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STEREOSPECIFIC REDUCTION OF HALOPERIDOL IN HUMAN TISSUES

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Abstract—In the current study, we have examined the catalytic activity and stereospecificity of haloperidol (HP) reductase activity in the cytosolic fractions of human brain and liver and in whole blood. The reductase activity was NADPH-dependent and inhibited by menadione, features typical of the ketone reductases (EC 1.2.1). The V_{max} in the brain was about 4-fold higher than in the liver. Moreover, the reaction was stereospecific in that only the *S*(−) enantiomer was detected in brain and blood and 99.2 ± 0.1% of the reduced HP (RHP) produced in the liver was *S*(−). The potential clinical implications of our results are unknown because until now all binding and pharmacodynamic studies with RHP have been performed with the racemate.

Haloperidol (HP§) {4-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone} (Fig. 1), a dopamine receptor (D_2) antagonist, is one of the most widely prescribed neuroleptic drugs [1]. Its major metabolite is reduced haloperidol (RHP) (Fig 1). In human beings, RHP is often found at concentrations equal to or higher than those of HP [2] and is reoxidized to HP [3]. Reduction of HP to RHP has been demonstrated in guinea pig kidney [4], human and guinea pig liver [5], and human erythrocytes [6]. The enzymes involved are NADPH-dependent, cytosolic, do not reduce aldehydes and are inhibited by certain ketone-containing substrates, characteristics consistent with them being ketone reductases. Ketone reductases (EC 1.2.1) metabolize a broad variety of xenobiotic and natural carbonyl-containing compounds [7] and are distributed throughout the body, including the brain [8]. Their role is thought to be detoxification of carbonyl-containing species.

Studies in animals given HP show that RHP is present in the brain at concentrations higher than those of HP. One hour after guinea pigs were given either 0.1 or 1.0 mg/kg HP, concentrations of RHP in the cerebellum, striatum and prefrontal cortex were consistently higher than those of HP [9]. In the occipital cortex of nine patients with schizophrenia who were taking HP prior to death, the concentrations of RHP were higher than those of HP in all but one subject [10].

Unlike the parent molecule, RHP is chiral. This fact may have pharmacodynamic as well as pharmacokinetic consequences. Although it is not a potent D_2 antagonist, racemic RHP has high affinity for the "HP-sensitive" sigma binding sites in the brain, K_i = 5.1 ± 2.3 nM [11]. Thus, enantioselective binding is likely to be most important for sigma sites. In addition, reoxidation of RHP to HP and other metabolic or elimination pathways may be stereoselective. Whether or not RHP is formed in the brain by ketone reductases or enters via the blood stream has not been studied; nor has the stereospecificity of the reductase reaction. The latter would be important if the enantiomers were shown to have different pharmacologic properties. In the current report, we demonstrate the catalytic activity of HP reductases and the stereospecific formation of RHP from HP in D_2-rich areas of human brain, and in human liver and blood.

MATERIALS AND METHODS

Materials. HP, RHP and chlorohaloperidol (CHP) were donated by Janssen Pharmaceutica (Belgium), the enantiomers of RHP by Dr J. Jaen, Parke-Davis (Ann Arbor, MI, U.S.A.) and SKF525-A by Dr L. Rivory. Daunorubicin was obtained from May and Baker (Dagenham, U.K.). Menadione, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and $NADP^+$ were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Methanol acetonitrile, hexane, heptane and isopropanol were supplied by Mallinckrodt (Paris, KY, U.S.A.). All other chemicals were of the highest purity available.

Human tissues. The studies were approved by the appropriate Ethics Committees. The caudate nucleus, putamen and substantia nigra from both sides of the brain were obtained at routine post-mortem examination within 8 hr of death from four males who had no history of HP therapy (age 38–81 years). Human liver was obtained from five renal transplant donors (age 16–48 years, one female and four males) who were maintained on life support systems until the organs were removed. The maximum warm ischaemia time was 21 min. All tissue samples were frozen immediately at −70°. Whole blood was collected from five healthy, medication-free volunteers (aged 20–30 years, one female and four males) into tubes containing EDTA.

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§ Abbreviations: HP, haloperidol; RHP, reduced HP; CHP, chlorohaloperidol; D_2, dopamine receptor.

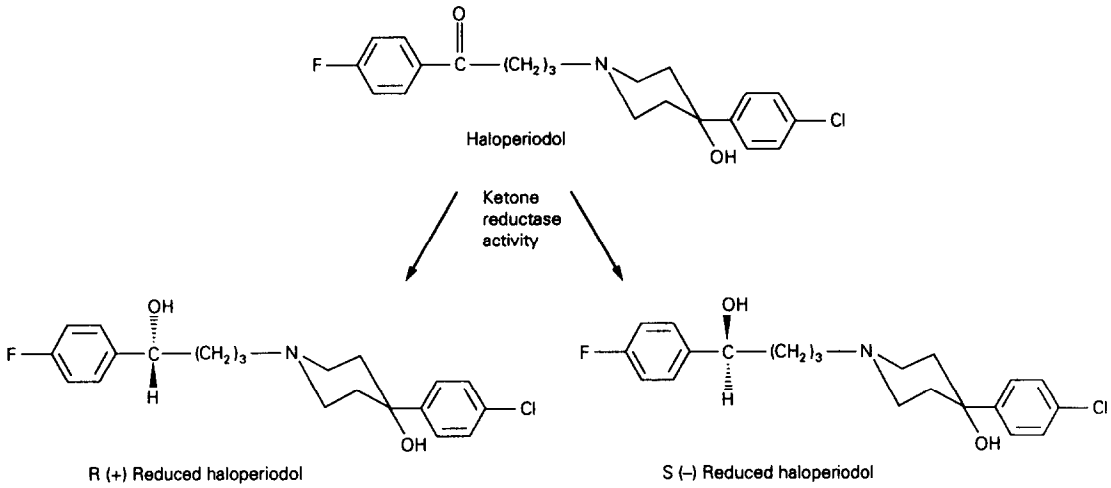

Fig. 1. Reduction of HP to the $R(+)$ and $S(-)$ enantiomers.

HPLC systems and mobile phases. The total amount of RHP produced was quantitated by HPLC as reported previously [12] (system A). Enantiomeric ratios were established using the HPLC method of Jaen *et al.* [13], in which the detection limit was 2 μg RHP injected (0.1 μg/mL × 20 μL) (system B). Briefly, these systems were: system A: a mobile phase comprised of 80 mM NaH_2PO_4:acetonitrile, pH 7.25 and mixed in a ratio of 65:25 containing 0.01% (w/v) alkaline cetrimide was delivered to a 3 μm reverse phase C18 column (Nova-pak® C18, 60 Å, 4 μm, internal dimensions 3.9 × 150 mm, Waters Associates, Milford, MA, U.S.A.) at a flow rate of 1.3 mL/min. Total RHP was recorded using an amperomeric electrochemical detector (Waters Associates) set at a potential of 0.97 V between the reference and working electrodes. System B: a mobile phase comprised of hexane:isopropanol (96:4, v/v) was delivered to a Chiralcel OJ cellulose chiral column (J.T. Baker Inc., Phillipsburg, NJ, U.S.A.) at a flow rate of 1.5 mL/min. Ratios of RHP enantiomers were determined using a UV detector (Waters Associates) monitoring absorbance at 220 nm.

Subcellular fractionation. All procedures were performed at 4°. Approximately 1–2 g samples from brain or liver were homogenized in 10 vol. of 20 mM Tris–HCl buffer pH 7.4, containing 1.15% KCl (w/v) using 3 × 5 sec strokes of a Brinkman Polytron, setting 7. The homogenate was centrifuged at 9000 *g* for 30 min and the supernatant decanted and centrifuged at 100,000 *g* for 60 min. Cytosolic protein was measured using the method of Lowry *et al.* [14] and bovine serum albumin as the standard. The cytosolic fractions were stored at −70° for no more than 3 months.

Assay for reductase activity. The assays were conducted in duplicate in 1 mL mixtures containing 200 μL cytosol or blood, an NADPH-generating system consisting of 2 mM $NADP^+$, 20 mM glucose-6-phosphate and 0.6 U glucose-6-phosphate dehydrogenase, and 10 mM $MgCl_2$ and 30 mM KCl in 0.2 mM phosphate buffer, pH 7.4. The concentration of cytosolic protein ranged between 0.2 and 0.5 μg/mL for the putamen and 2.3 and 2.9 μg/mL for the liver. The reaction was initiated at 37° by the addition of substrate (2.3–123 μM) and the samples mixed by rotation. The concentration of HP was limited to 123 μM by solubility. The reaction was terminated after the appropriate interval by the addition of 1 mL cold (0°) 0.25 M NaOH containing 350 nM CHP as the internal standard. This solution was mixed with 5 mL hexane-isopropanol (95:5, v/v), the aqueous layer frozen and the organic layer containing drug and metabolite decanted and evaporated under air at 60°. The extract was then reconstituted in the appropriate mobile phase and subjected to HPLC System A. The above reaction conditions gave rates that were linear with respect to protein concentration and time (15 min). The Michaelis–Menten constants were calculated by non-linear regression.

Inhibition of reductase activity. Duplicate mixtures, constituted and extracted as outlined above, and containing blood, or cytosol from the liver or one of the three areas of the brain, were incubated for 1 hr after the simultaneous addition of 94 μM HP and one of the following inhibitors, or in the absence of the NADPH generating system. The inhibitors used were; SKF-525A, 1 mM; menadione, 0.5 mM; and daunorubucin, 1 mM. The extracts were analysed using HPLC system A. Significant inhibition was assessed using a Student's *t*-test for paired samples.

Stereospecificity of HP reductase activity. Blood, or cytosol from the putamen or the liver was incubated as above in triplicate with 60 μM HP for 60 min and extracted similarly except that CHP was not added. The extract was subjected to HPLC system A, modified by the use of UV instead of electrochemical detection and a fraction collector. A 650 μL fraction corresponding to the retention time of RHP was collected, evaporated at 60° under air, resuspended in 600 μL 1 M NaOH and vortex mixed for 2 min. Then, 2 mL hexane-isopropanol

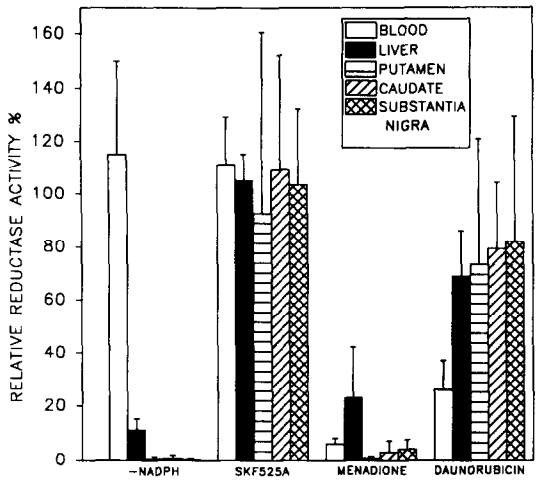

Fig. 2. HP reductase activity measured in the cytosolic fraction from the human brain (putamen, caudate nucleus and substantia nigra) and liver and in whole blood in the presence and absence of an NADPH-generating system, and with the generating system and a putative inhibitor (SKF525-A, 1 mM; menadione, 0.5 mM; daunorubicin, 1 mM). The results are expressed as a percentage of the activity measured in the presence of an NADPH-generating system and no inhibitors (mean ± SD, N = 4 for the brain, N = 5 for the liver and blood). The concentration of HP was 94 μM.

(96:4, v/v) was added, the solution mixed for 2 min, placed on dry ice and the resulting organic layer containing the RHP enantiomers evaporated to dryness at 60°. This final extract was reconstituted in 200 μL of hexane-isopropanol (96:4, v/v) and subjected to HPLC system B.

RESULTS

The rate of production of RHP from HP in the cytosol of the putamen exceeded that in the liver: $V_{max} = 4.8 \pm 2.7 (N = 4)$ and $1.5 \pm 0.6 (N = 5)$ μmol/g protein/min, respectively. The K_m for each tissue was similar: 0.30 ± 0.12 and 0.25 ± 0.14 mM, respectively. For these kinetic studies, the putamen was used instead of the caudate nucleus or substantia nigra because of its greater size. However, when the caudate and substantia nigra were studied in preliminary experiments, the kinetics of the reaction were very similar to those in the putamen. HP reductase activity in blood was four to five orders of magnitude lower than that in the liver or putamen.

In the absence of NADPH, a small degree of HP reduction occurred in the liver and virtually none in the brain. In the blood, RHP production was evident in the absence of the NADPH-generating system and was unaffected by its addition.

The effects of the various inhibitors on HP reductase activity in the blood, and in the cytosolic fraction from the three areas of the brain and from the liver are shown in Fig. 2. HP reductase activity is expressed as a percentage of the activity in the absence of inhibitors and the presence of the NADPH-generating system. The classical inhibitor of cytochrome P450 mono-oxygenases, SKF525-A, did not inhibit reductase activity, whereas menadione, a ketone reductase inhibitor, inhibited HP reduction in all the tissues studied. There was no significant difference in the inhibition pattern in the three regions of the brain ($P > 0.1$). Daunorubicin, a substrate for ketone reductases, although used at twice the concentration of menadione, inhibited HP reduction significantly in the blood and liver ($P < 0.001$ and $P < 0.025$ respectively) but not in the brain ($P > 0.1$).

Reductase activity in the putamen, liver and blood was stereospecific. More than 99% of the RHP produced in all three tissues was the $S(-)$ enantiomer. No $R(+)$ enantiomer was detected at all in the brain or blood, and only $0.8 \pm 0.1\%$ was evident in the liver in which the concentrations of cytosolic protein were 10-fold higher. The RHP generated catalytically was of greater enantiomeric purity than that of the individual $S(-)$ and $R(+)$ enantiomeric standards which were synthesized chemically and contained 2.8% $R(+)$ and 2.2% $S(-)$, respectively. The chromatograms obtained from incubations with brain cytosol, liver cytosol and blood, as well as those obtained from injection of the racemic RHP standard, are shown in Fig. 3 A, B, C and D, respectively. The insert to Fig. 3B demonstrates the 0.9% production of the $R(+)$ enantiomer detected in this liver. Of further interest in this chromatogram is an unidentified compound produced by all five livers that co-eluted with the RHP enantiomers from the C18 column but was resolved well from the separated enantiomers, resulting in a large, broad, slowly eluting peak. Due to its substantial size, its identity is a topic for further investigation. This peak was present in some of the incubations with blood but was too small to be quantified.

DISCUSSION

HP reductase activity was evident in the three human tissues studied: the cytosolic fractions of the brain and liver, and whole blood. The inhibition studies in all three tissues support the findings of others that the enzymes involved are ketone reductases [5, 6]. Daunorubicin appeared to inhibit HP reduction in the three tissues studied, but the inhibition was significant only in the liver and blood. HP reductase activity in human red blood cells has been reported previously [6] where, in contrast to our results, it was detectable in six out of eight blood samples in the presence of exogenous NADPH only. There is no obvious explanation for this apparent discrepancy.

HP reductase activity in the brain has not been reported previously. Korpi *et al.* [4], who examined the metabolism of HP in slices of guinea pig striatum and cerebellum, could not demonstrate any HP reductase activity. This may have been due to their use of a much lower concentration of HP (0.27 μM) than used in our study (2.3–123 μM). The same group demonstrated reductase activity in guinea pig liver and kidney at the lower substrate concentration. Therefore, guinea pigs may either be deficient in brain HP reductase or have much lower levels of

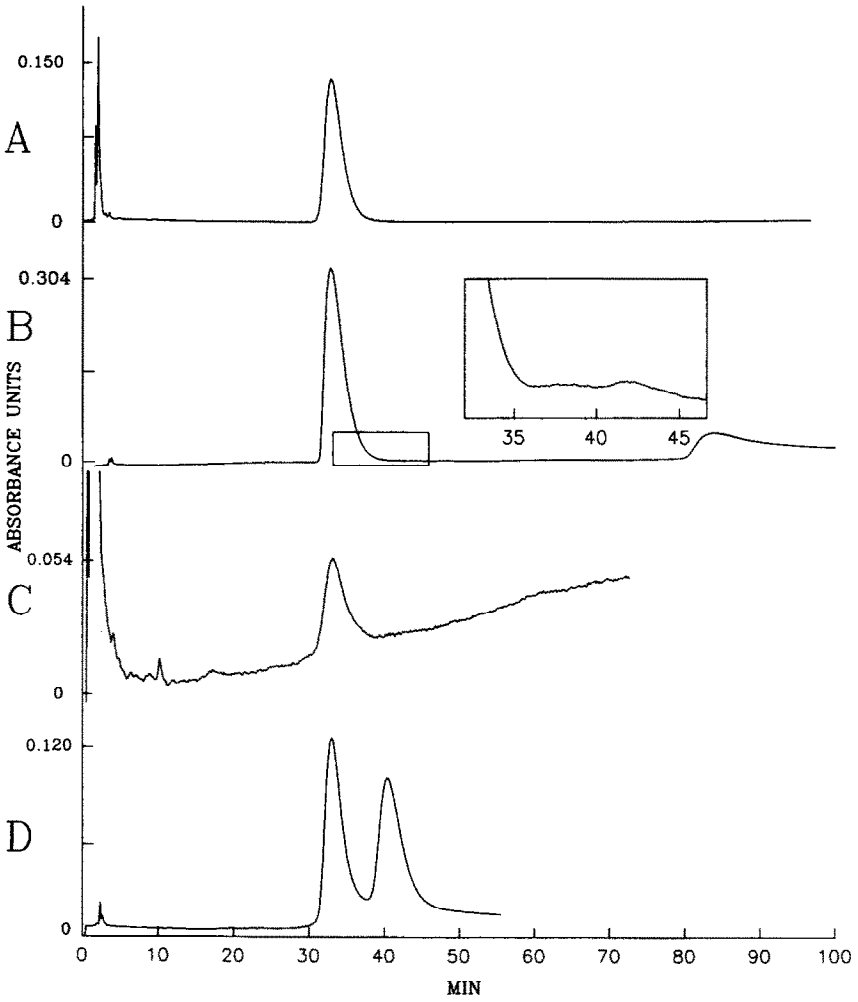

Fig. 3. HPLC chromatograms demonstrating the stereospecific reduction of HP to *S*(−)RHP in cytosol from the human putamen (A) and human liver (B) and in the blood (C). Note the small amount of *R*(+)RHP and the late eluting peak in the insert of (B). (D) shows the chromatogram of the racemic RHP standard.

activity in the brain compared to the liver or kidney. Miyazaki *et al.* [15] reported that, in rats dosed with radiolabelled HP and killed at intervals of up to 48 hr thereafter, radioactivity in the brain and liver was attributable largely to the presence of HP and not metabolites. In relation to the liver, they concluded that the metabolites formed were cleared rapidly from the organ. However, Korpi *et al.* [9] demonstrated higher concentrations of RHP than HP in guinea pig brain 1 hr after the administration of HP and a similar result was evident in post mortem human brains [10]. Our results suggest that the presence of the metabolite in the human brain could be due, at least in part, to the high catalytic activity of HP reductase in this organ.

The stereospecificity of HP reductase has not been reported previously. Essentially, only one enantiomer, the *S*(−), was formed in all three tissues. Stereoselective reduction of another butyrophenone (phenyl *n*-propyl ketone) was reported in the rabbit *in vivo* in 1954 [16]. In a more recent publication, a number of aryl-alkyl ketones were reduced to the *S* alcohol enantiomer (90–98%) in homogenates from rabbit and rat liver [17]. Reduction of HP to the *S*(−) enantiomer of RHP is consistent with the pattern seen for reduction of aryl-alkyl ketones in bacteria, and in the rat and rabbit [18]. According to the formula of Baumann and Prelog [19], if HP is oriented in a plane as shown in Fig. 4, formation of the *S* enantiomer is predicted. However, there are cases where such a prediction is incorrect, an example of which is the reduction of warfarin [20]. Not known at present is whether the stereospecific HP reductase activity observed in three human tissues *in vitro* results in the dominance of the *S*(−) RHP in the blood or tissues *in vivo*. For an answer to this question, a much more sensitive assay than the one available currently is required.

In summary, we have shown that HP reductase activity is present in the basal ganglia of the human

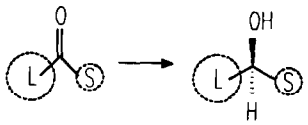

Fig. 4. The diagram demonstrating the application of the formula of Baumann and Prelog [19] to the steric reduction of a carbonyl compound. L and S refer to sterically larger and smaller groups, respectively, attached to the carbonyl. Once the carbonyl is aligned in the plane as depicted, the alcohol product is predicted to have the configuration shown.

brain, and in the liver and blood. The reactions are stereospecific in that only the $S(-)$ enantiomer, essentially, is produced. The V_{max} is about 4-fold higher in the brain than in the major organ of drug metabolism, the liver. We suggest that, at least in part, local ketone reductase activity may be responsible for the presence of RHP in the brain. The therapeutic or toxic implications of our results are unknown because until now all binding and pharmacodynamic studies with RHP have been performed with the racemate.

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