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A COMPARISON OF 6.14.ENDOETHENO-7-(1-HYDROXY-1-CYCLOHEXYLETHYL)-TETRAHYDRO-ORIPAVINE (A POTENT ANALGESIC DERIVED FROM THEBAINE) WITH MORPHINE

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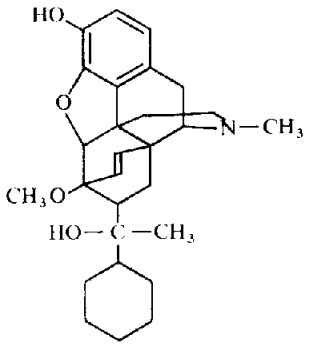
Abstract—The biological activities of M125 (6.14.endoetheno-7-(1-hydroxy-1-cyclohexylethyl)-tetrahydro-oripavine) and morphine have been compared by studying the changes produced by their repeated administration to rats in (1) the *in vitro* *N*-demethylating activity of the liver microsomal enzymes and (b) the pharmacological response to the drugs. M125 is *N*-demethylated by liver microsomal preparations under the same conditions required for the *N*-demethylation of morphine. After the repeated administration of M125 rats became tolerant to M125 itself and also to morphine. Similarly, morphine tolerant rats were tolerant to M125. The *in vitro* *N*-demethylation of both compounds was depressed in liver microsomal preparations from both M125 and morphine tolerant rats. After withdrawal of M125 from tolerant rats the pharmacological response of the animals to the drug and the liver microsomal *N*-demethylating activity returned to normal within twenty-five days. *N*-allyl normorphine inhibited the *N*-demethylation of morphine more strongly than that of M125 but the nature of the inhibition was the same in each case.

It is suggested that M125 produces its biochemical and pharmacological effects by similar mechanisms to morphine.

BENTLEY and Hardy[1] have described a series of potent analgesics derived from thebaine, the *N*-methyl members of which were shown by Lister[2] to have similar pharmacological properties to morphine in so far as their injection into laboratory animals produced analgesia, depression of the respiratory and cough centres, inhibition of gastrointestinal motility and lowering of body temperature. Further information about the biological similarities of these compounds and morphine has been sought in the present study.

Previous workers (see the review by Way and Adler[3]) have shown that morphine and compounds of similar chemical structure and pharmacological activity are all *N*-dealkylated *in vitro* by liver microsomal enzymes. The development of tolerance produced by the repeated administration of these compounds to animals and the subsequent recovery of the pharmacological response after withdrawal of the compound is accompanied by a depression and subsequent recovery of the *N*-dealkylating activity of the liver microsomal enzymes. The development of tolerance to one drug results in a cross-tolerance to the actions of related drugs and, similarly, the depression of the microsomal *N*-dealkylating activity extends to the *N*-dealkylation of related drugs. It has also been found that *N*-allyl normorphine (nalorphine) both antagonises

the pharmacological effects of morphine and related narcotics and inhibits their *N*-dealkylation by liver microsomal enzymes.



M125. 6.14. endoetheno-7-(1-hydroxy-1-cyclohexylethyl)-tetrahydro-oripavine.

M125 and morphine have been compared by studying the effects of their repeated administration to rats on (a) the *in vitro* *N*-demethylating activity of the liver microsomal enzymes and (b) the pharmacological response to the drugs.

METHODS

Drugs

Both M125 and morphine (as the hydrochlorides) were administered by s.c. injection into the ventral surface of the rat. M125 solutions for injection were obtained by the appropriate dilution, with water, of a stock solution of 1 mg/ml in 20% aqueous methanol. Morphine was given as an aqueous solution. Control animals were injected with saline, 1 ml/kgm.

Since methanol is converted to formaldehyde by the microsomal and soluble fractions of the liver cell the M125 solutions for the *in vitro* metabolic studies were made up in 0·01 M NaOH in 90% aqueous propylene glycol.

Animals

Adult male albino rats of the Porton strain (S.P.F.), weighing 150–180 g. were used in all the experiments.

Pharmacological testing

(a) *Shock avoidance test.* The apparatus consisted of a box which was divided into two 1 ft × 1 ft compartments, with an aperture in the partition to allow access from one compartment to the other. The base of the cage was made of parallel brass rods through which an electric shock could be applied to the rat through its feet, in either of the compartments independently. The stimulus used was a 45 V pulse of 10 msec. duration at a frequency of 50 pulses per second. The test procedure was to place the rat in one compartment, apply the stimulus to that compartment and measure the time taken for the rat to find the hole and pass to the unstimulated side of the apparatus. Each animal was given a series of six successive stimuli, with a pause of 45 sec between each stimulus, and the mean time to respond determined. The maximum period of any stimulus was 50 sec. The animals were trained to respond to the test and most learned

very quickly so that after three or four tests, given on successive days, the mean response time was less than 5 sec. This quick response was retained over several weeks, provided the rats were tested in the apparatus every 4–5 days. The results were more consistent if the feet of the rat were bathed in saline just before the test.

(b) *Tail clip test.* A large crocodile clip, with polythene covered jaws, was placed about 3 cm from the base of the rat's tail. A positive reaction was a *definite attack* on the clip within 20 sec of its application. If the animals did not respond within this time the clip was removed.

Biochemical methods

The rates of *N*-demethylation of M125 and morphine were determined as follows. The combined microsomal plus soluble fraction was prepared from liver, using a Potter–Elvehejem homogeniser as described previously.[5] For the nalorphine inhibition studies the microsomal preparations were obtained from the pooled livers of five rats homogenised in an M.S.E. Atomix blendor.[6] The incubation conditions were those employed by Leadbeater and Davies.[6] The rate of *N*-demethylation of both morphine and M125 was determined either by estimating the amount of formaldehyde or secondary amine (*N*-demethylated M125) formed during a 30 min incubation.[6]

RESULTS

The N-*demethylation of* M125 *by liver microsomes*

The rates of *N*-demethylation of M125 by four preparations of liver microsomes were determined (a) by the release of formaldehyde and (b) by the formation of secondary amine. Two preparations were of the combined microsomal and soluble fractions and two were of microsomes which had been washed free of the soluble fraction by repeated centrifuging at 105,000 *g* for 60 min and by resuspending the pellet of microsomes in 0·10 M Tris-HCl buffer (pH 7·50). In assaying the *N*-demethylating activity of the washed microsomes the NADP in the incubation medium was replaced by 10^{-3} M $NADPH_2$. The results, shown in Table 1, demonstrate that the

TABLE 1. *N*-DEMETHYLATION OF M125 BY PREPARATIONS OF LIVER MICROSOMES

Enzyme preparation	Rate of formation of formaldehyde (μ mole/g liver/hr)	Rate of formation of secondary amine (μ mole/g liver/hr)
Microsomes	1·21	1·24
	1·26	1·17
Combined microsome and soluble fractions	1·32	1·28
	1·39	1·31

amount of formaldehyde produced was equivalent to the amount of secondary amine formed, i.e. that M125 is *N*-demethylated, and that the enzymes bringing about the *N*-demethylation are situated in the microsomal fraction of the liver.

The cross-tolerance of M125 *and morphine*

Six rats were injected with M125 according to the following schedule:— days 1 and 2—20 μg/kg; days 3 and 4— 40 μg/kg; days 5 and 6—60 μg/kg; days 7 and 8—

80 μg/kg and days 9 to 14—100 μg/kg. The pharmacological response of the rats to standard test doses of M125 (25 μg/kg) and morphine (50 mg/kg) on days 11 and 14 respectively was determined in the Shock Avoidance Test. The animals were killed and liver microsomes prepared on day 15. A second group of rats was given a similar series of injections of morphine, rising from 20 to 100 mg/kg. The control group of rats was injected daily with saline.

The results of the Shock Avoidance Tests and also the rates of *N*-demethylation of M125 and morphine by the microsomal enzymes are shown in Table 2. The time to

TABLE 2. THE CROSS-TOLERANCE OF M125 AND MORPHINE. THE EFFECT OF THE REPEATED ADMINISTRATION OF M125 AND MORPHINE ON THE PHARMACOLOGICAL RESPONSE OF RATS, MEASURED IN THE 'SHOCK AVOIDANCE' TEST AND ON THE *N*-DEMETHYLATING ACTIVITY OF THE LIVER MICROSOMAL ENZYMES

Experiment	Number of Rats	Test Drug	'Shock Avoidance' test		Microsomal *N*-demethylation	
			Increase in the time to respond (sec)	Significance of the change with respect to controls	Rate of *N*-demethylation (μ mole HCHO/g liver/hr)	Significance of the change with respect to controls
Control	8	M125	32·1 ± 13·7	—	2·11 ± 0·69	—
		Morphine	42·2 ± 10·8	—	8·67 ± 2·01	—
M125 treated	6	M125	−1·2 ± 1·8	$P<0.01$	1·45 ± 0·44	$0.10>P>0.05$
		Morphine	1·7 ± 1·7	$P<0.01$	5·02 ± 1·56	$P<0.01$
Morphine treated	6	M125	6·1 ± 3·5	$P<0.01$	0·93 ± 0·12	$P<0.01$
		Morphine	0·4 ± 1·5	$P<0.01$	1·60 ± 0·83	$P<0.01$

The increase in the time to respond is the difference between the response times measured immediately before and 60 min after the injection of the standard dose of the test drug.
The significance of the difference between the data reported in this and subsequent Tables was determined by the Student *t* test.[7]

respond to the shock, measured 24 hr after the last injection, was not affected by pretreatment with either drug and the mean time to respond was 2·2 ± 1·9 sec. In the control animals, the effect of the standard doses of M125 and morphine was to increase the time to respond by 35·5 and 43·3 sec respectively. After repeated treatment with M125 the effects of M125 and morphine on the response time were completely abolished. Repeated treatment with morphine markedly reduced the increase in time to respond after a test dose of M125 and abolished the effect of the test dose of morphine. The *N*-demethylation of both M125 and morphine was depressed in liver microsomal enzymes from both M125 and morphine treated rats although in the case of M125 tolerant animals the depression of M125 demethylation was barely significant ($0.10 > P > 0.05$).

The failure of M125 to depress its own metabolism to a significant extent may have been due to the relatively small amounts of the drug which were administered. To test this hypothesis, five times the dose of M125, used in the previous experiment, was given to a group of six rats according to the following schedule:— day 1—2 × 100 μg/kg; day 2—2 × 150 μg/kg; day 3—2 × 200 μg/kg; day 4—2 × 250 μg/kg; day 5—300 μg/kg; day 6—400 μg/kg and day 7—500 μg/kg. The animals were killed on the eighth day and liver microsomes prepared. One rat died after the first two injections but the remainder survived the treatment and became tolerant to the drug in so far

as the catatonic effect of the last injection (500 μg/kg) disappeared within 4 hr compared with 6 hr after the first injection. The rates of *N*-demethylation of both M125 and morphine (shown in Table 3) by the microsomes from the treated rats were very significantly depressed ($P < 0{\cdot}01$).

TABLE 3. THE MICROSOMAL *N*-DEMETHYLATION OF M125 AND MORPHINE IN M125 TREATED RATS

Experiment	Compound	Number of rats	Rate *N*-demethylation (μ mole HCHO/g liver/hr)	Depression of *N*-demethylation (%)	Significance of the depression of *N*-demethylation
Control	M125	6	1·76 ± 0·22	—	—
	Morphine	6	6·68 ± 1·04	—	—
M125 Treated	M125	5	0·57 ± 0·24	67	$P<0{\cdot}01$
	Morphine	5	1·93 ± 0·62	71	$P<0{\cdot}01$

The withdrawal of M125 *from tolerant rats*

Twenty rats were given M125 daily in increasing doses according to the schedule described immediately above. The maximum dose of 500 μg/kg was repeated on days 8 and 9. A control group of twelve rats was given a similar injection schedule of saline. On the tenth day the analgesic effects of a test dose of M125 (40 μg/kg) were determined by using the Tail Clip test 30 min after the injection of the drug. All the treated animals were tolerant to M125 but none of the controls responded to the test (Table 4).

TABLE 4. THE EFFECT OF WITHDRAWAL OF M125 FROM TOLERANT RATS

Treatment of animals	Response to tail clip test		Microsomal *N*-demethylation of M125	
	Fraction responding before test dose of M125	Fraction responding after test dose of M125	No. of rats	μ mole HCHO/g liver/hr
Saline (controls)	12/12	0/12	6	1·60 ± 0·38*
Increasing doses of M125 over 10 days	20/20	20/20	10	0·52 ± 0·40*
Saline (controls)	6/6	0/6	5	2·01 ± 0·58†
Treated with M125 then withdrawn from drug for 25 days	9/10	0/10	10	1·82 ± 0·49†

* Significant difference in activity ($P<0{\cdot}01$).

† Difference in activity not significant ($P>0{\cdot}9$).

The control rats were fully catatonic and lay supine in their cages whereas the tolerant rats showed signs of excitation and hyperactivity.

Half the animals in each group were killed and microsomes prepared from their livers. The *N*-demethylating activity of the preparations from the tolerant rats was reduced by about 63 per cent towards M125. M125 was abruptly withdrawn from the remaining tolerant rats and when they were tested 25 days later none of them responded to the Tail Clip after the test dose of M125. The response of these animals was indistinguishable from that of the control rats which were of the same age and had been

kept under identical conditions to the experimental ones. The liver microsomes prepared from the experiment and control rats *N*-demethylated M125 at the same rate.

The inhibition of N-*demethylation by nalorphine*

The rates of *N*-demethylation of M125 and morphine were determined at various substrate concentrations in the presence and absence of 3×10^{-4} M nalorphine and the data fitted to the Lineweaver and Burk equation.[7] The equation of the best fitting line was determined by the method of least squares and the values of the Michaelis constant (K_m) and the maximum velocity of the reaction (V) were calculated from the equation. The results are shown in Table 5. The data for morphine have been published previously.[5]

TABLE 5. THE INHIBITION OF THE LIVER MICROSOMAL *N*-DEMETHYLATION OF M125 AND MORPHINE BY NALORPHINE

Substrate	Parameter	Preparation number	Control	In presence of 3×10^{-4}M Nalorphine	Significance of the change
M125	K_m (10^{-4}M)	1	2·66	3·87	
		2	2·89	3·60	
		3	1·72	2·38	0·05>P>0·02
		4	1·83	4·23	
		Mean	2·28 ± 0·51	3·52 ± 0·69	
	V (μ mole/g liver/hr)	1	5·88	3.71	
		2	4·63	3·88	
		3	5·10	4·73	0·6>P>0·5
		4	4·28	4·24	
		Mean	4·97 ± 0·58	4·64 ± 0·68	
Morphine	K_m (10^{-4}M)	1	6·22	18·21	
		2	8·25	19·40	
		3	4·63	42·28	P<0·01
		4	3·33	27·85	
		Mean	5·61 ± 1·83	27·87 ± 9·62	
	V (μ mole/g liver/hr)	1	16·95	10·24	
		2	23·02	7·78	
		3	19·32	11·45	P<0·01
		4	10·76	10·08	
		Mean	19·51 ± 2·20	9·89 ± 1·32	

Both K_m and V for the *N*-demethylation of morphine were significantly changed in the presence of nalorphine. In the *N*-demethylation of M125 the K_m was significantly increased in the presence of nalorphine but the significance of the decrease in V was doubtful ($0{\cdot}6 > P > 0{\cdot}5$) as determined by the Student t test. To establish whether nalorphine had reduced the maximum velocity of M125 demethylation the significance of the differences between the values of V in the presence and absence of nalorphine was examined by the method described by Wilkinson,[8] which showed that nalorphine significantly ($P < 0{\cdot}05$) depressed V for the *N*-demethylation of M125.

DISCUSSION

The object of this work was to determine whether M125 has similar biochemical and pharmacological action to morphine. The two compounds are similar biochemically in that M125 was shown to be *N*-demethylated by rat liver microsomes in

the presence of $NADPH_2$ and oxygen by the same system which *N*-demethylates morphine.[9] The *N*-demethylation of both M125 and morphine by liver microsomal enzymes was inhibited by nalorphine. The inhibition was qualitatively the same for both substrates—the K_m was increased and V was decreased. The inhibition was neither wholly competitive nor wholly non-competitive but a mixture of the two.[5] Quantitatively nalorphine inhibited the *N*-demethylation of morphine to a greater extent than that of M125. The difference between the inhibitory actions of nalorphine on the *N*-demethylation reactions may be due to the greater affinity of M125 for the active centre of the enzyme than morphine (the K_m for M125 demethylation is approximately half the K_m for morphine).

M125 and morphine were shown to be similar pharmacologically in that repeated administration of M125 to rats resulted in a decreased pharmacological response to a standard test dose of either M125 itself or morphine. Similarly, morphine tolerant rats were also tolerant to M125. However, the cross-tolerance between the two compounds was not complete since the response to a standard test dose of M125 was not completely abolished in rats which were fully tolerant to morphine. The development of tolerance to either M125 or morphine was accompanied by a depression of the *N*-demethylating activity of the liver microsomal enzymes towards both compounds. The extent of the depression of the microsomal demethylation of M125 in M125 tolerance varied with the dose of M125 administered. On withdrawal of M125 from tolerant rats the response of animals to the drug and the microsomal *N*-demethylating activity returned to normal within 25 days. Although the time course of the recovery was not followed in the experiment reported, the data were consistent with those of Cochin and Economon[10] for morphine withdrawal from tolerant rats. They found that sixteen days after withdrawal the pharmacological response was only 40 per cent of the control value whereas the *N*-demethylating activity of the liver microsomes had returned to normal.

M125 is approximately one thousand times more active pharmacologically than morphine. This great increase in potency produced by the insertion of the ethylene bridge and tertiary alcohol group into the morphine molecule suggests that the receptor sites for the analgesic drugs, in the central nervous system, may contain a locus which interacts with the polar alcoholic grouping, thus increasing the specificity of the action of M125. Beckett and Casy[11] have suggested that the analgesic receptor sites contain (a) an anionic site which forms an ionic bond with the basic centre of the drug, (b) a flat surface which binds the flat aromatic structure in the drug by Van der Waals forces and (c) a cavity which can accommodate a projecting hydrocarbon moiety of the drug molecule. If the receptor site is extended to include a fourth locus to interact with the alcoholic grouping then the complete interaction of a drug with the receptor will be very highly specific. Thus, M125, whose structure contains groups which could interact with all four loci of the proposed receptor, is one thousand times more active than morphine which does not have the tertiary alcohol group in the 7 position. The relative importance of the loci or of different combinations of loci, when only three of the four are occupied, in determining pharmacological activity remains to be elucidated. M125 is more lipid soluble than morphine, at physiological pH, and this may be an important factor in facilitating the access of the drug to the receptor, e.g. by increasing its penetration of membranes.

The microsomal *N*-demethylating enzyme system does not have the same degree

of specificity as the central nervous system receptors since M125 is metabolised at approximately one quarter the rate of morphine whereas it is a thousand times more active pharmacologically. Nevertheless, the mechanism by which the microsomal *N*-demethylating activity is depressed during the development of tolerance has a similar high specificity since the dose required to produce a depression of activity is one thousandth that of morphine. However, Cochin and Axelrod[12] showed that, during the repeated administration of morphine to rats, the development of tolerance and the depression of the liver microsomal *N*-demethylating activity did not occur in strict parallel. A similar lack of parallelism was found between the two effects during the repeated administration of M125 to rats: the rate of microsomal *N*-demethylation of M125 was not significantly depressed in animals which were fully tolerant to its pharmacological effects. Nevertheless the present work has emphasised the similarities between the mechanism which reduces the pharmacological response of the rat to the drug and that which depresses the *N*-demethylating activity of the liver microsomal enzymes.

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