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Note

Determination of 4-(2-di-*n*-propylaminoethyl)-7-hydroxy-2-(3H)-indolone and N-[2'-hydroxy-5'-(N,N-di-*n*-propylaminoethylphenyl)] methane sulfonamide in plasma by high-performance liquid chromatography with electrochemical detection

JAMES E. SWAGZDIS, RALPH GIFFORD and BRUCE A. MICO*

Biochemistry Section, Department of Drug Metabolism, Smith Kline and French Laboratories, P.O. Box 7929, 1500 Spring Garden Street, Philadelphia, PA 19101 (U.S.A.)

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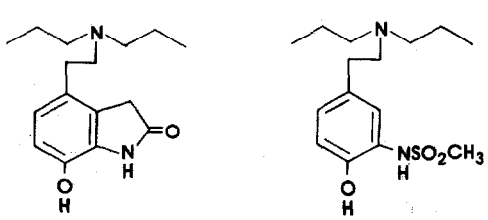
4-(2-Di-*n*-propylaminoethyl)-7-hydroxy-2-(3H)-indolone (SK&F 89124) (Fig. 1, I) and N-[2'-hydroxy-5'-(N,N-di-*n*-propylaminoethylphenyl)] methane sulfonamide (SK&F 85738) (Fig. 1, II) are novel, potent and selective dopamine$_2$ agonists currently under evaluation as potential antianginal and antihypertensive drugs. In preliminary in vivo animal studies, these compounds were found to be peripherally acting dopaminergic agents which decrease neurotransmission and produce hypotension with concomitant bradycardia [1, 2]. Since the pharmacokinetics of cardiovascular drugs have a dramatic influence on their therapeutic utility [3], we have examined the pharmacokinetics of I, II and related compounds in rats and dogs in order to select the dopamine$_2$ agonist with the greatest potential for desirable pharmacokinetics in humans. We report here a method for the determination of I and II in rat, dog and human plasma. The method involves sample preparation with liquid—solid



I II

Fig. 1. Chemical structures of I and II.

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extraction, reversed-phase high-performance liquid chromatography (HPLC) with an ion-pairing agent and electrochemical detection.

EXPERIMENTAL

Reagents

4-(2-Di-*n*-propylaminoethyl)-7-hydroxy-2-(3H)-indolone hydrochloride (Lot No. GG-10983-46A) and N-[2′-hydroxy-5′-(N,N-di-*n*-propylaminoethyl-phenyl)] methane sulfonamide fumarate (2:1), hydrate (Lot No. MSS-8857-276) were obtained from the Department of Medicinal Chemistry, Smith Kline and French Labs. (Philadelphia, PA, U.S.A.). Ammonium formate and formic acid were obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Ethylenediaminetetraacetic acid (EDTA), disodium salt, was purchased from Matheson, Coleman and Bell (Cincinnati, OH, U.S.A.) and sodium octyl sulfate was purchased from Bioanalytical Systems (West Lafayette, IN, U.S.A.). Acetonitrile and methanol, HPLC grade, and octadecyl, 3-ml LD solid-phase extraction columns were obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.).

Chromatographic and data systems

The chromatographic system consisted of a Beckman Model 110A pump equipped with a WISP Model 710B, and a Bioanalytical Systems Model LC-4B electrochemical detector. A glassy carbon working electrode with an Ag/AgCl reference electrode was used at a potential of +0.74 V in the oxidative mode with a range of 1.0 nA. The detector response as a function of oxidation potential was measured for each compound (hydrodynamic voltammogram). The detector response of I is at a plateau at +0.74 V, while the detector response of II is clearly in a linear range at this voltage. A Beckman Ultrasphere ODS 5-μm reversed-phase column (250 × 4.6 mm) was used. The mobile phase was acetonitrile—0.07 *M* ammonium formate buffer, pH 3.8 (20:80, v/v), containing 0.3% (w/v) EDTA and 0.005% (w/v) sodium octyl sulfate. The flow-rate was 1.0 ml/min and the column temperature was 26°C. Chromatographic data were collected in real time using the Beckman/CIS CALS data system; peak height ratios were calculated for each chromatogram after a baseline was defined by inspection.

Procedures

Commercially available solid-phase extraction columns were conditioned immediately before use with three column volumes of methanol followed by three column volumes of water. The appropriate internal standard, 200 ng in 100 μl water, was added to 1 ml of plasma standard or sample. The sample was then transferred and aspirated onto the extraction cartridge. The extraction column was washed with 10 ml of water, followed by 10 ml of 0.07 *M* ammonium formate buffer, pH 3.8. The compounds were then eluted with 4.5 ml of mobile phase. The eluate was diluted to a final volume of 5.0 ml; 50 μl of this eluate were injected onto the column.

The concentrations of I and II in plasma samples were quantitated by comparison of the peak height ratio drug to internal standard of the sample with those on a calibration curve obtained from spiked plasma standards. Each com-

pound was used as the internal standard for the quantitation of the other compound. Calibration curves were prepared by dissolving drug and internal standard in water. Appropriate amounts of I and II were added to plasma to provide a standard curve concentration range of 12.5—500 ng/ml (n=7). Peak height ratios were plotted versus drug concentration. The slope and intercept of the standard curve were estimated by weighted least-squares non-linear regression [4, 5]. The within-day and between-day precision and accuracy of these methods were estimated by performing replicate analyses (n=5) of spiked plasma samples at 25, 250 and 450 ng/ml on five different days. The within-day precision is the mean of the daily coefficients of variation at each concentration. The between-day precision was assessed by determining the coefficient of variation of the daily means at each concentration. The daily accuracy for each concentration and each day of validation is the ratio of the measured to the theoretical concentration. The overall accuracy of these methods was assessed by determining the mean daily accuracy at each concentration.

The recovery of drug from plasma was estimated by comparison of the peak heights of sets of extracted plasma samples with those of unextracted spiked water samples (25 and 450 ng/ml) diluted with mobile phase. The stability of I and II in frozen plasma (−80°C) was assessed by comparing the measured drug concentration after 0, 15, and 30 days of storage. Means, standard deviations and coefficients of variation were calculated using the subroutines available from the RS/1 data analysis software [6].

RESULTS AND DISCUSSION

Electrochemical oxidation was initially examined as a method for HPLC detection because of the chemical similarity of I and II to acetaminophen [7—9]. Hydrodynamic voltammograms of each compound indicate that I and II are susceptible to oxidation under moderate conditions. The detector response to I is at a plateau at +0.74 V with increasing oxidation potential while II was in the linear range of detector response with increasing oxidation potential at +0.74 V. Although the detector response of II is not ideal at this potential and is a possible source of error due to changes in peak height ratios with changing oxidation potential, validation data and experience indicate that the oxidation potential is stable under these conditions. To guard against possible errors due to changes in oxidation potential during sample analysis, we routinely used replicate injections of the extracted samples from the standard curve at the beginning, middle and end of a set of samples. With over 500 samples analyzed with this procedure, variation in oxidation potential has not been a problem to date. An oxidation potential of +0.74 was selected since it gave a good compromise between sensitivity and background noise.

Representative chromatograms of plasma from beagle dogs spiked with I and II are shown in Fig. 2B and C. Fig. 2D is a chromatogram of an actual determination of I concentration. The chromatographic conditions utilized yield baseline separation of I and II. Under the above conditions, the retention times of I and II were 6.0 and 9.5 min, respectively. The retention times of these compounds were very sensitive to the acetonitrile concentration and could be altered easily with small changes in the mobile phase composition. As

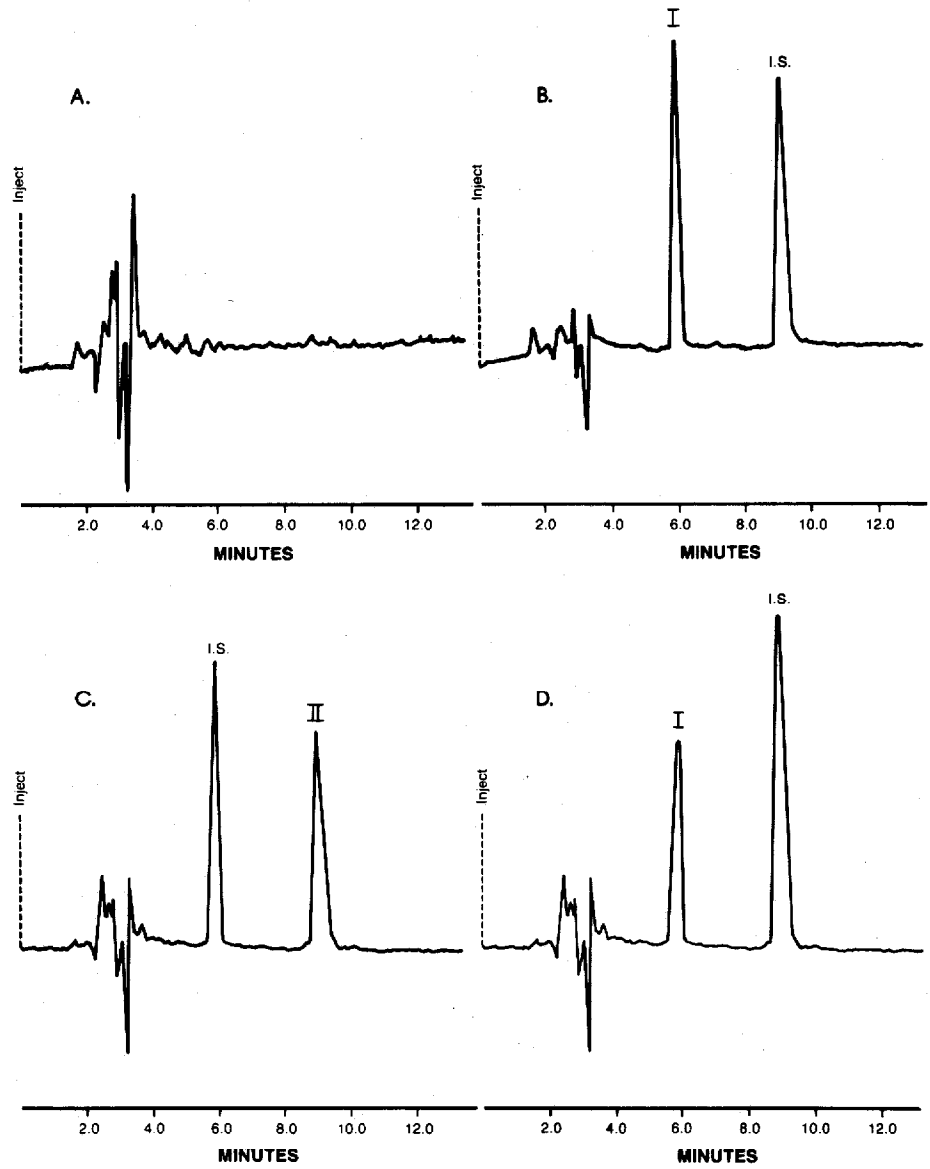

Fig. 2. HPLC—ED profiles of: (A) blank plasma; (B) plasma spiked with 250 ng/ml I and 200 ng/ml II; (C) plasma spiked with 200 ng/ml I and 175 ng/ml II; (D) determination of I in beagle plasma. See text for chromatographic conditions. I.S. = Internal standard.

shown in Fig. 2A, control samples of the plasma from dogs (n=25) show no interfering peaks. Control plasma samples from male Sprague—Dawley rats and humans were also free of interfering peaks. These chromatograms demonstrate that I and II can be used as internal standard for each other. A plasma concentration of 2 ng/ml is detectable (signal-to-noise ratio =4) and a concentration of 5 ng/ml of both I and II can be quantitated.

For each compound, the standard curves were linear over the range 12.5—500 ng/ml. Standard curves analyzed by unweighted linear regression yielded

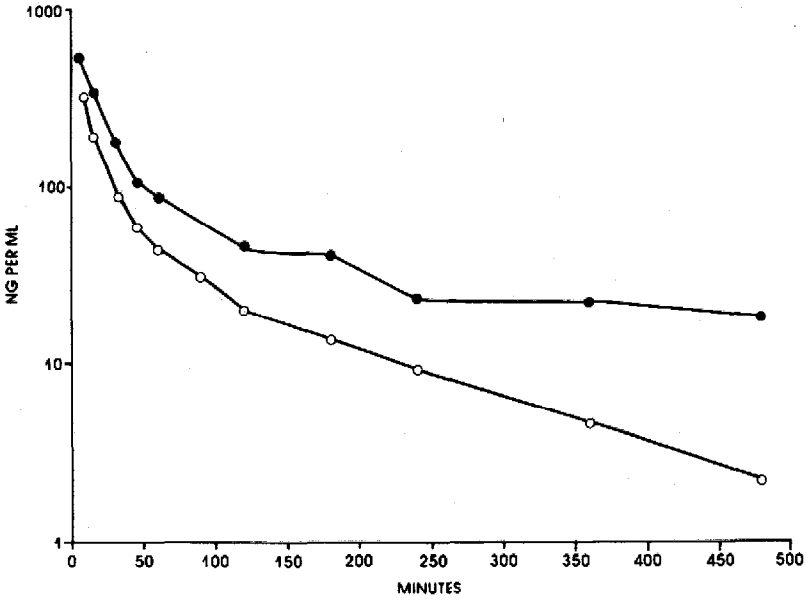

Fig. 3. Plasma concentration versus time profiles of I (○) and II (●) in the pentobarbital-anesthetized beagle dog after a 1 mg/kg intravenous bolus dose.

inferior and more variable results compared with analysis with weighted regression. Weights of $1/y$, $1/y^2$, and extended least squares yielded essentially equivalent results with $1/y^2$ being somewhat better. The recovery from plasma with both I and II is essentially 100%. Both compounds are stable in plasma at −80°C for at least 30 days.

With I as the analyte and II as the internal standard, the within-day precisions (the mean of the daily coefficients of variation) at 25, 250 and 450 ng/ml were 5.6, 3.4 and 3.3%, respectively. The mean accuracies at these concentrations were 101.6, 101.3 and 101.6%. The between-day precisions (the coefficient of variation of the daily means) at 25, 250 and 450 ng/ml were 6.6, 2.8 and 2.6%, respectively. With II as the analyte and I as the internal standard, the within-day precisions at 25, 250 and 450 ng/ml were 7.1, 3.3, and 1.7%, respectively. The mean accuracies were 99.7, 98.8 and 96.5% at these respective concentrations. The between-day precisions of this method were 4.7, 5.3 and 4.8% at 25, 250 and 450 ng/ml, respectively. Thus, these analytical procedures yield accurate and precise values over the concentration range studied.

The method described here has been utilized in pharmacokinetic studies of I and II in rats and dogs (Fig. 3). Although increased sensitivity was not required for our present needs, preliminary studies which use a small volume of acidified methanol to elute I and II from the extraction column indicate that improvements in sensitivity of ten-fold can be accomplished easily. In addition, since the separation of I and II is very good, use of a shorter, smaller-particle-size HPLC column should improve resolution, increase sensitivity and decrease analysis time.

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