Amino Acids (1999) 17: 401–413

Amino
Acids

Where does indolylacrylic acid come from?

E. Marklová

Department of Paediatrics, Laboratory of Inherited Metabolic Diseases, Faculty of Medicine & Faculty Hospital, Charles University, Hradec Králové, Czech Republic

Accepted January 29, 1998

Summary. In addition to the main catabolic routes of tryptophan (Trp), there exist minor and less thoroughly investigated pathways; one of these leads to indolylacrylic acid (IAcrA). IAcrA is a plant growth hormone, whereas its biological role in animals is still obscure, as is the way and site where it is formed in the organism. A two-stage production is likely: Intestinal microorganisms catabolize Trp to indole derivatives which are then absorbed and converted to IAcrA and its glycine conjugate, indolylacryloylglycine (IAcrGly). Our finding of IAcrGly in the urine of proven germ-free piglets points to the possibility that Trp can be converted to IAcrA without the intervention of intestinal microorganisms. Seasonal and age variations, influence of light and connection with photodermatoses have been reported. Besides other pathological conditions the differences in IAcrGly excretion relative to normal controls were especially pronounced in some myopathies, namely in boys with Duchenne muscular dystrophy.

Keywords: Amino acids – Indolylacrylic acid – Tryptophan – Indolylacryloylglycine

Introduction

Tryptophan (Trp) is one of the amino acids with the highest number of alternative degradation pathways. One of these pathways, which so far has been given insufficient attention, leads to indolylacrylic acid (IAcrA). IAcrA in plants has been known since 1949 (Gautheret) and is included among the growth hormones. Later on, it was found in animal urine (man, monkey, pig, rat, mouse) in the form of the glycine conjugate, indolylacryloylglycine (IAcrGly). Its role in animals is still obscure, as is the way and site where it is formed in the organism. A two-stage production is likely: intestinal microorganisms catabolize Trp to indole derivatives (e.g. indolylpropionic acid), which are then absorbed and converted in the liver or kidney to IAcrA and its glycine conjugate, IAcrGly (Smith et al., 1968). Another possible way of IAcrA production goes via hypaphorine (N-trimethyltryptophan). Conver-

sion of Trp to this betaine has been so far demonstrated in some plants only (family Fabaceae) (Hofinger et al., 1975) (Fig. 1).

The possibility of conversion of Trp to IAcrA, catalysed by the animal enzyme system[1] without the participation of intestinal bacteria, is suggested only by one finding mentioned in the literature (Mandell and Rubin, 1965).

Methods

The method for IAcrGly and IAcrA used formerly is based on their unusual behaviour on the Sephadex G-10 column and involves, in addition, urine extraction, two-dimensional cellulose TLC and UV spectrophotometry (Marklová and Hais, 1972). The methods used for indolylacetic acid (IAA) (Weisbach et al., 1959), 5-hydroxyindolylacetic acid (5-HIAA) (Udenfriend et al., 1955) and 3-hydroxyanthranilic acid (3-HAA) (Tompsett, 1959), consist of urine extraction, colour reaction with xanthydrol, α-nitroso-β-naphthol and 4-aminoantipyrine, resp., followed by spectrophotometric determination. Further study of IAcrGly and its potential precursors, which might be present in biological fluids

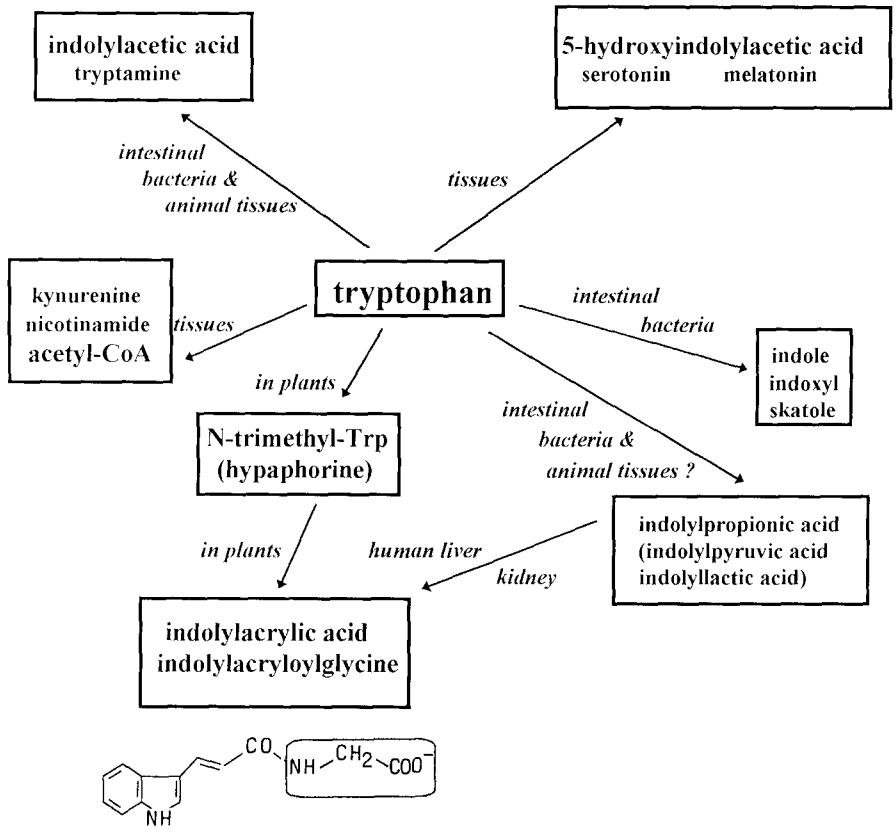


Fig. 1. Some pathways of tryptophan degradation

[1] There is no direct evidence for enzymic processes so far and non-enzymic processes cannot be excluded.

in exceedingly small quantities, required a new approach. We elaborated and introduced two methods utilising HPLC procedure (Marklová and Fojtášková, 1996; Marklová et al., 1996).

Hypaphorine was determined as follows: urine was twice extracted with n-butanol, combined organic layers evaporated, dissolved in the phosphate-citrate buffer pH 7.1 and incubated with urease (to prevent the overlapping of both HP and urea on TLC). The sample was then desalted on Dowex 50WX8, eluted with 2M ammonia and farther processed by TLC, as described by Hofinger et al. (1975) for the vegetal material. The Ehrlich and Dragendorff reagents were used for detection.

The method of Monboise et al. (1982) was used for the 3-methylhistidine (3-MeHis) quantitation in urine, combining TLC with the fluorimetric techniques.

Preferably 24h urine samples were used; the excretion of IAcrGly and other metabolites was expressed on creatinine basis.

Results

IAcrGly throughout the year and after irradiation

We have established (Marklová et al., 1975), that the same group of healthy adults excrete more IAcrGly during the summer (June–September), than during the winter months (November–March). We have attempted to investigate this seasonal increase by following the excretion pattern at monthly intervals throughout the year in 10 healthy persons, and then examining the influence of UV radiation by direct experiment. In spite of individual differences in skin sensitivity, all subjects exhibited a marked increase of IAcrGly excretion during the summer period (Fig. 2) as well as during the three days following the single dose irradiation (3 times the minimum erythema doses), Marklová and Hais (1978a).

IAcrGly in some skin diseases

Papers reporting the occurrence of the so-called Kimmig's light band can be considered as the first reports on IAcrGly in the dermatological literature,

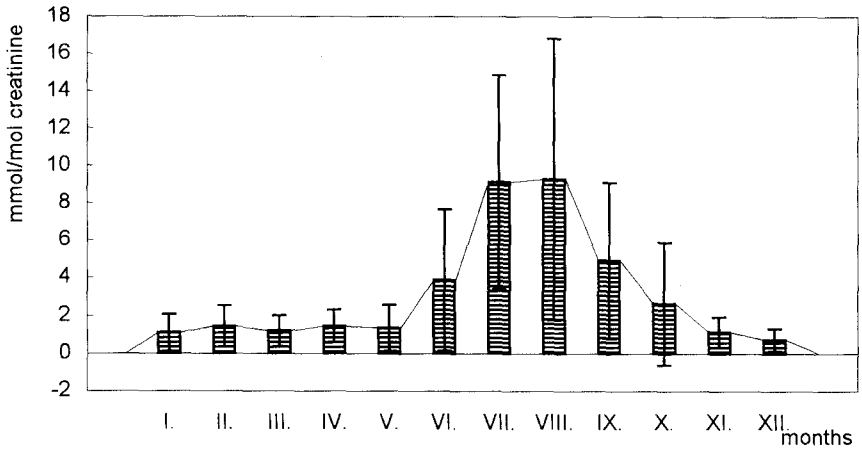


Fig. 2. IAcrGly excretion during a year in 8 adults and 2 children, means ± SD

since IAcrGly was later identified as the main chromogen of the band (Kimmig et al., 1958). The disagreement between the earlier papers and some other states concerning some skin affections, represented a challenge for us to clarify the situation.

In comparison with controls we observed significantly higher mean IAcrGly excretion in patients with chronic polymorphous light eruption (CPLE) and skin tuberculosis, but only in the winter (November–March) period. In summer (June–September) some well-marked increase in both the control and CPLE groups occurred, but the difference between controls and CPLE patients disappeared (Marklová et al., 1975), (Fig. 3).

Age variations of IAcrGly excretion

In addition to adults we followed the urinary excretion of IAcrGly in a large number of both healthy and diseased children. The finding of a difference between newborn infants and older children prompted the investigation of age differences in normal subjects. In winter period we checked the IAcrGly excretion in 235 controls of both sexes, children and adults, and an age dependence with a maximum excretion (and the highest scatter of the values) between ages 2 and 7 years were observed (Marklová and Hais, 1978b) (Fig. 4).

IAcrGly in muscular dystrophy

In addition to healthy controls we investigated groups of children, hospitalised for various disorders (e.g. scarlet fever, enterocolitis, epilepsy, psychomotor retardation). The differences in IAcrGly excretion relative to normal controls were especially pronounced in some myopathies, the highest

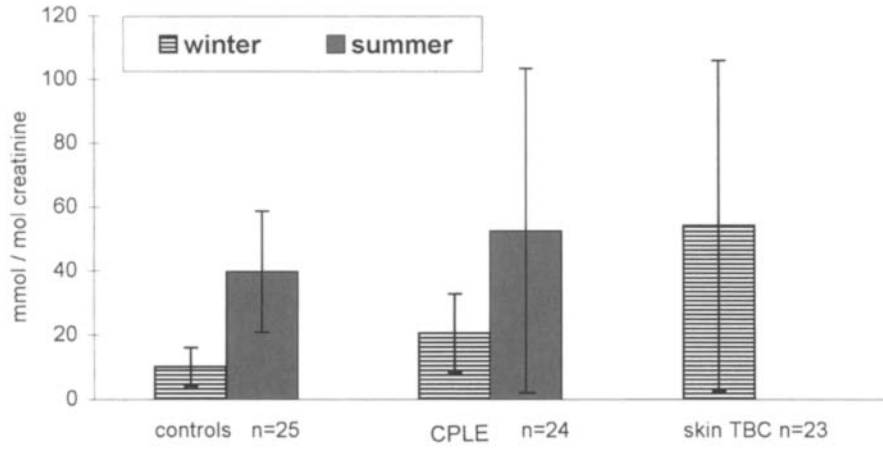


Fig. 3. IAcrGly excretion in some photodermatoses (mean ± SD); *CPLE* chronic polymorphous light eruption; *TBC* tuberculosis

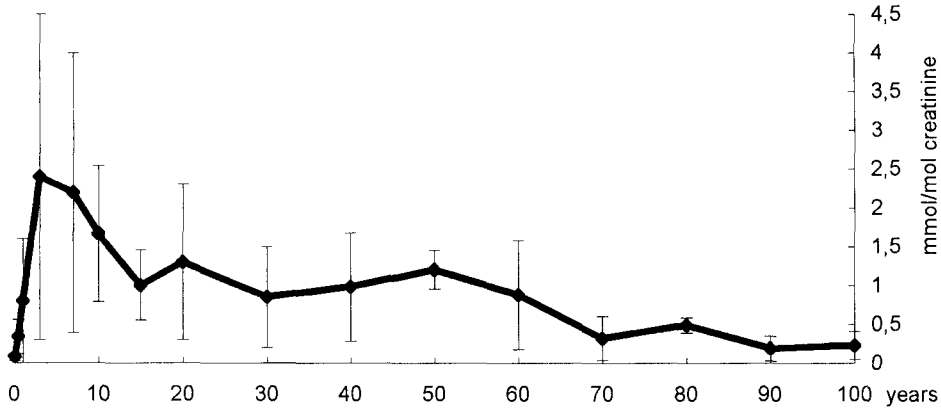


Fig. 4. Age variations of IAcrGly excretion in controls (mean ± SD, n = 235; tested in November–March)

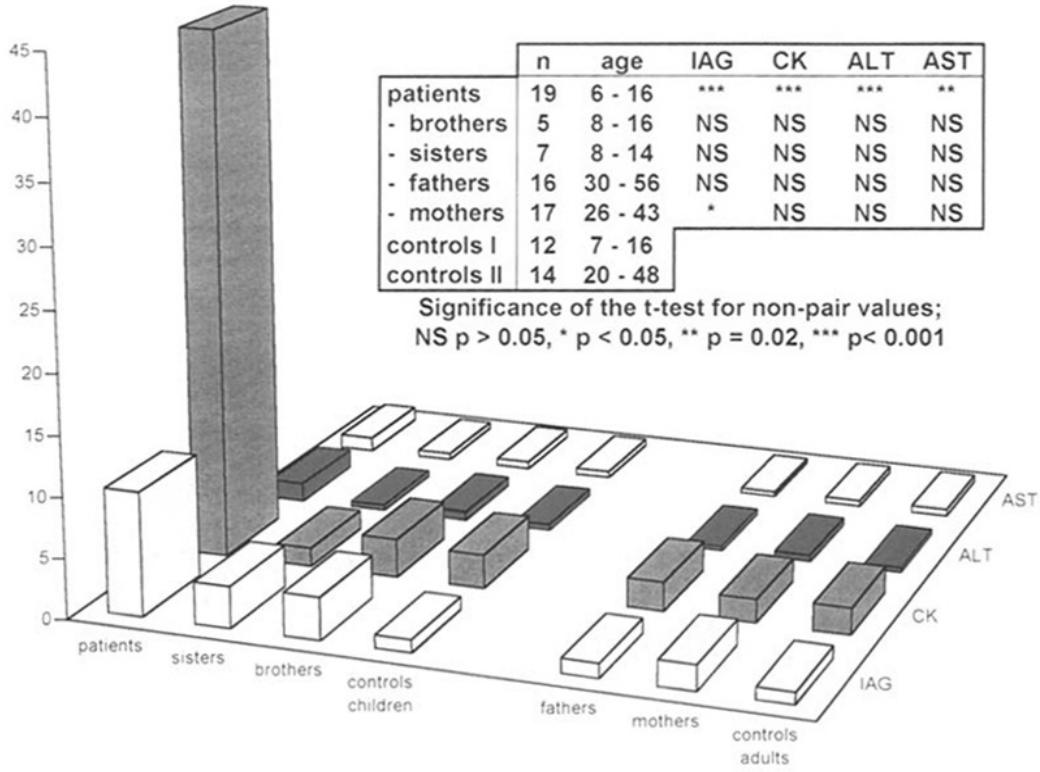
being in a group of 32 boys suffering from the X-linked Duchenne type of progressive muscular dystrophy (Marklová et al., 1986b). Looking for the possible causes of such an increase we checked for the correlation of IAcrGly values with 1) urinary 3-MeHis (an indicator of the muscle-turnover) excretion, 2) some other catabolites of Trp (IAA and 5-HIAA), 3) eventual hyperaminoaciduria, derived from the higher breakdown of muscle proteins or from an increased permeability of the defective membranes, 4) a degree of muscle involvement, body weight, age and serum enzymes (creatine kinase and transaminases) and 5) the type of diet and therapy. Farther, we expanded our investigations to other family members.

The prevailing higher 3-MeHis / creatinine index and the occasionally mild hyperaminoaciduria found in some patients do not correlate with the respective IAcrGly levels. No relationship of the IAcrGly values and the diet or therapy could be certified. No clear-cut correlation was recognized between urinary IAcrGly, IAA, 5-HIAA and the other factors followed, with the exception of the weak relation between the age, activity of creatine kinase and IAcrGly excretion in the group of patients ($p < 0.1$, Spearman's coefficient of correlation).

In addition to affected boys, higher mean IAcrGly excretion was found in their mothers ($p < 0.05$, t-test for non-pair values) (Fig. 5, 6).

IAcrGly and diet

We investigated the effect of composition and the way of nourishment on IAcrGly excretion, as the diet is one of the speculated factors, when considering the origin of IAcrGly and its precursors in animals. As follows from the search for IAcrA in some foodstuffs, this exogenous source does not participate considerably in the amount of IAcrGly in animal urine (traces of IAcrA being detected only in lentil and pea). No influences either of the food com-



	n	age	IAG	CK	ALT	AST
patients	19	6 - 16	***	***	***	**
- brothers	5	8 - 16	NS	NS	NS	NS
- sisters	7	8 - 14	NS	NS	NS	NS
- fathers	16	30 - 56	NS	NS	NS	NS
- mothers	17	26 - 43	*	NS	NS	NS
controls I	12	7 - 16				
controls II	14	20 - 48				

Significance of the t-test for non-pair values;
NS $p > 0.05$, * $p < 0.05$, ** $p = 0.02$, *** $p < 0.001$

Fig. 5. Variations of means in the families of patients with Duchenne muscular dystrophy: *IAG* indolylacryloylglycine (μmol/24 h), creatine kinase (*CK*), alanine aminotransferase (*ALT*) and aspartate-aminotransferase (*AST*) (μkat/l)

position (vegetarians, couples on exactly the same diet) or by the Trp loading have been observed, but complete elimination of Trp from the diet (therapeutic starvation) resulted in a marked decrease of IAcrGly in urine, similarly as with parenteral alimentation (Marklová et al., 1986a).

IAcrGly in urine of microbial gnotobionts

1) Treatment with antibiotics: Excretion of IAcrGly was tested in a group of 50 hospitalised patients with severe neutropenia, treated by selective decontamination of the intestinal tract on account to the protection from infectious complications. Usually we could follow a distinct decrease of IAcrGly excretion in the process of treatment (Marklová et al., 1986a).

2) Conventional and germ-free animals: Urine samples from 62 piglets or adult pigs of the miniature breed (Minnesota) of both sexes fed on various types of diet were studied. The set included a) conventional animals bred in the usual way, b) the selectively decontaminated animals (devoid of gram-negative aerobic intestinal bacteria, moulds and yeast), c) germ-free animals, kept from birth in a sterile environment, d) originally germ-free animals, associated additionally with certain microorganisms (Mandel et al., 1985). It

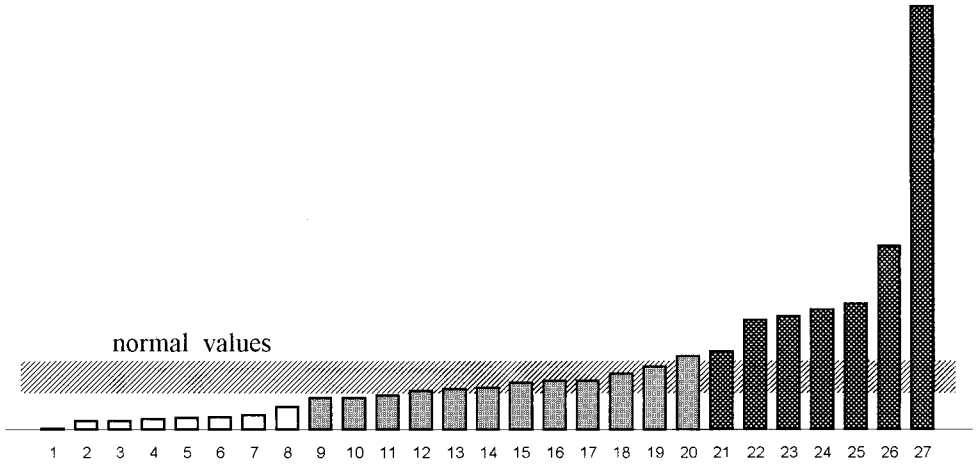


Fig. 6. Urinary IAcrGly excretion in various diseases (in % of the appropriate normal values; Ad – adults, Ch – children; 1–8 significantly lower, 21–27 significantly higher excretion of IAcrGly) *1* burns (first 5 days after injury) Ad, *2* brain tumours Ch, *3* bladder cancers Ch, *4* lymphomas Ch, *5* liver cirrhosis Ad, *6* leukaemia remission (no treatment) Ch, *7* leukaemia relapse (cytostatics) Ch, *8* selective decontamination with antibiotics Ad, *9* eczema Ad, *10* porphyria cutanea tarda Ad, *11* liver tumours Ch, *12* Down syndrome Ch, *13* psoriasis Ad, *14* vitiligo Ad, *15* psychomotor retardation Ch, *16* epilepsy Ch, *17* Down syndrome Ch, *18* CPLE adults (summer), *19* hyperpigmentation in pregnancy Ad, *20* enterocolitis Ch, *21* scarlet fever Ch, *22* Duchenne dystrophy – mothers, *23* skin tuberculosis Ad, *24* other muscular dystrophies Ch, *25* CPLE Ad (winter), *26* spinal atrophy Ch, *27* Duchenne dystrophy Ch

can be concluded that conventional animals excreted IAcrGly irrespective of age. In other groups age dependence of excretion was observed: in younger individuals urinary IAcrGly was below the detection limit whereas in older animals (over 3 months, i.e. adult) IAcrGly excretion was clear-cut. Surprisingly, IAcrGly was detected in urine of four demonstrably germ-free adult subjects. We could not find any connection between the IAcrGly excretion and the type of diet (Marklová et al., 1986a).

IAcrGly in burns

Some facts have drawn our attention to the skin as an organ which can influence the catabolism of Trp and thus the amount of IAcrGly produced: a) An increase of IAcrGly in the summer months, following insolation and UV irradiation and in some photodermatoses has been found. b) IAcrA is an analogue of imidazolylacrylic (urocanic) acid which is formed in the skin. c) Changes in the excretion of other Trp metabolites, especially those of the kynurenine pathway, were described in some skin diseases.

Skin damage in burns might be a model of a diffuse inflammatory-degenerative process (Binazzi and Calandra, 1975).

The mean excretion of the total group of 14 male burn patients (involved area 10–67% of the body surface) was significantly lower than that in the

controls ($p = 0.0008$) with the lowest average values on days 3–15 following injury (Fig. 6). Values of IAcrGly did not systematically correlate either with those of the two other Trp catabolites (IAA and 5-HIAA) in urine, or with the therapy (antibiotics, corticoids), parenteral nourishment or acute liver injury present in some patients (Marklová et al., 1993).

IAcrGly in malignant diseases

A number of investigations have brought the tryptophan metabolites of the kynurenine pathway under suspicion of being carcinogenic or cocarcinogenic, as some of those metabolites (e.g. 3-HAA) were reported as elevated in urine of some patients with bladder cancer. A certain carcinogenic effect has also been found in IAcrA (Sergeeva et al., 1979).

Not only did we fail to find a higher level of IAcrGly in patients with various types of malignant diseases as we expected, but in fact, lower excretion was observed. We found no significantly higher mean excretion of 3-HAA in adult bladder cancer patients. Hematological malignancies in children were further accompanied by low excretion of IAA and bladder cancers by a lower 5-HIAA level. We found no correlation of the metabolites tested in individuals of any patient group. In controls, however, IAcrGly and IAA did correlate (Marklová et al., 1992) (Fig. 7).

Hypaphorine

A method for analysis of hypaphorine (N-trimethyltryptophan, lenthicine, HP) in plants (Hofinger et al., 1975) was introduced, and a number of foodstuff of vegetable origin has been processed. HP was found in eight types of plant fruits (e.g. lentil, pea, bean, carrots, beetroot).

A procedure for HP determination in urine and other animal body tissues was worked out (see Methods). No traces of HP have been detected in the

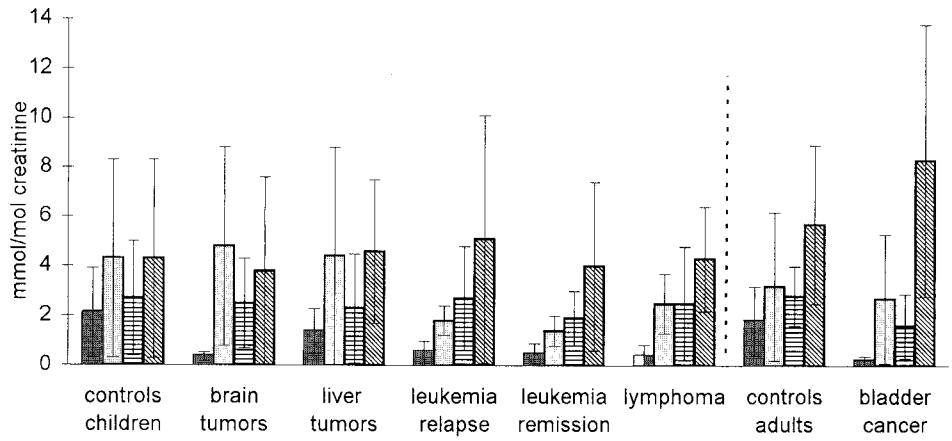


Fig. 7. IAcrGly and other Trp metabolites in two groups of controls and in patients with malignant diseases (means ± SD); the columns in order: IAcrGly, IAA, 5-HIAA, HAA

samples processed so far (Ehrlich and Dragendorff-positive spot on TLC, reminding that of the standard HP, has been observed in urine of two patients during the therapeutical starvation, without any further identification).

Parenteral loading with HP did not expressively increase the excretion of IAcrGly in mice (Marklová et al., 1986a).

Discussion

IAcrGly is a regular constituent of human urine (Marklová and Hais, 1972). Changes in its excretion have been observed under some physiological and pathological conditions (Fig. 6), but without any detailed knowledge of this metabolic pathway from tryptophan, those findings are difficult to explain.

So far it is generally assumed that bacterial activity in the intestine is most probably the main source of IAcrA in man, even though the possibility of endogenous formation directly from Trp by the human's own enzymatic system can not be excluded. In speculating on reasons for any changes in IAcrGly excretion one should consider that it may be due to changes in the formation or degradation of precursors (IAcrA, indolylpropionic acid), changes in the glycine conjugation of IAcrA, and changes in the renal excretion of the conjugate.

Seasonal variations and skin diseases

As the source of IAcrGly and its precursors in humans is still a matter for conjecture, it is difficult to give an unambiguous explanation for its increase in the summer since in addition to stronger sunlight other factors (temperature, diet) can be involved. The theory about the effect of light might be supported by the increase of IAcrGly following artificial UV irradiation. There may be some "summer" differences in the composition of food, thus either increasing the uptake of IAcrGly precursors or changing the intestinal bacterial flora. Apart from the eye or the pineal, light can act by influencing the skin, and this organ might then change the function of the intestine, liver or kidney. We have suggested a hypothesis based on the different catabolism of both trans and cis isomers of IAcrA, leading under some conditions to increased IAcrGly excretion (Marklová et al., 1975).

One can also speculate on the changes in Trp metabolism as a direct or mediated consequence of the pathological process of the skin, e.g. in photodermatoses. The disappearance of the difference between the controls and the CPLE group in summer, observed in our experiments, might be explained by the fact, that patients successfully avoided insolation.

The occurrence of abnormal intestinal functions in connection with photodermatoses have been described (Wiskemann and Kimmig, 1963).

When evaluating changes in the IAcrGly excretion, seasonal variations and the influence of light must be born in mind. That is why all our next investigations were strictly limited to the winter months (November–March).

Age differences

Low values of IAcrGly during the early months of life compared with later periods might be attributed to differences in dietary regime, intestinal flora and the enzymatic systems of the liver. The question as to why IAcrGly is formed and/or excreted more in the 2–7 year age group is difficult to answer even tentatively. Assuming that this phenomenon is related to the influence of sunlight (UV light) on the excretion of IAcrGly, it would have to be proven that children of this age group are more likely to spend longer periods outdoors, than others.

Duchenne dystrophy

Muscular dystrophies are a group of genetic diseases, characterised by degeneration of skeletal and often of cardiac muscles. The severe muscle devastating disorder, X-linked Duchenne type is due to the mutation in the dystrophin gene, which leads to the breakdown of the sarcolemmal dystrophin protein complex and to the muscle degeneration. One speculates about a membrane defect in muscle fibres, causing increased permeability. If other tissues including intestinal cells were similarly affected, the supply (exorption) of proteins to the intestinal bacteria producing IAcrGly precursors would be increased. It cannot even be excluded, that massive release of muscular proteins into the circulation might cause higher exsorption without any changes in the permeability of cells.

This hypothesis is somewhat contradicted by the lack of a well-marked hyperaminoaciduria in our patients. However, only amino acid analyzer has been used for the quantification, which is not very reliable procedure for Trp; other studies should verify the results.

Selective decontamination

Therapeutic selective decontamination of the intestinal tract in patients with neutropenia in most cases leads to a decrease of IAcrGly in urine; some exceptions observed might be possibly explained by more resistant strains present, or by higher endogenous production (?).

Germ-free animals

The finding of IAcrGly in the urine of 4 demonstrably germ-free animals is most revealing, since it testifies to the existence of an extraintestinal source of IAcrA. This source would come to the fore after the respective enzyme systems have become expressed. Our results suggest that intestinal microorganisms significantly participate in the production of IAcrA in minipigs (possibly in other animals as well); however, they are not its only source.

The formation of IAcrGly precursors in animal tissues should also be considered interpreting the increase in excretion of IAcrGly observed in some diseases (photodermatoses or muscular dystrophy).

Burns

One might consider some reasons for the decrease in IAcrGly excretion after a burn injury: Providing that intestinal flora is the main source of IAcrA precursors, then some derangement of intestinal absorption due to vasoconstriction and hypoxia should lead to a decrease of indolylacetic acid excretion, in addition to IAcrGly (owing to the similar origin, Weisbach et al., 1959), which in fact was not found. Speculation based on the decrease of Trp supply for intestinal microorganisms caused by parenteral nutrition following injury in some patients can not explain the observed decrease in those on a normal diet. Antibiotic treatment theoretically might influence the intestinal microorganism: In five of our patients penicillin was administered, which can hardly affect the situation; colimycin was used only in one case.

Pathological process of the skin in burns may lead to changes in Trp metabolism by a similar process, as speculated in photodermatoses.

Malignant diseases

There may be some inhibitory effects caused by chronic malignant process (the influence of hormones, enzyme inhibition, etc.), which are involved.

Hypaphorine

HP found in some plant fruits (of the tribe Fabaceae in particular) can not be theoretically excluded as an exogenous source of urinary IAcrGly. However, owing to its concentration (the highest being in the lentil seeds in amount approx. 50μg/g) and the daily intake of such a food, HP could not make any great contribution as a hypothetical precursor of IAcrA.

Various pathways of Trp catabolism

The assumption that the changes in the excretion of one of the Trp metabolites would be compensated for by lower or higher use of the remaining possible metabolic pathways (leading to IAA, 5-HIAA, or other metabolites) was not confirmed in our studies (Marklová et al., 1986b, 1992, 1993).

A certain correlation in the IAcrGly and IAA excretion was found in individuals of the control groups. This might be explained by the similarity in the origin of both metabolites, at least under physiological conditions (in IAA, two sources have already been proved – exogenous, e.g. intestinal, and endogenous, e.g. extraintestinal (Weisbach et al., 1959).

More informations about the catabolic pathway leading to IAcrA formation in animals (humans) would appreciably contribute to a better understanding of changes in the IAcrGly excretion, possibly exploitable in the clinical praxis.

Acknowledgement

This work was supported by the Czech National Research Council of Ministry of Health, grant No. 4097-3.

References

Binazzi M, Calandra P (1975) Tryptophan metabolism in skin diseases. Acta Vitamin Enzymol (Milano) 29: 161–163

Gautheret RJ, Raoul Y (1949) Recherches sur l'action conjugé de l'acide indol-acrylique et de tryptophane sur les cultures de tissues de Carottes et Topinambour. Bull Soc Chim Biol 31: 1635–1638

Hofinger M, Monseur X, Pais M, Jarreau FX (1975) Futher confirmation of the presence of indolylacrylic acid in lentil seedlings and identification of hypaphorine as its precursor. Phytochem 14: 475–477

Kimmig J, Sticherling W, Tschesche R, Urbach HG (1958) N-/(beta-)Indolyl-(3)-acryloyl/-glycin. Isolierung aus Harn und Synthese. Z Physiol Chem 311: 234–238

Mandel L, Talafantová M, Trebichovský I, Trávníček J, Koukal M (1985) Selective decontamination, induced colonisation resistance and connected immunological changes in piglets. Folia Microbiol 30: 312–318

Mandell AJ, Rubin RT (1965) Indolylacroyl glycine excretion in man. Life Sci 4: 1657–1664

Marklová E, Hais IM (1972) A method for the estimation of indolylacryloylglycine in urine. Clin Chim Acta 40: 455–460

Marklová E, Hais IM (1978a) Urinary excretion of indolyl-3-acryloylglycine throughout the year and under the influence of UV irradiation. Sbor věd Prací LF UK v Hradci Králové 21 5: 515–520

Marklová E, Hais IM (1978b) Age variations of indolyl-3-acryloylglycine excretion in healthy children. Sbor věd Prací LF UK v Hradci Králové 21 5: 521–525

Marklová E, Fojtášková A (1996) HPLC profiling of indolylacryloylglycine and its possible precursors in body fluids. J Chromatogr A 730: 133–137

Marklová E, Malina L, Hais IM (1975) Urinary excretion of indolyl-acryloylglycine in some skin affections. Clin Chim Acta 64: 273–280

Marklová E, Mandel L, Jebavý L, Sobotka L, Hais IM (1986a) Metabolism of indolylacrylic acid. I. Hypaphorine. II. Effect of the diet on indolylacryloylglycine excretion in urine. III. Effect of intestinal microbes on excretion of indolylacryloylglycine in urine. Sbor věd Prací LF UK v Hradci Králové [Suppl] 29/4–5: 435–455

Marklová E, Lichý J, Nesvadba Z, Hais IM (1986b) Urinary excretion of indolyl-3-acryloylglycine in Duchenne type muscular dystrophy. Sbor věd Prací LF UK v Hradci Králové 21 5: 527–531

Marklová E, Hak J, Pařízek J, Morávek P (1992) Urinary excretion of some metabolites of tryptophan in malignant diseases. Sbor věd Prací LF UK v Hradci Králové 35 3: 275–279

Marklová E, Klein L, Hais IM (1993) Urinary excretion of indolylacryloylglycine, indolylacetic acid and 5-hydroxyindolylacetic acid in burn patients. Sbor věd Prací LF UK v Hradci Králové [Suppl] 36/4–5: 299–303

Marklová E, Fojtášková A, Makovičková H (1996) HPLC methods for determination of tryptophan and its metabolites. Chem Listy 90 9: 732–733

Monboisse JC, PierreLee P, Bisker A, Pailler V, Randoux A, Borel JP (1982) Quantitation of the urinary methylhistidine isomers by a combination of thin-layer and fluorimetric techniques. J Chromatogr 233: 355–360

Sergeeva TI, Zharova EI, Raushenbach MO (1979) /Development of spontaneous leukemias induced by 3-indoleacrylic acid in a highly-leucemic mouse strain AKR Probl Gematol Pereliv Krovi 24: 44–45

Smith HG, Smith WRD, Jepson JB (1968) Interconversion of indolic acids by bacteria and rat tissue. Possible relevance to Hartnup disorder. Clin Sci 34: 333–342
Tompsett SL (1959) The determination in urine of some metabolites of tryptophan – kynurenine, anthranilic acid and 3-hydroxyanthranilic acid – and reference to the presence of o-aminophenol in urine. Clin Chim Acta 4: 414–419
Udenfriend S, Titus E, Weissbach H (1955) The identification of 5-hydroxy-3-indoleacetic acid in normal urine and a method for its assay. J Biol Chem 216: 499–505
Weisbach H, King W, Sjoerdsma A, Udenfriend S (1959) Formation of indole-3-acetic acid and tryptamine in animals. A method for estimation of indole-3-acetic acid in tissues. J Biol Chem 234: 81–86
Wiskemann A, Kimmig J (1963) Photodermatosen durch Arzneistoffe und endogene Sensibilisatoren. Internist 4: 509–513

Authors' address: Eliška Marklová, MD, Baarova 1374, CZ 500 02 Hradec Králové, Czech Republic, Fax+420-49-551 11 16, e-mail: marklova@lfhk.cuni.cz

Received August 19, 1997

Verleger: Springer-Verlag KG, Sachsenplatz 4–6, A-1201 Wien. – Herausgeber: Prof. Dr. Gert Lubec, Universitäts-Kinderklinik, Währinger Gürtel 18–20, A-1090 Wien. – Redaktion: Sachsenplatz 4–6, A-1201 Wien. – Hersteller: Satz und Umbruch: Best-Set Typesetter Ltd., Hong Kong; Offsetdruck: Novographic Druck G.m.b.H., Maurer-Lange-Gasse 64, A-1230 Wien. – Verlagsort: Wien. – Herstellungsort: Wien. – Printed in Austria.