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Note

Quantification of *R*-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-methyl-3-benzazepine in brain and blood by use of reversed-phase high-performance liquid chromatography with electrochemical detection

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Antipsychotic drugs are believed to act by blocking dopamine receptors [1—6]. These have been divided into two classes on the basis of specific biochemical characteristics: the D_1 class is linked to the dopamine-mediated stimulation of cyclic adenosine 5′-monophosphate (cAMP) synthesis, whereas the D_2 class (labeled by [^{3}H]spiperone) is not [7]. On the basis of pharmacological data, it is the D_2 class that has been felt to mediate both antipsychotic effects in man and various antidopaminergic behavioral effects in laboratory animals or man (cf. refs. 2—6). Based principally upon relative potencies in

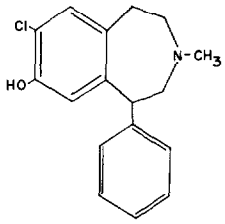

Fig. 1. Structure of *R*-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-methyl-3-benzazepine (I).

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several in vitro biochemical tests, *R*-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-methyl-3-benzazepine [SCH23390, I, Fig. 1] recently was reported to be the first selective D_1 antagonist [8—11]. The drug inhibited dopamine-sensitive adenylate cyclase with an IC_{50} (concentration required to get 50% inhibition) of ca. 1 n*M*, whereas concentrations three orders of magnitude higher were required to inhibit the binding of [^{3}H]spiperone. However, quite unexpectedly, I was found to be extremely potent in antidopaminergic behavioral tests, characteristics expected of D_2, but not D_1, dopamine receptor antagonists [12—14]. These data have spurred enormous interest in the pharmacology of I as a probe to study the neurobiology of dopamine receptors, and it is clear that quantification of the parent compound and any major metabolites will be an essential component of future studies.

This paper describes the application of reversed-phase high-performance liquid chromatography (HPLC) with electrochemial detection for the quantification of this drug in rat brain and serum. Using this method, intraperitoneal doses of I were found to be cleared from the circulation extremely rapidly, but to persist at significant concentrations in brain even when plasma concentrations were undetectable. This method should prove useful in understanding how pharmacokinetic events contribute to the pharmacodynamics of this extremely interesting drug.

EXPERIMENTAL

General

All glassware was pre-treated with a 5% solution of dichlorodimethylsilane in toluene, and then thoroughly rinsed with methanol. I was a gift of Schering (Bloomfield, NJ, U.S.A.) and chlorpromazine · HCl was a gift of Smith Kline and French (Philadelphia, PA, U.S.A.). Tetrabutylammonium chloride was purchased from Sigma (St. Louis, MO, U.S.A.), and HPLC-grade solvents and other reagents were supplied by Fisher (Pittsburgh, PA, U.S.A.).

Sample preparation

Rat serum. A 1-ml aliquot of plasma is transferred to a 10-ml round-bottom culture tube containing 100 ng of chlorpromazine which serves as an internal standard. The pH is adjusted by addition of 1 ml of 1 *M* sodium carbonate buffer (pH 9.5), and 3 ml of *tert.*-butyl methyl ether—hexane (1:1) are added. After mixing for 10 min on a wrist-action shaker (Burrel, Pittsburgh, PA, U.S.A.) and centrifugation at 2000 *g* for 5 min, the organic layer is transferred to a tube containing 1 ml of 0.1 *M* hydrochloric acid. Following shaking for another 10 min and centrifugation, the organic phase is aspirated and discarded. The aqueous phase is then washed with 2 ml of hexane and the hexane is discarded. The aqueous phase is made alkaline by addition of 1 ml of the carbonate buffer, and extracted again with 3 ml *tert.*-butyl methyl ether—hexane by shaking for 10 min. After centrifugation, the organic phase is transferred to a conical bottom glass tube and evaporated under a gentle stream of nitrogen at room temperature. The resulting residue is dissolved in 100 μl of mobile phase and 50—100 μl are injected into the HPLC system. A calibration

curve is prepared using 1-ml aliquots of drug-free serum to which varying (0, 0.5, 1.0, 2.5, 5.0, 10.0, or 20.0 ng) amounts of I and a fixed (100 ng) amount of chlorpromazine are added.

Rat brain. Each brain is homogenized in 2 ml of 0.2 *M* perchloric acid using a Brinkmann Polytron®, the homogenates are vortexed, and a 1-ml aliquot is added to 1.5-ml microcentrifuge tubes containing 50 ng of chlorpromazine (internal standard). After centrifugation (10 000 *g*) for 10–15 min at 4°C in a microcentrifuge (Fisher), the supernatant is transferred to a round-bottom tube and extracted as described above. For the quantification of I in brain tissue, a standard curve is prepared using brain tissue from untreated rats, which is homogenized in 0.2 *M* perchloric acid. Aliquots (1 ml) of the homogenate are transferred to microcentrifuge tubes containing 50 ng of internal standard (chlorpromazine) and I (0, 1, 2.5, 5, 10, or 25 ng/g), and extraced as above.

Chromatography

Mobile phase. The mobile phase used is a mixture of 0.1 *M* ammonium acetate (pH adjusted to 5.5 with glacial acetic acid)–acetonitrile (1:1). Tetrabutyl ammonium chloride is added to a final concentration of 1 m*M* to minimize the effects of secondary interactions between the protonated I (and also chlorpromazine) and unreacted silanol residues on the silica backbone of the adsorbent.

Equipment. A 250 mm × 4.0 mm I.D. stainless-steel column packed with 10-μm microparticulate silica (Si 100 RP-8, E. Merck, Darmstadt, F.R.G.) is used for the isocratic HPLC separations, which used a Laboratory Data Control Constametric III pump to generate a flow-rate of 1.5 ml/min. A glassy carbon working electrode (Bioanalytical Systems, TL-4) is maintained at a potential of +0.85 V (versus an Ag/AgCl reference electrode) by an LC-4 controller (Bioanalytical Systems). Cyclic voltammograms were obtained using a CV-1B appparatus (Bioanalytical Systems), a glassy carbon working electrode, and concentrations of each compound of 250 μ*M* in 0.1 *M* ammonium acetate buffer.

RESULTS

Cyclic voltammograms of I and chlorpromazine indicated peak anodic current responses at +730 and +810 mV, respectively, and supported the feasibility of electrochemical detection for recovery-monitored quantification of I. Typical chromatographic separations obtained with the procedure described above are shown in Fig. 2. Retention times of 3.1 min (I) and 5.5 min (chlorpromazine) permit a high daily throughput rate. No significant chromatographic interferences from any endogenous substances in either brain or blood were noted. The assay described has a lower working limit of detection of 0.5 ng I per ml of blood or brain supernatant. As is shown in Table I, the technique is sufficiently accurate and precise for routine use. In preliminary studies with this method, the absolute recovery of I across the whole working range of concentrations has averaged 82%.

An approximate time course of the absorption, distribution, and elimination of I was defined after intraperitoneal (i.p.) injection. As shown in Table II,

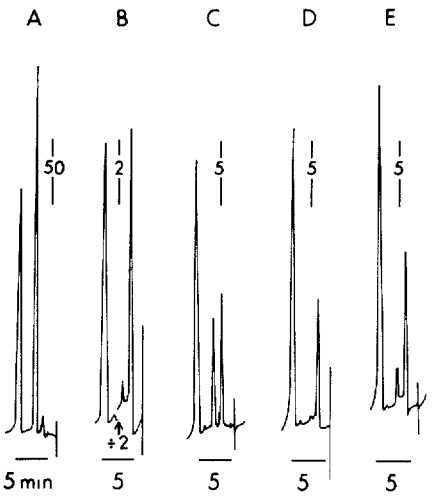

Fig. 2. Chromatogram of I and the internal standard chlorpromazine. The peaks for chlorpromazine (left; retention time ca. 5.5 min) and I (right; retention time ca. 3.5 min) can be noted in A. (A) 200 ng I and 200 ng chlorpromazine internal standard (500 nA/V scale). (B) Extraction of 0.5 ng I standard (20 nA/V scale). (C) Extraction of 10 ng I standard (50 nA/V scale). (D) Extracted plasma from drug-free rats. (E) Extracted plasma from rat sacrificed 60 min following administration of I.

TABLE I

RECOVERY, PRECISION AND ACCURACY OF HPLC MEASUREMENT OF I

These values represent data from five separate determinations made on the same day.

Concentration added (ng/ml)	Concentration found (ng/ml)	Coefficient of variation (%)	Absolute percentage recovery
2.5	2.9 ± 0.1	3.0	84.6
50	44.6 ± 1.3	3 0	78.3

TABLE II

TIME COURSE OF THE CONCENTRATION OF I IN BLOOD AND BRAIN AFTER INTRAPERITONEAL INJECTION

All rats were injected with 0 3 mg/kg I by intraperitoneal injection at various times before sacrifice. Each value represents the mean ± S E.M. for three animals.

Time (min)	Concentration	
	Blood (ng/ml of serum)	Brain (ng/g of tissue)
15	13.5 ± 5 4	8.4 ± 0.6
30	9.6 ± 0 7	11.0 ± 1 8
60	16.5 ± 3.6	9 1 ± 1.3
120	1.3 ± 0.6	7.1 ± 0.8

there is a rapid disappearance of I from the blood, but not the brain. Although formal pharmacokinetic studies have not yet been performed, the i.p. studies indicate that after the absorption phase, the plasma half-life of I appears to be very short, in the order of 20 min. Of greatest interest, however, is the fact that the brain concentrations of I (Table II) are still near maximal at the latest time points we have examined (2 h), whereas the plasma concentrations are undetectable (< 0.5 ng/ml).

DISCUSSION

Two major pharmacological issues concerning I have been addressed by the use of this method. First, Iorio and co-workers [8, 9] reported that after oral administration I had an ID_{50} (dose required to get 50% inhibition) of ca. 2.5 mg/kg against apomorphine-induced behaviors. Conversely, we [12, 13] have found that I, given i.p. or intracerebroventricularly (i.c.v.), was extremely potent (ID_{50} = 0.03 mg/kg i.p. and 1 μg i.c.v.) in antagonizing the behavioral effects of apomorphine. The psychopharmacological studies suggest extensive first-pass metabolism of I, and the approximate plasma half-life determined here is consistent with that notion. A likely hypothesis is that formal pharmacokinetic experiments will find that I given orally has an extremely large volume of distribution. Of particular importance to the large psychopharmacological literature that is already emerging with this drug is that the plasma half-life of I after parenteral administration is ca. 20 min and that, after an i.p. dose of 0.1 mg/kg, neither the parent compound nor any electrochemically active metabolites [e.g., *R*-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine] are detectable in blood 2 h after drug administration.

Conversely, there are significant amounts of I still present in brain after the drug has disappeared from the blood. It is interesting that preliminary biological experiments have shown that after i.p. administration of I, there is significant inhibition of amphetamine-induced locomotion in vivo for at least 8 h after treatment [15]. Moreover, dopamine-stimulated cAMP synthesis is also inhibited in membranes from animals pretreated 12 h prior to sacrifice [15]. The data presented here support the hypothesis that it is the parent compound and not, as frequently occurs with other drugs [16, 17], a metabolite, that is responsible for the antidopaminergic effects of I.

In summary, we have described a simple and sensitive method for extracting and quantifying I from blood or brain. Since this compound is likely to be extremely important in the study and definition of dopamine receptors, the method described should be of value in thorough studies of the pharmacology of this drug.

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