Biochemical Pharmacology, Vol. 37, No. 18, pp. 3526–3528, 1988.
Printed in Great Britain.

0006–2952/88 $3.00 + 0.00

Treatment of rats with glucagon, vasointestinal peptide or secretin has a different effect on bilirubin and *p*-nitrophenol UDP-glucuronyltransferase

(*Received* 14 *December* 1987; *accepted* 24 *March* 1988)

UDP-glucuronyltransferases catalyze the conjugation of several substrates with a glucuronyl moiety [1–4]. In the rat, transferases have tentatively been subdivided in two groups according to their perinatal development and their pattern of activation [3–5]. Recent separation studies have documented the existence of even more isoforms [6–8]. These enzymes are most abundantly present in the liver, which is the first organ on the venous outflow of the intestine where several gastrointestinal peptides are released in response to feeding or other stimuli. The question thus arises whether the liver is influenced by these hormones. Some of them have choleretic properties [9–12]; in addition glucagon [13] and secretin [14] were found to enhance bilirubin UDP-glucuronyltransferase activity in the rat. The effect of secretin was substrate-specific, since glucuronidation of *p*-nitrophenol, a substrate of the "late foetal" group, was unchanged [14].

The aim of the present study was to investigate whether vasoactive intestinal peptide (VIP) also has an effect on glucuronidation and whether glucagon and VIP, both belonging to the secretin family of polypeptides [15], have a specificity for the acceptor substrate used.

Materials and methods

Male Wistar R/A rats with a body weight of 210–260 g were prepared under pentobarbital anaesthesia (6 mg/100 g) with a catheter into the right jugular vein. They were restrained and kept overnight in ventilated and thermostated boxes, to keep their body temperature constant at 37.2° [16] while receiving an infusion of 5% glucose in 0.16 M NaCl at the rate of 0.6–0.8 ml/hr/100 g body wt. In the morning the following peptides dissolved in 0.16 M NaCl were infused over a 90 min period: glucagon (Novo Industries, Coopenhagen, Denmark) 1, 25, 50 or 150 μg/hr/100 g body wt; VIP 50, 150, 300 or 1000 ng/hr/100 g body wt or porcine secretin 0.1, 0.2, 0.4 or 0.8 clinical units (CU)/hr/100 g body wt (both obtained from G.I.H. Laboratories, Karolinska Institute, Stockholm, Sweden). Control animals received saline. After 90 min the rats were killed by cervical dislocation and exsanguination. Livers were quickly excised and perfused with 120–150 ml chilled 0.16 M KCl, blotted with filter paper and weighed. To prepare liver homogenates, approximately 1 g was weighed and minced on a glass support: the sample was then dropped into a Potter–Elvehjem homogenizer containing a known volume of 0.25 M sucrose containing 1 mM EDTA, adjusted to pH 7.4 and homogenized.

Liver homogenates containing 100 mg wet wt of liver per ml were assayed as such or after preincubation with an equal volume of a digitonin solution (Merck AG. Darmstadt, F.R.G.) or of UDP-*N*-acetylglucosamine (UDPNAG, Sigma, St. Louis, MO). To study the *in vitro* activation different concentrations of digitonin 0.3, 0.6, 0.9, 1.5 and 3 mg/ml final concentration) or of UDPNAG (0.5, 1.0, 1.5 and 2 mM) were incubated for 60 min at 4° with liver homogenate.

For the bilirubin UDP-glucuronyltransferase assay [17] the reaction mixture contained (final concentrations): 0.1 M Tris–HCL buffer, pH 7.8; 0.125 mM bilirubin dissolved in 0.125 mM human serum albumin (Belgian Red Cross); 7.5 mM $MgCl_2$ liver homogenate at an average concentration of 3 mg protein/ml in a total volume of 1.32 ml. Reaction was started by adding UDP-glucuronic acid at a final concentration of 3 mM.

Para-nitrophenol UDP-glucuronyltransferase was assayed in the 1500 g av. supernatant of liver homogenates, prepared at 4° in a L2-65 B Beckman centrifuge. The incubation mixture contained (final concentrations): 0.05 M Tris–HCl buffer, pH 7.4, 1.4 mM *p*-nitrophenol, 15 mM $MgCl_2$ and 5–10 mg of liver homogenate (with or without digitonin or UDPNAG in a final volume of 2 ml). Reaction was started by adding UDP-glucuronic acid at a final concentration of 5.5 mM. The decrease in absorbance was followed at 400 nm, at 27°. It is assumed that disappearance of *p*-nitrophenol is only due to conjugation, and enzyme activity is calculated using a molar extinction co-efficient of $18.1 \times 10^3\ M^{-1} \times cm^{-1}$ [18].

Protein was measured by a modified Lowry procedure [19].

Results and discussion

In non-activated liver preparations glucuronyltransferase activities were enhanced by pretreatment of the rats for 90 min with glucagon only (Tables 1 and 2). The other treatments had no significant effect; this was presumably due to the low enzyme activities obtained and the relatively wide range of the results.

Digitonin and UDPNAG activated bilirubin glucuronidation by an average factor of 4.5 and 3 (Table 1) and *p*-nitrophenol glucuronidation by 6.7 and 6 respectively in control rats (Table 2). Maximal activation by digitonin was reached at a concentration of 0.9–1.5 mg/ml; for UDPNAG this value was 1.5 mM. At this amount of digitonin glucagon produced a dose-related increase of transferase activity of 120% (bilirubin) and 145% (*p*-nitrophenol) at 150 μg/hr/100 g body wt (Tables 1 and 2). Treatment with VIP did not modify the glucuronidation of bilirubin but led to a dose-dependent increase up to 135% of *p*-nitrophenol glucuronidation (Tables 1 and 2). Treatment with secretin augmented the conjugation of bilirubin but not that of *p*-nitrophenol. Comparable results were obtained with UDPNAG activated preparations. The apparent selectivity of these gastrointestinal polypeptides in enhancing glucuronyltransferase activity towards bilirubin or *p*-nitrophenol might be related to (1) the known functional heterogeneity of the glucuronyltransferases in the rat [3–8, 20], (2) the differing physicochemical properties of the substrates used, which influence their permeation of the endoplasmic reticulum membrane to gain access to the catalytic enzyme, or (3) stimulation of different possible intracellular mediators of the enzymes such as calcium or cyclic AMP.

The gastrointestinal hormones investigated are released in blood after food intake. Hypothetically, one could envisage that these hormones activate the liver for a digestive or detoxifying role just as they stimulate other organs such as the pancreas or the intestine. To really appreciate the physiological relevance of the observed increases in enzyme activity one should have more data on the postprandial concentrations in the portal vein. In addition, further investigation of other enzyme activities such as cytochrome P450 [14] would be rewarding. To evaluate whether phase I and II enzyme reactions become activated, direct studies during feeding might be appropriate but seem cumbersome.

The highest amounts of glucagon used in the present study are 15–45-fold higher than those previously used to demonstrate its choleretic effect in rats [21], but they are below the dosage originally used to document the enhance-

Table 1. Hepatic bilirubin uridine diphosphate-glucuronyl transferase activities: effect of the treatment of rats with various gastro-intestinal hormones

	N	Liver preparations		
		Non-activated	Activated with digitonin 1.5 mg/ml	Activated with UDPNAG 1.5 mM
Untreated controls	18	0.064 ± 0.010	0.28 ± 0.02	0.18 ± 0.01
Pretreatment with:				
Glucagon: 1 μg	5	0.065 ± 0.007	0.25 ± 0.02	0.18 ± 0.01
25 μg	4	0.082 ± 0.019	0.28 ± 0.02	0.20 ± 0.01*
50 μg	5	0.077 ± 0.011	0.33 ± 0.03*	0.21 ± 0.02*
150 μg	5	0.089 ± 0.012*	0.34 ± 0.02*	0.19 ± 0.04
Secretin:0.1 cu	4	0.067 ± 0.010	0.27 ± 0.02	0.18 ± 0.01
0.2 cu	4	0.069 ± 0.011	0.30 ± 0.01*	0.20 ± 0.03
0.4 cu	5	0.081 ± 0.011	0.32 ± 0.02*	0.19 ± 0.02
0.8 cu	5	0.084 ± 0.014	0.33 ± 0.02*	0.20 ± 0.03
V.I.P.: 50 ng	4	0.073 ± 0.008	0.27 ± 0.02	0.15 ± 0.02
150 ng	5	0.074 ± 0.009	0.26 ± 0.02	0.15 ± 0.01
300 ng	5	0.071 ± 0.010	0.27 ± 0.03	0.13 ± 0.02
1000 ng	3	0.083 ± 0.012	0.26 ± 0.02	0.16 ± 0.02

The amount of the different polypeptides is given per hour/100 g body wt; they were administered for 90 min.

Mean values ± SD are given in nmol/min/mg protein.

* Difference from controls at $P < 0.05$ level.

ment of bilirubin UDP-glucuronyltransferase activity [13]. The 300 ng of VIP was equal to that given in dogs to demonstrate a choleretic response [12].

In summary, UDP-glucuronyltransferase activity towards bilirubin and *p*-nitrophenol was assayed in liver preparations obtained from rats pretreated for 90 min with an intravenous infusion of saline, or of various dosages of glucagon, VIP or secretin. Pretreatment with glucagon (1–150 μg/hr/100 g body wt) produced a dose-related increase of bilirubin and *p*-nitrophenol glucuronidation. VIP (50–1000 ng/hr/100 g body wt) only enhanced *p*-nitrophenol glucuronidation whereas secretin (0.1–0.8 CU/hr/100 g body wt) stimulated only bilirubin glucuronyltransferase. These results seem a further argument in favour of a functional heterogeneity of rat liver glucuronyltransferases, and confirm previous observations about the effect on G.I.

Table 2. Effect of gastrointestinal hormones on hepatic *p*-nitrophenol UDP-glucuronyltransferase (UDP-GT)

		N	Liver preparations		
			Non-activated	Activated with digitonin 1.5 mg/ml	Activated with UDPNAG 1.5 mM
Untreated controls		18	1.05 ± 0.27	7.10 ± 1.32	6.28 ± 0.68
Pretreatment with:					
Glucagon:	1 μg	5	1.37 ± 0.20	8.73 ± 0.69	6.64 ± 0.66
	25 μg	4	1.56 ± 0.23	9.43 ± 0.78	7.32 ± 0.61*
	50 μg	5	1.62 ± 0.24*	9.58 ± 0.91	7.83 ± 0.52*
	150 μg	5	1.47 ± 0.17	10.34 ± 1.53	8.49 ± 0.59*
Secretin:	0.1 cu	4	1.02 ± 0.18	6.89 ± 0.67	6.39 ± 0.44
	0.2 cu	4	1.00 ± 0.11	7.22 ± 0.82	6.16 ± 0.52
	0.4 cu	5	1.07 ± 0.18	7.31 ± 0.69	5.87 ± 0.39
	0.8 cu	5	0.97 ± 0.14	6.78 ± 0.45	5.69 ± 0.56
V.I.P.:	50 ng	4	0.95 ± 0.17	8.91 ± 0.83*	8.33 ± 0.88*
	150 ng	5	1.12 ± 0.27	10.03 ± 0.91*	8.52 ± 0.79*
	300 ng	5	1.22 ± 0.16	11.68 ± 0.89*	9.03 ± 0.81*
	1000 ng	3	1.35 ± 0.15	11.98 ± 1.09*	8.54 ± 0.74*

The amount of the polypeptides is given per hour/100 g body wt. and was administered over 90 min.

Mean values ± SD are given in nmol/min/mg protein.

* Difference from controls at $P < 0.05$ level.

hormones on hepatic enzymes involved in drug metabolism. The present data also suggest that the three polypeptides although belonging to the same hormone "family", interact via different intracellular mediators to produce the observed effects on the transferases.

Acknowledgements—Authors are indebted to R. Michiels, M. Crets and V. Baldassarre for help and to K.P.M. Heirwegh for stimulating discussions. The work was supported by the Foundation for Scientific Medical Research of Belgium.

*Gastroenterologia Clinica Medica 2
Policlinico Umberto I
00161 Rome, Italy
†Laboratory of Hepatology
Catholic University of Leuven
B-3000 Leuven, Belgium

G. L. RICCI*
J. FEVERY†

REFERENCES

1. Dutton GJ, *Glucuronidation of Drugs and Other Compounds*. CRC Press, Boca Raton, FL, 1980.
2. Okolicsanyi L, Frei F, Magnenat P and Naccarato R, Multiplicity and specificity of UDP-glucuronyltransferase. *Enzyme* **12**:658–673, 1973.
3. Bock KW, Josting D, Lilienblum W and Pfeil H, Purification of rat liver microsomal UDP-glucuronyltransferase. Separation of two enzyme forms inducible by 3-methyl-cholanthrene or phenobarbital. *Eur J Biochem* **98**:19–26, 1979.
4. Wishart GJ and Campbell MT, Demonstration of two functionally heterogeneous groups within the activities of UDP-glucuronosyltransferase towards a series of 4-alkyl-substituted phenols. *Biochem J* **178**:443–447, 1979.
5. Onishi S, Kawade N, Itoh S, Isobe K and Sugujama S, Postnatal development of uridine diphosphate glucuronyltransferase activity towards bilirubin and 2-aminophenol in human liver. *Biochem J* **184**:705–707, 1979.
6. Burchell B, Isolation and purification of bilirubin UDP-glucuronyltransferase from rat liver. *FEBS Letters* **111**:131–135, 1980.
7. Falamy CN and Tephly TR, Separation purification and characterization of three isoenzymes of UDP-glucuronyltransferase from rat liver microsomes. *Arch Biochem Biophys* **227**:248–258, 1983.
8. Matern H, Matern S and Gerok W, Isolation and characterization of rat liver microsomal UDP-glucuronyltransferase activity toward chenodeoxycholic acid and testosterone as a single form of enzyme. *J Biol Chem* **257**:7422–7429, 1982.
9. Thulin L, Effects of gastro-intestinal polypeptides on hepatic blood flow and splanchnic circulation. *Acta Chir Scand Suppl.* **441**:8–31, 1973.
10. Ricci GL, Michiels R, Fevery J and De Groote J, Enhancement by secretin of the apparently maximal hepatic transport of bilirubin in the rat. *Hepatology* **4**:651–657, 1984.
11. Ricci GL and Fevery J, The action of VIP on bile secretion and bile acid output in the non-anaesthetized Rat, *Biochem Pharmacol.* **34**:3765–3767, 1985.
12. Østergaard Thomsen O and Lersen JA, The effect of glucagon, dibutyrylic cyclicAMP and insulin on bile production in the intact rat and the perfused rat liver. *Acta Physiol Scand* **111**:23–30, 1981.
13. Constantinopoulos A and Matsaniotis N, Augmentation of uridine diphosphate glucuronyltransferase activity in rat liver by adenosine 3′, 5′-monophosphate. *Gastroenterology* **75**:486–491, 1978.
14. Ricci GL and Fevery J, Stimulation by secretin of bilirubin UDP-glycosyltransferase activities and of cytochrome P-450 concentration in rat liver. *Biochem J* **182**:881–884, 1979.
15. Said SI and Mutt V, Polypeptide with broad biological activity: isolation from small intestine. *Science* **169**:1217–1218, 1970.
16. Cooper B, Eakins MN and Slater TF, The effect of various anaesthetic techniques on the flow rate, constituents and enzymic composition of rat bile. *Biochem Pharmacol* **25**:1711–1718, 1976.
17. Heirwegh KPM, Van de Vijver M and Fevery J, Assay and properties of digitonin-activated bilirubin uridine diphosphate glucuronyltransferase from rat liver. *Biochem J* **129**:605–618, 1972.
18. Zakim D and Vessey DA, Techniques for the characterization of UDP-glucuronosyltransferase, glucose-6-phosphatase, and other tightly-bound microsomal enzymes. *Meth Biochem Anal* **21**:1–37, 1973.
19. Bensadouse and Weinstein D, Assay of proteins in the presence of interfering materials. *Anal. Biochem* **70**:241–250, 1976.
20. Wishart GL, Functional heterogeneity of UDP-glucuronyltransferase as indicated by its differential development and inducibility by glucocorticoids. *Biochem J* **174**:485–489, 1978.
21. Thulin L and Hellgren M, Choleretic effect of vasoactive intestinal peptide. *Acta Chir Scand* **142**:235–237, 1976.

Biochemical Pharmacology, Vol. 37, No. 18, pp. 3528–3531, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Ethanol modulation of rat alveolar macrophage superoxide production

(*Received* 6 *October* 1987; *accepted* 4 *March* 1988)

The production of superoxide (O_2^-) by phagocytic cells, such as the alveolar macrophage, is involved in both bacterial killing and inflammation [1]. Mechanisms involved with the limitation of O_2^- production are, therefore, of interest for two reasons: excessive production of extracellular O_2^- is potentially toxic to normal tissue; and factors that reduce the level of O_2^- production could reduce antibacterial effectiveness [2]. Stimulation of O_2^- production has been proposed to involve phosphatidylinositol turnover, intracellular Ca^{2+} mobilization and activation of protein kinase C [3, 4], although there is some debate about which processes are necessary or sufficient [5, 6]. Effects of long chain alcohols on neutrophils have been reported [7]; however, the effects of ethanol on alveolar macrophage O_2^- production have not been examined previously. In this investigation, we explored the observations that ethanol acted both as a weak agonist for O_2^- production and, more strikingly, as an inhibitor of O_2^- production, produced by