Pharmacology Biochemistry & Behavior, Vol. 24, pp. 1219–1223, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Endogenously Formed Norharman (β-Carboline) in Platelet Rich Plasma Obtained From Porphyric Rats

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Received 19 July 1985

SCHOUTEN, M. J. AND J. BRUINVELS. *Endogenously formed norharman (β-carboline) in platelet rich plasma obtained from porphyric rats.* PHARMACOL BIOCHEM BEHAV **24**(5) 1219–1223, 1986.—Porphyria was induced in adult male Wistar rats starved for 24 hr by SC injection of 400 mg/kg allylisopropylacetamide (AIA). The presence of porphyria was shown by measuring excretion of δ-aminolevulinic acid (δ-ALA) and porphobilinogen (PBG) into the urine during 24 hr after AIA administration. Plasma levels of glycine, serine and of a number of other amino acids were decreased in porphyric rats as compared to controls. Intraperitoneal injection of 2 mmol/kg serine 24 hr after AIA administration was used as an animal model for an acute psychosis, by measuring catalepsy scores 30 min after serine injection. The concentration of 5 different β-carbolines in platelet rich plasma (PRP) was measured using an HPLC-fluorometric method. An increase in the concentration of norharman (NH) in PRP, ranging from 0.57 nmoles/l in control rats to 1.88 nmoles/l in serine treated porphyric rats was found. The catalepsy duration was exponentially correlated with the NH concentrations in PRP. It is concluded that an elevated conversion of serine into glycine via serine hydroxymethyltransferase (SHMT) may be responsible for the enhanced NH biosynthesis.

Endogenous β-carboline β-Carboline Harmanes Psychosis Porphyria Amino acids HPLC
Rat model Catalepsy Porphyrins

INITIATED by the transmethylation hypothesis of Osmond and Smythies [11], several investigators have postulated the endogenous formation of β-carbolines from indolemines acting as "psychotogens" [3, 5, 6, 15]. However, until now, no increased levels of β-carbolines in schizophrenics could be detected [15]. The involvement of β-carbolines in a group of patients suffering from an episodic, schizo-affective psychosis of the manic-psychedelic type has been suggested previously. It was shown that within 2–3 hours after oral administration of serine to clinically recovered psychotic patients, psychotic symptoms reappeared [2,14]. An increased conversion of serine into glycine was proposed to be responsible for the production of β-carbolines, thus evoking psychotic symptoms.

It has been shown previously that IP injection of serine into porphyric rats causes catalepsy. This behavioural phenomenon was used as an animal model for the acute psychosis studied [16]. According to the hypothesis described above, it was proposed that in porphyric rats β-carbolines could be formed as a result of an increased serine to glycine conversion via the enzyme serine hydroxymethyltransferase (SHMT). Pearson *et al.* [13] showed that 1,2,3,4-tetrahydro-β-carboline (THBC) can be formed *in vitro* by SHMT if incubated with serine in the presence of tryptamine. This finding supports our hypothesis that an increased conversion of serine into glycine *in vivo* could give rise to the endogenous formation of β-carbolines.

The aim of the present study was to investigate whether an increased formation of β-carbolines occurred in porphyric rats treated with serine, and whether this is related to the cataleptic behaviour observed.

METHOD

Male Wistar rats (280–320 g) were starved for 48 hr. After 24 hr the starving rats were injected subcutaneously with 400 mg/kg allylisopropylacetamide (AIA, a generous gift of Hoffman-La Roche, The Netherlands), dissolved in 1 ml polyethyleneglycol (PEG), which had an approximate molecular weight of 200 (Baker Grade, Baker Chemicals). During the following 24 hr of starvation urines were collected in dark brown glass bottles to which 0.1 ml glacial acetic acid (Merck) was added before collection of the urine, in order to prevent δ-aminolevulinic acid (δ-ALA) breakdown. δ-ALA and prophobilinogen (PBG) were determined as described previously [16]. After the 48 hr starvation period a blood sample of 0.4 ml was taken by heart puncture under light ether anaesthesia just before the administration of serine, for determination of plasma amino acids. Amino acid analysis was performed using an LKB Model 4400 Amino

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TABLE 1

δ-AMINOLEVULINIC ACID (δ-ALA) AND PORPHOBILINOGEN (PBG) EXCRETION (nmoles/24 HR ± SEM) INTO URINE DURING 24 HR AFTER SC INJECTION OF 400 mg/kg ALLYLISOPROPYLACETAMIDE (AIA)

Porphyrin	Pretreatment Controls	AIA	% Change	2P<
δ-ALA	490 ± 32	898 ± 118	+ 83.2%	0.005
PBG	24.5 ± 3.5	753 ± 115	+ 2970%	0.001

Statistical calculations were performed using Student's *t*-test; (N=42).

TABLE 2

PLASMA AMINO ACIDS (μM ± SEM) IN STARVED PORPHYRIC RATS, MEASURED 24 HR AFTER AIA TREATMENT, JUST BEFORE SERINE INJECTIONS

Amino acid	Pretreatment Controls	AIA	% Change	2P<
glycine	451 ± 16	253 ± 14	−43.9%	0.0001
arginine	134 ± 3	80.0 ± 4.2	−40.3%	0.0001
tyrosine	95.8 ± 2.5	57.5 ± 1.8	−40.0%	0.0001
serine	293 ± 11	178 ± 4.4	−39.2%	0.0001
glutamic acid	86.7 ± 5.3	53.4 ± 6.7	−38.4%	0.0005
asparagic acid	12.3 ± 1.0	7.9 ± 0.4	−35.4%	0.0005
ornithine	55.6 ± 3.1	38.0 ± 2.7	−31.6%	0.0001
citrulline	50.5 ± 1.3	34.9 ± 1.7	−30.9%	0.0001
α-ABA	14.4 ± 1.0	10.3 ± 1.0	−28.5%	0.01
alanine	266 ± 9	194 ± 11	−27.1%	0.0001
threonine	212 ± 6	156 ± 5	−26.4%	0.0001
taurine	145.3 ± 7.2	110.7 ± 5.7	−23.8%	0.0001
methionine	49.9 ± 0.9	42.9 ± 2.1	−14.0%	0.002
histidine	55.9 ± 1.4	49.2 ± 2.0	−11.7%	0.01

Stastistical calculations were performed using Student's paired *t*-test (N=18).

TABLE 3

CATALEPSY DURATION (SEC), NH CONCENTRATION IN PLATELET RICH PLASMA (nM) AND SERINE AND GLYCINE PLASMA CONCENTRATION (μM) IN PORPHYRIC RATS 30 MINUTES AFTER 2 mmol/kg SERINE IP INJECTIONS

treatment	catalepsy	norharman	serine	glycine
Control	3.7 ± 2.1*	0.57 ± 0.03	267 ± 20	453 ± 32
AIA	4.1 ± 1.2	1.08 ± 0.19†	205 ± 20†	300 ± 30‡
Serine	13.3 ± 4.4†	1.63 ± 0.34†	778 ± 61§	409 ± 31
AIA + Serine	24.9 ± 4.2§¶	1.88 ± 0.85	663 ± 38§	319 ± 30‡

Statistical calculations were performed using the Mann-Whitney-U-test for the catalepsy data and Student's *t*-test for the other parameters. *Data represent mean ± SEM; N=9; †$p<0.05$, ‡$p<0.005$ and §$p<0.002$ vs. controls; ¶$p<0.005$ vs. AIA.

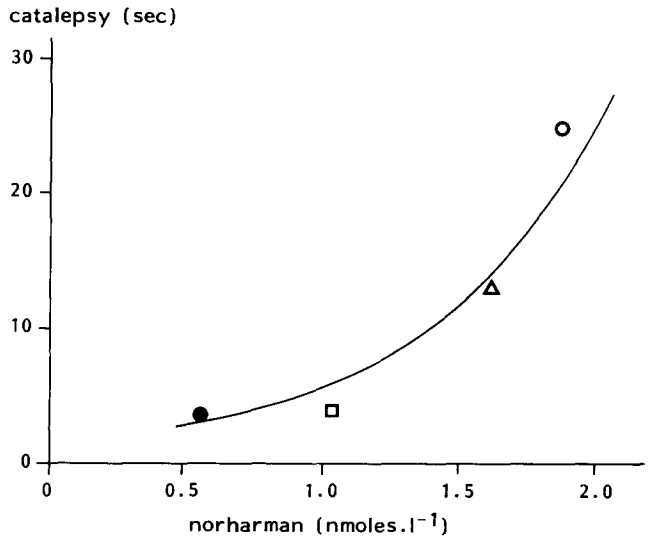

FIG. 1. Correlation of norharman concentration in PRP (nmoles/l) vs. catalepsy duration (sec). Each value represents the mean of 9 observation pairs. Curve fitting on the data in Table 3 indicated that an exponential fit showed best correlation (r=0.894). ●=control; □=AIA; △=serine; ○=AIA + serine.

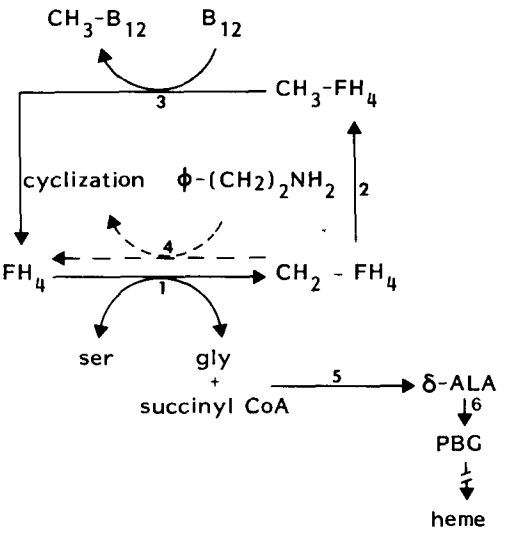

FIG. 2. Metabolic pathways of the one-carbon cycle, serine and glycine metabolism and the heme pathway. 1. Serine hydroxymethyltransferase (SHMT); 2. Methylenetetrahydrofolate reductase (CH_2-FH_4); 3. Methyltetrahydrofolate: homocysteine methyltransferase; 4. Non-enzymatic cleavage of CH_2-FH_4 to FH_4 and formaldehyde, followed by the Pictet-Spengler reaction; 5. δ-Aminolevulinic acid synthetase (δ-ALAS); 6. Porphobilinogen synthase (PBGS). ϕ=indoleamine or catecholamine.

Acid Analyzer according to the standard methods for determination of free amino acids in blood plasma, as described by Bruinvels and Pepplinkhuizen [3].

Twenty-four hours after AIA administration rats were injected intraperitoneally with 2 mmol/kg serine (Merck, biochemical grade) dissolved in 0.5 ml saline, or with the vehicle solely. Thirty minutes after serine injection, catalepsy was measured by placing the rat on a vertical grid with a grid size of 1.5×1 cm, about 10 above the cage floor. The time the animal did not displace one of its front or hind paws was recorded. This measurement was repeated twice and the mean duration was calculated. Immediately after the three catalepsy measurements 2 blood samples (0.4 ml and 10 ml for plasma amino acid and for β-carboline determination, respectively) were collected by heart puncture under light ether anaesthesia, and β-carbolines were determined in platelet rich plasma according to the method described by Schouten and Bruinvels [17].

RESULTS

The induction of porphyria was checked by measuring δ-aminolevulinic acid (δ-ALA) and porphobilinogen (PBG) excreted into 24 hr urine (Table 1). A significant increase in δ-ALA excretion, and a massive increase in PBG excretion was measured in the AIA pretreated rats, indicating that these rats had developed a severe porphyria.

Determination of plasma amino acids 24 hr after AIA-pretreatment revealed a significant decrease of about half of the plasma amino acids (Table 2). The greatest decrease was found for glycine (43.9%), while arginine, tyrosine and serine were decreased for about 40 percent. No significant differences in plasma concentration were measured for the amino acids not mentioned in Table 2.

The norharman concentration in platelet rich plasma (PRP) was significantly increased after treatment of rats with AIA or serine. Although AIA + serine treatment caused a further increase of NH concentration, the latter was not significantly different from controls due to the large standard error. The concentrations of 6-hydroxytetrahydro-β-carboline (6-OH-THBC), 6-methoxytetrahydro-β-carboline (6-MeO-THBC), tetrahydro-β-carboline (THBC) and 1-methyl-β-carboline (harman, 1-Me-BC) in PRP were below limits of detection (<4.6, <2.0, <4.3 and <0.5 pmol/ml, respectively).

Catalepsy was significantly increased after treatment with AIA + serine, as compared to both control and AIA treated rats, and after serine treatment when compared to control rats. However, rats treated solely with AIA did not show any cataleptic behaviour. During the cataleptic episode, catatonic phenomena like Straub tail were also observed (data not shown).

After serine injections, serine plasma levels were 3-fold increased when compared to controls and AIA pretreated animals (Table 3). Glycine plasma levels were decreased in AIA pretreated animals, and did not change significantly after serine administration.

When the duration of catalepsy was plotted against the norharman concentration in PRP, these data showed best fitting into an exponential curve (Fig. 1). No correlation was found between δ-ALA or PBG excretion and norharman PRP-levels during porphyria (data not shown).

DISCUSSION

Many suggestions have been put forward concerning the endogenous formation of β-carbolines and its relation to a number of behavioural disturbances [2, 5, 6, 15]. Previously, an animal model for an acute psychosis has been developed, using administration of serine to starved porphyric rats, assuming that β-carbolines could be formed endogenously under these circumstances [16]. The present experiments were designed in order to show the presence and to measure

the concentration of 5 different β-carbolines in serine treated porphyric rats using a chromatographic method where the artifactual formation of β-carbolines was excluded or at least below the limit of detection [17].

In the present study, a severe porphyria developed after AIA administration to starved rats, resembling acute intermittant prophyria (AIP) as indicated by the massive increase of δ-aminolevulinic acid (δ-ALA) and porphobilinogen (PBG) excretion into urine (Table 1). It has been shown that AIA depletes the heme pool by inducing cytochrome P 450 activity, thus causing strong induction of the enzyme δ-aminolevulinate synthase (δ-ALAS), the first enzyme in the heme pathway, in liver (Fig. 2) [7,19]. As a result, the demand for glycine will be increased in these porphyric animals. Indeed it has been shown that plasma glycine levels were decreased in porphyric rats, and a negative correlation was found between δ-ALA excretion and glycine plasma levels [16]. The present results show that plasma levels of serine and threonine were also significantly lowered during AIA-porphyria (Table 2), probably because the increased amount of glycine needed for porphyrin biosynthesis is produced from these amino acids by serine hydroxymethyltransferase (SHMT).

Since the Km values of SHMT for serine and glycine are low, the enzyme will be nearly saturated with its substrates *in vivo*. The direction of the equilibrium reaction will therefore be determined by the intracellular concentrations of each of the substrates [18]. In porphyria, where the demand for glycine is increased, glycine concentrations were significantly lowered and thus more serine must be converted into glycine (see Fig. 2). However, in the present experiments serine administration did not increase glycine plasma levels, and therefore does not favour an increased conversion of serine into glycine in both porphyric and non-porphyric animals (Table 3). An explanation for this failure may be that metabolic pathways which convert glycine into other products occur too fast in the rat to measure an increased formation of glycine in plasma 30 min after serine administration in contrast to results obtained in man [3].

The expected increased conversion of serine into glycine will simultaneously increase the production of CH_2-FH_4 from tetrahydrofolate (FH_4, Fig. 2) [2]. Studies of Pearson and Turner [13] showed that THBCs can be synthesized *in vitro* under physiological conditions from tryptamine in the presence of serine and FH_4 using partially purified SHMT. Methylenetetrahydrofolate, which is formed by this reaction, can non-enzymatically decompose into FH_4 and formaldehyde (HCHO). The latter substance can react with indoleamines forming THBCs via the Pictet-Spengler reaction, probably because other enzymes of the one-carbon cycle are not present in this preparation. Nevertheless, an increased conversion of serine into glycine *in vivo* may also result in the formation of β-carbolines [8]. The present results indeed show that norharman (NH) is formed in porphyric rats after serine injection, but instead of norharman one would rather expect the formation of THBC or of 6-hydroxy-THBC from the physiological substances tryptamine and 5-hydroxytryptamine, respectively. An explanation might be that the concentration in plasma of hydrogenated precursors of NH do not reflect concentrations in tissues where these compounds are formed. One may therefore assume that dehydrogenation of the formed THBC, which is known to occur in rats [4], accounts for the formation of NH.

There has been some discussion whether determination of THBCs in biological samples using extraction procedures are reliable methods to quantitate these compounds [1]. Careful studies using C-18 reverse-phase sample clean-up cartridges in order to minimize the use of organic solvents indicate that these compounds can be determined without any measurable artifactual formation of tetrahydro-β-carbolines [17]. In addition, artifactual dehydrogenation of THBCs in biological samples has, to our knowledge, not been reported to occur during work up procedures.

The present results show a positive correlation between catalepsy and plasma NH concentration as determined immediately after measurement of catalepsy. However, a relatively small increase of plasma NH resulted in a large behavioural response. Therefore, the question arises whether the plasma concentration of NH is causally related to the behavioural phenomena observed. It is not inconceivable that NH, which is a very lipophylic substance, easily enters the brain resulting in higher concentrations of NH in brain as compared to plasma. However, determination of NH in brains of porphyric rats have to be performed in order to support this suggestion. In addition, other substances like tetrahydroisoquinolines (THIQs) may be formed under the same conditions from catecholamines and these may also contribute to the cataleptic behaviour observed [9]. Nevertheless, some behavioural effects of NH resembling the behaviour observed in the present study, like a stiff tail, have been found by Morin *et al.* [10,11] after IP injections of 20 or 50 mg/kg NH in rats. Using the higher dose, they observed loss of righting reflex and a catatonic appearance with stiff and extended front and hind limbs. The authors also measured the *in vivo* concentrations of NH in rat brain cortex, which ranged from 4–16 μM after IP administration of 50–200 mg/kg NH, respectively. These results suggest a causal relationship between the increased plasma concentration of NH and the cataleptic behaviour observed in the present study. However, the brain concentrations reported by Morin [11] are much higher than the plasma concentration of endogenously formed NH in our animal model.

In conclusion, the present results show that in an animal model for acute psychosis using serine treated (AIA) porphyric rats, a correlation was found between NH PRP levels and the duration of catalepsy. In addition these results support the hypothesis that endogenous "psychotic" substances can be formed as a result of metabolic disturbances in serine and glycine metabolism. This metabolic disturbance and the endogenous formation of β-carbolines are held responsible for the psychedelic symptoms in episodic psychotic patients where the psychedelic symptoms could be reinduced in recovered patients by oral administration of serine [2,14].

ACKNOWLEDGEMENTS

During these studies we were supported by excellent technical assistance of Y. Dikkerboom, T. Adolfs and A. Edixhoven.

REFERENCES

1. Bosin, T. R., B. Holmstedt, A. Lundman and O. Beck. Presence of formaldehyde in biological media and organic solvents: Artifactual formation of tetrahydro-β-carbolines. *Anal Biochem* **128:** 287–293, 1982.
2. Bruinvels, J., L. Pepplinkhuizen, H. R. van Tuijl, P. Moleman and W. Blom. Role of serine, glycine, and the tetrahydrofolic acid cycle in schizoaffective psychosis. A hypothesis relating porphyrin biosynthesis and transmethylation. In: *Enzymes and Neurotransmitters in Mental Disease*, edited by E. Usdin, T. L. Sourkes and M. B. H. Youdim. New York: John Wiley, 1980, pp. 139–154.
3. Bruinvels, J. and L. Pepplinkhuizen. Impaired glycine-serine conversion and increased plasma taurine levels in episodic psychotic patients with psychedelic symptoms. *J Psychiatr Res* **18:** 307–318, 1984.
4. Greiner, B. and H. Rommelspacher. Two metabolic pathways of tetrahydronorharman (tetrahydro-β-carboline) in rats. *Naunyn Schmiedebergs Arch Pharmacol* **325:** 349–355, 1983.
5. Honecker, H., H. Coper, C. Fahndrich and H. Rommelspacher. Identification of tetrahydronorharman (tetrahydro-β-carboline) in human blood platelets. *J Clin Chem Clin Biochem* **18:** 133–135, 1980.
6. Kari, I., P. Peura and M. M. Airaksinen. Mass fragmentographic determination of tetrahydro-β-carboline in human blood platelets and plasma. *Med Biol* **57:** 412–414, 1979.
7. Marver, H. S., A. Collins, D. P. Tschudy and M. Recgciglh, Jr. δ-Aminolevulinic acid synthetase, II. Induction in rat liver. *J Biol Chem* **241:** 4323–4329, 1966.
8. McIsaac, W. M. Formation of 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline under physiological conditions. *Biochim Biophys Acta* **26:** 607–609, 1961.
9. Melchior, C. and M. A. Collins. The route and significance of endogenous synthesis of alkaloids in animals. *CRC Crit Rev Toxicol* **9:** 313–356, 1982.
10. Morin, A. M., I. A. Tanaka and C. G. Wasterlain. Norharman inhibition of [^{3}H]-diazepam binding in mouse brain. *Life Sci* **28:** 2257–2263, 1981.
11. Morin, A. M. β-Carboline kindling of the benzodiazepine receptor. *Brain Res* **321:** 151–154, 1984.
12. Osmond, H. and J. Smythies. Schizophrenia: A new approach. *J Ment Sci* **98:** 309–315, 1952.
13. Pearson, A. G. M. and A. J. Turner. The formation of β-carboline alkaloids mediated by serinehydroxymethyl transferase. *FEBS Lett* **98:** 96–98, 1979.
14. Pepplinkhuizen, L., J. Bruinvels, P. Moleman and W. Blom. Schizophrenia-like psychosis caused by a metabolic disorder. *Lancet* **1:** 454–456, 1980.
15. Rimon, R., M. M. Airaksinen, I. Kari, J. Gynther, E. Venalainen, L. Heikkila, J. Ryyppo and J. Palo. Pinoline, a β-carboline derivative in the serum and cerebrospinal fluid of patients with schizophrenia. *Ann Clin Res* **16:** 171–175, 1984.
16. Schouten, M. J. and J. Bruinvels. Serine and glycine-induced catalepsy in porphyric rats: An animal model for psychosis? *Pharmacol Biochem Behav* **19:** 5, 1983.
17. Schouten, M. J. and J. Bruinvels. High Performance Liquid Chromatography of tetrahydro-β-carbolines extracted from plasma and platelets. *Anal Biochem* **147:** 401–409, 1985.
18. Snell, K. Enzymes of serine metabolism in normal, developing and neoplastic rat tissues. *Adv Enzyme Regul* **22:** 325–400, 1984.
19. Unseld, A. and F. de Matteis. Destruction of endogenous and exogenous haem by 2-allyl-2-isopropylacetamide: Role of the liver cytochrome P-450 which is inducible by phenobarbitone. *Int J Biochem* **9:** 865–869, 1978.