EFFECT OF ANAESTHETIC AGENTS ON BILE FLOW AND BILIARY EXCRETION OF $^{131}$I-CHOLYLGLYCYLTYROSINE IN THE RAT†

C. O. MILLS, J. F. FREEMAN, P. J. SALT AND E. ELIAS

Anaesthetic agents used in animal research and clinically in patients may affect hepatobiliary function. Such agents used commonly include pentobarbitone, a barbiturate which has been used widely in hepatobiliary studies in the belief that it does not affect bile formation in the bile fistula rat [1—3]. This view was challenged recently [4]. Paumgartner and colleagues [5] have reported that another oxybarbiturate, phenobarbitone, increased bile flow in the rat, whilst Klaassen [6] reported that pentobarbitone may not be suitable for studies investigating the biliary excretion of drugs because its metabolism produces polar metabolites which compete for the hepatic transport of the drugs under investigation. Similarly, it has been reported that pentobarbitone anaesthesia should be used with caution while studying the effect of other agents on the rate of bile secretion [7]. This was confirmed in a study which reported that pentobarbitone diminished the hepatic transport of succinylsulphathiazole into the bile [8]. Other anaesthetic agents unsuitable for studies involving the biliary excretion of drugs include ether, which produced acidosis in the isolated rat liver [9] and chloralose [9].

Because of these conflicting reports we have investigated the effect of anaesthetic agents on bile flow and biliary excretion of $^{131}$I-cholylglycyltyrosine ($^{131}$I-cholylglycyltyr.). This novel bile acid has a very high first pass liver uptake, similar to that of the natural bile acid taurocholate [10], thus providing an index of hepatobiliary transport [11] under different anaesthetic conditions. We
studied Althesin, which is excreted in bile (70%) and urine (30%) [12–14], etomidate (urine (78%) and bile (13%) [15]), pentobarbitone (urine (72%) and bile (28%) [6]; metabolized in the liver before renal excretion [16]) and propofol (Diprivan) (kidneys (88%) and bile (2%) [17]).

MATERIALS AND METHODS

Materials

Pentobarbitone was obtained from May and Baker Ltd, Dagenham, Essex; Diprivan from ICI plc, Pharmaceutical Division, Macclesfield, Cheshire; etomidate from Janssen Pharmaceutical Ltd, Grove, Oxford; and Althesin from Glaxo Ltd, Harefield, Uxbridge. Portex polythene tubing was obtained from A. R. Horwell Ltd, London. 131I-cholylgly.tyr. was synthesized in our laboratory as described elsewhere [10].

Animal studies

Four groups each of six male Wistar rats (220–250 g) were anaesthetized with different anaesthetic induction agents. Pentobarbitone 50 mg kg⁻¹ was given by the intraperitoneal (i.p.) route. The other agents were given i.v. as a bolus injection followed by a constant infusion via a tail vein cannula: etomidate 1-mg bolus over 1 min followed by 2-mg h⁻¹ infusion; Althesin 3-mg bolus, 14.5-mg h⁻¹ infusion; propofol 3.3-mg bolus, 3.3-mg h⁻¹ infusion. After anaesthesia was established in each rat, laparotomy was performed and the common bile duct was cannulated with polythene tubing, after which the abdomen was closed using a suture. All animals were allowed to breathe room air spontaneously and, 1 h after induction of anaesthesia, the jugular vein was exposed and 131I-cholylgly.tyr. 5 µCi was injected in a volume of 200 µl of physiological saline. Bile was collected in pre-weighed tubes every 1 min for 10 min.

The aorta was cannulated using a 14-gauge cannula with injection valve and a Teflon catheter, and aortic blood samples were obtained after 1 h for analysis of acid–base balance and oxygenation using an ABL blood-gas machine (Radiometer, Copenhagen).

Measurements

The radioactive content of each bile aliquot (131I-cholylgly.tyr.) was determined by counting in a SP500 gamma counter and expressed as percentage of the dose administered. Summation of the dose in each tube gave the cumulative percentage of dose in bile in 10 min.

Bile was collected from the biliary fistula of the animals for the time intervals indicated for each experiment. Bile volume was measured and expressed as µl min⁻¹/100 g body weight.

Statistical analysis

All results are expressed as mean (SD). Student’s t test was used to determine the significance of differences between means.

RESULTS

Althesin and propofol produced the highest bile flow rates, which were significantly greater than that seen with pentobarbitone (P < 0.001). There was no significant difference between etomidate and pentobarbitone (fig. 1).

There was no significant difference in the cumulative biliary excretion of 131I-cholylgly.tyr. between etomidate or propofol and pentobarbitone, but excretion was greater in Althesin treated rats (P < 0.01) (fig. 2).

A summary of biliary kinetics of 131I-cholylgly.tyr. is shown in table I. Mean peak excretion rates for the different agents were 12–18% of
injected dose per minute. There was no significant
difference between the values for the different
agents. Peak excretion rates occurred at 3 min for
propofol and etomidate and at 4 min for pen-
tobarbitone and Althesin. The 50% biliary re-
tention time was computed from a linear cor-
relation of the logarithm of the cumulative dose in
bile against different time intervals between 1 and
4 min and extrapolation of the straight line to give
the time for the first 50% of the total biliary
excretion; it occurred between 4 and 5 min for all
agents, with no significant difference between
propofol, etomidate or Althesin and pentobar-
bitone. Only Althesin produced a cumulative
percentage dose in bile in 10 min which was
significantly different from that of pentobarbitone
($P < 0.01$) (table I).

Only propofol was associated with normal acid-
base status and oxygenation. Pentobarbitone ap-
proached normal values, whereas etomidate and
especially Althesin caused a metabolic acidosis
(table II).

### Table I. Biliary kinetics of $^{131}$I-cholylgly.tyr. (mean (SD)). $n = 6$. $*P < 0.01$; $**P < 0.001$

<table>
<thead>
<tr>
<th>Anaesthetic agent</th>
<th>Peak excretion (% dose min$^{-1}$)</th>
<th>Cumulative dose in bile in 10 min (%)</th>
<th>50% Biliary retention (min)</th>
<th>Bile flow ($\mu l$ min$^{-1}$/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentobarbitone</td>
<td>17.0 (1.4)</td>
<td>76.4 (3.2)</td>
<td>4.6 (0.04)</td>
<td>7.3 (0.3)</td>
</tr>
<tr>
<td>Etomidate</td>
<td>12.6 (8.3)</td>
<td>69.4 (17.6)</td>
<td>4.8 (0.05)</td>
<td>8.5 (0.5)</td>
</tr>
<tr>
<td>Propofol</td>
<td>17.6 (2.4)</td>
<td>74.1 (5.2)</td>
<td>4.5 (0.03)</td>
<td>14.1 (0.6)**</td>
</tr>
<tr>
<td>Althesin</td>
<td>18.0 (1.9)</td>
<td>82.3 (2.2)*</td>
<td>4.5 (0.05)</td>
<td>12.5 (0.05)**</td>
</tr>
</tbody>
</table>

### Table II. Mean (SD) acid-base and blood-gas status. $n = 6$ for each agent. BE = Base excess; SBE = standard base excess; SBC = standard base concentration

<table>
<thead>
<tr>
<th></th>
<th>Pentobarbitone</th>
<th>Etomidate</th>
<th>Althesin</th>
<th>Propofol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp. ($^\circ$C)</td>
<td>37.00 (0.50)</td>
<td>37.50 (0.60)</td>
<td>37.00 (0.50)</td>
<td>37.50 (0.50)</td>
</tr>
<tr>
<td>Hb (g dl$^{-1}$)</td>
<td>14.07 (5.38)</td>
<td>16.03 (0.84)</td>
<td>15.87 (1.39)</td>
<td>13.83 (4.00)</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 (0.03)</td>
<td>7.34 (0.07)</td>
<td>7.29 (0.03)</td>
<td>7.37 (0.01)</td>
</tr>
<tr>
<td>$P_{CO_2}$ (kPa)</td>
<td>5.70 (0.84)</td>
<td>5.56 (0.64)</td>
<td>5.38 (0.55)</td>
<td>5.94 (0.07)</td>
</tr>
<tr>
<td>$P_{O_2}$ (kPa)</td>
<td>9.12 (2.20)</td>
<td>8.86 (0.84)</td>
<td>8.24 (0.54)</td>
<td>9.07 (1.21)</td>
</tr>
<tr>
<td>$HCO_3^-$ (mmol litre$^{-1}$)</td>
<td>24.30 (3.30)</td>
<td>21.83 (3.25)</td>
<td>18.73 (0.98)</td>
<td>25.00 (0.64)</td>
</tr>
<tr>
<td>BE (mmol litre$^{-1}$)</td>
<td>-0.70 (2.91)</td>
<td>-3.73 (4.08)</td>
<td>-7.33 (0.74)</td>
<td>0.00</td>
</tr>
<tr>
<td>SBE (mmol litre$^{-1}$)</td>
<td>-0.57 (2.96)</td>
<td>-4.13 (2.80)</td>
<td>-6.80 (0.78)</td>
<td>0.00</td>
</tr>
<tr>
<td>Saturation (%)</td>
<td>89.73 (5.11)</td>
<td>90.10 (0.28)</td>
<td>86.97 (1.45)</td>
<td>91.10 (2.40)</td>
</tr>
<tr>
<td>SBC (mmol litre$^{-1}$)</td>
<td>23.90 (2.38)</td>
<td>21.23 (3.20)</td>
<td>18.27 (0.54)</td>
<td>23.87 (0.63)</td>
</tr>
</tbody>
</table>
We have studied two anaesthetic agents which are excreted mainly via the kidneys [15, 17], one which is excreted mostly by the liver [12–14] and pentobarbitone, which is excreted via both routes [6]. Pentobarbitone was administered i.p., whereas the other agents were given i.v., and this may have resulted in a different pattern of haemodynamic and ventilatory changes in the animals. However, evidence suggests that a similar biochemical profile was produced after administration of pentobarbitone either 50 mg kg⁻¹ i.p. or 35 mg kg⁻¹ i.v. Furthermore, the cumulative biliary excretion within 6 h was similar for either i.v. or i.p. administration of pentobarbitone [6, 7].

We have used the i.p. route because it is that used commonly for anaesthetic induction with pentobarbitone in hepatic transport studies. Althesin, which is known to be excreted in bile, caused a significant increase in bile flow compared with pentobarbitone, an effect which is compatible with an osmotic drag of fluid into bile in response to the presence of the drug. As anticipated for an agent excreted predominantly in urine, etomidate did not cause an increase in bile flow rate compared with pentobarbitone. However, propofol, which in common with etomidate is believed to be excreted mainly via the kidneys, caused a significant increase in bile flow rate relative to pentobarbitone (fig. 1). This propofol-induced choleretic would be compatible with an osmotic choleretic caused by active transport of propofol, its metabolites, or both, from the liver into the lumen of the biliary canaliculi [18, 19], as described for Althesin. Of the agents studied, etomidate and Althesin resulted in a greater deviation from normal acid-base status and oxygenation than did pentobarbitone, whereas propofol maintained normal acid-base status and oxygenation (table II). It was surprising that a diminution in bile flow rate resulting from acidosis which has been reported with ether [20] was not observed with Althesin. The biliary excretion of ¹³¹I-cholylglycine tyr. in rats treated with the different anaesthetic induction agents (fig. 2) demonstrated that the cumulative percentage dose in bile over 10 min was similar for etomidate, propofol and pentobarbitone. However, there was a slight but significant difference between pentobarbitone and Althesin, suggesting that Althesin-induced choleretic may enhance the biliary output of ¹³¹I-cholylglycine tyr., which is a bile salt derivative.

ACKNOWLEDGEMENT

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REFERENCES


