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The Metabolism of Phenolic Opiates by Rat Intestine

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1. A range of phenolic opiate agonists and antagonists undergo conjugation during passage across the rat intestine.

2. The efficiency of conjugation, mediated by gut UDP-glucuronyltransferase, is a function of the lipophilicity of the substrate.

3. Conjugation of lipophilic substrates such as buprenorphine and etorphine in rat intestinal mucosa is such that the gut wall must be regarded as the primary site of metabolism of these compounds after oral administration.

4. *N*-Dealkylation of the *N*-cyclopropylmethyl opiate, buprenorphine, has been demonstrated in rat gut sacs though dealkylation of *N*-methyl substrates was not detected.

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Introduction

A relative lack of oral activity has long been a problem associated with opiate drugs (Houde, Wallenstein & Beaver, 1965), low oral efficacy frequently being associated with the presence of a phenolic function. For example, the lowered availability of morphine after oral administration in man has been elegantly demonstrated in pharmacokinetic terms by Brunk and Delle (1975), who showed that plasma levels of unmetabolized drug following an oral dose of morphine were an order of magnitude lower than those obtained after the same dose administered by three different parenteral routes. It has been postulated that the low oral efficacy is due to poor absorption of the drug from the gastro-intestinal tract (Way & Adler, 1962), but studies in both animals and man have shown this not to be so (Cochin *et al.*, 1954; Brunk & Delle, 1975). It follows therefore that the low peripheral availability of morphine after oral administration is probably due to its metabolism during a 'first pass' through gut wall and liver.

The presence of a phenolic function in morphine-like compounds appears to be beneficial to their activity. It is, however, this functional group which appears to be responsible for the observed first pass effect in many cases, since conjugation takes place readily at this site. In the past, the major organ responsible for first pass metabolism has been assumed to be the liver, and hepatic metabolism of opiates has been extensively studied and reviewed (Way & Adler, 1960; Scrafani & Clouet, 1971). Although the drug-metabolizing capacity of the gut mucosa has been known for many years (Hartiala, 1973), it is only recently that the involvement of the intestinal wall in the biotransformation of opiates has been observed (Del Villar, Sanchez & Tephly, 1974).

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The capacity of the intestinal mucosa to carry out phase two metabolism of drugs is becoming the subject of an increasing amount of study and recent reports of the conjugation of phenol (Powell *et al.*, 1974), isoetharine (Williams *et al.*, 1974), isoprenaline (George, Blackwell & Davies, 1974), 1-naphthol (Bock & Winne, 1975), paracetamol and morphine (Josting, Winne & Bock, 1976) in whole animals have all established the gastro-intestinal tract as a major site of phase two metabolism. In the light of this work, a preliminary investigation of the role of the gut in the metabolism of three phenolic analgesics, dihydromorphine, etorphine and buprenorphine, was undertaken and leads to the speculation that lipophilicity might be a determining factor with regard to the efficiency of gut UDP-glucuronyl transferase activity (Rance & Shillingford, 1976). These studies, which have been extended to include a total of five opiates, dihydromorphine (I), naloxone (II), diprenorphine (III), etorphine (IV) and buprenorphine (V) (Fig. 1) are reported here in full.

(I) R = Me, R′ = H.

(II) R = allyl, R′ = OH, 6 = -one.

(III) R = cyclopropylmethyl, R′ = Me, X = CH_2CH_2.

(IV) R = Me, R′ = n-Pr, X = CH = CH.

(V) R = cyclopropylmethyl, R′ = t-Bu, X = CH_2CH_2.

Fig. 1. *Structures of opiates studied.*

Materials and methods

Radiochemical methods

[1,7,8(*n*)-^{3}H]Dihydromorphine (70 Ci/mmol) was supplied by the Radiochemical Centre, Amersham. [15, 16(*n*)-^{3}H]Diprenorphine (5·68 Ci/mmol), [15, 16(*n*)-^{3}H]etorphine (30 Ci/mmol) and [15, 16(*n*)-^{3}H]buprenorphine (28 Ci/mmol) were synthesized by the method of Lewis, Rance and Young, (1974). [15-^{3}H]Naloxone (4 Ci/mmol) was generously given by Professor H. W. Kosterlitz.

The radiochemical purity of all the labelled compounds was shown to be >95% by t.l.c. on Kieselgel F_{254} plates using (*a*) n-butanol–acetic acid–water (20 : 5 : 8), and (*b*) ethyl acetate–methanol (85 : 15) as developing solvents. Samples of drug solutions and biological fluids were diluted to 1·0 ml with water before addition to NE 260 (10 ml; Nuclear Enterprises Ltd.) for counting. Counting efficiencies were determined by internal standardization with [^{3}H]-hexadecane. All samples were counted in a Packard 2450 or a Packard 3003 scintillation spectrometer.

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Chromatographic methods

Plasma samples were diluted (50% v/v) with methanol to precipitate proteins which were removed by centrifugation. Samples of serosal fluids and plasma supernatants were applied to t.l.c. plates (0·25 mm Kieselgel HF_{254}, Merck Ag) and developed with solvents (*a*) or (*b*).

Authentic compounds and the *N*-dealkylated products, noretorphine and norbuprenorphine, were also applied to plates and visualized under u.v. light. Bands (1 cm) of silica gel were removed and counted in suspension in water (2 ml) and an aliquot (5 ml) of a solution of 2-(4′-t-butylphenyl)-5-(4″-biphenylyl)-1,3,4-oxadiazole (7·5 g) in Triton X–100 (334 ml) and toluene (666 ml).

Samples containing conjugated species were diluted with citrate buffers (pH 5·0; pH 6·8), *ca.* 100 units of β-glucuronidase (Type H1; Type 1; Sigma) or sulphatase (Sigma) added and the mixture incubated overnight. Saccharo-1,4-lactone was added in some cases to inhibit β-glucuronidase activity. The hydrolysates were extracted with chloroform (5 ml), the organic layer taken to small volume and applied to silica plates which were analysed for radioactivity as above.

Everted gut studies

The general procedure used was that of Wilson and Wiseman (1954). The rat was killed by cervical dislocation and a length of small intestine removed, everted and cut into segments (5 cm) which were kept in isotonic saline gassed with O_2–CO_2 (95 : 5) during subsequent manipulations. The intestinal segments were rinsed with isotonic saline to remove food and bacterial contaminants. Sacs 4 cm in length were filled with citrate–phosphate buffer (0·154 M, pH 7·4, 1·0 ml) containing glucose (0·5% w/v) and incubated at 37° in an aliquot (5 ml) of the same buffer solution containing the radiolabelled drug. Drug and metabolites in serosal fluid were separated by t.l.c. and assayed radiochemically as described above.

Absorption studies in situ

A modification of the method of Doluisio *et al.* (1970) was used. Albino male rats (Sprague–Dawley, Bantin and Kingman *ca.* 200 g), fasted overnight but allowed water *ad libitum*, were anaesthetized with urethane (25% w/v in saline). The small intestine was exposed by a midline abdominal incision and a polyethylene cannula (2·5 mm i.d., 3·5 mm o.d.) was inserted into duodenal and ileal ends. A syringe (50 ml) fitted with a three way tap was attached to the duodenal cannula and the intestinal lumen was cleared of particulate matter by slow introduction of solution from the syringe. A duplicate syringe was affixed to the ileal cannula and 0·15 M Sørensens phosphate buffer (pH 7·4, 10 ml) containing the radiolabelled drug was introduced into the small intestine. Portal venous blood was sampled by the introduction of a fine injection needle connected to a polythene cannula (Portex PP 25). Samples (0·2 ml) of portal blood were taken every 10 min and plasma separated by centrifugation. Plasma was assayed radiochemically for drug and metabolites as described above.

Results

The rate of transfer of the five opiate drugs across the intestinal mucosa of the rat everted gut sac is shown in Table 1, together with the percentage of the free

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Table 1. Absorption of ^{3}H-opiates *in vitro* in the rat everted gut sac preparation.

Drug	Drug luminal concentration (μg/ml)	Rate of absorption of drug-related material (μg drug equivalent/h)	% Free drug absorbed	%Dealkylation
Buprenorphine	1	$0{\cdot}32 \pm 0{\cdot}04$	$3{\cdot}4 \pm 0{\cdot}3$	$11{\cdot}5 \pm 1{\cdot}8$
	5	$2{\cdot}68 \pm 0{\cdot}10$	$15{\cdot}2 \pm 0{\cdot}4$	$22{\cdot}2 \pm 3{\cdot}6$
	10	$4{\cdot}25 \pm 0{\cdot}31$	$20{\cdot}0 \pm 1{\cdot}9$	$24{\cdot}6 \pm 1{\cdot}7$
Etorphine	1	$0{\cdot}39 \pm 0{\cdot}07$	$3{\cdot}3 \pm 0{\cdot}2$	—
	5	$2{\cdot}85 \pm 0{\cdot}08$	$6{\cdot}2 \pm 0{\cdot}4$	—
	10	$4{\cdot}25 \pm 0{\cdot}26$	$30{\cdot}1 \pm 4{\cdot}3$	—
Diprenorphine	1	$0{\cdot}91 \pm 0{\cdot}20$	$19{\cdot}3 \pm 2{\cdot}3$	$5{\cdot}2 \pm 0{\cdot}7$*
	5	$3{\cdot}70 \pm 0{\cdot}31$	$25{\cdot}9 \pm 0{\cdot}9$	$8{\cdot}3 \pm 3{\cdot}4$*
	10	$5{\cdot}92 \pm 0{\cdot}36$	$51{\cdot}3 \pm 4{\cdot}7$	$11{\cdot}6 \pm 1{\cdot}9$*
Naloxone	1	$0{\cdot}70 \pm 0{\cdot}04$	$56{\cdot}4 \pm 5{\cdot}1$	—
	5	$3{\cdot}33 \pm 0{\cdot}34$	$62{\cdot}4 \pm 1{\cdot}6$	—
	10	$4{\cdot}70 \pm 0{\cdot}68$	$61{\cdot}0 \pm 5{\cdot}0$	—
Dihydromorphine	1	$0{\cdot}22 \pm 0{\cdot}03$	$85{\cdot}5 \pm 0{\cdot}8$	—
	5	$1{\cdot}60 \pm 0{\cdot}11$	$86{\cdot}8 \pm 2{\cdot}4$	—
	10	$3{\cdot}22 \pm 0{\cdot}17$	$90{\cdot}7 \pm 1{\cdot}9$	—

Results are means ± S.E., $n \geqslant 4$.

*Unidentified metabolite, possibly the nor compound.

drug found in the serosal fluid in each case. The remainder of the radioactivity in the serosal fluid was present as polar conjugates in all cases. The conjugates were readily cleaved by β-glucuronidase and the hydrolysis was inhibited by saccharo-1,4-lactone indicating that the major conjugating species was glucuronic acid. The principle conjugated species formed in the intact rat after oral administration of buprenorphine is also the glucuronic acid conjugate (Jordan and Rance, unpublished). Hydrolysis by the sulphatase enzyme was low showing minimal sulphate ester formation with the substrates used. Chromatographic examination of the products of β-glucuronidase hydrolysis indicated the presence of the *N*-dealkylation product of buprenorphine (norbuprenorphine). No *N*-dealkylation was detected with the *N*-methyl compounds, dihydromorphine and etorphine, nor with naloxone which possesses an *N*-allyl function. With diprenorphine, which has, in common with buprenorphine, an *N*-cyclopropylmethyl group, an unidentified metabolite was detected in low amounts which might be the nor compound derived from this substrate. Table 2 presents the results of the studies carried out *in situ* using the two drugs (dihydromorphine and buprenorphine) of most widely different lipophilicity (Table 3). The results are expressed as total plasma levels of drug-related material present in the portal system together with the percentages of that material which were present as the unchanged drug.

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Table 2. Portal vein plasma levels of [^{3}H]buprenorphine, [^{3}H]dihydromorphine and conjugates after absorption of the drugs from the rat isolated small intestine *in situ*

Drug concentration in small intestine* (μg/ml)	Time (min)	Buprenorphine		Dihydromorphine	
		Plasma level of total radioactivity (μg buprenorphine equivalent/ml)	% free drug	Plasma level of total radioactivity (μg dihydromorphine equivalent/ml)	% free drug
1·0	10	0·06 (0·04–0·07)	—†	0·014 (0·009–0·016)	41·2 (34·1–50·1)
	20	0·11 (0·09–0·14)	—	0·024 (0·021–0·027)	38·8 (36·4–43·4)
	30	0·12 (0·09–0·15)	—	0·043 (0·036–0·050)	36·7 (31·0–42·1)
	40	0·08 (0·06–0·09)	—	0·048 (0·039–0·052)	40·2 (33·1–42·0)
	50			0·036 (0·028–0·040)	39·9 (37·0–45·7)
10·0	10	0·37 (0·30–0·66)	10·1 (3·3–13·4)	0·034 (0·028–0·036)	41·8 (32·6–53·5)
	20	0·55 (0·45–0·73)	14·2 (8·2–20·2)	0·044 (0·040–0·048)	35·4 (27·3–42·9)
	30	0·54 (0·40–0·65)	13·5 (9·6–20·1)	0·079 (0·072–0·084)	39·9 (37·6–43·1)
	40	0·61 (0·42–0·73)	9·5 (7·8–15·0)	0·086 (0·082–0·094)	41·6 (38·0–46·7)
	50	0·55 (0·32–0·78)	9·3 (6·6–15·5)	0·094 (0·090–0·098)	40·2 (40·0–45·9)

Results are means of 3 experiments. Ranges in parentheses.

* Total volume introduced into the small intestine was 10·0 ml in each case.

† No free drug detected.

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