Biochemical Pharmacology, Vol. 43, No. 11, pp. 2335–2340, 1992.
Printed in Great Britain.

0006–2952/92 $5.00 + 0.00
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DIFFERENTIAL INHIBITION OF HEPATIC MORPHINE UDP-GLUCURONOSYLTRANSFERASES BY METAL IONS

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(*Received* 17 *January* 1992; *accepted* 10 *March* 1992)

Abstract—The major metabolites of morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), possess significant pharmacological properties. Whilst both metabolites bind to μ-opioid binding sites, M6G is a potent agonist whereas M3G antagonizes some of the effects of morphine and M6G. An inter-species comparison of *in vitro* hepatic morphine processing was performed. The results showed that not all species were able to produce M6G whereas all those tested did produce M3G. Guinea-pig liver produced the greatest amounts of M6G and was therefore chosen as a model to study morphine glucuronidation in further detail. Inclusion of the detergent Brij 58 (0.33 mg/mg protein) and Mg^{2+} (15 mM) in the standard assay incubation gave optimal production of both M3G and M6G by guinea-pig liver homogenates. A number of metal ions were investigated for their ability to inhibit glucuronidation of morphine in both the 3- and 6-positions. Some metal ions, namely Cu^{+}, Cu^{2+} and Cd^{2+}, were able to inhibit the production of M3G without affecting glucuronidation at the 6-position. Taken together, these data provide further evidence for the existence of UDP-glucuronosyltransferase isoenzymes responsible for the metabolism of morphine. In addition these isoenzymes can be differentially modulated and therefore it is possible to alter the ratio of M3G:M6G formed during *in vitro* metabolic studies.

It has long been established that the major metabolic fate of morphine is glucuronidation by UDP-glucuronosyltransferase (UDPGT‡) yielding morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) as the major metabolites [1]. More recently, it has been demonstrated that these metabolites are present in plasma at significantly greater concentrations than morphine itself [2]. The metabolic process of morphine glucuronidation has been observed in homogenates of human liver [3] and brain [4], and in the microsomal fraction of rodent liver and brain [5].

Both M3G and M6G retain significant pharmacological properties and are therefore of importance. M6G has a 2-fold higher affinity for the μ-opioid binding site as compared with morphine [6] and is an extremely potent agonist, being analgesic both in laboratory animals [7] and in humans [8]. M3G retains a relatively high affinity for μ-sites *in vitro*, although 50-fold lower than that of M6G [6], and has been demonstrated recently to antagonize functionally some of the effects of morphine and M6G [9].

Considering the opposing pharmacological actions of M3G and M6G, the proportions of the two metabolites formed are clearly relevant. In adults, chronic oral morphine treatment has been shown to produce a plasma ratio of 5:1 for M3G:M6G [10]. Both children and neonates, at steady state levels following continuous morphine infusion, display a urine ratio of approximately 10:1 for M3G:M6G [11]. Similarly, *in vitro* studies have shown M3G to have a 10-fold faster rate of formation than M6G in human brain [5]. Thus, the primary metabolite of morphine, both peripherally and centrally, is M3G. The antagonistic action of M3G towards morphine and M6G could potentially be important in the development of tolerance, particularly on chronic administration since there would be a gradual accumulation of this metabolite. The ability to reduce the amount of M3G formed relative to M6G could therefore have a number of useful indications. Firstly, the length of analgesia obtained from a dose of morphine may be increased and, secondly, the problems of tolerance associated with repeated doses of morphine may be diminished.

The present study was designed to investigate the possibility of manipulating hepatic morphine metabolism in favour of M6G production. Guinea-pig liver was chosen from a range of species tested since this gave the greatest M6G production in the first instance. After optimization of morphine glucuronidation by guinea-pig liver, the effects of various metal cations upon both M3G and M6G production were investigated.

MATERIALS AND METHODS

UDP-glucuronic acid (UDPGA) (sodium salt) was obtained from the Sigma Chemical Co. (Poole, U.K.). Morphine hydrochloride was obtained from the Pharmacy at Walton Hospital (Liverpool). All

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‡ Abbreviations: M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; UDPGA, UDP-glucuronic acid; UDPGT, UDP-glucoronosyltransferase.

other chemicals were of analytical grade, and all solvents were HPLC grade.

Livers from sheep, cows and pigs were obtained from local abbatoirs immediately following death from a captive bolt. Other livers used were rat (Wistar), guinea-pig (Dunkin–Hartley) and mouse (MF1). A human liver sample was obtained from Fazakerley mortuary, Liverpool (time lag from death to post mortem no more than 7 hr). All tissues were from male subjects. Liver samples were chopped into cubes and then homogenized in 5 vol. (w/v) of ice-cold 125 mM sucrose with a Potter–Eveljhem homogenizer. Aliquots were stored at −80° prior to dilution immediately before use.

In order to assess the ability of liver homogenates from different species to form M6G, morphine glucuronidation was determined using a modification of the method of Miners *et al.* [12]. Liver homogenates (600 μg of total protein) were incubated at 37° with UDPGA (15 mM), $MgCl_2$ (2.8 mM), Tris–HCl (100 mM, pH 7.5) and morphine (1.5–500 μM) in a total volume of 2 mL. Incubations were pre-equilibrated for 20 min in a shaking water bath at 37° prior to the addition of substrate. Aliquots (100 μL) were removed at various time intervals (0–60 min) after addition of substrate and enzymatic action was terminated by addition of 100 μL of 12% (v/v) perchloric acid. The tubes were centrifuged at 15,000 *g* and 4° for 10 min, the supernatant was removed and applied directly to the HPLC system for analysis of morphine and its metabolites.

Following this preliminary study, metabolism of morphine was examined in greater detail in guinea-pig liver. In this instance, the final assay volume was 500 μL, with the various constituents present at the same concentrations, except for morphine which was present at a concentration of 3 mM. In addition, the assay was optimized for glucuronide production in terms of pH and also Mg^{2+}, the detergent Brij 58 and UDPGA, whose concentrations are given in the pertinent results section. Incubations, except in the case of kinetic experiments, proceeded for 60 min at 37°.

Analysis of morphine and its metabolites, M3G and M6G, was performed by HPLC employing fluorescence detection, according to the method of Venn and Michalkiewicz [13]. The HPLC system consisted of an LKB 2249 gradient pump with an LKB 2156 solvent conditioner and an LKB 2157 autosampler. A Merck-Hitachi F1050 fluorescence detector (excitation wavelength 280 nm, emission wavelength 335 nm) was coupled to a JCL6000 chromatography data system. The column used was a Techsphere 5C8 (250 mm × 4.6 mm i.d., 5 μm particle size, HPLC Technology, Macclesfield, U.K.) with an octyl pre-column. All solvents were of HPLC grade. Data were quantified by comparing the peak areas with those of standards run daily. The limit of detection for all three analytes was 1 ng/mL.

Protein concentration was measured by the method of Smith *et al.* [14] using crystalline bovine serum albumin as standard. Unless otherwise stated all results are expressed as mean ± SEM. Kinetic parameters such as K_m and V_{max} were calculated from Lineweaver–Burk plots derived from the Michaelis–Menten equation. A paired *t*-test was employed to assess the significance of differences between parameters measured; $P < 0.05$ was considered significant.

RESULTS

Source of UDPGT

Using the standard assay conditions, livers from all species tested (rat, mouse, pig, sheep, rabbit, guinea-pig and human) all produced M3G. However, rat, mouse and pig liver failed to produce measurable quantities of M6G, and sheep liver produced only a trace of M6G. In contrast, guinea-pig (0.26 μmol/g tissue/hr), rabbit (0.14 μmol/g tissue/hr) and human (0.13 μmol/g tissue/hr) liver were able to produce quantifiable amounts of M6G. Guinea-pig liver produced the most M6G and was therefore selected for further detailed study.

Optimization of morphine glucuronidation by guinea-pig liver

The assay conditions were varied in order to optimize glucuronide production by guinea-pig liver. The detergent Brij 58, at concentrations of 0.33 and 3.3 mg detergent/mg protein, significantly increased the formation of M3G by 3.2-fold ($P < 0.01$) and 2.6-fold ($P < 0.05$), respectively. In addition these concentrations of detergent significantly increased M6G formation by 5.0-fold ($P < 0.01$) and 4.2-fold ($P < 0.01$), respectively. Moreover, Brij 58 at a concentration of 0.33 mg/mg protein also resulted in a small, but significant, decrease in the ratio of M3G:M6G from 7.2 ± 1.4 in its absence to 5.3 ± 1.6 in its presence ($P < 0.05$, mean ± SEM of three observations). Another parameter varied was the

Table 1. Effect of metal ions on the ratio of M3G:M6G produced by guinea-pig liver homogenates

	M3G:M6G ratio	
Metal ion	0.3 mM	30 mM
Control	6.3 ± 1.8	5.8 ± 1.7
Cd^{+}	4.5 ± 1.6	5.2
Mn^{2+}	6.6 ± 2.0	5.3 ± 1.3
Zn^{2+}	6.4 ± 2.8	3.8, 4.2
Fe^{3+}	6.9 ± 2.3	—
Cr^{2+}	6.6 ± 2.1	—
Ni^{2+}	5.8 ± 1.9	—
Al^{3+}	6.6 ± 2.0	—
Fe^{2+}	6.6 ± 2.1	5.4 ± 1.9
Rb^{+}	6.5 ± 2.1	6.2 ± 1.8
Ba^{2+}	6.6 ± 2.2	5.7 ± 1.6
Cu^{+}	2.6 ± 0.7*	4.5, 4.7
K^{+}	7.4 ± 1.8	7.2 ± 1.7
Cu^{2+}	2.7 ± 0.8*	2.2
Sn^{2+}	7.1 ± 1.6	7.6

Values represent means ± SEM (N = 3) or individuals. — Represents complete enzyme inhibition. *$P < 0.05$ paired *t*-test compared with control.

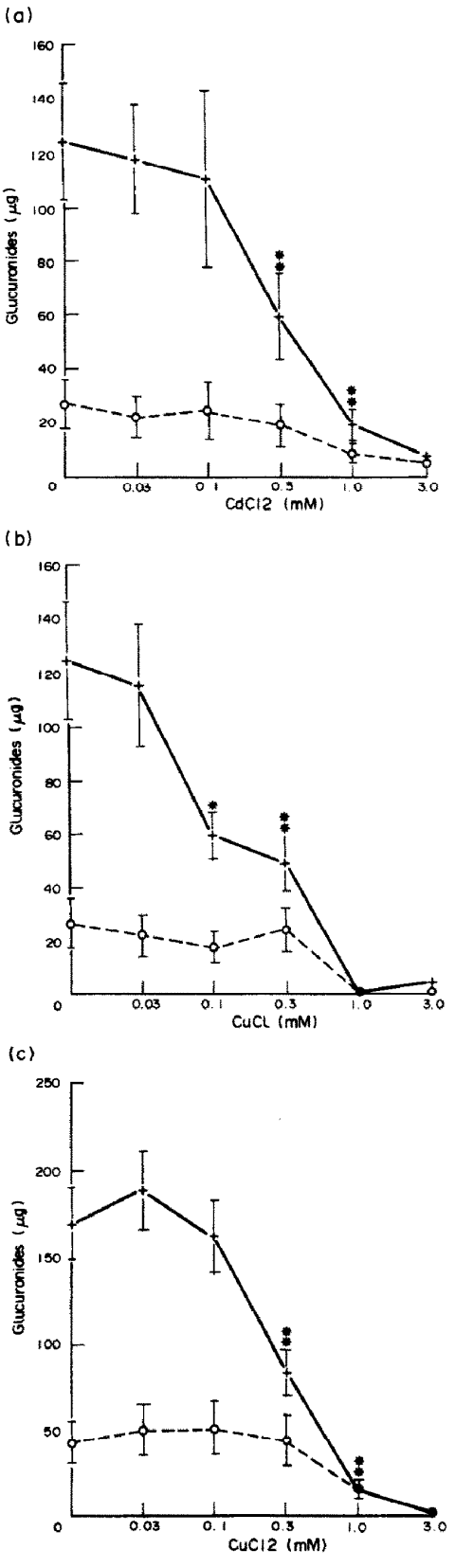

Fig. 1. (a) Effect of Cd^{2+} ions on the production of M3G (crosses) and M6G (circles) by guinea-pig liver homogenates. Guinea-pig liver homogenate (600 μg protein) was incubated at 37° in Tris–HCl (100 mM, pH 7.5), UDPGA (15 mM), $MgCl_2$ (15 mM), Brij 58 (0.33 mg detergent/mg protein) and morphine (3 mM) with increasing concentrations of Cd^{2+}. Results are expressed as micrograms of glucuronide formed during a 60-min incubation and are means ± SEM of three observations; *$P < 0.05$, **$P < 0.01$ paired t-test compared with control. (b) Effect of Cu^{+} ions on production of M3G (crosses) and M6G (circles). Assay conditions were as described above. Values are means ± SEM of three observations; *$P < 0.05$, **$P < 0.01$ paired t-test compared with control. (c) Effect of Cu^{2+} ions on production of M3G (crosses) and M6G (circles). Assay conditions were as described above. Values are means ± SEM of five observations; **$P < 0.01$ paired t-test compared with control.

concentration of Mg^{2+} ions. A 2-fold increase in both M3G and M6G production was observed when the control concentration of Mg^{2+} (2.8 mM) was increased to 15 mM. No significant alteration in the ratio of M3G:M6G occurred with different Mg^{2+} concentrations. UDPGA concentrations were also varied from 3 mM (1:1, UDPGA: morphine) to 60 mM (20:1, UDPGA:morphine). In this instance, the optimum UDPGA concentration was found to be 15 mM (corresponding to a 5-fold excess of UDPGA with respect to morphine). Thus, 15 mM UDPGA caused a significant increase in both M3G and M6G production compared to 6 mM UDPGA ($P < 0.01$, $N = 5$), but no difference was observed between 15 and 30 mM UDPGA in terms of either M3G ($P = 0.283$, $N = 5$) or M6G ($P = 0.31$, $N = 5$) formation. Finally, the assay was optimized in terms of pH. Incubations were performed at pH 7.25, 7.5 and 7.75; formation of M6G was significantly higher at pH 7.5 than 7.25 ($P < 0.05$, $N = 5$) but there was no difference in glucuronidation between pH 7.5 and 7.75 ($P = 0.198$, $N = 5$). The standard incubation conditions for further experiments were therefore modified to include 0.33 mg Brij 58/mg protein and 15 mM Mg^{2+}; UDPGA was present at 15 mM and the incubation pH was 7.5.

Modulation of hepatic morphine metabolism in the guinea-pig by metal cations

A range of metal ions (as chlorides so as not to introduce a novel anion) were tested for their ability to affect the course of UDPGT-catalysed glucuronidation of morphine by guinea-pig liver. Metal ions were added to the preparation at 0.3 and 30 mM, representing 10-fold less and 10-fold more than the concentration of morphine (3 mM). As can be seen from Table 1 most metals had no effect on the ratio of M3G:M6G at 0.3 mM, although a reduction in the ratio was observed with Cd^{2+}, Cu^{+} and Cu^{2+}, being significant with the latter two. On increasing the concentration of metal ion to 30 mM some ions still had no significant effect on morphine glucuronidation (e.g. Mn^{2+}, Fe^{2+}, Rb^{+}, Ba^{2+} and K^{+}) whereas others caused total inhibition of both M3G and M6G formation.

The three metals displaying greatest reduction of the M3G:M6G ratio at concentrations of 0.3 mM, namely Cd^{2+}, Cu^{+} and Cu^{2+} were studied in further detail by way of concentration–effect curves. Figure 1a shows Cd^{2+} to cause a concentration-dependent inhibition of M3G production over the range 0.03–3 mM. The EC_{50} for the inhibition of M3G formation

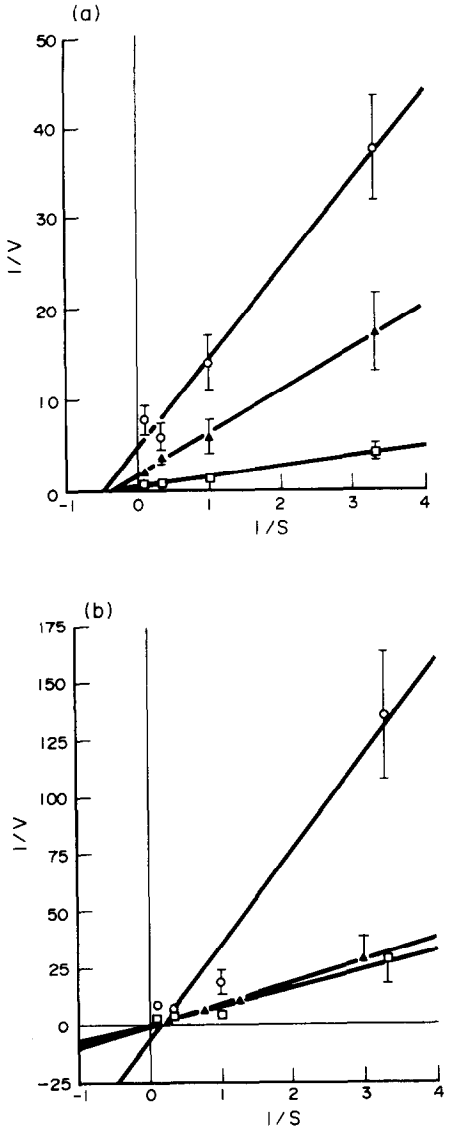

Fig. 2. (a) Lineweaver–Burk plots for M3G production in the absence (squares) or the presence of Cu^+ (circles) or Cu^{2+} (triangles) at a final concentration of 0.3 mM. Values are means ±SEM of five observations. (b) Lineweaver–Burk plots for M6G production in the absence (squares) or the presence of Cu^+ (circles) or Cu^{2+} (triangles) at a final concentration of 0.3 mM. Values are means ± SEM of five observations.

by Cd^{2+} was approximately 0.3 mM (Fig. 1a). However, glucuronidation of morphine in the 6-position was relatively unaffected by Cd^{2+} until a concentration of 1 mM, when a significant reduction in the ratio of M3G:M6G compared to the control was also observed (5.7 ± 1.7 to 3.4 ± 1.3, $P < 0.05$). Similarly, Cu^+ and Cu^{2+} caused dose-dependent inhibition of M3G production with EC_{50} values of approximately 0.1 and 0.3 mM, respectively (Fig. 1b and c). In both cases, inhibition of M6G production only occurred at concentrations greater than 0.3 mM.

Table 2. Kinetic data detailing the effect of Cu^+ and Cu^{2+} ions on the glucuronidation of morphine to M3G and M6G

	Control	Cu^+	Cu^{2+}
M3G			
K_m	1.14 ± 0.2	1.13 ± 0.1	2.28 ± 1.1
V_{max}	1.85 ± 0.2	0.23 ± 0.06**	0.60 ± 0.1*
M6G			
K_m	1.23 ± 0.2	1.47 ± 0.1	2.15 ± 1.1
V_{max}	0.52 ± 0.1	0.17 ± 0.05*	0.43 ± 0.05

Units for K_m are mM and V_{max} nmol/mg protein/min. Values represent means ± SEM (N = 5). *$P < 0.05$, **$P < 0.01$ paired t-test compared with control.

The ratio of M3G:M6G was significantly reduced by Cu^+ at 0.3 mM (6.3 ± 1.8 to 2.6 ± 0.7, $P < 0.05$) and by Cu^{2+} at both 0.3 mM (6.3 ± 1.8 to 2.7 ± 0.8, $P < 0.05$) and 1.0 mM (6.3 ± 1.8 to 1.1 ± 0.2, $P < 0.05$).

To determine the mechanism of the UDPGT inhibition mediated by Cu^+ and Cu^{2+}, kinetic experiments were employed in which rates of M3G and M6G production were determined at four morphine concentrations (0.3, 1, 3 and 10 mM). The velocity of M3G formation was dependent on substrate concentration and followed first order kinetics. A Lineweaver–Burk transformation of this data (Fig. 2a) showed that Cu^+ and Cu^{2+} acted as non-competitive inhibitors of M3G production. Both ions significantly reduced V_{max} for M3G production without affecting K_m (Table 2). The effects of copper ions on M6G formation are depicted in Fig. 2b. Again the reaction followed first order kinetics; however, only Cu^+ inhibited M6G production. The nature of this inhibition was confirmed to be non-competitive from the Lineweaver–Burk transformation of this data (Fig. 2b). In line with this, Cu^+ (0.3 mM) caused a significant decrease in V_{max} without affecting K_m (Table 2).

DISCUSSION

There is now overwhelming evidence that UDPGTs exist as a multigene family resulting in a range of isoenzymes which possess different, but closely related, chemical and physical properties [15]. This concept originated indirectly from studies demonstrating a differential inducibility of different transferases [16], and also chromatographic separation of solubilized enzymes [17]. Similarly, the absence of 1-naphthol glucuronidation in human fetal liver while morphine glucuronidation occurs is also suggestive of more than one form of UDPGT [18]. More recently, however, direct evidence of multiple UDPGTs has been confirmed from cDNA clones [19]. Indeed, at least 11 different forms of UDPGT have been identified; the form responsible for the glucuronidation of morphine being known as GT_2B, a 56 kDa enzyme [20].

Addition of 15 mM Mg^{2+} and 0.33 mg Brij 58/mg protein were found to produce optimal activity of guinea-pig liver UDPGT for both 3- and 6-

glucuronidation. The K_m for 3-glucuronidation was increased from 250 μM in inactivated liver homogenates to 1.1 mM. UDPGTs are latent enzymes and detergent activation has similarly been shown to decrease the affinity of morphine UDPGT in both human [21] and rat [20] liver microsomes.

The present data demonstrate, in the guinea-pig, a functional heterogeneity in the hepatic UDPGT activity responsible for the glucuronidation of morphine in the 3- and 6-positions. This conclusion is based upon a number of observations, the most important being the ability to modulate differentially the formation of the two metabolites M3G and M6G. Despite having similar affinities, the isoenzymes can be distinguished by the presence of certain metal ions. Further evidence for this heterogeneity is the observation that not all species are capable of producing both glucuronides. *In vitro* glucuronidation of morphine in the 3-position by human liver UDPGT has been demonstrated to possess biphasic kinetics, representing two forms of transferase activity, one of high affinity (K_m = 5.3 μM) but low capacity and the other of low affinity (K_m = 1.2 mM) but high capacity [21]. The kinetic properties outlined in Table 2 would appear to describe low affinity isoenzymes of UDPGT in guinea-pig liver.

The glucuronidation of morphine to form both M3G and M6G follows classic Michaelis–Menten kinetics. In the case of M3G production both Cu^+ and Cu^{2+} ions exert non-competitive inhibition on the rate of reaction. In contrast, only Cu^+ ions reduce the rate of 6-glucuronidation with Cu^{2+} ions having no effect on the reaction kinetics. Furthermore, these two metals (and also Cd^{2+}) can inhibit the total amount of M3G formed by over 50% before any significant effect is observed on M6G production. This leads to roughly a 2.5-fold reduction in the ratio of M3G:M6G. Taken together these observations provide strong evidence for a well-defined substrate specificity between different isoenzymes responsible for the two glucuronidations.

The possibility that the metal ion effects are indirect via steric blockage of the morphine molecule is unlikely. Firstly, the huge excess of Mg^{2+} ions has a stimulatory rather than an inhibitory effect on the reaction. Secondly, the most effective chelator of bidenate oxygen donors is Fe^{3+} [22], which was without effect on the course of morphine glucuronidation. A more likely explanation is that the metals are acting directly on the enzyme to mediate this inhibition, possibly via the formation of covalent bonds with suitable free residues on the enzyme molecule, or as allosteric inhibitors, attaching to a specific site on the enzyme. The latter appears less likely due to the difference in ionic size between Cu^+/Cu^{2+} and Cd^{2+}, combined with the ineffectiveness of Zn^{2+} and Ni^{2+} which are both very close in ionic size to Cu^+/Cu^{2+}.

In conclusion, the present study has provided further evidence that more than one UDPGT is responsible for the glucuronidation of morphine. In guinea-pig liver, these isoenzymes have sufficiently different properties to allow differential modulation of the production of M3G and M6G. If the properties observed in guinea-pig liver are also found in human morphine UDPGTs then this mechanism may be of potential use pharmacologically.

Acknowledgements—We would like to thank the Pain Relief Foundation (AJL) and the Sir Halley Stewart Trust (AM) for financial support.

REFERENCES

1. Yeh SY, Urinary excretion of morphine and its metabolites in morphine-dependent subjects. *J Pharmacol Exp Ther* **192**: 201–210, 1975.
2. Säwe J, Kager L, Svensson JO and Rane A, Oral morphine in cancer patients: *in vivo* kinetics and *in vitro* hepatic glucuronidation. *Br J Clin Pharmacol* **19**: 495–501, 1985.
3. Pacifici GM, Säwe J, Kager L and Rane A, Morphine glucuronidation in human fetal and adult liver. *Eur J Clin Pharmacol* **22**: 553–558, 1982.
4. Wahlström A, Winblad B, Bixo M and Rane A, Human brain metabolism of morphine and naloxone. *Pain* **35**: 121–127, 1989.
5. Wahlström A, Lundin L-G, Ask B and Rane A, The sensitivity to noxious heat in relation to brain and liver opioid glucuronidation in inbred strains of mice. *Pain* **38**: 71–77, 1989.
6. Chen ZR, Irvine RJ, Somogyi AA and Bochner F, Mu receptor binding of some commonly used opioids and their metabolites. *Life Sci* **48**: 2165–2171, 1991.
7. Shimomura K, Kanaat O, Ueki S, Ida S, Oguri K, Yoshimura H and Tsukamoto H, Analgesic effect of morphine glucuronides. *Tohaku J Exp Med* **105**: 45–52, 1971.
8. Osborne R, Joel S, Trew D and Slevin M, Analgesic activity of morphine-6-glucuronide *Lancet* **i**: 828, 1988.
9. Gong Q-L, Hedner T, Hedner J, Björkman R and Nordberg G, Antinociceptive and ventilatory effects of the morphine metabolites: morphine-6-glucuronide and morphine-3-glucuronide. *Eur J Pharmacol* **193**: 47–56, 1991.
10. McQuay HJ, Carroll D, Faura CC, Gavaghan DJ, Hand CW and Moore RA, Oral morphine in cancer pain: influences on morphine and metabolite concentration. *Clin Pharmacol Ther* **48**: 236–244, 1990.
11. Choonara IA, McKay P, Hain R and Rane A, Morphine metabolism in children. *Br J Clin Pharmacol* **28**: 599–604, 1989.
12. Miners JO, Lillywhite KJ, Matthews AP, Jones ME and Birkett DJ, Kinetic and inhibitor studies of 4-methylumbelliferone and 1-naphthol glucuronidation in human liver microsomes. *Biochem Pharmacol* **37**: 665–671, 1988.
13. Venn RF and Michalkiewicz A, Fast reliable assay for morphine and its metabolites using high-performance liquid chromatography and native fluorescence detection. *J Chromatogr* **525**: 379–388, 1990.
14. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC, Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**: 76–85, 1985.
15. Mulder GJ, Coughtrie MWH and Burchell B, Glucuronidation. In: *Conjugation Reactions in Drug Metabolism* (Ed. Mulder GJ), pp. 51–105. Taylor and Francis, London, 1990.
16. Bock KW, Fröhling W, Remmer H and Rexer B, Effects of phenobarbital and 3-methylcholanthrene on substrate specificity of rat liver microsomal UDP-glucuronyltransferase. *Biochim Biophys Acta* **327**: 46–56, 1973.
17. Bock KW, von Clausbruch UC, Joshing D and Henwälder H, Separation and partial purification of two

differentially inducible UDP-glucuronyltransferases from rat liver. *Biochem Pharmacol* **26**: 1097–1100, 1977.
18. Rane A, Sjöqvist F and Orrenius S, Drugs and fetal metabolism. *Clin Pharmacol Ther* **14**: 666–672, 1973.
19. Mackenzie PI and Haque SJ, Multiplicity and structure of UDP-glucuronosyltransferases as revealed by gene cloning. In: *Microsomes and Drug Oxidations* (Eds. Miners JO, Birkett DJ, Drew R, May B and McManus ME), pp. 271–278. Taylor and Francis, London, 1988.
20. Puig JF and Tephly TR, Isolation and purification of rat liver morphine UDP-glucuronosyltransferase. *Mol Pharmacol* **30**: 558–565, 1986.
21. Miners JO, Lillywhite KJ and Birkett DJ, *In vitro* evidence for the involvement of at least two forms of human liver UDP-glucuronosyltransferase in morphine 3-glucuronidation. *Biochem Pharmacol* **37**: 2839–2845, 1988.
22. Cotton SA, Some aspects of the coordination chemistry of iron (III). *Coord Chem Rev* **8**: 185–223, 1972.