Evidence for morphine-independent central nervous opioid effects after administration of codeine: Contribution of other codeine metabolites

Objective: **Our objective was to investigate whether codeine or one of its metabolites contributes substantially to central nervous effects independent from the cytochrome P450 (CYP) 2D6–mediated *O*-demethylation to morphine.**

Methods: **After oral administration of codeine, plasma concentrations of codeine and its metabolites, as well as pupil size as a measure of central nervous effects, were measured in 11 healthy volunteers representing poor, intermediate, extensive, and ultrarapid metabolizers for CYP2D6. Subsequently, the observed plasma morphine concentrations were mimicked by use of computerized morphine infusion, and the miotic effects were compared with those observed after codeine administration. The contribution of codeine, codeine-6-glucuronide, norcodeine, morphine, morphine-6-glucuronide, and normorphine to the miotic effects was analyzed by means of pharmacokinetic-pharmacodynamic modeling.**

Results: **The areas under the curve of the miotic effects after codeine were 1.7 ± 2 times greater than after morphine ($P < 0.01$). This contrasted to similar or even lower morphine concentrations after codeine than after morphine (area under the curve ratio, 0.5 ± 0.4; $P = .21$). A pharmacokinetic-pharmacodynamic fit of the miotic effects by use of morphine as the only active moiety was most significantly ($P < .0001$) improved when codeine-6-glucuronide as a second active moiety was added.**

Conclusion: **CYP2D6-dependent formation of morphine does not explain exclusively the central nervous effects of codeine. Codeine-6-glucuronide is the most likely additional active moiety. (Clin Pharmacol Ther 2006;79:35-48.)**

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Cytochrome P450 (CYP) 2D6 catalyzes the *O*-demethylation of codeine into morphine, which has an approximately 200 times higher affinity at μ-opioid receptors than codeine.[1,2] Therefore the effects of codeine are considered to depend largely on morphine formation.[3] Indeed, when CYP2D6 was not functional because of a loss of functional mutations or gene deletion[4-6] or pharmacologic inhibition,[7,8] codeine was virtually ineffective. However, inhibition of CYP2D6 by quinidine did not always completely extinguish the analgesic effects of 100 mg codeine despite a very low plasma morphine concentration.[9] Subjects with nonfunctional CYP2D6 had side effects from 170 mg codeine,[10] and in 4 patients with an inability to *O*-demethylate codeine, an analgesic effect of 90 mg codeine after dental surgery was reported.[11] In addition, codeine was proposed to produce cognitive impairment independent of measurable blood morphine concentrations.[12] Furthermore, the prolongation of gastrointestinal transit caused by 50 mg codeine was reported to not

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This study was supported by Dr Robert Pfleger Stiftung, Bamberg, Germany, and by the Robert-Bosch Foundation, Stuttgart, Germany.

Received for publication July 19, 2005; accepted Sept 12, 2005.

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0009-9236/$32.00

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doi:10.1016/j.clpt.2005.09.005

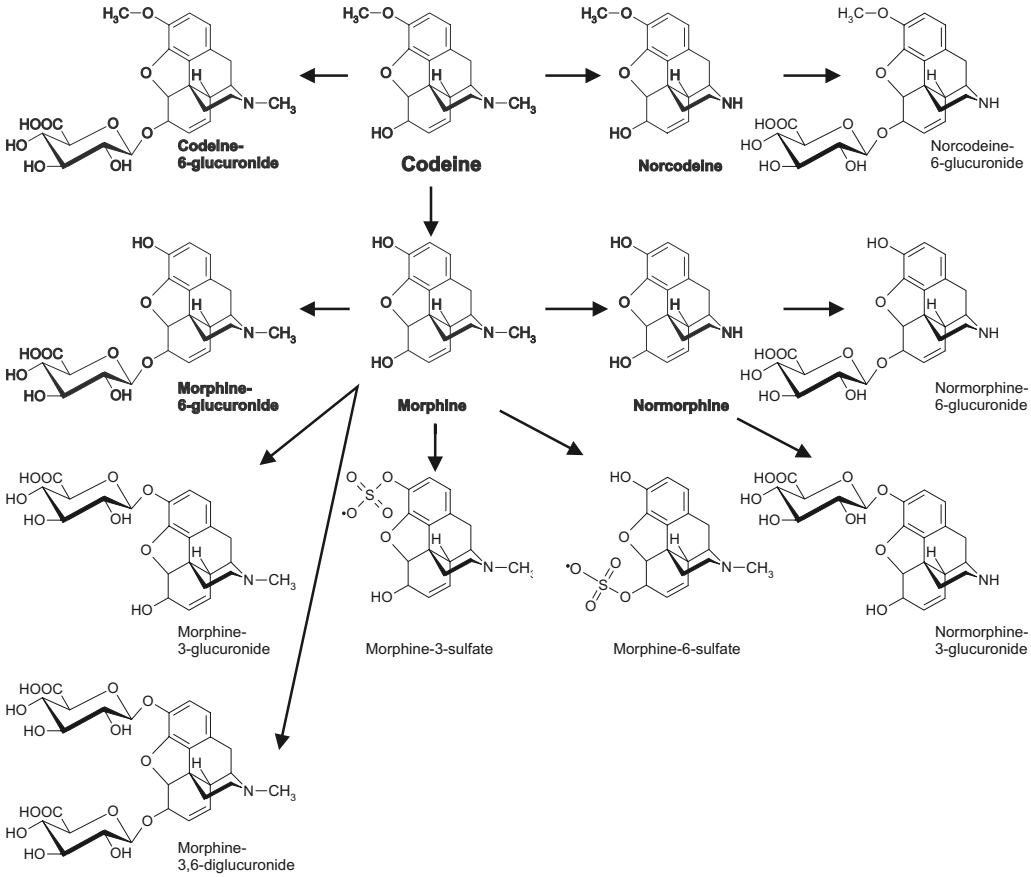


Fig 1. Metabolism of codeine, with the more important metabolites assessed in this study being shown in boldface type.

depend on the formation of morphine.[13] This suggests that plasma morphine does not fully explain the effects of codeine and that either codeine itself or one of its other metabolites contributes to the effects. The metabolites of codeine (Fig 1) have affinities at μ-opioid receptors comparable to or greater than that of codeine. Specifically, inhibition constant (K_i) values for D-Ala[2], *N*-MePhe[4], Gly-ol[5]-enkephalin (DAMGO) replacement are 0.6 ± 0.45 nmol/L for morphine-6-glucuronide (M6G), 1.2 ± 0.32 nmol/L for morphine, 4.7 ± 0.51 nmol/L for normorphine, 238.7 ± 91.5 nmol/L for codeine-6-glucuronide (C6G), 248.3 ± 101.1 nmol/L for codeine, and 266.9 ± 107.5 nmol/L for norcodeine.[14] Knowledge about the intrinsic activity of the codeine metabolites at μ-opioid receptors is incomplete, except for that of codeine and morphine, with K_i values of 1:50 for inhibition of forskolin-induced intracellular accumulation of cyclic adenosine monophosphate of 5517 nmol/L for codeine and 109 nmol/L for morphine.[2]

The morphine-independent opioid agonists (ie, codeine, C6G, and norcodeine) are especially candidates that may explain opioid effects after codeine administration independent from or in addition to the CYP2D6-dependent effects of morphine. This study investigated the issue of whether the effects of codeine are entirely mediated by morphine by comparing central nervous opioid effects in humans after administration of codeine and of morphine and relating the plasma concentrations of codeine and its important metabolites with μ-agonist activity to the observed effects.

The codeine effects reported as not being attributable to morphine formation consisted of a variety of opioid actions such as cognitive impairment,[12] sedation, dizziness, euphoria/dysphoria, headache, blurred vision, itching, flush,[10] or prolongation of gastrointestinal transit.[13] Analgesia has also been reported in this context,[11] but other investigators explicitly excluded it as a morphine-independent effect after codeine administration.[10] Thus, because analgesic effects were uncertain

to provide a basis to judge the contribution of codeine metabolites other than morphine to its effects and side effects such as sedation or headache often provide a weak data basis for exact quantification of opioid effects, we chose the pupil diameter as the target parameter for this study (for review, see reference 15). Pupil size has been found to be an acceptable surrogate for opioid effects[16-22] and had the advantage in the present context of an easily repeatable parameter to obtain frequent sensitive measurements of the opioid effect that could provide a suitable data basis for the presently used pharmacokinetic (PK)–pharmacodynamic (PD) modeling approach to the contribution of codeine metabolites to its effects.

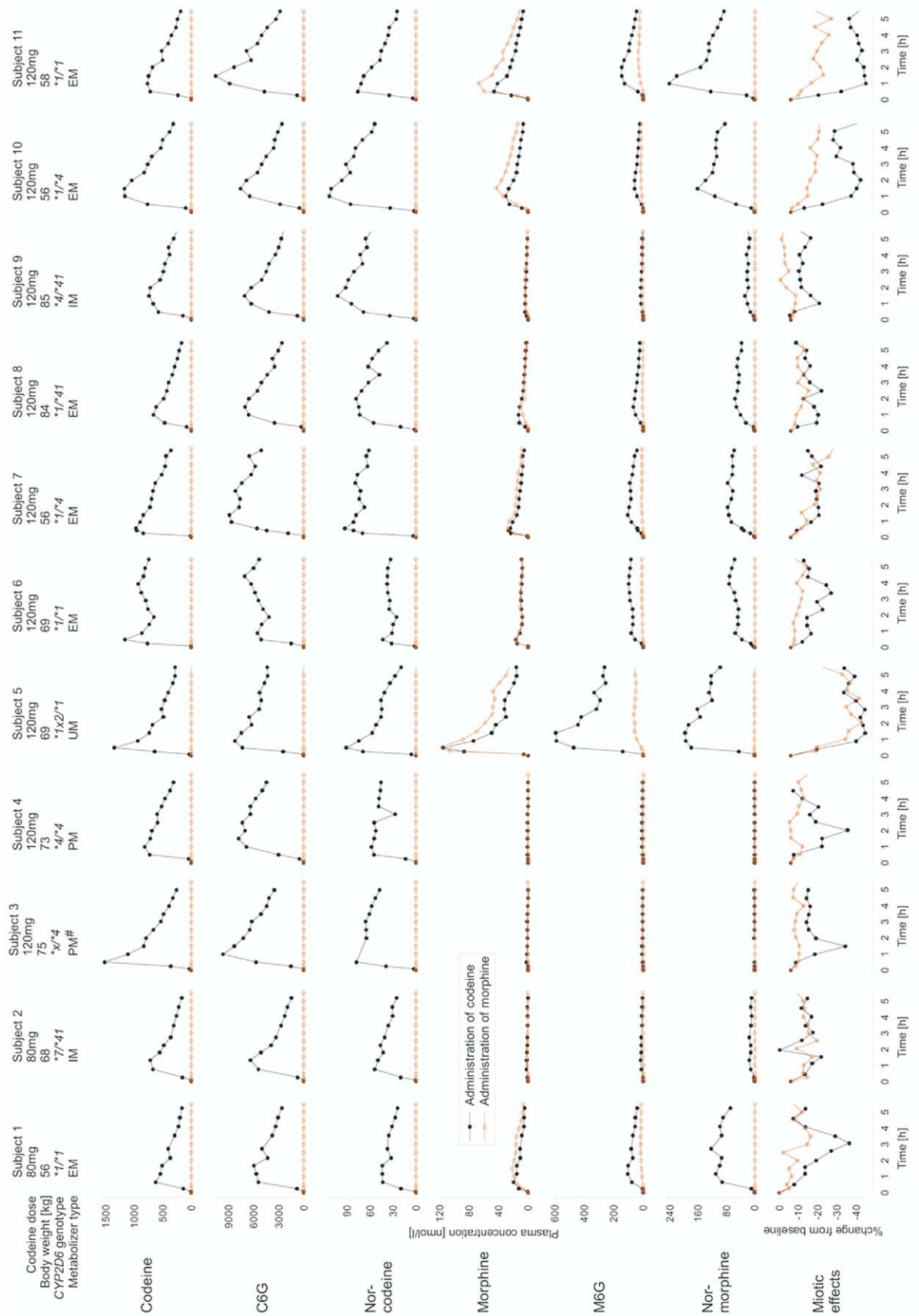
METHODS

Study design and medications. Sixteen healthy young volunteers (all of whom were white, although one had a relative of Arab origin in his ascending line) were recruited for this open-label study. On the first study day, subjects first received an oral dose of codeine after an overnight fast. Plasma concentrations of morphine were analyzed. Subsequently, the observed morphine plasma concentrations after codeine administration were mimicked by administration of morphine via computerized intravenous infusion. Because of the time required for analysis of the plasma concentrations and subsequent scheduling of the second study day, the washout phase between the 2 study occasions (codeine and morphine) was up to 4 months for particular subjects. Because of this long interval, 5 subjects were not available or not willing to participate at the second occasion, and therefore data from 11 subjects were ultimately available for comparisons of the codeine and morphine conditions (Table I). The miotic effects were compared between codeine and morphine administration. If the miotic effects after codeine administration were not greater than those after morphine administration, then morphine would fully explain the miotic effects after codeine. The subjects received a standard meal at 4 hours after administration of codeine or the start of the morphine infusion. Intake of nonalcoholic fluids was allowed at will. The health of the subjects was ascertained by physical examination and routine laboratory tests including markers of liver function such as γ-glutamyltransferase, ALT, and AST. The regular use of drugs including those of herbal origin was not allowed from 4 weeks before the study up to the end of the study period, with the exception of oral contraceptives, and 2 weeks before each study day, the use of over-the-counter drugs was prohibited. The study was conducted according to the Declaration of Helsinki on biomedical research involving human subjects, the protocol was approved by the University of Frankfurt Medical Faculty Ethics Review Board (Frankfurt, Germany), and the subjects provided written informed consent with regard to the study medications and procedures.

Table I. Subject demographics

	Sex	*Age (y)*	*Height (cm)*	*Weight (kg)*
Subject No.				
1	F	33	168	56
2	F	32	166	68
3	M	30	180	75
4	M	35	173	73
5	M	41	180	69
6	M	29	183	69
7	F	28	165	56
8	M	28	180	84
9	M	23	182	85
10	F	22	167	56
11	F	26	167	58
Mean ± SD		29.7 ± 5.4	173.7 ± 7.3	68.1 ± 10.7

Study medications. Clinical effects of codeine not accounted for by plasma morphine had been observed in experimental studies after oral codeine doses of 100 mg,[9] 170 mg,[10] and 50 mg.[13] In the clinical setting some effects of codeine apart from morphine formation[11] were reported after 90 mg oral codeine. To administer a dose in the range of the doses after which the effects aimed for in this study had been observed, subjects first received codeine as an oral solution (ct-Arzneimittel, Berlin, Germany) at doses initially set at a base level of 80 mg codeine but later increased to 120 mg because of small miotic effects in the first subjects. Because pairwise statistical comparisons of the codeine and morphine conditions and PK-PD modeling analyses were planned, equal doses in all subjects were not a prerequisite of the study, and therefore the experiments with lower codeine doses were not repeated at higher doses. On the second study day, the observed morphine plasma concentrations after codeine administration were mimicked by computerized infusion of morphine sulfate (Morphinsulfat GRY; GRY-Pharma, Kirchzarten, Germany) by use of computerized infusion controlled by STANPUMP[23] (available from Steven L. Shafer, MD[24]). The target morphine plasma concentrations were the individual morphine concentrations measured after codeine administration. To smoothly mimic the morphine concentrations after codeine, target concentrations were provided to STANPUMP for every 30 seconds, obtained by interpolation of the individually observed morphine concentration versus time courses af-

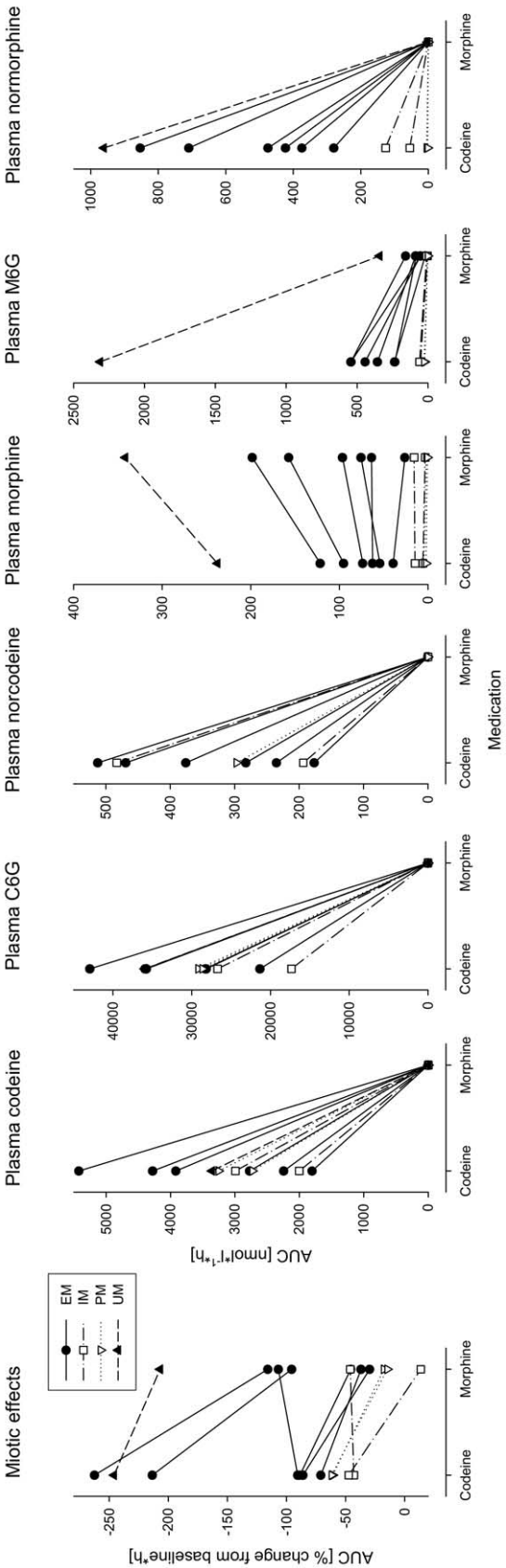

Codeine dose
Body weight [kg]
CYP2D6 genotype
Metabolizer type
Subject 1 80mg 56 *1/*1 EM
Subject 2 80mg 68 *1/*41 IM
Subject 3 120mg 75 *x/*4 PM#
Subject 4 120mg 73 *4/*4 PM
Subject 5 120mg 69 *1x2/*1 UM
Subject 6 120mg 69 *1/*1 EM
Subject 7 120mg 56 *1/*4 EM
Subject 8 120mg 84 *1/*41 EM
Subject 9 120mg 85 *4/*41 IM
Subject 10 120mg 56 *1/*4 EM
Subject 11 120mg 58 *1/*1 EM
Codeine
C6G
Nor-codeine
Morphine
M6G
Nor-morphine
Miotic effects
Plasma concentration [nmol/l]
%change from baseline
Administration of codeine
Administration of morphine
Time [h]

ter codeine, with linear interpolation for ascending concentrations and log-linear descending concentrations. Previously obtained PK parameters of morphine were adapted for each subject's body surface, which had been identified as a covariate for morphine pharmacokinetics,[25] and provided to STANPUMP together with the individual target morphine concentrations. On the basis of this information, STANPUMP controlling an infusion pump (Harvard Pump 22; Harvard Apparatus, South Natick, Mass) changed the infusion rate of morphine every few seconds to produce morphine plasma concentrations virtually identical to the target (ie, to those observed after codeine administration).

Measurement of pupil diameters. The open-label study design with codeine always being administered before morphine administration required a PD target parameter devoid of sequence effects and insensitive for placebo effects. These criteria were met by the pupil size in several previous studies. With our laboratory kept in the same unchanged condition, we did not observe a bias in pupil size measurements in the same subjects during different studies performed during the last few years. Measurements took place in a windowless room devoid of daylight. The subjects wore sunglasses when they had to leave the laboratory, which was allowed for not more than 5 minutes. During intervals between pupil measurements, the light in the laboratory was kept at 13.6 lux (average of 33 single measurements in random directions throughout the room). Three minutes before pupil diameter measurements were taken, lamps in the laboratory were turned off. The only sources of light were 2 computer screens; the subject's eyes were directed in a direction opposite to these screens. The light in the room had an intensity of 0.3 lux. During the measurements, the subject's eyes were exposed to a light of 10.8 lux emitted by the pupillograph ("CIP"; Amtech, Weinheim, Germany). Thirty seconds of adaptation to this light was found to be sufficient. Measurement of pupil diameter uses the different reflection of light by iris and pupil, which enables the charge-coupled device–line camera to detect the pupil margins. During measurements, the subject was sitting in front of the device with the chin and forehead suspended by the pupillograph to keep the pupil and sensor at a constant distance. The observer stood behind the device and, using an 8.9-cm video display, controlled the correct positioning of the sensors by moving them up and down or left and right until a horizontal line representing the measuring plane was placed exactly at the biggest diameter of the pupil. The resolution of the device was 0.05 mm. Each pupil diameter was the average of at least 5 repetitions of single measurements. Dark-adapted pupil diameters were determined at 0, 15, and 30 minutes and then every 30 minutes thereafter until the end of the 5-hour observation period.

Plasma concentrations of codeine and its important metabolites. Venous blood samples were collected immediately after each pupil size measurement, that is, approximately 3 minutes later than the timing of pupillography reported previously. The exact time of blood sampling was noted and used in the analyses. Plasma was separated within 30 minutes and stored at −20°C until analysis. Morphine and M6G concentrations were assayed by liquid chromatography–tandem mass spectrometry as described previously.[26] The concentrations of morphine-3-glucuronide (M3G) were not used because this metabolite has no agonist activity at μ-opioid.[27] Concentrations of codeine, C6G, norcodeine, and normorphine were determined similarly. The lower limit of quantification was 0.05 ng/mL for morphine and codeine, 0.1 ng/mL for C6G, and 0.2 ng/mL for the other analytes. The coefficient of variation over the calibration range of 0.05 (or 0.1 or 0.2) to 100 ng/mL was less than 15%. Samples with concentrations of analytes above the calibration curves were sufficiently diluted and reassayed. Quality control samples contained 0.1 (or 0.2 or 0.4), 5, and 50 ng/mL of each analyte. Randomly selected samples from each subject after codeine administration were reanalyzed during the analytic procedure after morphine administration. No differences in the results were observed, thus excluding sequence effects resulting from the analytic procedure.

Fig 2. Time courses of plasma concentrations of codeine and its important metabolites with μ-opioid agonist activity, as well as percent changes in pupil size, observed after oral administration of codeine and intravenous administration of morphine to healthy young volunteers. *Pound sign*, Nonwhite subject with a heterozygous genotype for the nonfunctional *CYP2D6*4* allele but an unambiguously identified poor metabolizer (PM) phenotype (dextromethorphan/dextrorphan metabolic ratio, 0.44).[28] EM, Extensive metabolizer; IM, intermediate metabolizer; UM, ultrarapid metabolizer; C6G, codeine-6-glucuronide; M6G, morphine-6-glucuronide.



Assessment of CYP2D6 function. The subject's CYP2D6 function was unknown during recruitment except for the ultrarapid metabolizer (UM) and 1 poor metabolizer (PM). The assignment of the subjects to 1 of 4 CYP2D6 phenotypes, that is, PM with extremely decreased or absent enzyme function, intermediate metabolizer (IM) with present but reduced enzyme function, extensive metabolizer (EM) with "normal" enzyme function, and UM with increased enzyme function, was based on both genotyping and phenotyping by use of oral administration of 30 mg dextromethorphan hydrobromide and by calculating the metabolic ratio (MR) of dextromethorphan/dextrorphan, a CYP2D6-dependent metabolite, from urine concentrations measured in the 8-hour collected urine,[28] administered in the period between the 2 study days. The urine concentrations of dextromethorphan and dextrorphan were assayed by a newly established HPLC–mass spectrometry assay (Appendix). Genotyping for relevant nonfunctional *CYP2D6* alleles (*CYP2D6*3*, **4*, **6*, **7*, and **8*) was performed with Pre-Developed TaqMan Assay Reagents Allelic Discrimination Kits (Applied Biosystems, Foster City, Calif). The functionally impaired *CYP2D6*41* allele was detected by HPLC analysis,[29] and quantitative real-time polymerase chain reaction analysis was performed to distinguish between *CYP2D6* gene amplifications and deletions (**5* allele).[30] Subjects with a dextromethorphan/dextrorphan MR of 0.3 or greater were classified as PMs.[28] In addition, the PM phenotype was predicted for carriers of 2 nonfunctional alleles. The IM phenotype was predicted when individuals were homozygously mutant for the **41* allele or heterozygous in combination with a nonfunctional allele,[29,31] the UM phenotype was predicted for carriers of more than 2 *CYP2D6* gene copies, and in all other cases a CYP2D6 EM phenotype was assumed. Because we did not test for the *CYP2D6*2* and **35* alleles, which are both

Fig 3. Individual values of areas under curve (AUC) versus time of percent change in pupil size as measure of miotic effects and plasma concentrations of codeine and its important metabolites with μ-opioid agonist activity, observed after oral administration of codeine and intravenous administration of morphine to healthy young volunteers, with different symbols for CYP2D6 phenotypes. Note that more negative values of the changes in pupil size indicate more pronounced decreases in pupil size (ie, a greater effect). The individual values after codeine and morphine administration are connected with lines, different for the CYP2D6 phenotypes.

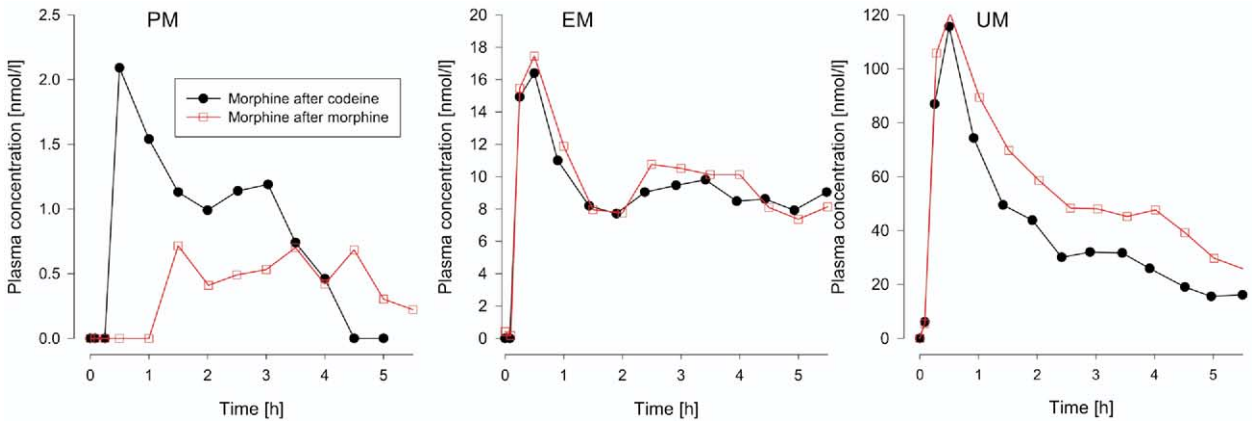


Fig 4. Mimicking of time courses of plasma concentrations of morphine after administration of codeine by computerized intravenous infusion of morphine by use of STANPUMP. Examples from subjects with the CYP2D6 PM (*left*), EM (*center*), and UM (*right*) phenotypes are shown.

associated with normal function, all individuals with wild type were classified as **1* carriers.

Data analysis. In a first exploratory analysis, the areas under the curve (AUCs) of the percent changes in pupil size or plasma concentration versus time curves obtained after codeine administration were compared with those obtained after morphine administration by means of Wilcoxon signed rank tests (SPSS 13; SPSS, Chicago, Ill) (by use of the "exact test" option, which provides a reliable estimate of the statistical significance regardless of the size, distribution, sparseness, or balance of the data). This provided an overview of whether the morphine plasma concentrations were similar at both conditions as intended, whether the miotic effects differed between conditions, and whether the plasma concentrations of opioids differed between conditions. In addition, comparisons between CYP2D6 phenotypes were performed by means of Kruskal-Wallis 1-way ANOVA.

The contribution of the opioids found in plasma after codeine administration to the miotic effects was analyzed by means of semiparametric PK-PD modeling.[32] The effect of each opioid was assumed to relate to its concentration at a theoretic effect compartment (Ce).[33,34] Equilibration between this effect compartment and the plasma compartment was assumed to follow a first-order transfer process, and thus Ce as a function of time (t) was described by the following:

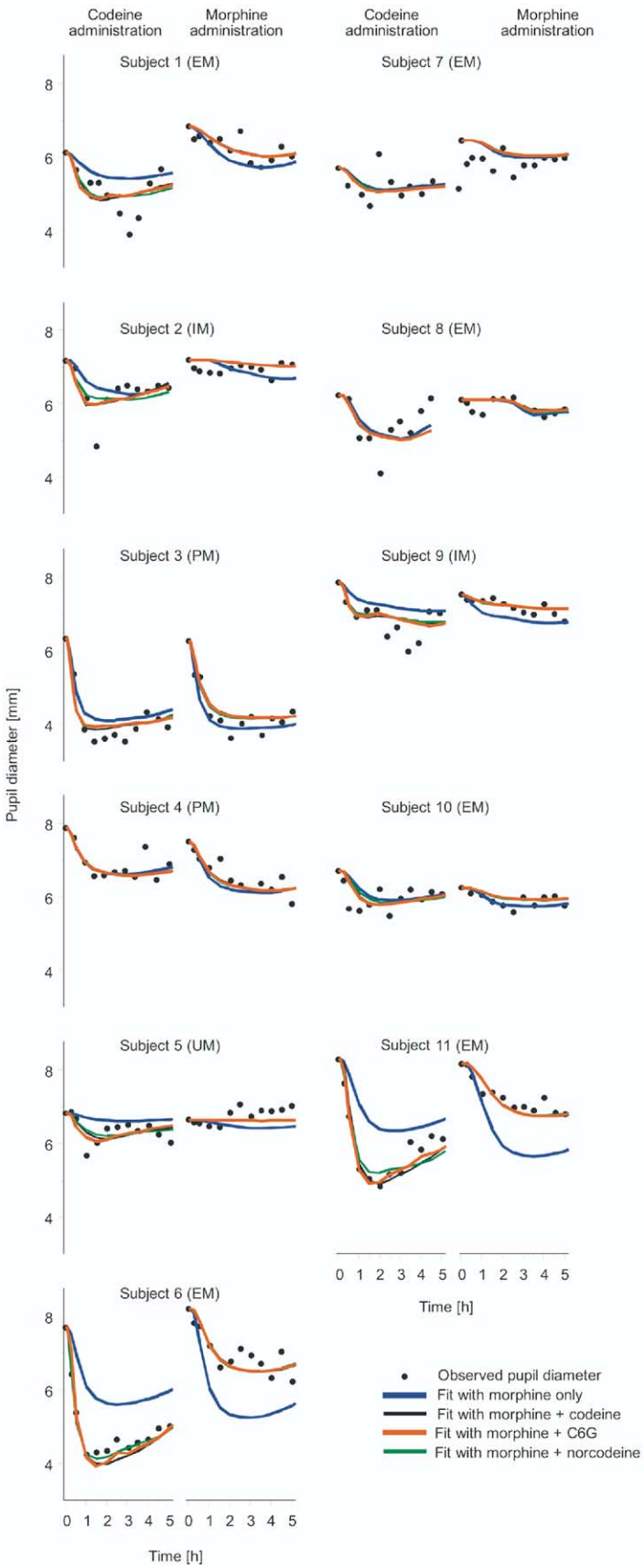
$$Ce(t) = Cp(t) * k_{e0} \cdot e^{-k_{e0} \cdot t} \quad (1)$$

where Cp(t) denotes the plasma concentration versus time profile, k_{e0} is the first-order transfer rate constant, and the asterisk denotes convolution of the 2 functions.

The semiparametric approach described the plasma concentrations by linear splines, that is, by straight connections of 2 neighboring data points of the observed plasma concentration versus time profiles that were available at sufficiently small sampling intervals. This avoided calculation of Ce(t) starting from a badly fit Cp(t), by use of the observed instead of modeled concentrations. Pupil diameters were assumed to be related to Ce by an E model for competitively acting full agonists:

$$\%\ \text{change in pupil size} = E_{max} \cdot \left(\frac{\frac{Ce_{morphine}}{EC_{50,morphine}} + \frac{Ce_{opioid\ 2}}{EC_{opioid\ 2}} + \cdots}{1 + \frac{Ce_{morphine}}{EC_{50,morphine}} + \frac{Ce_{opioid\ 2}}{EC_{opioid\ 2}} + \cdots} \right) \quad (2)$$

where E_{max} is the maximum possible percent change in pupil size from baseline and EC_{50} is the opioid concentration at half-maximum effect numerically denoting the opioid's potency. Further opioids could easily be added to the equation when needed. The PK-PD modeling was performed with NONMEM V software (version 1.1; GloboMax, Hanover, Md). The modeling process repeatedly used goodness-of-fit procedures, which were as follows: (1) the NONMEM objective function of minus 2 times the log likelihood (−2LL) and the χ^2 approximation with the number of degrees of freedom equal to the difference in the number of terms between 2 models (α level =.05); (2) the median absolute weighted residuals, calculated as (Measured − Predicted)/Predicted, and the mean of the individual mean absolute weighted residuals; and (3) visual in-


Codeine administration
Morphine administration
Codeine administration
Morphine administration
Subject 1 (EM)
Subject 7 (EM)
Subject 2 (IM)
Subject 8 (EM)
Subject 3 (PM)
Subject 9 (IM)
Subject 4 (PM)
Subject 10 (EM)
Subject 5 (UM)
Subject 11 (EM)
Subject 6 (EM)
Pupil diameter [mm]
8
6
4
0 1 2 3 4 5 0 1 2 3 4 5
Time [h]
Observed pupil diameter
Fit with morphine only
Fit with morphine + codeine
Fit with morphine + C6G
Fit with morphine + norcodeine

spection of the fits versus observed data. Log–normally distributed interindividual variances of the PK-PD link parameter k_{e0} and of the potency EC_{50} were used: $P_i = \theta_{i,TV} \cdot e^{\eta_i}$, where P_i is the value of the parameter of the individual, $\theta_{i,TV}$ is the typical value of this parameter in the population, and η is a variable accounting for the interindividual variability, with a mean of 0 and variance of ω^2. Interindividual variability was assigned to parameters of the final model in case this improved the fit. An assumption of normal rather than log-normal distribution of parameter values resulted in worse fits and was, therefore, rejected. The residual error ε was modeled by use of an additive error model. Proportional or combined proportional and additive error models resulted in worse fits and were rejected. Calculations were performed by use of "first-order conditional estimation" and "η-ε interaction." Ninety-five percent confidence intervals of parameter values were calculated from 100 runs of each of the 6 models (as discussed later) with data sets that were obtained by bootstrap resampling from the original data set,[35] by use of Wings for NONMEM (N. Holford, Auckland, New Zealand[36,37]). The 95% confidence intervals of the parameter values were obtained as the 2.5th and 97.5th percentiles of the results of the 100 model runs. Available computational power did not allow for more runs in a reasonable amount of time. An attempt to estimate E_{max} from the present data resulted in a maximum miotic effect of 100%, meaning that the pupils disappeared, which was unlikely to be true and probably a consequence of the small miotic effects observed in this study, which never came close to the maximum observed after administration of higher doses of morphine.[38] However, the maximum miotic effects of morphine were known for most of the subjects from previous studies. Therefore E_{max} was not estimated, and individual minimum pupil sizes available from observation after administration of higher morphine doses to the same subjects were used instead. For subjects without such values available, a previously obtained population central value[26] was taken. The underlying assumption of comparable interindividual degree of maximum miosis was supported by observations during previous studies with high doses of morphine in our laboratory (eg, reference 21). In addition, it could be assumed that after very high codeine doses the plasma concentrations of morphine theoretically reach levels associated with maximum miosis. Nevertheless, analysis performed with E_{max} free to be fitted yielded no results with respect to a contribution of opioids other than morphine to the miotic effects of codeine other than the chosen procedure of using more realistic values of E_{max} than calculated from the present data set. The model-building process started with describing miosis as an effect of morphine only. This was obtained by fixing the EC_{50} and k_{e0} values of the other opioids at 10^6 nmol/L and 10^{-5} h^{-1}, respectively. In subsequent modeling steps, for each of the other opioids, EC_{50} and k_{e0} were free to be fitted and the goodness of these fits was compared with that of the fit with morphine only to judge the contribution of opioid agonists other than morphine to the miotic effects observed after codeine administration.

RESULTS

Side effects were absent or consisted of very mild dizziness. On the basis of the dextromethorphan/dextrorphan MR of greater than 0.3, 2 subjects carried the CYP2D6 PM phenotype. One of them had 2 nonfunctional alleles (**4/*4*), whereas a heterozygous genotype (**1/*4*) was found for the second PM. This discrepancy most likely may be explained by the presence of an additional rare nonfunctional *CYP2D6* allele[39] corroborated by the fact that this subject had a nonwhite relative in his ascending family line. For all other subjects, phenotype prediction based on genotyping identified 2 IMs (**41/*4* and **41/*7*), 1 UM (**1x2/*1* [ie, 3 *CYP2D6* gene copies]), and 6 EMs (**1/*1*) (Fig 2). Observed plasma concentrations and miotic effects are shown in Fig 1.

The AUCs of the miotic effects and plasma concentrations of codeine and its opioid agonist metabolites (Fig 3) were greater after codeine than after morphine administration ($P = .005$ for miotic effects, $P = .001$ for plasma M6G, $P = .001$ for plasma normorphine, and $P < .001$ for plasma codeine, norcodeine, and C6G) except for the AUCs of plasma morphine, which were similar during both study days ($P = .21$) as intended.

Fig 5. Fits of pupil diameters (*circles*) after administration of codeine and morphine, either by use of morphine as only active moiety or by use of morphine together with codeine or its *N*-demethylated or glucuronidated metabolites as 2 concomitant active moieties. The metabolites of morphine are omitted. Note that the fits with C6G, codeine, and norcodeine as active moieties second to morphine are almost superimposed.

Table II. Parameters of semiparametric population pharmacokinetic-pharmacodynamic models of miotic effects observed after administration of codeine and morphine

	Morphine				
	*k_{eO} (h^{-1})**		*EC_{50} (nmol/L)*		
Second opioid to morphine	*Population central value*	*95% CI*	*Population central value*	*95% CI*	*Interindividual variability (% CV)*
—	0.51	0.38-0.83	16	10.2-30.4	128
Codeine	0.35	0.18-0.77	28.2	15.9-71.5	162
C6G	0.38	0.21-0.8	29.1	15.6-74.7	163
Norcodeine	0.41	0.24-0.83	29.5	15.3-68.2	165
M6G	0.52	0.29-1.11	138	15-250.1	307
Normorphine	0.46	0.23-0.93	28.7	14.7-62.6	172

k_{e0}, First-order transfer rate constant; EC_{50}, opioid concentration at half-maximum effect numerically denoting opioid's potency; CI, confidence interval of parameter obtained as 2.5th and 97.5th percentiles of results of 100 model runs by use of bootstrap resampling with NONMEM; CV, coefficient of variation in population; −2LL, minus 2 times log-likelihood (NONMEM objective function used as parameter of goodness of fit).

*Interindividual variability of k_{e0} was not part of the final models.

The plasma concentrations of morphine observed after codeine and after morphine were within the same range of magnitude (Fig 4). However, there was a general tendency toward higher plasma morphine concentrations after morphine than after codeine, except in the PMs, in whom the computerized infusion had difficulty achieving the very low morphine target concentrations and obtained lower-than-intended concentrations of morphine. The AUCs of the miotic effects after codeine were 1.7 ± 2 times greater than after morphine (P =.005). This contrasted to similar or even lower morphine concentrations after codeine than after morphine (AUC ratio, 0.5 ± 0.4; P =.21 for the difference between morphine AUCs after codeine and morphine administration). Interestingly, the factor by which the AUCs of the miotic effects after codeine were more pronounced than after morphine was greater in PMs (3.8 ± 0.5) compared with EMs (2 ± 0.7), although this was not statistically significant. Normalization of AUCs of the miotic effects to the respective AUCs of the morphine plasma concentrations did not improve the significance level. As expected, the AUC of the plasma concentrations for morphine, M6G, and normorphine (P =.002), as well as of the miotic effects (P =.006), differed significantly among CYP2D6 phenotypes, with the highest AUC being in the single UM and the smallest AUCs being in both PMs. However, AUCs of codeine, C6G, and norcodeine were similar among various CYP2D6 phenotypes (P =.21).

Fitting the change in pupil size as an agonistic effect of morphine and a second opioid (Fig 5) resulted in a highly significant improvement of fit (P <.0001) with every opioid as compared with the fit with morphine as the only opioid that produced miosis (Table II). The greatest improvement of fit was obtained when C6G was the second active moiety in addition to morphine, followed by codeine and norcodeine, which provided improvements of fit similar to those with each other, whereas normorphine and M6G provided the smallest, although still highly significant, improvement of fit (−2LL columns in Table II). Combinations of 3 active opioids such as morphine, codeine, and norcodeine or morphine, codeine, and C6G resulted in further significant improvements of fit (decrease in −2LL of >20 as compared with the fits with 2 active opioids). However, the modeling became less reliable for these multiple parameter fits as indicated by an increasing proportion of unsuccessful fits or by very differing results with slightly changing parameter start values. The results were, therefore, restricted to 2 opioids.

DISCUSSION

The data clearly show a greater miotic effect after administration of codeine compared with administration of morphine despite a tendency toward higher plasma morphine concentrations after morphine administration. This provides, for the first time, a quantitative basis for the repeatedly reported clinical effects of codeine not explained by plasma morphine.[9-13]

The hints in the literature about clinical activity of codeine despite nonfunctional CYP2D6[9-13] made it unlikely that the second active moiety in addition to morphine was among the metabolites of morphine (ie, M6G or normorphine). This would require a function-

Second opioid							
k_{e0} (h^{-1})*		EC_{50} (nmol/L)					
Population central value	*95% CI*	*Population central value*	*95% CI*	*Interindividual variability (% CV)*	*−2LL*	*Change in −2LL relative to morphine only*	*Residual variance ε*
—	—	—	—	—	32.62	—	0.359
2.31	1.36-4.66	2420	1074-6642	129	−701.25	−733.87	0.124
9.88	3.1-40.2	24,100	11,135-50,770	124	−719.45	−752.12	0.109
2.65	1.64-5.33	265	122-786	132	−704.38	−737	0.108
21.1	1.62-45.61	182	121-471	150	−620.266	−652.889	0.124
15.8	2.29-85.09	212	110-486	164	−695.174	−727.797	0.109

ing CYP2D6 to first form morphine, which subsequently can be metabolized into M6G and normorphine. Therefore, despite their much higher plasma concentrations after oral codeine administration than after intravenous morphine administration, which was probably caused by the first-pass metabolism of codeine extending to morphine (ie, glucuronidation immediately after *O*-demethylation), M6G and normorphine were the least likely candidates for being the second active moiety in addition to morphine that contributes to the effects of codeine. Indeed, both M6G and normorphine provided the smallest improvement of fit of the miotic effects in the current data set. In addition, there was no hint in the data that the additional miotic effects of codeine not attributable to morphine depended on CYP2D6 function, which further strengthens the hypothesis that a morphine-independent codeine metabolite had caused these effects. With respect to M3G, the results support its omission from this report. Its plasma concentrations after codeine were also much higher than after morphine, by the same extent and for the same reason as for M6G. With M3G having effects, if any, contrary to those of morphine[27] and having only a marginal affinity to opioid receptors,[40,41] it should have acted against more miosis after codeine than after morphine, which is contrary to our observation.

Likely active moieties for this effect were codeine itself and its *N*-demethylated or glucuronidated metabolites. Among these, C6G provided the greatest improvement of the fit of the miotic effects as a function of opioid concentrations, followed by both codeine and norcodeine. C6G is the main metabolite of codeine, and its contribution to the central nervous effects observed after codeine administration has been previously proposed on the basis of chemical structure analysis.[42] Moreover, C6G has been shown to produce analgesic effects after intravenous administration to rats.[43] However, the line of evidence favoring C6G as the cause of the miotic effects in addition to morphine is based on goodness of fit and also supported by agreement with the literature.[42,43]

Our approach was importantly based on intraindividual comparison of the miotic effects of comparable morphine concentrations in the presence or absence of codeine and its other metabolites. Therefore a different opioid action in subjects with different ethnic backgrounds[44] might have increased the overall interindividual variability of the PK-PD parameter estimates, but unless one assumes that the relative activity of morphine and C6G (or the other codeine metabolites) greatly differs among races, it is unlikely that this has influenced the conclusion that morphine was not the only active moiety by which the miotic effects were explained. Indeed, the exclusion of the subject with a different ethnic background did not change this result. That is, morphine alone provided significantly fewer good fits as compared with those produced by adding a second active moiety, and C6G again provided the greatest improvement of fit when included as an active moiety second to morphine in the fit of the miotic effects.

Before finally rejecting codeine or norcodeine as the active moiety on the basis of our statistical evidence, one has to consider that statistical evidence suggested a

contribution of systemically available M6G to the effects of morphine to a degree ranging between less than 0.1% and 66% when based on a modeling analysis of data obtained after administration of morphine only.[45] However, when M6G was directly administered at doses that produced M6G plasma concentrations similar to those found after these morphine doses, it produced no measurable effect.[46] By analogy, both codeine itself and norcodeine, which provided almost the same improvement of fit as C6G (Fig 5), cannot be ruled out as second active moieties for the miotic effects of codeine. Thus, although favored by our analysis and supported by data obtained in laboratory animals,[43] a final judgment about the contribution of C6G to the observed morphine-independent central nervous system effects after administration of codeine can probably be made only after direct administration of C6G to humans, analogous to the quantification of the contribution of M6G to the effects seen after morphine administration.[38]

The estimated population PK-PD parameter values of $k_{e0,morphine}$ (Table II) agree with those of 0.25 h^{-1} and 0.18 h^{-1} previously estimated.[20,26,38] In contrast, the estimated population PK-PD parameter value of $k_{e0,M6G}$ of 0.52 h^{-1} (Table II) is completely different from that of 0.08 h^{-1} or 0.11 h^{-1} observed for the miotic effects after intravenous administration of M6G.[26,38] An explanation for this difference is that the currently obtained values are not correct because M6G was not involved in the miotic effects, thus further supporting the rejection of M6G as an active moiety. Values of the transfer half-life between plasma and the effects site for codeine, norcodeine, and normorphine are not available in the literature, and the correctness of the current estimates, therefore, cannot be judged on the basis of previously published information. The value of $EC_{50,morphine}$ obtained with C6G, codeine, and norcodeine, as well as with normorphine, corresponded to the values of 26.7 nmol/L and 34.4 nmol/L previously obtained for the miotic effects.[26,38] However, when morphine was used as the only active moiety, it appeared to be more potent, with an $EC_{50,morphine}$ of 16 nmol/L. This may be interpreted as an indication that the second active moiety was missing from the first fit and all of the effects were attributed to morphine, which resulted in an apparent increase in its potency. The fit with M6G as the second active moiety resulted in an apparent low potency of morphine, with an $EC_{50,morphine}$ value of 138 nmol/L, indicating that M6G was the false second opioid to morphine. This assumption is supported by the comparably high potency of M6G, with an $EC_{50,M6G}$ value of 138 nmol/L, which contrasted to previously estimated values of 745 nmol/L and 743 nmol/L for the miotic effects of M6G.[26,38] Respective values for the other opioids are not available, and therefore the current estimates, especially for C6G, need to be verified in further investigations with direct administration of C6G.

This study focused on the effects of codeine not attributable to morphine. Nonetheless, its results do not at all contradict the general view that the effects of codeine are mainly mediated by morphine.[47] The estimated low potency of C6G also emphasizes that the identified effect not attributable to morphine consisted only of a small fraction of the total effects observed after codeine administration, the main effects being mediated by morphine.

In conclusion, by comparison of the central nervous effects of codeine and morphine in the presence of similar morphine plasma concentrations, we provide quantitative evidence that codeine has effects independent of its *O*-demethylation. On the basis of statistical rather than direct evidence, the most likely active moiety for this effect in addition to that attributable to morphine is C6G, although norcodeine and even codeine itself remain further candidates as mediators of the repeatedly observed codeine effects despite inactive CYP2D6.[15]

We dedicate this article to Professor Kay Brune, Institute of Experimental and Clinical Pharmacology, University of Erlangen-Nuremberg, Germany, on the occasion of his 65th birthday.

There are no conflicts of interest.

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APPENDIX

Analysis of dextromethorphan and dextrorphan

Dextromethorphan and its metabolite dextrorphan were assayed by HPLC–mass spectrometry by use of an HP Series 1100 LC-MS system (Hewlett-Packard, Waldbronn, Germany) with a binary pump, degasser, autosampler, and mass-selective detector equipped with an electrospray source. Urine (0.5 mL) was spiked with deuterated internal standards (25 μL of a standard mix containing 1.0 μg D_3-dextrorphan and 50 ng D_3-dextromethorphan) and diluted with 0.5 mL acetate buffer (0.2 mol/L, pH 5) containing β-glucuronidase/arylsulfatase (8000 Fishman units). Enzymatic hydrolysis of the glucuronides and sulfates was performed for 16 hours at 37°C. Subsequently, 0.5 mL saturated sodium carbonate was added, and the samples were extracted with 6 mL hexane/ethyl acetate (50:50 [vol/vol]). The organic phase was evaporated to dryness in a stream of nitrogen, and the residue was dissolved in 80 μL of the mobile phase (water/acetonitrile, 83:17 [vol/vol], with 1% acetic acid). Five microliters was used for liquid chromatography–mass spectrometry analysis. The mobile phases used for HPLC were as follows: 1% acetic acid in water (A) and 1% acetic acid in acetonitrile (B). HPLC separation was achieved on a Hypurity Elite C18 column (50 × 2.0 mm internal diameter; particle size, 5 μm) (Thermo Hypersil, Dreieich, Germany) at a flow rate of 0.25 mL/min by use of a gradient program as follows: 17% B for 2 minutes, with a linear increase to 40% B for 6 minutes and 40% B for 1 minute, and then re-equilibration at 17% B for 4.5 minutes until the next sample was injected. The mass spectrometer was operated in the selected ion monitoring mode by use of the respective MH^+ ions, mass-to-charge ratio (*m/z*) 258 for dextrorphan, *m/z* 261 for D_3-dextrorphan, *m/z* 272 for dextromethorphan, and *m/z* 275 for D_3-dextromethorphan. Quantification was performed by weighted (1/x) linear regression by use of the peak area ratios against the amount of the substance. The calibration curves were linear over the concentration ranges of 0.25 to 25 μg/mL for dextrorphan and 6.25 to 2500 ng/mL for dextromethorphan. The limits of quantification were 6.25 ng/mL for dextromethorphan and 0.25 μg/mL for dextrorphan, with coefficients of variation of 2.4% and 0.7%, respectively. Intra-assay and interassay variations at 3 different concentrations (12.5, 500, and 2500 ng/mL for dextromethorphan and 0.5, 5, and 25 μg/mL for dextrorphan) were better than 4.7% for dextromethorphan and 3.4% for dextrorphan. Accuracy, expressed by the bias, ranged between 2.2% and 7.8% for dextromethorphan and between −3.3% and 3.5% for dextrorphan.