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Modification of taurocholate stabilised choleresis by secretin and glucagon in calves

Modyfikujące działanie sekretyny i glukagonu na wydzielanie żółci stabilizowane taurocholanem sodowym u cieląt

Calves bile contains in profound quantity sodium taurocholate (4, 6, 14, 24, 27) which exerts a much greater choleretic effect than remainder bile acids present in their bile. During constant intraportal infusion of this compound bile flow is stable (3). In such conditions an additional introduction to the infused solution of another substance makes possible to find liver response which maintains normal secretion (10, 16). In preliminary study on cannulated calves it has been shown that glucagon alone was unable to stimulate bile secretion. In such condition secretin, on the other hand, stimulates bile flow rich in bicarbonates (11, 12, 17). According to current concepts (8, 9, 21) hepatic bile secretion is mainly the result of active secretion of bile salts into the canaliculi and subsequent flow of water and electrolytes. It has become clear that there are canalicular and ductular choleresis and although the choleretic action of glucagon has been studied extensively (15, 18, 19) the exact mechanism by which it acts is not known yet. The aim of these studies was to compare the influence of glucagon and secretin on bile secretion in calves in a condition when bile flow is higher then minimal as a result of sodium taurocholate (TCHNa) infusion.

MATERIALS AND METHODS

All studies were conducted on four female calves aged between 12 and 15 weeks and weighing 100 to 118 kg. The calves were starved for a day before the operation, then anaesthesia was induced with intravenous brietal sodium and maintained with a mixture of halothane and oxygen in a closed circuit. Each calf had been prepared several weeks earlier by cholecystectomy, and installation of a common bile duct, portal and duodenal cannulas. These organs were approached through an incision immediately behind and parallel to the last rib on the right side. Polyethylene tubes (i.d. 3.0 mm, o.d. 4.0 mm and i.d. 1.2 mm, 1.8 mm) were placed in the common bile duct just proximal to the pancreatic duct and vena porte through small mesenteric tributaries respectively. Plexiglass cannule was introduced into the lumen of the duodenum opposite to entries of bile ducts and anchored with a purse-string suture.

EXPERIMENTS

The calves were deprived of bile 24 h before each experiment. The sodium taurocholate (TCHNa) was dissolved in saline and was infused at a rate 50 μM/min a variable speed pump into the portal vein. In addition the solution of secretin 2 IU/min and glucagon 5 μg/min was infused simultaneously with TCHNa in some experiments.

The first series of studies was designed to give some indication of the effect on bile formation of infusion of TCHNa into portal vein. Two 10 min samples of bile were collected from each of four calves, then TCHNa was infused at 50 μM/min for at least 1 h and until the rate of bile flow had become stabilised, then bile was collected for six periods, each of 10 min. These calves were allowed to recover for 3-4 days, then were used for experiments in the second series.

The second series was designed to assess the nature and time relations response after addition for the first 20 min 2 IU/min secretin (diluted in normal saline) to infusion of 50 μM/min TCHNa. The last series was performed to assess the effects of stabile infusion TCHNa together with glucagon given in doses 5 μg/min. Two 10 min control samples of bile were taken, then infusions of TCHNa were given for 60 min and glucagon for the first 20 min while six consecutive 10 min samples were collected.

ESTIMATIONS

Bicarbonate content and pH were determined by means of acid-base analyser (Plastomed), chloride by electrometric titration, total bile acids by enzymatic assay using 3 hydroxysteroid dehydrogenase as described elsewhere (5). Values quoted are mean ± SEM. Differences between mean values were assessed statistically using Student t-test. Other calculations are explained as necessary in the text.

RESULTS

BILE FLOW (FIG. 1)

Control calves had biliary flow of 10.26 μl/min/kg. The flow of bile increased during infusion TCHNa and reached a mean 16.24 μl/min/kg at 30 min and remained almost stabile throughout all infusion period (Fig. 1). When secretin was introduced at rates 2 IU/min to the infusion of TCHNa a highly significant ($p < 0.05$) increase in bile flow to 21.26 μl/min/kg was observed. Therefore, the increase in flow during TCHNa infusion was 5.98 μl/min/kg and with an additional secretin administration was 5.02 μl/min/kg. There was a significant depression ($p < 0.05$) of bile flow to 8.76 ± 1.23 μl/min/kg during the postsecretin period. Also glucagon given to TCHNa infusion brought about an increase in bile flow although a choleresis was not the same order of magnitude in comparison to produced by TCHNa and secretin. In Figure 1, which depicts three experiments, it can be seen that secretin produced abrupt changes in bile flow while glucan choleresis is smaller as regards magnitude but lasts longer.

BILE SALT CONCENTRATION AND OUTPUT

As shown in Table 1 the concentration of bile salts was the highest during TCHNa infusion together with glucagon. Bile salts concentration in these series of experiment ranged between 44.76 and 62.54 μM/ml and was significantly ($p<0.05$) higher than during TCHNa infusion alone. The maximal bile salts concentration achieved with TCHNa and secretin was considerably lower (8.47 μM/ml) than with TCHNa alone. In all collection periods the bile salts excretion rate during TCHNa infusion at a rate 50 μM/min was comparable (56.92 and 47.93 μM/min) to the rate of administration (Fig. 2). Thus the intravenous infusion of TCHNa proved to be a satisfactory way of establishing a state of constant and reproducible bile salt excretion rate during which other changes could be more readily interpreted. As anticipated, the secretin infused together with TCHNa produced a marked decrease of bile salts secretion up to 13 μM/min. Quite the opposite effect produces glucagon which brought about a highly significant increase of bile salts secretion (64.80 μM/min) in comparison to TCHNa alone (47.93 μM/min). In all three experiments the secretion of bile salts was much greater during TCHNa infusion together with glucagon than in the absence of glucagon.

BICARBONATE CONCENTRATION AND SECRETION

The mean bile concentration of bicarbonate during control period was 35.50 ± 3.51 μM/ml (Tab. 1). The infusion TCHNa produced a small but stable increase in bicarbonate concentration which never exceeded 40.50 μM/ml. The maximal bicarbonate concentration achieved with glucagon 29.90 μM/ml was considerably lower than with secretin 53.80 μM/ml. Bicarbonate output (Fig. 3) increased in response to all three series. With secretin the response was highest (89.84 μM/min) but with glucagon the bicarbonate output rose only insignificantly.

CHLORIDE ANIONS

The infusion of TCHNa did not cause any significant change in the concentration of chloride (Tab. 1), but in each experiment addition of secretin was associated with a significant ($p < 0.05$) decrease in the concentration of chloride from the value of 103.34 μM/ml to 79.98 μM/ml. Glucagon introduced to the infusion of TCHNa produced a statistically significant increase in chloride concentration (Tab. 1) up to 130.54 μM/ml. Chloride output (Fig. 4) increased with TCHNa infusion, especially seen with simultaneous infusion of glucagon when the highest chloride response was observed (217.10 μM/min). But when the results were examined by analysis of variance it was not possible to demonstrate any significant interactions between the responses to glucagon and those to TCHNa.

Tab. 1. Effects of natrium taurocholate (TCHNa) infusion alone and with glucagon (+G) or secretin (+S) on composition of bile in calves (n=6). Data expressed as a mean ± S.E.M. * $p < 0.05$, ** $p < 0.01$ vs. control (0) values

min	pH			HCO_3^- (μ/M/ml)			Cl^- (μ/M/ml)			Bile salts (μ/M/ml)		
	TCHNa	+G	+S	TCHNa	+G	+S	TCHNa	+G	+S	TCHNa	+G	+S
0	7.58 ±0.05	7.62 ±0.05	7.70 ±0.08	33.20 ±2.31	37.40 ±3.45	38.23 ±3.67	103.23 ±9.67	106.34 ±12.32	103.34 ±7.89	12.45 ±1.76	9.87 ±1.02	18.51 ±1.23
10	7.60 ±0.06	7.69 ±0.09	7.78 ±0.11	37.40 ±3.71	39.40 ±4.32	46.21 ±6.43	98.78 ±7.35	116.89 ±12.09	96.59 ±6.57	36.57** ±4.56	46.56** ±5.98	10.47 ±0.94
20	7.69 ±0.08	7.61 ±0.08	8.26** ±0.09	39.70 ±5.23	31.54 ±6.11	59.65** ±5.79	105.27 ±18.67	122.85 ±16.03	76.09* ±3.94	37.87** ±7.23	52.89** ±7.32	4.45* ±0.26
30	7.71 ±0.10	7.57 ±0.07	8.20** ±0.07	36.65 ±3.98	22,34* ±1.67	56.23** ±7.42	95.89 ±8.45	126.79* ±13.32	75.32* ±5.78	29.11* ±4.65	44.76** ±6.43	4.78* ±0.56
40	7.61 ±0.06	7.72 ±0.06	7.91* ±0.08	40.50 ±6.61	29.98 ±3.34	53.83** ±5,45	109.23 ±14.41	130,54** ±13.48	79.98 ±6.89	28.72* ±5.76	52.71** ±6.98	8.47* ±0.84
50	7.78 ±0.07	7.69 ±0.05	7.86 ±0.08	36.86 ±2.98	39.60 ±5.76	48.34* ±3.89	110.25 ±12.76	116.93* ±10.28	91,69 ±7.78	31.76** ±4,59	62.54** ±5.87	4.60* ±0.67
60	7.80 ±0.07	7.69 ±0.07	7.82 ±0.08	37.98 ±4.25	35.32 ±2.76	41.95 ±5.67	112.42 ±18.11	112.51 ±9.76	98.94 ±4.69	29.63** ±5.34	50.81* ±3.24	9.98 ±0.76

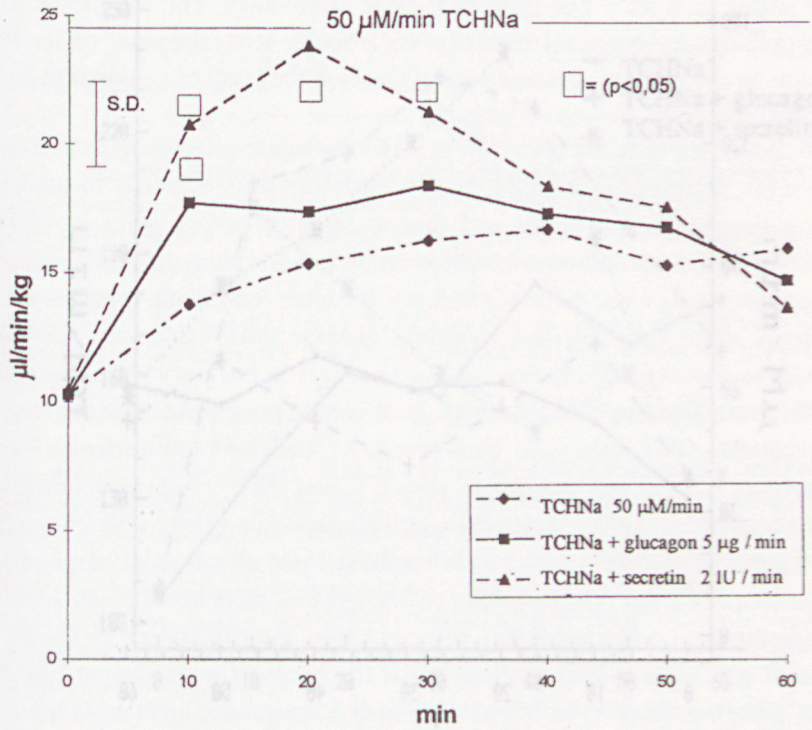

Fig. 1. Time dependent changes in bile flow (mean * SD, n=6) in calves during TCHNa alone and with glucagon or secretin infusions. A continuous horizontal line represents rate and duration of bile salt infusion

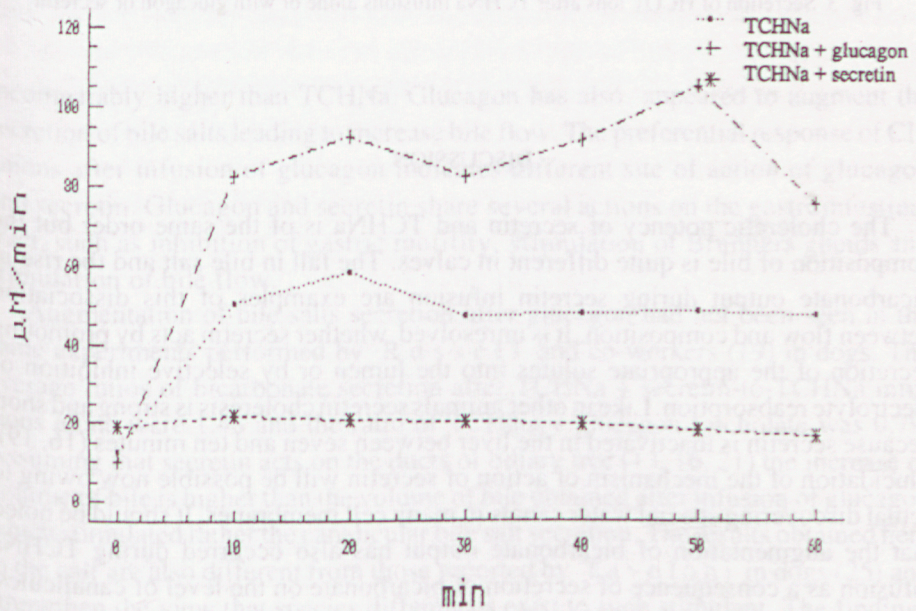

Fig. 2. Secretion of total bile salts (TBS) after TCHNa infusions alone or with glucagon or secretin in bile of calves

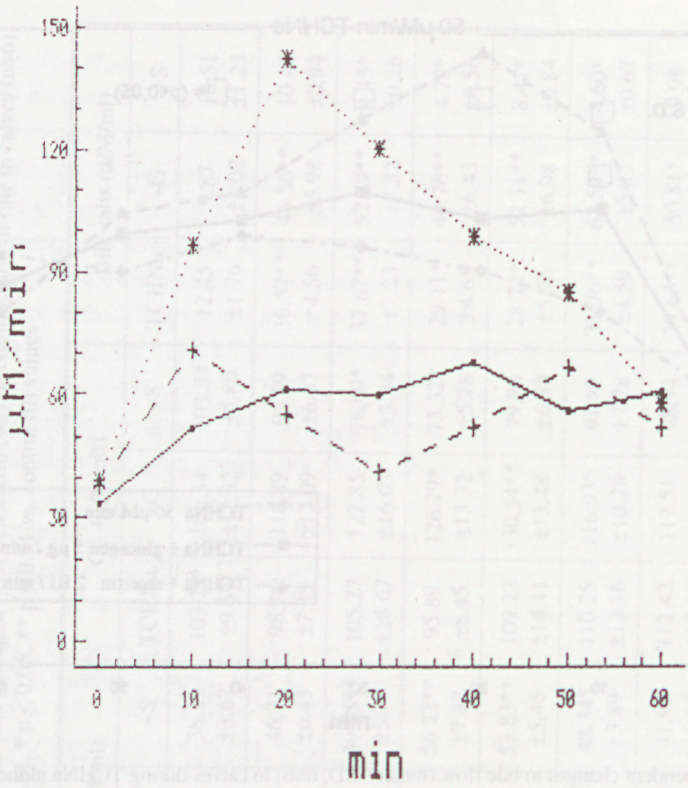

Fig. 3. Secretion of HCO_3^- ions after TCHNa infusions alone or with glucagon or secretin

DISCUSSION

The choleretic potency of secretin and TCHNa is of the same order but the composition of bile is quite different in calves. The fall in bile salt and the rise in bicarbonate output during secretin infusion are examples of this dissociation between flow and composition. It is unresolved whether secretin acts by promoting secretion of the appropriate solutes into the lumen or by selective inhibition of electrolyte reabsorption. Like in other animals secretin choleresis is strong and short because secretin is inactivated in the liver between seven and ten minutes (16, 19). Elucidation of the mechanism of action of secretin will be possible now owing to actual discovering special water canals in many cell membranes. It should be noted that the augmentation of bicarbonate output has also occurred during TCHNa infusion as a consequence of secretion of bicarbonate on the level of canalicules, driven by active transport of bile salts (1, 4, 13, 20, 23, 26). Although glucagon was weak in choleretic action, its potency to stimulate CL^- anions output in bile was

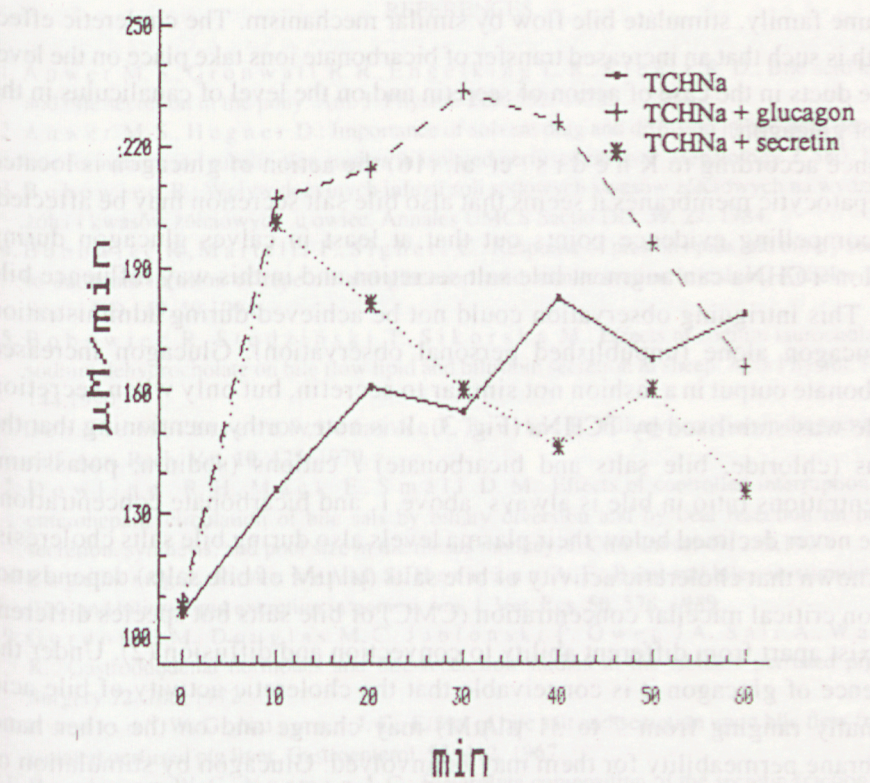

Fig. 4. Secretion of CL^- ions after TCHNa infusions alone or with glucagon or secretin

incomparably higher than TCHNa. Glucagon has also appeared to augment the secretion of bile salts leading to increase bile flow. The preferential response of CL^- anions after infusion of glucagon indicates different site of action of glucagon and secretin. Glucagon and secretin share several actions on the gastrointestinal tract, such as inhibition of gastric motility, stimulation of Brunners glands and stimulation of bile flow.

Augmentation of bile salts secretion after glucagon had not been seen in the same experiments performed by R u s s e l l and co-workers (19) in dogs. The average ratios of bicarbonate secretion after TCHNa + secretin-to-TCHNa infusions alone were 1.48 and the ratio of TCHNa + glucagon-to-cholate was 0.79. Assuming that secretin acts on the ducts of biliary tree (13, 16, 21) the increase of volume of bile is higher than the volume of bile obtained after infusion of glucagon which stimulated rather the canalicular bile salt secretion. The results obtained here in the calf are also different from those reported by T a v o l o n i in dogs (25) and strengthen the view that species differences exist to such stimulant. The findings reported by other authors (15, 18) indicate that secretin and glucagon, belonging to

the same family, stimulate bile flow by similar mechanism. The choleretic effect of both is such that an increased transfer of bicarbonate ions take place on the level of bile ducts in the case of action of secretin and on the level of canaliculus in the case of glucagon.

Since according to K h e d i s et al. (16) the action of glucagon is located on hepatocytic membranes it seems that also bile salt secretion may be affected. Our compelling evidence points out that at least in calves glucagon during infusion TCHNa can augment bile salt secretion and in this way influence bile flow. This intriguing observation could not be achieved during administration of glucagon alone (unpublished personal observation). Glucagon increased bicarbonate output in a fashion not similar to secretin, but only when secretion of bile was stabilised by TCHNa (Fig. 3). It is noteworthy mentioning that the anions (chloride, bile salts and bicarbonate) / cations (sodium, potassium) concentrations ratio in bile is always above 1, and bicarbonate concentrations in bile never declined below their plasma levels also during bile salts choleresis. It is known that choleretic activity of bile salts (µl/µM of bile salts) depends not only on critical micellar concentration (CMC) of bile salts but species differences exist apart from different ability to convection and diffusion (2). Under the influence of glucagon it is conceivable that the choleretic activity of bile acid (normally ranging from 7 to 31 µl/µM) may change and on the other hand membrane permeability for them may be involved. Glucagon by stimulation of chloride and bile salts secretion and leaving bicarbonate secretion unaffected exerts almost the opposite influence to secretin and such action of this preferentially metabolic hormone may point to rather hepatocytic action. On the assumption that bile formation is linearly dependent on bile acid secretion (2, 7, 22, 24) the bile salt concentration should be the same during normal bile flow and during choleresis produced by administration of glucagon. Also in rat the bile flow even decreased while the bile acid concentration increased after taurocholic acid administration. In our experiments the increase of bile salts output was much greater than the increase of bile flow after infusion of glucagon. It appears that biliary flow in the calf is not entirely dependent on bile acid secretion, especially when glucagon is introduced. The observed in our experiments increase in bicarbonate concentration after TCHNa infusion may prove that some of these anions are secreted on the level of canaliculus driven by bile salts.

In conclusion, these studies have demonstrated that, regardless of bile flow stimulated by secretin, bile acid concentrations in bile were increased under the influence of bile salts infused together with glucagon. It is suggested that glucagon acts on the canalicules not only by stimulation of bicarbonate but also by bile salts secretion.

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STRESZCZENIE

W badaniach przeprowadzonych na cielętach kaniulowanych do żyły wrotnej i przewodu żółciowego wspólnego oznaczono odpowiedź wydzielniczą wątroby na dwa często podobnie działające hormony – glukagon i sekretynę w warunkach stałego podawania taurocholanu sodowego (TCHNa). Hormony i TCHNa infundowano do żyły wrotnej rozpoczynając 24 godz. po przerwaniu jelitowo-wątrobowego krążenia soli żółciowych, gdy endogenne wydzielanie soli żółciowych i spowodowane nim wydzielanie żółci jest niskie i stosunkowo stałe. TCHNa podawano we wszystkich doświadczeniach w tempie 50 µM/min. w ciągu 1 godz. W części doświadczeń dodatkowo w okresie pierwszych 20 min. infuzji TCHNa podawano sekretynę (2 IU/min) lub glukagon 5 µg/min. Po rozpoczęciu infuzji TCHNa wydzielanie żółci wzrastało i osiągało względnie wyrównany poziom 15 µl/min/kg. Wydzielanie osiągało maksymalne wartości (21,26 µl/min/kg) podczas infuzji sekretyny. Również glukagon powodował podniesienie wydzielania żółci, ale o wiele mniejsze w porównaniu do wartości osiągniętych po TCHNa + sekretyna. Infuzje glukagonu związane były z statystycznie istotnym ($p < 0,05$) wzrostem wydzielania jonów Cl^- (217,10 µM/min) oraz soli żółciowych (101,21 µM/min). Wydzielanie soli żółciowych po infuzjach sekretyny ulegało istotnemu ($p < 0,01$) zmniejszeniu (21,45 µM/min) przy równoczesnym zwiększeniu wydzielania wodorowęglanów. Zmiany spowodowane wpływem obu hormonów w wydzielaniu i składzie żółci wskazują, iż glukagon działa u cieląt na poziomie kanalików, zwiększając sekrecję soli żółciowych i w konsekwencji objętość żółci, sekretyna zaś (jak u innych gatunków zwierząt) wywiera efekt choleretyczny na poziomie przewodów żółciowych.