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Transformation of thebaine to oripavine, codeine, and morphine by rat liver, kidney, and brain microsomes*

(NADH/NADPH/cytochrome P-450/high-pressure liquid chromatography/radioimmunoassay)

HARUYOSHI KODAIRA AND SYDNEY SPECTOR

Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

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ABSTRACT **Thebaine, an intermediate of morphine biosynthesis in the poppy plant, *Papaver somniferum*, was transformed to oripavine, codeine, and morphine by rat liver, kidney, and brain microsomes in the presence of an NADPH-generating system. The formation of morphine, codeine, and oripavine was identified by a specific RIA, HPLC, and GC-MS. Thebaine also gave rise to four other compounds, which for the moment are unidentified. NADH dramatically increased the formation of both codeine and morphine when used together with an NADPH-generating system, especially in liver microsomes. NADPH is essential in the formation of oripavine from thebaine and morphine from codeine, while NADH is critical in the conversion of thebaine to codeine and from oripavine to morphine. Carbon monoxide or SKF 525A inhibited the conversion, indicating a role of cytochrome P-450. These results provide evidence for the enzymatic *in vitro* conversion by mammalian tissues of thebaine to morphine. The pathway is similar to that which exists in plants.**

Morphine, a potent and effective analgesic compound, was originally found in the poppy plant, *Papaver somniferum*. Its analgesic effect is thought to be a consequence of its interaction at the μ receptor (1). Morphine has a higher affinity for that site than the endogenous opioid peptides, though the alkaloid also can bind to the κ and δ receptors (2).

Recently morphine was identified in toad skin in our laboratory (3), and subsequently Goldstein *et al.* (4) have reported its presence in beef brain and adrenal gland. Furthermore, Donnerer *et al.* (5) also reported on the presence of morphine and codeine in various mammalian tissues and the increase of these alkaloids in the tissues after the intravenous administration of intermediates in morphine biosynthesis in the plant. Therefore, there may be a synthetic pathway in mammals for the formation of the opiate alkaloids. However, it is still uncertain whether the opiate alkaloids found *in vivo* were formed enzymatically. To definitively demonstrate it, direct evidence for the enzymatic formation of the alkaloids in mammalian tissues is required. Therefore, thebaine was investigated regarding its transformation to codeine and morphine in various mammalian tissues *in vitro* and the different cofactor requirements in this biotransformation. Also, *in vitro* studies were initiated to gain insight into the biosynthetic pathway and whether mammalian tissues utilize the same pathway as the poppy plant.

METHODS

Materials. Male Sprague–Dawley rats weighing 200–350 g were used for the preparation of microsomes and cytosol fraction. (−)-Thebaine (Hoffmann–La Roche) crystallized from methanol was free of morphine, codeine, and oripavine as measured by HPLC and RIA. (−)-Oripavine, a gift from E. Brochmann-Hanssen (University of California, San Francisco), was purified by HPLC, and was free of morphine. (−)-Codeine (Merck) was also free of morphine. Morphine hydrochloride and ^{125}I-labeled morphine (90–150 Ci/mmol; 1 Ci = 37 GBq) were from Hoffmann–La Roche. SKF-525A, a cytochrome P-450 inhibitor, was from Hoffmann–La Roche. The following reagents were obtained from Sigma: NADP (Sigma grade), NADPH (type X), NAD (grade V-C), NADH (grade III), $MgCl_2$, glucose-6-phosphate dehydrogenase (type XI), and D-glucose 6-phosphate. Other reagents were purchased from Baker or Sigma.

Preparation of Microsomes and Cytosol Fraction. The rats were killed by decapitation, and the tissues were removed, rinsed, and chilled on ice. The organs were homogenized in 3 vol of 0.15 M KCl/0.05 M Tris·HCl buffer (pH 7.4) in a glass–Teflon homogenizer. The homogenate was centrifuged at 9000 × *g* for 20 min at 4°C. The supernatant was centrifuged at 105,000 × *g* for 60 min at 4°C. The microsomal pellet was resuspended in the same buffer and used for the incubations (see below), or the suspension was stored at −80°C and used within 1 month. The 105,000 × *g* supernatant was used as the cytosol fraction. Protein was determined by using the Bio-Rad protein assay based on the Bradford method (6) with bovine serum albumin as a standard.

Incubation Conditions. Crystallized thebaine was dissolved in methanol and then in 0.15 M KCl/0.05 M Tris·HCl buffer (pH 7.4). A portion (300 μl) of the thebaine solution containing 65% (vol/vol) methanol was added to the incubation mixture containing 30 mg of microsomal or cytosolic protein of rat liver, brain, or kidney, and an NADPH-generating system [1 mM NADP, 5.5 mM glucose 6-phosphate, 10 units of glucose-6-phosphate dehydrogenase, and 5.0 mM $MgCl_2$ in a final volume of 10 ml of 0.15 M KCl/0.05 M Tris·HCl buffer (pH 7.4); this represents the complete system]. Incubation was carried out at 37°C. The final concentration of the precursors thebaine, oripavine, or codeine used in these studies was 1 mM. Fig. 3 shows the time course for the conversion of thebaine by rat liver microsomes. From these time-course studies, the incubation period in each experiment was kept at 2 hr (Tables 2–5).

Extraction Procedures. The incubations were terminated by the addition of 10 ml of acetone and the mixture was then saturated with potassium acetate (13 g), which causes the organic phase to separate (7, 8). After the addition of the solid salt, the pH was about 9.6. The aqueous incubation medium was extracted three times with 5 ml of acetone, separated, and added to the original acetone extract (total volume 25 ml). The combined organic phase was evaporated to a small volume (1–2 ml) at 35°C under a stream of oxygen-free nitrogen and placed in a SpeedVac concentrator

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*Some of these results have been communicated in preliminary form (14).

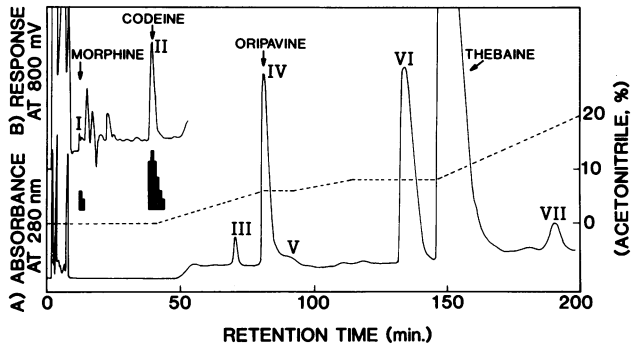

FIG. 1. Reverse-phase HPLC chromatograms of compounds formed from thebaine by rat liver microsomes. Extracted sample (60–100 μl) was injected into the HPLC system equipped with a LiChrosorb RP-18 column. ---, Acetonitrile concentration. Effluent was monitored by UV detector at 280 nm (trace A), by an electrochemical detector at 800-mV oxidation potential (trace B), and by specific RIA for morphine and codeine (hatched bar graphs represent the immunoreactivity). Arrows indicate the positions of standard morphine, codeine, oripavine, and thebaine in the HPLC system.

(Savant) and evaporated to dryness. Then 700 μl of 0.1 M pyridine/acetic acid (pH 4.7) was added to the residue, and the whole was mixed, sonicated, and centrifuged at 14,000 × g for 30 min. The clear portions (4–150 μl) of the centrifuged samples were directly applied to the HPLC system (see below).

HPLC Procedure. HPLC was carried out using a Beckman model 322 system. A LiChrosorb RP-18 column (0.4 × 25 cm, 5-μm particles, Merck) was operated with a flow rate of 1.45 ml/min and a solvent gradient [solvent A, 0.1 M pyridine/acetic acid (pH 4.7); solvent B, 0.1 M pyridine/acetic acid (pH 4.7) containing 20% (vol/vol) acetonitrile] changing from 0% to 100% B in 200 min. This system was used for the preparative isolation of formed compounds (Fig. 1). The effluent was monitored by a UV detector (model 786, Micromeritics, Norcross, GA) at 280 nm and also simultaneously by the amperometric electrochemical detector model LC-4A (Biochemical Analytical Systems, West Lafayette, IN) at an oxidation potential of 800 mV vs. a Ag/AgCl electrode. Specific RIA for morphine and codeine was also used for the monitoring (see below). In addition, morphine, codeine, and oripavine fractions obtained from the reverse-phase HPLC were analyzed with the HPLC system using a Whatman Partisil 10 SCX column (0.46 × 25 cm) and a solvent system with 0.1 M acetic acid/pyridine (pH 3.5) for purification and confirmative identification (Fig. 2). The purified samples thus obtained were analyzed by GC-MS or NMR for further identification. In routine analytical assay, the isocratic mobile phase of 30% solvent B for codeine and oripavine separation or of 0% solvent B for morphine separation was used for the reverse-phase HPLC system described above. Under these conditions, retention volumes for morphine, codeine, and oripavine were 15.9 ml, 17.4 ml, and 31.9 ml, respectively. After the elution of these compounds, the solvent was changed to 0.2 M pyridine/acetic acid (pH 4.7) containing 50% (vol/vol) acetonitrile for washing the column and then re-equilibrated with the starting solvent (30% B or 0% B). Oripavine concentration was determined from calibration curves constructed for extracted microsome standards by measuring the UV or electrochemical peak heights at three or four different concen-

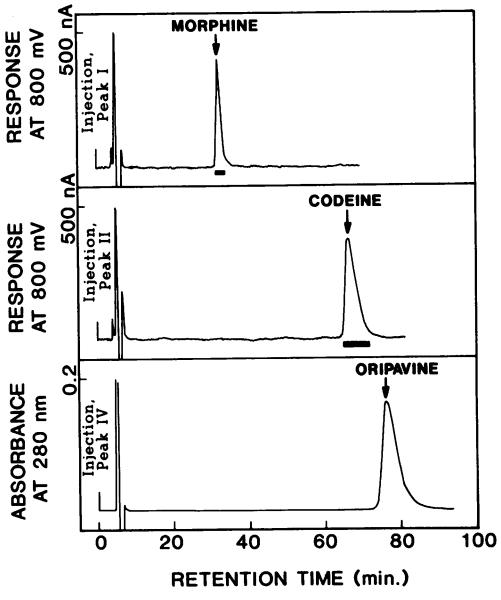

FIG. 2. Ion-exchange HPLC chromatograms of collected peaks I, II, and IV from the reverse-phase HPLC. Collected peaks from the reverse-phase HPLC were injected into the HPLC system equipped with Whatman Partisil 10 SCX column. Effluent was monitored as described for Fig. 1. The chromatograms are the results of the repeated HPLC. Arrows indicate the positions of standard morphine, codeine, and oripavine in the HPLC system, and solid bars indicate the positions of morphine and codeine immunoreactivity.

Table 1. GC-MS of peaks I, II, and IV formed from thebaine by rat liver microsomes

Peak	Molecular and fragment ions
I	429 (M^+; 100), 414 (36), 401 (19), 371 (13), 356 (8), 324 (15), 287 (20), 236 (69), 196 (43)
II	371 (M^+; 100), 356 (21), 343 (20), 313 (28), 298 (7), 282 (16), 266 (10), 229 (45), 196 (68), 178 (75)
IV	369 (M^+; 41), 354 (100), 338 (7), 313 (37), 296 (20), 281 (9), 267 (7), 253 (14), 207 (11), 174 (11)

Peaks I, II, and IV purified as described in Fig. 2 were collected, trimethylsilylated, and then analyzed by GC-MS equipped with an OV-1 capillary column. The mass fragment patterns of peaks I, II, and IV were completely identical to those of authentic trimethylsilylated morphine, codeine, and oripavine, respectively. M^+ indicates the molecular ion, and numbers in parentheses are relative intensities (%).

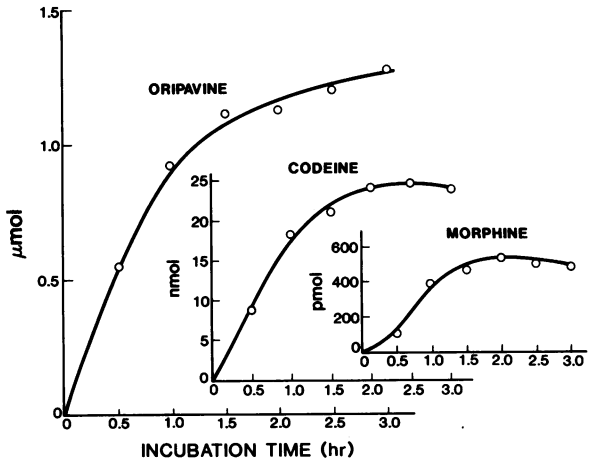

FIG. 3. Time course of the formation of oripavine, codeine, and morphine from thebaine in rat liver microsomes. Thebaine was incubated with rat liver microsomes in the presence of an NADPH-generating system plus NADH for the time indicated. Total amounts of oripavine, codeine, and morphine formed at each incubation time are shown. Each point is the mean of the results from three experiments.

Table 2. *In vitro* formation of oripavine, codeine, and morphine from thebaine by rat liver microsomes

Exp.	Conditions	Opiate formation, pmol/hr per mg of protein		
		Oripavine	Codeine	Morphine
A	Complete system (1 mM NADP)	15,200 ± 1800	57.1 ± 7.2	0.936 ± 0.09
	+ NADP (1 mM)	17,000 ± 1200	62.7 ± 3.8	1.03 ± 0.13
	+ NADPH (1 mM)	20,000 ± 2000	179 ± 28*	2.45 ± 0.21*
	+ NAD (1 mM)	19,000 ± 1800	105 ± 12*	1.51 ± 0.12*
	+ NADH (1 mM)	20,400 ± 2100	384 ± 23*	6.73 ± 0.56*
B	− NADP + NADPH (1 mM)	13,900 ± 1900	62.1 ± 5.1	1.36 ± 0.24
	− NADP + NADH (1 mM)	4,490 ± 500*	102 ± 11*	0.496 ± 0.09*
	− NADP + NAD (1 mM)	3,300 ± 400*	2.20 ± 0.33*	0.062 ± 0.01*
C	− NADPH-gen. sys. + NADPH (2 mM)	14,800 ± 2100	15.8 ± 1.6	0.316 ± 0.05
	− NADPH-gen. sys. + NADP (2 mM)	4,000 ± 900*	4.64 ± 0.52*	0.096 ± 0.01*
	− NADPH-gen. sys. + NADH (2 mM)	4,380 ± 800*	39.1 ± 4.1*	0.235 ± 0.04
	− NADPH-gen. sys. + NAD (2 mM)	3,970 ± 700*	2.35 ± 0.39*	0.059 ± 0.02*
D	− NADPH-gen. sys.	<50	<0.005	<0.001
	− Microsomes	ND	ND	ND
	− Microsomes + cytosol	272 ± 32	ND	ND
	− Microsomes + cytosol + NADH (1 mM)	333 ± 29	ND	ND
	Denatured microsomes	ND	ND	ND

Incubation was carried out with thebaine (1 mM), 30 mg of microsomal or cytosolic protein from rat liver, and an NADPH-generating system (1 mM NADP, 5.5 mM glucose 6-phosphate, 10 units of glucose-6-phosphate dehydrogenase, and 5.0 mM $MgCl_2$) in a final volume of 10 ml of 0.15 M KCl/0.05 M Tris·HCl buffer (pH 7.4) at 37°C for 2 hr. This represents the complete system. In Exp. A, supplemental NADP, NADPH, NAD, or NADH (1 mM each) was added to the complete system. In Exp. B, NADP was replaced by NADPH, NADH, or NAD (1 mM each) in the complete system. In Exp. C, the NADPH-generating system (gen. sys.) was replaced by NADPH, NADP, NADH, or NAD (2 mM each). In Exp. D, conditions were varied as indicated. Cytosol indicates 30 mg of cytosol protein. Denatured microsomes had been heated at 90°C for 4 min before incubation with the complete system. Each value is the mean ± SEM of three to six determinations. ND, not detectable.

*$P < 0.05$ that the value is the same as that obtained under the conditions given in the first line of that experiment.

trations. The recovery of oripavine added to microsomes was 80–90% (from RP-18 column).

Morphine and Codeine RIA. Morphine and codeine formed were determined on the basis of their competition with ^{125}I-labeled morphine for antibodies developed against 3-carboxymethylmorphine conjugated to bovine serum albumin (3). Codeine reacts as well as morphine with the antiserum [IC_{50} (mean ± SEM) 0.073 ± 0.005 pmol]. Fractions (0.5–1 min) were collected from the reverse-phase or ion-exchange HPLC, and an aliquot was evaporated to dryness and assayed. The recovery of morphine and codeine added to microsomes (from RP-18 column) was 80–90%.

RESULTS

Identification of Morphine, Codeine, and Oripavine Formed from Thebaine. Thebaine was incubated with rat liver microsomes in the presence of an NADPH-generating system plus NADH to identify formed compounds. As shown in Fig. 1, seven peaks were detected. Peaks I, II, and IV were identified as morphine, codeine, and oripavine, respectively, by HPLC and GC-MS using authentic compounds (Fig. 2 and Table 1). Peak IV was also confirmed to be oripavine by 400-MHz proton NMR comparison (in completely deuterated pyridine) with the authentic sample (data not shown). Peaks III, V, and VI were homogeneous, but the structures as yet are not known. Peak VII is an unstable compound. Morphine formed from oripavine or codeine was also identified by RIA, HPLC, and GC-MS in the same procedure.

***In Vitro* Synthesis of Morphine, Codeine, and Oripavine from Thebaine in Rat Liver, Brain, and Kidney Microsomes.** The time course for the formation of oripavine, codeine, and morphine from thebaine in rat liver microsomes is seen in Fig. 3. Maximal formation of codeine occurred between 2 and 2.5 hr, while oripavine formation was still increasing even after 3 hr. The maximal formation of morphine occurred at 2 hr and then formation gradually decreased.

Table 3. *In vitro* formation of oripavine, codeine, and morphine from thebaine by rat brain and kidney microsomes

Tissue	Conditions	Opiate formation, pmol/hr per mg of protein		
		Oripavine	Codeine	Morphine
Brain	Complete system	5.48 ± 0.91	0.195 ± 0.04	0.028 ± 0.003
	+ NADH (1 mM)	5.74 ± 0.86	0.226 ± 0.07	0.029 ± 0.005
	− NADPH-gen. sys.	ND	ND	ND
Kidney	Complete system	205 ± 24	0.280 ± 0.11	0.031 ± 0.006
	+ NADH (1 mM)	380 ± 37*	0.305 ± 0.12	0.032 ± 0.008
	− NADPH-gen. sys.	<4.0	ND	ND

The complete system included 30 mg of microsomal protein of brain or kidney. Other conditions are the same as described for Table 2. Each value is the mean ± SEM of three determinations. ND, not detectable.

*$P < 0.05$ compared to the complete system with kidney microsomes.

Table 4. *In vitro* formation of morphine from codeine by rat liver, brain, and kidney microsomes

Tissue	Conditions	Morphine formation, pmol/hr per mg of protein
Liver	NADPH (2 mM)	10,200 ± 1800
	NADH (2 mM)	4,200 ± 542*
	− Cofactor	<50
Brain	NADPH (2 mM)	14.0 ± 0.9
	NADH (2 mM)	5.71 ± 1.13*
Kidney	NADPH (2 mM)	274 ± 20
	NADH (2 mM)	52.5 ± 7.6*
	− Cofactor	<4.0

Incubation was carried out with codeine (1 mM), 30 mg of microsomal protein of rat liver, brain, or kidney, and NADPH (2 mM) or NADH (2 mM). Other conditions are the same as described for Table 2. Each value is the mean ± SEM of three determinations. *$P < 0.05$ compared to the NADPH condition for that tissue.

The formation of oripavine, codeine, and morphine from thebaine requires an NADPH-generating system in liver microsomes (Table 2). Maximal synthesis occurs in the presence of an NADPH-generating system containing 1 mM NADP (complete system). The addition of NADP (1 mM) failed to further enhance the conversion of oripavine, codeine, or morphine (Table 2, Exp. A). However, addition of NADPH or NADH to the complete system dramatically increased both codeine and morphine formation. NADH increased the codeine and morphine formation to 6–7 times that in the complete system and about 2 times that in the complete system plus NADPH. In addition, when an NADPH-generating system was replaced by NADPH or NADH, specific oripavine (and morphine) formation occurred with NADPH, and specific codeine formation with NADH (Table 2, Exp. C). This tendency was also observed in the case of replacement of NADP by NADPH or NADH in the complete system (Table 2, Exp. B). Liver cytosol does not seem to transform thebaine to codeine or morphine under the present conditions (Table 2, Exp. D).

As shown in Table 3, rat brain and kidney can convert thebaine to oripavine, codeine, and morphine. In contrast to the liver, the complete system of brain and kidney microsomes failed to increase the formation of codeine or morphine when NADH was added.

Effect of NADPH and NADH in the Transformation of Codeine or Oripavine to Morphine by Rat Liver, Brain, and Kidney Microsomes. Although each tissue converts codeine to morphine at a different rate, the cofactor requirement is the same—namely, NADPH is most effective (Table 4). Table 5 shows that rat liver, brain, and kidney microsomes all have the capability to convert oripavine to morphine. In this biotransformation, NADH is the more effective cofactor, especially in liver microsomes. Upon the addition of NADH to the complete system, the formation of morphine dramatically increased. However, in brain and kidney microsomes the increase of morphine formation was not significant even after the addition of NADH to the complete system.

Inhibition of Oripavine, Codeine, and Morphine Formation from Thebaine by SKF-525A or Carbon Monoxide. SKF-525A (0.5 mM final concentration) inhibited the formation of oripavine, codeine, and morphine by rat liver microsomes in the presence of an NADPH-generating system plus NADH (1 mM) more than 95%. Carbon monoxide gas (99.9%) under the same conditions produced a 70–90% inhibition.

DISCUSSION

These *in vitro* studies show that rat liver, kidney, and brain microsomes have the capability of converting thebaine, an intermediate of morphine biosynthesis in plants, to oripavine, codeine, and morphine in a system that requires NADPH generation. Since NADH can markedly increase both codeine and morphine synthesis from the precursor, thebaine, when used in conjunction with the NADPH-generating system, there may be a different cofactor requirement, and more than one isozyme may be involved in this transformation. In fact, the formation of oripavine from thebaine and morphine from codeine requires NADPH as a cofactor (Tables 2 and 4), while the formation of codeine from thebaine and morphine from oripavine requires NADH as a cofactor (Tables 2 and 5). Morphine biosynthesis from thebaine in mammalian tissues can occur by at least two routes, which are similar to those of the poppy: (*i*) via codeine, thebaine → neopinone → codeinone → codeine → morphine; and (*ii*) via oripavine, thebaine → oripavine → 3-*O*-demethylated neopinone → morphinone → morphine (9) (Fig. 4). All of the intermediates described above have not yet been identified in these biotransformations. However, from our results it is reasonable to assume that morphine can be synthesized by both routes. Either pathway requires the 3- and 6-*O*-demethylation of thebaine. The methyl group on the 3 position is on a phenolic structure, while that on the 6 position is on an enolic structure, and the 6-*O*-demethylation reaction is followed by the migration of a double bond (see Fig. 4). Therefore, the structural difference and the different cofactor requirements for the 3- and 6-*O*-demethylations indicate that different enzymes are involved in the transformation. In plants, it is known that there are different enzymes responsible for the 3- and 6-*O*-demethylations of

Table 5. *In vitro* formation of morphine from oripavine by rat liver, brain, and kidney microsomes

Tissue	Conditions	Morphine formation, pmol/hr per mg of protein
Liver	Complete system	2.29 ± 0.70
	+ NADH (1 mM)	15.3 ± 2.3*
	− NADPH-gen. sys. + NADPH (2 mM)	1.51 ± 0.25
	− NADPH-gen. sys. + NADH (2 mM)	3.28 ± 0.48†
	− NADPH-gen. sys.	ND
Brain	Complete system	0.135 ± 0.04
	+ NADH (1 mM)	0.201 ± 0.03
Kidney	Complete system	0.275 ± 0.09
	+ NADH (1 mM)	0.341 ± 0.13
	− NADPH-gen. sys.	ND

The complete system included 30 mg of microsomal protein of liver, brain, or kidney and 1 mM oripavine. Other conditions are the same as described for Table 2. Each value is the mean ± SEM of three determinations. ND, not detectable.
*$P < 0.05$ compared to complete system.
†$P < 0.05$ compared to − NADPH-generating system + NADPH.

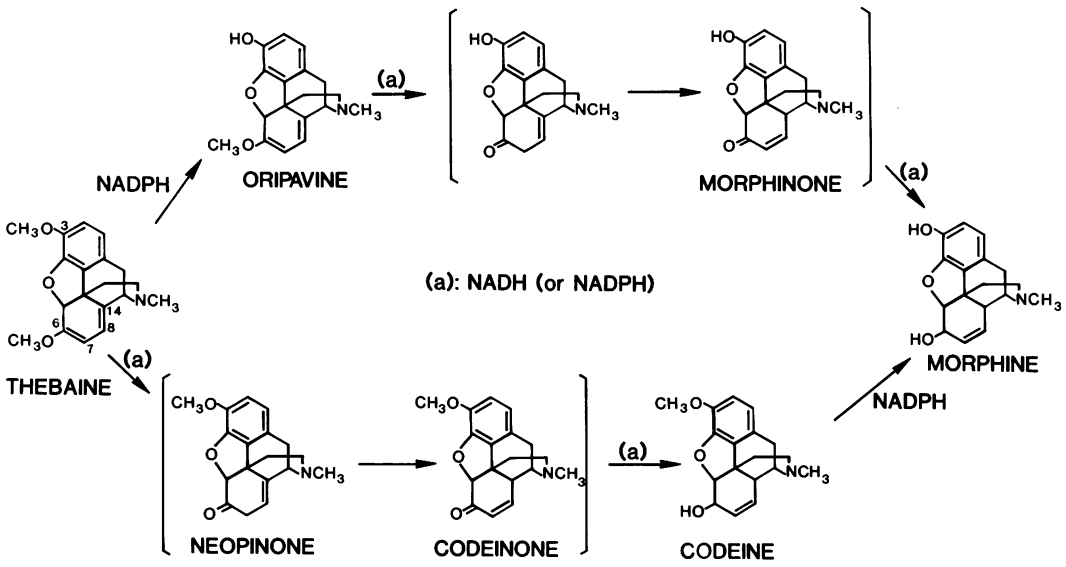

FIG. 4. Possible morphine biosynthetic pathways from thebaine in mammalian tissue and their cofactor requirements.

thebaine and even the enzymes responsible for 3-*O*-demethylation of thebaine and codeine are different (10–12), but the cofactor requirement for these enzymatic conversions is not known.

In the present *in vitro* experiments, the low concentration of morphine formed from thebaine, especially in brain and kidney microsomes, may be due to (*i*) the multistep reaction described above and the instability of the intermediates and (*ii*) the further metabolism of morphine. In fact, in the case of a one-step biotransformation such as from thebaine to oripavine, or codeine to morphine, the formation of each compound was higher even in brain or kidney microsomes (Tables 2–4). Another possibility for the low yield of morphine from thebaine is that the formation *in vitro* could be regulated or inhibited by some unknown factors.

Whether the microsomal enzymatic reactions described above are specific or nonspecific for thebaine has to be resolved. However, an enol ether such as the 6-*O*-methyl group of thebaine or oripavine is rare in nature, but the formation of codeine from thebaine and morphine from oripavine actually did occur *in vitro*. There might be specific enzyme systems responsible for these conversions in the microsomes. Yamano *et al.* (13) reported the presence of an enzyme in guinea pig liver cytosol that exhibits specificity for the 6-dehydrogenation of morphine. Rat liver cytosol failed to convert thebaine to codeine or morphine (Table 2, Exp. D). Therefore, the cytosol lacks the enzyme necessary for codeine and morphine biosynthesis from thebaine. Carbon monoxide and SKF-525A significantly inhibited formation of oripavine, codeine, and morphine, indicating that cytochrome P-450 may be involved in the biotransformation.

The presence of morphine and codeine in mammalian tissue can be explained by the fact that these compounds are enzymatically synthesized there. In plants thebaine is biosynthesized from two phenethyl residues, both being derived from tyrosine (9), and this precursor may be the same in mammalian tissues. Therefore, finding an intermediate for morphine and codeine biosynthesis in mammalian tissues will provide further explanation for the origin of these alkaloids. It is also most important to understand how morphine and codeine synthesis is regulated and what the rate-limiting step in their synthesis is.

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1. Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E. & Gilbert, P. E. (1976) *J. Pharmacol. Exp. Ther.* **197**, 517–532.
2. Kosterlitz, H. W. (1985) *Proc. R. Soc. London Ser. B.* **225**, 27–40.
3. Oka, K., Kantrowitz, J. D. & Spector, S. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1852–1854.
4. Goldstein, A., Barrett, R. W., James, I. F., Lowney, L. I., Weitz, C. J., Knipmeyer, L. L. & Rapoport, H. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5203–5207.
5. Donnerer, J., Oka, K., Brossi, A., Rice, K. C. & Spector, S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4566–4567.
6. Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254.
7. Matkovich, C. E. & Christian, G. D. (1973) *Anal. Chem.* **45**, 1915–1921.
8. Duquette, P. H. & Holtzman, J. L. (1979) *J. Pharmacol. Exp. Ther.* **211**, 213–218.
9. Brochmann-Hanssen, E. (1985) in *The Chemistry and Biology of Isoquinoline Alkaloids*, eds. Phillipson, J. D., Roberts, M. F. & Zenk, M. H. (Springer, Berlin), pp. 229–239.
10. Brochmann-Hanssen, E. & Okamoto, Y. (1980) *J. Nat. Prod.* **43**, 731–735.
11. Hodges, C. C., Horn, J. S. & Rapoport, H. (1977) *Phytochemistry* **16**, 1939–1942.
12. Brochmann-Hanssen, E. & Wunderly, S. W. (1978) *J. Pharmacol. Sci.* **67**, 103–106.
13. Yamano, S., Kageura, E., Ishida, T. & Toki, S. (1985) *J. Biol. Chem.* **260**, 5259–5264.
14. Kodaira, H. & Spector, S. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 2983 (abstr.).