Effect of Cytochrome P450 2D1 Inhibition on Hydrocodone Metabolism and its Behavioral Consequences in Rats

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ABSTRACT

Humans that lack cytochrome P450 2D6 (CYP2D6) activity may have an altered risk of drug dependence or abuse because this enzyme is important in the metabolism of some drugs of abuse, including hydrocodone. In rats, hydrocodone conversion to hydromorphone is catalyzed by CYP2D1, the rat homolog of the human CYP2D6. To determine the impact of impaired hydromorphone formation on the behavioral effects of the parent compound, hydromorphone-induced analgesia and hyperactivity, hydrocodone discrimination and self-administration were examined in male Wistar rats, with or without pretreatment with CYP2D1 inhibitors (quinine and budipine). In vivo, quinine (20 mg/kg) and budipine (10 mg/kg) produced a marked suppression in brain and plasma hydromorphone levels detected after the peripheral administration of hydrocodone, thus confirming that the doses used suppressed CYP2D1 activity. In contrast, CYP2D1 inhibition had no impact on the analgesic or discriminative stimulus effects of hydrocodone, nor did this type of manipulation alter hydrocodone self-administration. The effects of quinine on the locomotor activating effects of hydrocodone were subtle at best. Because inhibition of CYP2D1 in this rat strain is proposed to be a useful animal counterpart for studying the impact of CYP2D6 polymorphism in humans, these data suggest that differences in CYP2D6 phenotype will have limited influence on the drug response to hydrocodone after nonoral administration. This has recently been verified in a study showing that inhibition of hydrocodone biotransformation to hydromorphone does not affect measures of abuse liability. Therefore, hydrocodone’s behavioral effects are most likely attributable to its own intrinsic effects at mu opioid receptors.

HC (dihydrocodeinone) is a semisynthetic opioid related structurally to codeine. It is used therapeutically as an antitussive agent, and is only available on prescription. The addictive liability of HC seems more comparable to morphine, than to codeine. HC is similar to morphine in inducing euphoria in former morphine addicts, and completely relieves the signs of morphine abstinence (Himmelsbach, 1941). Not surprisingly, HM has powerful mood-enhancing properties (Preston et al., 1989) and it produces similar discriminative stimulus effects to those elicited by other abused opioids, e.g., morphine (Jasinski, 1977).

Based on binding studies using rat tissues, the question has been raised whether HC depends on metabolic conversion to HM for its opiate effects. Chen et al. (1991) observed that HM was 33 times more potent than HC in displacing 3H-DAMGO, a highly specific mu-receptor ligand, in homogenates of rat whole brain less cerebellum. Earlier, Hennies et al. (1991) also compared HC and HM in rat brain homogenates. HM was 37 times more potent than HC against 3H-dihydromorphine (mu-receptor ligand) and 3H-ethyketocyclazocine (kappa receptor ligand) in rat whole brain homogenates. BM was 7 times more potent than HC at the mu receptor and 37 times more potent at the kappa receptor. These authors (Hennies et al., 1988) also compared HC and HM using tail flick analgesia to radiant heat. They found parallel dose-response curves, with HM being more than four times more potent than HC (ED50 = 0.28 mg/kg and 1.18 mg/kg, respectively). Even greater (20–200 times) differences

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ABBREVIATIONS: CYP2D6, cytochrome P450 2D6; CYP2D1, cytochrome P450 2D1; EM, extensive metabolizer; PM, poor metabolizer; HC, hydrocodone; HM, hydromorphone; PLL, paw lick latency; QN, quinine; BUD, budipine; FR, fixed ratio.
in analgesic potency were observed by Plummer et al. (1990) after intrathecal administration using hot plate and tail flick tests. Because HM exhibits greater potency in these assays than HC, it is plausible that the HM formed after HC administration contributes significantly to the overall pharmacological effects observed.

The enzyme that mediates the formation of HM from HC (CYP2D6) is absent in approximately 7% of Caucasians as a result of inherited mutations in the CYP2D6 gene (for recent review, see Kroemer and Eichelbaum, 1995). As a result, the pharmacokinetics of formed HM is profoundly different in these people (PM) compared to the rest of the population (EM). We (Otton et al., 1993) observed that the partial metabolic clearance to HM after a 10 mg oral HC bitartrate was 725% faster in EM than in PM (28.1 + 10.3 vs. 3.4 + 2.4 ml/hr/kg). Furthermore, pretreatment of EM with quinidine (a selective inhibitor of CYP2D6 activity) on another occasion reduced their clearance to levels similar to that seen in PM. HM Cmax was 445% higher in EM than PM and in EM when pretreated with quinidine. In this study, subjective and physiologic measures were taken coincidentally with blood sampling over the first 2 hr postdosing. No statistically significant differences in physiologic measures were observed. However, during the first hour after dosing, EM reported more positive opiate effects and fewer unpleasant opiate effects compared to PM, and on the test day when their CYP2D6 activity was inhibited by quinidine pretreatment (Otton et al., 1993). However, a larger (n = 25), double-blind, placebo-controlled trial failed to find any phenotypic differences in the abuse liability of HC (Kaplan et al., 1996). This finding was surprising in view of other data indicating that PM obtain slightly less analgesia than EM after codeine, which is metabolized to morphine by CYP2D6 (Yue et al., 1989; Chen et al., 1991).

Studies in rats (Cleary et al., 1994; Ritchie et al., 1990) have demonstrated that codeine analgesia (tail flick test) was related to the extent of codeine converted to morphine. In rats, this reaction is catalyzed by the rat homolog of human CYP2D6, CYP2D1 (Zysset et al., 1988; Mikus et al., 1990; Barham et al., 1994). Cleary et al. (1994) showed that codeine did not produce analgesia in Dark Agouti rats, a strain deficient in CYP2D1 activity and the animal counterpart of the human PM phenotype (Al-Dabbagh et al., 1981). Furthermore, codeine analgesia was attenuated in Sprague-Dawley rats (an EM counterpart) by pretreatment with 40 mg/kg s.c. quinine, a selective inhibitor of CYP2D1 activity (Kobayashi et al., 1989).

The only behavioral studies that have specifically compared the effects of a psychoactive drug in rats with functional and deficient CYP2D1 have used amphetamines. Enhanced stereotypy from amphetamine (Freeman and Sulser, 1974) and methamphetamine (Suzuki et al., 1984; 1986) have been observed in Sprague-Dawley rats after inhibition of the 4-hydroxylation of these drugs, either by CYP2D1 inhibitors or by other nonspecific P450 inhibitors. Similarly, we (D. M.Tomkins, unpublished data) observed that CYP2D1 inhibition was associated with significant increases in the duration of amphetamine-induced hyperactivity and the discriminative stimulus effects of amphetamine in Wistar rats (an EM counterpart). Furthermore, budipine, a novel antiparkinsonian drug (Jackisch et al., 1993), which potently inhibits CYP2D1 activity in vitro (Ki = 130 nM; D. Wu, unpublished data) suppressed amphetamine self-administration behavior in this rat strain. The behavioral profiles showed good correlation with the effect of CYP2D1 inhibition on the pharmacokinetic profile of amphetamine. In our study, the same strategy has been used to determine the impact of CYP2D1 inhibition on the analgesic, locomotor activating and discriminative stimulus effects of HC, as well as HC self-administration behavior. The effects of the CYP2D1 inhibitors, budipine and quinine, on plasma and brain levels of HC and HM were also examined to confirm that the doses used in the behavioral studies were sufficient to suppress CYP2D1 activity.

Methods

Animals and Housing

Except where stated, subjects were experimentally naive male Wistar rats (Charles River, Quebec, Canada) that were individually housed in a temperature controlled environment (22-24°C) on a 12-hr light/dark cycle. Rats used in the locomotor, analgesia and pharmacokinetic studies were given free access to food and water. Rats used in the drug discrimination and self-administration studies were given free access to water but were maintained on a restricted diet of approximately 16 g of food per day (Lab Diet, Richmond, IN) made available in the home cage after each test session. In addition, the drug discrimination rats could earn up to a maximum of 2.25g of food pellets (dustless precision pellets, Bio-Serv) during the training and test sessions. Before initiating these studies all animals were handled during a 1-wk habituation period to the animal housing facility.

Drugs and Injections

All drug solutions were prepared daily. Doses are expressed as the free base and were administered in a 2 ml/kg volume. HC bitartrate (Du Pont Merck Pharma, Mississauga, Ontario, Canada), HM hydrochloride (Knoll Pharmaceuticals, Mount Olive, NJ) and quinine hydrochloride (Sigma Chemical Co., St. Louis, MO) were dissolved in saline. Budipine hydrochloride (Byk Gulden Pharmazeutika, Konstanz, Germany) was administered in a 0.5% methyl cellulose solution.

Experiment 1: Effect of CYP2D1 inhibition on plasma and whole brain levels of HC and HM

Five groups of five rats (body weights of 350–400 g) were injected with either HC (3 mg/kg, s.c.), then killed by decapitation at 30 or 60 min postinjection, or HM (0.5 mg/kg, s.c.) and then killed at 30, 60 or 90 min later. In four other groups, HC administration (3 mg/kg, s.c.) was preceded by either quinine (20 mg/kg, i.p., 60-min pretreatment time) or budipine (10 mg/kg, i.p., 30-min pretreatment time) and the animals were killed either 30 or 60 min later. Trunk blood was collected into heparinized tubes. Plasma was separated by centrifugation (10 min at 3000 rpm) and stored at -20°C until analysis. Immediately after decapitation, whole brains were removed, the cerebellum dissected out, washed with ice-cold saline and homogenized in distilled water (1 g/2 ml). Unchanged HC, and free and total HM (i.e., conjugated plus unconjugated) were assayed using a previously described method (Otton et al., 1993), except that acetonitrile (4 ml) was used to extract the drugs from plasma (2 ml) or brain homogenate (2 ml) saturated with sodium chloride. After the samples were vortexed for 5 min and centrifuged at 3000 rpm for 10 min, the acetonitrile extracts were dried under nitrogen gas and then the residues were resuspended in acetonitrile (0.4 ml). The acetonitrile extracts were evaporated to dryness under nitrogen gas and resuspended in 0.2 ml 0.05% phosphoric acid. An aliquot was analyzed by high-performance liquid chromatography using tandem UV detection (for HC and its internal standard, oxycodone) and electrochemical detection (for HM and its internal standard, nalorphine). The limit of detection was 1 and 25 ng/ml plasma or g brain tissue for HM and HC, respectively.
Experiment 2: Effect of CYP2D1 inhibition on HC analgesia

Before the experimental studies, the animals were habituated to the hot plate until stable responding was established following the methodology outlined by Paronis et al. (1993). The test procedure consisted of placing the rats individually in a plastic cage with an aluminum floor. The cage was situated in a hot water bath to maintain the plate at 51°C. The latency to hind paw lick or jump was recorded as the PLL. Immediately after each response was observed or after 60 sec, whichever occurred first, the animal was removed from the apparatus. In the first part of the study eight rats were allocated to one of four treatment sequences such that each animal received all treatments of HC (0, 6, 8 and 10 mg/kg) using a balanced design. On test days the rats were given four habituation trials separated by at least 45 min. Ten min after the final trial the rats received s.c. injections of either saline or HC (6–10 mg/kg) and were reexposed to the test apparatus 30 min later. Each test session was separated by at least 48 hr. For the CYP2D1 inhibition studies, the same animals were reallocated to one of six treatment sequences (after a 2-wk washout period) in which quinine (20 mg/kg i.p., 60 min pretreatment) or budipine (10 mg/kg i.p., 30 min pretreatment) was administered before the HC injection. For comparative purposes, the effect of the opioid antagonist naloxone (1 mg/kg i.p., 30 min pretreatment) on the analgesic effects of HC was also examined.

Experiment 3: Effect of CYP2D1 inhibition on HC- and HM-induced hyperactivity

Locomotor activity was assessed using clear Plexiglas boxes (40 x 40 x 20 cm), each of which was placed within the arena of an OptoVarimex activity meter (Columbus Instruments, Columbus, OH). Locomotor activity was calculated as either the total number of locomotor counts (first study) that were manually recorded from the activity meters or the distance traveled (cm) within each 20-min sample period and stored on an IBM-compatible computer using the Auto-Track software package (Columbus Instruments) (second study). Distance traveled data were not available for the initial study due to computer malfunction. In the first part of this study, two groups of rats (n = 12/group) were placed individually in the activity boxes for 48 hr. For the CYP2D1 inhibition studies, the same animals were reallocated to one of six treatment sequences so that the first group received injections of HC (0, 0.1, 0.3, 0.5 and 1 mg/kg s.c.), whereas the second group were injected with HM (0, 0.01, 0.03, 0.05 and 0.1 mg/kg s.c.). Doses were administered using a randomized design such that all animals received all treatments. On test days, the rats were placed in the activity boxes; 1 hr later the allocated drug treatment was administered using a randomized design such that all animals received each treatment of HC (0, 6, 8 and 10 mg/kg) using a balanced design. On test days the rats were given four habituation trials separated by at least 45 min. Ten min after the final trial the rats received s.c. injections of either saline or HC (6–10 mg/kg) and were reexposed to the test apparatus 30 min later. Each test session was separated by at least 48 hr. For the CYP2D1 inhibition studies, the same animals were reallocated to one of six treatment sequences (after a 2-wk washout period) in which quinine (20 mg/kg i.p., 60 min pretreatment) or budipine (10 mg/kg i.p., 30 min pretreatment) was administered before the HC injection. For comparative purposes, the effect of the opioid antagonist naloxone (1 mg/kg i.p., 30 min pretreatment) on the analgesic effects of HC was also examined.

Experiment 4: Effect of CYP2D1 inhibition on the discriminative stimulus properties of HC

The first series of discrimination experiments were conducted at the Addiction Research Foundation. The test apparatus consisted of four identical test chambers (22 x 22 x 28 cm, LxWxH; Med Associates Inc., St. Albans, VT) each containing two response levers mounted 7 cm above a grid floor and positioned either side of the pellet dispenser. Reinforcement delivery was controlled by a microcomputer interface (Med Associates) linked to a 386sx IBM computer. The rats were trained in groups of four with each rat always being placed in the same designated test chamber. They were initially trained to lever press for food reinforcement (45-mg dustless precision pellet) under an FR-1 schedule during twice daily sessions, from Monday to Saturday. Each session began immediately after the animal was placed in the test chamber and the signal light was turned on. Drug discrimination training began once stable responding for food was observed following the procedure outlined in Higgins et al. (1994). Rats were given daily discrimination trials, with either morphine (3 mg/kg s.c.) or saline (2 ml/kg s.c.) injected 30 min before each session. The rats could obtain food reinforcement by responding selectively on one of the two levers after morphine or on the alternative lever after saline administration. To control for positional preferences, the treatment assigned to a particular lever was counterbalanced. Responses on the inappropriate lever were recorded but had no programmed consequences. Initially, each response on the correct lever resulted in the presentation of a food pellet. Once responding was stable, the number of responses required for each food pellet was gradually increased to an FR-10 schedule. Once the FR-10 schedule was attained, each animal received only one daily training session that lasted either 15 min or until the animal had acquired 50 food pellets. Training on this schedule was continued until the following criteria were met on six consecutive sessions. Animals must have made no more than 16 lever presses preceding the delivery of the first reinforcement with 95% of the total responses thereafter on the drug-appropriate lever. During discrimination training, morphine and saline trials were presented on a fixed 20-day schedule, i.e., DVVDV, VDIDD, VDVDD, VDVDV (where V = vehicle; D = morphine).

During the experimental phase of the study, test sessions during which both levers were active, were conducted on Wednesdays and Saturdays. Training trials were continued on the intervening days to ensure that discrimination was maintained. Initially, generalization curves for different doses of morphine (0.3, 0.1, 1.5, 3, and 5 mg/kg/2 ml), HM (0.03, 0.075, 0.1 and 0.2 mg/kg/2 ml) and HC (0, 0.1, 0.3, 1, 2 and 3 mg/kg/2 ml) were established. The ability of HC and HM to substitute for the morphine training dose was examined at two time points, 30 and 60 min. Finally, the effect of the CYP2D1 inhibitor, budipine (10 mg/kg/2 ml; 30-min pretreatment), on the ability of HC (30-min pretreatment) to substitute for morphine was examined (n = 4). All injections in this experiment were given s.c.

In a second drug discrimination experiment conducted at the Center for Drug and Alcohol Studies, nine male Sprague-Dawley rats (COBS CD, Charles River, Wilmington, MA) were trained to discriminate morphine (2.5 mg/kg i.p., 15-min pretreatment) from saline in a similar manner to that described above with the exceptions that correct lever responding was reinforced under an FR-32 schedule and that incorrect responses reset the FR schedule. Test sessions were carried out once animals had reached criteria on the preceding Monday or Thursday training session of the first FR on the correct lever and 85% or more responding was on the drug appropriate lever for the entire session. Initially, a morphine generalization curve was established, and then the ability of HC to substitute was examined at doses ranging from 0.5 to 8 mg/kg i.p. (15-min pretreatment). This was followed by a redetermination of the HC dose-effect curve, except that the CYP2D1 inhibitor, quinine (20 mg/kg i.p.), was administered to the rats 75 min before the test session.

Experiment 5: Effect of CYP2D1 inhibition on HC self-administration

Testing was conducted in eight identical operant chambers (22 x 22 x 28 cm, LxWxH; Med Associates Inc.) each containing two response levers mounted 7 cm above a grid floor and positioned either side of the pellet dispenser. Stimulus lights were mounted 5
cm above each lever and a house light was positioned at the back of the chamber. The house light was illuminated throughout the session except during the time-out phase of the self-administration procedure. Reinforcement delivery was controlled by a microcomputer interface (Med Associates) linked to a 386sx IBM computer. These studies were conducted following the procedure outlined in Higgins et al. (1994) and are therefore only briefly summarized here.

Rats were initially trained to lever press for food reinforcement until stable responding on an FR-5 schedule was attained. Nine rats were surgically implanted with an i.v. catheter into the right jugular vein under acepromazine (10 mg/kg i.p.) and ketamine (100 mg/kg intramuscularly) anesthesia. The catheter was exteriorized on the back of the animal between the scapulae. The catheters were infused daily with 0.1 ml heparinized saline (30 IU/ml) to help maintain patency. After 7 days recovery, self-administration sessions were begun. The rats were trained to press a designated lever to receive an infusion of HC (0.16 mg/kg body weight/infusion) during 60-min test sessions. The reinforcement conditions were initially set such that one lever press resulted in a 0.05 ml infusion of HC over a 5-sec period. For a 60-sec period after each infusion the houselight was switched off and the levers were inactivated. Any lever presses made during this time were recorded but not reinforced. When an animal had displayed stable responding under the FR-1 schedule, the schedule of reinforcement was gradually increased to FR-5. Drug testing began once rates of self-administration had stabilized at an average of 10 infusions/session. The effect of CYP2D1 inhibition on HC self-administration over a range of HC drug levels (0.02, 0.04, 0.08, 0.16 and 0.32 mg/kg/infusion) was then assessed during single daily test session. Budipine (10 mg/kg; 30 min pretreatment), quinine (15 mg/kg; 60 min pretreatment) or saline was administered before the start of the test session. All animals in the study received each treatment using a counterbalanced design.

Data Analysis

For the pharmacokinetic study, Student’s t tests were used to determine the statistical significance of time and inhibitor pretreatment on the plasma and brain levels of HC and HM. For the locomotor activity experiments, to determine the effect of CYP2D1 inhibition on the time course of HC- and HM-induced hyperactivity a two-way analysis of variance was used, with time and treatment as the main effects. Where appropriate, significant main effects were followed by individual mean comparisons using planned contrasts. For the drug discrimination studies ED50 and confidence limits were determined using Litchfield-Wilcoxon analysis with the Pharmacological Calculation System software for the IBM computer.

Results

Experiment 1: Effect of CYP2D1 Inhibition on Plasma and Brain Levels of HC and HM. After peripheral administration of HC, the HC level in the brain was higher at 30 min, compared to 60 min postinjection (table 1). Furthermore, the brain levels of HC were higher than those seen in the plasma at equivalent time points (table 1). Pretreatment with the CYP2D1 inhibitor, budipine, but not quinine, resulted in a significant elevation in plasma HC levels (table 1). Thirty min post-HC administration, the brain-free HM level was 3.3 ng/g; this level was increased 2-fold at 60 min postinjection (P < .05). Although plasma free HM levels were similar to those in the brain, total plasma HM increased by 14- to 20-fold at 30 and 60 min, respectively (fig. 1).

Pretreatment with budipine, before HC administration resulted in a decrease in the brain HM levels by 100 and 91% at 30 and 60 min, respectively (fig. 1). Decreases in brain and plasma HM levels by 70 and 94%, respectively, were also observed after pretreatment with quinine at the corresponding times (fig. 1). Significant decreases in free and total plasma HM levels by budipine or quinine were also observed, although the differences were not as pronounced as those seen in the brain.

After the peripheral administration of HM, HM levels in the brain were 37 ng/g at 30 min, and then decreased gradually at 60 and 90 min (table 2). In contrast, there was a more rapid decrease in plasma free HM concentrations over this time period (P < .005). Total HM plasma concentrations also decreased at 60 and 90 min, but these failed to reach statis-

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>Quinine</th>
<th>Budipine</th>
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<tbody>
<tr>
<td>30</td>
<td>587.9 ± 44.8</td>
<td>568.4 ± 46.3</td>
<td>681.7 ± 82.6</td>
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<tr>
<td>60</td>
<td>328.9 ± 35.4</td>
<td>266.2 ± 77.8</td>
<td>418.8 ± 49.4</td>
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</table>

Note: Data are presented as the mean ± S.E.M. n = 5 male Wistar rats per group. * Compared with brain; \* compared with plasma control; ‡ compared with the corresponding groups at 30 min. **P < .01, ***P < .005.
was an overall significant effect of time (F_{6,150} = 10.3, P < .001; fig. 2). Pretreatment with neither quinine nor budipine alone produced any significant effects on paw lick latency, nor did they alter the analgesic effects of HC. In contrast, the opioid antagonist naloxone, completely abolished the antinociceptive effects of a high dose (10 mg/kg) of HC.

Experiment 2: Effect of CYP2D1 Inhibition on HC Analgesia. HC produced a significant dose dependent antinociceptive effect in the hot plate test for analgesia (F_{9.67} = 14.9, P < .001; fig. 2). Pretreatment with neither quinine nor budipine alone produced any significant effects on paw lick latency, nor did they alter the analgesic effects of HC. In contrast, the opioid antagonist naloxone, completely abolished the antinociceptive effects of a high dose (10 mg/kg) of HC.

Experiment 3: Effect of CYP2D1 Inhibition on HC and HM-Induced Hyperactivity. The effects of HC and HM on the locomotor activity in Wistar rats is shown in figure 3. One-way analysis of variance with repeated measures revealed a significant dose effect for both HC (F_{4,44} = 10.2, P < .001) and HM (F_{4,44} = 5.6, P < .001). HM was approximately 10-fold more potent than HC in producing hyperactivity in these animals. From this study, doses of 0.05 mg/kg HM and 0.5 mg/kg HC were selected for the CYP2D1 inhibition studies.

In rats pretreated with quinine (20 mg/kg) and HC, there was an overall significant effect of time (F_{8,150} = 14.9, P < .001) and treatment (F_{5,150} = 3.3, P < .02) and a significant interaction between treatment condition and time (F_{25,150} = 4.8, P < .001). Post hoc analysis revealed that HC produced a small but significant increase in locomotor activity between 40 to 60 min postinjection times compared to saline treated controls (fig. 4). Pretreatment with quinine did not suppress locomotor behavior alone, but did attenuate the mild hyperactivity response to HC (fig. 4). In rats pretreated with quinine (20 mg/kg) and HM, there was an overall significant effect of time (F_{25,175} = 7.9, P < .001) and treatment (F_{5,175} = 4.0, P < .01) and a significant interaction between treatment condition and time (F_{25,175} = 1.6, P < .05). Post hoc analysis revealed that in contrast to HC, HM produced a more sustained increase in locomotor behavior, which was not attenuated by pretreatment with quinine (fig. 4).

Experiment 4. Effect of CYP2D1 Inhibition on the Discriminative Stimulus Properties of HC. The generalization curves for HM and HC from the 3 mg/kg morphine training dose in male Wistar rats are presented in figures 5 and 6. In the case of HM, the discriminative stimulus effects were moderately greater when the drug was given 60 min before the test session, compared to 30 min (ED$_{50}$ values [95% confidence limits] were 0.09 (0.06–0.13) and 0.15 (0.09–0.22) mg/kg, respectively). In contrast, the discriminative stimulus effects of HC were similar when the drug was administered either as a 30 min or 60 min pretreatment (ED$_{50}$ values [95% confidence limits] for the 30 and 60 min pretreatment times were 0.5 [0.2–1.2] and 0.7 [0.3–1.7] mg/kg, respectively). HM was approximately 7-fold more potent than HC and 13 times more potent than morphine (ED$_{50}$ = 1.3 (0.9–0.8) mg/kg) in these generalization tests. Inhibition of CYP2D1 activity by pretreating the animals with budipine

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>30 Min</th>
<th>60 Min</th>
<th>% Change</th>
<th>90 Min</th>
<th>% Change</th>
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<td>HC</td>
<td>3.3 ± 0.2</td>
<td>6.8 ± 1.1*</td>
<td>+106</td>
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<td>Plasma(f) (ng/ml)</td>
<td>3.2 ± 0.2</td>
<td>4.0 ± 0.4</td>
<td>+25</td>
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<td>Plasma(t) (ng/ml)</td>
<td>46.0 ± 8.3</td>
<td>93.2 ± 21.7</td>
<td>+103</td>
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<td>Brain/plasma (f)</td>
<td>1.1 ± 0.1</td>
<td>1.87 ± 0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HM</td>
<td>37.3 ± 1.7</td>
<td>29.5 ± 4.6</td>
<td>−20.9</td>
<td>28.4 ± 2.4a</td>
<td>−23.9</td>
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<tr>
<td>Plasma(f) (ng/ml)</td>
<td>42.6 ± 3.2</td>
<td>19.4 ± 3.3b</td>
<td>−54.5</td>
<td>8.7 ± 0.6b</td>
<td>−79.6</td>
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<tr>
<td>Plasma(t) (ng/ml)</td>
<td>133.9 ± 24.9</td>
<td>95.5 ± 10.9</td>
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<td>77.3 ± 12.6</td>
<td>−42.3</td>
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<tr>
<td>Brain/plasma(f)</td>
<td>0.89 ± 0.08</td>
<td>1.61 ± 0.28</td>
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Data are presented as the mean ± S.E.M.; f, free; t, total. *p < 0.05, **p < 0.005.
did not decrease the ability of HC to substitute for morphine, nor alter its response-rate reducing effects (fig. 6).

In male Sprague-Dawley rats trained to discriminate 2.5 mg/kg morphine from saline, both morphine and HC produced similar effects to that observed in Wistar rats described above, despite the different route of administration for HC (fig. 7). Pretreatment with the CYP2D1 inhibitor quinine alone (20 mg/kg) resulted in saline appropriate responding (data not shown). When quinine was administered before HC, there was no attenuation of its morphine-like discriminative stimulus effects, despite quinine being a potent CYP2D1 inhibitor in this strain (Cleary et al., 1994). If anything, there was a small leftward shift in the generalization curve. This was most evident at the lowest dose of HC examined (0.5 mg/kg, i.p.), where five of six animals tested made 75% or more of the total responses on the morphine appropriate lever, compared to only two of six in nonquinine pretreated animals (fig. 7).

Experiment 5. Effect of CYP2D1 Inhibition on HC Self-Administration. In rats trained to lever press for 0.16 mg/kg infusion HC, alteration of the drug infusion concentration produced a significant change in the total number of infusions taken during the 60-min test session (F<sub>4,32</sub> = 4.7, P = .004) (fig. 8). When the dose was reduced, the rats showed a compensatory increase in the number of infusions taken. Pretreatment with either quinine (F<sub>1,24</sub> = 2.04, NS) or budipine (F<sub>1,24</sub> = 1.0, NS) failed to modify HC self-administration behavior at any of the HC doses over the 1-hr test session (fig. 8). The accuracy of responding (percentage of lever presses on the active lever) was approximately 90% throughout the experiment and was not altered by either different drug infusion doses or by pretreatment with the CYP2D1 inhibitors.

Discussion

In humans, variation in the metabolic conversion of HC to HM does not appear to be related to the opiate effects of HC (Kaplan et al., 1996). This may be due to the relatively small contribution that this CYP2D6 pathway makes to the overall elimination of this drug. In EM, clearance of HM accounts for only 4.6% of the total metabolic clearance of HC (Otton et al., 1993). It is possible that metabolites formed by other, less variable cytochromes P450 enzymes may possess opiate-like activity or that the parent compound itself, has sufficient opiate properties such that the formation of HM is not essential for the pharmacological activity of HC. Earlier studies have shown that rats do not N-demethylate HC, and that the
major metabolic reaction is HM formation (Cone and Darwin, 1978). Therefore, it appeared that the rat was a better model in which to assess the extent that HM contributes to the analgesic and behavioral effects of HC. This was examined in our study by establishing animal counterparts of human PM and EM phenotypes by comparing the effects of HC in the same rat strain, with or without previous administration of a CYP2D1 inhibitor. This approach was used previously to demonstrate differences in the behavioral effects of another CYP2D1 substrate, amphetamine (D. M. Tomkins, unpublished data).

Initially, we confirmed that CYP2D1 inhibition did indeed inhibit the conversion of HC to HM. Pretreatment with either quinine or budipine decreased plasma levels of free HM by approximately 70% and was associated with a complete absence of detectable levels of HM in whole brain (fig.1). In contrast to the marked effects of CYP2D1 inhibition on HC metabolism, there were at best, only subtle changes, in the pharmacological effects of HC after this type of manipulation. At the doses used, neither budipine, nor quinine, altered the dose response curve for the antinociceptive effects of HC (fig. 2). Because HC-induced analgesia was reversed by the opioid antagonist, naloxone, it is doubtful that impaired performance in this task due to repeated testing on the hot plate would account for these negative findings. Cleary et al. (1994), have recently shown that CYP2D1 inhibition also failed to alter the analgesic effects of oxycodone, despite blocking its conversion to oxymorphone, a metabolite with greater affinity for opioid mu receptors. They raised three potential explanations for these findings. First, they suggested that because complete inhibition of CYP2D1 activity was not achieved, enough of the more active metabolite was formed to produce an analgesic effect. This would be an unlikely explanation of our HC data because budipine and quinine, at the doses used, reduced plasma concentrations of free HM by approximately 70%. The plasma HM levels in the CYP2D1 inhibited animals are considerably lower than the plasma HM levels achieved after administration of a dose of HM known to elicit similar antinociceptive effects (on comparison of ED50 for the analgesic effect of HM from Hennies et al., 1988 and Plummer et al., 1990, with our pharmacokinetic data following administration of 0.5 mg/kg HM shown in table 2). Furthermore, this does not account for the observation that the antinociceptive effects of lower doses of HC were not altered, despite the fact that one would predict in this construct that the analgesic effects elicited by threshold doses of HC would be more susceptible to CYP2D1 inhibition. A second explanation offered for the oxycodone data (Cleary et al., 1994) was that inhibition of CYP2D1 activity results in
increased levels of the parent compound of sufficient order of magnitude to compensate for reduced formation of the more active product. Again, our pharmacokinetic data suggest this to be unlikely in the case of HC, because both quinine and budipine failed to alter HC-induced analgesia, whereas only budipine produced a significant increase in plasma and brain levels of HC. Furthermore, it is unlikely that the increased levels of HC observed in budipine pretreated animals is sufficient to compensate for the more pronounced reductions in HM levels, particularly when the differences in their affinities for the mu-opioid receptors is taken into consideration (Hennies et al., 1988; Chen et al., 1991). A final explanation, which at present cannot be ruled out, is that an alternative metabolite is formed that exhibits potent analgesic effects. However, what these data do demonstrate is that O-deethylation is not an essential step to produce an analgesic response to HC.

It is evident from the pharmacokinetic data that quinine and budipine differ moderately in their effects on the metabolism of HC as outlined above. In terms of their mechanism of action, both quinine and budipine have been shown to be potent inhibitors of rat CYP2D1 activity (Otton et al., 1984; Kobayashi et al., 1989; present study). Quinine’s inhibitory activity may be mediated, at least in part, by an interaction with the ferric form of the CYP 2D1 enzyme (Murray, 1984), rather than simply acting as a competitive substrate of this enzyme (Guengerich et al., 1986). In contrast, although budipine’s interaction with the CYP has not been extensively examined, its potent inhibition of [3H]GBR-12935 binding to CYP2D1 suggests that it is a competitive substrate (Niznik et al., 1990). However, because both compounds are potent inhibitors of CYP2D1 activity, it would seem unlikely that these different mechanisms of action at the CYP2D1 enzyme would account for the differences reported here. Alternatively, because HC is metabolized via a number of different pathways (Cone and Darwin, 1978), it is possible that budipine, but not quinine, may also inhibit one or more of these alternative metabolic routes leading to an accumulation of the parent compound.

Locomotor activity, drug discrimination and drug self-administration studies are animal models used to predict the abuse liability of drugs in humans with relatively good success. In both the drug discrimination and self-administration studies, CYP2D1 inhibition did not attenuate the behavioral effects of HC. These data do not correlate with the human study reported by Otton et al. (1993), in which CYP2D6 inhibition did alter subjective reports of “good drug” effects during the first hour after administration. One possible explanation that may account for these discrepant findings is that if the central metabolism of HC plays a major role in regulating these behaviors, then the failure of quinine and budipine to modify these behaviors may be due to an inability to block central metabolism. This has been proposed previously, because it is known that the diastereoisomer quinidine does not penetrate the blood brain barrier at doses similar to quinine (Agon et al., 1988). However, this explanation seems improbable because our in vivo pharmacokinetic studies clearly show that brain levels of HM are markedly reduced after CYP2D1 inhibition (fig. 1). In line with the results of the analgesia studies, these data suggest that HC has significant pharmacological properties in its own right and does not require conversion to HM. This is further supported in the drug discrimination procedure by the observation that the generalization curves for HC in morphine trained rats were superimposable at both 30 and 60 min pretreatment, meanwhile, plasma levels of HM increased 100% between these two time points (table 2). New evidence supporting the fact that HC exhibits greater opioid agonist activity than previously thought is now coming to light (France et al., 1995).

In summary, although we have demonstrated that inhibition of CYP2D1 activity in male Wistar rats has a pronounced effect on the pharmacokinetic profile of HC and its biotransformation to the more active metabolite, HM, we found little evidence that this has a significant impact on the pharmacological and behavioral effects of the parent drug. Because inhibition of CYP2D1 in this rat strain is proposed to be a useful animal counterpart for studying the impact of CYP2D6 polymorphism in humans, these data suggest that, differences in CYP2D6 phenotype will have limited, if any, influence on the drug response to HC. Kaplan et al. (1996) have recently verified this, showing that inhibition of HC biotransformation to HM does not affect measures of abuse liability. Therefore, the behavioral effects of HC are most likely attributable to its own intrinsic effects at mu opioid receptors.

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References


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