Relationship of Pharmacokinetic and Metabolic Parameters to the Absence of Physical Dependence Liability with Thebaine-^{3}H

Thebaine (pk_a, 8.15), a congener of morphine (pk_a, 8.05) is devoid of analgesic activity and physical dependence liability[1,2]. Primarily a central stimulant, it produces strychnine-like convulsions in experimental animals[3–6] (LD_{50} in rabbits 14 mg/kg, average fatal dose in mice 31 mg/kg). Although the comparative pharmacology of thebaine has been studied[6–9], the basic mechanism of the lack of its physical dependence liability remains obscure. The implications of pharmacokinetic and metabolic factors on the little or no potential for physical dependence on thebaine are discussed here.

Materials and methods. Thebaine-^{3}H was prepared by catalyzed exchange tritium labeling of thebaine in dimethyl formamide-tritiated water of high specific activity (Amersham/Searle). Labile tritium was removed by lyophilization after mixing with methanol and the brownish residue subjected to repeated chromatography on neutral alumina, several precipitations from dilute acid solution, charcoal decolourisation and preparative sequential ITLC (Gelman instant thin layer chromatography media, silica gel impregnated glass fibre sheets, 20 × 20 cm) with solvent systems: *n*-butanol-acetic acid and water (35:3:10, v/v) and benzene-ethyl acetate-methanol and conc. ammonia (80:20:1.2:0.1, v/v). The final product was checked for its radiochemical purity by melting point, and buffered paper and thin layer chromatography in different solvent systems. Thebaine-^{3}H was diluted with nonradioactive material to a sp. act: 1.2 μCi/mg for injection solution.

Male Wistar rats (110–150 g) were injected s.c. with a 5 mg/kg dose of thebaine-^{3}H, the brain and blood collected at different periods of time by previous techniques[10–12]. Free thebaine-^{3}H was determined in diluted (1:5) 2 ml aliquots of plasma or brain homogenates (20% in 0.5 *M* HCl) containing 1 ml nonradioactive thebaine carrier (500 μg/ml). The pH was adjusted to 10–10.5 with dilute NaOH, the solution buffered with 2 ml K_3PO_4 buffer pH 10.4 and extracted with 15 ml chloroform by shaking for 15 min. The organic phase was centrifuged, washed with 4 ml 4% K_2HPO_4 and 10 ml aliquots of organic phase evaporated in counting vials. The residue was dissolved in 0.5 ml methanol and radioactivity counted with 10 ml toluene-phosphor solution. The counts were corrected for quenching using toluene-^{3}H as an internal standard. This method (minimal sensitivity 10–20 ng) provided in vitro, 95 ± 3% recovery of thebaine from biological materials in the concentration range 50–1000 ng. Other methods for assay of total radioactivity, acid-hydrolysis of conjugated metabolites were similar to those described earlier[10,13]. Binding of thebaine with rat plasma proteins and human albumin (fraction V, Sigma Chemical Co., St. Louis, Mo.) was studied using Amicon ultrafiltration membrane cones.

Results and discussion. The mean values on the concentration of thebaine and half-life in rat brain and plasma

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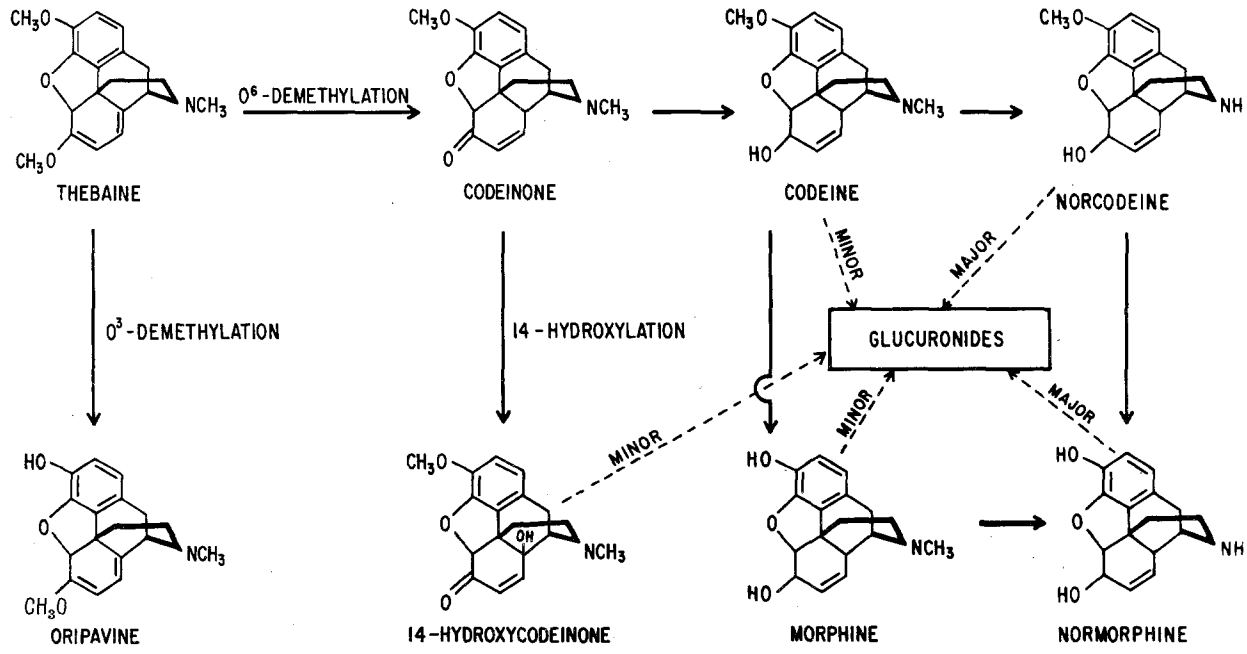

Metabolic pathways of thebaine.

Distribution[a] and half-life of thebaine-^{3}H in rat brain and plasma following a 5 mg/kg (free base) dose by s.c. injection

	0.5 h	1 h	2 h	4 h	6 h	16 h	24 h	48 h	Half-life (h)
Brain (free drug)	936.8 ± 31.0	815.8 ± 21.9	438.3 ± 17.6	192.0 ± 48.5	60.5 ± 7.6	60.3 ± 3.8	ND[c]	ND	1.50
Plasma (free drug)	1012.5 ± 16.5	1076.0 ± 56.7	558.0 ± 39.4	144.7 ± 15.6	85.7 ± 9.8	61.8 ± 5.8	ND	ND	1.05
Conjugated metabolites in plasma	0	0	0	0	249.3 ± 34.4	116.7 ± 34.0	428.0 ± 85.1	566.8 ± 113.0	
B/P[b]	0.92	0.76	0.78	1.33	0.71	0.97	—	—	

[a] Data represent mean value ± S.E.M. (ng/g wet brain weight or ng/ml plasma) of 4 determinations from 2 animals at each time period. [b] B/P represents the ratio of mean brain to plasma concentrations. [c] ND denotes not-detectable drug.

at different times after a single s.c. 5 mg/kg dose are given in the Table. The brain to plasma ratio for thebaine at 0.5 h was approximately 5 times that with morphine[10, 14]. Higher concentrations of thebaine in brain could arise due to the greater lipid-solubility of thebaine as compared to morphine (apparent partition coefficient of thebaine-^{3}H and morphine-N-methyl-^{14}C in 1-octanol/M/15 phosphate buffer pH 7.4 in conc. range 1–100 µg/ml were 8.32 and 1.03, respectively). Thebaine had a faster rate of penetration into and egression from the CNS than morphine and its levels in brain were not sustained as long as those of morphine[10, 15–17]. In contrast to drugs of high dependence liability e.g., morphine[10,18] and methadone[11, 13], thebaine did not persist in rat brain for a prolonged period. The elevation and decline of CNS levels of thebaine in brain coincided roughly with those in plasma and indicated that unlike morphine and methadone, it was predominantly present in the extracellular sites in the CNS and probably bound in a freely reversible manner to the brain tissue.

Binding of drugs to plasma proteins is an important factor in the duration and intensity of pharmacological action[19]. Rat plasma proteins and human albumin (5% in M/15 phosphate buffer pH 7.4) bound thebaine to the extent of 66.74 ± 2.03 (S.E.) and 27.34 ± 1.07 (S.E.)% respectively at 5 different concentrations in the range 1–100 µg/ml and this binding was found to be relatively independent of thebaine concentration in this range (morphine binding[20] to plasma proteins, 30 ± 10%). The greater binding of thebaine compared to morphine could possibly be due to a closer interaction of its ring structure with proteins by short range molecular forces, because amino groups in basic drugs have been reported[21, 22] to play a minor role in such binding. Further α and β-lipoproteins also account in large measure for the binding of lipophilic thebaine molecules.

The mean percentage of free thebaine-^{3}H excreted in 96 h urine and feces of 4 male rats following 5 mg/kg s.c. dose were 16.65 ± 2.05 and 4.34 ± 1.27 (S.E.)%; total radioactivity values in urine and feces were 43.38 ± 3.92 and 8.42 ± 1.86 (S.E.)% respectively. Column chromatography of pooled rat urine on Amberlite XAD-2 and ITLC (silica gel) by techniques previously described[13, 23, 24] established the presence of free codeine, norcodeine, normorphine, morphine and 14-hydroxycodeinone as some of the minor metabolites, and oripavine and glucuronide conjugates of normorphine and norcodeine as major metabolites. Some of these minor metabolites were also detected in brain 1 h

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ollowing a 5 mg/kg s.c. dose. Although evidence for codeinone as a metabolite was not obtained in this study, the formation of norcodeine and codeine would implicate its formation as an intermediate. Except for the formation of norcompounds and glucuronide conjugated detoxication products, the metabolic pathways of thebaine (Figure) have some interesting similarities to the biogenetic sequence of opium alkaloids in poppy plant[25-27].

This study demonstrates that rapid metabolism, elimination and lack of persistence of thebaine in rat brain conceivably do not give rise to cellular adaptation in the CNS, consequently thebaine possesses very low potential for tolerance and physical dependence. Repeated administration of thebaine, however, could lead to some accumulation in brain of small quantities of its minor metabolites e.g., norcodeine, normorphine, codeine and morphine, which may be responsible for the low grade dependence recently reported in monkey by the self-administration technique[28].

Zusammenfassung. Rasche Metabolisierung und Ausscheidung sowie Eliminierung von Thebain im Rattenhirn verursacht keine biochemische Änderung der Zellen im Zentralnervensystem, woraus die geringe physiologische Gewöhnung resultieren dürfte.

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Ileal Absorption of Disodium Ethane-1-Hydroxy-1,1-Diphosphonate (EHDP) and Disodium Dichloromethylene Diphosphonate(Cl_2 MDP) in the Chick

The therapeutic potential of the diphosphonate group of compounds dictates that pertinent and relevant information be obtained on their metabolic fate. These compounds have been shown to inhibit the precipitation and dissolution of calcium phosphate in vitro[1-4]. In vivo they were found to prevent soft tissue calcification[1,4] and in certain conditions bone calcification[5-10]. Furthermore they can also diminish bone resorption[1,3,7,9,11]. Because of these properties, a diphosphonate, disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP), has been used successfully in clinical conditions involving either abnormal calcification such as calcinosis universalis[12] and myositis ossificans progressiva[13,14], or increased bone turnover such as Paget's disease[15].

The knowledge of the metabolism of these compounds, and especially their intestinal absorption, therefore became of importance. In a recent multi-species study, MICHAEL et al.[16] noted that EHDP, when fed as part of the diet or given by gavage, was only slightly absorbed in the rat, rabbit and monkey ($< 10\%$). In the dog, EHDP absorption was, on the average, about 20% in young animals and about 14% in old ones, and it was suggested that nearly all of the absorption occurred in the stomach[16]. In man, absorption is in the range of a few percent. The values differ, however, greatly from one individual to another. It is possible that the uneven therapeutic response is due to this variation in absorption. Therefore a better understanding of the latter process is necessary. In the present experiments we have studied the absorption of two diphosphonates by ligated chick ileum.

Experimental. White Leghorn cockerels, in the weight range of 150–238 g, were fasted overnight. In the absorption phase, the chicks were anesthetized with ether, a laparotomy performed, and the ileum exposed. The lumen of the ileum was rinsed with saline, followed by a stream of air to remove excess saline. The length of the ileal segment was from the remnant of the yolk sac to the proximal attachment of the cecal horns. 1 ml of the dosing solution, at pH 7.2, composed of 150 m*M* NaCl, 2 m*M* K_2HPO_4, either 8 m*M* EHDP or 8 m*M* Cl_2MDP, was injected into the lumen between ligations; the concentration of ^{14}C-EHDP and ^{14}C-Cl_2MDP was 0.2 μc/ml[17]. At 15 min post-injection, the chick was killed by pentobarbital (Nembutal®) and the ligated segment removed and its length determined. The luminal contents were allowed to flow into a graduated tube from a cut end and the lumen subsequently rinsed with 30–35 ml of phosphate buffer (150 m*M* NaCl, 2 m*M* K_2HPO_4, pH 6.8), and all tubes were made up to 40 ml. The intestinal tissue was weighed, cut into small pieces and transferred to a homogenizing tube. Phosphate buffer was added in an amount equal to 3 times the weight of the tissue and the tissue thorougly homogenized with a Potter-Elvehjem homogenizer (teflon pestle). 1 ml aliquots of the gut homogenate

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