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EFFECT OF MONOAMINE OXIDASE INHIBITORS ON FORMATION OF MORPHINE GLUCURONIDE*

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Abstract—The inhibition of the formation of morphine glucuronide *in vitro* by five monoamine oxidase inhibitors is described. Pargyline and phenelzine at 1·0 and 5·0 × 10^{-4}M concentration and tranylcypromine at 5 and 10 × 10^{-4}M concentration were competitive inhibitors of morphine glucuronidation, while isocarboxazide was a mixed-type inhibitor. Nialamide, in the concentrations studied, did not significantly inhibit the formation of morphine glucuronide.

A PREVIOUS study concerning the effect of pargyline on the analgesic effect of morphine demonstrated that this action was enhanced in rats treated acutely with pargyline but reduced in animals receiving chronic injections of the drug.[1] Data obtained in studies *in vitro* indicated that: (1) pargyline at a concentration of 1·0 × 10^{-4}M inhibited the formation of morphine glucuronide by 50 per cent when the morphine concentration was 1·25 × 10^{-4}M, and (2) the liver microsomal glucuronyl transferase activity of rats treated with pargyline daily for 7 and 8 days was increased about 40 per cent of that of the saline control rats.[1]

The present study was undertaken to determine if other monoamine oxidase inhibitors (MAOI) were also capable of inhibiting morphine glucuronide formation and, if so, to determine the nature of this inhibition.

METHODS

Materials. Uridine diphosphate glucuronic acid (UDPGA) as the ammonium salt and bacterial glucuronidase were obtained from Sigma Chemical company. Pargyline HCl was obtained through the courtesy of Abbott Laboratories, phenelzine sulfate from Warner–Lambert Research Institute, tranylcypromine sulfate from Smith, Kline & French Laboratories, nialamide from Pfizer inc., and isocarboxazide from Hoffmann-La Roche Inc.

Pargyline HCl, phenelzine sulfate and tranylcypromine sulfate were dissolved in distilled water. Nialamide was made to dissolve in water with the addition of HCl. The resultant solution had a pH of 4·5–5·0. Isocarboxazide was dissolved in a spectrophotograde Cellosolve. The same amount of water or Cellosolve (0·5 ml) was added to the control samples.

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Animals. Holtzman male rats, weighing 190–210 g, were kept in constant environmental conditions and maintained on Wayne Lab-blox (Allied Mills, Inc.) and tap water *ad lib.* throughout the course of the experiment. They were allowed at least 2–3 days acclimation before use in the experiments.

Preparation of microsomes. The rats were killed by a blow on the head and the livers were removed immediately and immersed in ice-cold 1·15% KCl solution. They were then blotted dry, weighed, placed in cold 1·15% KCl solution, and minced with scissors. The minced livers were homogenized in cold 1·15% KCl solution using a Potter–Elvehjem homogenizer having a Teflon plunger. During this process, the tissue grinder was placed in an ice-water bath and a 20 per cent homogenate was prepared. The homogenate was centrifuged at 10,000 *g* for 20 min in an International refrigerated centrifuge and the supernatant (microsomal and soluble cytoplasmic fraction) was carefully removed. The mitochondria, nuclei and unbroken cells were discarded. The supernatant was then centrifuged at 105,000 *g* for 60 min in a Spinco ultracentrifuge. The resultant pellet was resuspended in 1·15% KCl and centrifuged at 105,000 *g* for 60 min.[2] Microsomes, equivalent to 1 g (wet weight) of liver, were suspended by gentle homogenization in 1 ml of 1·15% KCl.

Protein determination. The protein content of the washed microsomal suspension was determined in duplicate according to the method of Lowry *et al.*[3] with crystalline bovine serum albumin used as a standard.

Assay of glucuronyl transferase. The formation of morphine glucuronide was studied according to the method of Axelrod and Inscoe,[4] except that the concentration of UDPGA was 6·7 μmoles instead of 0·6 μmole. All other concentrations of microsomes, KCl, $MgCl_2$ and Tris buffer were the same as those described by Axelrod and Inscoe,[4] but the final volume was 2 ml instead of 4 ml. Incubations were carried out in 40-ml tubes in a Dubnoff shaker at 37° for 30 min under an atmosphere of air. The incubation mixture contained 0·5 ml microsomes, $5{\cdot}0 \times 10^{-3}$M $MgCl_2$, $5{\cdot}0 \times 10^{-2}$M Tris (hydroxymethyl) aminomethane buffer with a pH of 8·0, $1{\cdot}25 \times 10^{-4}$M *N*-^{14}C-methyl-morphine, 0–10×10^{-3}M MAOI, with and without 6·7 μmoles UDPGA, and water to make a final volume of 2 ml. The concentrations of UDPGA, 0·6 and 0·8 μmole, used for morphine glucuronide formation *in vitro* by previous workers[4,5] were found to be too low in our experiments. The K_m of UDPGA for the formation of morphine glucuronide was $2{\cdot}42 \pm 0{\cdot}12 \times 10^{-4}$M. The incubation was terminated by placing the tubes in a boiling water bath for 10 min, and free morphine in the incubated samples was determined according to the procedure described previously.[6] Liver preparations from three animals were used at each morphine concentration. The incubations were run in duplicate. To study the nature of inhibition of the formation of morphine glucuronide by MAOI, the method used was the same as that described above except that varying concentrations of morphine were used.

Calculations. The amount of the glucuronide of morphine formed from the washed microsomes was calculated from the average difference between the duplicate samples containing UDPGA and those without UDPGA. All morphine was recovered from the incubated mixture containing UDPGA when autoclaved at 17–20 lb for 1 hr in 2·2 N HCl or when incubated with β-glucuronidase at 37° for 20 hr, confirming the results of Axelrod and Inscoe.[4] The disappearance of morphine thus is the measurement of glucuronide formation. Enzyme activity was expressed as the amount of the glucuronide of morphine formed per 30 min per milligram of protein.

Statistics. Separate analyses of variance were performed on the data obtained for each concentration of morphine. A least significant differences test[7] was then performed on the mean values for the formation of morphine glucuronide from samples containing the various concentrations of MAOI. Kinetic data were plotted according to the method of Lineweaver and Burk.[8] Each point was the mean of three experiments. Each experiment was conducted with the microsomes obtained from one animal for all concentrations of the substrate, an inhibitor and control. The best-fitting straight line was determined by the method of least squares. Michaelis–Menten constants, K_m and V_{max}, were determined by the method of Wilkinson[9] using a Linc-Eight digital computer. The program used was a modification of the Fortran program published by Cleland.[10]

RESULTS

Effect of MAOI on the formation of morphine glucuronide by rat liver microsomes. Four of the five MAOI studied in this series inhibited the formation of morphine glucuronide as shown in Table 1. Only nialamide was inactive in the concentrations studied. Among the MAOI studied, pargyline was the strongest glucuronyl transferase inhibitor. Pargyline in $1{\cdot}0 \times 10^{-4}$M concentration inhibits the formation of morphine glucuronide by about 50 per cent.

TABLE 1. INHIBITION OF THE FORMATION OF MORPHINE GLUCURONIDE BY MONOAMIDE OXIDASE INHIBITORS IN RAT LIVER MICROSOMES*

	MAOI concn ($\times 10^{-3}$M)			
	0	0·1	1·0	10·0
Inhibitor	Morphine glucuronide formed (nmoles/30 min/mg protein)			
Pargyline	12·44 ± 0·85	6·40† ± 0·35	1·73† ± 0·44	0·20† ± 0·20
Phenelzine	12·44 ± 0·85	11·36 ± 0·72	8·23† ± 0·53	2·31† ± 0·19
Isocarboxazide	12·44 ± 0·85	12·18 ± 1·23	9·10† ± 0·87	3·83† ± 0·41
Tranylcypromine	12·44 ± 0·85	12·46 ± 0·68	12·08 ± 0·67	6·52† ± 1·31
Nialamide	15·20 ± 2·31	15·13 ± 2·26	14·82 ± 2·75	13·94 ± 3·84

* Details of the incubation mixture and conditions are given in the text. Each value represents the mean ± S. E. with an N of three rats.

† Significant difference from the control at $P < 0{\cdot}05$ using the least significant difference procedure.[7]

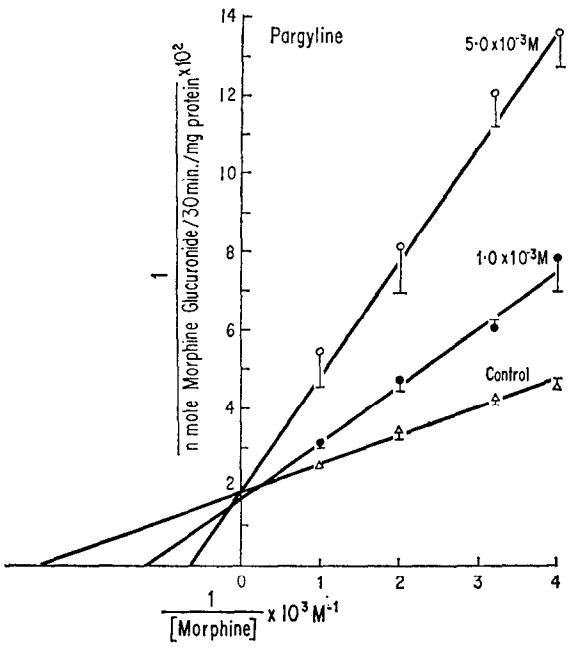

FIG. 1. Lineweaver–Burk plot of morphine concentration versus rate of glucuronide formation with varying concentrations of pargyline. The points and bars represent the mean and S. E. with N = 3.

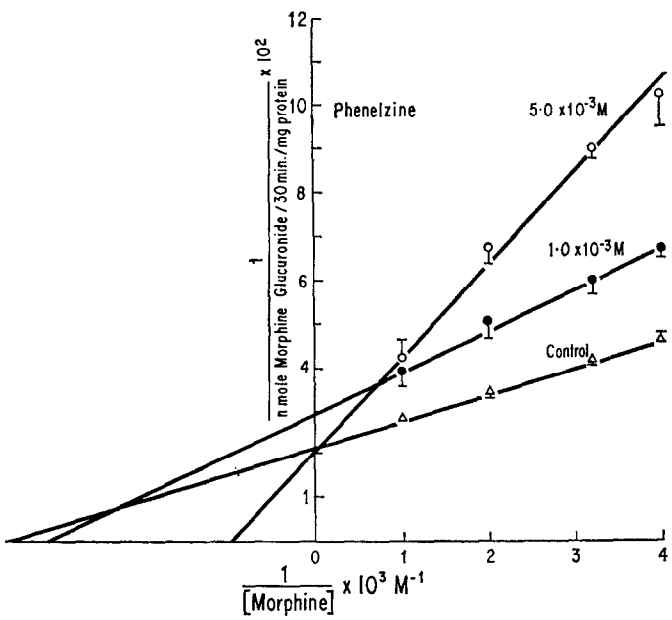

FIG. 2. Lineweaver–Burk plot of morphine concentration versus rate of glucuronide formation with varying concentrations of phenelzine. The points and bars represent the mean and S. E. with N = 3.

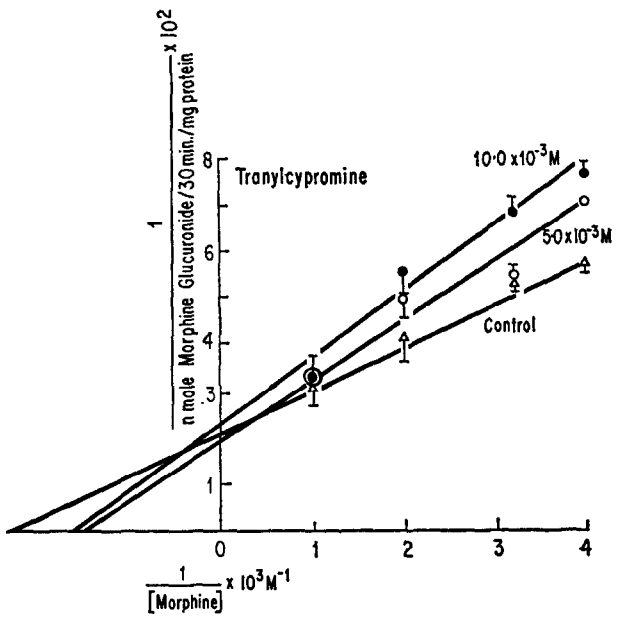

FIG. 3. Lineweaver–Burk plot of morphine concentration versus rate of glucuronide formation with varying concentrations of tranylcypromine. The points and bars represent the mean and S. E. with N = 3.

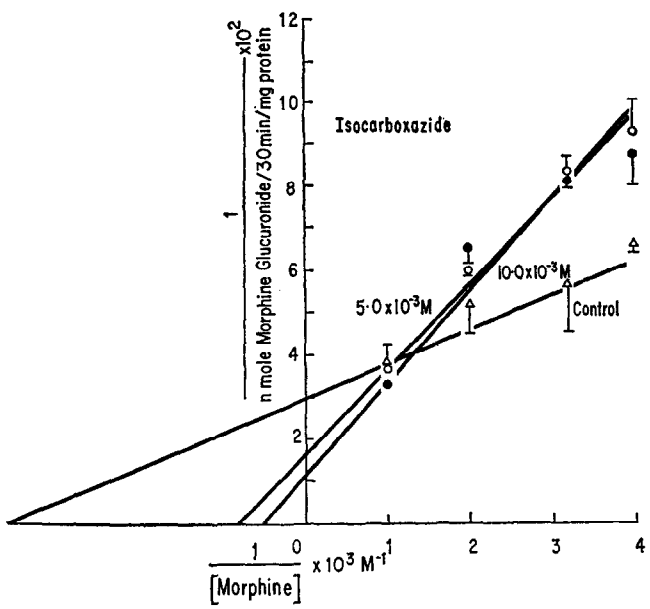

FIG. 4. Lineweaver–Burk plot of morphine concentration versus rate of glucuronide formation with varying concentrations of isocarboxazide. The points and bars represent the mean and S. E. with N = 3.

Kinetic studies of the formation of morphine glucuronide. The results of the effect of MAOI at 1·0 and 5·0 $\times$ 10^{-3}M or 5 and 10 $\times$ 10^{-3}M concentrations on the rate of the formation of morphine glucuronide in a range of morphine concentrations (2·5 to 10 $\times$ 10^{-4}M) have been plotted in Figs. 1–4 by the method of Lineweaver and Burk.[8] The inhibition produced by 1·0 and 5·0 $\times$ 10^{-3}M pargyline and phenelzine and by 5·0 and 10·0 $\times$ 10^{-3}M tranylcypromine was competitive, as indicated by a constant V_{max} (Table 2). Isocarboxazide, 5·0 and 10·0 $\times$ 10^{-3}M, exhibited a mixed type of inhibition. The slope of the curve obtained by the method of Lineweaver and Burk[8] for isocarboxazide at the higher concentration (10·0 $\times$ 10^{-3}M) is smaller than that at the lower concentration (5·0 $\times$ 10^{-3}M). This unusual observation is probably due to the limited solubility of isocarboxazide in water.

TABLE 2. EFFECT OF MAOI ON THE FORMATION OF MORPHINE GLUCURONIDE

Inhibitor	Conc (mM)	K_m $\times$ 10^{-4}M*	V_{max}* (nmoles morphine glucuronide/30 min/mg protein)
Pargyline	0	3·94 ± 0·585	53·70 ± 3·53
	1·0	8·54 ± 1·654†	59·00 ± 6·92
	5·0	16·02 ± 7·113	53·60 ± 21·53
Phenelzine	0	2·74 ± 0·484	44·81 ± 2·94
	1·0	3·21 ± 1·467	34·15 ± 5·37
	5·0	10·65 ± 3·774	48·83 ± 10·10
Nialamide	0	3·36 ± 1·478	46·74 ± 6·96
	5·0	6·05 ± 1·740	53·85 ± 8·00
	10·0	6·19 ± 2·304	49·89 ± 7·73
Tranylcypromine	0	4·31 ± 2·113	47·65 ± 9·82
	5·0	6·48 ± 1·539	50·44 ± 6·60
	10·0	6·10 ± 1·584	43·02 ± 5·79
Isocarboxazide	0	2·66 ± 1·018	33·72 ± 5·46
	5·0	12·36 ± 1·830†	61·03 ± 6·43†
	10·0	21·59 ± 5·152†	97·68 ± 20·52†

* K_m and V_{max} were determined by the method of Wilkinson.[9] Each value represents the mean ± S.E. with an N of three rats.

† Significantly different from control at $P < 0·05$.

Conjugation of morphine in the liver microsomes of rats in the absence of UDPGA. Data obtained in one set of experiments show that 0·189 and 0·036 μmole morphine disappeared from the incubation medium containing 0·25 μmole ^{14}C-methyl-morphine with and without UDPGA respectively. After acid hydrolysis or incubation with β-glucuronidase, the amounts of free morphine reappearing were 0·233 and 0·237 μmole respectively. The data suggest that some conjugation of morphine occurs with the washed liver microsomes of rats in the absence of UDPGA. The amount of conjugated morphine formed in the absence of UDPGA was proportional to the amount of morphine present (Table 3).

TABLE 3. CONJUGATION OF MORPHINE IN 10 mg OF WASHED LIVER MICROSOMES OF RATS IN THE ABSENCE OF UDPGA

No. of animals	3	9	3	18	15	15	15
Morphine added (μmoles)	0·125	0·25	0·375	0·50	0·625	1·0	2·0
Conjugated morphine formed (nmoles)*	7·27 ±0·91	10·75 ±3·42	15·39 ±6·48	23·91 ±2·32	37·85 ±3·19	77·42 ±7·63	99·70 ±9·78

* Calculated from the difference in the disappearance of free morphine from samples with and without microsomes in the absence of UDPGA. Values are means ± S.E.

DISCUSSION

The MAOI, i.e. pargyline, phenelzine, tranylcypromine and isocarboxazide, were shown to inhibit morphine glucuronide formation. This is not particularly surprising, since Hargreaves[11] has shown that some MAOI are capable of inhibiting the glucuronide formation of bilirubin and *O*-aminophenol by rat and rabbit liver homogenates.

Nialamide has been shown to potentiate the analgesia of morphine in rats[12] and rabbits,[13] but not in mice.[12] Nialamide has also been shown to inhibit the glucuronide formation of *O*-aminophenol and bilirubin in rat liver slices and rabbit homogenates. In the present study, nialamide had no significant effect on the formation of morphine. glucuronide. This discrepancy may be due to: (1) precipitation of nialamide out of the incubation medium (pH 8·0), or (2) alteration of the pH of the incubation medium by addition of nialamide HCl solution (pH 4·5).

The finding that these agents inhibit the formation of morphine glucuronide can readily explain the results obtained in the experiments *in vivo* of Yeh and Mitchell,[1] Rogers and Thornton,[14] and Mustala and Jounela.[15] Yeh and Mitchell[1] reported that the analgesic effect of morphine was potentiated by an acute dose of pargyline in rats. Mustala and Jounela[15] reported that the toxic effects of morphine were potentiated by a single dose of pargyline in mice. Rogers and Thornton[14] reported that the toxicity of morphine in mice was potentiated by an acute injection of tranylcypromine or iproniazid.

A detailed discussion of the reasons for favoring inhibition of morphine glucuronidation as the mechanism has been developed in a previous paper.[1]

While we cannot, of course, speak about other potent analgesic agents, the data reported here together with the literature cited in Ref. 1 strongly indicate that the interaction of MAOI with morphine is due primarily, if not entirely, to the alteration in the metabolism of this analgesic drug.

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