Estimation of Harmine and Its Derivatives by HPLC: Correlation of Brain Harmine Levels with Jumping Behavior in Rats

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Abstract—Brain harmine was simply and sensitively determined by high performance liquid chromatography (HPLC), and the relationship between brain harmine concentrations and the jumping behavior induced by harmine and apomorphine was investigated. The concentration of harmine and the peak height in HPLC showed a good correlation, and the detection limit was 0.05 pmole harmine. In rats treated with 2, 5 and 10 mg/kg of harmine, the concentrations of harmine in the brain cortex were 9.0, 21.3 and 43.1 nmole/g tissue (wet wt.), respectively. The regional brain harmine concentrations and the jumping activity measuring by a scoring system increased with increasing doses of harmine administered, and there was a direct relation between the harmine concentrations and the jumping activity. This relation was observed in washed membranes in which about 40% of the harmine in whole homogenate was present. Tissue subfractionation showed that 30% of the harmine in whole brain homogenates was in the P2 fraction, and of this, 70% was located in the synaptosomes. The brain homogenate did not metabolize harmine and no metabolite was detected in the brain 10 min after treatment with harmine and apomorphine. These results suggest that harmine itself, but not its metabolites, may be responsible for inducing the jumping behavior and that the jumping activity is correlated with brain harmine concentrations.

Harmine (1-methyl-7-methoxy-β-carboline) is a tremogenic alkaloid and induces tremors in rats and mice (1-4). We have reported that administration of harmine in combination with apomorphine induces violent jumping in rats instead of a tremor. This behavior was induced by harmine when the central dopaminergic system was activated by apomorphine or L-DOPA plus benzerazide, and the intensity of the jumping behavior depended on the dose of harmine administered (5). Harmine microinjected into the caudate nucleus as well as by subcutaneous injection caused a tremor in rats (1), and harmaline, an analog of β-carboline, induced a tremor which depended on harmaline concentration in the brain (6). Therefore, this study was undertaken to elucidate whether harmine itself was involved in the jumping behavior or whether the concentration of harmine or its metabolites in brain were related to the jumping behavior. We developed a simple and sensitive assay method for harmine and the metabolites in rat brain.

The present paper deals with an assay method for harmine which uses high performance liquid chromatography (HPLC) equipped with spectrofluorometer as a detector, and we describe our results on the brain harmine concentration of rats as determined by this method. In addition, the relationship between harmine concentrations in the brain and the jumping behavior induced by harmine and apomorphine are described.

Materials and Methods

Drugs: Harmine, harmaline, harmane, norharmane, harmol and harmalol were purchased as the respective hydrochloride salts from Sigma Chemical Co., Ltd. (St. Louis, U.S.A.). Ethyl β-carboline-3-
carboxylate (3-CCE) was obtained from Aldrich Chemical Co. (Milwaukee, U.S.A.). These compounds were dissolved in 0.1 N HClO₄-25% methanol solution for the HPLC assay. Apomorphine hydrochloride was obtained from Woelm Pharma GmbH & Co. (Eshwege, West Germany). Methamphetamine hydrochloride (product of Dainippon Pharmaceutical Co., Ltd., Osaka) was a gift from Dr. S. Suzuki (Chiba University). All other chemicals were of reagent grade. In in vivo experiments, harmine, apomorphine and methamphetamine were dissolved in 0.9% NaCl solution and injected s.c. or i.p. to rats.

**Determination of harmine by HPLC:** The system (Japan Spectroscopic Co., Ltd., Tokyo, Japan) consisted of a model BIP-1 pump, a model VL-614 injector, and a Finepak SIL C₁₈ column (4.6×150 mm). A model UVIDEC-100-V UV spectrophotometer and a model FP-210 spectrofluorometer were used as detectors. The excitation wavelength was 320 nm, and the emission wavelength was 420 nm. The mobile phase consisted of a mixture of methanol and 0.2% phosphoric acid (60:40), and it was run at 1.0 ml/min. The column was maintained at room temperature during the runs. Two to ten μl of samples in duplicate were injected into the HPLC. A standard curve was generated by dissolving varying amounts of harmine in 0.1 N HClO₄-25% methanol solution.

**Dissection and tissue preparation:** Male Wistar rats (Japan Rat Co., Ltd., Urawa, Japan) weighing 200–230 g were housed in an air-conditioned room with a 12 hr light-dark cycle and given a commercial diet and water ad libitum. Ten min after treatment with harmine (2–10 mg/kg) and apomorphine (2 mg/kg), s.c., rats were sacrificed by microwave irradiation at 1.5 kW for 1.3 sec using 5 kW microwave equipment (model NJE 2601, New Japan Radio Co., Ltd., Tokyo, Japan). Experiments were carried out from 10:00 a.m. to 1:00 p.m. The brain was removed quickly and dissected into the following regions: cerebral cortex (CTX), frontal cortex (FTX), hypothalamus (HY), corpus striatum (CS), hippocampus (HIP), cerebellum (CEL), midbrain (MB) and pons-medulla oblongata (PO). The tissues were homogenized in 0.1 N HClO₄-25% methanol and mixed well to extract the harmine. After standing for at least 30 min in ice with shaking every 10 min, the mixtures were centrifuged at 16,000×g for 5 min at 4°C. The resulting supernatants were filtered using 0.45 μm microfilters, and the aliquots (2–10 μl: equivalent to 100–500 μg tissue weight) were applied to the HPLC. The tissues from control rats (no harmine and apomorphine) were homogenized in 0.1 N HClO₄-25% methanol solution containing 10⁻⁶ M harmine to estimate the recovery of harmine.

Methamphetamine was given i.p. to rats at a dose of 1 or 2 mg/kg 20 min before harmine injection in order to activate the central dopaminergic system. Rats were killed 20 min after the harmine injection, and the harmine concentrations of the brain were determined as described above.

Washed membranes were prepared according to the method of Kuriyama et al. (7). Briefly, the brain tissues were homogenized in 0.05 M Tris-citrate buffer, pH 7.0, and centrifuged at 45,000×g for 20 min at 2°C. The resulting pellets were washed three times with ice-cold buffer, by resuspension and centrifugation at 45,000×g for 15 min at 2°C. The final pellets were suspended in 0.1 N HClO₄-25% methanol solution for harmine determination.

**Tissue subfractionation** was carried out by the method of De Robertis et al. (8). The brain tissues were homogenized with 0.32 M sucrose and centrifuged at 600×g for 10 min, and the supernatant was centrifuged at 12,000×g for 20 min to obtain the crude mitochondrial (P₂) fraction. The resulting supernatant was centrifuged at 100,000×g for 60 min to separate the microsomal fraction. The P₂ fraction was centrifuged using a discontinuous sucrose gradient (1.2 M, 0.8 M and 0.32 M) to separate myelin, synaptosomes and the mitochondrial fraction. Harmine in each fraction was extracted with 0.1 N HClO₄-25% methanol solution, and the extract was applied to the HPLC column.

**Measurement of jumping activity:** The jumping behavior was assessed according to the method described in the previous paper (5). Groups of 5 rats were placed in the observation cage (41×25×15 cm) at least
30 min before injections of drugs to allow for adaptation to the new environment. Fine vibration of the cage induced by the jumping movements of the rats was detected by Minor Tremor Pick-up (MT-3T, Nihon Kohden Kogyo Co., Ltd., Tokyo, Japan) attached under the center of the cage. The vibrations were amplified and continuously recorded using an inkwriting-oscilloscope (Nihon Kohden Kogyo Co., Ltd., Tokyo, Japan). The recorded data were scored for quantitative and objective analysis by the scoring system (0, 1, 2, 3 and 4). Data was scored at 2 min intervals and averaged every 10 min. Different groups of rats were used for determination of harmine concentration and jumping activity.

All data in the tables were expressed as the mean±S.E., and statistical significance was evaluated by Student’s t-test at the 0.05 level of probability.

**Results**

**Structures and properties of β-carbolines:**

Figure 1 shows the structures, the excitation (Ex) and emission (Em) wavelengths, relative peak heights and retention times of harmine and several β-carboline analogs. Excitation and emission wavelengths of harmine and its desmethyl analog, harmol, were shorter than those of the other analogs. Under standard assay conditions (320 nm (Ex) and 420 nm (Em)), only harmol showed a high peak height (252% of harmine) with a different retention time from harmine. Harmane, harmalol and β-CCE had 15–21% of the peak height of harmine, norharmane had 4% and harmaline did not show any peak under these conditions.

**Elution pattern of harmine and its analogs:**

A representative elution profile of harmine standard (2 pmole), a mixture of harmine (2 pmole), harmane (2 pmole), harmol (1 pmole) and harmaline (2 pmole), and a brain sample are shown in Fig. 2A, B and C, respectively. The results shown indicate that harmine can be separated from the other β-carboline analogs and other components in the brain using this HPLC procedure. Harmine standard added to the brain sample coeluted with harmine from the brain extract (Fig. 2D). The retention times of harmine standards or harmine from different brain samples varied by 5% or less.

| Compound    | R1 | R2 | R3 | R4       | Ex (nm) | Em (nm) | Relative peak ht. (%) | Retention time (min) |
|-------------|----|----|----|---------|---------|---------|-----------------------|----------------------|
| Harmine     | CH₃ | H  | H  | CH₃O    | 320     | 420     | 100                   | 8.6                  |
| Harmaline   | CH₃ | H₂ | H₂ | CH₃O    | 380     | 480     | 0                     | 9.6                  |
| Harman      | CH₃ | H  | H  | —       | 370     | 430     | 15                    | 6.6                  |
| Norharmane  | H   | H  | H  | —       | 375     | 445     | 4                     | 7.0                  |
| Harmol      | CH₃ | H  | H  | OH      | 330     | 420     | 252                   | 4.0                  |
| Harmalol    | CH₃ | H₂ | H₂ | OH      | 380     | 480     | 19                    | 4.0                  |
| β-CCE       | H   | COO-| H  | —       | 380     | 460     | 21                    | 8.6                  |

Fig. 1. Structure of β-carbolines, excitation and emission wavelengths and elution properties from the ODS column. Peak heights of all the β-carbolines were estimated under the standard assay conditions for harmine set at 320 nm (Ex) and 420 nm (Em), and they were expressed as a percentage of the peak height of harmine.
Standard curve and recovery of harmine from brain homogenates: Figure 3A demonstrates the results of a typical standard curve. It can be seen that the application of increasing amounts of harmine (1–15 pmole) to the column resulted in a corresponding linear increase in peak height ($r=0.999$, higher concentrations were not tested). The detection limit was 0.05 pmole harmine.

Recoveries of a known amount of harmine added to various regions of brain tissues were only 60% in extraction by 0.1 N HClO$_4$ solution. With increasing amount of methanol added to 0.1 N HClO$_4$, the recoveries of harmine from the brain homogenates increased: for example, in CTX homogenate, the recoveries were 64% by 5% methanol, 72% by 10% methanol, 77% by 20% methanol and 87% by 25% methanol containing 0.1 N HClO$_4$ solution. The recoveries of harmine from various regions extracted by 25% methanol containing 0.1 N HClO$_4$ were 87% in CTX; 88% in FTX, HY and HIP; 91% in CS; 94% in CEL; and 83% in MB and PO. Good linearity between the peak height and harmine concentration was observed in the range of 1–10 pmole of harmine present in the homogenate. Figure 3B shows a typical curve for CTX homogenate ($r=0.998$). The detection limit in brain...
Harmine extracted from brain homogenates was stable in 0.1 N HClO₄-25% methanol solution at least 1 week in a refrigerator and 6 months in a freezer (-40°C). After incubation of harmine with brain homogenates for 90 min at 37°C, no metabolite of harmine was detected. This indicates that harmine was not metabolized by the brain tissue in this condition. On the contrary, liver homogenates metabolized harmine to produce three metabolites including harmol (data not shown).

Although harmine has been reported to be found in the mammalian CNS (9, 10), we could not detect harmine in brain tissue using the standard assay conditions for harmine. This was probably due to the small amount of the brain used (100-250 μg wet weight).

Regional brain harmine concentrations after treatment with harmine: Table 1 represents the harmine concentrations in various brain regions of rats treated with 2, 5 and 10 mg/kg of harmine in a combination with 2 mg/kg of apomorphine. The harmine concentration increased with increasing doses of harmine injected; at a dose of 2 mg/kg, harmine concentrations ranged from 8.23 to 13.0 nmole/g tissue weight; concentrations ranged from 19.59 to 26.27 at a dose of 5 mg/kg; and they ranged from 38.27 to 50.6 in rat brain treated with 10 mg/kg of harmine. A major regional difference was observed: at a dose of 2 mg/kg, HY had a significantly higher concentration compared to the other 7 regions. FTX showed significantly higher harmine concentration than CS and MB in 5 mg/kg harmine-treated rats and significantly higher concentration than MB and HY in 10 mg/kg harmine-treated rats. Rats treated with harmine alone had 31-40% lower brain harmine concentrations in all regions except HIP, although the decreases did not reach statistical significance, compared to the rats treated with harmine and apomorphine (data not shown).

Table 2 indicates the result of subcellular fractionation of rat brain cortex treated with 10 mg/kg of harmine and 2 mg/kg of apomorphine. About 30% of the total harmine in homogenates was observed in the nuclear fraction, another 30% in the P₂ fraction, 18% in microsomes and 19% in the supernatant fraction. Seventy percent of the harmine in the P₂ fraction was found in synaptosomes.

When the tissues were homogenized in 0.1 N HClO₄-Na₂S₂O₅-EDTA solution for determination of monoamines, brain monoamines such as dopamine and serotonin and

| Regions | 2 (avg.) | 5 (avg.) | 10 (avg.) | Correlation coefficient* |
|---------|---------|---------|----------|------------------------|
| CTX     | 8.97±0.44 (6) | 21.35±1.58 (6) | 43.06±2.25 (6) | 0.985 |
| FTX     | 9.62±0.56 (6) | 26.27±1.94 (6)*** | 50.60±3.39 (6)*** | 0.976 |
| CS      | 8.23±0.48 (6) | 20.46±1.48 (6) | 41.35±2.55 (6) | 0.984 |
| HIP     | 9.51±0.66 (6) | 22.78±1.88 (6) | 43.45±2.49 (6) | 0.979 |
| CEL     | 8.56±0.70 (6) | 23.04±1.61 (6) | 41.60±2.82 (6) | 0.989 |
| MB      | 8.37±0.84 (6) | 19.59±1.37 (6) | 38.27±2.22 (6) | 0.982 |
| PO      | 8.78±0.60 (6) | 22.00±1.83 (6) | 44.20±2.57 (6) | 0.983 |
| HY      | 13.0±0.35 (6)** | 24.40±1.90 (6) | 41.60±1.82 (6) | 0.977 |

Rats were given harmine (2, 5 or 10 mg/kg) and apomorphine (2 mg/kg) simultaneously. Ten min later, rats were killed, and brain harmine concentrations were determined. The values are expressed as nmol/g tissue wet weight±S.E. Numbers in parentheses are numbers of rats used. In another group of rats treated with harmine and apomorphine, jumping activity was scored as described in Materials and Methods (average for 10 min±S.E.). *Correlation coefficient was estimated between harmine concentrations and jumping scores. **HY shows significantly higher value than the other 7 regions. ***FTX is higher than CS and MB in 5 mg/kg harmine-treated rats, and it is higher than MB and HY in 10 mg/kg harmine-treated rats (P<0.05).
A rat was given 10 mg/kg of harmine and 2 mg/kg of apomorphine. Ten min later the rat was sacrificed, and the brain cortex was dissected out. Subfractionation and determination of harmine concentration were carried out as described in Materials and Methods. Values are expressed as the mean±S.E. (number of rats used).

Changes in dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, were observed in the rats treated with harmine and apomorphine.

Relation between brain harmine concentration and jumping activity: Rats exhibited a violent jumping behavior 3–4 min after the simultaneous injection of harmine and apomorphine. However jumping behavior was never induced by harmine or apomorphine alone (5). The jumping activity increased with increasing doses of harmine injected; a few rats jumped at a dose of 2 mg/kg, and every rat jumped with short duration at a dose of 5 mg/kg. With 10 mg/kg of harmine, every rat showed violent jumping during the 10 min observation period. The jumping activity was quantified by the scoring system described in the Materials and Methods. The highest jumping activity was observed about 10 min after harmine injection, and the activity decreased gradually. The mean scores in rats treated with 2, 5 and 10 mg/kg of harmine 10 min later were 1.35±0.33, 1.78±0.25 and 3.48±0.29, respectively. A good correlation between the brain harmine concentrations and the jumping scores was observed in every region (Table 1, right column and Fig. 4A). This correlation between harmine concentrations and jumping activity was also observed in washed membranes prepared by resuspension and centrifugation three times.

![Fig. 4](image-url) The relation between brain harmine concentration and jumping score. Rats were given 2, 5 or 10 mg/kg of harmine with 2 mg/kg of apomorphine. Ten min later, rats were killed, and the brain homogenate was determined. Intensity of jumping behavior was measured as described in Materials and Methods. Relation between jumping score and (A) harmine concentrations in the homogenate, (B) harmine concentrations in washed membranes prepared by resuspension and centrifugation three times.

### Table 2. Distribution of harmine in subcellular fractions

| Subfraction       | Harmine conc. (nmole/g tissue wet wt.) | %     |
|-------------------|----------------------------------------|-------|
| Cerebral cortex   |                                        |       |
| Homogenate        | 30.74±1.31 (9)                         | 100.0 |
| Nuclear fraction  | 9.14±1.03 (9)                          | 29.7  |
| P₂ fraction       | 8.99±0.32 (9)                          | 29.2  |
| myelin            | 0.44±0.09 (3)                          | 1.4   |
| synaptosomes      | 6.33±0.61 (5)                          | 20.6  |
| mitochondria      | 0.51±0.17 (3)                          | 1.7   |
| Microsomes        | 5.43±0.30 (9)                          | 17.7  |
| Cell sap          | 5.99±0.36 (8)                          | 19.5  |
| **total**         |                                        | 96.1  |
Methamphetamine was given to rats 20 min before harmine (5 mg/kg) injection. Rats were killed 20 min later, and the brain harmine concentrations were determined as described in Materials and Methods. Values are expressed as nmole of harmine/g tissue wet weight (mean±S.E.). Numbers in parentheses are the numbers of rats used. *Significant difference from the 1.0 mg/kg methamphetamine treated-rats.

Membranes from all the regions; and as seen in Fig. 4B, about 40% of the harmine in the homogenate remained in the membranes despite strong washing. The total amount of harmine in the brain was estimated to be about 60 nmole (15 μg), which was equivalent to 0.7% of the harmine injected to rats.

In rats pretreated with 2 mg/kg of methamphetamine followed by a 10 mg/kg harmine injection, 75% of the group showed jumping behavior during a 20 min observation period; however, the onset of jumping was delayed compared to the rats treated with apomorphine. At a dose of 1 mg/kg methamphetamine, rats hopped forward occasionally. Methamphetamine alone enhanced locomotor activity, but did not show any jumping behavior. As presented in Table 3, the brain harmine concentrations after treatment with harmine and methamphetamine tended to increase with increasing amount of methamphetamine injected, in spite of the injection of an equal dose of harmine.

The assay method for harmine by HPLC presented in this paper was simple and sufficiently sensitive to determine the harmine concentration of rat brain treated with harmine and apomorphine. Harmine was separated by an ODS column from other β-carboline analogs and the biological materials in the brain using excitation and emission wavelengths of 320 nm and 420 nm, respectively. By increasing the amount of methanol in the mixture of 0.1 N HClO₄ and methanol, the recovery of harmine from the tissues increased to 94%.

Regional brain harmine levels after treatment with harmine and apomorphine revealed that the concentration of brain harmine increased with increasing doses of harmine injected. It was found that there was a close relationship between brain harmine levels and the jumping activity, i.e., jumping activity increased with increasing concentrations of harmine in the brain. This demonstrates that the level of jumping behavior induced by harmine and apomorphine is directly related to the harmine concentration in the brain. This relation was observed in washed membranes, in which about 40% of the harmine in the homogenate still remained even though the membranes were vigorously washed three times.

Harmine was not metabolized in the brain tissues in vitro: no metabolites were detected in brain extracts of rats treated with harmine and apomorphine. This differed from the result in the liver in which harmine was metabolized.
metabolized to produce harmol and its glucuronide or sulfate (13). A small amount of harmol was detected 20 min after the injection of harmine and apomorphine. This harmol may be derived from the liver. These findings strongly suggest that harmine itself, but not the metabolites, may be responsible for inducing the jumping behavior.

The results of subfractionation of the brain tissue shows that harmine has high affinity for membrane fractions. The fact that most of the harmine in the P2 fraction is located in the synaptosomes suggests that harmine may play a role in the nerve terminals. There were regional differences of harmine concentration in the brain, but we could not demonstrate the exact area in which harmine would act and produce its specific behavioral effect. Cox and Potkonjak (1) have demonstrated that a microinjection of harmine into the caudate nucleus and substantia nigra caused a tremor, and they speculated that those areas were involved in the generation of a tremor.

It was observed that the brain harmine levels in rats treated with harmine plus apomorphine were 31–40% higher than those in rats treated with harmine alone. Pretreatment with methamphetamine at a dose of 2 mg/kg caused the jumping behavior in rats which showed increased brain harmine concentrations compared to rats treated with 1 mg/kg of methamphetamine, in spite of the injection of an equal dose of harmine. It is not clear whether or not an activation of the dopaminergic system by apomorphine or methamphetamine may contribute to this elevation of brain harmine concentration, but the hypothesis is very interesting because the jumping behavior was never induced by harmine alone, and induction of the jumping behavior by harmine required the activation of the central dopaminergic system by apomorphine or L-DOPA plus benserazid (5).

β-Carbol ine compounds have been proposed as a candidate for the endogenous ligand of the benzodiazepine receptor (14, 15), which is tightly associated with the GABA-Cl ionophore complex (16). Robertson et al. (14) have reported that harmine displaced specifically-bound [3H]nitrazepam from the benzodiazepine receptor, but had a very low affinity for the receptor (IC50 of approximately 200 nM). He has also demonstrated that a tremor induced by harmaline depended on the harmaline concentration in the brain and suggested that harmaline produced tremor at least partly as a result of its interaction with the benzodiazepine receptor (6). In preliminary experiments, we have found that [3H]-diazepam binding to the benzodiazepine receptor decreased in the corpus striatum of rats treated with 10 mg/kg of harmine and 2 mg/kg of apomorphine. It suggests that the benzodiazepine receptor in the corpus striatum may be partly concerned with the behavioral effect by harmine. It is well-known that nigrostriatal dopaminergic neurons are regulated by striato-nigral GABAergic neurons. The finding that intrastriatal microinjection of GABA and the related compounds affected dopamine receptor activity in the striatum has also been reported (17). Therefore, it is possible that harmine may affect dopaminergic neurons directly or via GABAergic neurons by interacting with the benzodiazepine receptor and consequently producing the jumping behavior.

References
1 Cox, B. and Potkonjak, D.: An investigation of the tremorgenic actions of harmine in the rat. Eur. J. Pharmacol. 16, 39–45 (1971)
2 Kelly, D.M. and Naylor, R.J.: Mechanisms of tremor induction by harmine. Eur. J. Pharmacol. 27, 14–24 (1974)
3 Kelly, D.M. and Naylor, R.J.: The importance of extrapyramidal function for the induction and antagonism of harmine tremor. Eur. J. Pharmacol. 32, 76–86 (1975)
4 Costall, B., Kelly, D.M. and Naylor, R.J.: The importance of 5-hydroxytryptamine for the induction of harmine tremor and its antagonism by dopaminergic agonists assessed by lesions of the midbrain raphe nuclei. Eur. J. Pharmacol. 35, 109–119 (1976)
5 Takashi, K. and Kuga, T.: Role of brain monoamine systems in the jumping behavior induced in rats by the combination of harmine and apomorphine. Japan. J. Pharmacol. 31, 677–688 (1981)
6 Robertson, H.A.: Harmaline-induced tremor: the benzodiazepine receptor as a site of action. Eur. J. Pharmacol. 67, 129–132 (1980)
7 Kuriyama, K., Kurihara, E., Ito, Y. and Yoneda, Y.: Increase in striatal \(^{[3]}\)H-muscimol binding following intrastriatal injection of kainic acid: a denervation supersensitivity phenomenon. J. Neurochem. 35, 343-348 (1980)

8 De Robertis, E., De Lores Arnaiz, G.R. and Alberich, M.: Subcellular distribution of adenyl cyclase and cyclic phosphodiesterase in rat brain cortex. J. Biol. Chem. 242, 3487-3493 (1967)

9 Shoemaker, D.W., Cummins, J.T., Bidder, T.G., Boettger, H.G. and Evans, M.: Identification of harmane in the rat arcuate nucleus. Naunyn Schmiedebergs Arch. Pharmacol. 310, 227-230 (1980)

10 Rommelspacher, H., Damm, H., Straub, S. and Schmidt, G.: Ethanol induces an increase of harmane in the brain and urine of rats. Naunyn Schmiedebergs Arch. Pharmacol. 327, 107-113 (1984)

11 Honecker, H. and Rommelspacher, H.: Tetrahydronorharmane (tetrahydro-\(\alpha\)-carboline), a physiologically occurring compound of indole metabolism. Naunyn Schmiedebergs Arch. Pharmacol. 305, 135-141 (1978)

12 Bidder, T.G., Shoemaker, D.W., Boettger, H.G., Evans, M. and Cummins, J.T.: Harmain in human platelets. Life Sci. 25, 157-164 (1979)

13 Sietkin, T.A. and DiStefano, V.: A model of harmine metabolism in the rat. J. Pharmacol. Exp. Ther. 174, 456-462 (1970)

14 Robertson, H.A., Barker, G.B., Coutts, R.T., Benderly, A., Locock, A. and Martin, I.L.: Interactions of \(\beta\)-carbolines with the benzodiazepine receptor: structure-activity relationships. Eur. J. Pharmacol. 76, 281-284 (1981)

15 Rommelspacher, H., Hanz, C., Borbe, H.O., Fehske, K.J., Muller, W.E. and Wollert, U.: Benzodiazepine antagonism by harmane and other \(\beta\)-carbolines in vitro and in vivo. Eur. J. Pharmacol. 70, 409-416 (1981)

16 Olsen, R.W.: GABA-benzodiazepine-barbiturate receptor interactions. J. Neurochem. 37, 1-13 (1981)

17 Richards, J.G. and Mohler, H.: Benzodiazepine receptors. Neuropharmacology 23, 233-242 (1984)