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# Stimulation by Secretin of Bilirubin UDP-Glycosyltransferase Activities and of Cytochrome *P*-450 Concentration in Rat Liver

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The activity of bilirubin UDP-glucuronyltransferase in liver tissue was increased 1.5-fold after 90 min of secretion administration (4 i.u./h per kg body wt.) in anaesthetized Wistar rats biopsied half-hourly over a period of 2 h. In unanaesthetized R/A Wistar rats, activities of liver enzymes were assayed after administration secretin for 1 h. Bilirubin UDP-glycosyltransferase activities and cytochrome *P*-450 concentration were increased, but *p*-nitrophenol UDP-glucuronyltransferase and UDP-glucose dehydrogenase activities remained unchanged.

In several animal species, the effects on the liver of administration of the gastrointestinal polypeptide hormone secretin have been studied mainly with regard to bile flow. Minimal choleretic changes have been reported for rat liver, and this has been assumed to represent a general poor response of the organ to the hormone (Debray *et al.*, 1962; Forker *et al.*, 1967; Forker, 1977). No effort has been made to investigate its effects on the hepatocyte or on bilirubin secretion.

During our work on the effects of secretin it became evident that the overall hepatic transport of bilirubin was increased (G. L. Ricci & J. Fevery, unpublished work). Therefore we decided to investigate the influence of secretin on hepatic bilirubin UDP-glycosyltransferase activities and on potentially related components of the detoxification chain.

## Materials and Methods

### *Treatment of animals*

Normal male Wistar-R rats and inbred R/A rats weighing 250–350 g were left without food but with free access to water the night before the operation. The jugular vein was cannulated under pentobarbital anaesthesia (60 mg/kg body wt.), and a continuous intravenous infusion of 5% (w/v) glucose in 0.16 M-NaCl was given. Body temperature was kept at 37.5°C.

Two different sets of experiments were planned.

*Serial biopsy technique.* To study the time necessary for any effect of secretin to be seen, several liver specimens were taken from the same animal. The animals were kept under anaesthesia on a thermostatically controlled plate throughout the whole experiment. The abdomen was re-opened 30–45 min after the cannulation. A control sample was obtained by excising a small cylinder of liver tissue by using a cylindrical device inserted deeply into one of the lobes supported on the finger of the operator. The device was made by sharpening the edge of a piece of plastic tubing (4 mm internal diam.). The biopsy samples, weighing 45–110 mg, were immediately washed in ice-cold 0.25 M-sucrose containing 1 mM-EDTA, wiped with filter paper placed in glass tubes and frozen at −50°C in acetone/solid $CO_2$. Bleeding at the site of biopsy was minimal, and a blood clot completely filling the puncture site was promptly observed. The abdominal cavity was closed by stitches with metal thread. Immediately after this biopsy ('zero'-time sample), an infusion of secretin (Boots Co., Nottingham, U.K.) corresponding to 4 i.u./h per kg body wt. was given for 2 h to three rats. After 30, 60, 90 and 120 min, the stitches were removed and another fragment of liver was obtained. In two rats an additional dose of pentobarbital had to be given (5 mg intraperitoneally). The overall bleeding, as estimated by examination of the peritoneal cavity at the end of the experiments, was less than 1.5 ml, and all animals survived. In three other rats secretin was given intraperitoneally and they received the glucose/saline mixture intravenously. Controls ($n = 3$) received only glucose in saline and were biopsied at the same time intervals.

*Studies with non-anaesthetized Wistar R/A rats.* In order to define more quantitatively the changes induced by secretin, animals were operated on under the conditions mentioned above and kept in restraining cages for 18 h. During the recovery period they received intravenous glucose in saline. Rats were divided into two groups: (*a*) controls ($n = 6$), which received only glucose in saline, and (*b*) test animals ($n = 6$), which received in addition pure natural pig secretin, 4 i.u./h per kg body wt. (batch no. 17831; G.I.H. Research Unit, Karolinska Institutet, Stockholm, Sweden). Experiments were done in parallel, with three test rats and three controls being used at the same time. Animals were all killed within 15 min after a 60 min infusion of secretin. Livers were quickly perfused with chilled 0.16 M-KCl and excised; kidneys were removed as well and stored at −20°C.

*Handling of liver homogenates*

In the first series, the biopsy samples were detached from the glass tube, weighed while still frozen, chopped with a scalpel blade and homogenized in a hand-operated glass homogenizer with 9 vol. of 0.25 M-sucrose containing 1 mM-EDTA. The tubes were left for 15–20 min on ice, and parts of the homogenate were transferred to other tubes for glucuronyltransferase assay and protein determination. In the second series, after the liver had been weighed and rinsed, a homogenate (250 mg/ml) was prepared in 0.25 M-sucrose containing 1 mM-EDTA. Part of this was diluted with a suspension of digitonin in sucrose/EDTA solution to a final concentration of 50 mg of liver/ml, with a digitonin/protein ratio of 3.2:1 After 30 min incubation at 0°C the fully activated mixtures were used (Fevery *et al.*, 1972) for assay of bilirubin UDP-glycosyltransferase and *p*-nitrophenol UDP-glycosyltransferase activities. Another part of the liver homogenate was centrifuged for 10 min at $9000g_{av.}$ in a refrigerated Beckman model L-50 ultracentrifuge. The supernatant fraction was diluted 1.5-fold with 0.16 M-KCl and centrifuged for 1 h at $105000g_{av.}$. The final supernatant was used for assay of UDP-glucose dehydrogenase activity. Cytochrome *P*-450 concentration in the microsomal fraction was determined after adjustment of the protein content to an optimal concentration of 1 mg/ml.

*Experiments performed in vitro*

These were done to test whether the presence of secretin itself in the medium could account for the changes seen. Both types of secretin were added in concentration of 0–0.32 i.u./g of liver (homogenate) and of 0–$3.5\times10^{-3}$ i.u./mg of microsomal protein. The preparations were incubated for 1 h at 0 and 37°C. For glucuronyltransferase, mixtures were assayed both with and without digitonin activation. In the latter group, secretin was incubated before digitonin in one set and after digitonin activation in another set of experiments.

*Assay methods*

UDP-glycosyltransferase activities were assayed with bilirubin as acceptor substance, by methods previously described (Heirwegh *et al.*, 1972; Fevery *et al.*, 1972). In the kidneys, only the cortical part was used and in the incubation time was 30 min. The assay of *p*-nitrophenol UDP-glucuronyltransferase was a modification of the methods described by Mulder (1970) and Zakim & Vessey (1973). Incubation mixtures (1 ml) contained the following components (final concns.): Tris/HCl buffer, pH 7.4 (0.05 M-HCl), 1.6 mM-*p*-nitrophenol, 7.5 mM-$MgCl_2$ and digitonin-activated liver homogenate (1–1.5 mg of protein). After pre-incubation for 1 min at 37°C the reaction was started by addition of 2 μmol of UDP-glucuronic acid. After 1 and 7 min incubation, 100 μl of the solution was transferred to tubes containing 2 ml of ice-cold 4% (w/v) trichloroacetic acid. After brief centrifugation, 1 ml of the supernatant solution was made alkaline with 0.05 ml of 10 M-KOH and the absorbance read at 400 nm. Assuming that the decrease in absorbance was due exclusively to conjugation of *p*-nitrophenol, the concentration

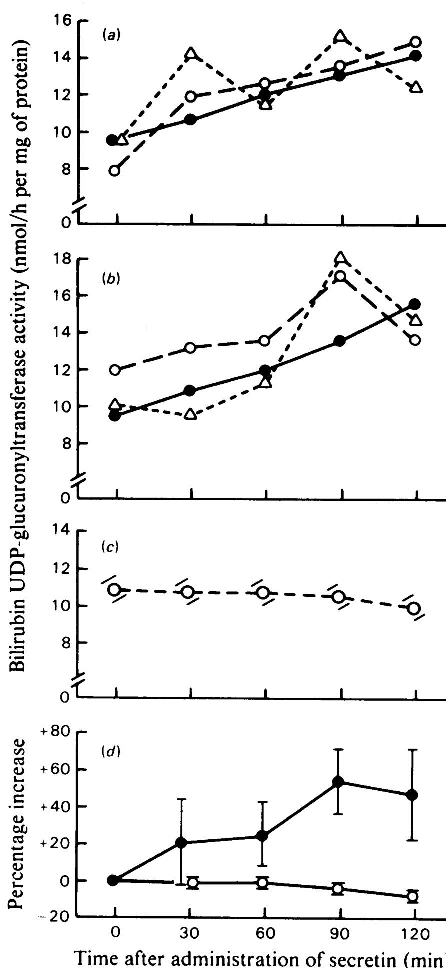


Fig. 1. *Stimulation of bilirubin UDP-glucuronyltransferase activity by secretin in anaesthetized rats*

Bilirubin UDP-glucuronyltransferase activity in digitonin-activated rat liver homogenates is given before and after 30, 60, 90 and 120 min of intravenous (*a*) or intraperitoneal (*b*) administration of secretin (4 i.u./h per kg body wt.); individual results for three rats are shown. Control rats (*c*) received glucose in saline; the values for three rats were all within the areas of the points shown. Panel (*d*) shows the percentage increases in different groups (means ± S.D.): ●, secretin-treated; ○, control.

of glucuronide formed was calculated from $\varepsilon = 18.1\ \text{litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

The cytochrome *P*-450 assay method was essentially that described by Omura & Sato (1964), but the pH adopted was 7.4 {0.1 M-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] adjusted to pH 7.4} and all solutions were adjusted to the same protein concentration (1 mg/ml). A double-beam Beckman model 25 spectrophotometer was used to record the differential spectra. The activity of UDP-glucose dehydrogenase was obtained by recording at 340 nm the initial rate of formation of NADH in 1 ml of incubation mixture containing the following components (final concns.): glycine/NaOH buffer, pH 8.7 (0.1 M-NaOH), 1 mM-$NAD^{+}$ and 0.5–1 mg of cytosol protein (Gainey & Phelps, 1972). In addition, 5 mM-$MgCl_2$ was present in the test sample and 12.5 mM-EDTA in the blank, as a striking dependence on $Mg^{2+}$ had been noted (G. L. Ricci & J. Fevery, unpublished work). Reagents were preheated for 2 min at 30°C and the reaction was started by adding 1 $\mu$mol of UDP-glucose to the test cuvette.

All results are given as means ± 1 S.D.

## Results

In anaesthetized rats secretin infused either intraperitoneally or intravenously produced a significant increase of bilirubin UDP-glucuronyltransferase activity after 90 and 120 min of treatment (Fig. 1). As compared with the activities in the initial pre-infusion samples, the percentage increases were 54.7 ± 17.9% ($n = 6$) at 90 min ($P < 0.005$) and 47.4 ± 25.1% at 120 min ($P < 0.01$). Intravenous infusion of glucose/NaCl mixture without secretin had no effect. More complete investigations were done with two series of six non-anaesthetized rats (Table 1). After 60 min of secretin infusion the activities of UDP-glucuronyltransferase, UDP-glucosyltransferase and UDP-xylosyltransferase (assays with bilirubin as the acceptor substrate) were increased by 28.7 ± 3.3%, 33 ± 10% and 22 ± 4% respectively, as compared with the control animals. The relative proportions of the three bilirubin transferase activities, 1:0.20:0.26, were identical for secretin-treated and control animals. The activity of *p*-nitrophenol UDP-glucuronyltransferase was unchanged. Cytochrome *P*-450 concentration was also increased by 27.1 ± 9.7% ($n = 5$) in the secretin-treated group. In contrast, the activity of the cytosol enzyme UDP-glucose dehydrogenase was unaltered. In the kidney cortex bilirubin UDP-glucuronyltransferase activity was 3.09 ± 0.39 nmol of bilirubin conjugated/h per g of liver for controls ($n = 6$) and 3.01 ± 0.44 in the secretin-treated group ($n = 6$). No significant changes were detected in UDP-glucuronyltransferase activity or cytochrome *P*-450 concentration when secretin was added *in vitro* to the homogenate or to the microsomal preparation.

## Discussion

Secretin, a gastrointestinal hormone released after ingestion of a meal, stimulates the pancreatic and bile-duct secretion of water and bicarbonates (Chenderovitch, 1976; Johnson, 1977). It is noteworthy that in certain species a change in bile flow and bilirubin output was found after feeding (Strasberg *et al.*, 1974; Austin *et al.*, 1978; Merle *et al.*, 1978) We have now demonstrated a stimulation of bilirubin conjugation in rat liver after administration of secretin (4 i.u./h per kg body wt.).

With bilirubin as acceptor substrate, parallel increases in the transfer ratio of glucuronyl, glucosyl and xylosyl residues were noted after treatment with secretin. In contrast, no significant change was found with *p*-nitrophenol. This different behaviour suggests specificity of the effect of secretin for different acceptor substrates and adds to the recently documented

Table 1. *Enzyme pattern in liver and kidney in unanaesthetized R/A rats after intravenous administration of secretin*
Secretin (4 i.u./h per kg body wt.) was given for 60 min; controls received only glucose in saline. Experimental details are given in the text. UDP-glycosyltransferase activities with bilirubin are expressed in nmol/h per g of protein, and with *p*-nitrophenol in nmol/10 min per mg of protein. UDP-glucose dehydrogenase activity is given in nmol/min per mg of cytosol protein, and cytochrome *P*-450 concentration in nmol/mg of microsomal protein. Means ± S.D. are given. Abbreviation: n.s., not significant.

| | Liver | | | | | | Kidney |
|---|---|---|---|---|---|---|---|
| | With bilirubin | | | With *p*-nitrophenol | | | With bilirubin |
| | UDP-glucuronyltransferase | UDP-glucosyltransferase | UDP-xylosyltransferase | UDP-glucuronyltransferase | UDP-glucose dehydrogenase | Cytochrome *P*-450 | UDP-glucuronyltransferase |
| Controls (6) | 19.14 ± 1.54 | 4.10 ± 0.30 | 5.80 ± 0.21 | 304 ± 45 | 2.64 ± 0.68 | 56.8 ± 10.1 | 3.09 ± 0.39 |
| Proportions ... | 1 : | 0.20 : | 0.28 | | | | |
| Treated (6) | 24.23 ± 2.15 | 5.44 ± 0.55 | 7.07 ± 0.29 | 301 ± 58 | 2.39 ± 0.69 | 72.2 ± 7.0 (5) | 3.01 ± 0.44 |
| Proportions ... | 1 : | 0.20 : | 0.26 | | | | |
| *P* values ... | < 0.001 | < 0.01 | < 0.005 | n.s. | n.s. | < 0.02 | n.s. |

heterogeneity of UDP-glucuronyltransferases (Wishart, 1978). However, specificity with regard to the sugar donors was not apparent. In the experiments where biopsy samples were obtained from rats kept under anaesthesia the activities of bilirubin UDP-glucuronyltransferase were lower than when the enzyme was assayed on liver homogenates obtained from rats fully recovered from anaesthesia. We attribute this to: (*a*) possible depressive effects of anaesthesia (Cooper *et al.*, 1976) and (*b*) the presence of blood protein in the biopsies. Ischaemia, which may have occurred to some extent, might also decrease enzyme activity. Bilirubin UDP-glucuronyltransferase activity was unchanged in the kidney cortex of treated rats, suggesting that the stimulation is specific for the liver. UDP-glucose dehydrogenase was assayed because its activity is essential for synthesis of activated glucuronic acid. No change was noted in the activity of this cytosol enzyme.

An increase of the concentration of another microsomal component, cytochrome *P*-450, was also observed. As this protein is involved in hydroxylation of drugs and endogenous substances, one could presume that this increase represents an adaptation to metabolism of secretin itself. However, secretin probably does not enter the liver cells, but, as described for other hormones, only hits and runs away (Curtis *et al.*, 1976). This raises the question of how the effects observed are mediated. Several gastrointestinal hormones increase the concentration of cyclic AMP in the liver and other organs (Desbuquois *et al.*, 1973; Bataille *et al.*, 1974). Among these, secretin, glucagon and vasoactive intestinal polypeptide share several amino acid sequences. It has been reported that in the rat glucagon also increased hepatic bilirubin UDP-glucuronyltransferase activity, presumably mediated by cyclic AMP, as dibutyryl cyclic AMP had a similar effect (Constantinopoulos & Matsaniotis, 1978). This is not yet known for secretin, but the fact that secretin added *in vitro* was ineffective seems to support further the hypothesis that mediating substances are released *in vivo*. Secretin itself could well be a poly-releaser of other hormones, but at least in man at a dosage of 0.5–2 i.u./h per kg body wt. it did not change the glucagon concentration in peripheral blood (Shima *et al.*, 1978).

The dosage of secretin used in our work somewhat exceeded the amount assumed to give a physiological response in man but was 5–10-fold lower than dosages previously used in the rat and the dog (Wheeler & Mancusi-Ungaro, 1966; O'Maille *et al.*, 1966; Forker *et al.*, 1967). For man, Häcki *et al.* (1977) reported that 0.3–0.4 i.u./h per kg body wt. elicited a pancreatic bicarbonate secretion of 50% of the maximal output. However, we are dealing here with an increase in enzyme activities, produced in a relatively short time, in an animal and in an organ traditionally thought to be poorly sensitive to secretin. It has still to be assessed in terms of normal physiology whether the effects observed are physiological or pharmacological. Secretin released from the duodenum is first delivered to the liver via the portal vein, and only later to the pancreas via the general circulation. Presumably, some process(es) in the liver become stimulated by gastrointestinal hormones, and this may represent an important step in controlling the metabolism of food products and endogenous compounds by enzymes of the endoplasmic reticulum.

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