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OCCURRENCE AND PARTITION OF THE ß-CARBOLINE NORHARMAN IN RAT ORGANS

Durk Fekkes and Willem T. Bode

Section Pathophysiology of Behaviour, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

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Summary

The ß-carboline norharman was determined in plasma, brain, liver, kidney, spleen, heart and lung of the rat using HPLC with fluorescence detection. In order to improve the speed and sensitivity of this assay an earlier published sample clean-up extraction procedure and HPLC method were adjusted. Norharman was found to be present in plasma as well as in all organs tested, concentrations in organs being about 80 times higher than those in plasma. Intraperitoneal injections of 2 and 100 mg/kg norharman showed that the partition of norharman between organs and plasma is about 3. Only the highest dose was found to have behavioural effects, viz. alerting reactions, a decrease in motor and exploratory activity, sedation, loss of righting reflex and after 30 min complete muscle relaxation, but no catatonia was observed. Norharman was found to be metabolized by the liver with a half live of about 20 min, whereas all other organs tested did not show any norharman clearing capacity. The results suggest that norharman is not likely the cause of psychosis, but a natural sedative and by- or coproduct of a more primary biochemical derangement.

The ß-carboline or harmala alkaloids are long known and used by diverse cultures such as South American indians for their psychotropic e.g. hallucinations inducing properties (1). One of these ß-carbolines, viz. norharman (ß-carboline), causes behavioural phenomena in rodents, that are thought to be equivalent to psychotic behaviour in humans eg. catatonic or cataleptic states (2-4). Recently, norharman was found in the plasma of a group of episodic psychotic patients at an average concentration of 0.54 nM, while this compound was neither detectable in healthy controls nor in nonsymptomatic patients (5). A special feature of these patients is that their psychotic symptoms could be induced after the ingestion of the amino acid serine (6).

Corresponding author: Dr. D. Fekkes, Section Pathophysiology of Behaviour, room Ee 1371, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

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and the system was thermostated at 45° C. One binary gradient was used to separate tryptamine, 5-hydroxytryptamine, 5-methoxytryptamine, THBC, 6-hydroxy-THBC, 6-methoxy-THBC (all obtained from Sigma), norharman and harman. Buffer A consisted of 50 mM $(NH_4)_2HPO_4$ and 10% (v/v) isopropanol (Merck, Lichrosolv quality), adjusted to pH 8.7 with NH_4OH and filtered through a .45 µm HA filter (Millipore). Buffer B consisted of 100 mM $(NH_4)_2HPO_4$ and 30% isopropanol (v/v), adjusted to pH 5.0 with H_3PO_4 and filtered through a .50 µm FH filter (Millipore). The buffers were prepared in Milli-Q quality water. The following gradient was used. From starting until 3 min: B = 5%, from 3 until 12 min a linear gradient to B = 80%, from 12 until 20 min isocratic at B = 80%, from 20 until 20.5 min back to B = 5% . The total run time was 27 min. The compounds were detected using an Aminco SPF500 ratio fluorometer fitted with a 35 µl flow cell. During the first 14.5 min an excitation wavelength of 290 nm (bandwidth 10 nm) and emission wavelength of 345 nm (bandwidth 40 nm) was used to detect tryptamine and derivatives and THBC and derivatives. An interval between 14.5 and 15.5 min was used to adjust to an excitation wavelength of 375 nm (bandwidth 15 nm) and an emission wavelength of 452 nm (bandwidth 40 nm) to detect harman and norharman and was maintained until end of run.

Quantitation took place by measuring peak-height using an Omega-2 integration program (Perkin Elmer) interfaced to the fluorometer.

Results

Behavioural studies. Intraperitoneal injection of norharman in a dose of 2 mg/kg resulted in no observable alteration of behaviour during the timespan of 1 hour. No catalepsy was observed neither directly nor from the grid catalepsy measurement. Treated and untreated rats all moved away from the grid within 2 seconds. Intraperitoneal injection of the high dose of 100 mg/kg norharman resulted in marked behavioural changes. Within 5 min alerting reactions of whiskers were observed, and reactions that anthropomorphically interpreted can be seen as starteling reactions. After 5 min, movement and exploratory behaviour quickly diminished. Strong pupil constriction (myosis) was observed. After 10 min there was a total loss of righting reflex and total muscle relaxation. At the moment of catalepsy measurement, the muscle relaxation was complete and no reactions were observed. For this reason it was impossible to place the animals on the grid to grab it. After one hour the status was unchanged. To stress the point we were ourselves impressed by the high degree of muscle relaxation; if taken up the rats resembled little bags filled with fluid. Yet respiration, heart beat and other vital functions remained intact. In a single experiment we injected 10 mg/kg (i.p.) of the benzodiazepine receptor blocker RO 15-1788, before injecting a dose of 100 mg/kg norharman and blocked with it all behavioural effects described before.

Chromatography and extraction. The use of fully endcapped Zorbax C8 material made the addition of the ion-pair former sodium octylsulfate in the HPLC solvents unnecessary (7). With this HPLC procedure it was also possible to determine all 5 ß-carbolines together with their 3 precursor amines in a single run (Figure 1A). During a period of 4 days at regular intervals 9 measurements of

standards were performed. The mean retention times (± coefficient of variation) for the various compounds were as follows: 5-hydroxytryptamine, 2.84 (0.56); 6-hydroxy-THBC, 3.36 (0.57); 5-methoxytryptamine, 6.01 (0.38); tryptamine, 6.95 (0.27); 6-methoxy-THBC, 10.54 (0.22); THBC, 12.01 (0.21); harman, 17.81 (0.08) and norharman, 19.51 (0.07). Fluorescence was linear up to 10 μM for each component. The detection limit for harman and norharman at a signal to noise ratio of 3 were 0.15 and 0.3 pmol, respectively. These values were lower than those previously published (7). With the adjusted extraction procedure the use of large amounts of $NaClO_4$ in the extraction buffer (7) was no longer necessary. This resulted in a faster solid phase extraction and the residue could be taken up in a much smaller volume, which provides increased sensitivity. As in the earlier study (7), no artefactual formation of ß-carbolines during the extraction procedure was found. Since the chromatographic properties of harman and norharman are very similar, we choose harman as internal standard. As can be seen in Figures 1B and 1C, the endogenous concentration of harman in the rat tissues was negligible with regard to exogenous harman (Figure D).

With our HPLC system it was also possible to detect 5-hydroxytryptamine, tryptamine, THBC and harman in rat plasma (data not shown). Using the chromatographic method described by Schouten and Bruinvels (7) large peaks were observed in plasma samples at retention times of 7-10 min, obscuring tryptamine. With the system

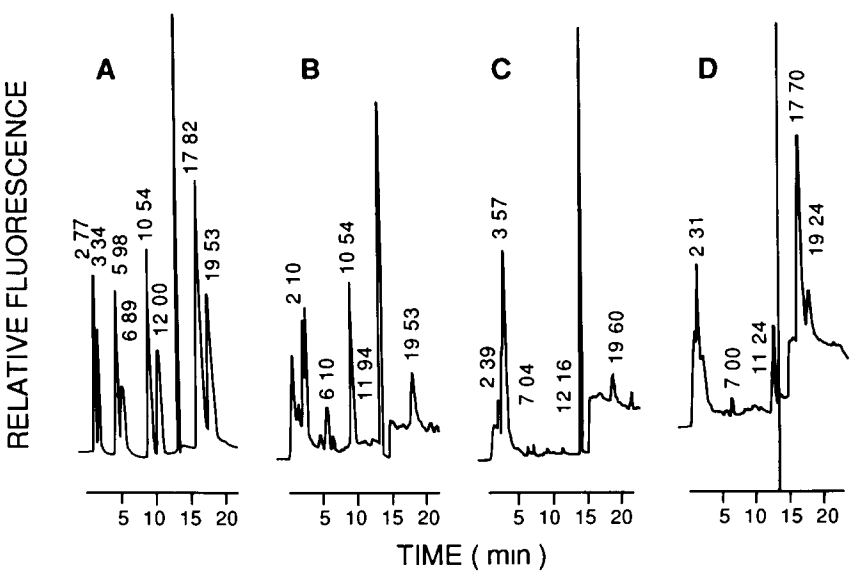

FIG. 1
Liquid chromatograms of authentic compounds (A), control rat liver extract (B), control rat brain extract (C), and control rat brain extract with added harman as internal standard (D). Peaks at retention times 17.70-17.82 and 19.24-19.60 correspond to harman and norharman, respectively. See for the retention times of the other compounds the Results section.

described above large peaks were interfering with 6-methoxy-THBC. From this effect it could already be inferred that those peaks were coeluting substances. However, using vacutainer tubes containing EDTA instead of thrombotect tubes, these peaks were absent. Therefore, we concluded that thrombotect tubes are not the right choice for this determination and that vacutainer tubes containing EDTA should be used.

Distribution and partition studies. Norharman was found to be present in rat plasma and in all organs of the C-group (Table I). The measured plasma levels were about twice as high as those described by Schouten and Bruinvels (2,7). This elevation can certainly not be attributed to artefactual formation, but rather to a higher sensitivity and accuracy of our method. Brain norharman concentrations were about 55 times those of plasma. Additional evidence for the identity of norharman in these rat tissues was obtained by measuring the column effluent at an excitation wavelength of 300 nm (the other excitation peak of norharman) and an emission wavelength of 445 nm. The ratio of the signals at both fluorometer settings was determined in the samples and compared with authentic norharman. In one control plasma the positive identification of norharman was confirmed by liquid chromatography-mass spectrometry using a thermospray system and an HP 5988A mass spectrometer (data not shown). Concentrations of norharman in other organs were 70-100 times those of plasma. In the L-group, 10% of norharman injected was recovered in plasma and 46% in the organs. For the H-group these figures were 12 and 40%, respectively. These data were calculated under the assumptions of homogeneous partition and no metabolic transformation of norharman to occur. In the L-group brain norharman concentrations were 4 times those of plasma, while in the H-group this difference was somewhat smaller (Table I). Comparing the findings of Morin et al. (4) with those in the H-group for brain norharman levels, we found a 40-fold saturation if the partition time is to be extended from 2 to 60 min.

TABLE I
Occurrence and Partition of Norharman in Plasma and Organs of the Rat.

Location	C-group (n=6)	L-group (n=6)	H-group (n=5)
Heart	0.071 ± 0.006	4.12 ± 1.22	167 ± 103
Brain	0.060 ± 0.025	5.64 ± 1.70	231 ± 154
Liver	0.085 ± 0.008	6.50 ± 2.14	272 ± 125
Lung	0.106 ± 0.063	4.35 ± 1.29	260 ± 154
Spleen	0.112 ± 0.047	8.13 ± 3.19	324 ± 163
Kidney	0.108 ± 0.035	5.52 ± 1.65	233 ± 108
Plasma	0.0011 ± 0.0003	1.30 ± 0.45	76 ± 43

C-group: rats injected (i.p.) with saline. L- and H-group: rats injected (i.p.) with 2 and 100 mg/kg norharman, respectively. Values are means ± SD and are expressed in nmol/g for organs and in μM for plasma.

In the preincubation experiments we found THBC to be stable in all organs and plasma (data not shown). We also could not detect any norharman formation in the organs incubated with THBC.
Norharman was also stable in all organs and plasma except liver. Concentrations of norharman added to the L-group liver to a concentration equivalent to the H-group and H-group liver showed clearance with a half life of about 20 min in these incubations (Figure 2). The fact that there is no difference observed between the pretreated groups indicates that no induction of metabolizing enzymes occurs under the conditions chosen.

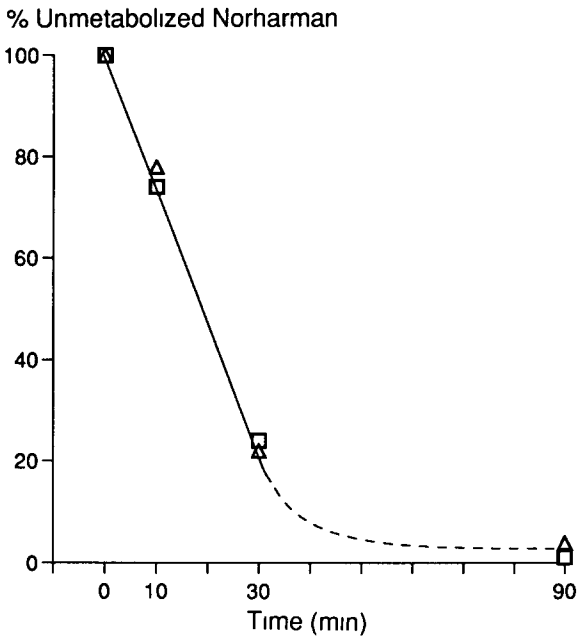

FIG. 2
Effect of preincubation on norharman concentration in liver homogenates of rats injected with 100 mg/kg norharman (□, H-group) or with 2 mg/kg norharman after adding an amount of norharman equal to H-group concentrations (△, L-group). Other details are decribed in the Methods section. Results are expressed as percent of amount recovered in control tubes without incubation, and are mean values of three tests, each assayed in duplicate.

Discussion

It seems unequivocal that norharman is a naturally occurring substance in all studied tissues of the rat. One might therefore ask whether norharman is a metabolic waste product or that a physiological function must be attributed to norharman. The last possibility is more likely because distinct and different binding sites for norharman were described by Pawlik and Rommelspacher (8). Endogenous concentrations were in the same order as the K_D for

mitochondrial membrane fractions (46 ± 8 nM), but higher than the K_D for synaptosomal membranes (1.55 ± 0.7 nM). It was also established that ß-carbolines (9) and more specific norharman (10) have high affinities for benzodiazepine receptors. In adult male Wistar rats, i.p. doses of 100 mg/kg norharman first induced alerting reactions and after 5 min sedation, decreased motor and exploratory behaviour, loss of righting reflex and eventually complete muscle relaxation were observed. In contrast to the study of Morin et al. (3) with mice, we observed no catatonia. The effect of norharman is probably species dependent, because Morin et al. (4) also did not mention catatonic appearance when they injected Sprague-Dawley rats with norharman. Although the observed alerting reactions seen within 5 min may result from activation of specific ß-carboline receptors, the behaviour modulating and pharmacological action of norharman seen after 5 min suggest that this compound is a benzodiazepine agonist. This is supported by the observation that the latter effects could be blocked by the benzodiazepine receptor antagonist RO 15-1788. From this point of view norharman does not seem likely to be a candidate for promoting psychotic phenomena but more a natural sedative or possibly a psychodysleptic substance. If lowering of consciousness may be attributed to norharman these phenomena could be counteracted by a benzodiazepine antagonist.

Norharman was found to be present in plasma of patients during episodes of acute psychosis at concentrations of 0.54 ± 0.10 nM (mean ± SEM), whereas concentrations in control subjects were below limit of detection (2). Measurements we performed with the above mentioned modified HPLC-fluorometric and sample clean-up system showed that actual concentrations in plasma of acute psychotic patients can be 2 to 4 times those found by the quoted authors, while norharman levels in controls were found to be 0.2 ± 0.2 nM (11). The differences are attributed to the greater sensitivity of our method. One reason is that the number of theoretical plates for norharman with our method is much larger and consequently the detection limit smaller.

To fit the findings of the animal model for psychosis proposed by Schouten and Bruinvels (2) with the quantities described by Morin et al. (3), an enrichment of norharman in brain with a factor of at least 2000 must occur. However, clearance of norharman by the liver may also cause plasma norharman concentrations to be underestimated. The ratio we found as endogenous distribution for brain : plasma was 55, for the L-group this was 4.3 and for the H-group 3.0. This is a more homogeneous partition and especially at the active range there is no large enrichment of brain with norharman versus plasma. The difference between endogenous concentrations in plasma and different organs however is remarkable and may point at endogenous synthesis. Our levels of norharman in brain were about 40 times higher than those found by Morin et al. (3). Combined with liver clearance of norharman observed, we conclude that our values are within peak range, whereas the values of Morin et al. were far from a partition equilibrium. Although it may be possible that in the animal model and in patients an enrichment of norharman occurs in brain regions due to local synthesis, this does not seem likely. First of all it is unlikely that there should be such a preference for this reaction in brain, more so because porphyria induction, which has a high incidence in the earlier memtioned psychotic patients (6), is mainly connected

with liver and heamatopoetic tissues (12,13). Second, serine in brain is mainly synthesized from glucose via 3-phosphoglycerate or from glycine with tetrahydrofolate as cofactor and passes the blood-brain barrier by a low affinity and presumably glutathione dependent uptake system (14). Combined with our finding that only liver metabolizes norharman lets us assume that the liver is the primary locus under the conditions mentioned by Schouten and Bruinvels. We believe that other compounds formed e.g. isoquinolines are better candidates, because their second order rate constants for the Pictet-Spengler condensation is much higher (15), their action being on all neurotransmitter systems that are implied in psychosis (16) and the dopamine or analogue tetrahydroisoquinolines administered intracerebroventricularly in low doses cause pronounced effects e.g. repetitive purposeless responses (16), the analogue in man being a DSM-III-R diagnostic criterium for schizophrenia (17). This however does not mean that isoquinolines are the primary cause of psychosis, but we believe that analogue to the models of theoretical physics e.g. bootstrap and S-matrix hypothesis, local alterations in intermediary metabolism synergetically induced by inheritance, mental factors and diet as different system levels interacting, cause states of specific pathways that result in psychosis. So considered we maintain that isoquinolines are better candidates of being causative for psychotic phenomena. We can point at pathways we consider primary e.g. the gamma-glutamylcycle, pentose phosphate cycle, one-carbon cycle, cytochrome P-450 complex related systems, redoxpotential (especially NADPH availability), (form)aldehyde metabolizing systems, their influencing and being influenced by amino acid and sugar metabolism, folate metabolism and the influence of these systems on scavenger systems, and neurotransmitter- and receptor systems. It is also possible to describe these synergetic intermediary networks for manic depression, types of schizophrenia, other psychiatric states and in greater detail for degeneration psychosis with psychedelic symptoms, but it is at this point beyond the scope of this paper. The value of the finding of norharman during psychotic states and the fact that the model mimics those states however is not to be underestimated, because it supplies theoretical information on the location in the above mentioned pathways where derailments are suspected.

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