

# Somatostatin inhibits the effect of secretin on bile flow and on hepatic bilirubin transport in the rat

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**SUMMARY** Increasing amounts of porcine secretin (0.05 to 2.00 clinical units/h/100 g body wt) given to rats during a continuous infusion of bilirubin, increased bile flow and the apparent maximal biliary excretion of bilirubin ('T<sub>m</sub>'). This increment was caused by an enhanced biliary output of bilirubin monoconjugates. The effect was dose dependent but maximal at a secretin infusion of 0.80 CU. Somatostatin 0.2 and 0.8 µg/h/100 g body wt caused a dose related inhibition of the hepatic effects of secretin both on bile flow and on biliary output of bilirubin conjugates. As secretin elicits the release of somatostatin, a feed-back system could be envisaged whereby the somatostatin released stops the effects of secretin.

An increasing number of gastrointestinal polypeptide hormones are being documented. A vast amount of information concerning their functions and mode of action on stomach, gut and pancreas is available<sup>1,2</sup> but only a limited number of studies have dealt with hepatic effects,<sup>3-8</sup> although the liver is the first organ placed on the venous effluent of the GI hormone secreting organs.

In the rat, secretin produces a mild but short lasting choleresis.<sup>7</sup> Because secretin as well as other gastrointestinal hormones elicit the release of somatostatin<sup>9</sup> and as the latter was shown to be cholestatic in rat<sup>6</sup> and dog,<sup>10,11</sup> our aim was to test the hypothesis that somatostatin could block or interfere with the hepatic effects of secretin. A type of feed back system could be envisaged whereby the secretin released as a response to feeding, stimulates the secretion of somatostatin which in its turn would end the effects of secretin.

## Methods

### ANIMALS

Male Wistar R-rats weighting 280-340 g were used. Approximately 20 min after anaesthesia with sodium

pentobarbital (6 mg/100 g body wt, ip) the bile duct and the right jugular vein were cannulated (catheters with internal to external diameters of 0.3-0.7 mm and 0.5-0.9 mm respectively, Biotrol Pharma, Paris, France) while the animals were kept on heating plates. Rats were then put in restraining cages and placed in thermostatically controlled boxes for three hours to allow recovery from anaesthesia and to maintain body temperature at 37.1 (0.3) °C; during this period they received 5% glucose in 0.16 M NaCl iv at the rate of 1.2 ml/h/100 g body wt. The jugular catheter was connected by a three way tap to two different perfusers (Braun, Melsungen, Germany) to allow later injection of different solutions. Bile was collected in the dark in preweighed plastic tubes over 10 min periods.

### BILIRUBIN TRANSPORT MAXIMUM ('T<sub>m</sub>')

Unconjugated bilirubin-IX<sub>α</sub> (containing less than 5% of non- $\alpha$  isomers) (Sigma, St Louis, MD) was solubilised in 150 mM NaOH. The final pH of the bilirubin solution was brought to pH 9.5 just before the injection. Three hours after surgical preparation, rats were given a constant infusion of bilirubin at a rate of 256 nmol/min/100 g body wt after a priming dose a 3.42 µmol/100 g body wt to obtain a maximal secretory rate of bile pigments ('T<sub>m</sub>').<sup>12,13</sup> The onset of bilirubin loading is referred to as time zero. In this condition the output of conjugates reaches a steady state from approximately 20 min onwards. The

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output from 40 to 60 min after the beginning of bilirubin loading is taken to represent bilirubin-Tm in the animal under investigation.

#### POLYPEPTIDE INFUSIONS

After measurement of bilirubin-Tm (40–60 min period), while bilirubin was further administered as above, an infusion of secretin (GIH Laboratories, Karolinska Institute, Stockholm, purchased from KabiVitrum, The Netherlands) was administered at the rate of 0.05, 0.2, 0.4, 0.8 or 2.0 CU/h/100 g body wt (clinical units). To study the inhibitory effect of somatostatin on secretin, somatostatin (a gift from Ayerst Benelux, Brussels) 0.2 µg or 0.8 µg/h/100 g body wt was administered respectively together with one of the following rates of secretin: 0.1, 0.8 or 2.0 CU/h/100 g body wt. Polypeptides were dissolved in 0.16 M NaCl containing 0.5% human serum albumin and given at the volume rate of 3.5 ml/h per animal.

#### ANALYTICAL PROCEDURES

The volume of bile secreted per 10 min was weighed. Biliary bilirubin conjugates were assayed by diazo-cleavage with ethylanthranilate at pH 2.7<sup>14</sup> within 30 min of collection of the samples. The composition of bile pigments was analysed by tlc after alkaline methanolysis.<sup>15</sup> Unconjugated bilirubin and its mono- and di-methylesters were quantified either by densitometry (Vitatron, Dieren, The Netherlands) or by scraping the silica gel off the plate and by resuspending the pigments in chloroform:methanol (50:50, vol:vol) to measure optical absorbance at 436 nm.

Table Bile flow before and during the administration of different polypeptides. The measurements were done at bilirubin-Tm: 40–60 min (for the control period) and 90–110 min (for the polypeptides) after the onset of bilirubin infusion

Bile flow: µl/min/100 g body wt			
Secretin: CU	Secretin alone n:	Associated with somatostatin 0.2 µg   n:	Associated with somatostatin 0.8 µg   n:
0 (control) period)	15 5.85 (0.22)	14 5.93 (0.31)	14 5.78 (0.23)
0.10	—	3 6.05 (0.26)	3 5.57 (0.29)
0.20	3 6.24 (0.27)	—	—
0.40	2 6.71 (0.32)†	4 6.28 (0.31)*§	3 5.92 (0.27)§
0.80	3 6.95 (0.38)‡	3 6.53 (0.27)†	4 5.87 (0.30)§
2.00	3 6.89 (0.35)‡	4 7.05 (0.30)‡	4 6.09 (0.20)‡§

Statistical significance was evaluated by a Wilcoxon's test (rank sum): difference from control: \*p<0.05; †p<0.01; ‡p<0.005; difference from secretin alone: §<0.05; ||all doses of polypeptides are per hour/100 g body wt.

Results are given as mean ± 1 standard deviation. Statistical significance was evaluated by a Wilcoxon's rank-sum test for paired data, to compare the basal (Tm) and experimental (somatostatin) period within each animal.

#### RESULTS

Secretin produced a dose related increase of bile flow (Table). The stimulating effect of secretin was maximal at the rate of 0.8 cu/h/100 g body wt. Because the pigment concentration in bile was com-

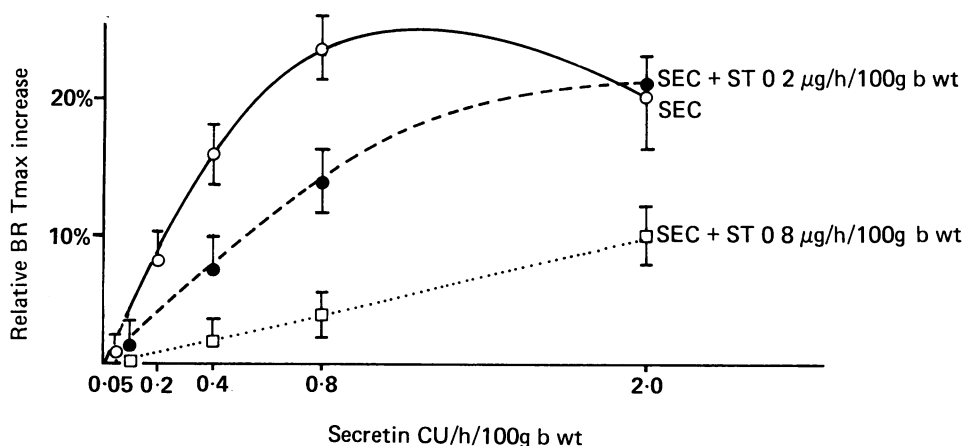


Figure Effect of different infusion rates of secretin, administered alone or together with one of two rates of somatostatin (0.2 µg or 0.8 µg/h/100 g body wt) during an already established bilirubin-Tm. The excess increase in bilirubin output (mean (1 SD); n=2–4 for each point) relative to the 'basal' period of bilirubin-Tm – that is, the bilirubin output at 40–60 min from the beginning of bilirubin loading are given.

parable in all animals studied (mean value  $\pm 1$  SD; 27.5 mM (2.1)), the biliary bilirubin output varied in parallel with the bile flow. The enhancement in bilirubin Tm was due to an increased secretion rate of monoconjugates; their fractional amount rose from 52% (2) in controls to 57% (2) at 0.8 cu of secretin.

Somatostatin interfered in a dose dependent fashion with the stimulatory effects of secretin (Table). The relative increases in bilirubin output produced by secretin without or with somatostatin were plotted as a function of the infusion rate of secretin (Figure). Using somatostatin 0.2  $\mu$ g/h/100 g body wt, higher amounts of secretin were required to obtain the same increase in bilirubin-Tm than with secretin alone. With a higher dose of somatostatin, all rates of secretin failed to reach the level of bilirubin-Tm obtained with secretin alone. Somatostatin thus exerts a dose dependent inhibition on the effects of secretin.

### Discussion

The administration of secretin to rats during a steady, apparent maximal biliary bilirubin output (hepatic transport maximum or 'Tm') leads to an increase of this biliary output. This enhanced level can be the result of an increased bile flow or to enhanced formation of conjugates or to a combination of both. Previous experiments have shown that secretin increases hepatic bilirubin UDP-glucuronyltransferase activity<sup>6</sup> and bilirubin diglucuronide excretion<sup>8</sup> but only when rats have been pretreated for 90–120 min by the polypeptide. On the other hand, in the rat secretin induces a short lasting and not very pronounced enhancement of bile flow,<sup>7</sup> but which is also paralleled by an augmented maximal secretory rate (the present results).

Impurities in the administered secretin preparation or prolonged collection periods<sup>16,17</sup> or the use of rats still under anaesthesia<sup>17</sup> or below physiological body temperature have led to controversies whether secretin is choleric or not in the rat.<sup>16–19</sup> Anaesthesia or low body temperature induce cholestasis<sup>20,21</sup> but our rats, having recovered at least for three hours from the anaesthesia and having a normal body temperature, showed a short lasting choleresis under purified porcine GIH secretin; it was dose related and the maximal increment of bile flow amounted up to 15–20% (Table). This choleresis was paralleled by an enhanced maximal output of bilirubin monoconjugates without any alteration of the diconjugate excretion, in contrast with what is seen after a 90–120 min pretreatment with secretin.<sup>7</sup> The fact that the choleric effect of secretin tended to fade out despite continuation of the polypeptide<sup>7</sup> has led us to examine the hypothesis that secretin might stimulate

release of an inhibitory peptide such as somatostatin. The latter indeed decreases bile flow<sup>10,11</sup> but in itself had no effect on bilirubin-Tm.<sup>22</sup> The present studies demonstrate that somatostatin hampers in a dose dependent fashion the effects exerted by secretin on bile flow and maximal bilirubin output. These data are compatible with our hypothesis that secretin, released in response to feeding, produces an enhanced bile flow but at the same time triggers somatostatin release. The latter then counteracts the choleric effect of secretin. As a result only a short-lasting choleresis is observed after secretin. This hypothesis and its pathophysiological relevance will have to be confirmed by direct measurements of concentrations of somatostatin in the portal vein during secretin infusion or during feeding. From a comparison made with data obtained by others, however, it seems that the presently infused amounts are not out of proportion. In the rat<sup>23</sup> and dog<sup>24</sup> somatostatin concentrations in peripheral blood approximate 0.23–0.30 ng/ml whereas portal vein concentrations are 0.495 ng/ml.<sup>23</sup> An infusion of somatostatin in the dog at a rate of 0.15  $\mu$ g/h/100 g body wt led in the dog to a two-fold increase of the portal vein concentration.<sup>24</sup> In the present experiments rats received a comparable (0.20  $\mu$ g) and a four-fold higher (0.80  $\mu$ g) amount of somatostatin. With regard to the secretin infusion, an amount of 0.03 CU/h/100 g body wt given in man leads to plasma levels as obtained during acidification of the duodenum<sup>25</sup> whereas 0.27 CU seems to lead to a maximal stimulation of pancreatic bicarbonate.<sup>25</sup> For comparison, our rats received amounts of secretin between 0.10 and 2.00 CU with a maximal effect on bile flow at a rate of 0.80 CU. Thus, the dosages presently used appear only slightly supra-physiological.

At this moment it is clear that somatostatin interacts with the hepatic effects of secretin on bile flow and maximal hepatic bilirubin transport. Whether somatostatin would also inhibit the release of secretin in response to feeding, besides having an end organ inhibitory effect, cannot be deduced from the present experiments.

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