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Identification of Tetrahydronorharmane (Tetrahydro-β-carboline) in Human Blood Platelets

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Summary: The present paper shows that tetrahydronorharmane (tetrahydro-β-carboline) exists in human platelets. The concentration of tetrahydronorharmane in platelets from 10 ml platelet rich plasma was in the range of 9.3 to 25.6 pmol (n=8). Ingestion of tryptamine hydrochloride (n=4) three times daily for three consecutive days and of 19.6 mmol *D,L*-tryptophan (n=7) the evening (10 p. m.) before the blood collection did not lead to an increase of tetrahydronorharmane in platelets.

Nachweis von Tetrahydronorharman (Tetrahydro-β-carbolin) in menschlichen Thrombocyten

Zusammenfassung: In der vorliegenden Arbeit wird das Vorkommen von Tetrahydronorharman (Tetrahydro-β-carbolin) in menschlichen Thrombocyten nachgewiesen. Der Gehalt der Thrombocyten aus 10 ml plättchenreichem Plasma an Tetrahydronorharman lag in dem Bereich von 9,3 bis 25,6 pmol (n=8). Einnahme von Tryptaminhydrochlorid (n=4) dreimal täglich über einen Zeitraum von drei Tagen und von 19,6 mmol *D,L*-Tryptophan (n=7) am Abend (22^{00}) vor der Blutabnahme führte nicht zu einer Zunahme des Tetrahydronorhormon-Gehalts in den Thrombocyten.

Introduction

There is good evidence that tetrahydronorharmane exists in the urine of man and rats as well as in the forebrain of rats (1, 2). Tetrahydronorhamane produces pharmacological effects in rats, which are similar to those of serotonin-like drugs and antagonistic to those of dopamine (3). Furthermore it could be established in in vivo and in vitro experiments that tetrahydronorharmane inhibits high affinity uptake of serotonin and noradrenaline into a synaptosome-rich fraction from the brain of rats and mice (4, 5, 6). Moreover, tetrahydronorharmane apparently increases the release of serotonin from brain slices of rats, whereas a release of dopamine is inhibited (7). With respect to the uptake, storage and metabolism of serotonin and catecholamines, blood platelets have quite similar properties to those of neuronal tissue. Therefore, it seems of interest to investigate whether tetrahydronorharmane is a physiological constituent of human platelets and can be used as a peripheral indicator of central mechanisms.

Materials and Methods

Tryptamine-HCl and *D,L*-tryptophan were purchased from Merck AG, Darmstadt, FRG, [^{3}H]acetic anhydride 126–296 GBq/mmol (3, 4–8 Ci/mmol) from Amersham Buchler, Braunschweig, FRG. Tetrahydronorharmane was synthesized using the method of *Vejdelek* et al. (1961) and described elsewhere (4, 8).

Blood platelets were obtained from physically and mentally healthy volunteers (age 27–53 years of both sexes). In one series of experiments the subjects ingested 19,6 mmol *D,L*-tryptophan the evening before (10 p. m.) collection of the blood samples.

In another experiment a group of 8 persons received the same meals and beverages for three consecutive days. The food consisted of various kinds of meat, vegetables, fruits, bread etc. Tea and coffee served as beverages. Alcohol was not allowed. In addition to each breakfast, lunch, and dinner 4 subjects of this group ingested 1 mmol tryptamine hydrochloride for three days.

Preparation of platelets

Blood samples were collected at 8 a.m. by dropping venous blood (25 ml) from the cannula into plastic tubes containing 5 ml of ACD-stabilizer (USP X VIII formula B) as anticoagulant. The samples were centrifuged for 30 min at 180 *g* at 25 °C. A constant aliquot of platelet-rich plasma (10 ml) was removed, and the number of platelets was counted (280–320 · 10^9/l) Thereafter the platelet-rich plasma was further fractionated by centrifugation for 15 min at 12 000 *g* to give the platelet pellet and the plasma.

Assay

The platelet pellet was homogenized by sonication in 3 ml trichloroacetic acid (50 g/l 0.01 mol/l HCl) containing 28.3 μmol semicarbazide HCl. After centrifugation at 17 000 *g* for 10 min the supernatant was transferred to a glass-stoppered 50 ml centrifuge tube. The residue was washed with 3.5 ml 0.1 mol/l HCl and the suspension centrifuged again. Both supernatants were

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combined and made basic (pH 10.3) with NaOH and saturated with NaCl. This mixture was extracted twice with 30 ml each of nitrogen-saturated diethylether, pretreated with semicarbazide HCl. The organic phase was transferred to another test tube and evaporated. The dried residue was dissolved in 200 μl methanol and spotted on silica gel TLC-plates (Merck) in a line (15 cm) under nitrogen. The tubes were washed twice with 200 μl diethylether which was added to the spot. For identification purposes authentic tetrahydronorharmane was spotted separately on the plate.

Thin layer chromatography

The plates were developed in the solvent system chloroform/acetone/diethylamine (volumes, 50 ml + 40 ml + 10 ml). In a previous study, the possibility was excluded that, under the experimental conditions used, various amines and other physiologically occurring substances interfered with tetrahydronorharmane (1). Also dopamine, noradrenaline, and adrenaline have different R_f values in the applied solvent system. The area which ran isographic to authentic tetrahydronorharmane was scraped off. After the addition of 1.5 ml water saturated with NaCl and adjusted to pH 10.3 with sodium hydrogencarbonate the compound was eluted into 6 ml distilled diethylether. An aliquot of 5 ml of the organic phase was transferred to a glass stoppered vial and evaporated to dryness under a stream of nitrogen. Thereafter, the sample was acetylated by a solution of 15 nmol [^{3}H]-acetic anhydride (185 GBq/mmol) in 245 μl distilled benzene and 5 μl pyridine. The samples were incubated for 45 min at 75 °C in a glycerol bath. The organic phase was evaporated to dryness and the residue taken up in 1 ml saturated NaCl solution, pH 10.3, containing Na_2CO_3. The mixture was vortexed and treated with 6 ml distilled diethylether. The organic phase was removed and dried under a stream of nitrogen. The residue was dissolved in 200 μl of methanol. The solution was spotted on silica gel plates together with unlabelled N-acetylated tetrahydronorharmane as carrier. The plate was developed again by chloroform/acetone/diethylamine (volumes, 50 ml + 40 ml + 10 ml). In control experiments it was shown that the carrier did not become labelled by tritium exchange during chromatography. N-acetyl-tetrahydronorharmane was made visible by UV-light at 254 nm. The area which ran isographic with the carrier was scraped off and transferred to a scintillation vial containing 1 ml methanol to elute tetrahydronorharmane from silica gel. Thereafter, the radioactivity of the sample was determined by liquid scintillation spectroscopy (Packard 3380). Samples which contained water instead of platelets were run through the whole procedures for blank values.

The amount of tetrahydronorharmane in the platelets was calculated by an external standard method. Two samples of authentic tetrahydronorharmane (58 and 116 pmol) were acetylated in duplicate as described above. In control experiments, it had been ascertained that the acetylation of tetrahydronorharmane by radiolabelled acetic anhydride proceeds approximately linearly from 5.8 to 1160 pmol tetrahydronorharmane.

For the determination of the recovery of tetrahydronorharmane different amounts of authentic tetrahydronorharmane were added to the platelet samples. These samples were run through the whole procedure.

Results

Tetrahydronorharmane is a physiological constituent of human platelets. Table 1 demonstrates the tetrahydronorharmane content in platelets of 8 healthy subjects. Ingestion of tryptophan or tryptamine obviously did not lead to an increase of tetrahydronorharmane.

The blank values showed good reproducibility and their deviation was low (400 ± 13 Bq; n=6). By the method

Tab. 1. Tetrahydronorharmane (pmol) in human platelets isolated from 10 ml platelet-rich plasma. The control subjects were not on a diet. Four subjects ingested 1 mmol tryptamine hydrochloride after breakfast, lunch and dinner for three consecutive days. 7 subjects received 19.6 mmol *D,L*-tryptophan the evening (10 p.m.) before collection of the blood samples. Values in parentheses are from repeated determinations.

Control	Tryptamine load	Tryptophan load
M.B. ♀ 25.8		
C.F. ♀ 10.3		
G.S. ♂ 7.7		19.2
R.K. ♀ 22.5 (22.2–22.6)		26.4
H.C. ♂ 10.0	9.4	21.0 (17.4–24.4)
H.R. ♂	48.6	38.0
B.W. ♀ 12.8	11.1	22.4 (21.7; 21.9; 23.6)
J.B. ♀ 22.8	9.8	17.5
H.H. ♂ 9.6		14.7 (14.3–15.1)
x̄ ± s 15.2 ± 9.1	19.8	22.7 ± 10.0

applied the smallest amount of endogenous tetrahydronorharmane labelled with [^{3}H]acetic anhydride was easily detectable. Approximately 400 Bq (net amount) is equivalent to 5.8 pmol tetrahydronorharmane.

The extraction efficiency of tetrahydronorharmane (n=5) ranged between 51 and 55%. The tetrahydronorharmane concentrations presented were corrected for recovery. The tetrahydronorharmane content in plasma is not listed in the table since the recovery varied between 23 and 45%. Considering these variations the content of tetrahydronorharmane in 10 ml plasma is in the same order of magnitude as that in the platelets of the respective volume of plasma.

Discussion

Tetrahydronorharmane is formed in vitro by condensation of tryptamine with formaldehyde (9, 10, 11, 12). Both substances are present in the mammalian organism (13, 14). Therefore, it was conceivable that the synthesis of tetrahydronorharmane also occurs in human tissue. In fact *Honecker* et al. (1) ascertained the presence of tetrahydronorharmane not only in rat brain but also in human urine. The identification of the substance was confirmed by chromatography in different solvent systems, cocrystallisation, isotope dilution, and mass spectrometry (1). The amount of 9.3 to 25 pmol tetrahydronorharmane in platelets isolated from 10 ml platelet-rich plasma is low compared with that of serotonin, but is in the same order as the amount of catecholamines (15, 16). On account of the low concentration of tetrahydronorharmane in the platelets a mass spectrum could not be performed. But because an identical methodological procedure was used for tetrahydronorharmane determination in urine and platelets it is certain that the measured compound represents tetrahydronorharmane. It is likely that the storage sites create a concentration

gradient between platelets and plasma for tetrahydronorharmane, which is mainly transported into the platelets by diffusion as described for catecholamines by *DaPrada & Picotti* (16).

In rats tryptophan (735 μmol/kg) and tryptamine (765 μmol/kg) caused a twofold and twentyfold increase of tetrahydronorharmane concentration in the forebrain 90 min after i.p. administration (1).

In humans, however, ingestion of tryptophan (19.6 mmol *D,L*-tryptophan, the evening before the collection of blood) or of tryptamine (1 mmol in the morning, noon, and evening for three consecutive days) did not lead to an increase of tetrahydronorharmane in platelets. The failure of the tryptophan or tryptamine load to induce an increase of the concentration of tetrahydronorharmane in men may be explained by the lower doses of the precursor applied, compared to the experiments with rats.

Since it remains to be elucidated whether tetrahydronorharmane acts by itself or by modulating the effects of biogenic amines, it is not clear whether the amount of tetrahydronorharmane is high enough to exert any function. However, it is conceivable that alterations of the content of tetrahydronorharmane in platelets reflect changes in the brain. Studies to investigate tetrahydronorharmane in platelets of patients with mental disorders are in progress.

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