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Short communication

Determination of dopaminergic prodrugs by high-performance liquid chromatography followed by post-column ion-pair extraction

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Abstract

One possibility to optimize the therapeutic application of dopaminergic compounds with a catechol function is the reversible protection of this moiety using a prodrug approach. Important features in this respect are a proper chemical stability in the gastrointestinal tract, an adequate release rate after arrival in the blood stream or the possibility to cross the blood–brain barrier. A HPLC method was developed to measure the hydrolysis of prodrugs of dopamine and epinine directly. The method is based on reversed-phase separation followed by post-column ion-pair extraction with a fluorescent counter-ion. The separation of di-isobutyryl esters of dopamine and epinine is obtained within 10 min while the more hydrophobic dopaminergic esters, di-benzoyl and di-pivaloyl dopamine, are retained for 30 min. The precision of the assay measuring 160 ng dibudop and 100 ng ibopamine was 1.2 and 1.0%, respectively. The detection limit of all prodrugs tested was approximately 10 ng.

Keywords: Dopaminergic compounds

1. Introduction

The catecholamine dopamine has proven to be of therapeutic value for central as well as peripheral disorders. However, dopamine cannot cross the blood–brain barrier, the peripheral effects are short living and the oral bioavailability is poor. To overcome these limitations novel dopamine receptor agonists as well as prodrugs have been developed. Except for kinetic purposes, modification of dopamine appeared to be of interest to obtain receptor and/or organ selectivity [1].

The prodrug approach involves the reversible protection of functional groups in the particular agent. In the case of dopamine, both the catechol- and amino-group have been protected successfully using different pro-moieties [1–3]. Screening the rate of hydrolysis of new dopaminergic drugs in body fluids like plasma, in gastric and intestinal contents as well as in organ homogenates seems a suitable

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approach to obtain a first impression on the cleavability of the developed compounds [2–4]. To date, the measurement of catechol protected prodrugs of dopaminergic compounds in-vitro is commonly conducted by measuring the generation of the parent drug instead of determining the disappearance of the prodrug. Of note, the interpretation of the rate of parent drug generation can be corrupted by chemical instability and/or enzymatic conversion of the drug.

Therefore, we adapted an assay method to measure catechol protected dopaminergic compounds directly. This assay is based on reversed-phase high-performance liquid chromatography followed by a post column ion-pair extraction with a fluorescent counter ion [5–7].

2. Material and methods

2.1. Chemicals

The di-pivaloyl, di-benzoyl and di-isobutyryl (di-budop) ester of dopamine were synthesized according to Borgman et al. [8]. The purity was checked with NMR, IR and melting point.

The di-isobutyryl ester of epinine (ibopamine) and the di-phosphate esters of dopamine and epinine were kindly provided by the Zambon group (Milan, Italy).

1,4-Dioxane, NaH_2PO_4 and orthophosphoric acid (35%) of pro-analysis quality were obtained from Merck (Darmstadt, Germany). 1,2-Dichloroethane of analytical reagent quality was purchased from Lab-Scan (Dublin, Ireland). 9,10-Dimethoxyanthracene-2-sulphonate (DAS), obtained from Fluka Chemie (Buchs, Switserland), was subjected to Soxhlet extraction with dichloroethane before use. 1-Heptane-sulfonic acid sodium salt 98% was obtained from Janssen Chimica (Beerse, Belgium).

2.2. Apparatus

For the HPLC post-column ion-pair extraction method, a Jasco 880 PU pump (Tokyo, Japan) was used for the delivery of the mobile phase and a similar pump was used for the post-column delivery of the organic extractant. The sample was injected with an Jasco 851-AS autosampler (Jasco Model 851-AS). For post-column extraction, the organic solvent was introduced by a stainless steel low-dead-volume-T-piece. After the extraction in a stainless steel capillary coil (1 m×0.25 mm I.D.) with a coil diameter of 9 mm, the organic layer was separated from the aqueous HPLC eluent by a laboratory-made phase separator and delivered to a fluorimetric detector (Jasco Model 820 EP). The required organic flow through the detector was obtained by adjusting the backpressure in the phase separator with a SGE BMVC-1 micro-control needle valve BMVC-1 (Ringwood, Australia). The chromatograms were analyzed with Jones Chromatography JCL6000 HPLC peak integration software (Littleton, CO, USA).

High speed centrifugation was performed in a MSE micro centaur centrifuge (UK).

2.3. Bioanalytical method

The analysis was essentially performed according to Paanakker [7]. Briefly, the HPLC eluent (flow-rate 0.5 ml/min) consisted of 35% dioxane and 65% buffer, containing 0.11 m*M* DAS (9,10-dimethoxy-anthracene-2-sulphonate), 0.11 m*M* HSA (1-heptane-sulfonic acid) and 0.1 *M* NaH_2PO_4 adjusted to pH 3.0 with orthophosphoric acid. The separation was performed on a Millipore Waters Nova-Pak C_{18} column (15 cm×3.9 mm I.D.) guarded by a Millipore Waters μBondapak C_{18} Guard-Pak (Milford, MA, USA).

For post-column extraction, the organic solvent dichloroethane was introduced with a flow-rate of 1 ml/min. The fluorescence was detected at 380-nm excitation and 452-nm emission wavelength.

2.4. Precision of the method

The precision of the method was established for the prodrugs, dibudop and ibopamine and is expressed in the mean intra- and inter-assay coefficients of variation (C.V.). The area under the curve of three standard amounts dibudop (20, 80 and 160 ng) and ibopamine (20, 50 and 100 ng) were analyzed five times on three consecutive days.

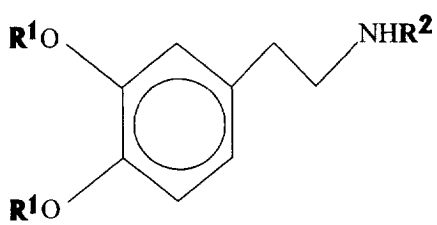
2.5. Prodrug hydrolysis experiments in vitro

The hydrolysis of the prodrugs in plasma was studied at 37°C. Of the drug, 100 μg was added to 1 ml human plasma–phosphate buffer (pH 7.4) (50:50, v/v). After different time intervals, the incubate was diluted ten times with dioxane to stop the reaction and to precipitate the protein. After high speed centrifugation for 0.5 min, 20 μl of the sample was injected on the HPLC. As a control, the drugs were incubated in 100% phosphate buffer (pH 7.4) at 37°C.

3. Results

Fig. 1 shows the chemical structures of the prodrugs of dopamine and epinine used in this study. Representative chromatograms of dibudop and di-pivaloyl dopamine are shown in Fig. 2.

The capacity factor on the column was 3.3, 3.4, 10.9 and 11.7 for ibopamine, dibudop, di-pivaloyl dopamine and di-benzoyl dopamine, respectively. In practice at the indicated flow-rates, the separation of the di-isobutyryl esters of dopamine and epinine was obtained within 10 min, while the more hydrophobic dopaminergic esters, di-benzoyl and di-pivaloyl dopamine were retained for about 30 min. The present method appeared not to be applicable for the analysis of the highly hydrophilic di-phosphate esters of dopamine and epinine.



	R^1	R^2
dibudop:	$(CH_3)_2CHCO$	H
di-pivaloyl dopamine:	$(CH_3)_3CCO$	H
di-benzoyl dopamine:	C_6H_5CO	H
di-phosphate dopamine:	H_2PO_4	H
ibopamine:	$(CH_3)_2CHCO$	CH_3
di-phosphate epinine:	H_2PO_4	CH_3

Fig. 1. Chemical structures of dopamine and epinine prodrugs.

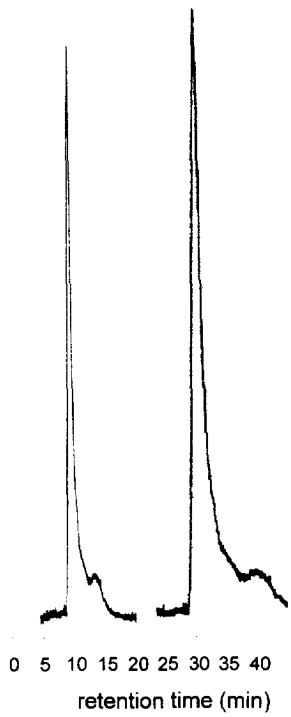


Fig. 2. Representative chromatograms of dibudop and di-pivaloyl dopamine.

The precision of the assay was established for the prodrugs dibudop and ibopamine. The intra-assay coefficient of variation of the analysis varied between 1.2% (at an amount of 160 ng) and 13.6% (at 20 ng) for dibudop and between 1.0% (at 100 ng) and 6.2% (at 20 ng) for ibopamine. The inter-assay coefficient of variation varied to a comparable degree with 1.2% (at an amount of 160 ng) and 8.3% (at 20 ng) for dibudop and 1.1% (at 100 ng) and 5.4% (at 20 ng) for ibopamine. The linear regression was calculated from the peak-areas (y) versus injected amount in nanograms (x).

For dibudop, the calibration line was represented by the equation:

$$y = (4.2E + 3)x + (15.1E + 3)\, r$$

$= 0.9995$ for assayed amounts between 20 and 160 ng.

For ibopamine this was:

$$y = (11.6E + 3)x + (5.4E + 3);\, r$$

$= 0.9996$ for assayed amounts between 20 and 100 ng.

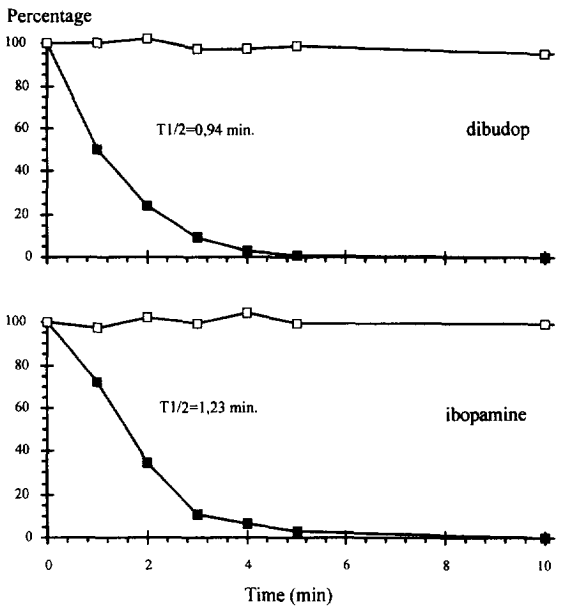


Fig. 3. Rate of in vitro hydrolysis of dibudop and ibopamine in human blood plasma (■) and phosphate buffer, pH 7.4 (□) at 37°C (in duplicate).

The detection limit was arbitrarily defined as the signal noise ratio of four. The analysis appeared to be somewhat more sensitive for ibopamine and di-benzoyl dopamine compared to dibudop and di-pivaloyl dopamine with detection limits of respectively 5 and 10 ng.

Incubation studies at 37°C showed that dibudop and ibopamine were both stable in phosphate buffer for at least 120 min. In human plasma however, a rapid hydrolysis of both prodrugs was observed with half-lives of 0.94 and 1.2 min for dibudop and ibopamine, respectively (Fig. 3).

4. Discussion

Reversed-phase high-performance liquid chromatography (RP–HPLC) followed by post column ion-pair extraction with a fluorescent counter ion combines the selectivity of RP–HPLC and the sensitivity of fluorimetric detection [5–7,9]. The method was initially developed for the detection of tertiary amines and quartenary ammonium compounds. However, as indicated previously [9,10], it is also applicable for analysis of primary and secondary amines. In this study, we adapted the method to detect catechol protected prodrugs of dopamine and epinine containing primary and secondary amino-groups. With the chosen composition of the eluent, there was only a slight difference in column retention between the di-isobutyryl ester of dopamine and epinine. In contrast, after the introduction of a more hydrophobic pro-moiety, such as di-benzoyl or di-pivaloyl, the retention clearly changed.

The sensitivity of the method could be increased by reduction of the post-column organic flow. At the same time however, a reduction of the flow reduced the precision. Like most studies using post-column extraction [5–7,9], the chromatographic peaks in the present study were rather broad and asymmetrical in the lower detection range. In spite of this, the correlation between the amount of prodrug and peak-area was accurate enough to allow a detection down to 10 ng of prodrug.

The limitation of the present method is evident, realizing the inability to measure the di-phosphate esters of dopamine and epinine. These prodrugs are highly hydrophilic and therefore poorly extracted into the organic layer. However, analysis of more hydrophilic prodrugs might be possible choosing a less lipophilic organic solvent for extraction.

Dibudop and ibopamine are prodrugs designed to pass the gastro-intestinal tract intact and subsequently to be rapidly converted into the active parent drug after arrival in the bloodstream [1]. In this study we showed that, while being stable in phosphate buffer (pH 7.4), the two prodrugs were indeed rapidly hydrolyzed in plasma. This very rapid hydrolysis of dibudop and ibopamine may largely hinder the analysis of the in-vivo plasma kinetics of these two prodrugs.

We conclude that the method presented here allows a detailed study of hydrolysis rates for catechol protected dopaminergic compounds in-vitro. Very likely, the method can also be used for the detection of prodrugs of other catecholamines like adrenaline and nor-adrenaline.

Acknowledgments

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