Excretion of Sulfobromophthalein in Rats with Iodomethane-Induced Depletion of Hepatic Glutathione

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Summary. Male urethane-anesthetized Wistar rats with biliary fistulas were infused for 60 min i.v. with sulfobromophthalein (BSP) or BSP-glutathione conjugate (BSP-GSH) at 594 nmol/100 g/min. Thirty minutes prior to the start of the infusion, 20 mg/kg iodosmethane, dissolved in olive oil, was given into the duodenum. The control received oil only. At the start of the infusion the hepatic concentration of GSH was 0.96 ± 0.23 mg/g liver in the iodosmethane-treated animals versus 1.93 ± 0.13 mg/g liver in the control (P < 0.001).

When unconjugated BSP was infused, the excretion of total BSP (unconjugated plus conjugated) was markedly lower in the iodosmethane-treated group than in the control. This difference was due solely to differences in biliary appearing conjugated BSP; the excretion of unconjugated BSP was identical in both groups. The different excretion patterns were paralleled by equal hepatic accumulation of total BSP in both groups. The ratio of unconjugated BSP/BSP-GSH in the liver was about twice as high after pretreatment with iodosmethane than in the control group.

When BSP-GSH instead of BSP was infused, the excretion rates of this dye were identical in both groups. The maximal transport capacity (Tm) was double that observed with infusion of unconjugated BSP in control animals. There is indirect evidence that BSP and BSP-GSH might have different excretion pathways.

Key words: Biliary excretion - BSP - Glutathione-conjugation capacity.

INTRODUCTION

The way of BSP from plasma to bile involves several steps: hepatic uptake and storage, conjugation with glutathione, and excretion into bile. Although the conjugation step is not a prerequisite for the excretion, recent investigations from this laboratory (Schulze and Czok, 1974, 1975) indicate that it may be the rate limiting step for the overall transport of BSP from blood to bile: When BSP was infused at 594 nmol/100 g/min in rats during 100 min, the resulting excretion of total BSP (i.e. unconjugated plus conjugated) first increased up to 20–30 min and subsequently declined almost linearly (Schulze and Czok, 1975). The course of the curve was determined mainly by the excretion of BSP-GSH. Contrarily, the amounts of biliary appearing unconjugated BSP were much smaller and varied within a narrow range. Although the biliary excretion of BSP-GSH declined with time, the hepatic concentration of this metabolite concurrently rose. Since the hepatic concentration of unconjugated BSP increased in parallel, it was concluded that the unconjugated dye inhibited BSP-GSH from being excreted and that the rate of conjugation was insufficient to prevent the accumulation of the unconjugated fraction from surpassing a critical value in the liver. It was further postulated that the conjugation step facilitated the excretion of BSP firstly by supplying BSP-GSH which is more easily excreted than BSP and secondly by lowering the hepatic concentration of unconjugated BSP which inhibits the excretion of BSP-GSH.

Although these conclusions fitted the experimental data, it seemed worthy of exploration whether they could be substantiated by results obtained from similar experiments on rats with reduced hepatic glutathione after pretreatment with iodosmethane.

Particularly, it was of interest to us, to what extent the hepatic accumulation of conjugated and unconjugated BSP in animals with impaired conjugation capacity would differ from that in control animals and whether these differences could be related to differences in the excretion pattern of the dye. Furthermore, we hoped to get more information about the type of inhibition displayed by unconjugated BSP...
on the biliary excretion of BSP-GSH. None of these questions has been dealt with in the thorough investigations of Priestly and Plaa (1970), the first to examine the excretion of BSP after the depletion of hepatic glutathione with iodomethane. According to these authors, the substance seems to have no influence upon the S-aryltransferase which catalyzes the conversion of BSP to BSP-GSH.

**METHODS**

**Animals.** Male Wistar rats were used throughout. The animals were maintained on Altromin® standard diet and tap water ad libitum.

**Surgical Procedure and GSH-Depletion.** After anesthetizing the rats with 1 g/kg ethylurethane i.p., the left jugular vein and the bile duct were cannulated with PVC P2 and P1 tubing, respectively. The body temperature was maintained at 37°C by a heating pad controlled via a thermostat. Iodomethane was given intraduodenally at a dosage of 20 mg/kg via a catheter 30 min after the ending of the surgical procedure and 30 min prior to the start of the infusion. The substance was freshly prepared by dissolving it in olive oil (2%, v/v). Iodomethane is conjugated to GSH (Johnson, 1965, 1965a, b) and thereby reduces the hepatic concentration of the monoothiol.

**Infusion of BSP and BSP-GSH.** Sulfobromophthalein disodium salt (BSP) or the BSP glutathione conjugate (BSP-GSH) was dissolved in 0.2 M tris-HCl (pH 7.4) and infused at a rate of 594 nmol/100 g/min.

**Analytical Methods.** The glutathione (GSH) content of the liver was determined by the method of Grunert and Phillips (1951). Presuming the specific gravity to be 1, the bile flow was determined gravimetrically every 10 min.

The BSP concentration in the bile and in the serum were measured colorimetrically at 578 nm after an appropriate dilution of the samples with alkalized sodium chloride solution (100 ml of 0.9% NaCl plus 5 ml of 10% NaOH). Biliary appearing BSP and metabolites of BSP were separated by thin-layer chromatography (10 µl bile on Merck® precoated silica gel 60-254, layer thickness 0.25 mm; solvent: tertiary butylic alcohol/distilled water, 3:1) (Sardini et al., 1969). BSP or its metabolites were eluted with alkaline sodium chloride and measured at 578 nm. The extinction coefficients of unconjugated and conjugated BSP were assumed to be identical (Combes, 1965; Whelan et al., 1970). According to Whelan et al. (1970), besides unconjugated BSP (BSP) two metabolite fractions were assumed: BSP-glutathione including traces of BSP-diglutathione (BSP-GSH) and BSP-cysteinylglycine plus BSP-cysteine (BSP-CG).

To estimate the hepatic content of BSP and metabolites of BSP 6 animals each, depleted and non-depleted, were sacrificed after 20, 40 and 60 min of infusion with BSP. A modification of the technique of Whelan et al. (1970) and Whelan and Combes (1971) was employed as described elsewhere (Schulze and Czok, 1975).

**Statistical Analysis.** The results are expressed as means ± S.E. of at least 6 animals. The statistical significance of the differences between means was determined by Student's t-test for independent means.

**RESULTS**

To elucidate the influence of the intraduodenal application of iodomethane on the hepatic concentration of GSH, one group pretreated with iodomethane (n = 6) and another group given olive oil (n = 6) were sacrificed at the moment when otherwise the infusion with BSP or BSP-GSH was started. In contrast to the oil-treated group, which displayed a GSH concentration of 1.93 ± 0.13 mg/g liver, the average concentration in the animals treated with iodomethane was reduced to 0.96 ± 0.23 mg/g liver (P < 0.001).

Figure 1a illustrates the bile flow in dependence on the different (pre)treatments. In the group pretreated with olive oil the bile flow during the subsequent infusion with BSP was higher throughout than in that given oil plus iodomethane (P < 0.05 30–60 min after starting the BSP infusion). When the solvent tris-HCl was infused in iodomethane-pre-treated rats, the bile flow remained constant.

When BSP-GSH instead of BSP was infused (Fig. 1b), the pretreatment with iodomethane led to no significant reduction in bile flow. Compared with BSP, the infusion of BSP-GSH produced a much greater choleresis. As seen from Figures 2a and b, during infusion with BSP the biliary concentration of BSP total (unconjugated plus conjugated) is much smaller after pretreatment with iodomethane (Fig. 2b) than after the application of olive oil (Fig. 2a) (P < 0.05 from 10–40 min of BSP infusion). Likewise, the hepatic concentration of BSP-GSH in the iodomethane-pre-treated group was below that of the control group (P < 0.005–0.001 from 10–60 min). Contrarily, in iodomethane-treated animals, the biliary concentration of unconjugated BSP ranged above that in the control group (P < 0.01 from 20–60 min). No significant differences between both groups were observed in the biliary concentrations of BSP-CG. When BSP-GSH was infused (Fig. 2c), the biliary concentration of this dye amounted to 24–25 nmol/µl and then reached a plateau; the concentrations were equal in GSH-depleted and non-depleted animals (P < 0.05).

Figures 3a and b depict the excretion of BSP and metabolites of BSP. Owing to the differences in bile flow and the biliary concentration of the dye, the excretion in the GSH-depleted group (Fig. 3b) was markedly less than that of the non-depleted group (Fig. 3a). This applied to the excretion of BSP total (conjugated plus unconjugated) (P < 0.05 from 10–60 min) and to the excretion of BSP-GSH as well (P < 0.01 from 10–60 min). Contrarily, the excretion of unconjugated BSP did not differ significantly in both groups. When BSP-GSH instead of BSP was infused, the resulting excretion of this dye (Fig. 3c) was statistically identical in GSH-depleted and non-depleted animals.