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Effect of Electrical Stimulation of the Vagus Nerves on Bile Secretion in Anaesthetized Sheep

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Abstract

Bile was collected before and during electrical stimulation of the vagus nerves in acute experiments on sheep with ligated cystic ducts. Most stimuli caused no change in bile formation, but a 10-V, 10-Hz stimulus caused a slight increase in bicarbonate output. Neither the response to infused secretin nor the maximum rate of bile salt transport by liver cells changed during vagal stimulation. It was concluded that the vagal innervation of the liver is not likely to play a major role in the regulation of bile formation in sheep.

Introduction

Major differences exist between species in the relative importance of different factors in the regulation of bile formation (Shaw and Heath 1972, 1975; Caple and Heath 1975). In sheep, the rate of flow of bile is considerably higher than in dogs, and both bile salts and secretin appear to play a significant role in the regulation of bile formation (Heath 1970; Heath *et al.* 1970; Caple 1972; Caple and Heath 1972). Although the rate of transport of bile salts by liver cells is an important regulator of bile secretion in sheep, the capacity of these transport mechanisms is limited (Heath *et al.* 1970).

The role of the vagus nerves in the regulation of bile formation has been studied extensively in dogs, but conflicting results have emerged. For example, electrical stimulation of the vagus nerves elicited an increase in bile production in the experiments of Tanturi and Ivy (1938), but not in those of Kaminski *et al.* (1974). Sham-feeding or administration of insulin or 2-deoxyglucose has been used in dogs in efforts to increase vagal activity (Fritz and Brooks 1963; Powell *et al.* 1965; Pissidis and Bombeck 1973). These stimuli have been shown to produce a choleresis but, as pointed out by Pissidis and Bombeck (1973), this could have been caused indirectly by the release of substances from the gastrointestinal tract. In the experiments of Kaminski *et al.* (1974), however, direct vagal stimulation did not appear to cause release of either cholecystokinin or secretin from the gut. Despite the interest in the effects of the vagus nerves on bile formation in dogs, information on such effects is not available from sheep or from other herbivorous animals.

In this study, electrical impulses were used to stimulate the vagus nerves in the sheep thorax, and studies were made on the effects of this stimulation on the formation of bile. Observations were made on the flow and composition of bile, on the response to infused secretin, and on the ability of the liver cells to transport bile salts into bile.

Materials and Methods

Crossbred ewes and wethers which weighed 30–40 kg were used. Normality of liver function was assessed from the clearance of sulphobromophthalein (BSP); all sheep used had a BSP half-time of less than 2·7 min (Heath 1969).

Each sheep was starved for 24 h, then anaesthesia was induced with pentobarbitone sodium and maintained with halothane for the duration of the experiment; the sheep was then destroyed.

Each surgical preparation involved a right paracostal incision, ligation of the cystic duct, and cannulation of the common bile duct proximal to the entry of the pancreatic duct. A drainage tube was placed in the abomasum, and a ligature was placed around the pylorus to prevent abomasal contents from entering the duodenum and influencing release of endogenous hormones. One or two cannulae for the infusion of solutions were placed in the portal vein through mesenteric tributaries. The dorsal and ventral trunks of the vagus nerve were approached through an incision at the eighth right rib. The nerve trunks were exposed and cut, then platinum electrodes in perspex mountings were attached to the distal ends.

Stimulation was applied to the vagus nerve trunks with an Electronic Square Wave Stimulator (C. F. Palmer (London) Ltd), which delivered 10-ms pulses at 5, 10 or 20 V, and 5, 10 or 20 Hz.

Sodium taurocholate (Sigma Chemical Co., catalogue number T-0750) was infused into the portal vein at 60 μmol/min to maintain a constant supply of bile salts to the liver cells during bile deprivation. This rate of infusion was maintained throughout the experimental periods except during studies on the maximum rate of hepatic transport; 90 μmol/min was infused for those studies (this had been shown previously to be higher than the maximum rate of transport by the sheep liver). In experiments on secretin, a preparation from the Karolinska Institutet (batch 17431) was infused into the portal vein at 0·88 clinical units/min. During the experiments on each sheep 1 litre of a multiple electrolyte solution (Normosol-R, Abbott Laboratories) was given through a catheter in the jugular vein.

All bile samples were collected under paraffin oil, and each was collected for a period of 5 min.

Plan of Experiments

In the first experiment changes in bile flow and composition were measured in each of four sheep in response to vagal stimulation at the following levels: 5 V and 5 Hz; 10 V and 10 Hz; 10 V and 20 Hz; 20 V and 10 Hz; 20 V and 20 Hz. Bile samples were collected for two control periods, then vagal stimulation was applied for 20 min. The bile which flowed during the first 5 min of stimulation was discarded, and then three bile samples were collected during the last 15 min of stimulation.

In the second experiment four sheep were used to examine the effect of vagal stimulation on the hepatic response to secretin. Changes in bile flow and composition were tested in each sheep in response to each of the following stimuli: vagal stimulation with 10 V and 10 Hz; infusion of secretin; vagal stimulation during infusion of secretin. Bile samples were collected for two control periods then each stimulus was applied for 20 min. Three samples of bile were collected during the last 15 min of stimulation.

In the third experiment studies were made on the effect of vagal stimulation on the maximum rate of hepatic bile salt transport. In six sheep sodium taurocholate was infused at 90 μmol/min into the portal vein for a total of 65 min. After the bile salts had been infused for 35 min, two control samples of bile were collected. The vagus nerve was then stimulated for 20 min with 10 V and 10 Hz in three of the sheep; the other three were used as controls to determine if any changes in bile production were associated with increasing duration of bile salt infusion. Three bile samples were collected during the last 15 min from each of the sheep given the bile salt infusion.

Analysis

All bile samples were analysed for bicarbonate (Segal 1955), chloride (Sanderson 1952) and total cholates (Irving *et al.* 1944).

Analysis of variance was used to estimate the significance of differences between means. Results are expressed as mean ± s.e. of mean.

Results

Stimulation of the vagus nerves was associated with little or no change in bile formation.

In the first experiment, no significant responses could be demonstrated to stimuli of 5 V and 5 Hz, 10 V and 20 Hz, 20 V and 10 Hz or 20 V and 20 Hz. When the vagus nerves in these four sheep were stimulated with 10 V and 10 Hz, bile flow did not alter significantly but as is shown in the following tabulation the output of bicarbonate did increase:

	Control period	During stimulation	
Bile flow (ml/min)	0·84±0·091	1·01±0·044	(n.s.)
Bicarbonate output (μmol/min)	31±3·6	38±2·2	($P < 0·025$)

In the second experiment, the infusion of secretin was associated with an increase in bile flow and bicarbonate output but this response was not modified by stimulation of the vagus nerves (Table 1). Vagal stimulation alone did not change bile flow or composition (Table 1).

Table 1. Effect of vagal stimulation at 10 V, 10 Hz and 10-ms pulse width on the response of the sheep biliary tract to secretin infused at 0·88 clinical units/min

Four sheep were used and each treatment was applied to each animal. Sodium taurocholate was infused into the portal vein at 60 μmol/min and a constant infusion of an electrolyte solution was given into a jugular vein throughout the experimental period

Treatment	Bile flow (ml/min)		Bicarbonate output (μmol/min)	
	Before treatment	During treatment	Before treatment	During treatment
Vagal stimulation	0·87±0·054	0·87±0·036	28±1·1	29±1·0
Secretin infusion	0·88±0·046	1·39±0·146	31±1·6	81±11·0
Vagal stimulation during secretin infusion	0·93±0·035	1·30±0·069	33±1·7	77±5·6

Table 2. Effect of vagal stimulation at 10 V, 10 Hz and 10-ms pulse width on the maximum rate of transport of bile salts by the sheep liver

Sodium taurocholate was infused into the portal vein at 90 μmol/min and a constant infusion of an electrolyte solution was given into a jugular vein throughout the experimental period. Three sheep in a control group were prepared and treated as for three experimental sheep, but they did not receive vagal stimulation

Treatment	Bile salt concn (μmol/ml)		Bile salt output (μmol/min)	
	Control period	Treatment period	Control period	Treatment period
No vagal stimulation	66±3·6	67±2·7	74±7·7	69±4·5
Vagal stimulation	60±3·2	65±2·4	58±3·7	64±2·5

In the third experiment the output of bile salts was less than the rate of infusion (Table 2), and this was taken to indicate that the transporting capability of the liver cells had been exceeded. The output of bile salts did not change during vagal stimulation.

Discussion

The only change observed during vagal stimulation was an increase in bicarbonate output. However, this occurred only at one level of stimulation, and was observed

only in the four sheep used in the first experiment; no significant effect occurred in the four sheep used in the second experiment (Table 1). Furthermore, the increase that was observed was small when compared with the effect of exogenous or endogenous secretion (Heath 1970; Caple and Heath 1972), and would seem to be of little physiological significance. The results of the second and third experiments did not indicate a role for the vagus nerves in determining either the hepatic response to secretin or the maximum rate of transport of bile salts by the liver.

It could be suggested that the inability to demonstrate important effects of vagal stimulation on bile formation in these sheep may have resulted from inadequate stimulation of the vagus nerve trunks. This is considered unlikely, as the same technique has been shown to be effective in stimulating pancreatic enzyme output, and gall bladder motility, in anaesthetized sheep (Reynolds 1972; Pass 1975). Furthermore some comparable experiments done recently on dogs yielded similar negative results (Kaminski *et al.* 1974).

These studies support the hypothesis that impulses in the vagus nerves have little direct effect on the formation of bile, at least in sheep.

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