Journal of Chromatography, 162 (1979) 433—438
Biomedical Applications

CHROMBIO. 257

Note

Mass fragmentographic determination of 4-amino-3-*p*-chlorophenylbutyric acid (baclofen) in cerebrospinal fluid and serum

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(Received May 11th, 1978)

Baclofen (4-amino-3-*p*-chlorophenylbutyric acid) is a γ-amino-butyric acid (GABA) analogue which, unlike the natural amino acid, is capable of passing the blood-brain barrier. Baclofen is generally assumed to be a GABA agonist but the compound has also been suggested to antagonize the effects of substance P [1].

Clinically baclofen is used in the treatment of multiple sclerosis and other spastic conditions [2]. Recently baclofen was suggested as being beneficial also in the treatment of schizophrenia, preferentially in combination with conventional neuroleptic drugs [3]. Other authors, however, have not been able to confirm this claim [4—7].

For the achievement of an optimal pharmacotherapy with baclofen, an analysis of the relationships between therapeutic effects and drug concentration in the body fluids may be valuable. A gas chromatographic method for the determination of baclofen using electron capture detection has been described [8]. However, for the determination of baclofen concentrations in cerebrospinal fluid (CSF) the latter method is insufficient [9]. Therefore a more specific and sensitive method with high precision is required.

In the present paper we describe a mass fragmentographic method for the determination of baclofen. The method has been applied in a preliminary analysis of drug levels in serum and CSF of baclofen treated patients. The analytical steps are shown in Fig. 1.

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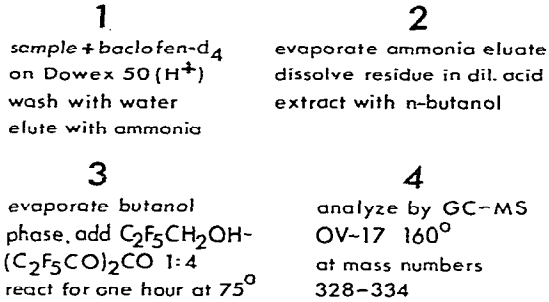

Fig. 1. Scheme of the analytical procedure.

EXPERIMENTAL

Materials

Baclofen-d_4 (2.5 mg) [10] was dissolved in 100 ml of water. An aliquot of this solution was diluted 10 times and 100 μl (250 ng of the compound) were added to all samples as internal standard. Authentic baclofen (Hässle-Ciba-Geigy, Mölndal, Sweden) was dissolved in water. A stock solution containing 1 mg in 100 ml was used. After two ten-fold dilutions the solution was used for the preparation of calibration curves. Standard solutions for CSF and serum contained 0–100 ng and 0—200 ng of baclofen respectively.

Dowex 50W-X4 (200—400 mesh) in the protonated form was treated with an excess of aqueous ammonia, washed with water to neutrality, regenerated with an excess of 4 *M* HCl and finally washed with water to neutral pH. This treatment removed impurities that interfered with the analysis. The pentafluoropropionic anhydride was purchased from Produktkontroll, Stockholm, Sweden, and 2,2,3,3,3-pentafluoropropanol was obtained from Columbia Organic Chemicals, S.C., U.S.A. Other chemicals were of analytical grade and were obtained from standard sources.

Sample preparation

Procedure. The deuterated standard (250 ng) was added to 1 ml of CSF or serum and the samples were mixed (Fig. 1). Columns of Dowex 50 (15 × 5 mm I.D.) were prepared in disposable pipettes plugged with a small amount of glass wool. The volume of the column above the resin corresponded to approximately 2 ml. The columns were washed with two portions of water after which the samples were allowed to flow through with gentle air pressure when required. The effluent and three washings with 2 ml of water were discarded. Baclofen was eluted with 2 ml of 10% ammonia, which was collected in small conical test tubes. The samples were blown to dryness in a stream of nitrogen gas with gentle heat from a hair drier. After evaporation, the samples were redissolved in 1 ml of 10^{-4} *M* HCl and extracted with 2 ml of *n*-butanol. The butanol phases were transferred to conical test tubes and evaporated as above. When the residue was completely dry, 100 μl of a 4:1 mixture of pentafluoropropionic anhydride and pentafluoropropanol was added. The test tubes were closed by glass stoppers and kept at 75° for 1 h. After removal of excess reagent in a desiccator at reduced pressure 25 μl of ethyl acetate containing 2%

pentafluoropropionic anhydride was added. The samples were analysed by gas chromatography—mass spectrometry (GC—MS) as described below.

GC—MS conditions

The analyses were performed on a Finnigan 3200 gas chromatograph—mass spectrometer system equipped with a vacuum diverter and operated in the electron-impact mode. An OV-17 column (1.5 m × 2 mm I.D.) was used for the GC separation. The column temperature was 160—170° with a helium gas flow of about 20 ml per min. The electron energy was set at 50 eV. Other spectrometer parameters were as reported elsewhere [11]. The mass numbers routinely monitored were 328 and 334, which have a relative intensity in the mass spectra of about 40% and correspond to the loss of pentafluoropropionamide from the molecule [10]. There is no loss of label in that process. The base peak in the region 170—500 is 273 but it retains no label. The molecular peak is not discernible.

Peak heights were determined manually and the ratios between non-labelled and labelled compounds were determined. The data from the standard solutions were used for the calculation of the equation of the calibration line. Unknown samples were then interpolated according to this equation using a small desk calculator.

Sampling of CSF and serum

Serum and CSF were collected from psychotic patients by venipuncture and lumbar puncture. Samples were taken before the morning dose after the patients had been treated for 2 and 4 weeks with baclofen (Lioresal®, Hässle-Ciba-Geigy; 10 mg, 3 times daily). Blood and CSF were centrifuged and all samples were frozen to −20° within 1 h.

Serum was also collected from a healthy, male volunteer who received 20 mg baclofen in a single oral dose. The compound was given at 8 a.m. with the subject fasting. Venous blood samples were removed as indicated in Table I.

RESULTS AND DISCUSSION

From preliminary experiments, using the more easily available baclofen-d_2 as internal standard, it was found that the mass number 332 (which must be used due to the ^{37}Cl present in non-labelled baclofen) was unsuitable. Compounds present in serum and CSF interfered at this mass number and could not be separated chromatographically without excessive prolongation of the retention time.

Therefore we decided to use a d_4-standard which was found to be more satisfactory. The calibration curves invariably show good linearity with a correlation coefficient usually higher than 0.99. When baclofen was added to samples of plasma and CSF a satisfactory recovery and experimental error was obtained (Table II). Preliminary investigations on serum and CSF sampled from patients treated with baclofen indicate that the levels are of the magnitude added to the samples shown in this table. Analysis of CSF and serum from untreated patients gave blank values of ± 0.5 ng/ml and ± 2 ng/ml, respectively.

Typical mass fragmentograms from serum and CSF of baclofen treated sub-

TABLE I

AMOUNTS OF BACLOFEN FOUND IN SERUM FROM A HEALTHY SUBJECT RECEIVING A SINGLE ORAL DOSE OF BACLOFEN 20 mg

The deviation from mean is the difference between the mean and either of the determined values.

Time from start of experiment (h)	Baclofen (ng/ml)	Deviation from mean (ng)
0	-1.9	0.4
1	3.1×10^2	0.2×10^2
2	260	2
3	197	3
4	150	2
6	92	4
8	68	2
12	36.2	0.6
24	7.7	0.1

TABLE II

STANDARD DEVIATION AND RECOVERY OF ASSAY OF BACLOFEN ADDED TO PLASMA AND CSF

Sample (n=5)	Amount added (ng/ml)	Found (ng/ml)		Found as % of added
		Mean	S.D.	
CSF	6.7	6.7	0.7	100
	13.3	13.2	0.9	99
Plasma	33.5	32.5	2.1	97
	100	96.9	3.2	97

jects are shown in Fig. 2. It can be seen from the mass fragmentograms that some interfering compounds are present both in serum and CSF. For serum, it was necessary to include a waiting period of a few minutes in order to elute a slowly moving component from the column. During that period the flow was diverted to the separator pump by the use of the vacuum diverter. A part of this peak can be seen in Fig. 2. The waiting period, however, did not cause an undue prolongation of the analysis time.

To determine the applicability and precision of the procedure for determination of baclofen levels in serum, the compound was given in a single dose to a healthy volunteer. Blood samples were removed as indicated in Table I and

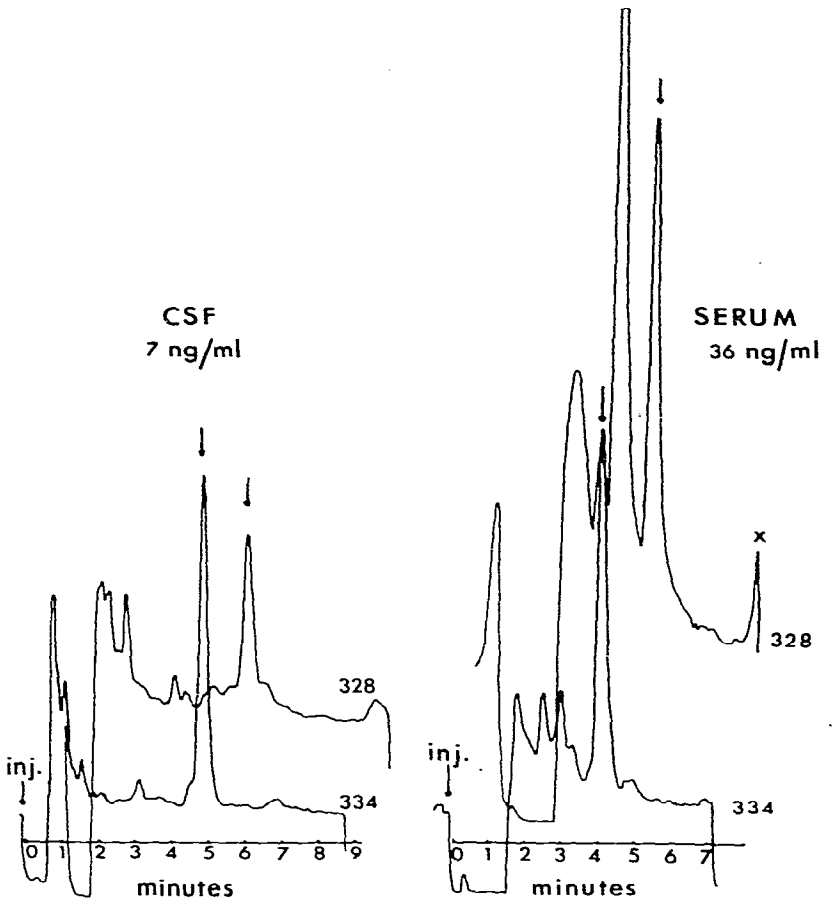

Fig. 2. Typical mass fragmentograms of CSF and serum extracts from subjects receiving baclofen. Arrow indicates retention time of baclofen. x is a slowly moving compound possibly interfering with the analysis.

serum was analysed in duplicates. The mean percentage deviation was 2.4 ± 1.6% and the correlation between the two sets of analyses was 0.995. The fact that there was a negative amount of compound at zero time may be due to a compound giving a small contribution at mass number 334, thus making the ratio 328:334 smaller than for the pure standard.

Being a 4-aminocarboxylic acid, there is a possibility of baclofen cyclizing to give the corresponding lactam. However, solutions of baclofen in water, kept at 4° for three months, did not show any change in their concentration. Therefore lactamization should be very slow, at least in water solutions at pH 7.

In summary, the method described in this paper for the determination of baclofen in serum and in CSF showed a high reproducibility and sensitivity. In CSF baclofen could be determined in concentrations down to about 5 ng/ml. In preliminary experiments the drug concentration in CSF from baclofen treated patients has been shown to be above this concentration. As compared to the previously described GC method [8] the present method has an approximately 4-fold increase in sensitivity.

ACKNOWLEDGEMENTS

The present study was supported by grants from the Swedish Medical Research Council (No. 14X-03560), National Institutes of Health, Bethesda, Maryland, U.S.A. (MH 27254-02), Hässle-Ciba-Geigy, Mölndal, Sweden, Hoffmann-La Roche, Basle, Switzerland, Magnus Bergvalls Stiftelse and Karolinska Institutet.

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