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THE ROLE OF THE GUT IN THE METABOLISM OF STRONG ANALGESICS

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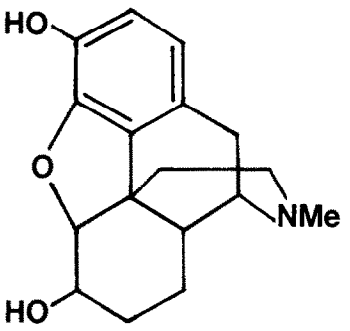
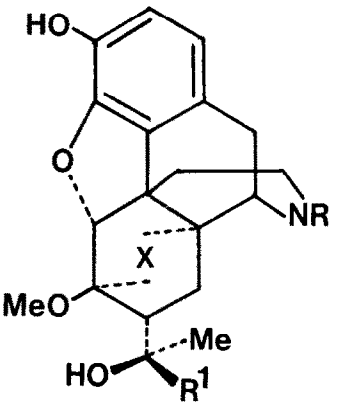
The presence of a 3-phenolic function appears to be an essential structural requirement for the activity of morphine-like, strong analgesics. Such a polar function introduces problems after oral administration of these compounds as phase two metabolism takes place very rapidly. A marked "first pass" metabolism effect is thus observed resulting in poor oral activity in many cases.[1] In the past, the major site of metabolism has been assumed to be the liver. Evidence is now beginning to accumulate, however, that the role of the gut in the metabolism of xenobiotic substances may well be more important than first thought.

Oral administration of morphine in man has been shown to provide very low levels of free drug in the peripheral circulation due to rapid metabolism of the drug.[2] It has been further suggested that conjugation may take place in the mucosal cells of the small intestine as well as in the liver, the slow absorption of morphine from the gastro-intestinal tract enabling a conjugation reaction to occur in the cells of the intestinal mucosa during absorption.[3]

The significance of intestinal conjugation is at present under review. Recent reports on the conjugation of phenol[4], 1-naphthol[5], isoetharine[6] and isoprenaline[7] have all established the gastro-intestinal tract as a major site of phase two metabolism.



(1)



(2) R=Me, R^1=^{n}Pr, X = -CH=CH-

(3) R=CH_2-cyclopropyl, R^1=^{t}Bu, X= $-CH_2-CH_2-$

In the present study, we have investigated the role of the gut in the metabolism of three phenolic analgesics, dihydromorphine[8] (1), etorphine[9] (2) and buprenorphine[10](3), which are subject to "first pass" metabolism but have widely differing physicochemical properties.

Materials and Methods

[1,7,8 n - ^{3}H] - Dihydromorphine was supplied by the Radiochemical Centre, Amersham and had a specific activity of 54 μCi/mg. [15, 16 -^{3}H] - Etorphine and [15, 16 - ^{3}H] - buprenorphine were synthesised by the method of Lewis, Rance and Young [11], and had specific activities of 58 μCi/mg and 200 μCi/mg respectively. Samples of drug solutions and biological fluids were diluted to 1.0 ml with water before addition to NE260 (10 ml; Nuclear Enterprises Ltd.) for counting. Efficiencies were determined by internal standardisation.

Plasma samples were diluted (50% v/v) with methanol to precipitate proteins which were removed by centrifugation. Protein free samples were applied to thin layer plates (0.25 mm Kieselgel HF_{254}, Merck Ag) and eluted with n-butanol:acetic acid:water (20:5:8) or ethyl acetate:methanol (85:15) as solvent. Authentic etorphine, buprenorphine and dihydromorphine were also applied to plates and visualised under UV light (254 nm). Bands of silica gel were removed from the plates and counted in suspension in water (2 ml) and an aliquot (5 ml) of a solution of butyl PBD (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), a few units of β - glucuronidase (Type H1, Type 1; Sigma) or sulphatase (Sigma) added and the mixture incubated overnight. Saccharo -1,4-lactone was added to inhibit the β- glucuronidase activity. The hydrolysate was extracted with chloroform (5 ml), the organic layer was taken to small volume and applied to tlc plates which were analysed for radioactivity as above.

The general procedure used for everted sac studies was that of Wilson and Wiseman[12]. The rat was killed by cervical disclocation and a length of ileum removed, everted and cut into four segments (5 cm) which were kept in isotonic saline gassed with O_2:CO_2 (95:5) during subsequent manipulation. The intestinal segments were rinsed with isotonic saline to remove food and bacterial contaminants. Sacs 4cm in length were filled with citrate-phosphate buffer (0.154M 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. Samples of gut serosal fluid were analysed for the labelled drug and metabolites.

For in situ absorption studies, male Wistar-albino rats, fasted overnight but with access to water were anaesthetised with urethane (25% w/v), a midline abdominal incision was made and the small intestine isolated and cannulated at the duodenal and ileal ends with polyethylene cannulae (2.5 mm i.d. ; 3.5 mm o.d.). After clearing the particulate matter from the intestinal lumen by a slow perfusion of saline at 37°, 0.15 M Sorensons phosphate buffer (pH 7.4, 10 ml) containing the radiolabelled drug was introduced into the intestine. The portal vein was cannulated by introducing a fine injection needle connected to a polythene cannula. Blood samples (0.2 ml) were taken at timed intervals. Plasma samples were analysed for the labelled drug and metabolites.

Table 1

In vitro absorption of [^{3}H] buprenorphine, [^{3}H] etorphine and [^{3}H] dihydromorphine by an everted gut sac preparation (*Mean ± SEM n ≥ 4)

Drug	Drug luminal concentration (μg/ml)	Total drug related material absorbed* (μg drug equivalent/h)	Free drug absorbed %	% Dealkylat
Buprenorphine	1	0.32 ± 0.04	3.4 ± 0.3	11.5 ± 1.8
	5	2.68 ± 0.10	15.2 ± 0.4	22.2 ± 3.6
	10	4.25 ± 0.31	20.0 ± 1.9	24.6 ± 1.7
Etorphine	1	0.39 ± 0.07	3.3 ± 0.2	-
	5	2.85 ± 0.08	6.2 ± 0.4	-
	10	4.25 ± 0.26	30.1 ± 4.3	-
Dihydromorphine	1	0.22 ± 0.03	85.5 ± 0.8	-
	5	1.60 ± 0.11	86.8 ± 2.4	-
	10	3.22 ± 0.17	90.7 ± 1.9	-

Results

The rate of transfer of buprenorphine, etorphine and dihydromorphine across the intestinal barrier of the everted gut sac and the nature of the absorbed species are summarised in Table 1. Enzymic hydrolysis was effected by β-glucuronidase and inhibited by saccharo-1,4-lactone. Minimal hydrolysis of the conjugate with the sulphatase preparation was observed. This indicates that the major species present in each case was a glucuronide of the unchanged drug. In addition, N-dealkylation of buprenorphine was shown to take place in the everted gut sac to yield the nor-metabolite (3; R=H) in significant amounts. N-dealkylation of the other two drugs was not detected.

Portal vein plasma levels of free and conjugated buprenorphine obtained during the *in situ* absorption studies are shown in Table 2.

Table 2

Portal vein plasma level of [^{3}H] - buprenorphine after absorption of the drug from rat intestine (in situ) (*Mean ± SEM n ⩾ 3)

Buprenorphine dose introduced into the small intestine	Time (min)	Plasma level of drug related material (ng buprenorphine equiv/ml)*	Free drug absorbed %
1 µg/ml:10 ml	10	0.06 ± 0.02	-
	20	0.11 ± 0.02	-
	30	0.12 ± 0.02	-
	40	0.08 ± 0.01	-
10 µg/ml: 10 ml	5	0.37 ± 0.15	10.1 ± 3.6
	10	0.55 ± 0.09	14.2 ± 3.5
	20	0.54 ± 0.08	13.5 ± 3.3
	30	0.61 ± 0.09	9.5 ± 2.8
	40	0.55 ± 0.12	9.3 ± 3.1

Discussion

The absorption of buprenorphine and etorphine in the everted gut sac preparation appears to be essentially similar both in rate and nature of absorbed species. Conjugation with glucuronic acid on passage through the intestinal wall accounted for 95% of the absorbed radioactivity at the lowest concentration studied (1 µg/ml). The capacity of the enzyme system responsible for the conjugation reaction in the gut sac preparation appears to be limited, as at higher mucosal drug concentrations relatively higher levels of free drug were observed on the serosal side of the membrane. A similar pattern of metabolism by rat intestine has been demonstrated for buprenorphine in situ. After introduction of the drug into the small intestine at a concentration of 1 µg/ml, essentially all the drug related material appearing in the portal vein was in the conjugated form. At a higher concentration, (10 µg/ml) some free buprenorphine

was observed, though the percentage of the unconjugated species was lower than that observed in vitro.

In contrast to the situation with buprenorphine and etorphine, the absorption of dihydromorphine appears to follow a distinctly different pathway. At all concentrations examined, dihydromorphine showed very little metabolism in the everted sac and crossed the intestinal barrier essentially unchanged.

Table 3.

Partition coefficients of dihydromorphine, etorphine and buprenorphine[1]

Drug	Log N heptane/phosphate buffer pH 7.4
Buprenorphine	1.78
Etorphine	0.15
Dihydromorphine	-5.0

Examination of the partition coefficients (heptane/phosphate buffer, pH 7.4) shown in table 3 indicate that, in contrast to buprenorphine and etorphine, dihydromorphine is hydrophilic in character. Since the latter drug is far less efficiently conjugated by the intact gut wall, this observation leads to speculation that lipophilicity might be a determining factor with regard to the efficiency of gut UDP-glucuronyl-transferase activity.

The results of this study suggest that the role of the liver in the deactivation of strong analgesics after oral administration has been overemphasised, for it has been shown that this organ is not essential for the "first pass" metabolism of the lipophilic compounds buprenorphine and etorphine in rat. Dihydromorphine appears not to be readily conjugated by rat intestine and the liver must be regarded as the prime site of metabolism of this relatively polar compound.

Further evidence has thus been obtained that a major pathway exists in the gastro-intestinal tract for the protection of the whole organism from the potential toxic effects of xenobiotic substances and that a determinary factor in the efficiency of this pathway is the lipophilicity of the substance.

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