0026-895X/90/030294-05$02.00/0

MOLECULAR PHARMACOLOGY, 38:294-298

Photoaffinity Labeling of Rat Liver Microsomal Morphine UDP-Glucuronosyltransferase by [^{3}H]Flunitrazepam

JACQUES THOMASSIN and THOMAS R. TEPHLY
Department of Pharmacology, The University of Iowa, Iowa City, Iowa 52242
Received October 4, 1989; Accepted March 15, 1990

SUMMARY

Benzodiazepines have been shown to competitively inhibit morphine glucuronidation in rat and human hepatic microsomes. Flunitrazepam exerted a potent competitive inhibition of rat hepatic morphine UDP-glucuronosyltransferase (UDPGT) activity (K_i = 130 μM). It has no effect on the activity of *p*-nitrophenol, 17β-hydroxysteroid, 3α-hydroxysteroid, or 4-hydroxybiphenyl UDPGTs. Because flunitrazepam is an effective photoaffinity label for benzodiazepine receptors, studies were performed in solubilized rat hepatic microsomes and with partially purified preparations of morphine UDPGT to determine the enhancement of flunitrazepam inhibition and binding to morphine UDPGT promoted by exposure to UV light. Under UV light, flunitrazepam inhibition was markedly enhanced. UV light exposure also led to a marked increase in binding of [^{3}H]flunitrazepam to microsomal protein, which was protected substantially by preincubation with morphine. Testosterone, androsterone, and UDP-glucuronic acid did not protect against UV-enhanced flunitrazepam binding, and morphine did not reverse flunitrazepam binding once binding had occurred. As morphine UDPGT was purified, a good correlation was found between the increases in specific activity of morphine UDPGT and flunitrazepam binding to protein. Chromatofocusing chromatography showed that flunitrazepam bound only to fractions containing active morphine UDPGT, and no binding to 4-hydroxybiphenyl UDPGT was observed. Fluorography of a sodium dodecyl sulfate-polyacrylamide electrophoresis gel of solubilized hepatic microsomes that had been treated with [^{3}H] flunitrazepam under UV light revealed a band with a monomeric molecular weight between 54,000 and 58,000. This monomeric molecular weight compares favorably with the reported monomeric molecular weight of homogeneous morphine UDPGT (56,000). These studies suggest that flunitrazepam binds rather selectively to the morphine binding site of morphine UDPGT and may prove to be a useful probe for this enzyme.

Glucuronidation of xenobiotics is one of the most important reactions in drug metabolism. Glucuronide formation is catalyzed by numerous UDPGTs in the endoplasmic reticulum, where UDPGA serves as the glucuronic acid donor. Of the many drugs metabolized via glucuronidation, morphine is one of the most important and its conjugation is of considerable interest. Morphine UDPGT has been purified to homogeneity from rat liver microsomes and, unlike many other UDPGTs, it possesses a relatively narrow substrate specificity (1).

Very few inhibitors of UDPGT-catalyzed reactions, other than alternative substrates, have been identified. However, several types of substances that are not substrates for glucuronidation and that possess *N*-alkyl moieties are effective inhibitors of morphine UDPGT. del Villar *et al.* (2) demonstrated that cyproheptadine was a potent competitive inhibitor of morphine glucuronidation in rabbit liver microsomes, and del Villar *et al.* (3) and Vega *et al.* (4) have shown that a number of benzodiazepines are potent competitive inhibitors of morphine glucuronidation in rat liver microsomes. These workers have also shown that other UDPGT-catalyzed reactions, such as *p*-nitrophenol, testosterone, and estrone glucuronidation, are not inhibited by benzodiazepines (3–5) in rat liver microsomes. It would appear that benzodiazepines are relatively specific inhibitors of morphine UDPGT. More recently, the competitive inhibition of morphine glucuronidation by benzodiazepines in human liver microsomes has been demonstrated (6).

Of the benzodiazepines, flunitrazepam was shown by Vega *et al.* (4) to be an especially potent inhibitor of morphine glucuronidation. It has also been used as a photoaffinity probe for peripheral-type benzodiazepine receptors (7). Mohler *et al.* (8) demonstrated that reversible binding of [^{3}H]flunitrazepam was transformed to covalent binding by UV light irradiation. We have explored the interaction of flunitrazepam with rat liver microsomal morphine UDPGT and report on the potent inhibition and UV light-promoted binding of this substance to morphine UDPGT.

Materials and Methods

Chemicals. UDP-D-[U-^{14}C]glucuronic acid (319 mCi/mmol) was purchased from ICN Pharmaceuticals, Inc. (Irvine, CA). Sep-Pak C-18

This work was supported by the National Institutes of Health, Grant GM-26221.

ABBREVIATIONS: UDPGT, UDP-glucuronosyltransferase; UDPGA, UDP-glucuronic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; DMSO, dimethyl sulfoxide.

minicolumns were obtained from Waters Associates (Milford, CA). Dithiothreitol, 4-hydroxybiphenyl, androsterone, testosterone, *p*-nitrophenol, *p*-[^{14}C]nitrophenol (1–10 mCi/mmol), flunitrazepam, L-α-phosphatidylcholine (egg yolk, type XI-E), and morphine-3-glucuronide were obtained from Sigma Chemical Co. (St. Louis, MO). Morphine sulfate was acquired from Merck and Co., Inc. (Rahway, NJ). Emulgen-911 was a gift from Kao-Atlas Ltd. (Tokyo, Japan). [4-^{14}C]Testosterone (50 mCi/mmol), [1,2-^{3}H]androsterone (40–60 Ci/mmol), and [^{3}H]flunitrazepam (101 Ci/mmol) were obtained from New England Nuclear Co. (Boston, MA). Trisacryl-DEAE was purchased from IBF Biotechnics (Villeneuve la Garenne, France). Reagents for protein analysis were obtained from Bio-Rad (Richmond, CA). Chromatofocusing materials were obtained from Pharmacia Fine Chemicals (Piscataway, NJ).

Animals. Female Wistar rats (200–250 g) were obtained from Charles River Laboratory (Wilmington, MA). In certain experiments, rats were treated with phenobarbital intraperitoneally (80 mg/kg) each day for 4 days and were killed 24 hr after the last dose.

Preparation of microsomes and morphine UDPGT. Microsomes were prepared as described previously (9). Morphine UDPGT was purified by Trisacryl-DEAE chromatography or by chromatofocusing chromatography after anion exchange chromatography as described by Puig and Tephly (1).

Assays. The glucuronidation of morphine, 4-hydroxybiphenyl, testosterone, androsterone, and *p*-nitrophenol was determined according to the methods previously reported (1, 9). Protein was determined using the Bio-Rad method, with bovine serum albumin as a standard (10).

UV light irradiation studies of morphine UDPGT activity. Rat liver microsomes were solubilized with Emulgen-911 (0.8 mg/mg of protein). Partially purified preparations obtained from hepatic microsomes were prepared as described by Puig and Tephly (1). Morphine UDPGT activity was analyzed at 37°, in the presence and absence of flunitrazepam, after exposure at 4° to either ambient light, darkness, or UV light. Enzyme preparations (0.95 ml) were kept at 4° and placed in either a 1.5-ml quartz cuvette or a 5.0-ml beaker. Flunitrazepam was dissolved in DMSO (50 μl) when it was added. DMSO alone served as a vehicle control. At various times, 20 μl of incubation mixture were removed for analysis of morphine 3-glucuronide formation (1). UV light exposure was carried out using two different systems, one with low intensity and one with high intensity light. Experiments shown in Table 1 were carried out in a cuvette using a UV light apparatus commonly employed for viewing thin layer chromatography plates (MFD; Ultraviolet Light Products, Inc., San Gabriel, CA). This apparatus delivered 4600 μW/cm². Experiments demonstrated that UV light at 2546 Å provided maximal effects in promoting inhibition of morphine UDPGT activity by flunitrazepam. In all other experiments, a UV light sun lamp (model RSK6A; General Electric) was used, and exposure was carried out at 15.0 cm from the light source in 5.0-ml beakers at 4°, with stirring. This lamp emitted more than 60% of the maximal irradiation at 2546 Å, with an intensity of 92,000 μW/cm² at a distance of 15 cm.

Studies on [^{3}H]flunitrazepam binding to microsomal protein or partially purified UDPGTs. Various amounts of enzyme were mixed with [^{3}H]flunitrazepam, in a final volume of 1.0 ml, and photoirradiation studies were carried out as described above. In order to demonstrate protection by morphine of flunitrazepam binding, morphine sulfate (5.0 mM) was preincubated with enzyme at 4° for 10 min in the dark, before addition of flunitrazepam and irradiation with UV light. At various times following incubation with flunitrazepam or flunitrazepam and morphine, 200-μl aliquots were removed from reaction mixtures and added to small Eppendorf centrifuge tubes, following which 500 μl of 10% trichloroacetic acid were added. The tube was gently vortexed and 100 μl of this mixture were added to a filtration chamber (Millipore) and filtered by vacuum through a Whatman GF/C glass microfiber filter (2.4 cm). Filters had been presoaked with 50 mM Tris·HCl buffer, 1 mM EDTA, pH 7.4. Protein precipitated on the filter was washed with 4 ml of the same buffer and rinsed with 1.0 ml of absolute methanol. No radioactivity was removed by further washing procedures. A linear relationship between bound radioactivity and protein concentration was observed. Filters were then removed, dried at room temperature, and added to scintillation vials. Scintillation cocktail (Budget Solve) was added, the mixture was shaken gently for about 10 sec, and radioactivity was determined using standard scintillation counting technology.

PAGE and autoradiography. PAGE was performed in the presence of SDS, as described by Laemmli (11). Samples were applied to the gel in a buffer consisting of 0.8% SDS and 0.3% mercaptoethanol, after boiling in a water bath for 10 min. Commercial molecular weight standards (Rainbow protein molecular weight markers, ^{14}C-labeled; Amersham) were boiled for 1 min and used to calculate the molecular weight of the band revealed by fluorography. After electrophoresis, the gel was fixed for 30 min in a solution of isopropanol, acetic acid, and water (25:10:65), soaked in Amplify (Amersham) for 30 min, and dried. Finally, the dried gel was exposed to Hyperfilm-^{3}H (Amersham) for 8 weeks at −70°.

TABLE 1

Effect of UV light on the inhibition of morphine and 4-hydroxybiphenyl UDPGTs by flunitrazepam

Morphine UDPGT activity was eluted from Trisacryl-DEAE at 60 mM NaCl; 4-hydroxybiphenyl UDPGT was eluted at 75 mM NaCl from the Trisacryl-DEAE column. Activity is expressed for each enzyme after 15 min of incubation with either UV light or ambient light exposure. Control activity represents rates obtained after incubation of substrate alone under ambient light for 20 min, where no preincubation was employed. When the effect of flunitrazepam (2.5 mM) was studied, a 15-min preincubation with enzyme (0.6 mg of protein/ml) was used, after which aliquots were removed for morphine UDPGT activity determination, as described in Materials and Methods. Concentrations of substrates used were 10 mM morphine and 0.5 mM 4-hydroxybiphenyl. Each value represents the mean of three experiments ± standard deviation.

Treatment	Activity	
	Morphine glucuronidation	4-Hydroxybiphenyl glucuronidation
	nmol/min/mg of protein	
Control	27.5 ± 4.8	20.2 ± 10.1
Flunitrazepam in ambient light	15.9 ± 3.8	19.2 ± 6.9
Flunitrazepam with UV light	6.9 ± 1.4	19.7 ± 7.2

Results

Kinetic studies were first performed in ambient light using solubilized rat liver microsomes or partially purified preparations of morphine UDPGT. Competitive inhibition was produced by flunitrazepam, with an inhibition constant (K_i) of 130 μM under ambient conditions. These results are similar to those of other workers (2, 3). The inhibition observed was reversible, because preincubation of enzyme either with flunitrazepam or with flunitrazepam and morphine for 30 min yielded the same degree of inhibition.

With a partially purified preparation of morphine UDPGT (obtained after anion exchange chromatography), flunitrazepam (2.5 mM) produces about a 40–50% inhibition of the rate of morphine glucuronide formation under ambient light in the presence of 10 mM morphine (Table 1). However, flunitrazepam (2.5 mM) in the presence of UV light produced an 80% inhibition of morphine UDPGT activity (Table 1). UV light exposure of enzyme in the absence of flunitrazepam but in the presence of DMSO had no significant effect on its activity (data not shown). Table 1 also shows that flunitrazepam has no effect on 4-hydroxybiphenyl glucuronidation in ambient light or after irradiation with UV light.

Whereas an approximate IC_{50} for flunitrazepam of 2.5 mM

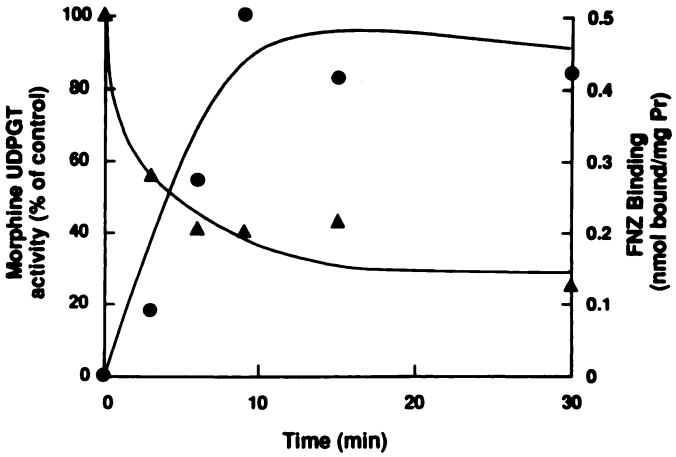

Fig. 1. Time-dependent inhibition of morphine glucuronidation and binding of [^{3}H]flunitrazepam (*FNZ*) to solubilized hepatic microsomes from rats treated with phenobarbital, in the presence of UV light. Control morphine UDPGT activity was 13 nmol/mg of protein/min. Inhibition studies were carried out using 100 μM flunitrazepam, and 10 mM morphine was added after UV light exposure for determination of the rate of morphine glucuronidation. Each *point* represents the mean of duplicate samples. In binding studies, 50 μM flunitrazepam (12,430 dpm/nmol) was employed. Reaction mixtures contained 3 mg/ml solubilized microsomal protein, and 20 or 200 μl were removed at various time periods for analysis of either morphine glucuronidation or [^{3}H]flunitrazepam binding, respectively. ▲, Morphine glucuronidation; ●, [^{3}H]flunitrazepam binding. These results were obtained from a single experiment but this experiment was performed three times.

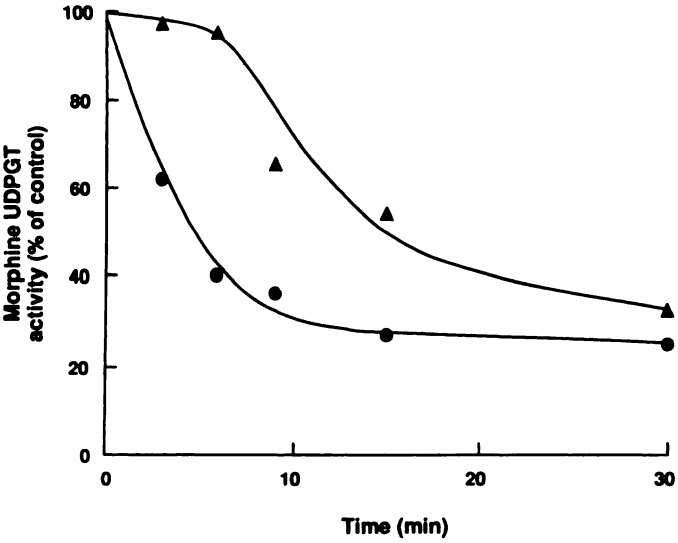

Fig. 2. Protection of flunitrazepam inhibition of morphine glucuronidation by morphine. ●, Flunitrazepam (100 μM); ▲, flunitrazepam plus morphine (10 mM). Control activity was 13 nmol/mg of protein/min. When morphine was used with flunitrazepam, reactions were preincubated for 10 min before exposure to UV light. Solubilized microsomes obtained from livers of phenobarbital-treated rats were employed. Each *point* represents the mean of duplicate samples from a single experiment. This experiment was performed three times.

was found in ambient light (Table 1), an approximate IC_{50} of 100 μM was obtained when solubilized hepatic microsomes were incubated with flunitrazepam under UV light (Fig. 1). Fig. 1 shows a typical experiment where the time-dependent inhibition of morphine glucuronidation by flunitrazepam (100 μM) under UV light was studied. Fig. 1 also shows a time-dependent increase in incorporation of [^{3}H]flunitrazepam under UV light

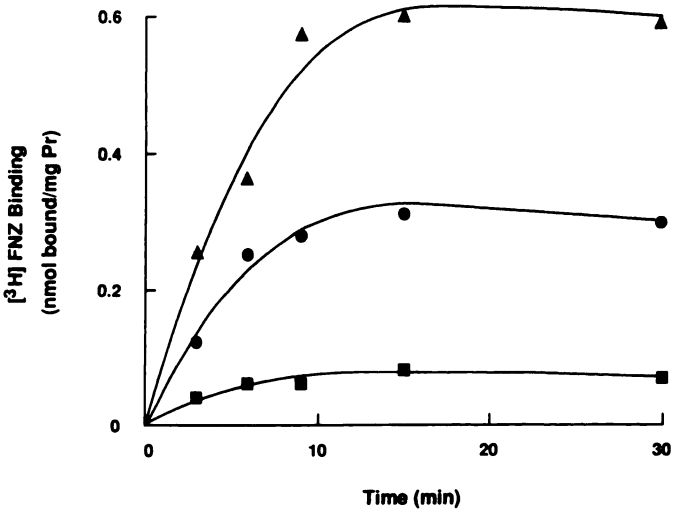

Fig. 3. Protection by morphine of [^{3}H]flunitrazepam ([^{3}*H*]*FNZ*) binding to solubilized hepatic microsomal protein. Conditions were similar to those described for Fig. 1. ▲, Flunitrazepam (50 μM); ●, flunitrazepam plus morphine (10 mM); ■, flunitrazepam (50 μM) exposed to ambient light. Each *point* represents the mean of duplicate samples but this experiment was performed three times.

TABLE 2

Effect of substrates for other UDPGTs on [^{3}H]flunitrazepam binding to solubilized microsomes after UV irradiation

FNZ concentration was 50 μM; incubation under UV light was 10 min. Testosterone and androsterone were dissolved in DMSO. Each value represents the mean of duplicate samples from one experiment and this experiment was performed twice.

Treatment	[^{3}H]Flunitrazepam bound
	nmol/mg of protein
[^{3}H]Flunitrazepam	0.5
Testosterone (5 mM) + [^{3}H]flunitrazepam	0.5
Androsterone (5 mM) + [^{3}H]flunitrazepam	0.44
UDPGA (5 mM) + [^{3}H]flunitrazepam	0.5

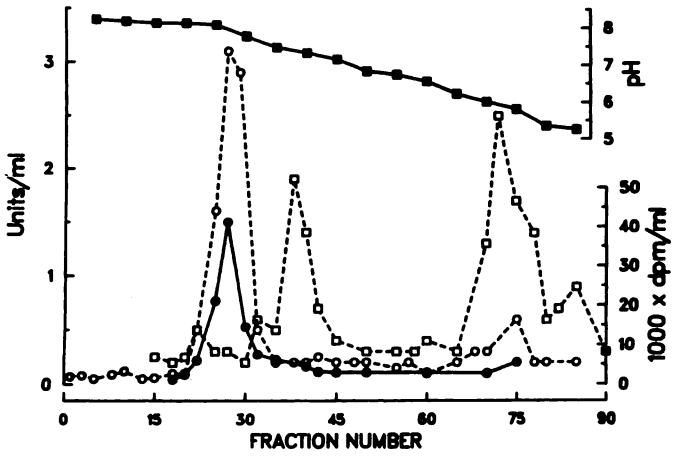

Fig. 4. Chromatofocusing chromatography of morphine and 4-hydroxybiphenyl UDPGTs. Fractions obtained from Trisacryl-DEAE containing morphine and 4-hydroxybiphenyl UDPGTs were pooled and dialyzed overnight. Approximately 15 mg of protein were applied to a chromatofocusing column previously equilibrated at pH 8.4, and a pH gradient from 8.4 to 5.5 was generated. ○, Morphine glucuronidation; □, 4-hydroxybiphenyl glucuronidation; ●, [^{3}H]flunitrazepam (400 μM) binding.

TABLE 3
Morphine UDPGT activity and [^{3}H]flunitrazepam binding

Morphine UDPGT source	Morphine UDPGT activity	[^{3}H]Flunitrazepam binding[a]
	nmol/min/mg of protein	*nmol/mg of protein*
Solubilized microsomes	10	3
Trisacryl-DEAE	33	6
Chromatofocusing	80	20

[a] 400 μM flunitrazepam.

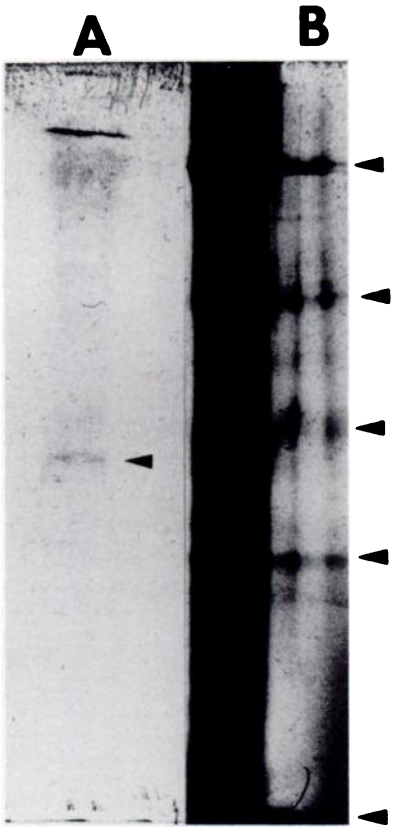

Fig. 5. SDS-PAGE fluorograph of solubilized rat hepatic microsomes photoaffinity labeled with [^{3}H]flunitrazepam. *Lane A*, solubilized hepatic microsomes (100 μg) from untreated rats, incubated with [^{3}H]flunitrazepam (400 μM) and exposed to UV light for 6 min. *Lane B*, molecular weight standards from the same gel. The molecular weight standards from *bottom* to *top* were carbonic anhydrase, 30,000; ovalbumin, 46,000; bovine serum albumin, 69,000; phosphorylase b, 92,500; and myosin, 200,000.

exposure. Under the conditions employed, maximal inhibition and binding were observed between 6 and 9 min.

Fig. 2 shows data from a typical experiment where morphine protects against the UV light-enhanced inhibition of morphine glucuronidation over a 6-min time of exposure. Morphine appears to be destroyed by light, because incubation of morphine for 7 min in UV light led to the abolishment of protection. Fig. 3 shows that morphine protects against the UV light-enhanced binding of [^{3}H]flunitrazepam to solubilized hepatic microsomes. When morphine was added following flunitrazepam binding, no reversal of binding occurred (data not shown). Also, it can be seen that flunitrazepam binding to microsomal protein in ambient light was minimal. Similar results have been obtained using a partially purified preparation of morphine UDPGT obtained from anion exchange chromatography. These results suggest that flunitrazepam binds to the morphine binding site on morphine UDPGT.

The effects of testosterone, androsterone, and UDPGA on [^{3}H]flunitrazepam binding to solubilized microsomal protein are shown in Table 2. No protection was observed. These results suggest that flunitrazepam does not interact with sites on UDPGTs binding testosterone, androsterone, and 4-hydroxybiphenyl. Furthermore, flunitrazepam does not interact with UDPGA binding sites, which are present on all UDPGTs. We have also shown that flunitrazepam does not inhibit the glucuronidation of testosterone, androsterone, and *p*-nitrophenol, as has been shown, in part, by others (2, 3).

In order to determine the specificity of flunitrazepam binding to morphine UDPGT, a purification procedure was carried out for this enzyme, as described previously (1). Fig. 4 shows results obtained after the chromatofocusing step of that procedure. Only fractions containing active morphine UDPGT bound [^{3}H]flunitrazepam. For comparison, 4-hydroxybiphenyl UDPGT activity and [^{3}H]flunitrazepam binding were monitored. Fractions containing 4-hydroxybiphenyl UDPGT activity did not bind [^{3}H]flunitrazepam. In addition, other fractions eluting before morphine UDPGT, which usually contain 17β-hydroxysteroid UDPGT (testosterone), did not bind [^{3}H]flunitrazepam.

A summary of the binding of [^{3}H]flunitrazepam and the specific activity of morphine UDPGT is shown in Table 3. A good correlation can be seen between the increase in specific activity of morphine UDPGT and the amount of [^{3}H]flunitrazepam bound to protein.

Fig. 5 represents a fluorograph of an SDS-PAGE gel of solubilized rat hepatic microsomes that had been exposed to [^{3}H]flunitrazepam. A band appears that possesses a monomeric molecular weight in the range of 54,000 to 58,000. Previously, the monomeric molecular weight of rat hepatic morphine UDPGT was determined to be about 56,000 (1). No other prominent bands appear in this molecular weight range.

Discussion

The glucuronidation of xenobiotics is catalyzed by a family of UDPGTs in rat liver microsomes, where an extensive overlap of substrate specificity is usually observed. Thus, specific inhibitors of glucuronidation reactions have been particularly difficult to devise and identify, and very few are known. It is important and surprising to find that benzodiazepines appear to inhibit only one UDPGT, that responsible for the glucuronidation of morphine. This enzyme, morphine UDPGT, is somewhat unique among UDPGTs in that it possesses a narrow substrate specificity (1). Of the benzodiazepines, flunitrazepam is a useful substance for study, in that it is not a substrate for glucuronidation, it is a potent competitive inhibitor of morphine glucuronidation, and it can be used as a photoaffinity probe. After UV light exposure, a remarkable increase in inhibitory potency occurs; morphine protects against this inhibition by flunitrazepam, within certain time constraints. Flunitrazepam binding to solubilized microsomal protein and to purified morphine UDPGT was demonstrated, and morphine protected against flunitrazepam binding. Other reactions of hepatic UDPGTs, such as those carried out by *p*-nitrophenol, 17β-hydroxysteroid, 3α-hydroxysteroid, and 4-hydroxybiphenyl UDPGTs, are not inhibited by flunitrazepam, and substrates for these enzymes do not protect against flunitrazepam binding. It is particularly interesting that UDPGA also had no effect on

flunitrazepam binding, suggesting that flunitrazepam specifically binds to the morphine binding site of morphine UDPGT.

The hypothesis that flunitrazepam specifically binds to morphine UDPGT is strengthened by observations carried out with preparations of morphine UDPGT throughout a typical purification procedure. As the specific activity of morphine UDPGT increased throughout anion exchange and chromatofocusing chromatography, a good agreement was found in the degree of enhancement of flunitrazepam bound to protein fractions containing active morphine UDPGT. These experiments indicate that flunitrazepam binds to protein where active morphine UDPGT is recovered. We have observed that flunitrazepam binding to fractions containing morphine UDPGT is substantially diminished with time, as the activity of morphine UDPGT is diminished. Thus, flunitrazepam appears to bind only to active morphine UDPGT. This probably reflects the importance of the conformation of the enzyme in flunitrazepam binding and may, indeed, be a clue to an understanding of the well known lability of morphine UDPGT. In addition, binding to fractions where other UDPGTs are found was not observed.

Puig and Tephly (1) reported previously that homogeneous morphine UDPGT possesses a monomeric molecular weight on SDS-PAGE of about 56,000. Analysis of a fluorograph of solubilized rat hepatic microsomes treated with flunitrazepam and UV light showed an identifiable band between molecular weights of 54,000 and 58,000. This agrees roughly with the subunit molecular weight of morphine UDPGT. In addition, this fluorograph shows a lack of other flunitrazepam-binding protein bands between molecular weights of 50,000 and 60,000, where other important rat hepatic microsomal proteins such as the UDPGTs and cytochromes P-450 should appear if they bind flunitrazepam. This may not be surprising, because not all proteins that react with flunitrazepam exhibit photoactivation or enhanced inhibition as seen with morphine UDPGT. We have found that one human UDPGT that catalyzes the glucuronidation of tertiary amines is inhibited by flunitrazepam (12) but this inhibition is not enhanced by UV light exposure.

Recently, identification of a peripheral-type benzodiazepine receptor has been made for liver by Parola *et al.* (13). These interesting studies appear to have been carried out in preparations that exclude the microsomal fraction of the liver homogenate. The SDS-PAGE gels presented in their work show a binding protein for benzodiazepines whose monomeric molecular weight is 19,000. Thus, it appears that the peripheral benzodiazepine receptor of liver, as identified by these workers, is not morphine UDPGT. However, the techniques employed by Parola *et al.* (13) are quite different from those used in the current report, and more work is needed to completely exclude this possibility.

The utility of a photoaffinity label of the active binding site of morphine UDPGT has great potential. It may be possible, through purification of the morphine UDPGT labeled with [^{3}H]flunitrazepam, to determine the amino acid sequence(s) of the binding site of morphine UDPGT. Indeed, labeling the protein early in the purification scheme should greatly assist in the purification procedure itself, because the measurement of radioactivity is more convenient and rapid than is the determination of the rates of morphine glucuronidation. Should flunitrazepam binding prove to be as specific as it seems, binding studies on small amounts of microsomes isolated from tissues may be a useful way of predicting the level of morphine UDPGT in a tissue. The procedure should also be useful in screening a large number of potential substrates for morphine UDPGT that may react with the morphine binding site and in determining binding affinities relative to morphine. In this respect, we have already obtained preliminary data showing that naloxone is a potent protector of flunitrazepam binding.

Acknowledgments

The authors wish to acknowledge Jack Burke for photographic assistance and Mitchell Green for assistance with photography and helpful discussions in preparation of the manuscript.

References

1. Puig, J. A., and T. R. Tephly. Isolation and purification of rat liver morphine UDP-glucuronosyltransferase. *Mol. Pharmacol.* **30**:558–565 (1986).
2. del Villar, E., E. Sanchez, and T. R. Tephly. The inhibition of morphine UDP-glucuronosyltransferase in rabbit liver microsomes by cyproheptadine. *Life Sci.* **21**:1801–1806 (1977).
3. del Villar, E., E. Sanchez, M. E. Letelier, and P. Vega. Differential inhibition by diazepam and nitrazepam of UDP-glucuronosyltransferase activity in rats. *Res. Commun. Chem. Pathol. Pharmacol.* **33**:433–447 (1984).
4. Vega, P., M. Carrasco, E. Sanchez, and E. del Villar. Structure activity relationship in the effect of 1,4-benzodiazepines on morphine, aminopyrine and oestrone metabolism. *Res. Commun. Chem. Pathol. Pharmacol.* **44**:179–198 (1984).
5. Vega, P., G. Gaule, E. Sanchez, and E. del Villar. Inhibition and activation of UDP-glucuronosyltransferase in alloxatic diabetic rats. *Gen. Pharmacol.* **17**:641–645 (1986).
6. Rane, A., J. Sawe, G. M. Pacifici, J. O. Svenson, and L. Kager. Regioselective glucuronidation of morphine and interaction with benzodiazepines in human liver. *Adv. Pain Res. Ther.* **8**:57–64 (1986).
7. Snyder, S. H., A. Verma, and R. R. Trifiletti. The peripheral-type benzodiazepine receptor: a protein of mitochondrial outer membranes utilizing porphyrins as endogenous ligands. *FASEB J.* **1**:282–288 (1987).
8. Mohler, H., M. K. Battersby, and J. G. Richards. Benzodiazepine receptor protein identified and analyzed in brain tissue by a photoaffinity label. *Proc. Natl. Acad. Sci. USA* **77**:1161–1170 (1980).
9. Falany, C., and T. R. Tephly. Separation, purification and characterization of three isozymes of UDP-glucuronosyltransferase from rat liver microsomes. *Arch. Biochem. Biophys.* **7**:248–258 (1983).
10. Bradford, M. A rapid and sensitive method for the quantification of microgram amounts of protein utilizing the principle of protein dye binding. Anal. Biochem. **72**:248–253 (1976).
11. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T$_4$. *Nature* (*Lond.*) **227**:680–685 (1970).
12. Styczynski, P. B., B. L. Coffman, M. D. Green, and T. R. Tephly. Studies on quaternary ammonium-linked glucuronidation in human liver microsomes. *Pharmacologist* **31**:131 (1989).
13. Parola, A. L., C. W. Putnam, D. H. Russell, and H. E. Laird. II. Solubilization and characterization of the liver peripheral-type benzodiazepine receptor. *J. Pharmacol. Exp. Ther.* **250**:1149–1155 (1989).

Send reprint requests to: Dr. T. R. Tephly, Department of Pharmacology, Bowen Science Building, The University of Iowa, Iowa City, IA 52242.