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Harman (1-methyl-β-carboline) is a natural inhibitor of monoamine oxidase type A in rats

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Abstract

Harman (1-methyl-β-carboline) displaces [^{3}H]pargyline in vitro from high affinity binding sites on membranes from cerebral cortex, provided that experimental conditions are chosen under which [^{3}H]pargyline labels selectively monoamine oxidase type A. Norharman (β-carboline) is a much weaker displacing compound. It is well known that the type A enzyme can be blocked irreversibly in vivo by treatment of rats with clorgyline. Under these conditions no specific binding of [^{3}H]harman and [^{3}H]pargyline to monoamine oxidase type A was detected in brain, whereas the specific binding was reduced to 5% in liver tissue. The in vitro and ex vivo experiments suggest that there is a specific binding site for harman on monoamine oxidase type A, thereby extending earlier in vitro findings. It has been postulated that harman operates as a natural inhibitor of monoamine oxidase type A in mammals. The present study demonstrates that harman and norharman occur in rat brain, blood plasma, heart, kidney and liver. It further shows that pretreatment with clorgyline induces a time-dependent increase in the blood plasma levels of harman, suggesting the displacement of harman from the enzyme in tissue with its subsequent delivery into the blood. These findings strongly support the hypothesis based on in vitro experiments, that harman binds reversibly to the active site of monoamine oxidase type A in vivo. Dietary sources for mammalian harman play probably only a minor role, because the concentrations in beer and wine as well as other foodstuffs are too low to contribute substantially to endogenous levels of harman.

Key words: Harman; Norharman; β-Carboline; Monoamine oxidase; Pargyline; (Rat)

1. Introduction

Several research groups have demonstrated the presence of harman in human blood plasma and tissues of other species, by using various separation and detection methods (Bidder et al., 1979; Bosin and Faull, 1988a; Bosin et al., 1989; Rommelspacher et al., 1984, 1990, 1991b). Harman is formed from tryptamine and pyruvate with harmalan as an intermediate compound (Gynther et al., 1986; Susilo and Rommelspacher, 1987, 1988). Acetaldehyde, the metabolite of ethanol, presumably interferes with the biosynthesis of harman by interacting with thiamine pyrophosphate, the coenzyme of the pyruvate-dehydrogenase enzyme complex. Acetaldehyde binds to thiamine, which disturbs the function of this enzyme (Takabe and Itokawa, 1983). Thus, high levels of acetaldehyde could inhibit the conversion of pyruvate to acetylcoenzyme A, which would facilitate the formation of the 1-methyl-β-carbolines.

Harman inhibits monoamine oxidase (EC 1.4.3.4) subtype A in vitro by reversible binding with high affinity ($K_D \sim 3$ nM) to the active site of the enzyme. Harman is not oxidized by monoamine oxidase A. Specific [^{3}H]harman binding is displaceable by substrates of the enzyme, like 5-hydroxytryptamine and other biogenic amine neurotransmitters, as well as by potent and selective inhibitors of monoamine oxidase A (May et al., 1991a,b).

Therefore, it was of interest to investigate systematically the characteristics of harman binding to monoamine oxidase A under in vitro, ex vivo and in vivo conditions. Firstly, harman was tested in vitro as a

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displacer of [^{3}H]pargyline binding under experimental conditions in which the radioligand labels monoamine oxidase A only. A further objective of developing the [^{3}H]pargyline assay was to extend our previous work to a structurally different ligand. Secondly, a clorgyline treatment schedule was applied to investigate the binding to monoamine oxidase A, assessed by ex vivo quantitation of monoamine oxidase A and B by the aid of the [^{3}H]pargyline binding assay, as well as of monoamine oxidase A by using the previously described [^{3}H]harman binding assay (May et al., 1991a). Thirdly, the monoamine oxidase A-selective treatment schedule with clorgyline was used to determine some properties of harman in vivo in order to support the hypothesis that harman acts as a natural inhibitor of monoamine oxidase type A. Since norharman (β-carboline) differs chemically from harman only by the absence of the 1-methyl substituent and binds to monoamine oxidase A with much lower affinity, it was measured simultaneously to ascertain the postulated function of harman.

2. Materials and methods

2.1. Animals and animal treatment

Male Wistar rats (animal farm Hagemann Boesingfeld/Germany), weighing 160–180 g on arrival to the laboratory, were used throughout the study. The rats were housed in groups of four in macrolon cages (60 × 38 × 20 cm) and were supplied with standard rat chow (Altromin 1324) and tap water ad libitum.

Clorgyline hydrochloride (10 mg/kg body weight referring to the free base) dissolved in saline and 15 min later harman (0.1 mg/kg body weight) and saline, respectively, were injected i.p. The animals were decapitated after certain time periods as specified in the text.

2.2. [^{3}H]Harman and [^{3}H]pargyline binding

The tissues were rapidly dissected, frozen in liquid nitrogen and stored at −80°C until use. Hippocampus, cerebral cortex and liver tissue were used for binding experiments. Both assays were performed with tissue of single animals, but the hippocampi of 3–4 clorgyline-treated rats were pooled. The thawed tissues were homogenized with glass-Teflon homogenizers in 100 volumes (v/w) of 50 mM Tris-HCl, pH 7.4, and centrifuged 10 min at 40 000 × g_{av}. The pellets were washed once again under the same conditions. The final pellets were rehomogenized at a concentration of 20 mg and 70 mg original wet weight per ml for controls and clorgyline-treated animals, respectively. Samples of 100 μl of the homogenate (~ 120 and 420 μg of protein) were used for the binding assays.

The [^{3}H]harman binding assay determining affinity (K_D) and density (B_{max}) of binding to monoamine oxidase A was performed as described previously (May et al., 1991a), but with minor modifications. Briefly, the homogenate was coincubated 90 min at 0°C in 0.3 ml of 50 mM Tris-HCl, pH 7.4, with six concentrations of [^{3}H]harman (0.4–12 nM), each in duplicate, in the absence and presence of 1.0 μM clorgyline (total and nonspecific binding). The coincubation procedures allowed competitive binding of clorgyline and [^{3}H]harman at the active site of monoamine oxidase A (see Results). Clorgyline was used in a concentration approximately 100-fold higher than the K_i value (May et al., 1991a). The binding was terminated by rapid filtration through GF-B filters (presoaked in 0.1% polyethyleneimine) followed by two washings with 5 ml of ice-cold 50 mM Tris-HCl, pH 7.4. The radioactivity of the dried filters of each sample was determined by liquid scintillation spectrometry (counting efficiency about 50%; Packard, 1900C). Results were calculated by the use of Scatchard plots (Scatchard, 1949).

The [^{3}H]pargyline binding assay (Kalaria and Harik, 1987), which was used to determine monamine oxidase A and B densities in various brain regions and the liver, was performed in a modified fashion. The homogenate was preincubated 30 min at 37°C in a volume of 150 μl of 50 mM Tris-HCl, pH 7.4, with and without several irreversible MAO inhibitors. Radioligand binding was started by the addition of 50 μl [^{3}H]pargyline (final concentration 550 nM, specific activity 3–4 Ci/mmol) and terminated after 120 min of incubation at 37°C as described above for [^{3}H]harman binding. Increasing concentrations of the potent monoamine oxidase inhibitors pargyline, D-(+)-deprenyl, L-(−)-deprenyl, MDL 72,974 and clorgyline were tested in displacement studies (Fig. 1). The displacement of [^{3}H]pargyline binding by the selective monoamine oxidase B inhibitors MDL 72,974 (Palfreyman et al., 1988) and L-(−)-deprenyl (Knoll and Magyar, 1972), as well as by the selective monoamine oxidase A inhibitor clorgyline (Johnston, 1968), revealed biphasic curves with plateau concentrations at 10–50 nM (MDL 72,974), 20–50 nM (L-(−)-deprenyl), and 20–500 nM (clorgyline) (Fig. 1). Therefore, single plateau concentrations of 25 nM MDL 72,974, 40 nM L-(−)-deprenyl and 100 nM clorgyline (nonspecific binding), each in triplicate, were chosen to calculate monoamine oxidase A and monoamine oxidase B concentrations as the differences from [^{3}H]pargyline binding without inhibitor (total binding).

The density of monoamine oxidase A and B binding sites was calculated in pmol per mg of protein. The protein content was determined with bovine serum albumin as the standard (Lowry et al., 1951).

2.3. Measurement of norharman and harman in brain, liver, kidney, heart and blood plasma

Brain, liver kidney, heart: Only silanized glassware was used. Tissue was homogenized in 10 vols. of a mixture of ice-cold acetonitrile, distilled water and 6 M hydrochloride 40:37:3, containing 0.1 mM EDTA. The homogenate was divided into four 4 ml aliquots (~ 400 mg tissue per aliquot). Standard solutions of 0.2 ng norharman and harman were added to one sample and of 0.5 ng to another one. The samples were centrifuged 10 min, at $27460 \times g_{av}$ and 4°C, in a Sorvall centrifuge (RC5C). The first supernatant was decanted and the pellet was washed once with 4 ml of the cocktail. Both resulting supernatants were combined and 300 μl of 5 M KOH was added. Thereafter, the samples were lyophilized (~ 40 h). The dry residue was dissolved in 8 ml of 10 mM potassium phosphate buffer, pH 9.5 (cartridge buffer), and adjusted to pH 9.5–10.5. Then 15 ml of distilled ether (distilled within the preceding 5 days) was added. The sample was stirred, centrifuged in a table centrifuge and the organic phase was removed with a pipette. Ether (15 ml) was added to the remaining sample and the procedure was repeated. The two ether phases were combined and dried under low pressure. The residue was dissolved in 50% methanol (400 μl) and centrifuged before being injected into the HPLC apparatus (Hewlett-Packard, HP1090, fluorometric detection, HP1046A); 100 μl aliquots of each sample were analyzed under three different elution conditions. Method 1: flow 1.5 ml/min, 55% methanol/45% ammonium acetate buffer, 20 mM, pH 8, octane sulfonic acid, 2.5 mM, gradient elution with 51.7% methanol at 11 min. Method 2: flow 1.5 ml/min, 10% acetonitrile (90%) and 90% ammonium acetate buffer, pH 9, octane sulfonic acid, 2.5 mM, isocratic for 4 min, then within 30 s to 43% acetonitrile followed by isocratic elution. Method 3: flow 1.5 ml/min, 38.9% acetonitrile (90%) and ammonium acetate buffer, 50 mM, pH 9, containing 2.5 mM octane sulfonic acid (isocratic conditions). The respective retention times for norharman and harman were: (1) 8.6 and 10.1 min; (2) 10.4 and 11.0 min; (3) 7.0 and 8.0 min.

The recovery of internal standard was 85–90%. The glassware was washed thoroughly with methanol before usual laboratory washing. The glassware was only used for the determination of β-carbolines. These precautions prevented contamination of the probes.

Further details have been published earlier (Rommelspacher et al., 1991b). The same publication also contains a description of the method used for the determination of norharman and harman in blood plasma and a mass spectrum analysis and other analytical investigations which prove the identity of the measured compounds.

2.4. Measurement of norharman and harman in beer and wine

Beer: 5 ml of beer was adjusted to pH 7.8–8.5 with NaOH and mixed with 5 ml methanol. Then, the sample was centrifuged in a table centrifuge and 100 μl of the supernatant was applied to the HPLC apparatus and eluted by using 37% acetonitrile (90%) and 10 mM ammonium acetate buffer, pH 8, containing 2.5 mM octane sulfonic acid at the beginning of the run and 63% acetonitrile after 12 min (method 1). The method 2 utilized different proportions of the same components: 0 min 15% acetonitrile, 14 min 55% acetonitrile.

Wine: 4 ml samples were adjusted to pH 7.5–8.5 and concentrated under low pressure. The dry residue was dissolved in 4 ml of sodium phosphate buffer (10 mM, pH 8) and applied to cartridges (phenyl, J.T. Baker, Phillipsburg, USA). The β-carbolines were eluted by methanol, which was concentrated under low pressure. The dry residue was dissolved in 2 ml methanol. An aliquot of 100 μl was applied to the HPLC apparatus and eluted isocratically with acetonitrile 32% (method 1). A second aliquot was eluted by a continuous gradient: 0 min 20% acetonitrile, 15 min 48% acetonitrile (method 2).

2.5. Data analyses

All data were analyzed by using Hewlett-Packard table computers (series 200/300). Scatchard analyses were calculated with multipurpose computer programs. The competition experiments were analyzed, applying a nonlinear, unweighed least-squares procedure, for one and two classes of saturable binding sites (Wiemer et al., 1982).

All results are presented as means ± S.E.M. of n experiments. Significant differences ($P < 0.05$) were calculated by Student's t-tests.

2.6. Chemicals

[^{3}H]Harman (specific activity 20–24 Ci/mmol) was custom-labeled by Amersham, Amersham, UK. [^{3}H]-Parygline (specific activity 25.0 and 40.4 Ci/mmol) was purchased from NEN, Germany. Parygline, D-(+)-deprenyl, L-(−)-deprenyl and clorgyline were purchased from Biotrend, Cologne, Germany. MDL 72,974 was a gift from Merell Dow Research Institute, Cincinnati, OH, USA.

Harman hydrochloride and norharman hydrochloride were purchased from Sigma Chemie, Deisenhofen, Germany. The organic chemicals were of the highest purity grade available for fluorometric determinations (Lichrosolv, Merck, Germany); the other chemicals were of reagent grade.

3. Results

3.1. Binding experiments

The concentrations of displacer defining the nonspecific binding in the in vitro [^{3}H]pargyline binding study were chosen according to the findings of displacement experiments (Fig. 1, Table 1). Plateaus could be determined in the curves of [^{3}H]pargyline binding displacement by clorgyline (Fig. 1B), L-(−)-deprenyl (Fig. 1A) and MDL 72,974 (Fig. 1B), which makes it possible to estimate separately the quantity and proportions of monoamine oxidase A and B. As the three selective inhibitors display biphasic displacment curves (Fig. 1), IC_{50} values for [^{3}H]pargyline binding to monoamine oxidase A and B could be determined separately (Table 1). However, the less selective monoamine oxidase inhibitors pargyline and D-(+)-deprenyl possessed uniphasic curves (Hill coefficients about unity) with IC_{50} values of 32 nM and 16 nM, respectively (Fig. 1A). The displacement of specific [^{3}H]pargyline binding by harman and norharman was tested separately under monoamine oxidase B (presence of 100 nM clorgyline) and monoamine oxidase A (presence of 40 nM L-(−)-deprenyl) conditions (Table 1). Harman is obviously a potent and selective monoamine oxidase A inhibitor, whereas norharman is 2 orders of magnitude less potent at monoamine oxidase A and is nonselective according to its monoamine oxidase A and B binding characteristics. With respect to the assessment of the binding inhibiting potency of the displacing compounds, it should be kept in mind that a certain portion of [^{3}H]pargyline binds irreversibly during the incubation period.

Table 1
Displacement of [^{3}H]pargyline from binding sites on monoamine oxidase A and B

Compound	IC_{50} (μM)	
	Monoamine oxidase A	Monoamine oxidase B
L-(−)-Deprenyl	0.41 ±0.15	0.0038 ± 0.0011
MDL 72,974	0.90 ±0.30	0.0048 ± 0.0035
Clorgyline	0.0053 ± 0.0025	3.12 ±0.15
Harman	0.25 ±0.10	> 100.0
Norharman	34.0 ±5.0	14.0 ±6.0

Further binding experiments were performed under ex vivo conditions utilizing membranes from cerebral cortex, hippocampus and liver of naive and clorgyline-treated (10 mg/kg, killed 2 h after injection) rats.

Table 2 shows the results of [^{3}H]harman binding to monoamine oxidase A and of [^{3}H]pargyline binding to monoamine oxidase A and B. Obviously, the B_{max} values determined for reversible [^{3}H]harman binding to monoamine oxidase A correlate well with the corresponding monoamine oxidase A densities ($r = 0.996$, $P < 0.001$), but not with monoamine oxidase B densities ($r = 0.42$, $P > 0.05$) determined for irreversible [^{3}H]pargyline binding (Table 2). Furthermore, the density of [^{3}H]pargyline binding to monoamine oxidase A does not correlate with the corresponding values for monoamine oxidase B ($r = 0.4$, $P > 0.05$), whereas the respective values for monoamine oxidase B determined for the different displacers correlate very well with each other ($r = 1.000$, $P < 0.001$). After clorgyline treatment, monoamine oxidase A binding was no longer detectable in the brain tissue, whereas in the liver a reduction of approximately 95% was determined in

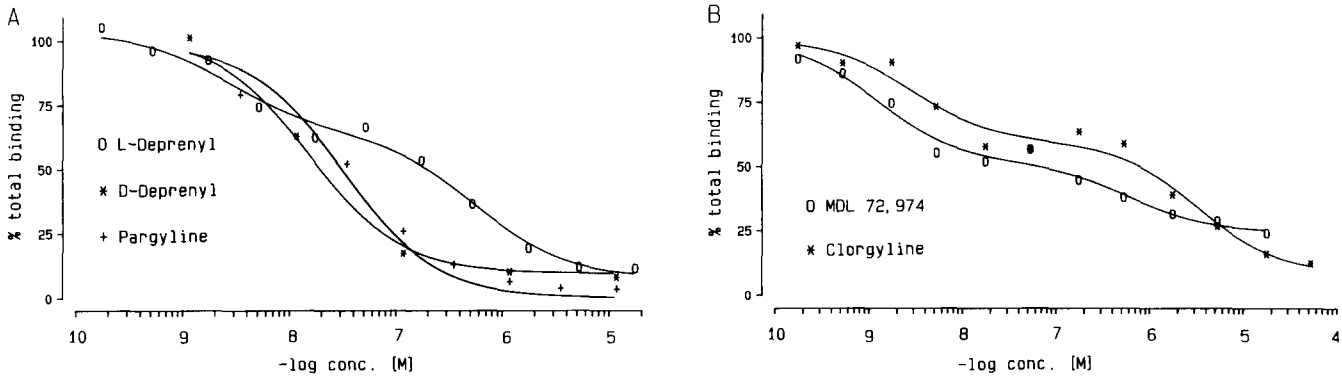

Fig. 1. Displacement of total [^{3}H]pargyline binding to membranes of rat cerebral cortex is shown under conditions for labeling monoamine oxidase A and B simultaneously (550 nM [^{3}H]pargyline, 2 h incubation at 37°C). The potent and selective monoamine oxidase A inhibitor clorgyline as well as the potent and selective monoamine oxidase B inhibitors L-(−)-deprenyl and MDL 72,974 (Cesura and Pletscher, 1992) had biphasic displacement curves, enabling the separate calculation of IC_{50} values for monoamine oxidase A and B, which are described in Table 1. The less selective monoamine oxidase inhibitors pargyline and D-(+)-deprenyl (Cesura and Pletscher, 1992) had monophasic displacement curves with IC_{50} values mentioned in Results. Single concentrations of 100 nM chlorgyline as well as of 40 nM L-(−)-deprenyl and 25 nM MDL 72,974 were chosen in further experiments for determining the characteristics (binding density: Table 2; displacement studies by harman and norharman: Table 1) of [^{3}H]pargyline binding to monoamine oxidase A and B, respectively.

Table 2
Determination of densities of monoamine oxidase A and B in membranes by binding studies

	[^{3}H]Harman binding		[^{3}H]Parygline binding		
	Clorgyline Monoamine oxidase A K_D (nM)	B_{max} (pmol/mg protein)	Clorgyline Monoamine oxidase A (pmol/mg protein)	L-(−)-Deprenyl Monoamine oxidase B (pmol/mg protein)	MDL 72,974 Monoamine oxidase B (pmol/mg protein)
Controls					
– Hippocampus (9)	3.14 ± 0.18	4.31 ± 0.09	3.78 ± 0.43	4.34 ± 0.23	4.89 ± 0.31
– Cerebral cortex (3)	3.14 ± 0.04	4.40 ± 0.36	4.40 ± 0.15	3.72 ± 0.30	4.03 ± 0.51
– Liver (3)	3.95 ± 0.26	8.41 ± 1.30	7.93 ± 0.71	17.06 ± 1.71	18.75 ± 2.08
Clorgyline-treated					
– Hippocampus (3)	n.d.		0.50 ± 0.41	5.64 ± 0.53	6.00 ± 0.78
– Cerebral cortex (3)	n.d.		n.d.	3.61 ± 0.47	4.33 ± 0.50
– Liver (4)	5.23 ± 0.90	0.52 ± 0.14	0.30 ± 0.18	14.31 ± 2.93	15.96 ± 4.81

n.d. = not detectable.
Means ± S.E.M. of 3–9 experiments (numbers in parentheses) in each group of controls and clorgyline-treated (10 mg/kg, 2 h) rats are presented. Specific [^{3}H]harman binding to monamine oxidase A was determined by Scatchard analysis (Scatchard, 1949) with 1 μM clorgyline as displacer to determine the nonspecific binding. Specific [^{3}H]pargyline binding was calculated by subtracting nonspecific binding measured with plateau concentrations (see Fig. 1) of 100 nM clorgyline (monoamine oxidase A), 40 nM L-(−)-deprenyl and 25 nM MDL 72,974 (monoamine oxidase B).

both binding assays. In all tissues, however, no decrease in the density of [^{3}H]pargyline binding to monoamine oxidase B was observed either with L-(−)-deprenyl or with MDL 72,974 used to define the nonspecific binding. Therefore, the clorgyline treatment schedule used in the in vivo experiments (10 mg/kg, 2 h) seems to inhibit monoamine oxidase A selectively and irreversibly, but not monoamine oxidase B. To assess the possibility that traces of clorgyline that were not irreversibly bound to monoamine oxidase A after the washing procedure contaminate the membrane homogenates and therefore bias the assays of radioligand binding, we tested the effect of 8 nM clorgyline ($K_i \sim 8$ nM) on in vitro [^{3}H]harman binding, using cerebral cortex membranes. Clorgyline had the characteristics of a competitive inhibitor by increasing the K_D from 3.91 ± 0.54 nM ($n = 3$; $r = -0.97 \pm 0.01$) to 8.63 ± 0.11 nM ($n = 3$; $r = -0.94 \pm 0.02$), with no change of the B_{max} (3.57 ± 0.24 and 3.47 ± 0.47 pmol/mg protein). No specific [^{3}H]harman binding was detectable in the

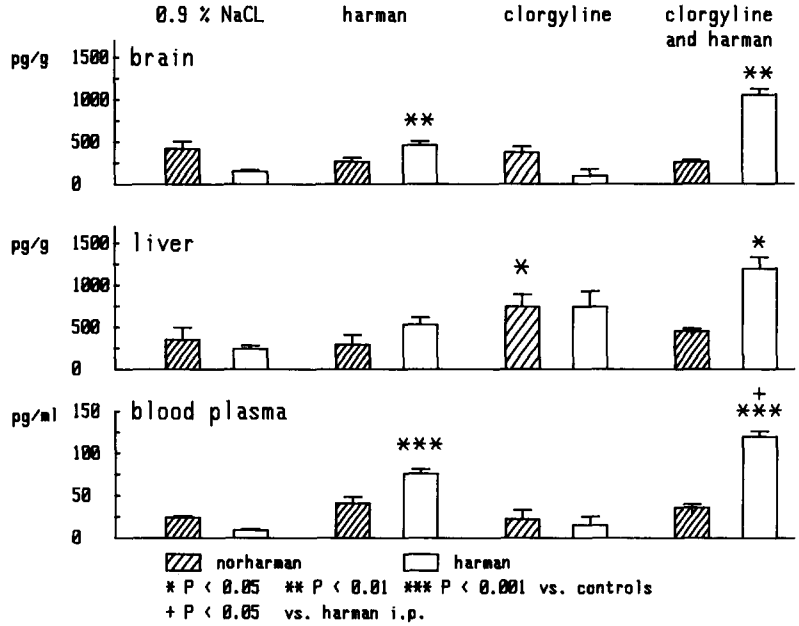

Fig. 2. Concentrations of norharman and harman in brain, liver and blood plasma of rats 2 h after intraperitoneal injection of saline and harman, respectively. Clorgyline was administered 135 min before decapitation. The values are the means ± S.E.M. of the number of experiments given in the text.

brain tissue of the clorgyline-treated rats; however, the data of the liver were comparable (Table 2). A very slight and non-significant increase in the K_D value was found (3.95 to 5.23 nM), suggesting that no relevant amount of unbound clorgyline was left in the tissue homogenates.

3.2. Norharman and harman levels

The experiments were designed to demonstrate that harman occurs naturally in various tissues and to find out whether the in vitro inhibition of monoamine oxidase A activity by harman can be demonstrated in vivo as well. The levels of norharman were measured concomitantly (Fig. 2). A low dose of harman (0.1 mg/kg) was injected to determine the amount necessary to elicit an increase in its levels. The experiments were also designed to examine whether norharman can be formed from harman by demethylation. This would represent an alternative biosynthetic pathway to the condensation of tryptamine and formaldehyde (Greiner and Rommelspacher, 1984).

Fig. 2 demonstrates that both harman and norharman occur naturally in brain, liver and blood plasma from rats. The concentration of norharman amounted to 292.4 ± 55.3 pg/g (23) in brain, 390.2 ± 60.7 pg/g (13) in liver, 891.4 ± 87.7 pg/g (5) in heart, 625.3 ± 47.1 pg/g (4) in kidney and 31.0 ± 4.6 pg/ml (20) in blood plasma (values are the means ± S.E.M. of the number of experiments given in parentheses). The respective values for harman in saline-treated rats were 147.4 ± 36.2 pg/g (6) in brain, 242.9 ± 64.0 pg/g (5) in liver, 215.8 ± 41.7 (5) in heart, 1049 ± 36.8 (3) in kidney and 8.57 ± 3.1 pg/ml (9) in blood plasma. Treatment with authentic harman increased concentrations of harman in brain (458.8 ± 72.1 pg/g (4)) and blood plasma (75.6 ± 8.0 pg/ml (4)). It is noteworthy that injection of harman did not elicit a significant increase in harman in liver tissue. Pretreatment with clorgyline, a specific and irreversible inhibitor of monoamine oxidase A, induced a further increase in harman levels which reached statistical significance in blood plasma (120.0 ± 12.7 pg/ml (6); Fig. 3).

The findings showed no evidence that norharman is formed from harman by demethylation. Based on the observation that alcoholics have increased levels of norharman and that many have increased levels of harman also, it could be speculated that alcoholic beverages are a source of the β-carbolines. As shown in Table 3, norharman can be detected in beer, with relatively high levels in dark beer, but only traces are found in wine and liquor. Harman has been found in both beer and wine and traces of it in liquor. The concentrations of both β-carbolines are so low that even a liter of the drink would not be sufficient to cause a detectable increase in the tissue levels. The identity of the extracted compounds was ascertained by mass spectrometry. The peak isographic of norharman was identified for Guiness beer and Erdinger beer and with respect to harman for Chardonnay wine. As we have already published several mass spectra (Rommelspacher et al., 1991a,b), we will not present further spectra.

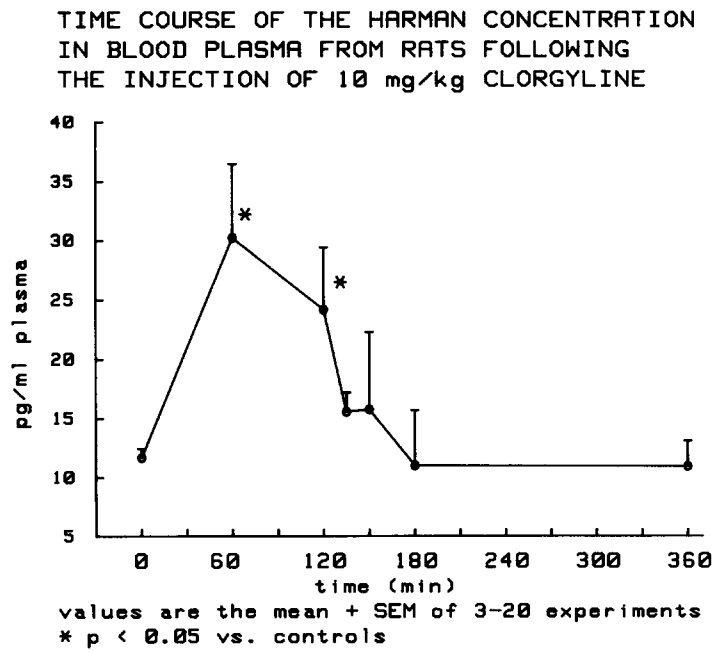

Fig. 3. Time course of the harman concentration in blood plasma from rats following the injection of clorgyline (10 mg/kg) at timepoint zero.

Table 3
Levels of norharman and harman in beer and wine

	ETOH (Vol.%)	Norharman (ng/ml)	Harman (ng/ml)
Beer brand			
Becks	4.7	4.8	1.7
Erdinger Weizenbock	7	11.7	5.4
Schultheiss	5	4.2	1.3
Guiness	5	22.7	3.2
Budweiser (CSFR)	5	4.6	2.4
Tuborg	4.8	4.9	1.9
Groterjan	0	2.7	0.7
White wine			
Chardonnay	12.5	d.l.	3.6
Bordeaux	12	n.d.	2.9
Müller-Thurgau	10	d.l.	7.8
Red wine			
Beuajolais	12	n.d.	1.3
Chianti	12	n.d.	2.5
Bordeaux	11.5	n.d.	2.0

n.d. = not detected; d.l. = near detection limit.
Values are the means of two independent experiments. Two different HPLC methods were applied. Neither norharman nor harman was detected in liqueur.

Another series of experiments was conducted to investigate the harman-liberating effect of the irreversible monoamine oxidase A inhibitor clorgyline. Firstly, the time course was determined in blood plasma. Application of clorgyline induced an increase in harman levels that lasted for about 2 h (Fig. 3). In the second study, a single time point (135 min) was chosen for experiments designed to find out the sources of the additional harman in plasma. In brain, the levels tended to decrease (86.7 ± 29 pg/g (3); $P < 0.08$, Fig. 2), whereas the levels in liver tissue tended to increase (741 ± 499 pg/g (3); $P < 0.09$). The concomitant measurements of norharman revealed no change in brain (365.5 ± 72 pg/g (3)), but an increase in liver (737 ± 147 (3); $P < 0.05$).

4. Discussion

The natural occurrence of condensation products of neurotransmitter metabolites and α-ketoacids or aldehydes is now well established (for review Brossi, 1991, 1993; Rommelspacher et al., 1991a). With respect to both the tetrahydroisoquinolines and the β-carbolines, the identity of the extracted compounds from human blood plasma and urine as well as from rat urine has been proven by mass spectrometry and by thorough chemical analyses. The two β-carbolines harman and norharman have not yet been demonstrated concomitantly in rat tissue. Therefore, we adapted the established method for human blood plasma to rat tissue and found harman and norharman in whole rat brain, heart, kidney and liver. Bosin et al. (1989) found harman in rat lung. No data were presented in this study with respect to norharman. In human platelets only harman was detected, whereas rat platelets contained neither of the β-carbolines (Schouten and Bruinvels, 1985). In a following study, norharman was detected in platelet-rich plasma from rats (Schouten and Bruinvels, 1986). Others have detected higher levels of harman in the brain of Sprague-Dawley rats than we did in the present study (410 ± 0.05 pg/g; Bosin et al., 1989). All these findings support an uneven organ distribution and a total brain concentration of the β-carbolines harman and norharman below 500 pg/g (~ 2.8 pmol/g) in rat brain. Higher levels would suggest artifactual formation and/or mixture with adjacent eluting compounds on the HPLC column. The diet could be an explanation for small differences. Neither harman nor norharman was found in meat extract, but both could be detected in fried meat depending on the duration of frying (norharman: 8.7 ng/g of wet weight raw product (6 min); 19.3 ng/g (13 min); harman: 3 ng/g (6 min); 4.8 ng/g (13 min); Gross, 1990). We and others have found low levels of harman and norharman in alcoholic beverages (Table 3) (Adachi et al., 1991; Bosin and Faull, 1988b). A source of appreciable amounts of harman are some brands of soy sauce (250 ng/ml) and wheat vinegar (730 ng/ml; Adachi et al., 1991). Harman (3.3–5.8 μg/g) and norharman (12.3–14.1 μg/g) were found in the smoke of tobacco and were formed by tryptophan pyrolysis (Poindexter and Carpenter, 1962). It should be realized that 100 μg/kg body weight causes approximately a doubling of the endogenous concentration (Fig. 1), thereby more or less excluding a significant contribution of exogenous β-carbolines to the endogenous levels.

In vitro binding experiments have revealed a reversible, single high-affinity binding site for [^{3}H]harman (K_D ~ 3 nM) on monoamine oxidase A in the brain of rat (May et al., 1991a) and the primate species marmoset (May et al., 1991b). Furthermore, the specific [^{3}H]harman binding site on monoamine oxidase A was also found in visceral organs of the rat (e.g. liver, heart, lung) (May et al., 1991b).

The potency of the reversible monoamine oxidase A inhibitor harman to displace [^{3}H]pargyline binding from monoamine oxidase A is in accordance to the former, determined potency as an inhibitor of monoamine oxidase A activity (Nelson et al., 1979; May et al., 1991a). Norharman was far less potent and even inhibited [^{3}H]pargyline binding to monoamine oxidase B with slightly higher preference (Table 1), in accordance with its enzyme-inhibiting properties (May et al., 1991a).

The monoamine oxidase A binding properties of harman were further supported by the ex vivo experiments in the present study. The clorgyline treatment schedule for rats produces a selective, irreversible

(mechanism-based) inhibition of monoamine oxidase A (Cesura and Pletscher, 1992) in brain and liver, as assessed by the in vitro [^{3}H]pargyline binding method. In accordance with these results, specific [^{3}H]harman binding was not detectable in brain, but was ~95% reduced in the liver. Specific [^{3}H]pargyline binding to monoamine oxidase B was not affected by the clorgyline treatment, suggesting a selective inhibition of monoamine oxidase A under the conditions of the treatment.

The main aim of the present study was to determine the postulated role of harman as an endogenous in vivo inhibitor of monoamine oxidase A. The affinity and selectivity of harman for the monoamine oxidase A binding site in vitro was much higher than that of norharman (Table 2). Thus, norharman levels can serve as controls for the effects of harman. Theoretically, if harman binds in vivo preferentially to monoamine oxidase A, one would expect an increase in the harman levels in blood plasma due to in vivo displacement of the endogenous harman by clorgyline in other tissues. This was actually observed (Fig. 3). In contrast, in monoamine oxidase A containing tissues like liver and brain, one would expect a decrease in the harman concentration. A trend to a decrease was found in the brain. In the liver, however, a slight increase was observed (Fig. 2). This could be explained by different mechanisms.

The calculated concentrations of [^{3}H]harman and [^{3}H]pargyline binding sites on monoamine oxidase A are about 150 pmol/g whole brain and 550 pmol/g liver. This means that the concentration of harman (0.8 pmol/g brain tissue) is at least 100-fold lower than the enzyme concentration. If harman competes with clorgyline for common monoamine oxidase A binding sites, possibly >99% of these sites would have to be occupied by clorgyline before a decrease in harman concentrations could be detected in these tissues. Monoamine oxidase A in blood vessel membranes could also contribute to the increase in harman in blood plasma following treatment with clorgyline. For example, in rat brain, a high density of monoamine oxidase A was found in microvessels (Kalaria and Harik, 1987).

An explanation of the small increase in harman in liver tissue would be the inhibition of microsomal cytochrome *P*450 enzyme(s) by clorgyline (Dupont et al., 1987). Therefore, as metabolic degradation was probably inhibited, the harman concentration increased slightly in vivo. Increased levels of norharman support this view. There is evidence that norharman can be metabolized only in the liver (Fekkes and Bode, 1993).

The main question remains, whether harman plays a crucial role as an endogenous monoamine oxidase inhibitor. A comparison of the measured endogenous concentrations of harman (low nmol/kg tissue range) with the in vitro dose-response (inhibition) curves (Rommelspacher et al., 1991a) would suggest that this is not probable, since concentrations of about 1 μmol/kg tissue are required to inhibit 80–90% of the enzyme activity. This percentage of inhibition is necessary for a significant effect in vivo (Cesura and Pletscher, 1992). If harman is unevenly distributed, compartmentalized and enriched in high concentrations in monoaminergic synapses, this mammalian alkaloid could play a significant role as an endogenous monoamine oxidase A inhibitor. This assumption is supported by a study describing a significant increase in the levels of 5-hydroxytryptamine and noradrenaline in the brain of rats after in vivo administration of reversible monoamine oxidase inhibitors of the β-carboline group (e.g. harmaline, harmine; Pletscher et al., 1959).

A recent study questions that concentrations of harman in the μmol/kg tissue range are necessary to elicit significant and reliable effects (Ergene and Schoener, 1993). Sixty single neurons of the nucleus accumbens were recorded: 85% were sensitive to harman. Of these, 55% were excited by concentrations between 10^{-11}–10^{-9} M, whereas 45% were inhibited at 10^{-8}–10^{-6} M. The nucleus accumbens plays an important role in behavioral reinforcement and reward. The significance of these findings is underlined by the observation that chronic infusion of harman into the third ventricle of rats elicits an increase in voluntary ethanol intake (Rommelspacher et al., 1987). It remains to be demonstrated, however, whether these effects are elicited by inhibition of monoamine oxidase A as harman also binds to other binding sites/receptors in the brain, e.g. to the high-affinity [^{3}H]norharman binding sites (Pawlik and Rommelspacher, 1988), as well as to the receptors of benzodiazepine/γ-aminobutyric acid, opioid and 5-hydroxytryptamine (Strömbom et al., 1992).

In conclusion, there is good evidence from in vitro, ex vivo and in vivo experiments that harman inhibits monoamine oxidase A specifically and reversibly. The concentration is so low that only a small percentage of the enzyme is inhibited under physiological conditions.

However, there may be high levels in some brain regions and possibly in some neuronal compartments, which could explain the high sensitivity of neurons of the nucleus accumbens to the application of harman and the increase in the voluntary intake of ethanol in rats. Dietary sources do not contribute significantly to endogenous β-carboline levels, supporting the view that harman is a natural inhibitor of monoamine oxidase A.

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