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Note

Enantioselective high-performance liquid chromatographic determination of baclofen after derivatization with a chiral adduct of *o*-phthaldialdehyde

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Baclofen (4-amino-3-*p*-chlorophenylbutyric acid), a skeletal muscle relaxant used in the treatment of spastic disorders, is administered clinically as a racemic mixture. In animals, *R*(−)-baclofen (Fig. 1) is the more potent isomer responsible for the $GABA_B$-mimetic action [1–5]. *S*(+)-Baclofen interferes with the binding of *R*(−)-baclofen, and has been proposed to act as an antagonist at the $GABA_B$-receptors [6,7]. Pharmacokinetic properties of the separate enantiomers have not yet been described. To be able to examine the optical purity of baclofen preparations, a reliable analytical method is required, for pharmacokinetic purposes to be extended to measurements in biological fluids.

Stereoselective analysis of a mixture of enantiomers is difficult. In high-performance liquid chromatography (HPLC), separation is possible through the use of chiral eluents or chiral stationary phases or through derivatization with chiral reagents [8,9]. For optical resolution of the baclofen enantiomers for preparative purposes, Weatherby et al. [10] used a chiral mobile phase. This paper describes the first results of a modification of our recently developed assay for racemic baclofen, based on derivatization with *o*-phthaldialdehyde (OPA) in the presence of thiol compounds [11]. The optically inactive thioglycolic acid was substituted for N-acetyl-L-cysteine (NAC), a method originally developed for the optical resolution of amino acids [12,13].

EXPERIMENTAL

Chemicals

o-Phthaldialdehyde and N-acetyl-L-cysteine were obtained from Merck (Darmstadt, F.R.G.) and *R,S*-, *R*(−)- and *S*(+)-baclofen were a gift from Ciba-

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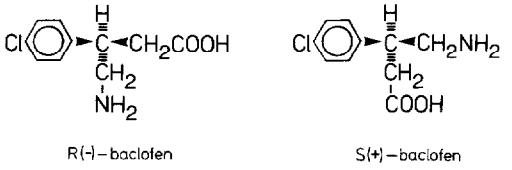

Fig. 1. Enantiomers of baclofen.

Geigy (Basle, Switzerland). Solutions were prepared in distilled water. All glassware was rinsed with distilled water prior to use. All other chemicals were of analytical-reagent grade and were used without further purification.

Chromatographic system

The chromatographic system was similar to that described previously [11]: a reversed-phase system with on-column concentration and fluorimetric detection (excitation at 340 nm, emission at 460 nm). The only differences were the analytical column (Chrompack Cp-Spher C_8, 25 cm; Cat. No. 28310) and solvent B [43–50% (v/v) methanol, 2.5% (v/v) tetrahydrofuran and 54.5–47.5% (v/v) phosphate buffer (pH 8.5)].

Pre-column derivatization procedure

For the preparation of the OPA–NAC reagent, boric acid (0.62 g) and NAC (1.12 g) were dissolved in 20 ml of water and adjusted to pH 10.4 with sodium hydroxide. OPA (250 mg) dissolved in 3 ml of methanol was added and diluted with water to a final volume of 25 ml. This solution was kept at 4 °C and prepared freshly every week.

To 0.9 ml of a sample solution of baclofen containing 5–500 ng was added 0.2 ml of OPA–NAC reagent and the mixture was allowed to stand for 25 min at 80 °C. The solution was then cooled in ice for 1 min and 25 μl of 60 m*M* sodium acetate solution were added. After mixing, 1.0 ml was taken for direct injection into the HPLC system.

RESULTS AND DISCUSSION

Fig. 2 shows chromatograms of the OPA–NAC derivatives of *R*(−)- and *S*(+)-baclofen. A blank run did not show interfering substances (Fig. 2a). The detection limit is approximately 2 ng (Fig. 2c). The fluorescent derivatives of *R*(−)- and *S*(+)-baclofen have capacity ratios of 2.7 and 3.1, respectively, and a resolution of 1.0. The calibration graphs for both derivatives showed good linearity between peak heights and concentrations in the range 20–200 ng per injection ($r^2 > 0.99$). The precision of the determination was measured for three different concentrations of each enantiomer (35, 90 and 205 ng/ml) ($n = 4$) and the coefficients of variation were less than 4%.

Derivatization of baclofen with the OPA–NAC reagent was not as simple and rapid as described for a series of amino acids [12,13]. If the same reaction conditions were chosen as for the amino acids, following injection of racemic baclofen

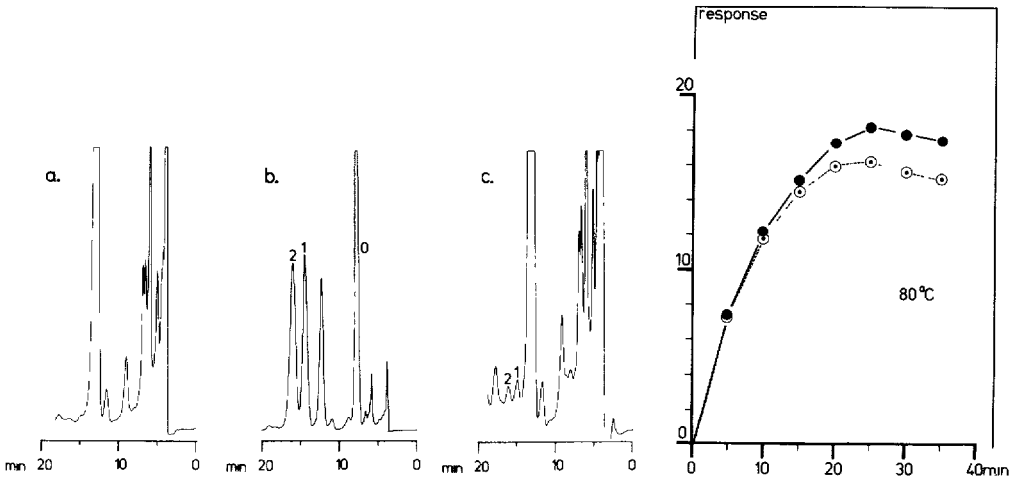

Fig. 2. Chromatograms of OPA–NAC derivatives of $R(-)$- and $S(+)$-baclofen. The injected sample contained (a) 0 ng (blank), (b) 410 ng and (c) 4.0 ng of R,S-baclofen. Peaks: 1 = $R(-)$-baclofen; 2 = $S(+)$-baclofen; 0 = unresolved reaction product.

Fig. 3. Example of the OPA–NAC derivatization with baclofen as a function of reaction time at 80 °C. The injected sample contained 40 ng of R,S-baclofen; the fluorescence was measured as peak height. ●, $R(-)$-Baclofen; ○, $S(+)$-baclofen.

three peaks were observed on the chromatogram that were not present in the blank sample. Under these conditions (3 min at room temperature), peak 0 (Fig. 2b) had the highest fluorescent response, its stability being comparable to that of the previously described OPA–NAC primary amine derivatives, i.e., a decomposition half-life of about 3 h at room temperature [12]. The other two components on the chromatogram, corresponding to derivatives of R-(−)- and S-(+)-baclofen (Fig. 2, peaks 1 and 2) gave a maximum response beyond a reaction time of 7 h at room temperature. At 80 °C this time could be reduced to 25 min (Fig. 3), with reproducible results.

The reaction mechanism is not clearly understood. An explanation could be the formation of 1,3-dithio-substituted isoindoles, due to autoxidation [14]. As all OPA-derived isoindoles have limited stability depending on parameters not yet fully known, the reaction time and temperature should be kept constant [15,16].

Additional studies in biological fluids are currently being carried out.

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