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Developmental Regulation of Codeine Analgesia in the Rat

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Background: **Codeine analgesia is dependent on metabolism to morphine. Metabolic capability is genetically determined in rats and humans, and individuals can be classified as extensive or poor metabolizers, as determined by the extent of production of morphine. Codeine is often given to infants and children. The aim of this study was to investigate the effects of developmental age on codeine analgesia in rats.**

Methods: **The effects of codeine were compared with those of morphine using withdrawal reflex responses to mechanical stimuli (with and without inflammation) and to noxious heat in two strains of rats (Sprague–Dawley and Dark Agouti) that have been used to model human metabolic phenotypes because of marked differences in enzyme activity. Effects of the opioids were compared at 3, 10, and 21 days of age and in adult rats.**

Results: **Consistent age-related changes in the efficacy of codeine relative to morphine were noted for both strains of rats. For the extensive metabolizer (Sprague–Dawley) strain, codeine efficacy was substantially lower at 3 days of age ($P < 0.001$), but there was no difference between the effects of codeine and morphine for 10- and 21-day-old rats and adults ($P > 0.05$). Poor metabolizers (Dark Agouti strain) also had comparatively low efficacy for codeine compared with morphine in 3-day-old rats and in adults ($P < 0.001$). In 10- and 21-day-old Dark Agouti rats, there was no difference between either drug ($P < 0.05$).**

Conclusions: **Codeine analgesia is developmentally regulated, with low efficacy in the early postnatal period. Effects in the adult rat were not predictive of efficacy in development in either strain, which has important implications for further study and, possibly, for clinical use.**

CODEINE is considered to be a moderate-potency opioid, and it is often suggested for the treatment of pain in the neonatal period, infancy, and childhood.[1] Codeine is metabolized to morphine by O-demethylation, and available data strongly suggest that codeine analgesia is wholly or mostly dependent on morphine production.[2–4]

Conversion of codeine to morphine is catalyzed in humans by the cytochrome P450 enzyme CYP2D6, for which a large number of different genetic variants are known to exist, leading to a wide spectrum of metabolic capabilities within and between populations.[5,6] Both laboratory and human studies have confirmed that individuals with a limited capacity for this reaction also have less analgesia.[7–9] Individuals can be phenotyped as extensive metabolizers with normal enzyme activity or poor metabolizers with very little or no activity. Actually, a range of activities is usual within a population, leading to a wide variation in the amount of morphine produced. This may explain the low efficacy of codeine observed in many laboratory and clinical studies and the very high number needed to treat that has been calculated for codeine from systematic reviews.[2,10,11]

The rat is an established model of nociceptive development, and the female Sprague–Dawley and Dark Agouti strains of rats have been extensively used to model human extensive metabolizer and poor metabolizer phenotypes, respectively, in studies of codeine analgesia.[7,12,13] Because codeine is used clinically in pediatric practice, the aim of the current study was to investigate the influence of postnatal development on the antinociceptive effects of codeine in Sprague–Dawley and Dark Agouti rats.

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Materials and Methods

Female Sprague–Dawley and Dark Agouti adult rats (63–70 days old) and rat pups aged 3, 10, and 21 days were obtained from University College London Biologic Services (London, United Kingdom). Experiments were performed under license in accordance with Home Office regulations (Home Office, London, United Kingdom).

Opioids and Injection Techniques

Morphine sulfate (1 mg/ml), codeine phosphate (60 mg/ml), and naloxone 20 (μg/ml) were diluted in sterile normal saline to achieve the required dose at a uniform volume of 10 ml/kg. Drugs were given by the intraperitoneal route into the left lower quadrant of the abdomen with the animal awake and securely restrained in all experiments. The injections were administered using a 0.5-, 1-, or 2-ml syringe, depending on the volume to be injected, and a 30-gauge needle.

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Table 1. Force and Log_e of Each Numbered von Frey Hair

von Frey Hair No.	Force, g	log_e
1	0.015	−4.12
2	0.04	−3.22
3	0.06	−2.81
4	0.12	−2.12
5	0.17	−1.77
6	0.36	−1.02
7	0.55	−0.59
8	0.92	−0.08
9	1.37	0.31
10	2.2	0.79
11	3.4	1.22
12	5.5	1.71
13	10.5	2.35
14	17.1	2.84
15	25.8	3.25
16	37	3.61
17	63.5	4.15
18	105	4.65
19	157	5.06
20	248	5.51

Mechanical Thresholds

Hind limb flexion withdrawal thresholds to mechanical stimulation were determined using calibrated von Frey hairs, as described previously.[14] The stimulus was applied to the dorsal surface of the hind paw three times at 1-s intervals for each von Frey hair, starting with the lowest intensity stimulus, until a reflex was elicited, and the threshold was recorded as the von Frey hair number (table 1). The initial or baseline threshold was determined, the opioid to be tested was then injected (time = t_0), and further thresholds were determined at intervals of 15 min up to 90 min (t_{90}) and thereafter at 120 min (t_{120}) and 150 min (t_{150}), unless the threshold had previously returned to baseline. In older animals, whose baseline thresholds were close to the highest available von Frey hair force, if the mechanical threshold was not reached during testing, a von Frey hair number of 21 was assigned to allow visual comparison, but this value was not included in the analysis. To simplify comparison of thresholds and threshold differences between the age groups, data are expressed as von Frey hair number rather than force or percentage change. This approach converts the threshold values to a linear scale and allows changes in thresholds to be compared even if the original baselines were not the same.[15]

Carrageenan Inflammation

An inflammatory response was induced in the left hind paw by intraplantar injection of a carrageenan solution, 1%. This has been shown to be a reliable method of producing experimental hypersensitivity or allodynia in previous studies.[16–18] Before carrageenan injection, mechanical sensory thresholds using von Frey hairs were measured in both the ipsilateral hind limb and the contralateral (*i.e.*, right) hind limb. Carrageenan (1 μl/g) was subcutaneously injected on the plantar surface of the left hind paw during general anesthesia using a mixture of halothane, 2–4%, in 100% oxygen. After carrageenan administration, ipsilateral mechanical sensory thresholds decreased with time to a minimum at 3 h, with this reduction remaining constant up to at least 6 h (when measurements were terminated). Experiments investigating the effects of study drugs on carrageenan hypersensitivity were therefore conducted after 3 h.

Noxious Heat

The time to withdrawal of the limb in response to noxious heat was determined using a hot water bath, as described previously.[19] The hind paw was placed into the water, and the time to withdrawal of the limb was recorded using a stopwatch accurate to 0.1 s. The average of three repetitions was recorded, with a 5-min gap allowed between each recording. An initial pilot study was carried out to determine the optimal temperature of the water for each age group: *i.e.*, the temperature that gave an easily recordable and reproducible baseline plus an increase from baseline after opioid administration that was long enough to show a significant change but not so long as to not produce the withdrawal. The optimal temperature was 45°C for 3-day-old rats and 48°C for 10- and 21-day-old rats. Initial or baseline thermal latency was determined, and the time to withdrawal was recorded every 15 min up to 90 min and again at 120 min after injection of opioid or saline.

Dose and Age Groups

The effects of codeine on mechanical sensory thresholds were compared at the following postnatal ages and doses for Sprague–Dawley rats: postnatal day 21: 7.5, 10, and 15 mg/kg; postnatal day 10: 7.5, 10, and 15 mg/kg; and postnatal day 3: 7.5, 10, 15, and 30 mg/kg. Controls received morphine (2 mg/kg) and saline at each age; this morphine dose produced a robust submaximal reduction in threshold without obvious sedation. Species (Sprague–Dawley and Dark Agouti strains) and sensory modalities (inflammatory hypersensitivity and noxious heat) were compared further using codeine (10 mg/kg) and morphine (2 mg/kg) at doses with approximately equivalent effects (figs. 1 and 2). Naloxone reversibility was also tested at postnatal days 3 and 10 using simultaneous intraperitoneal injections of opioid and naloxone at 20% of the dose of either codeine or morphine after carrageenan-induced hypersensitivity.

Statistical Analysis

Data were analyzed using Microsoft Excel 2000 (Microsoft Corporation, Redmond, WA) and the statistical software Graphpad Prism 3.0 (Graphpad Software, Inc., San Diego, CA). Threshold data were analyzed in von Frey hair units according to a logarithmic transformation, as described previously, or in seconds for heat.[15,19,20] For analysis, the data from each experiment

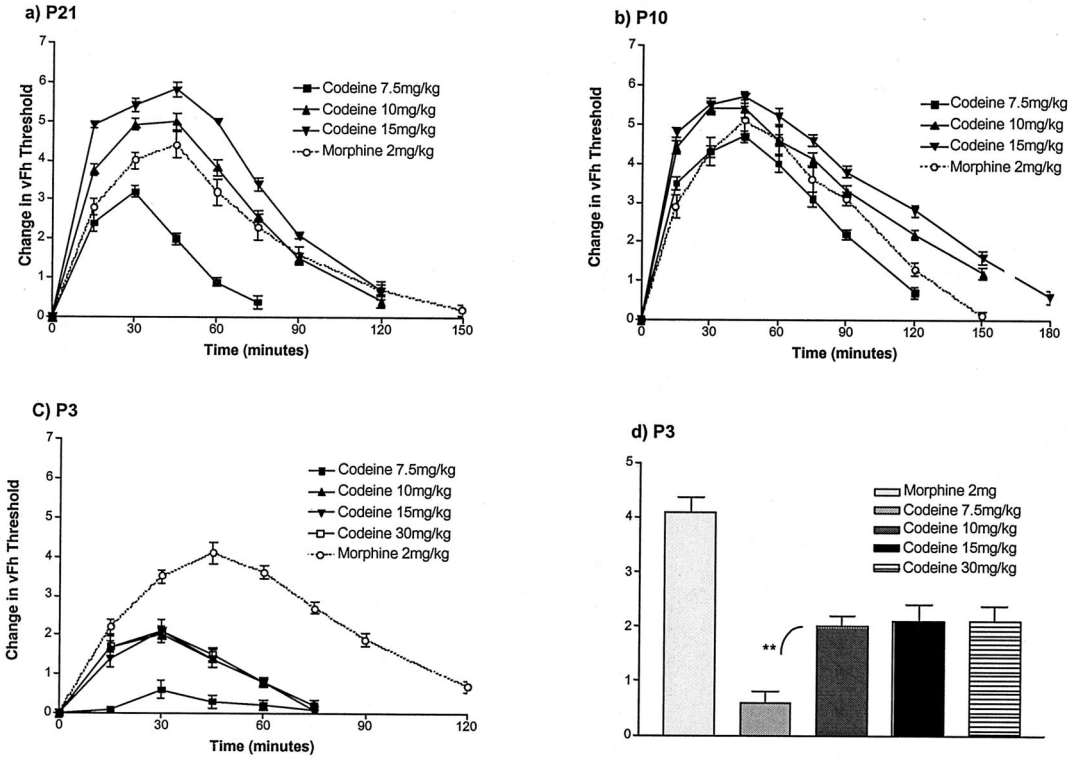

Fig. 1. Effects of codeine and morphine on mechanical sensory thresholds for Sprague–Dawley rats (the extensive metabolizer group). Time course of the effect on postnatal days 21 (P21, *a*); 10 (P10, *b*); and 3 (P3, *c*). (*d*) Peak effects on P3 for morphine and codeine. ** = highly significant ($P < 0.001$). vFh = von Frey hair.

were also converted when possible into two summary statistics: peak effect (defined as the largest change in mechanical threshold from baseline) and area under the curve (representing a summary of the strength and duration of response). Continuous variables were compared using ANOVA with posttest Bonferroni corrections. Categorical data were analyzed using the chi-square test. For all comparisons, $P < 0.05$ was considered significant. When determining the mechanical threshold for adult rats, the postopioid threshold was sometimes greater than the maximum available force that did not also cause damage to the skin of the foot (von Frey hair number 21). In this case, the data were analyzed by comparison of the proportion of animals in each group with thresholds higher than could be elicited by the strongest stimulus at the 45-min time point using the chi-square test.

Results

The mean weights of the rats according to age and species are given in table 2.

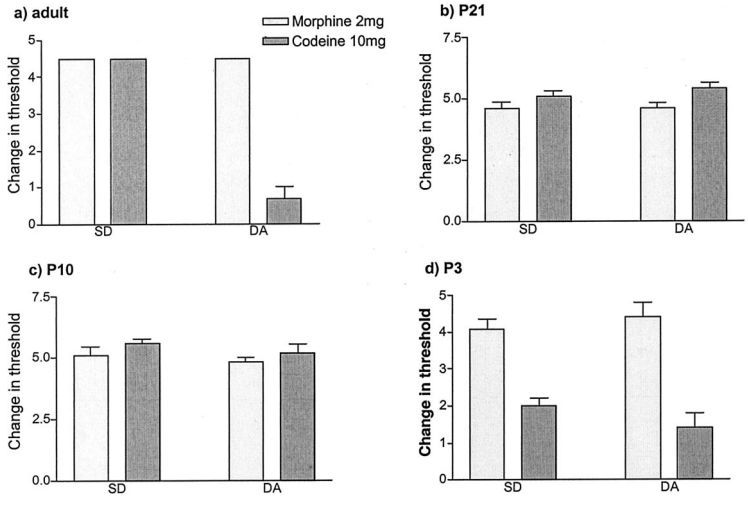

Fig. 2. Comparison of the effects of morphine (2 mg/kg) and codeine (10 mg/kg) on mechanical thresholds for Sprague–Dawley (SD, the extensive metabolizer group) and Dark Agouti (DA, the poor metabolizer group) rats. P = postnatal day.

Table 2. Mean Weights ± SEM of Rats at Each Age

	Mean Weight (g) ± SEM	
Postnatal Day	SD	DA
3	9.47 ± 0.17	8.86 ± 0.17
10	23.05 ± 0.29	14.49 ± 0.27
21	48.81 ± 0.62	44.45 ± 1.36
63–70	235.5 ± 11.1	147.8 ± 2.58

DA = Dark Agouti rats; SD = Sprague–Dawley rats.

Postnatal Development of Mechanical Sensory Thresholds

Figure 3 shows the mean (baseline) sensory thresholds to mechanical stimulation for both Sprague–Dawley and Dark Agouti rats on postnatal days 3, 10, and 21 and when fully mature (postnatal days 63–70). Thresholds increased with postnatal age for both strains. Comparison between the strains showed differences in threshold on postnatal day 3 (9.75 *vs.* 8.4, respectively) and postnatal day 10 (11.48 *vs.* 9.22, respectively) ($P < 0.001$), but no difference was found on postnatal day 21 or postnatal days 63–70 ($P > 0.05$).

Effect of Codeine: Sprague–Dawley Strain

Mechanical Sensory Thresholds. Figure 1, a–c, shows the effects of codeine (7.5–15 mg/kg) on the mechanical thresholds for Sprague–Dawley rat pups at postnatal days 3, 10, and 21, and 30 mg/kg at postnatal day 3, with morphine 2 mg/kg given to controls. There was a significant interaction between age and drug as well as a significant effect of age and drug dose on both peak effect ($P < 0.0001$) and area under the curve ($P < 0.0001$). The response to codeine on postnatal day 3 was significantly less at all doses than on postnatal days 10 or 21 ($P < 0.001$). In addition, on postnatal day 3, a maximum or ceiling effect of codeine was observed with 10 mg/kg, which was not increased by higher doses of 15 or 30 mg/kg (these being ≈ 50% less effective than morphine control values) (fig. 1d) ($P < 0.001$). On postnatal days 10 and 21, responses to morphine (2 mg/kg) and codeine (10 mg/kg) were approximately equivalent (area under the curve, $P > 0.05$), and these doses were therefore used in subsequent experiments.

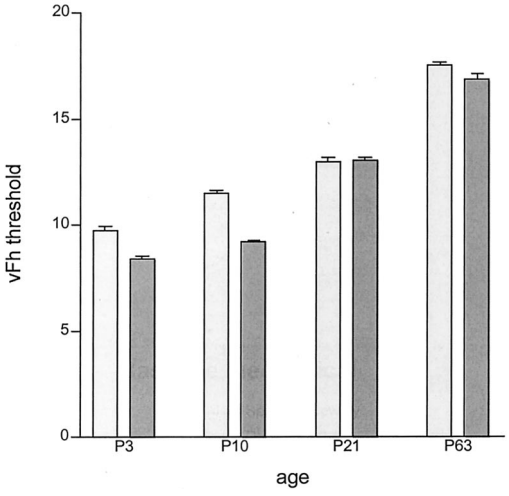

Fig. 3. Baseline mechanical thresholds expressed as von Frey hair (vFh) number at increasing developmental age. Sprague–Dawley rats (*dark gray bars*) are the extensive metabolizer group, and Dark Agouti rats (*light gray bars*) are the poor metabolizer group. P = postnatal day.

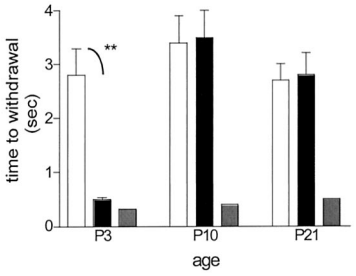

Fig. 4. Latency of withdrawal in seconds from noxious heat for Sprague–Dawley rats (the extensive metabolizer group) on postnatal days 3 (P3), 10 (P10), and 21 (P21). *Open bars* = morphine 2 mg/kg; filled bars = codeine 10 mg/kg; *gray bars* = saline; ** = highly significant ($P < 0.001$).

Withdrawal Latency to Noxious Heat. The withdrawal latency of the hind limb from a heated water bath increased with postnatal age; the mean latencies on postnatal days 3, 10, and 21 were 0.31, 0.45, and 0.53 s. Figure 4 shows the maximum latencies after intraperitoneal injection of codeine (10 mg/kg). Codeine was also much less effective at increasing the latency on postnatal day 3 than on postnatal days 10 and 21 ($P < 0.001$).

Mechanical Thresholds after Inflammatory Hypersensitivity. Figure 5 shows the effects of intraperitoneal codeine, morphine, and saline on carrageenan hypersensitivity in Sprague–Dawley rat pups on postnatal days 3, 10, and 21. Carrageenan inflammation induced hypersensitivity as a decrease in threshold for the three ages tested. The maximum reduction in threshold after carrageenan hypersensitivity was age-dependent; the reduction in threshold was less on postnatal day 3 than on both postnatal days 10 and 21 ($P < 0.001$), as

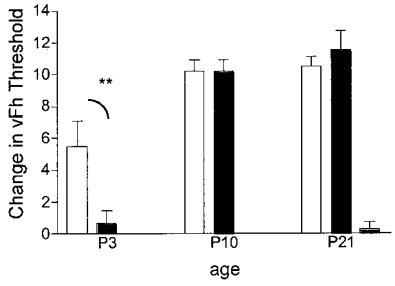

Fig. 5. Effects of morphine 2 mg/kg (*open bars*) and codeine 10 mg/kg (*filled bars*) on mechanical thresholds reduced by carrageenan-induced hypersensitivity for Sprague–Dawley rats (the extensive metabolizer group) on postnatal days 3 (P3), 10 (P10), and 21 (P21). *Gray bars* = saline; ** = highly significant ($P < 0.001$). vFh = von Frey hair.

shown previously.[18,20] In comparison with morphine, on postnatal day 3, codeine (10 mg/kg) had little effect on carrageenan hypersensitivity ($P < 0.001$); in contrast, the effects of codeine and morphine were similar on both postnatal days 10 and 21 ($P > 0.05$).

Naloxone Reversibility. Naloxone delayed and significantly attenuated the increase in threshold after the injection of morphine and codeine on postnatal day 10 and the increase in threshold after morphine injection on postnatal day 3 ($P < 0.01$). On postnatal day 3, there was no increase in threshold for animals treated with codeine with or without naloxone.

Effect of Codeine: Dark Agouti Strain

Figure 2 shows the mechanical threshold changes for codeine at 10 mg/kg for adult Dark Agouti and Sprague–Dawley rats and rat pups at ages 3, 10, and 21 days compared with those for morphine at 2 mg/kg. As expected (fig. 2a), codeine had much less of an effect than morphine on mechanical thresholds for the Dark Agouti adults, but the effects of codeine and morphine were similar for Sprague–Dawley adults. On postnatal days 10 and 21 (fig. 2b and c), the effect of codeine was similar to that of morphine, and no difference was found between the strains ($P > 0.05$ for all groups). At the youngest age (fig. 2d), codeine was significantly less effective than morphine in both strains ($P < 0.001$), with no difference between the two strains ($P > 0.05$).

Discussion

Although the importance of genetic determinants on the efficacy of codeine are well recognized and have been reviewed recently, to our knowledge, the influence of development has not previously been studied.[2–4] In the current study, we found that postnatal age had a profound effect on the efficacy of codeine in two strains of rats (Sprague–Dawley and Dark Agouti) that have been used extensively as models of human "extensive" and "poor" metabolic phenotypes for codeine. In both Sprague–Dawley and Dark Agouti strains, the efficacy of codeine is low in early development and increases with age to postnatal day 21. In the Sprague–Dawley strain (extensive or normal metabolic capacity), the efficacy of codeine is maintained after this time; in the Dark Agouti strain, the efficacy in adults is low. In addition, in the youngest Sprague–Dawley rats, the maximum efficacy appears to be independent of dose: *i.e.*, a ceiling effect is observed, which is not changed by increasing the dose of codeine.

Investigation of analgesic efficacy in development is complicated by changing sensory processing in early life.[12,21] Because of this, we used three experimental paradigms of pain and nociception: mechanical sensory thresholds with and without inflammatory hypersensitivity and the withdrawal latency to noxious heat. Codeine analgesia is thought to be due to metabolism to morphine, and for this reason, the effects of codeine were compared with those of morphine at all ages. The threshold of the cutaneous flexion withdrawal reflex to mechanical, and other, stimuli is well established as a tool in pain research and has been extensively used in the assessment of antinociceptive agents.[22,23] The current study confirms that in young rat pups, as in human infants, the flexion withdrawal reflex can be elicited by a weaker mechanical stimulus than that required in adults and that the force of the stimulus required to elicit the reflex increases with age.[14,24,25] Despite its lower threshold, in common with the adult reflex, the neonatal reflex clearly demonstrates a hypersensitivity response to injury with a reduction in threshold, which can be reversed by analgesics such as opioids and local anesthetics.[18–20,26] The withdrawal response to noxious heat, as in the tail-flick test and with the hot plate and hot water bath, has also been frequently used in pain research and in assessment of antinociceptive agents. The latency of withdrawal increases with age, which is consistent with previous studies showing that lower temperatures are required and withdrawal times are shorter for younger animals, and that opioids are capable of prolonging latencies at all ages.[19,27] A number of factors could account for underlying differences in basal response: developmental changes in skin thickness and texture, differences in the amount of subcutaneous fat, relatively deeper positioning of thermal nociceptors in older animals, and differences in afferent fiber function and their central connections.[27]

The current study confirms that the analgesic response to codeine relative to morphine in mature (63- to 70-day-old) Sprague–Dawley and Dark Agouti rats differed between the two species. In the Sprague–Dawley wild- or normal-type rats, codeine and morphine had similar efficacies, with a potency ratio of 5:1. In Dark Agouti adults, the response to codeine was significantly less than that in Sprague–Dawley rats, in contrast to the effect of morphine (which was similar in both). This is in agreement with previous studies and is consistent with the assumption that metabolism of codeine to morphine is responsible for the analgesic effect of codeine, because the adult Dark Agouti rat is known not to be able to make this conversion.[7,13]

The effects of codeine relative to morphine were also age-dependent. In the youngest rats, regardless of species, the response to codeine (at a range of doses) was much less than that to morphine. One potential explanation of the decreased effect after codeine injection found for the 3-day-old neonatal rats is the low activity of the O-demethylase reaction, which converts codeine to morphine. Total enzyme activity in Sprague–Dawley rats on postnatal day 3 has been shown to be about one fifth of the activity attained in later development.[28] This also explains the lack of increased effect of codeine when using higher doses, up to 30 mg/kg at this age, because metabolic capacity may not be changed by an increase in

substrate availability. As in adults, Dark Agouti infant rats might have been expected to have a poor response to codeine; paradoxically, a relative increase in efficacy was observed at 10 and 21 days. The development of enzyme activity in Sprague–Dawley rats has been shown to increase dramatically after birth to peak on postnatal days 20–30, decreasing to intermediate values therafter.[28,29] To our knowledge, the development of O-demethylase activity in Dark Agouti rats has not been investigated; however, these results suggest that, as with Sprague–Dawley rats, enzyme activity is low at birth. Apparent relative increases in the rate and extent of drug metabolism for humans between 2 and 7 yr have been attributed to an increased liver-to-body mass ratio in this age group.

This type of developmental effect may also be a factor influencing the efficacy of codeine in the Dark Agouti rat on postnatal days 10 and 21. In humans, it is known that O-demethylase activity is also low in the neonatal period and reaches only 25% of adult levels by 5 yr of age[30–32]; however, little is known about the interactive influence of postnatal development and phenotype on codeine metabolism or efficacy. Metabolic activity may not be the only influence on the effects of codeine; other factors may also be important contributors to the species- and age-related differences observed here, such as substrate concentration, maturational changes in renal and other excretory pathways, or differences in sensory development (*e.g.*, receptor distribution or function). Furthermore, potential developmental changes in P-glycoprotein, an efflux transporter protein in endothelial cells of the central nervous system, may also affect opioid efficacy.[33] For human neonates and infants, the efficacy of codeine has not been investigated, to our knowledge. The influence of genotype, phenotype, morphine production, and adverse effects has similarly not been studied, but the currently available evidence suggests that developmental factors may be important. Until such studies have been performed, the use of codeine in the very young is questionable.

In summary, the effects of codeine are both developmentally and genetically regulated in the rat. Marked age-dependent differences in the response to codeine exist that are profoundly influenced by strain, with generally low efficacy in the neonatal period followed by differing developmental patterns depending on genotype. These findings highlight the complexity of factors determining the efficacy of codeine and have important implications for further study and clinical use.

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