corticotrophic and opioidergic peptides to increase brain benzodiazepine (BNZ) receptors (Gomar *et al.*, 1993, 1994). Besides potentiating brain inhibitory neurotransmission, aMT also blocks glutamatergic-dependent brain excitability (Castillo-Romero *et al.*, 1993; Escames *et al.*, 1996), thus acting as an antiexcitotoxic compound. Therefore, the relative balance between the excitotoxic properties of KYN, 3HK and other excitatory amino acids, and the neuroprotective effects of KYNA and XA and aMT may be relevant in the pathogenesis of seizure disorders. We report here on the results of our studies into the relationships between aMT and the kynurenine pathway metabolites in normal children and in children with febrile or epileptic convulsions. Our aim was to determine whether alterations in central nervous system tryptophan metabolism could be related to the occurrence of seizures, and also to distinguish between febrile and non-febrile seizures.

Materials and methods

A total of 118 infants and children from the University of Granada Hospital were studied. Their parents were fully informed and their authorization obtained, as was permission from the hospital's Ethical Committee, in accordance with the 1983 revised Helsinki Declaration of 1975. A history was prepared and a complete clinical examination carried out for all the children involved; anthropometric measurements were noted and a routine biochemical analysis was also made. Depending upon their clinical diagnosis, the children, aged 35·1 ± 31·9 months (mean ± SD, range 1 month–12 years), were divided into three main groups in order to investigate possible differences in the daily pattern of aMT secretion. Each of these groups was then divided into two subgroups (diurnal and nocturnal) depending upon the time at which each child was admitted to hospital and sampled. If the time of the sample was in the period between 0900 and 2100 h the child was grouped in the diurnal group, otherwise the child was included in the nocturnal group (samples obtained between 2100 and 0900 h).

The control group (CG) contained 39 children who were hospitalized because of non-endocrine, non-psychological and non-neurological diseases; most were in hospital for hernias or for a health check-up. These children had normal psychomotor and somatometric development, normal clinical and routine biochemical findings and had no history either of obstetric and/or perinatal difficulties that might represent neurological risk factors or of neurological or endocrine illness in the family. They were age-, gender- and weight-matched with children in the other groups. Each child in all the convulsive groups was sampled once only during the study, on admission to hospital. One child from the control group was sampled at a similar time of day or night in order to compare this child's data with that of his counterpart in the convulsive group. It was not always possible to match the times of sampling between the convulsive and control children exactly but we are satisfied that the times were close enough to make the comparisons adequate and valid. The control group was divided into two subgroups: a diurnal control group (DCG), comprising 22 children, each sampled once in the period between 0900 and 2100 h, and a nocturnal control group (NCG), comprising 17 children sampled between 2100 and 0900 h.

The patients belonging to the convulsive groups were admitted to the University of Granada Hospital with convulsive pathology. The febrile convulsive group (FG) comprised 51 children with the following neurological manifestations (WHO) (Gastaut, 1983): seizures produced during febrile states, with poorly defined signs and symptoms. Table 1 shows the criteria for inclusion in the FG group. On the basis of both the clinical examination and seizure characteristics of each patient, 41

Table 1 Criteria for the inclusion of children with febrile convulsions

Type of convulsion	Inclusion criteria
Typical febrile convulsion (simple or benign) (80–85%)	Temperature increase >38°C Extracerebral infectious illness Lack of neurological signs Age 6 months to 5 years Generalized and symmetrical tonic-clonic convulsion Duration <15 minutes Only one convulsion per febrile episode Rapid normalization of EEG without drug treatment Family history of febrile convulsions (25%) Recidivism (33%)
Atypical febrile convulsion (complicated) (10–15%)	Age <6 months or >5 years History of encephalopathy or acute fetal–neonatal distress Family history of epilepsy Focal or hemilateral convulsion Duration >15 minutes Prolonged postconvulsive period Apparently normal EEG Repeated seizures Recidivism (50%)
Post-vaccination febrile convulsion (0–5%)	Appearance about 2 weeks post-vaccination Partial or generalized seizures Lasting effects (epilepsy, psychomotor retardation, hemiparesis)
Concomitant febrile convulsion	Febrile convulsion during acute infection of the CNS
Releasing febrial convulsion	Clinical appearance of latent epilepsy due to rise in temperature

individuals of FG were classified as having typical febrile convulsions and 10 as having atypical febrile convulsions. From their clinical characteristics 34 children showed generalized tonic-clonic convulsions, eight showed hypertonic convulsions, four showed hypotonic convulsions and five showed focal convulsions (three of which led to secondary generalization). Two patients in this group were under previous treatment with phenobarbitone and one with sodium valproate. During the acute seizure twelve patients were treated with diazepam. The FG group was divided into two subgroups: one of 26 patients corresponding to the diurnal febrile convulsive group (DFG) and one of 25 patients making up the nocturnal febrile convulsive group (NFG).

The FG group was considered separately from the epileptic convulsive group (EG), which included 28 children classified as epileptics according to WHO criteria (Commission on Classification and Terminology, 1989). This group included nine patients with partial tonic-clonic convulsions, two with generalized tonic-clonic convulsions of focal origin, 10 with hypotonic convulsions and seven with focal convulsions. Nineteen patients from this group did not receive any anticonvulsant treatment until they were sampled. Nine patients were treated before the convulsive episodes: four with sodium valproate, two with sodium valproate + phenobarbitone, one with sodium valproate + clonazepam and two with phenobarbitone. None of the patients in this group developed febrile symptoms associated with their convulsions. The EG group was also divided into two subgroups: a diurnal epileptic group (DEG), comprising 18 patients and a nocturnal epileptic group (NEG), comprising 10 patients.

Peripheral blood samples (5 ml) were collected from patients in both convulsive groups upon admission into hospital, between 1 hour and 3 hours after seizures, the duration of the convulsion, the time lapse between the end of the convulsion (Table 2) and the time of day were all noted. Due to ethical considerations only one sample was taken from each individual. According to the time of sampling the children were grouped into two periods of 12 hours each (diurnal and nocturnal groups). As far as possible, each child in the control group was sampled at the same time as each from the convulsive groups. The blood samples were centrifuged at 3000 *g* for 10 minutes and plasma was separated and frozen at −20°C until assay. Urine was collected from 0900 to 2100 h (diurnal groups) and from 2100 to 0900 h (nocturnal groups) in the control and convulsive groups. The urine volume was noted and an aliquot was frozen at −20°C until assay.

Table 2 Characteristics of the control and convulsive groups, and duration of convulsion and time elapsed from convulsion to sampling (mean ± SEM).

Group	n		Age (months)	texp	Duration of convulsion (min)	Time elapsed (min)
	Males	Females				
CDG	15	7	44·00 ± 7·74		—	—
				0·56 (ns)		
CNG	12	5	37·70 ± 10·01		–	
DFG	11	15	27·07 ± 3·77		10·42 ± 2·18	111·15 ± 21·10
				1·9 (ns)		
NFG	10	15	18·56 ± 2·05		11·65 ± 1·86	134·00 ± 15·53
DEG	10	8	59·50 ± 9·16		19 83 ± 3·42	106·38 ± 22·93
				1·46 (ns)		
NEG	7	3	38·00 ± 12·08		30·40 ± 23·16	122·00 ± 34·84

texp, t values obtained from statistical analysis

The concentration of plasma and urine aMT was determined by RIA (WHB, Bromma, Sweden). This method has been validated elsewhere for the direct measurement of aMT in human plasma and urine (Fernández *et al.*, 1990). Pooled human plasma serially diluted with assay buffer gave displacements parallel to those of aMT standards. The intra- and interassay coefficients of variation were 11·3% and 16·3%, respectively. Recovery of aMT, as assessed by the standard addition method, gave a value of 84·4% and sensitivity was 5 ng/l.

Standard reagents (KYN, 3HK, KYNA, XA and 3HANA) of the highest available purity were bought from Sigma for kynurenine metabolite determinations. These metabolites were determined by thin-layer chromatography according to Coppini *et al.* (1959), slightly modified by us (Narbona *et al.*, 1986), using 60 F254 silica gel plates (Merck). Briefly, 20 μl of standard mixture containing 4 μg of each standard or 100 μl of urine was applied to the chromatographic plate and quickly dried to avoid sample diffusion. The plate was then developed with the eluent (butanol : formic acid : distilled water, 40 : 5 : 55) without being previous saturated. After a development time of 4 hours, the solvent front reached 10 cm and the chromatography was stopped. The chromatogram was then dried in hot air and the spots were exposed by ultraviolet light (360 nm) in a photomatic Uvatom 75 (Atom). The spots were identified by their respective R_f values. Peak areas for appropriate standards covering the dynamic range, spotted in duplicate on each TLC plate, were used to construct a standard curve. Peak areas calculated for samples of urine were converted into the quantity of each kynurenine metabolite via the corresponding standard curve. Peak areas were always calculated using the square of the peak height (Narbona *et al.*, 1986) instead of the original formula (peak area = peak height × horizontal width of one-half peak height; Freer *et al.*, 1979). The relationship between the sample and standard concentration and the square of the peak height is linear and always results in correlation coefficients of above 0·99. The chromatographic method is highly sensitive, even when there are marked concentration variations among the five metabolites studied. The TLC method was subject to quality control (Freer *et al.*, 1979). Within-run (within-plate) analytical variation for the kynurenine standards of eight replicates at every concentration of each metabolite was determined, and the coefficients of variation thus obtained were between 6·5% and 8·5%. In precision studies on the urine samples from a control group (eight replicates in each case) the coefficients of variation were similar to those obtained for the within-run analytical variation of the standards (6·7–8·9%). The detection limit, i.e. the minimum amount of a compound that must be present in the chromatogram to allow reproducible results = sensitivity, was 0·3 μg for XA and 1·0 μg for KYN, 3HK, KYNA and 3HANA, respectively. The values obtained for each compound (in μg/100 μl of urine) were multiplied by 10 and divided by the child's weight (in kg). Thus, the results were expressed in μg/ml · kg.

All results are expressed as mean ± SEM. Plasma and urine aMT are expressed in ng/l. The results were analysed by Dunnet's *t*-test for multiple comparisons. Statistical analysis also included Pearson's correlation coefficient and correlation analysis.

Results

The results obtained in the control group are shown in Table 3. Melatonin production increased significantly during the night ($P < 0·001$) concomitantly with a significant decrease in the

Table 3 Day and night production of melatonin, kynurenine and kynurenine metabolites in normal and in convulsive children.

	Control group		Febrile group		Epileptic group	
Compound	DCG	NCG	DFG	NFG	DEG	NEG
Methoxyindoles (ng/l)						
Melatonin (plasma)	26·5 ± 2·0*	53·2 ± 5·2	75·6 ± 13·3	94·3 ± 12·2	33·1 ± 3·8*	88·5 ± 17·76
Melatonin (urine)	56·5 ± 5·9	63·9 ± 7·5	67·4 ± 8·7	84·5 ± 14·7	56·0 ± 9·2	67·8 ± 21·9
Kynurenines (μg/ml.kg)						
L-kynurenine	8·2 ± 1·3*	0·7 ± 0·2	2·4 ± 0·3	5·4 ± 1·6	2·6 ± 0·5	1·8 ± 0·3
3-hydroxykynurenine	24·5 ± 4·2*	4·1 ± 0·6	17·4 ± 3·3	42·3 ± 13·6	24·5 ± 12·5	9·9 ± 2·4
Kynurenic acid	24·7 ± 4·5*	5·5 ± 0·9	52·1 ± 8·9	53·3 ± 17·9	33·1 ± 16·7	124·6 ± 45·4
Xanturenic acid	25·3 ± 3·2*	8·7 ± 1·1	143·4 ± 29	119·0 ± 37	108·2 ± 25·2	136·1 ± 84·5
3-hydroxyantranilic acid	1·9 ± 0·2*	1·6 ± 0·3	2·1 ± 0·3	4·5 ± 1·2	3·5 ± 0·7	1·9 ± 0·4

Data are expressed as mean ± SEM. *$P > 0.001$ vs. night.

urinary excretion of kynurenine metabolites ($P < 0.001$). These results suggest the existence of two different profiles for tryptophan metabolism: a nocturnal profile for methoxyindole production (aMT) and a diurnal profile for kynurenine production.

The group of patients with febrile convulsions showed a completely different pattern of aMT and kynurenine metabolite production compared to controls (Table 3). In these groups the diurnal/nocturnal differences between the tryptophan metabolites seen in the control group disappeared. There was an increase in diurnal aMT production, whereas the production of 3HK, KYNA and XA increased both during diurnal and nocturnal periods. A similar pattern of tryptophan metabolite secretion was found in the group of patients with epileptic convulsions (Table 3). The significant difference between diurnal and nocturnal aMT levels found in the control group persisted in the epileptic group ($P < 0.001$) but was absent in the febrile group.

The percentage alterations in the presence of these compounds in the febrile and epileptic convulsive groups were compared to those in the control group (Fig. 1). A significant increase in diurnal aMT secretion can be seen in children with febrile (284% ± 50%, $P < 0.001$) but not epileptic (124% ± 14%) convulsions, but with no changes in their urine contents (119% ± 15% and 99% ± 16%, febrile and epileptic convulsions, respectively). It can also be seen in Fig. 1 that there was a significant decrease in KYN (29% ± 3% and 32·3% ± 7%, $P < 0.01$) and an increase in XA (566% ± 104% and 446% ± 87%, $P < 0.001$) in the febrile and epileptic diurnal groups, respectively. The other tryptophan metabolites remained unchanged, showing values of 3HK, 70·7% ± 13% and 99% ± 51%; KYNA, 210% ± 36% and 133% ± 67%; 3HANA, 104% ± 15% and 180% ± 71%, corresponding to the febrile and epileptic diurnal groups, respectively. During the night aMT increased in both the febrile and epileptic groups (177% ± 22% and 166% ± 36%, respectively; $P < 0.01$) but with no change to their urine contents (132% ± 23% and 106% ± 34%, febrile and epileptic groups, respectively) (Fig. 2). During the night there was also a significant increase in all the kynurenine metabolites in both the febrile and epileptic groups (KYN, 801% ± 248%, $P < 0.001$ and 236% ± 44%, $P < 0.01$; 3HK, 1026% ± 330%, $P < 0.001$ and 241% ± 58%, $P < 0.01$; KYNA, 958% ± 321%, $P < 0.001$ and 2241 ± 716%, $P < 0.001$; XA, 1370% ± 660%, $P < 0.001$ and 1567% ± 62%, $P < 0.001$, respectively). No changes in 3HANA were found 277% ± 73% and 119% ± 22%, nocturnal febrile and epileptic groups, respectively). A difference did exist between these groups, however; while the increase in these metabolites was similar in the febrile group, in the epileptic group the most significant increase was found in KYNA and in XA.

To analyse further the relationship between the methoxyindole and kynurenine metabolic pathways, we made a correlation analysis between their metabolites. The results of these comparisons are set out in Table 4, where the correlation coefficient and the statistical significance in each of the variable pairs can be seen. These data suggest: first, that in the control group, plasma aMT showed a significant inverse correlation with KYN ($P < 0.01$) and 3HK ($P < 0.05$); secondly, that in the febrile and epileptic groups the correlation level disappeared; and lastly, that when the relationship between the different kynurenines was analysed, significant correlation coefficients were found both in the control group and in the febrile and epileptic groups.

Discussion

Tryptophan is the main indole derivative, being especially abundant in high-quality biological proteins. Organisms use

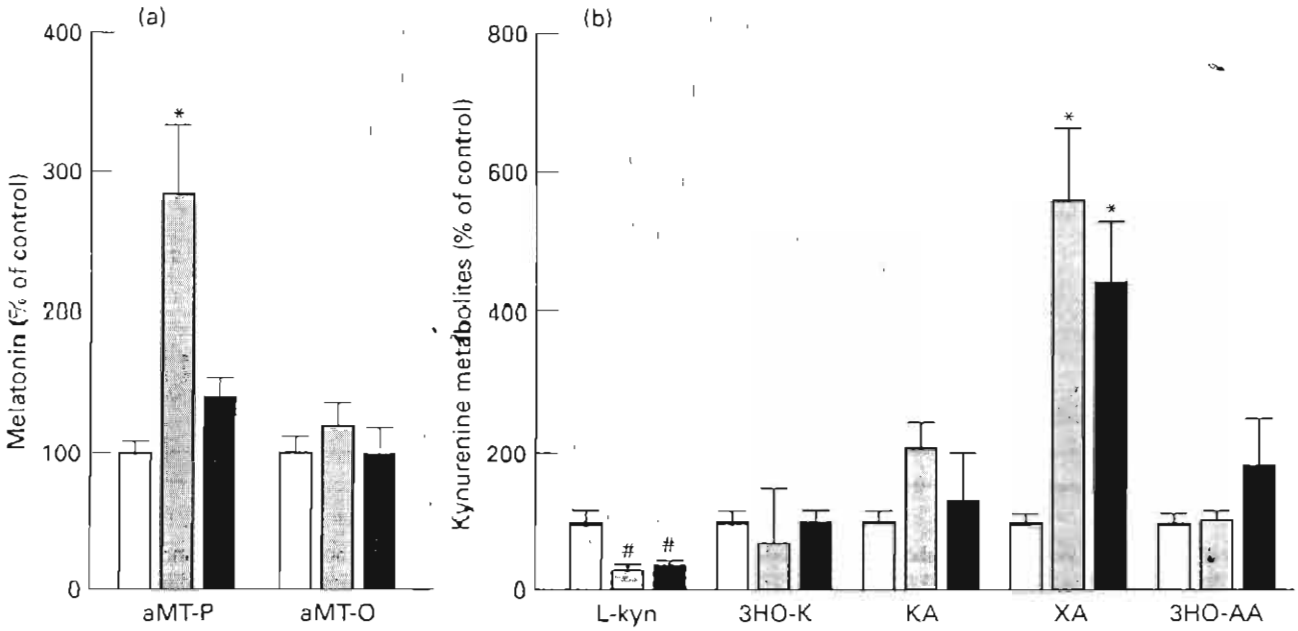

Fig. 1 Percentage changes in (a) diurnal methoxyindole pathway metabolites and (b) kynurenine pathway metabolites in the diurnal febrile group (▨), the diurnal epileptic convulsive group (■) and the diurnal control group (□). aMT-P: plasma melatonin; aMT-O: urine melatonin; L-kyn: L-kynurenine; 3OH-K: 3-hydroxykynurenine; KA: kynurenic acid; XA: xanturenic acid; 3HO-AA: 3-hydroxyindoleacetic acid. *$P < 0.001$ *vs.* DCG; #$P < 0.01$ *vs.* DCG.

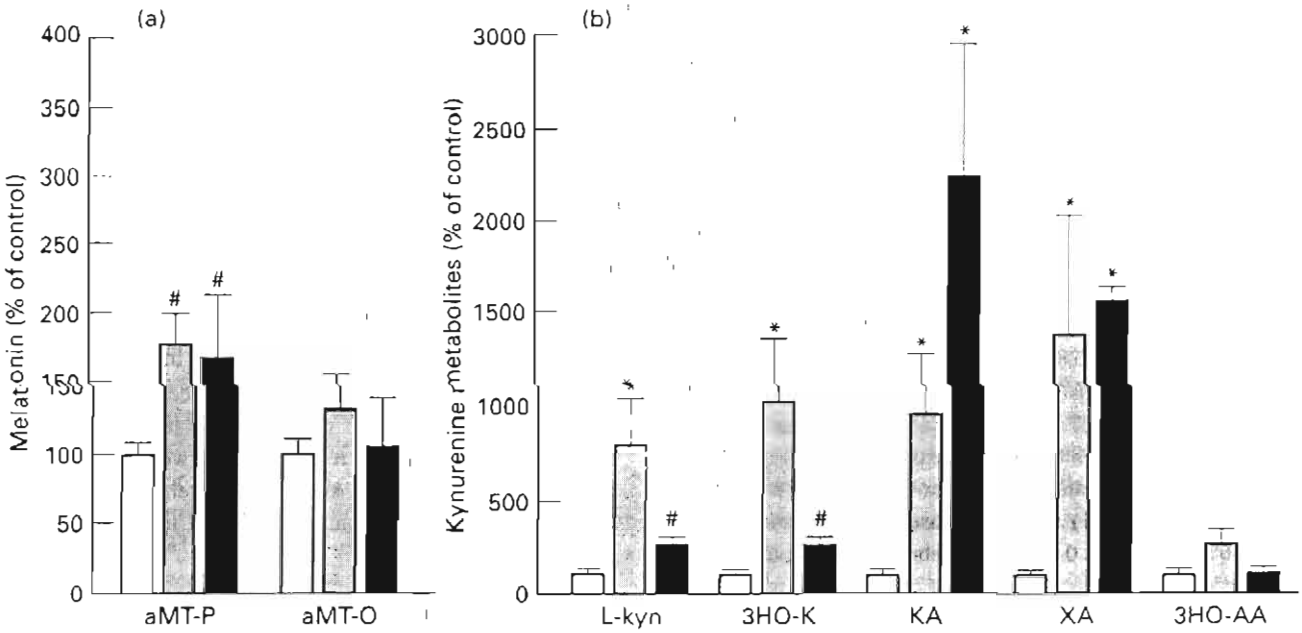

Fig. 2 Percentage of changes in (a) the nocturnal methoxyindole pathway metabolites and (b) kynurenine pathway metabolites in the nocturnal febrile group (▨), the nocturnal epileptic convulsive group (■) and the nocturnal control group (□). Abbreviations are as in Fig. 1. *$P < 0.001$ *vs.* NCG; #$P < 0.01$ *vs.* NCG.

Table 4 Correlation coefficients (r) and significance levels of each pair of related variables in each of the studied groups.

Pair of variables	DCG	NCG	DFG	NFG	DEG	NEG
aMT-P/aMT-O	−0·25	0·78**	0·65**	0·35	0·53	0·67
aMT-P/L-Kyn	−0·68*	−0·27	0·15	−0·16	−0·11	−0·19
aMT-P/3HO-K	−0·51	−0·34	0·11	0·004	0·001	−0·11
aMT-P/KA	−0·28	−0·16	0·13	0·10	0·001	−0·13
aMT-P/XA	−0·32	−0·004	−0·21	0·002	−0·007	−0·005
aMT-P/3HO-AA	−0·35	−0·20	0·0006	0·001	−0·19	−0·006
aMT-O/L-Kyn	−0·59	−0·44	0·24	−0·13	−0·13	0·005
aMT-O/3HO-K	−0·37	−0·45	0·26	−0·10	0·003	0·10
aMT-O/KA	−0·25	−0·22	0·37	0·16	0·002	0·17
aMT-O/XA	−0·24	0·14	0·15	0·002	−0·10	−0·004
aMT-O/3HO-AA	−0·54	−0·26	0·0005	0·002	−0·25	0·008
L-Kyn/3HO-K	0·85*	0·79**	0·91**	0·32	0·37	0·84*
L-Kyn/KA	0·64*	0·78**	0·85**	0·0007	0·27	0·72
L-Kyn/XA	0·47	0·43	0·46	0·17	0·66*	0·44
L-Kyn/3HO-AA	0·66*	0·68*	0·71**	0·30	0·67*	0·88**
3HO-K/KA	0·76*	0·84**	0·89**	0·47	0·97**	0·91**
3HO-K/XA	0·62*	0·51	0·33	0·69**	0·85**	0·80*
3HO-K/3HO-AA	0·65*	0·89**	0·51*	0·78**	0·36	0·80*
KA/XA	0·80*	0·74*	0·65**	0·93**	0·83*	0·72
KA/3HO-AA	0·62*	0·83**	0·53**	0·85**	0·30	0·61
XA/3HO-AA	0·61*	0·48	0·59*	0·97**	0·46	0·56

aMT-P: plasma melatonin; aMT-O: urine melatonin; L-kyn: L-kynurenine; 3HO-K: 3-hydroxykynurenine; KA: kynurenic acid; XA: xanturenic acid; 3HO-AA: 3hydroxyantranilic acid.
*$P < 0·01$; **$P < 0·001$.

four main pathways, with different enzyme systems, to metabolize tryptophan (Narbona *et al.*, 1994). We have analysed two of these catabolic pathways: the kynurenine pathway, which is considered to be the most important metabolic destination of tryptophan; tryptophan pyrrolase cleaves the pyrrolic ring of the amino-acid producing formylkynurenine, which is rapidly hydrolyzed to KYN and its metabolites (Snyderman *et al.*, 1961), and the methoxyindole pathway, which produces N-acetylserotonin and aMT, both important biological amines. The action of N-acetyltransferase on 5-hydroxytryptamine is the limiting step in this synthesis. The main source of these amines is the pineal gland, which helps to control biological rhythms and brain activity (Cardinali, 1981; Reiter, 1981, 1983; Acuña-Castroviejo *et al.*, 1995).

Intracerebroventricular injection of KYN or 3HK in mice produces convulsions (Lapin, 1978), which may be counteracted by some of its metabolites, such as KYNA, XA and 3HANA. These kynurenine metabolites act as endogenous anticonvulsants. Thus, the increased excretion of some of these kynurenines might represent the expression of an endogenous compensatory mechanism during convulsions (Lapin, 1981). It has also been suggested that in some pathological situations in which an imbalance between kynurenine and its antagonists exists, with increasing KYN levels, an excitatory proconvulsant condition may occur (Lapin, 1981). With regard to this point, increased urinary excretion of both XA and KYNA during convulsions has been reported (Rodríguez *et al.*, 1990; Molina *et al.*, 1991).

Our results show significant diurnal/nocturnal changes in the urinary concentrations of KYN, 3HK, KYNA and XA in febrile and epileptic patients compared to normal children. In the control group all kynurenine metabolites, apart from 3HANA, were significantly lower during the night than during the day. In the febrile and epileptic groups, on the other hand, significant increases in both diurnal and nocturnal production of 3HK, KYN and XA took place. These changes explain the disappearance of the diurnal/nocturnal differences in these metabolites in the convulsive groups compared to controls. Similarly, plasma aMT and its urinary excretion increased in the febrile group both during the day and night, and only in the epileptic group was there any significant diurnal/nocturnal difference in plasma aMT.

When the values of tryptophan metabolites were expressed as a percentage change, a significant reduction in KYN and an increase in XA excretion compared to the control group was found during the day in the convulsive groups. During the night, however, there was a significant increase in KYN 3HK, KYNA

and XA in both the febrile and epileptic groups. As far as aMT levels are concerned, the results showed an increase in aMT production in the febrile group during the day and an increase in both the febrile and epileptic groups during the night.

The data reveal that in the convulsive groups there was a decrease in the proconvulsant metabolite KYN during the day whereas 3HK, another proconvulsant metabolite, was unchanged. During the day the anticonvulsants aMT (in the febrile group) and XA (in the febrile and epileptic groups) also increased. During the night, however, all the proconvulsant and anticonvulsant metabolites, except for 3HANA, increased. These data point towards the existence of a dissociation between the diurnal and nocturnal production of tryptophan metabolites: in normal children tryptophan is mainly metabolized during the day via the kynurenine pathway, thus producing higher levels of kynurenine metabolites and lower levels of aMT. During the night, tryptophan metabolism switches to the methoxyindole pathway, resulting in an increase in aMT and a concomitant decrease in kynurenines. In the convulsive children this diurnal/nocturnal equilibrium disappeared, perhaps due to some modification to the enzyme activity in the metabolic pathways, resulting in a significant increase in nocturnal tryptophan metabolites. This nocturnal metabolic increase might be related to the existence of a circadian rhythm in seizure activity, which appears to be higher during the night (Champney *et al.*, 1993).

Both pro- and anticonvulsant tryptophan metabolites were higher in the convulsive groups. Nevertheless, the higher nocturnal increase in the anticonvulsants KYNA and XA in the epileptic compared to the febrile group still remains to be clarified. Perhaps a chronic seizure status, such as epilepsy, induces a permanent alteration in tryptophan metabolism, deviating it to the production of anticonvulsant metabolites. This hypothesis points to an up-and-down regulatory mechanism for the tryptophan metabolic pathways, i.e. that the organism adapts itself over a period of time to counteract hyperexcitatory brain activity. The data also suggest that the changes in tryptophan metabolites are not the origin of the convulsions, but a consequence of them (Heyes *et al.*, 1994). Thus, measurements of tryptophan turnover in these patients may throw more light on the relationship between cerebral activity and tryptophan metabolism.

It has been shown that of the intermediary metabolites in the kynurenine pathway only KYN and 3HK are actively transported across either the blood–brain barrier or the cerebrospinal fluid barrier (Fukui *et al.*, 1991). KYNA, 3HANA and XA seem barely to cross the blood–brain barrier and are therefore not expected to contribute significantly to the brain pool under normal conditions. Our observations revealed that during seizures both the metabolites to which the blood–brain barrier is permeable and those that normally do not cross the barrier to any significant extent increased in urine. The extracerebral metabolism of kynurenines cannot be ruled out since a diurnal variability in the activity of hepatic tryptophan dioxygenase exists and consequently changes in the level of tryptophan metabolites in urine might reflect changes in hepatic metabolism. It is highly likely, however, that changes in systemic kynurenine pathway metabolism exert a considerable influence on changes in brain kynurenine metabolites such as KYNA, which do not normally cross the blood–brain barrier (Heyes, 1993). Several changes are also known to occur in brain cells following convulsive injury, including infiltration by macrophages and glial proliferation. Invading macrophages rather than microglia seem to constitute a major source of cerebral kynurenine metabolites (Alberati-Giani *et al.*, 1996). Thus, the changes in kynurenine metabolite excretion that we have observed probably reflect similar alterations in its presence in the brain. During seizures the normal distribution of kynurenine metabolites between the brain and its peripheries is altered because the blood–brain barrier is breached and thus become more permeable (Petito *et al.*, 1977).

Although Heyes *et al.* (1994) recently reported that no differences between tryptophan metabolite concentrations could be put down to the time of day at which the samples were collected, our observations record significant diurnal/nocturnal differences in the production of these metabolites. This discrepancy may be due to the fact that Heyes *et al.* (1994) compared data from samples taken between 0600 and 1200 h vs. 1200 and 1800 h vs. 1800 and 2000 h, whereas we compared the data collected from 0900 to 2100 h vs. 2100 to 0900 h. An alteration in the aMT circadian rhythm has recently been shown to exist in convulsive pathology (Molina-Carballo *et al.*, 1994a,b), and a 5-hour phase-shift delay in the aMT nocturnal peak has also been detected in epileptic children (Champney *et al.*, 1995). As far as the kynurenine pathway is concerned, a similar alteration in the nocturnal peak of its metabolites may well occur and collecting the samples between 0600 and 1200 h might mask the circadian nature of their production. These observations then could well explain the lack of correlation between KYN and KYNA previously reported (Heyes *et al.*, 1994). Our data showed a significant inverse correlations between aMT and KYN and 3HK in the control group. These correlations suggest the existence of a balance between anti- and proconvulsant tryptophan metabolite production. The disappearance of these correlations in convulsions further supports the hypothesis of an uncoupling of tryptophan metabolic pathways in seizure pathology.

The pro- and anticonvulsant tryptophan metabolite compounds seem to produce their effects by acting on brain NMDA-subtype glutamatergic receptors. Electrophysiological studies have demonstrated that aMT plays a physiological role in the inhibition of striatal NMDA receptor activity in rat brain

(Castillo-Romero *et al.*, 1993; Escames *et al.*, 1996). This indoleamine inhibits brain nitric-oxide–synthase activity (Pozo *et al.*, 1994) and counteracts nitric-oxide-dependent, free-radical production. The recently described free-radical scavenger properties of aMT supports the role suggested for this neurohormone as an efficient cell protector (Reiter *et al.*, 1994; Acuña-Castroviejo *et al.*, 1995; Reiter *et al.*, 1995). Thus, the increase in plasma aMT during convulsions without changes in urinary aMT excretion points to an increase in plasma aMT metabolism, which may be due to the 'suicidal' properties of this molecule after its antioxidant action (Reiter *et al.*, 1994, 1995).

In summary, relationships between the tryptophan metabolic pathways do exist. According to the needs and requirements of the organism and its condition, some tryptophan metabolic pathways will be stimulated while others will be inhibited in order to arrive at the required effect. Although generalized disturbances in kynurenine pathway metabolite levels take place in convulsions, our data do not support the ideal of KYN or 3HK playing any part in the triggering or the maintenance of febrile and/or epileptic convulsions in children. The greatest support is for the possibility that an imbalance between the methoxyindole and kynurenine tryptophan metabolic pathways may help to produce brain excitotoxicity and convulsions.

Acknowledgements

This work was partially supported by the Junta de Andalucía and the Hospital Universitario of Granada. We thank our colleague Dr J. Trout for revising our English text.

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