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PHARMACODYNAMIC STUDIES WITH (−)-3-PHENOXY-*N*-METHYLMORPHINAN IN RATS

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Abstract—Analgesia and brain and plasma concentrations of (−)-3-phenoxy-*N*-methylmorphinan (PMM) and its metabolites were determined in rats administered 50 mg/kg of ^{3}H-labeled PMM p.o., an approximate ED_{50}. Unchanged PMM and two active metabolites, levorphanol and a different phenol, *p*-hydroxylated on the 3-phenoxy group (*p*OH-PMM), were present in brain at concentrations greater than in plasma. Analgesia was observed from 1 to 6 hr and was associated with brain concentrations of 400–1400 ng/g of PMM, 190–300 ng/g of *p*OH-PMM, and 16–27 ng/g of levorphanol. The presence of 58% of the administered dose as unchanged PMM in the gastrointestinal tract at 6 hr may reflect slow absorption and explain the persisting brain concentrations of PMM and its metabolites as well as the prolonged analgesia. Analgesia may have been due to the presence in brain of only PMM, *p*OH-PMM or levorphanol, or to the combined activity of two or three of these substances. Administration of the approximate ED_{50} of ^{3}H-labeled levorphanol (0.1 mg/kg, s.c., or 6 mg/kg, p.o.) resulted in brain levorphanol concentrations (11–18 ng/g) close to those observed when PMM was administered p.o. at 50 mg/kg. After administration of an approximate subcutaneous ED_{50} of [^{3}H]*p*OH-PMM of 24 mg/kg, the brains contained *p*OH-PMM (1500–4100 ng/g) and levorphanol (60–100 ng/g); these levorphanol concentrations were higher than those found after administration of the approximate ED_{50} of PMM or levorphanol. The findings indicate that brain levorphanol concentrations resulting from administration of PMM or *p*OH-PMM to rats may account for the analgesic activity observed, i.e. that PMM and *p*OH-PMM may act as prodrugs for levorphanol.

(−)-3-Phenoxy-*N*-methylmorphinan (PMM) is a new analgesic drug which is at least as potent as codeine in the rat tail-flick test [1]. PMM was reported to have less physical dependence liability than codeine [1] and twice the duration of analgesic activity of codeine [2].

It was observed that levorphanol was excreted in the urine of rats treated intraperitoneally with 25 mg/kg of PMM [3]. The analgesic activity of levorphanol in rats is stronger and longer lasting than that of morphine [4] and the addiction potential of levorphanol in man is greater than that of codeine [5]. Additional metabolites of PMM found previously were norlevorphanol in rat urine [3], (−)-3-phenoxymorphinan (norPMM) from rat liver *in vitro*† and (*p*-hydroxy)phenoxy-*N*-methylmorphinan (*p*OH-PMM) in dog bile and feces [6]. The structures of PMM and its metabolites are shown in Fig. 1. The binding affinities of codeine, PMM and levorphanol are 10.0, 5.0 and 0.004, reflecting the considerable differences in the ED_{50} values [2], determined in the rat tail-flick test.

The present study was undertaken to correlate the brain concentration of PMM and its pharmacologically active metabolites with analgesia. To achieve this goal, the concentrations of drug and metabolites in rat brain and plasma after administration of the approximate ED_{50} or [^{3}H]PMM hydrochloride, [^{3}H]levorphanol tartrate and [^{3}H]*p*OH-PMM hydrochloride were measured.

MATERIALS AND METHODS

Analgesia testing. The tail-flick test, as described by Dewey and Harris [7], was used to measure the analgesic activity produced following the oral administration of [^{3}H]PMM and unlabeled PMM. The intensity of a radiant heat stimulus was set so that normal predrug reaction time latency was 2–5 sec. A cut-off time of 10 sec was used. Each rat was tested before drug administration and at a single time interval after drug administration.

The differences between initial and post-treatment reaction times (tail-flick latency) were tested for statistical significance by analysis of variance and Student's *t*-test. The analgesic activity was also expressed as a percentage of the maximum possible response (MPR) at each time interval, calculated from the formula described by Dewey and Harris [7]. The ED_{50} was then determined by plotting % MPR vs log dose.

$$\%\ \text{MPR} = \frac{\text{test latency} - \text{control latency}}{\text{cut-off time} - \text{control latency}} \times 100$$

Animal experiments. Male Sprague–Dawley rats from the Charles River Breeding Labs (Wilmington, MA) were used in all experiments. For the determination of the ED_{50} values of PMM hydrochloride, levorphanol tartrate and *p*OH-PMM hydrochloride,

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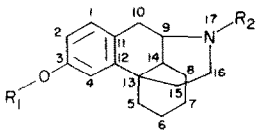
† J. J. Kamm and A. J. Szuna, personal communication.



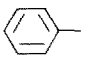
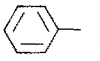
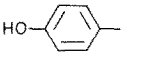
COMPOUND	R_1	R_2
PMM		CH_3
norPMM		H
pOH - PMM		CH_3
LEVORPHANOL	H	CH_3
NORLEVORPHANOL	H	H

Fig. 1. Structures of PMM and its metabolites.

each compound was administered in aqueous solution at four dose levels to five rats (115–165 g) per dose level. One hour after oral administration of PMM hydrochloride, or subcutaneous administration of levorphanol tartrate or *p*OH-PMM hydrochloride, analgesia was measured by the tail-flick test.

For the study with [^{3}H]PMM hydrochloride, analgesic activity and the plasma and brain concentrations of labeled PMM and metabolites were measured in the same rats. These rats received 50 mg/kg of nonradioactive or ^{3}H-labeled PMM hydrochloride p.o. Male rats weighing 115–165 g were employed in groups of six. Analgesia was measured before dosing (four rats were given labeled PMM and two were given unlabeled PMM) and again after 0.5, 1, 2, 3, 4, or 6 hr. Groups of six control rats (given saline) were similarly studied using the same time intervals. After analgesia was measured at the end of each time interval, the rats that had received labeled PMM were anesthetized with ether, and blood was withdrawn from the inferior vena cava. Plasma was promptly prepared by centrifugation, and aliquots (0.1 ml) were taken in duplicate to determine total plasma radioactivity. The remainder was stored frozen at −17°. The brains were excised immediately, weighed, frozen in dry ice, and stored frozen at −17°.

For studies with levorphanol and *p*OH-PMM, rats weighing 125–152 g were dosed in groups of five or six with [^{3}H]levorphanol (p.o. or s.c.) or [^{3}H]*p*OH-PMM (s.c.). Analgesia was not measured in these groups; each animal was killed after 60 min and plasma and brain samples for the analysis of drug and metabolites were obtained as described above.

Thin-layer chromatography. Chromatograms of brain and plasma extracts were run on silica gel 250 μm plates (type 60 F-254, Merck, obtained from Brinkmann Instruments, Westbury, NY) with one of the following solvent systems: (1) ethyl acetate–acetic acid–methanol–deionized water (15:1:1:2); (2) dichloromethane–*n*–propanol–methanol–conc. ammonium hydroxide (70:20:8:2); (3) chloroform–methanol–conc. ammonium hydroxide (93:15:2); (4) ethyl acetate–conc. ammonium hydroxide–methanol–heptane (4:1:1:2, upper phase); (5) chloroform-methanol-*n*-propanol-conc. ammonium hydroxide (80:10:10:2); (6) dichloroethane–methanol–conc. ammonium hydroxide (70:28:2); (7) chloroform–methanol–conc. ammonium hydroxide (75:4.5:0.5); and (8) chloroform–acetic acid–methanol (18:1:1). All proportions were v/v. Separations achieved with these solvent systems were described previously [8]. Reference compounds were cochromatographed with each plasma or brain extract. The various morphinans were visualised under short-wave u.v. light. The silica gel zones containing the radioactive compounds were scraped and the scrapings were suspended in 3.0 ml of deionized water and 10 ml of Aquasol (New England Nuclear Corp., Boston, MA) for radioassay.

The plasma and brain concentrations of ^{3}H-labeled PMM, *p*OH-PMM, levorphanol or norlevorphanol in ng/ml or ng/g were calculated from the total ^{3}H-concentration (dpm/ml or dpm/g) × fraction extracted × fraction extracted that migrated on t.l.c. with authentic PMM, *p*OH-PMM, levorphanol or norlevorphanol ÷ the specific activity (dpm/ng) of the administered drug.

Compounds and radioactive assays. Unlabeled PMM hydrochloride, norPMM and *p*OH-PMM and *p*OH-PMM hydrochloride were synthesized by Dr. E. Mohacsi [2].

The ^{3}H-labeled drugs were synthesized by the Roche Radioisotope Synthesis Laboratory with the tritium label on carbon atom 2. The ^{3}H-labeled PMM was 97% pure when assayed with solvent system 1 but the purity declined on storage. The radioactive PMM was purified by t.l.c. with solvent system 2. The sample was 98% pure when judged by t.l.c. with solvent systems 3 and 4. Tritium was measured with a model 2660 liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL). Conversions of counts per min to disintegrations per min were automatically computed by the external standardization method.

^{3}H-Labeled levorphanol was purified before use by preparative t.l.c. with solvent system 3. To reduce the possibility of radiochemical decomposition,

200 μg of unlabeled levorphanol was added before the purification by t.l.c. Thin-layer chromatography with solvent system 5, followed by radioassay of the segmented silica gel, indicated 98% purity. The purified [^{3}H]levorphanol tartrate was administered s.c. at 0.1 mg/kg (sp. act. 485 μCi/mg) or p.o. at 6 mg/kg (sp. act. 36 μCi/mg).

Radioactive *p*OH-PMM was purified using a model ALC/GPC-204 liquid chromatograph (Waters Associates, Milford, MA) equipped with a 4 × 30 mm μBondapak C18 column and a u.v. detector at 254 nm. Acetonitrile–deionized water–glacial acetic acid (99:99:2, by vol.) was used as solvent at a flow rate of 0.4 ml/min. The fraction that contained the [^{3}H]*p*OH-PMM was reduced to dryness in a stream of nitrogen. The purity of the residue was 94% and it contained no levorphanol. [^{3}H]*p*OH-PMM was administered subcutaneously at 24 mg/kg (sp. act. 2.2 μCi/mg, free base).

Isolation and assay of PMM and its unconjugated metabolites in plasma. To 1–2 ml of rat plasma were added 50 μg each of PMM, norPMM, levorphanol and norlevorphanol and 25 μg of *p*OH-PMM. The pH was adjusted to 10.8 by adding 1 ml of 0.2 M Na_3PO_4 solution and 50 μl of 1 M NaOH. PMM and its conjugated metabolites were extracted twice with 6 ml of diethyl ether. The ether phases were combined, and the ether was evaporated in a stream of nitrogen. The residue was dissolved in 0.4 ml of methanol and 10 or 20 μl was assayed for total ^{3}H. Aliquots were chromatographed with solvent systems 3 and 5.

To 1.5 to 3 ml of plasma of rats that received [^{3}H]*p*OH-PMM were added 50 μg each of *p*OH-PMM, levorphanol and norlevorphanol as nonradioactive carrier. Only levorphanol and norlevorphanol were added to the plasma of rats that received [^{3}H]levorphanol. The extracts of unconjugated compounds were assayed by t.l.c. with solvent system 5.

The aqueous phase was freed of ether in a stream of nitrogen. The samples were incubated overnight at 37° at pH 5.0 with Glusulase (0.2 ml; Endo Laboratories, Garden City, NY). The hydrolyzed metabolites were extracted at pH 10.8 with ether. After pooling, the ether was evaporated. Each residue was assayed for ^{3}H and subjected to t.l.c.

Isolation and assay of PMM and its unconjugated metabolites in brain. Each brain was homogenized with 4 ml of deionized water. Aliquots (0.1 or 0.2 ml) of the homogenate were assayed to determine total ^{3}H in each brain. To improve recoveries and to visualize brain metabolites on t.l.c. plates, 125 μg each of PMM, norPMM, levorphanol and norlevorphanol and 62.5 μg of *p*OH-PMM were added as carrier. The ether extracts, prepared at pH 10.8, were combined, and the ether was evaporated. The residue was dissolved in 4.5 ml of methanol and 50 μl was assayed for ^{3}H. The remainder, after addition of 0.5 ml of water and 2 drops of 5 M HCl, was extracted twice with 5 ml of hexane to remove nonradioactive materials from the extracts. A precipitate that formed in the methanol phase was separated by centrifuging; the pellet contained only 1–3% of the total ^{3}H and was discarded. The methanol extract was reduced to dryness and the residue was suspended in 0.3 ml of ethanol. Insoluble material was removed by centrifugation. The pellet contained less than 2% of the total ^{3}H and was discarded. Aliquots of the ethanol extracts were chromatographed with solvent systems 2, 3 and 6.

Each brain of rats that had received [^{3}H]levorphanol was fractionated as described, except that 125 μg each of levorphanol and norlevorphanol was added as nonradioactive carrier. In addition, *p*OH-PMM was added to brain homogenates of rats treated with [^{3}H]PMM. All extracts were chromatographed with t.l.c. solvent system 5.

The recovery of PMM, *p*OH-PMM and levorphanol from plasma and brain was tested by reisolating known amounts of [^{3}H]PMM, [^{3}H]*p*OH-PMM or [^{3}H]levorphanol added to control plasma (2.0 ml each) or brain homogenates (5.0 to 6.0 ml each). Mean recoveries of PMM from plasma and brain were 103 and 95% respectively. Mean recoveries of *p*OH-PMM from plasma and brain were 97 and 83% respectively. Mean recoveries of levorphanol from brain and plasma were 94 and 97% respectively. No corrections for incomplete recoveries were made.

Assay of intestinal PMM and its metabolites. Each entire gastrointestinal tract (G.I.T.) including contents was homogenized with 6–7 vol. of 1 M sodium phosphate, pH 11.0, with a model SDT Tissumizer (Tekmar, Cincinnati, OH) and extracted with ether. Aliquots of the homogenates, the ether extracts, and the aqueous residues were assayed for ^{3}H-content. The ether phases were combined and reduced to 10–20 ml. Addition of up to an equal volume of methanol caused formation of a precipitate. The precipitates were found to contain less that 1% of the ^{3}H and were discarded. Estimates of extracted PMM and metabolites were obtained by t.l.c. with solvent system 2 using silica gel GF plates (Analtech, Newark, DE). After scanning with a Packard model 7201 radiochromatogram scanner, the radioactive bands were scraped and radioassayed. For confirmation, ether extracts were also chromatographed with solvent systems 5, 6, 7 and 8.

RESULTS AND DISCUSSION

Determination of ED_{50} *values of PMM hydrochloride, levorphanol tartrate and p*OH-*PMM hydrochloride.* One hour after drug administration, the following ED_{50} values were obtained in the rat tail-flick test: PMM hydrochloride p.o., 59 mg/kg; levorphanol tartrate p.o., 6.1 mg/kg, and s.c., 0.07 mg/kg; and *p*OH-PMM hydrochloride s.c., 24 mg/kg.

Studies with PMM. The analgesic response 1–6 hr after oral administration of 50 mg/kg of PMM is depicted in Fig. 2 (top). The tail-flick latency values shown are combined means from rats treated with nonradioactive and ^{3}H-labeled PMM and represent the differences between pre-dose values and post-dose scores.

In all of the six groups treated with vehicle alone, the reaction times after treatment were comparable to those occurring before treatment and the variability was very low. PMM was not effective 30 min after treatment. At all other intervals, 50 mg/kg of PMM produced effects that ranged from 30 to 84%

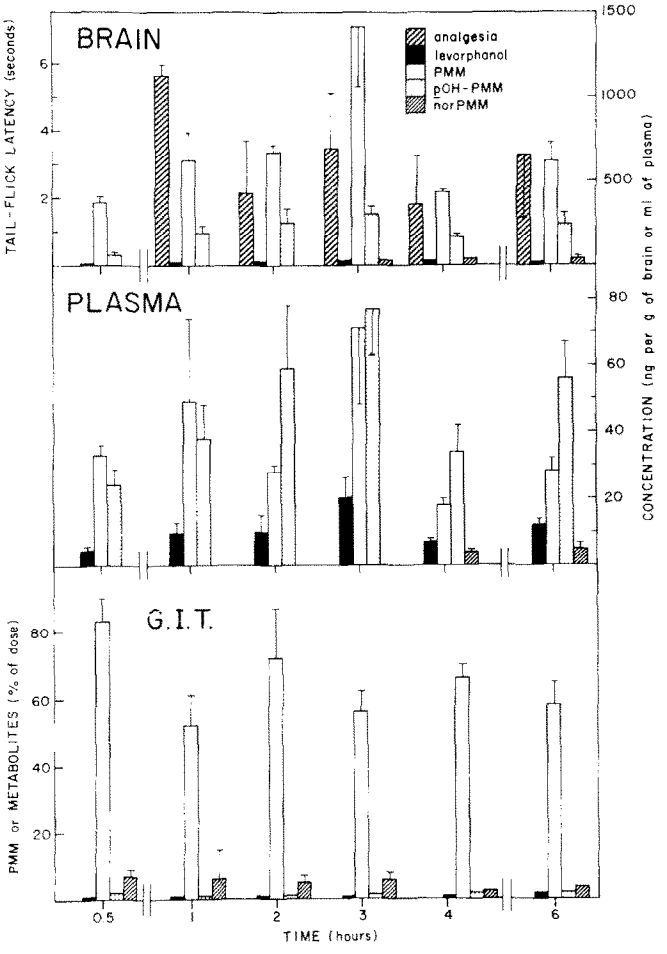


Fig. 2. Analgesia and concentrations of PMM and its conjugated metabolites in brain, plasma and gastrointestinal tract after a single oral dose of 50 mg/kg of PMM hydrochloride. Means (± S.E.M.) of tail-flick latencies (upper panel) represent differences between pre-drug and post-drug values. The brain (upper panel) and plasma concentrations (center) are in ng/g or ng/ml (mean ± S.E.M.) whereas PMM and metabolites in G.I.T. plus contents (bottom) are in percent of dose.

of the maximum possible analgesic response. Thus, there were fluctuations in the analgesic response. The S.E.M. shown in Fig. 2 suggest that no differences existed between the responses at 2, 3, 4 and 6 hr, and this was confirmed by the results of the *t*-test. The effect at 1 hr was significantly greater that the effects at 2 hr ($P < 0.05$), 3 hr ($P < 0.05$) and 4 hr ($P < 0.01$), but not at 6 hr ($P > 0.05$).

The parent drug predominated in brain at all time intervals (Fig. 2, top) and *p*OH-PMM was present as the major metabolite in brain. Omitting the 30-min data, a stable ratio of PMM to *p*OH-PMM of 3.6 ± 0.4 was found, with the concentrations of PMM and *p*OH-PMM highest at 3 hr. Relatively now concentrations of brain levorphanol were found in all animals. At 30 min, the mean levorphanol concentration was 8 ng/ml, rising to 16 ng/ml at 1 hr and reaching a maximum of 27 ng/ml at 3 hr. The tail-flick latencies at different times after treatment appeared to vary more than did the levorphanol concentrations. Nevertheless, when an analysis was made of the correlation between tail-flick latencies and brain concentrations of levorphanol in individual animals, with the values from all time intervals combined, there was a significant correlation between these two variables. A stable ratio of 37.3 ± 3.8 of PMM to levorphanol was determined from 1 to 6 hr. NorPMM first appeared at 3 hr, whereas norlevorphanol and an unidentified plasma metabolite were not detected in brain.

Unlike the pattern in brain, *p*OH-PMM was the major plasma conponent from 2 to 6 hr after PMM was administered, accompanied by smaller amounts of PMM (Fig. 2, center). Levorphanol was present at all time intervals but always at smaller concentrations than PMM. NorPMM and norlevorphanol became measurable 3 hr after dosage but were present at smaller concentrations than the other metabolites. An unknown metabolite gradually accumulated and at 6 hr exceeded the concentration of the parent drug. As in brain, PMM and its metabolites rose to apparent peak concentrations at 3 hr, and except for the 30-min and 1-hr data, the ratio of PMM to its major metabolites did not appear to change with time. The ratio of PMM to *p*OH-PMM in plasma was 0.65 ± 0.07 (± S.E.M.) and of PMM to levorphanol was 2.9 ± 0.3.

Comparison of plasma and brain data revealed a

Table 1. Correlations between analgesic activity and brain concentrations of PMM and metabolites in rats given 50 mg/kg, p.o., of [^{3}H]PMM

Comparison*	Product–moment correlation‡
Tail-flick latency vs concentrations of PMM	0.610
Tail-flick latency vs concentrations of *p*OH-PMM	0.539
Tail-flick latency vs concentrations of levorphanol	0.507
Tail-flick latency vs sum of concentrations of PMM and *p*OH-PMM	0.649

* In all cases, nineteen pairs of data were compared.
‡ Cf. Ref. 10. All values ≥ 0.389 are significant at $P < 0.05$ and all values ≥ 0.528 are significant at $P < 0.01$.

considerable uptake of PMM by brain. The brain to plasma concentration ratios between 1 and 6 hr averaged 24.4 ± 1.6, a ratio very much greater than for *p*OH-PMM (4.7 ± 0.4) or levorphanol (1.9 ± 0.2).

Stable ratios of brain PMM to its active metabolites, levorphanol and *p*OH-PMM, suggest that any correlation between the analgesic activity and brain concentrations of unchanged drug would also be true for the two metabolites. The degree of correlation between analgesia (measured either by tail-flick latency or percentage of maximum possible response) and the brain PMM concentrations was analyzed by computing product-moment correlation coefficients [9]. A significant correlation coefficient (r 0.61) was found for the correlation between brain concentrations of PMM and analgesic activity (Table 1). Although significant correlation coefficients were also computed between analgesic activity and brain concentrations of *p*OH-PMM, levorphanol, and the sum of PMM plus *p*OH-PMM, the lowest correlation value was to levorphanol alone and the highest to the sum of PMM and *p*OH-PMM. Multivariate regression analysis indicated that it is not possible to definitely ascribe the analgesic activity to only one of the three compounds. Accordingly, the analgesic activity observed may have been due to the presence in brain of only one active substance, PMM, *p*OH-PMM, or levorphanol, or to the combined activity of any two or all three.

Because the analgesia persisted for 6 hr, measurements of unchanged PMM and several metabolites in the G.I.T. were made during this 6-hr period (Fig. 2, bottom). During the entire observation period, unchanged PMM was present in amounts greater than 50% of the dose. This finding suggests that slow absorption of PMM is responsible for the persistent plasma and brain concentrations of drug metabolites and for the 6-hr duration of analgesic activity. The major intestinal metabolite was norPMM which was present in amounts of 4.8 ± 0.6% of the dose; this value is the mean (± S.E.M.) of all twenty rats at the various time intervals. The presence of norPMM in the G.I.T. may be due to *N*-demethylase activity in the mucosa of the duodenum [10]. Bacterial origin of norPMM, however, cannot be excluded in view of reports of *N*-demethylation by intestinal bacteria from rodents, e.g. imipramine [11] and methamphetamine [12]. Intestinal levorphanol was present at concentrations of less than 1% of the dose.

Studies with levorphanol. The brain levorphanol concentrations observed in this study were relatively small (10–20 ng/g). However, Misra *et al.* [13] reported brain concentrations of 572 ng/g in rats after subcutaneous administration of 5 mg/kg of levorphanol. Assuming proportionality of brain concentration to dose, the subcutaneous ED_{50} of 0.07 mg/kg of levorphanol would produce brain concentrations of 8 ng/g, which is close to those found after administration of the PMM ED_{50}. We therefore

Table 2. Brain concentrations of levorphanol in rats 1 hr after treatment with [^{3}H]PMM hydrochloride, [^{3}H]levorphanol tartrate or [^{3}H]*p*OH-PMM hydrochloride

Drug	ED_{50} (mg/kg)	Dose (mg/kg)	Route of administration	Mean levorphanol concentrations in brain (ng/g)	No. of rats
PMM hydrochloride	59	50	p.o.	16 ± 8	4
Levorphanol tartrate	6.1	6.0	p.o.	18 ± 5	6
Levorphanol tartrate	0.07	0.1	s.c.	11 ± 0.5	5
*p*OH-PMM hydrochloride	24	24	s.c.	53 ± 29	3

Table 3. Brain and plasma concentrations of *p*OH-PMM and metabolites and levorphanol and metabolites 60 min after a single oral dose of [^{3}H]*p*OH-PMM or [^{3}H]levorphanol

	Drug and route of administration	Dose (mg/kg)	N	Mean concentration (± S.E.M., ng per g or ml)				
				Free			Conjugated	
				*p*OH-PMM	levorphanol	norlevorphanol	levorphanol	norlevorphanol
	Levorphanol							
Brain	p.o.	6	6		18 ± 5	ND*	NA†	NA
Plasma					ND‡	ND‡	341 ± 68	6.5 ± 0.7
Brain	s.c	0.1	5		11 ± 0.5	ND‡	NA	NA
Plasma					2.9 ± 0.3	0.11 ± 0.01	12.6 ± 0.9	0.36 ± 0.05
	*p*OH-PMM							
Brain	s.c.	24	5	2400 ± 851§	53 ± 29	ND‖	NA	NA
Plasma				340 ± 60	22 ± 11	23 ± 11	NA	NA

* Not detectable in five out of six brains and in four out of six plasma samples. Limits of detection 1 ng per g or ml.
† Not analyzed.
‡ Limits of detection 0.07 ng per g or ml.
§ Two rats died within 60 min after dosing.
‖ Limits of detection 10 ng per g or ml (limits of detection vary with specific activity: see text).

tested whether ED_{50} values of levorphanol would also produce brain levorphanol concentrations of 10–20 ng/g. Brain concentrations of levorphanol 1 hr after 6 mg/kg p.o. and 0.1 mg/kg s.c., which approximate the ED_{50} by each route, are shown in Table 2. Despite the 60-fold difference in doses, brain levorphanol concentrations did not differ significantly ($P > 0.05$, Student's *t*-test) and were within the 10–20 ng/g range. The findings indicate that PMM may act as a prodrug of levorphanol.

The levorphanol studies are presented in greater detail in table 3. After oral administration of 6 mg/kg of ^{3}H-labeled levorphanol tartrate, norlevorphanol was detected in only one rat brain. Unconjugated levorphanol or norlevorphanol in plasma was barely measurable by t.l.c. in only two of the six rats. Even if one assumes that the small amounts of radioactivity extracted from the plasma of the remaining four rats represent only levorphanol, the highest levorphanol plasma concentration for all six rats would be 3.1 ± 0.4 ng/ml. This maximal value compared to the mean concentration of levorphanol in brain (18 ± 5 ng/g) indicates considerable uptake of levorphanol by the brain. The predominant fraction of the plasma radioactivity was conjugated levorphanol. Norlevorphanol was also present in plasma, mostly in the conjugated form.

Table 3 also lists the brain and plasma concentrations of levorphanol after subcutaneous administration of 0.1 mg/kg of [^{3}H]levorphanol tartrate. Levorphanol represented 95% of the radioactivity extracted from brain while norlevorphanol was not detected.

Studies with pOH-*PMM*. Because *p*OH-PMM is a major metabolite of PMM, and in view of the finding that *p*OH-PMM is converted to levorphanol*, brain and plasma concentrations of *p*OH-PMM and its metabolites levorphanol and norlevorphanol were determined after a single subcutaneous dose of *p*OH-PMM. The results are summarized in Table 3. The brain concentrations of *p*OH-PMM and/or levorphanol were several times greater than those observed at doses approximating the ED_{50} of PMM or levorphanol. However, the observed death of two out of five rats suggested that the dose of *p*OH-PMM administered may have exceeded the ED_{50}. Norlevorphanol was not present at measurable concentrations. It is interesting that *p*OH-PMM concentrations in brain are 7-fold greater than in plasma, indicating uptake by the brain. Levorphanol concentrations in brain were 2.4-fold larger than those in plasma, which is similar to the 1.9-fold difference in levorphanol concentrations found after PMM administration and the 3.8-fold difference seen after subcutaneous administration of levorphanol (Table 3). Norlevorphanol plasma concentrations, almost identical to those of plasma levorphanol, appeared to be higher than brain concentrations of norlevorphanol, suggesting a low norlevorphanol uptake by brain.

In conclusion, these studies provide evidence that the analgesic activity of PMM is due to its metabolite, levorphanol. This contention requires further support by studies of the analgesic activity of PMM in rats treated so as to stimulate or inhibit the formation of levorphanol from PMM. Also to be investigated is the possibility that the analgesic activity of PMM is greatly reduced in species in which there is no significant metabolism of PMM to levorphanol.

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* J. J. Kamm and A. J. Szuna, personal communication.

REFERENCES

1. W. L. Dewey, M. D. Aceto, L. S. Harris and E. L. May, in *Problems of Drug Dependence*, Proceedings of the Thirty-Ninth Annual Scientific Meeting, pp. 111–20 (1977).
2. E. Mohacsi, W. Leimgruber and H. Baruth, in *Problems of Drug Dependence*, Proceedings of the Forty-First Annual Scientific Meeting, p. 77. NIDA Research Monograph 27 (1979).

3. J. J. Kamm, A. Szuna and E. Mohacsi, *Pharmacologist* **21**, 173 (1979).
4. K. Fromherz, *Archs int. Pharmacodyn. Thér.* **85**, 387 (1951).
5. H. Isbell and H. F. Fraser, *J. Pharmac. exp. Ther.* **107**, 524 (1953).
6. F-J. Leinweber, A. J. Szuna, A. C. Loh, T. H. Williams, G. J. Sasso and B. A. De Barbieri, *Pharmacologist* **22**, 278 (1980).
7. W. L. Dewey and L. S. Harris, in *Methods in Narcotics Research* (Eds. S. Ehrenpreis and A. Neidle), Vol. 5, pp. 101–8. Marcel Dekker, New York (1975).
8. F-J. Leinweber, A. J. Szuna, T. H. Williams, G. J. Sasso and B. A. De Barbieri, *Drug Metab. Dispos.* **9**, 284 (1981).
9. A. W. Snedecor and W. A. Cochran, *Statistical Methods*, Sixth Edn, pp. 172–7. Iowa State Press, Ames, IA (1967).
10. F. B. Thomas, N. Baba, N. J. Greenberger and D. Salsburey, *J. Lab. clin. Med.* **80**, 548 (1972).
11. R. Minder, F. Schnetzer and M. H. Bickel, *Naunyn-Schmiedebergs Arch. Pharmak.* **268**, 347 (1971).
12. J. Caldwell and G. M. Hawksworth, *J. Pharm. Pharmac.* **25**, 422 (1973).
13. A. L. Misra, N. L. Vadlamani, R. Bloch and S. J. Mulé, *Res. Commun. Chem. Path. Pharmac.* **7**, 1 (1974).