The role of small intestine and kidney in bromosulphthalein conjugation

D. V. DATTA, SARBAN SINGH, P. N. CHHUTTANI AND C. R. NAIR
Division of Hepatic Diseases and Experimental Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

(Received 17 June 1976)

Summary

1. The roles of the kidney and the small intestine in the conjugation of bromosulphthalein have been compared with that of the liver.

2. Studies with homogenates indicated that the liver has a higher bromosulphthalein-glutathione-conjugating activity than the intestine and kidney. The reduced glutathione content of the liver is greater than that of the small intestine and kidneys, which contain comparable amounts.

3. Experiments in vitro with tissue slices confirmed that the three organs are able to extract significant amounts of bromosulphthalein. In carbon tetrachloride-induced hepatic necrosis the uptake and conjugation of the dye by the liver were reduced but were increased in the kidney and unchanged in the small intestine.

4. After an intravenous injection of bromosulphthalein, the percentages conjugated in the liver, kidney and gut were similar.

Key words: bromosulphthalein, conjugation, kidney, small intestine.

Introduction

The conversion of bromosulphthalein into conjugated bromosulphthalein in vivo has been well documented. This transformation involves the bromosulphthalein-glutathione (BSP-GSH)-conjugating enzyme (Combes & Stakelum, 1961) or glutathione-S-aryltransferase (Grover & Sims, 1964). Studies in our laboratory have shown that BSP-GSH\(^{11}\)-conjugating activity in human liver tissue is markedly reduced in acute and chronic liver diseases (Datta, Singh, Samata, Saha, Mukherjee, Nirankari, Chhuttani & Nair, 1973c). Nevertheless, conjugated bromosulphthalein appears in the plasma of patients with liver disease after its infusion (Carbone, Grodsky & Hjelte, 1959). This could arise after conjugation from the liver or from extrahepatic conjugation. We have demonstrated that tissue homogenates of kidney and gastrointestinal tract possess significant amounts of bromosulphthalein-conjugating enzyme (Datta, Singh & Chhuttani, 1973a) and, in this paper, the role of these tissues in the overall conjugation of bromosulphthalein is reported.

Material and methods

Adult male guinea-pigs (300–500 g) from the stock colony of the Institute animal house were used in the experiments. Three different studies were carried out.

In the first experiment, fed animals were killed under ether anaesthesia. The liver, kidney and small intestine were excised and rapidly chilled in ice. Weighed portions of these organs were cut into small pieces and homogenized in ice-cold pyrophosphate buffer (0·1 mol/l; pH 8·5; Boyland & Chasseaud, 1968). Homogenates of liver (1%, w/v) and kidney (2·5%, w/v) were prepared in a Potter-
Elvehjem homogenizer with Teflon pestle. For the intestinal homogenate, the small intestine was flushed with ice-cold sodium chloride solution (150 mmol/l; saline) and divided into three equal portions. The portion next to the stomach was cleaned with ice-cold saline, and an homogenate (2.5%, w/v) was prepared in pyrophosphate buffer as described above. The tissue homogenates were centrifuged at 30000 g for 50 min at 0°C. The supernatant fraction was used as the enzyme source as preliminary studies had shown that nearly 90% of the activity was confined to this fraction (Datta, Singh & Chhuttani, 1973b).

The BSP-GSH-conjugating enzyme activity was estimated by the method of Datta et al. (1973). It has been shown that the enzyme activity in the different segments of the intestine is identical. Zero-order kinetics were observed for the small intestine, kidney and liver extracts. The reduced glutathione content in each tissue was measured by the method of Ball (1966). The tissue proteins were estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

In the second set of investigations, liver and kidney slices (1 g) were prepared by the method of Cohen (1957) and intestinal segments were cut from the proximal end of the small intestine. The slices and intestinal segments were weighed and transferred immediately into a flask containing 4 ml of isotonic Krebs–Henseleit phosphate buffer, pH 7.4 (Umbreit, Burris & Stauffer, 1957), to which 1.2 mg of bromosulphthalein was added. No reduced glutathione was added to the incubation system. The flasks were thoroughly shaken every 5 min for 45 min in a water bath at 37°C, with air as gas phase. The enzyme activity of the tissues was stopped by the addition of saturated ammonium sulphate solution (0.36 ml) to the medium; the slices were then repeatedly rinsed with buffer and homogenized in 5 vol. of ethanol and centrifuged at 2500 rev./min. for 15 min. The ethanolic supernatant was concentrated in vacuo.

The total bromosulphthalein in the tissue was estimated by the method of Rosenthal & White (1952) and the results were expressed as μmol of bromosulphthalein/g of slices. The bromosulphthalein conjugates were estimated after chromatographic separation on Whatman no. 4 filter paper (Combes & Stakelum, 1961). The results were expressed as pmol of bromosulphthalein conjugated/}

### Table 1. Bromosulphthalein-glutathione-conjugating enzyme activity and reduced glutathione content of tissue homogenates from guinea-pigs

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Liver</th>
<th>Kidney</th>
<th>Intestine</th>
<th>Liver</th>
<th>Kidney</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.210±0.065</td>
<td>0.026±0.003</td>
<td>0.036±0.019</td>
<td>2.55±0.38</td>
<td>1.50±0.40</td>
<td>1.61±0.67</td>
</tr>
</tbody>
</table>

Results are expressed as mean values±sd. BSP = bromosulphthalein.

### Table 2. Total and conjugated bromosulphthalein in slices of guinea-pig liver, kidney and intestine

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Total BSP (μmol/g)</th>
<th>Conjugated BSP (μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>1</td>
<td>0.226</td>
<td>0.215</td>
</tr>
<tr>
<td>2</td>
<td>0.215</td>
<td>0.238</td>
</tr>
<tr>
<td>3</td>
<td>0.263</td>
<td>0.263</td>
</tr>
<tr>
<td>4</td>
<td>0.263</td>
<td>0.273</td>
</tr>
<tr>
<td>5</td>
<td>0.298</td>
<td>0.238</td>
</tr>
<tr>
<td>6</td>
<td>0.298</td>
<td>0.263</td>
</tr>
<tr>
<td>Mean</td>
<td>0.263</td>
<td>0.250</td>
</tr>
<tr>
<td>±sd</td>
<td>0.034</td>
<td>0.022</td>
</tr>
</tbody>
</table>
of slices. All the tissues were weighed immediately in a single-pan electrical balance.

In studies in vitro, the animals received carbon tetrachloride (1 ml/kg) in mineral oil (1:9, v/v) by the intraperitoneal route. After 24 h they received an intravenous injection of bromosulphthalein (5 mg/kg). The volume injected ranged from 0.2 to 0.3 ml. Preliminary studies showed that the dose of carbon tetrachloride used was sufficient to induce histologically demonstrable acute hepatic necrosis. The control animals received a similar volume of mineral oil without carbon tetrachloride. Exactly 10 min after the administration of bromosulphthalein, blood was withdrawn from the aorta, and the liver, kidney and intestine were removed and analysed for total and conjugated bromosulphthalein and reduced glutathione as in the earlier experiments. Statistical analysis was performed according to Fisher & Yates (1967); all results are expressed as mean values ± 1 SD.

Results

Table 1 shows that the enzyme activities of homogenates of kidney and intestine are of the same order and are approximately one-seventh of that of the liver. The reduced glutathione present in both the kidney and intestine is more than one-half that in the liver so that the amounts of endogenous substrate present in these tissues are more than adequate for conjugation of bromosulphthalein and the reduced enzyme activity cannot be explained by a deficiency of substrate.

The slice experiments were conducted in order to study the bromosulphthalein-conjugating efficiency of intact liver, kidney and intestine cells (Table 2). The mean uptakes of bromosulphthalein by liver, kidney and intestine after 45 min of incubation were 0.263 ± 0.034, 0.250 ± 0.022 and 0.263 ± 0.029 mol/g of tissue respectively. The mean values for bromosulphthalein conjugates in liver, kidney and intestine were 0.198 ± 0.042, 0.095 ± 0.030 and 0.107 ± 0.031 μmol/g of slices respectively, indicating that the capacities of the kidney and intestine for conjugation were approximately equal but were only half that of the liver.

Table 3 gives the results of the studies in vivo. Carbon tetrachloride administration produced a significant elevation of plasma bromosulphthalein when compared with control values (P<0.01) and a small rise in conjugated bromosulphthalein (P<
0.01). This treatment also caused a decrease in hepatic uptake ($P < 0.01$) and an increase in renal uptake ($P < 0.01$), although the total bromosulphthalein content in the intestinal tissues remained unchanged. Similarly the conjugated bromosulphthalein content of the liver was significantly reduced as the result of treatment with carbon tetrachloride, and was significantly increased in the kidney, although there was no change in the intestine.

Discussion

The present study has demonstrated that both reduced glutathione and the BSP-GSH-conjugating enzyme are present in the kidney and small intestine although in smaller concentrations than in the liver. All these organs therefore have the ability to conjugate bromosulphthalein.

The liver actively participates in the clearance of bromosulphthalein from the circulation but there has been no good evidence that extrahepatic conjugation is normally involved. Rosennau, Carbone & Grodsky (1959) detected significant amounts of conjugated bromosulphthalein in the serum of hepatectomized dogs but failed to observe any conjugate in the serum of animals that had been hepatectomized and nephrectomized. This suggested that the kidney was able to take up and conjugate bromosulphthalein in the absence of the liver. The present experiments provide support for this hypothesis since it has been shown that the kidney and intestine as well as the liver can take up bromosulphthalein in vitro and in vivo and that these extrahepatic tissues have BSP-GSH-conjugating activity, although it is only half of that in the liver.

From the results of the carbon tetrachloride experiments it is possible to speculate that in acute hepatic damage, when the liver fails to clear the dye efficiently from the circulation, the retention of bromosulphthalein in the plasma results in an increased uptake of the dye by the kidney and intestine and synthesis of conjugated dye occurs. In addition, there is regurgitation of conjugated bromosulphthalein formed in the liver into the plasma, and the dye is excreted in the urine.

In these investigations the results for uptake of bromosulphthalein have been expressed per g of tissue (Table 3). The roles of the different tissues are, however, dependent on the actual weights of the whole organ. Since the relative proportions by weight of the liver, kidney and intestine in the normal animal are approximately 7:2:5, it is apparent that the liver is the most important organ. In cirrhosis, the weight of the liver will be reduced and as the uptake by the kidney has increased it will obviously play a more important part in the metabolism of bromosulphthalein than normal. The role of the intestine is unchanged; nevertheless, it would appear to be involved. These studies throw some doubt on the generally accepted view that clearance of bromosulphthalein is a specific test of hepatic function.

Acknowledgment

The authors are grateful to Professors Billing and Dewan, and others who gave editorial advice.

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