Biochemical Pharmacology, Vol. 42, No. 3, pp. 469–473, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

CONJUGATION OF 1-NAPHTHOL IN THE GASTRIC MUCOSA OF GUINEA PIGS

MICHAEL SCHWENK,*† CHRISTA LINZ* and A.-B. KOBUSCH‡
*Abteilung Allgemeine Pharmakologie, Medizinische Hochschule Hannover, Konstanty-Gutschow Str. 8, D 3000 Hannover 61, Germany; and ‡Institut für Toxikologie, Universität Tübingen, Wilhelmstr. 56, D 7400 Tübingen, Germany

(*Received* 23 *August* 1990; *accepted* 16 *February* 1991)

Abstract—1-Naphthol was conjugated in tissue pieces of the gastric wall to naphthol glucuronide (85%) and naphthol sulfate (15%). There was no regioselectivity in different parts of the stomach. In separated gastric mucosal cell populations, the activities of both transferases were highest in the mucous cell fraction (apparent V_{max} of glucuronidation: 0.83 nmol/mg protein/min; apparent V_{max} of sulfation: 0.11) with only little, or no activity in chief cells and parietal cells. Immunohistochemically glucuronosyltransferase 1 was predominantly localized in surface mucous cells. In conclusion, the gastric mucosa is an important organ for phase II biotransformation.

The gastrointestinal mucosa has a considerable capacity to conjugate drugs and foreign compounds with sulfate and glucuronic acid [1]. The conjugation reactions have been intensively studied in the intestine with regard to enzyme activities, localization of the enzymes within the villus-crypt axis, and effects on pharmacokinetics (c.f. Ref. 2). However, there is at present only very little information on conjugation reactions in the stomach. The major data come from Hartiala and co-workers [1, 3–6] who measured glucuronosyltransferase activities. They showed that *o*-aminophenol is glucuronidated in tissue slices of different parts of the stomach and *p*-nitrophenol is efficiently glucuronidated in gastric mucosal homogenate of different species. But there is no information about the cell type responsible for gastric glucuronidation and about the efficiency of gastric drug sulfation.

We, therefore, studied gastric mucosal glucuronidation and sulfation in more detail, using tissue preparations and isolated gastric mucosal cells from the guinea pig. We selected 1-naphthol as substrate, which is a commonly used model compound for glucuronosyltransferase as well as sulfotransferase reactions in the gastrointestinal tract [2, 7].

MATERIALS AND METHODS

Chemicals. 1-[1-^{14}C]Naphthol (2.0 GBq/mmol) was obtained from the Radiochemical Center (Amersham, U.K.). Collagenase type I (*Clostridium histolyticum*) was from the Sigma Chemical Co. (St Louis, MO, U.S.A.), collagenase (*Clostridium histolyticum*) from Serva (Heidelberg) and pronase E from Merck (Darmstadt). Minimum essential medium with Earl's salts (MEM) was from Sigma. Silica gel G thin layer foils and all standard chemicals were obtained at the highest purity from Merck.

Animals and pretreatment. Male guinea pigs (250–280 g), obtained from Hagemann (Extertal, F.R.G.), were kept in a 12 hr day and night rhythm and received a commercially available diet (Altromin®) and water *ad lib.*

Preparation of tissue pieces and isolated cells. Animals were killed with a blow on the neck. The abdominal cavity was opened and the stomach was excised and opened along the major curvature. Food residues were removed under a flow of saline. Tissue pieces were prepared by excising segments of about 30 mg wet weight from the gastric wall. Gastric mucosal cells were isolated according to the method described by Soll [8] as modified by Sewing *et al.* [9]. The whole mucosa, except small areas of about 1 cm^2 around the cardia and the pylorus, was scraped off and passed through a metal sieve. After centrifugation (2 min, 100 *g*), the sediment was incubated in 40 mL of isolation medium (70 mM NaCl, 20 mM Na_2CO_3, 1.5 mM Na_2HPO_4, 5 mM KCl, 1.5 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM glucose) supplemented with 50 mM HEPES, 1.2 mg collagenase I (Sigma) and 16 mg collagenase (Serva) at 37° under a stream of carbogen while stirring. The pH was kept at 7.4 by repeated addition of a solution of 5% Na_2CO_3. After 10 min the material was centrifuged (2 min, 100 *g*) and the mucosal fragments in the sediment were resuspended in 40 mL of the collagenase-containing isolation medium, additionally supplemented with 10 mg pronase E and 0.1% bovine serum albumin, and incubated at pH 7.4 under a stream of carbogen while stirring. Incubation was stopped when microscopic control showed complete separation into isolated cells (about 70 min). The suspension was filtered through nylon cloth (mesh width 180 μm) and washed twice by centrifugation (5 min, 300 *g*). Cells were then resuspended in 5 mL of isolation medium, containing 25 mM HEPES (pH 7.4) and 0.1% bovine serum albumin, and separated according to cell size by zonal centrifugation in the Beckman elutriation system. Applying four different flow rates (5, 10, 15

† To whom correspondence should be addressed.

Table 1. Four fractions of separated gastric mucosal cells

Fraction	Mean diameter* (μm)	Yield† (10^6 cells)	Prevalent cell type	Purity‡ (%)
1	5.1	10	Endothelial cells	80
2	11.5	60	Mucous cells	70
3	12.5	40	Chief cells	55
4	21.6	20	Parietal cells	80

* Measured by resistance changes in capillary.
† Average yield from one guinea pig.
‡ Percentage of prevalent cell type in each fraction.

and 38 mL/min) at constant sedimentation velocity (240 *g*), four fractions with elutriation volumes of 100, 250, 350 and 200 mL were collected, each containing an enriched cell population (Table 1).

Cell diameter was measured by electrical resistance changes in a capillary (Casy II, Schärfe System, Reutlingen, F.R.G.). Viability of each fraction was checked with regard to oxygen consumption and trypan blue exclusion, and cells were only used if viability was >90% (trypan blue). Endothelial cells were identified by positive immunofluorescence with vimentin and ultrastructural morphology. The amount of mucous cells in each fraction was assessed by *N*-acetyl-[^{14}C]glucosamin incorporation, the amount of chief cells by acid-catalysed protease activity (pepsinogen/pepsin) [10] and the amount of parietal cells by histamine-stimulated uptake of radiolabeled aminopyrine [9].

Incubations. Tissue pieces (30 mg wet weight) or isolated cells (1 mg protein) were incubated in 2 mL MEM (containing 0.1 mM cystein and 1 mM sulfate), supplemented with 20 mM HEPES (pH 7.4) at 37° while shaking. After 10 min preincubation, 5 μL of a 30% ethanolic solution of 1-[1-^{14}C]naphthol was added (tissue pieces: 500,000 cpm, 6 μmol/L; isolated cells; 50,000–500,000 cpm, 0.8–106 μmol/L. In the case of tissue pieces, 200 μL of supernatant were withdrawn after 5, 10 and 20 min, then pieces were immediately homogenized in the presence of 200 μL ice-cold MEM. In the case of isolated cells, 200 μL of cell suspensions were withdrawn at various times.

Analysis of naphthol conjugates. The samples of 200 μL were transferred to 400 μL of a mixture of methanol and chloroform (3:1). After shaking 3 min, another 600 μL of chloroform was added, samples were centrifuged and 20 μL of each phase were used for determination of total conjugates by liquid scintillation counting. Fifty microlitres of the aqueous phases were applied to silica gel thin layer chromatography foils and 1-naphthol glucuronide was separated from 1-naphthol sulfate in a solvent system, consisting of *n*-butanol:0.01 M Tris: propionic acid (75:14:1.1). The R_f values were 0.26 for 1-naphthol glucuronide and 0.69 for 1-naphthol sulfate [7]. The radioactive bands were detected in a radioactivity scanner (Rita 90, Raytest, Straubenhardt, F.R.G.).

Immunolocalization of glucuronosyltransferase. Cryostate sections from the gastric wall were incubated with a rabbit anti-glucuronosyltransferase 1 antibody (kindly supplied by K. W. Bock, Tübingen) and successively exposed to peroxidase-conjugated anti-rabbit antibodies to visualize cell types containing glucuronosyltransferase as described by Fischer *et al.* [11]. Specificity of the reaction was checked by immunoplot analysis of UDPGT polypeptides [11, 12] in microsomes, prepared from each of the four cell fractions.

Protein was determined with the Biuret method. Experiments were performed in duplicates with five to six different preparations.

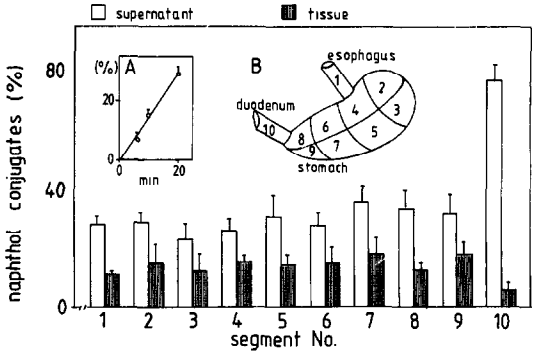

Fig. 1. 1-Naphthol conjugation in different parts of the stomach. Tissue pieces (30 mg wet weight), obtained from different regions of the stomach and the neighbouring areas of the esophagus and duodenum, were incubated with [^{14}C]naphthol (6 μM). The bars show conjugates (expressed in per cent of added naphthol) in the medium and in homogenized tissue after 20 min. Insert A: Time course of conjugates in the supernatant of tissue piece 1. Insert B: Regions from where the tissue pieces have been taken. Values are the means of 5 independent preparations (±SD).

RESULTS

Naphthol was conjugated by tissue pieces of the gastric wall at a constant rate for at least 20 min (Fig. 1A). If the mucosa was scraped off and only the muscularis and serosa were incubated, no conjugation was detectable. Gastric wall pieces from different areas of the stomach and from the esophagus exhibited similar conjugation rates (Fig. 1), amounting to about 40% of the rates in the duodenal slices. At the end of the experiment (20 min), gastric pieces had released 60% and duodenal pieces 91%

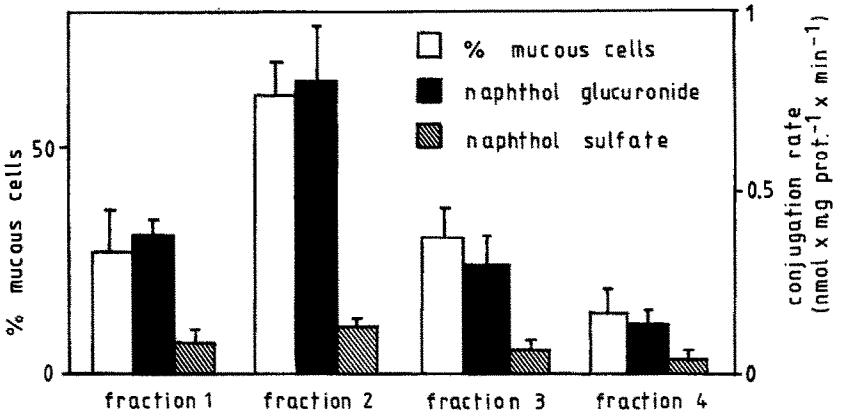

Fig. 2. Glucuronidation and sulfation in different cell fractions. Four gastric cell populations were incubated with [^{14}C]naphthol (30 μM) for 5 min. Bars show the portion of mucous cells in each fraction, and turnover rates for naphthol glucuronidation and sulfation (N = 5 ± SD).

of the conjugates into the medium. Thin layer chromatography of the gastric samples identified 82–87% of the conjugates as glucuronide and 13–18% as sulfate conjugates.

Separated gastric mucosal cells were employed to see if the conjugating activity is associated with a peculiar cell type of the gastric mucosa. Cells produced naphthol glucuronide and sulfate in the same proportions, as tissue pieces. Fraction 2 (enriched with mucous cells) exhibited the highest activities for both reactions (Fig. 2). Fraction 1 (enriched with endothelial cells), fraction 3 (mixed cells) and fraction 4 (enriched parietal cells) exhibited lower conjugating activities. In all fractions, the activities were proportional to the respective content of mucous cells.

To get information about the histological localization of the glucuronidating cell type, cryostate sections were prepared from the corpus region of the gastric wall. The sections were exposed to antibodies against glucuronosyltransferase I and subsequently to peroxidase-conjugated anti-antibodies. The whole lining of mucous surface cells, as well as isolated clusters of cells deeper in the glands, showed a positive reaction (Fig. 3). Control tissue, treated only with the second antibody, was negative. Immunoblot analysis of glucuronosyltransferase I in microsomes, isolated from each of the four cell fractions, showed the typical bands in the 54,000 Dalton region with highest intensity in cell fraction 2.

To study conjugation kinetics in mucous cells in more detail, experiments were made with different 1-naphthol concentrations in cell fraction 2 (Fig. 4). At all concentrations, glucuronidation was the prevailing reaction. There were no shifts of glucuronidation to sulfation ratios during 60 min incubations. Initial rates, determined from the slopes of the curves after 1 min of incubation, were used to construct the inserted Lineweaver–Burk plots. The apparent kinetic constants for glucuronidation were $K_m = 6.2$ (μM) and $V_{max} = 0.83$ (nmol/mg protein/min), those for sulfation were $K_m = 5.2$ (μM) and $V_{max} = 0.11$ (nmol/mg protein/min).

DISCUSSION

In contrast to other extrahepatic organs, such as the intestine, lungs and the kidneys, the stomach has been widely ignored as a drug metabolizing organ, probably as a consequence of the findings of various investigators, including our own with aminopyrine and ethoxycumarin as substrates (unpublished), that monooxygenase-dependent drug metabolism was very slow or even below the limits of detection [5, 6]. This blocked further interest in gastric biotransformation reactions although a pronounced glucuronosyltransferase activity had already been described 35 years ago [3]. We performed the present study in order to get more detailed information about the phase II reactions of the stomach, using 1-naphthol, which is glucuronidated in UDPGA-supplemented microsomal systems, and glucuronidated and sulfated in isolated cells and tissue preparations of various organs [2, 7], with no evidence for formation of other metabolites at a relevant rate.

The present data support earlier findings with tissue slices and mucosal homogenate [3–6], that the gastric mucosa exhibits glucuronosyltransferase activity. They also demonstrate considerable sulfotransferase activity.

The glucuronosyltransferase and sulfotransferase activities were confined to the gastric mucosa and not detectable in the muscularis or serosa. Mucous cells were identified as the predominant cell type for sulfation and glucuronidation, but minor activities in the other cell types cannot be excluded due to the contamination of cell fractions 1, 3 and 4 with mucous cells. The outstanding role of mucous cells for gastric mucosal phase II biotransformation was verified by immunohistochemical localization of glucuronosyltransferase. The enzyme was predominantly localized in mucous surface cells, and to a smaller extent in scattered cells, deeper in the gastric glands. The latter might represent mucous neck cells, or a mixed pepsinogen/mucous cell type which has been described in the guinea pig [13]. The confinement of glucuronosyltransferase to individual

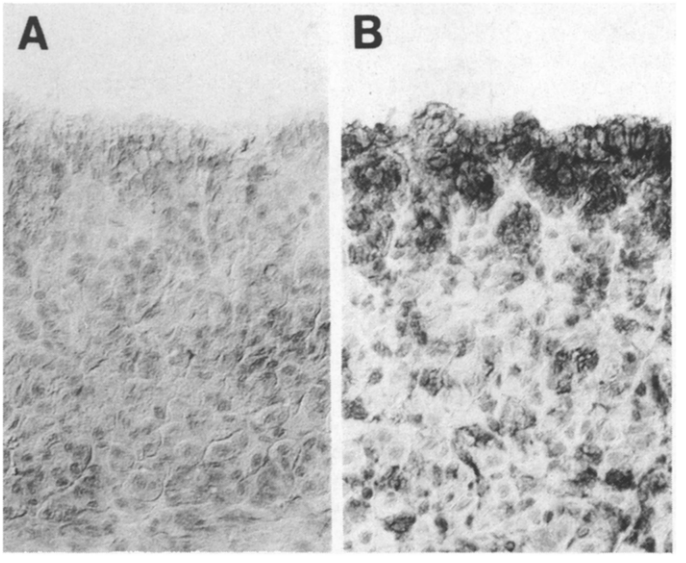

Fig. 3. Immunolocalization of UDP-glucuronosyltransferase. Freeze sections from the gastric mucosa were incubated with buffer (A = control) or with anti-glucuronosyltransferase 1 antibodies (B) and successively exposed to peroxidase-conjugated anti-rabbit antibodies. Positive reaction is mainly localized in surface mucous cells. Most cells in the gastric glands are negative.

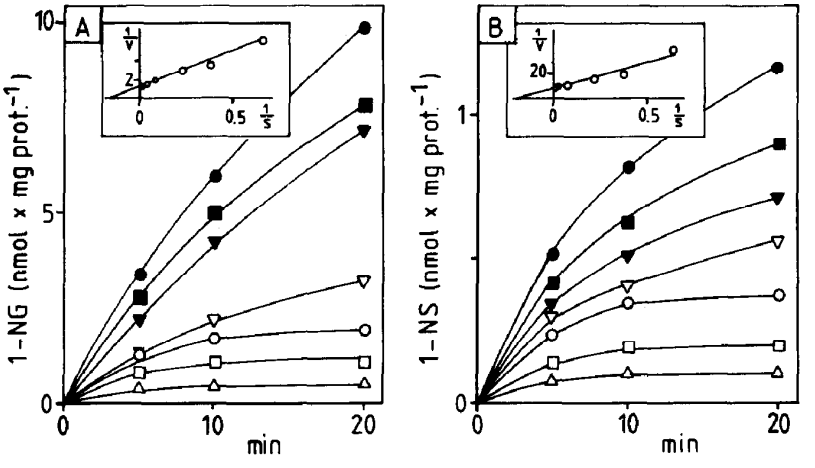

Fig. 4. Kinetics of 1-naphthol conjugation in separated mucous cells. [^{14}C]Naphthol was added to suspensions of enriched mucous cells (1 mg protein/2 mL MEM) at the following concentrations: (△) 0.8 μM, (□) 1.6 μM, (○) 2.6 μM, (▽) 4.6 μM, (▼) 12.4 μM, (■) 31.4 μM, (●) 106 μM. Graph A: naphthol glucuronide (NG); graph B: naphthol sulfate (NS). The inserts show the respective Lineweaver–Burk plots; v is expressed as nmol/mg protein/min and s is expressed as μM naphthol. The graphs show mean values of duplicate determinations in one experiment; the coefficients of variation in six independent experiments were <0.22.

cell types in epithelial tissues has also been observed in liver, intestine and kidney [14]. In the case of the stomach, one may consider the localization in the mucosal surface as the first line of defence against intruding foreign compounds.

The lack of a regioselectivity of 1-naphthol conjugation suggests that all gastric mucous cells exhibit similar conjugating enzyme activities, independent of their localization in the corpus-pylorus or major curvature–minor curvature axis. Comparison with data from an earlier work [7] shows that the maximal velocity of glucuronidation (apparent V_{max} value) in gastric mucous cells is comparable to that in isolated hepatocytes, and the maximal velocity of sulfation amounts to about 30% of the rate in hepatocytes. Thus, gastric mucous cells belong to the major 1-naphthol conjugating cell type in this species, in accordance with the findings of Aitio *et al.* [5].

In conclusion, the data show that the gastric mucosa

is a major organ for extrahepatic glucuronidation and sulfation. The enzymes are predominantly localized in the surface mucous cells. Further studies are required to elucidate the isoenzyme patterns in these cells and to investigate the effect of the gastric first pass biotransformation on the bioavailability of drugs.

Acknowledgement—The authors wish to thank Prof. Bock for providing the antibody against glucuronosyltransferase I, and Mrs A. Bauer and Mrs I. Hackbarth for excellent technical assistance.

REFERENCES

1. Hartiala KJ, Metabolism of hormones, drugs and other substances by the gut. *Physiol Rev* **53**: 496–534, 1973.
2. Schwenk M, Glucuronidation and sulfation in the gastrointestinal tract. *Prog Pharmacol Clin Pharmacol* **7**: 156–169, 1989.
3. Hartiala KJ, Studies on detoxication mechanism III. Glucuronide synthesis of various organs with special reference to the detoxifying capacity of the mucous membrane of the alimentary canal. *Ann Med Exptl Biol Fenniae* **33**: 240–245, 1955.
4. Hänninen O, Aitio A and Hartiala K, Gastrointestinal distribution of glucuronide synthesis and the relevant enzymes in the rat. *Scand J Gastroenterol* **3**: 461–464, 1968.
5. Aitio A, Hietanen E and Hänninen O, Mucosal drug metabolism and drug-induced ulcer. In: *Experimental Ulcer* (Ed. Gheorghiu T), pp. 17–21. G. Witzstock, Baden-Baden, 1975.
6. Hietanen E, Mucosal and hepatic metabolism during the spontaneous disappearance of salicylate-induced gastric erosions. *Am J Digest Diseases* **20**: 31–37, 1975.
7. Schwenk M and Locher M, 1-Naphthol conjugation in isolated cells from liver, jejunum, ileum, colon and kidney of the guinea pig. *Biochem Pharmacol* **34**: 697–701, 1985.
8. Soll AH, The action of secretagogues on oxygen uptake by isolated mammalian parietal cells. *J Clin Invest* **61**: 370–380, 1978.
9. Sewing K-Fr, Harms P, Schluz G and Hannemann H, Effect of substituted benzimidazoles on acid secretion in isolated and enriched guinea pig parietal cells. *Gut* **24**: 557–560, 1983.
10. Raufman J-P, Sutliff VE, Kasbekar DK, Jensen RT and Gardner JD, Pepsinogen secretion from dispersed chief cells from guinea pig stomach. *Am J Physiol* **247**: G95–G104, 1984.
11. Fischer G, Ullrich D and Bock KW, Effects of *N*-nitrosomorpholine and phenobarbital on UDP-glucuronosyltransferase in putative preneoplastic foci of rat liver. *Carcinogenesis* **6**: 605–609, 1985.
12. Bock KW, Schirmer G, Green MD and Tephly TR, Properties of a 3-methylcholanthrene-inducible phenol UDP-glucuronosyltransferase from rat liver. *Biochem Pharmacol* **37**: 1439–1443, 1988.
13. Bouhours D, Bouhours J-F and Bryon P-A, Association of glycoproteins and pepsinogen in the secretory granules of fundic epithelial cells isolated from guinea pig stomach. *Biochim Biophys Acta* **672**: 288–296, 1981.
14. Chowdhury JR, Novikoff PM, Chowdhury NR, Novikoff AB, Distribution of UDPglucuronosyltransferase in rat tissue. *Proc Natl Acad Sci USA* **82**: 2990–2994, 1985.