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Precipitated withdrawal following codeine administration is dependent on CYP genotype

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Abstract

The role of metabolic polymorphism in the development of physical dependence to codeine was assessed in cytochrome *P*450 2D2 (CYP2D2) deficient Dark Agouti and CYP2D2 intact Sprague-Dawley rats by assessment of the severity of naloxone precipitated withdrawal after codeine and morphine administration. Plasma morphine concentrations after codeine were significantly higher ($P < 0.01$) in Sprague-Dawley than in Dark Agouti rats with metabolic ratios of 0.71 ± 0.27 and 0.07 ± 0.04, respectively. Withdrawal after codeine resulted in significantly greater hypothermia (3.5–4 °C, $P < 0.0001$) in Sprague-Dawley animals compared to the other groups. Body weight loss was similar for all groups ranging from 6.2 ± 0.4 to 8.2 ± 0.6 g. When strain and treatment data were combined, a relationship between body temperature and plasma morphine concentration could be described by the inverse Hill equation ($r^2 = 0.76$, $EC_{50} = 556 \pm 121$ ng/ml, $n = 2.9 \pm 1.5$). These data indicate that dependence and withdrawal after codeine administration are dependent on its bioconversion to morphine.

Keywords: Codeine; Morphine; CYP2D2; Opioid dependence; Opioid withdrawal

1. Introduction

The *O*-demethylation of codeine to morphine is mediated by cytochrome *P*450 2D6 (CYP2D6) in humans (Sindrup and Brøsen, 1995) and CYP2D1 in rats (Mikus et al., 1991a). Between 5% and 10% of Caucasians lack CYP2D6 functional activity and are termed poor metabolisers, while the remainder are termed extensive metabolisers. Female Dark Agouti rats and female Sprague-Dawley rats are the animal counterparts of the human poor and extensive CYP2D6 metaboliser phenotypes, respectively (Al-Dabbagh et al., 1981). In vitro liver microsome studies (Kahn et al., 1985; Larrey et al., 1984; Gonzalez et al., 1987; Mikus et al., 1991a,b) have confirmed differences in the abilities of these strains to metabolise a number of xenobiotics which are CYP2D6 substrates. More recent studies demonstrating low levels of hepatic CYP2D2 expression in the female Dark Agouti rat (Yamamoto et al., 1998; Schulz-Utermoehl et al., 1999) support the previous functional evidence, and have determined that the isoform involved is CYP2D2 and not CYP2D1, as previously believed.

Codeine, with an affinity for the μ-opioid receptor 200–3000 times less than morphine (Pert and Snyder, 1973; Chen et al., 1991), is considered to have little or no intrinsic antinociceptive or analgesic activity (Sindrup and Brøsen, 1995). It has substantially lower antinociceptive activity in Dark Agouti rats and in Sprague-Dawley rats pretreated with quinine (a potent CYP2D2 inhibitor) when compared to untreated Sprague-Dawley rats (Cleary et al., 1994). In humans, Mikus et al. (1997) showed an attenuation of the decrease in gastrointestinal motility from codeine in CYP2D6 poor metabolisers compared to extensive metabolisers. Codeine is a widely available drug used for pain relief and cough suppression, but is also a drug of abuse (Mattoo et al., 1997). Although several authors have shown that human poor metabolisers obtain little pain relief from codeine (Sindrup and Brøsen, 1995), the influence of this genetic polymorphism on codeine dependence has not been investigated. While Sellars and Tyndale (2000) have noted that pharmacogenetic variation in drug metabolism can modify the risk of dependence developing, this has yet to be evaluated for codeine.

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The aim of this study was to investigate the effect of the CYP2D2 genetic polymorphism on codeine dependence in a rat model. Female Dark Agouti and Sprague-Dawley rats were administered morphine or codeine via a constant infusion over 7 days and the magnitude of their withdrawal measured when the opioid antagonist, naloxone, was administered. Relationships between plasma concentration of codeine and morphine and withdrawal were also examined.

2. Materials and methods

2.1. Animals

Aged matched, 12–14-week-old female Sprague-Dawley (220–300 g) and Dark Agouti rats (180–200 g) were obtained from Animal Services, Adelaide University. They were housed individually at 22 ± 2 °C on a 12/12-h light/dark cycle with free access to food and water. Ethical approval was obtained from the Adelaide University Animal Ethics Committee.

2.2. Drugs

Morphine tartrate (Tasmanian Alkaloids, Westbury, Australia), codeine phosphate (Glaxo, Boronia, Australia), naloxone hydrochloride (Sigma, St. Louis, MO, USA), methohexitone sodium (Brietal®, Eli Lilly, Sydney, Australia), trimethoprim/sulfadiazine (Tribrissen®, Jurox, Sydney, Australia), lignocaine (Astra Pharmaceuticals, Sydney, Australia) and sodium pentobarbitone (Nembutal®, Rhône Merieux, Sydney, Australia) were administered as described below. Morphine tartrate and codeine phosphate were dissolved in 0.9% sodium chloride for use in osmotic minipumps as described below. Sodium methohexitone (50 mg/ml) was dissolved in 0.9% sodium chloride.

2.3. Experimental protocol

Morphine tartrate and codeine phosphate were administered via osmotic minipumps (model 2 ML1, Alza, Palo Alto, USA) at a rate of 0.5 μl/h for 7 days. Concentrations were adjusted to allow delivery of morphine tartrate at a dose of 20 mg/kg/day (equivalent to 52 μmol base) and codeine phosphate at 200 mg/kg/day (equivalent to 501 μmol base). Each minipump was incubated for 4 h at 37 °C in 0.9% saline prior to insertion in the animal. The pumps were implanted subcutaneously in the neck region between the scapulae. Animals were anaesthetised with a mixture of methohexitone and pentobarbitone 1:10 at a dose of 5 ml/kg i.p. An area of the skin was shaved and a 1-cm midline incision was made in the neck. The pump was inserted and the cut closed with two suture clips. The clips were removed 4 days after the operation.

2.4. Measurements

Separate groups of Sprague-Dawley and Dark Agouti animals ($n = 4–8$) were implanted with pumps which delivered either morphine tartrate or codeine phosphate. At the end of 7 days, the animals were weighed and core body temperature measured prior to the administration of naloxone hydrochloride 10 mg/kg s.c. Core temperature and body weight measurements continued for 4 h following naloxone, at 15 min intervals for the first 90 min and then half-hourly for the remainder of the experiment. Body weight was monitored using an electronic balance (Arlec, Malaysia) with an error of less than 1 g at 200 g. Core body temperature was measured with a flexible thermistor probe attached to a digital thermometer (Anritherm model HL500, Amiritsu Meter, NJ, USA). The tip of the probe was inserted 5 cm into the rectum of the rat and left in place for 45 s to obtain a stable reading. The thermometer had an error of less than 0.1 °C at 37 °C. At the end of the observation period, each animal was anaesthetised and a blood sample was collected from the abdominal aorta. These samples were centrifuged and the plasma stored at −20 °C for determination of morphine and codeine concentrations as previously described (Chen et al., 1989). The pumps were in situ and still operational until the end of blood sampling.

2.5. Data analysis

2.5.1. Pharmacodynamic measures

Due to variations in initial body weight, bodyweight loss was calculated as the percentage decrease in weight from the pre-withdrawal value for each animal at each time point.

2.5.2. Pharmacokinetic measures

In the animals dosed with codeine, the metabolic ratio for metabolism to morphine was determined by the ratio of the plasma concentrations of morphine to codeine. Assuming complete bioavailability from the subcutaneous route of administration, the total systemic clearance values of codeine ($CL_{codeine}$) and morphine ($CL_{morphine}$) were calculated as the dose rate (codeine or morphine) divided by the steady state plasma concentration of codeine [$C_{codeine}$] or morphine [$C_{morphine}$]. The fraction of the systemically available subcutaneous dose of codeine converted to morphine (fm) was calculated (Rowland and Tozer, 1995) as:

$$\mathrm{fm} = \frac{[C_{morphine}](CL_{morphine})}{[C_{codeine}](CL_{codeine})} \quad (1)$$

2.5.3. Plasma concentration–response relationships

The relationship between the effect (E) (body temperature and percent body weight loss at 4 h) and plasma

concentration (C) was assessed using the Hill Eq. (2) or inverse Hill Eq. (3) by non-linear least squares regression:

$$E = \frac{E_{max} C^n}{EC_{50}^n + C^n} \quad (2)$$

$$E = E_{max} - \frac{E_{max} C^n}{EC_{50}^n + C^n} \quad (3)$$

where E_{max} is the maximum effect, n is the slope factor and EC_{50} the concentration corresponding to 50% of E_{max}.

2.5.4. Statistics

Data were subjected to a one- or two-way analysis of variance (ANOVA) as required with Tukey's post hoc test. Mann–Whitney *U*-test was used for comparison of metabolic ratios. Data are represented as mean ± S.E.M., $n = 4–8$. Values of $P < 0.05$ were considered significant.

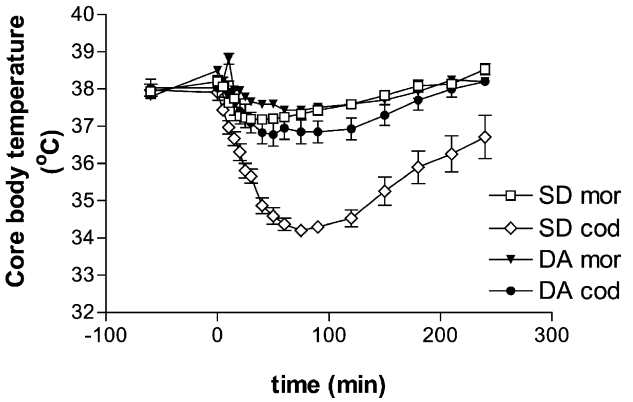

Fig. 2. Core body temperature in female Sprague-Dawley and Dark Agouti rats treated with morphine (mor) or codeine (cod) for 7 days and administered naloxone at time 0. Means ± S.E.M. $n = 4–8$. Sprague-Dawley (cod) was significantly different from Dark Agouti (cod) at all time points between 5 and 210 min ($P < 0.05$, $P < 0.001$).

3. Results

3.1. Plasma concentrations and pharmacokinetics

The plasma concentrations of codeine and morphine in both strains are shown in Fig. 1. There was no significant difference ($P > 0.05$) in plasma codeine concentrations between the strains when codeine was administered or in plasma morphine concentrations when morphine was administered ($P > 0.05$). When codeine was administered, plasma morphine concentrations were significantly lower ($P < 0.05$) in Dark Agouti compared to Sprague-Dawley rats. Plasma codeine concentrations exceeded morphine concentrations in both strains, but this difference was only significant in the Dark Agouti rats ($P < 0.01$). The metabolic ratio of plasma morphine to plasma codeine concentrations after codeine administration was significantly different between the two strains (Sprague-Dawley: 0.71 ± 0.27; Dark Agouti: 0.07 ± 0.04; $P < 0.01$). The total systemic clearance of morphine in the Sprague-Dawley rats was 80 ± 10 ml/min/kg ($n = 8$) and in the Dark Agouti rats 73 ± 12 ml/min/kg ($n = 8$) ($P > 0.05$). The total systemic clearance of codeine in the Sprague-Dawley rats was 64 ± 9 ml/min/kg ($n = 7$) and in the Dark Agouti rats 37 ± 6 ml/min/kg ($n = 4$) ($P < 0.05$). Using the median values for total systemic clearance and plasma concentrations, in the Sprague-Dawley rats, the fraction of the systemically available codeine converted to morphine was 0.64 and in the Dark Agouti rats was 0.13.

Comparisons of plasma morphine concentrations across the four groups of animals showed a significant difference, $F(3,23) = 7.01$, $P < 0.01$. Post hoc tests revealed significant differences between the Sprague-Dawley group administered codeine and each of the other three groups ($P < 0.05$).

3.2. Withdrawal responses

3.2.1. Core temperature

Measurement of core body temperature after naloxone administration in morphine-treated animals showed a decrease in core temperature of 0.5–1 °C ($P < 0.01$) which returned to control values by 4 h (Fig. 2). There was no difference between the two strains following morphine

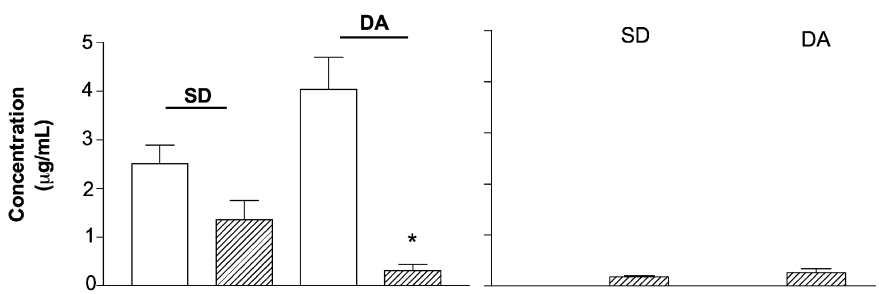

Fig. 1. Mean plasma concentrations of morphine (hatched bars) and codeine (open bars) in female Sprague-Dawley and Dark Agouti rats. These are steady state concentrations measured while the osmotic minipumps are still active. Left panel shows the concentrations after codeine administration and the right panel after morphine administration. The error bars indicate S.E.M., $n = 4–8$. * Significantly different from Sprague-Dawley morphine after codeine administration ($P < 0.05$).

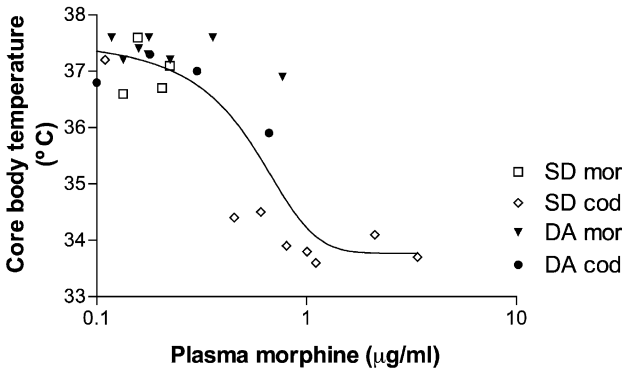

Fig. 3. The relationship between body temperature 4 h after naloxone challenge in female Sprague-Dawley and Dark Agouti rats treated with either codeine or morphine, and plasma morphine concentration ($r^2 = 0.76$, $EC_{50} = 556 \pm 121$ ng/ml, $n = 2.9 \pm 1.5$). The inverse Hill equation was fitted to the data.

treatment ($P > 0.05$). In contrast, naloxone precipitated withdrawal after codeine administration resulted in a large difference between the two strains ($P < 0.0001$). For Dark Agouti rats, the decrease in temperature was similar to the decrease found in the morphine-treated group, but Sprague-Dawley animals treated with codeine had a large (3.5–4 °C) drop in temperature. The hypothermia in this group of animals was still apparent 210 min after naloxone administration, when compared to initial values ($P < 0.05$) (Fig. 2).

3.2.2. Body weight

Weight loss after naloxone administration was similar in all four groups. The total loss in weight ranged from 6.2 ± 0.4 g for morphine-treated Dark Agouti rats to 8.2 ± 0.6 g for codeine-treated rats of the same strain. There were no statistically significant differences between the groups ($P > 0.05$).

3.3. Plasma concentration–effect relationships

When the data from both Sprague-Dawley and Dark Agouti animals with either treatment were combined, a relationship between body temperature and plasma morphine concentrations at 4 h could be described by the inverse Hill equation ($r^2 = 0.76$, $EC_{50} = 556 \pm 121$ ng/ml, $n = 2.9 \pm 1.5$, Fig. 3). There was no relationship between decrease in body temperature and plasma codeine concentration nor between weight loss and either plasma morphine or codeine concentrations using the Hill equation (r^2 values < 0.32).

4. Discussion

It is now accepted that the pharmacological effects of codeine are likely to be mediated through its *O*-demethylated metabolite, morphine. The evidence comes from two models: (i) the genetic model in humans who are genotypic poor metabolisers, and in the Dark Agouti/Sprague-Dawley rat counterparts, and (ii) from metabolic inhibition studies in which the *O*-demethylation of codeine has been inhibited with drugs such as quinidine/quinine, which render(s) the human/animal phenotypically poor metabolisers. In both species, codeine has been shown to be a pro-drug for antinociception (Cleary et al., 1994; Sindrup and Brøsen, 1995), and in humans for the effects on gut motility (Mikus et al., 1997). We are not aware that the influence of these genetic polymorphisms has been assessed in the withdrawal syndrome, which has been reported after cessation of codeine-containing syrups in humans (Mattoo et al., 1997), administration of naltrexone to mice pretreated with codeine (Etemadzadeh et al., 1988) and administration of naloxone to codeine-treated rats (Suzuki et al., 1984).

Temperature reduction and weight loss consistently occur in acute opioid withdrawal in rats (Bhargava, 1977). We showed that there was a relationship between plasma morphine concentration and temperature drop in response to precipitated withdrawal. There was no similar relationship between plasma codeine concentration and withdrawal, lending further support to the evidence implicating morphine as the active moiety when codeine is given. A similar relationship could not be demonstrated for weight loss, suggesting temperature reduction may be a more sensitive index of withdrawal than weight reduction.

The slope factor (n) for temperature reduction in this study is relatively large, indicating a steep plasma concentration–effect relationship. We are not aware that such a relationship has been reported previously for withdrawal in an animal model. However, our results are in accord with the steep brain morphine concentration–effect relationship, which has been reported for antinociception in the male adult hooded Wistar rat ($n = 2.42 \pm 0.53$) (Van Crugten et al., 1997a) and the aged male hooded Wistar rat ($n = 2.5 \pm 1.2$) (Van Crugten et al., 1997b) These authors also administered the active metabolite of morphine, morphine-6-glucuronide, and concluded that it is also likely to exhibit a steep concentration–effect relationship for antinociception. Steep plasma concentration–effect relationships have also been reported for methadone. These have been observed for withdrawal, direct opioid effects, mood and antinociception in humans (Inturrisi et al., 1987; Dyer et al., 1999; 2001), and for antinociception in the rat (Garrido et al., 1999). Overall, these studies indicate that a steep concentration–effect relationship is likely to be a general phenomenon for μ-opioid receptor agonists.

Plasma morphine concentrations were low in the Dark Agouti rat after codeine administration and similar to those achieved after morphine, although the doses were about 10-fold different. As anticipated, significantly higher plasma morphine concentrations were achieved in the Sprague-Dawley strain compared to the Dark Agouti strain following codeine dosing. The 10-fold difference in plasma

morphine to codeine metabolic ratios between the two strains shown here is in agreement with the 10-fold difference reported for the codeine intrinsic clearance in liver microsomes from Sprague-Dawley and Dark Agouti rats (Mikus et al., 1991a). Yamamoto et al. (1998) reported a 17-fold difference in CYP2D-dependent debrisoquine 4-hydroxylation activity between Sprague-Dawley and Dark Agouti rats. There is increasing evidence to indicate that the polymorphic debrisoquine 4-hydroxylase activity in the Dark Agouti and Sprague-Dawley rat strains is due to differences in CYP2D2 expression rather than CYP2D1 (Yamamoto et al., 1998; Schulz-Utermoehl et al., 1999). Despite a 30–40-fold lesser expression of CYP2D2 in female Dark Agouti than female Sprague-Dawley rats (Schulz-Utermoehl et al., 1999), the Dark Agouti strain in the present study was still able to generate plasma morphine concentrations equivalent to those achieved after morphine administration. These low concentrations were clearly sufficient to produce withdrawal as measured by both endpoints.

Our values for total systemic clearance of codeine in the female Sprague-Dawley rat (64 ± 9 ml/min/kg) were similar to those obtained by Xie and Hammarlund-Udenaes (1998) in the male Sprague-Dawley rat (77 ml/min/kg). These workers also found higher total codeine than morphine concentrations in plasma after codeine administration. Unbound concentrations, as measured by microdialysis, were equivalent. The time course of brain morphine concentrations is likely to be pivotal in interpreting the effects of codeine. Xie and Hammarlund-Udenaes (1998) were unable to detect morphine in brain microdialysate after intravenous codeine administration of 10, 20 mg/kg or an infusion designed to achieve a plasma concentration of 2500 ng/ml, but could measure it in brain homogenate. Chen et al. (1990) were also able to measure morphine in brain homogenate 30 and 60 min after intraperitoneal codeine, but could only detect it at 60 min, and then at much lower concentrations, after intraperitoneal morphine. The plasma to brain concentration ratio of morphine in rats given morphine subcutaneously was about 3–4:1 (Van Crugten et al., 1997a,b), indicating that this relatively polar molecule partitions less easily into brain. Our values for the total systemic clearance of morphine in the two rat strains (80 ± 10 and 73 ± 12 ml/min/kg) are similar to those reported by others (range 37–108 ml/min/kg), as reviewed by Milne et al. (1996).

Our results indicate that codeine-induced dependence and withdrawal, which have been reported to occur in people abusing codeine or taking it for chronic pain (Sproule et al., 1999), are a consequence of morphine dependence. Thus, in the rat, the CYP2D2 deficiency appears to protect against the development of codeine-induced physical dependence. This phenomenon has yet to be investigated in humans. However, it has been demonstrated that inhibition of CYP2D6 reduces the subjective effects of codeine (Kathiramalainathan et al., 2000) and decreases codeine intake by long-term users (Romach et al., 2000). Thus, variation in CYP2D6 activity could help explain individual differences in the propensity to develop codeine dependence, while modification of the activity of the enzyme could play a role in treatment.

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