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LEVELS OF 5-HYDROXYTRYPTOPHOL IN CEREBROSPINAL FLUID FROM ALCOHOLICS DETERMINED BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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Abstract—A quantitative gas chromatographic–mass spectometric method using a deuterated analogue as internal standard was developed for the analysis of 5-hydroxytryptophol in cerebrospinal fluid. The analytical procedure involves the addition of the internal standard and 5-hydroxyindole to the cerebrospinal fluid followed by extraction into chloroform and derivatization with pentafluoropropionic anhydride. 5-Hydroxytryptophol levels in the CSF of male alcoholics and a control group were examined. During ethanol intoxication the mean level, 10.4 ± 4.4 pmoles/ml, was significantly ($P < 0.001$) higher than in the controls, 3.31 ± 0.94 pmoles/ml. The following morning the mean had decreased ($P < 0.01$) to 4.46 ± 1.81 pmoles/ml. The level after 8 days was 4.70 ± 2.47 pmoles/ml, which is lower than during intoxication ($P < 0.01$). The levels found during the recovery from ethanol intoxication was not statistically different from the levels in the control group. These results indicate that serotonin metabolism in the central nervous system is affected by ethanol consumption.

Serotonin (5-hydroxytryptamine, 5HT) is catabolized by the action of monoamine oxidase. The immediate product formed is 5-hydroxyindole acetaldehyde (5HIAld). This compound is rapidly transformed either by oxidation to 5-hydroxyindole acetic acid (5HIAA) or by reduction to 5-hydroxytryptophol (5HTOL). The reduced product 5HTOL, has been identified as a metabolite of exogenously administered 5HT [1] and has also been shown to occur endogenously in the urine and cerebrospinal fluid (CSF) of man [1–3].

Studies of 5HT metabolism have shown that ethanol consumption induces an increased urinary excretion of 5HTOL [4, 5]. This was proposed to be caused by a shift of 5HT metabolism from 5HIAA to 5HTOL, mainly due to an increased $NADH/NAD^+$ ratio in the liver, resulting from ethanol metabolism.

Investigations of 5HIAA levels in CSF during the post-intoxication phase of alcoholics have shown unaltered or decreased levels [6–8]. In delerious states elevated 5HIAA levels have been reported [9]. In this paper we report results of a study of the levels of 5HTOL in the CSF of alcoholics, both during and after ethanol intoxication, as well as those of a control group.

MATERIALS AND METHODS

Chemicals. The 5HTOL was obtained from Sigma Chemical Co. (St. Louis, MO); the 5-hydroxyindole (5HInd) from Schuchardt AG (München, GFR) and the pentafluoropropionic anhydride (PFPA) from Produktkontroll AB (Solna, Sweden). The internal standard, 5-hydroxy-(α, α, β, β-$^2H_4$) tryptophol (5HTOL-$^2H_4$), was synthesized in the laboratory, by a procedure reported elsewhere [10]. All other chemicals used were of analytical grade. Stock solution of 5HTOL, 5HTOL-$^2H_4$ and 5HInd were prepared in 95% ethanol and stored at −18°.

CSF samples. Male alcoholic inpatients at the Alcoholic Clinic, Karolinska Hospital, were investigated. All fulfilled the criteria of dependence according to WHO, 1964 [11]. The age range was 28–76 yr. Apart from alcohol dependence, none had any serious somatic or psychiatric disorders. As far as could be determined the patients had received no prescribed medication prior to arrival at the hospital and were treated by either Amobarbital 0.6 g/day, Oxazepam 60 mg/day or Metylperon 300 mg/day (a butyrophenone drug) in a double-blind manner. No other drugs or alcohol were allowed during the period in hospital and patients were checked by toxicological analyses of urine for drugs and alcohol during the period of CSF sample collection.

In the first group of patients, CSF was collected immediately after admittance (day 1) to hospital between 10.00 a.m. and 5.00 p.m. These patients were still intoxicated. In the second group lumbar puncture was performed the first morning after admittance (day 2) at 8.00 a.m. when patients were abstinent but sober. The third group was investigated at 8.00 a.m. after 8 days in hospital. In the latter two groups patients had received no food during the previous 8 hr.

In the control group, CSF was collected from 10 patients on different neurological investigations. The patients received no prescribed medication and were of mixed sexes (5 males and 5 females). The age

range was 27–70 yr, mean 44. The CSF was withdrawn between 10.00 a.m. and 1.00 p.m.

The lumbar punctures were performed with the patients in a sitting position and 12 ml of CSF was withdrawn. The CSF was shaken, and within 30 min centrifuged at 2000 *g* for 15 min and immediately placed under storage at −20°.

Preparation of CSF samples. Aliquots of 1.0 ml of CSF were pipetted into 15 ml glass stoppered tubes and 53.7 pmoles 5HTOL-$^2H_4$, 13.2 nmoles 5HInd, 400 mg NaCl and 0.10 ml 4 M HCOOH (containing 0.5 mM of ascorbic acid) were added. After addition of 7.0 ml of $CHCl_3$, the samples were shaken for 10 min and centrifuged at 1000 *g* for 5 min. The $CHCl_3$ layers were transferred to clean tubes and evaporated to dryness under a stream of nitrogen.

The residues were treated with 50 μl of 1% (v/v) triethylamine solution in benzene and 50 μl of PFPA at 60° for 15 min. After cooling, the excess reagent was evaporated under a stream of nitrogen. The residues were dissolved in 1.0 ml of benzene and extracted with 0.20 ml of H_2O. The benzene layers were transferred to clean tubes and evaporated to dryness under a stream of nitrogen and redissolved in 10 μl of ethyl acetate. These extracts were analysed by GCMS.

The reproducibility of individual analysis of 5HTOL was determined by analysing eight 1.0 ml aliquots of a pool of CSF. The pool consisted of lumbar CSF obtained from various sources.

Preparation of standard samples. For quantitation purposes standard samples containing 5HTOL (0–17.0 pmoles) were prepared in 1.0 ml of an aqueous solution of: $NaH_2PO_4 \cdot H_2O$ (0.57 mM), $Na_2HPO_4 \cdot 12H_2O$ (0.12 mM), $MgCl_2 \cdot 6\ H_2O$ (0.40 mM), $CaCl_2 \cdot 2\ H_2O$ (0.49 mM), KCl (3.0 mM), NaCl (120 mM), $NaHCO_3$ (25 mM) and human serum albumin (250 mg/l). The samples were prepared for 5HTOL analysis as described above for CSF samples.

Gas chromatography–mass spectrometry. The analysis of the CSF extracts were performed using an LKB 2091 gas chromatograph–mass spectrometer operating in a computer controlled multiple ion detection mode.

The gas chromatographic separations were achieved using a 12 m SE 52 WCOT glass capillary column (I.D. 0.30 mm). Helium was used as a carrier and make-up gas. Splitless injections were carried out using a 'moving needle' device. The gas chromatographic conditions were: injector heater 200°, column temperature 160°, column flow rate ~2 ml/min and make-up gas flow rate ~12 ml/min. Aliquots of 2 μl of the samples were injected and an initial delay of 2 min in opening the valve was used to avoid contamination of the ion source. Under these conditions, the retention time of 5HTOL-$(PFP)_3$ and 5HTOL-$^2H_4$-$(PFP)_3$ was approximately

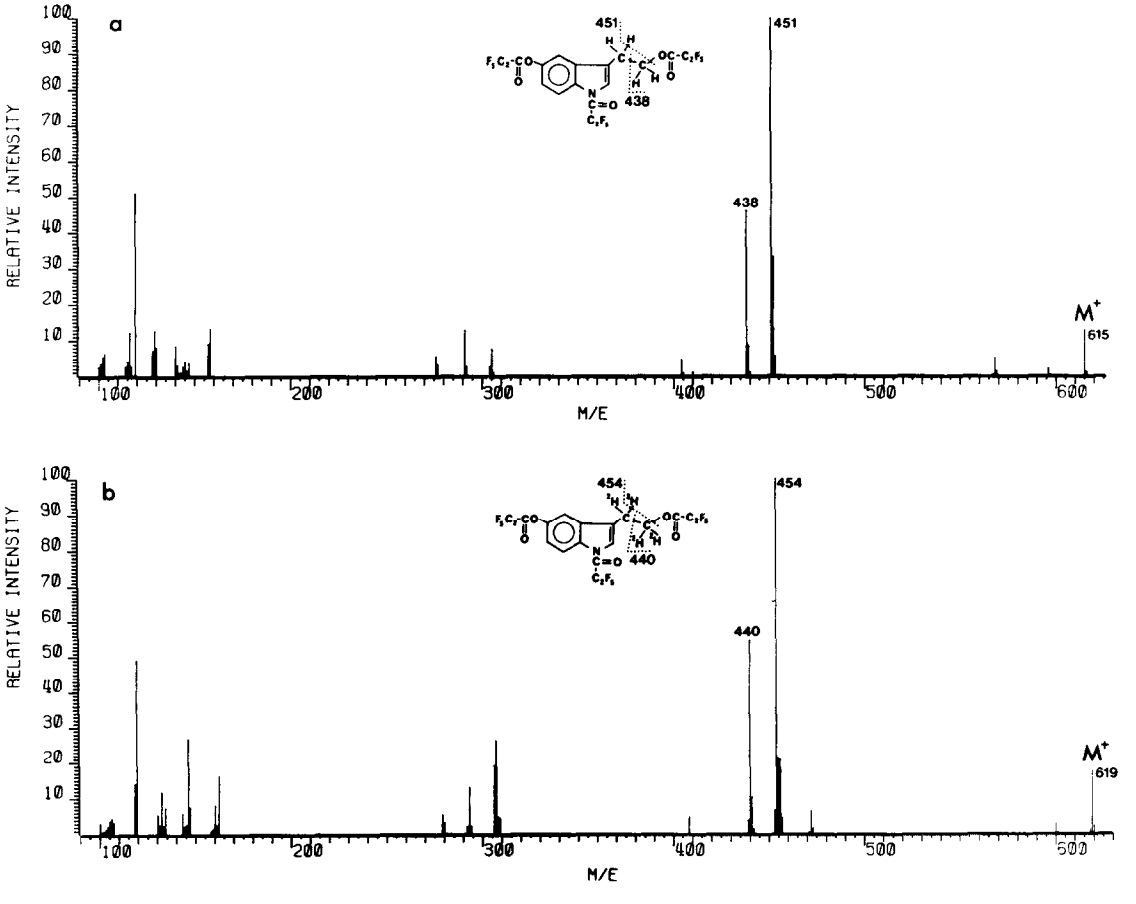

Fig. 1. Electron impact mass spectra of 5HTOL (a) and 5HTOL-$^2H_4$ (b) as PFP derivatives.

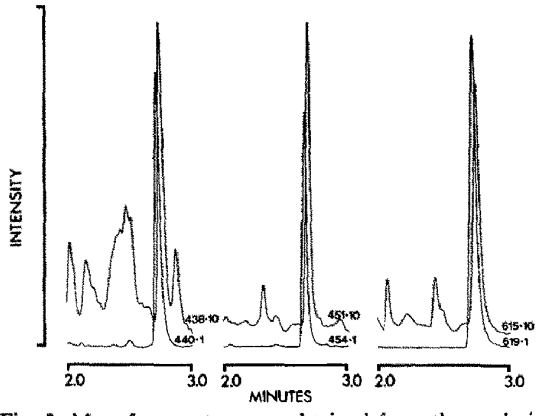

Fig. 2. Mass fragmentograms obtained from the analysis of a CSF extract. The mass numbers and amplifier gain factors are indicated in the figure.

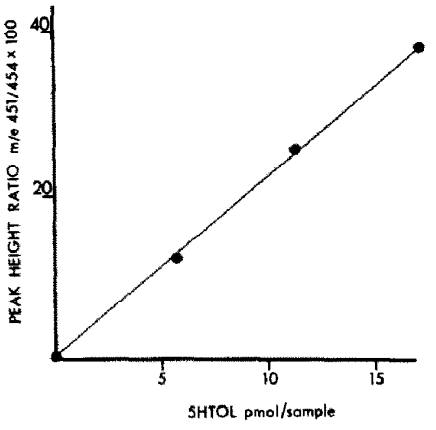

Fig. 3. Calibration curve used for the quantitative determination of 5HTOL.

2.7 min. The mass spectrometric conditions were: separator temperature 230°, ion source temperature 230°, electron energy 50 eV, and trap current 50 μA.

Quantitation. Calibration curves were constructed by plotting the peak height ratios (m/e 438/440, 451/454, 615/619) of the standard samples against the 5HTOL concentration. The 5HTOL levels were then determined from the peak height ratios of each CSF sample by reference to the calibration curve.

RESULTS

Mass spectra of 5HTOL-(PFP)$_3$ and 5HTOL-$^2H_4$-(PFP)$_3$. The electron impact mass spectra of the PFP derivatives of 5HTOL (Fig. 1a) and 5HTOL-$^2H_4$ (Fig. 1b) confirm the presence of three PFP groups per molecule. Both compounds show significant molecular ions $(M)^{+\cdot}$, at m/e 615 and 619, respectively. The base peaks at m/e 451 and 454 are formed in a β-elimation process. In addition, prominent ions formed in a β-cleavage fragmentation process are present at m/e 438 and 440.

Identification of 5HTOL in CSF. The multiple ion detection of 5HTOL was performed by monitoring the ion intensities at m/e 438, 451 and 615 for 5HTOL and m/e 440, 454 and 619 for 5HTOL-$^2H_4$, using separate injection for each ion pair; m/e 438/440, 451/454 and 615/619. The chromatograms obtained from the analysis of CSF extracts (Fig. 2) show that CSF contains a compound possessing both the same retention time and relative ion intensities as authentic 5HTOL.

The determination of the reproducibility of individual 5HTOL analysis (Table 1) showed that approximately the same level was found for all three ion pairs, with an experimental error less than 6 per cent.

Quantitation of 5HTOL in CSF of alcoholics and controls. The quantitation of 5HTOL in the CSF of alcoholics and controls utilized the peak height ratios at m/e 451/454. A typical calibration curve (obtained at m/e 451/454) is shown in Fig. 3. As seen, the curve is a straight line over a concentration range of 0-17.0 pmole/ml of 5HTOL and intercepts the *Y*-axis near the origin.

The analysis of CSF from the control group showed a mean value of 3.31 ± 0.94 pmole/ml (Table 2). No correlation of the 5HTOL level to either sex or age were observed.

The analyses of CSF taken from alcoholics during ethanol intoxication (day 1), revealed significantly higher levels of 5HTOL in comparison with those of the controls (Table 2). The following day (day 2), when the patients were sober, the 5HTOL levels had decreased significantly. The mean level was higher than in the controls but not statistically different. At day 8, the mean level of 5HTOL was similar to the one found at day 2. Further, the levels were significantly lower than during intoxication but not statistically different from the control group.

DISCUSSION

The result obtained using multiple ion detection confirms earlier reports on the presence of 5HTOL

Table 1. Reproducibility of 5HTOL analysis in CSF

	5HTOL*		
	m/e 438/440	m/e 451/454	m/e 615/619
Pooled CSF	6.34 ± 0.25	6.08 ± 0.33	6.01 ± 0.09
Experimental error (% of mean	3.9	5.5	1.5

* The results are expressed in pmoles/ml ± S.D. (N = 8).

Table 2. Levels of 5HTOL in the CSF of alcoholics and controls

Subject	5HTOL*	N
Control group	3.31 ± 0.94	10
Alcoholics, day 1	10.4 ± 4.4†	10
Alcoholics, day 2	4.46 ± 1.81‡	8
Alcoholics, day 8	4.70 ± 2.47‡	7

* The results are expressed in pmole/ml ± S.D.
† Differs from control group ($P < 0.001$).
‡ Differs from alcoholics, day 1 ($P < 0.01$).

in human CSF (Table 1 and Fig. 2). Almost identical levels were found for all ion pairs used, indicating a high specificity of the method. As previously reported [3] considerable losses (90–95 per cent) of 5HTOL occur during the extraction procedure, unless certain precautions are taken. In the earlier study, addition of human serum (0.10 ml/ml CSF) was employed to minimize losses. The present method involves the addition of 5HInd as a carrier which provided a high recovery of 5HTOL.

The level of 5HTOL in the CSF of the control group is in agreement with levels previously reported in patients suffering from various neurological disorders [3]. Comparison with the reported levels of 5HIAA in CSF of healthy volunteers [12], 102–107 pmole/ml, shows that 5HTOL is a minor 5HT metabolite.

The decline of the 5HTOL level in CSF following recovery from the ethanol intoxication suggests that the presence of ethanol is a main factor contributing to the elevated 5HTOL levels. The mean level in the alcoholics was still higher following abstention from alcohol for one week, indicating that other factors may also be involved.

It is widely assumed that the CSF monoamine metabolites originate from CNS. This has previously been used as one approach to the study of monoamine metabolism in brain in connection with various psychiatric disorders and the effect of ethanol intoxication [7, 8, 12]. If the assumption is correct, the elevated levels found in the present study during and after ethanol intoxication may reflect an increased rate of formation of 5HTOL in the CNS. This can be thought of as arising either from an increased 5HT turnover or a shift in product formation from 5HIAA to 5HTOL, or both.

Evidence of an increased 5HT turnover in brain has been reported to occur in animals during long-term ethanol intoxication [13]. Kinetic studies of the enzymes involved in the metabolism of 5HIAld have shown that ethanol, through the action of acetaldehyde, is capable of partly inactivating the pathway leading to the formation of 5HIAA [14]. This inactivation may be the result both of competitive inhibition of aldehyde dehydrogenase by acetaldehyde [15] and of an increased NADH concentration in brain [16]. The inactivation will result in an increased 5HIAld level, inducing a new steady state where the increase of 5HTOL formation equals the loss of aldehyde dehydrogenase activity. Thereby, a shift from 5HIAA to 5HTOL is produced. It therefore seems reasonable to assume that both an increased 5HT turnover and a shift of product formation contributes to elevate 5HTOL levels in CSF during the intoxication.

With regard to the post-intoxication phase, previous studies of 5HIAA levels in CSF have shown unaltered or decreased levels [6, 8]. Other mechanisms than the ones discussed for the intoxication phase must therefore be considered to explain the elevated levels of 5HTOL found after recovery from ethanol intoxication. Both inactivation of aldehyde dehydrogenase and activation of alcohol reductase may be involved. Still another factor to be considered is the transport of 5HTOL from the CSF.

The significance of an increased production of 5HTOL is not known. In animals, 5HTOL has been implicated in the control of sleeping time and temperature regulation but this has not been investigated in man [17–19]. The present study may be interpreted as providing evidence for the presence of acetaldehyde and elevated 5HIAld levels in the CNS during ethanol intoxication. These aldehydes may condense with brain monoamines to form compounds which increase voluntary intake of ethanol in rats [20].

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REFERENCES

1. V. E. Davis, J. L. Cashaw, J. A. Huff and H. Brown, *Proc. Soc. exp. Biol. Med.* **122**, 890 (1966).
2. H. C. Curtius, M. Wolfensberger, U. Redweik, W. Leimbacher, R. A. Maibach and W. Isler, *J. Chromat.* **112**, 523 (1975).
3. S. Takahashi, D. D. Godse, A. Naqvi, J. J. Warsh and H. C. Stancer, *Clin. Chim. Acta.* **84**, 55 (1978).
4. V. E. Davis, H. Brown, J. A. Huff and J. L. Cashaw, *J. Lab. clin. Med.* **69**, 132 (1967).
5. A. Feldstein, H. Hoagland, H. Freeman and O. Williamson, *Life Sci.* **6**, 53 (1967).
6. E. K. Orenberg, V. P. Zarcone, J. F. Renson and J. D. Barchas, *Life Sci.* **19**, 1669 (1976).
7. N. Kato, S. Takahashi, N. Tani, N. Iwase and K. Odani, *Alcoholism: Clinical and Experimental Research* **3:1**, 24 (1979).
8. J. C. Ballenger, F. K. Goodwin, L. F. Major and G. L. Brown, *Arch. Gen. Psychiat.* **36**, 244 (1979).
9. C. M. Banki and M. Vojnik, *J. Neurol. Neuros. Psychiat.* **41**, 420 (1978).
10. T. Hesselgren and O. Beck, *J. labelled Compds. Radiopharmac.*, in press.
11. WHO 1964. World Health Organization Technical Report Series, 273, p. 3 (1964).
12. G. Sedvall, B. Fyrö, B. Gullberg, M. Nybäck, F. Wiesel and B. Wode-Helgodt, *Br. J. Psychiat*, in press.
13. P. J. Griffiths, J. M. Littleton and A. Ortiz, *Br. J. Pharmac.* **50**, 489 (1974).
14. R. J. S. Duncan and T. L. Sourkes, *J. Neurochem.* **22**, 663 (1974).
15. R. J. S. Duncan and K. F. Tipton, *Eur. J. Biochem.* **22**, 538 (1971).
16. J. W. Ridge, *Biochem. J.* **88**, 95 (1963).
17. A. Feldstein, F. H. Chang and J. M. Kurcharski, *Life Sci.* **9**, 323 (1970).
18. R. G. Taborsky, *Experientia* **27**, 929 (1971).
19. I. Barofsky and A. Feldstein, *Experientia* **26**, 990 (1970).
20. C. L. Melchior and R. D. Myers, in *Alcohol and Aldehyde Metabolizing Systems* (Eds. R. G. Thurman, J. R. Williamson, D. R. Drott and B. Chance), Vol 3, pp. 545–554. Academic Press, New York (1977).