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Effect of *K*-Strophanthin on Bile Secretion and Hepatic Perfusion in the Isolated Rat Liver (40471)

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It is currently believed that an active sodium transport system, mediated by a hepatocytic membrane-bound Na^+,K^+-ATPase, may drive a portion of bile across the canalicular membrane, thereby determining the bile acid independent bile secretion. This view has evolved mainly from the experimental evidence that inhibitors of sodium transport markedly diminish canalicular bile flow without impairment of bile acid secretion in the rabbit (1) and in the isolated perfused rat liver (2). However, conflicting results also have been reported which make this hypothesis controversial. Thus, Graf and Peterlik (3) found that ouabain was choleretic in the isolated perfused rat liver while Russell and Klaassen (4) observed no effect on bile flow when the glycoside was administered to rats, rabbits or dogs. Additionally, in the isolated perfused rat liver (2), ouabain causes a severe diminution in hepatic perfusion rate, thus making it impossible to rule out that the inhibition of bile acid independent bile flow is partly the result of the impaired hemodynamics.

In an attempt to gain more information on the effect of cardiac glycosides on bile secretion and to better elucidate the relationship of the circulatory disturbances to the inhibition of bile flow, the effect of portal infusions of *k*-strophanthin on canalicular bile secretion and hepatic perfusion was studied in the isolated perfused rat liver.

Methods. Male Sprague-Dawley rats (Charles River strain, Morini, Italy), weighing 270–300 g were used as blood and liver donors in these studies. The animals were given free access to food and water prior to being sacrificed. Basically, perfusion and hepatectomy techniques were those of Miller (5). Liver donors were anesthetized with sodium pentobarbital (50 mg/kg, ip) and the liver was exposed by midline and subcostal incisions of the abdomen. After dissecting the liver from the main gastric, duodenal and splenic ligaments, the bile duct was cannulated (PE 50 tubing) and the vena cava ligated. The portal vein then was cannulated and the liver flushed with a portal injection of 5–10 ml of oxygenated Krebs-Ringer bicarbonate solution containing heparin (100 U/ml) until blood was cleared from all the lobes. Care was taken not to manipulate the hepatic artery. The thorax was then opened and the vena cava cannulated. Both portal and cava veins were cannulated with glass cannulae (id = 2 mm). Subsequently, the liver was carefully removed and transferred to the perfusion chamber with a maximum ischemic period of 3–4 min. Liver perfusions were carried out through the portal vein at a constant perfusion pressure of 15 cm of blood with a mixture (100–150 ml) of two parts of defibrinated and subsequently heparinized blood drawn from etherized rats and one part of Krebs-Ringer bicarbonate solution containing fraction V bovine serum albumin (Fluka AG) (3 g/100 ml) and glucose (100 mg/100 ml). The perfusate, the hematocrit of which averaged 25%, was adjusted to a pH of 7.44 with NaOH (0.1 *N*) at 37.5° and maintained throughout the study in the range 7.35–7.45 with $NaHCO_3$ (0.5 *N*). Oxygenation was provided by a mixture of 95% O_2–5% CO_2 at a gas flow rate of 500 ml/min. Perfusate temperature was scrupulously maintained at 37.5 ± 0.2°. Bile was collected in small preweighed vials over 10-min intervals. Hepatic blood flow was monitored by timed collections of cava outflow. Liver weights were obtained at the end of the perfusion after removing cannulae and excess tissue.

[^{14}C]erythritol (Amersham, London, sp act = 50 mCi/mmole) was used to estimate canalicular bile flow (6, 7). The labelled compound was freshly dissolved in 1 ml of Krebs-Ringer bicarbonate solution and added (2.5–

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3.5 μCi) to the perfusate 10-min prior to the beginning of the perfusion. Bile and plasma samples (50–100 μl) were prepared for counting as already reported (2). Quenching was corrected with external autostandardization. The biliary clearance of [^{14}C]erythritol was calculated as the product of bile flow and bile-to-plasma ratio of ^{14}C activity and expressed in μl min^{-1} g^{-1} liver. Following a 30 min equilibration period, to establish maximal rates of bile and perfusate flow, *k*-strophanthin (Fluka AG) was infused through the portal vein inflow tubing at the doses of 0.5, 2.0, 4.0 or 8.0 mg min^{-1} for the remainder of the perfusion. The glycoside was freshly dissolved in Krebs-Ringer bicarbonate solution containing 3% albumin and 0.1% glucose. Control livers received a constant infusion of the vehicle throughout the study.

Experimental data were analyzed for statistical differences by using Student's *t* test at 0.05 level. Regression line equation was calculated by computer by the least square method and slope and *y*-interept obtained as mean ± SD.

Results. In control experiments bile flow rate ranged from 1.64, maximal value obtained at the third 10-min collection, to 1.21 μl min^{-1} g^{-1} liver, at the end of 2-hr perfusion. Portal infusion of *k*-strophanthin significantly reduced the rate of bile flow when compared to controls (Fig. 1). With the lowest dose (0.5 mg min^{-1}) a slight but significant decrease was noted only in the last two 10-min collection periods, whereas with 2, 4 and 8 mg min^{-1} the inhibitory effect appeared 10–30 min after the glycoside infusion was started. With the highest dose the secretion of bile was practically blocked within 30 min. As shown in Table I, the bile-to-plasma ratios of [^{14}C]erythritol, both in control and *k*-strophanthin-treated livers, were approximately equal to one and never exceeded the value 1.28 throughout each study. Accordingly, canalicular bile flow was the same or somewhat higher than total bile flow.

As observed for bile flow, hepatic perfusate flow diminished remarkably when *k*-strophanthin was administered (Fig. 2). The effect appeared dose-related and roughly paralleled that observed on the rate of bile secretion. With the lowest *k*-strophanthin dose a 40% decrease in perfusate flow rate was noted at the end of the perfusion. With the highest dose, perfusate flow decreased 60–70% 10 min after the glycoside infusion started and declined to 0.6–0.3 ml min^{-1} g^{-1} liver (10–5% of control values) within 20–30 min.

Figure 3 illustrates the relationship between bile flow and hepatic perfusate flow during *k*-strophanthin administration. When perfusate flow declines from 5.5 to 3.0 ml min^{-1} g^{-1} liver, bile flow slightly diminishes. Conversely, for values of blood flow ranging from 3.0 to 0.15 ml min^{-1} g^{-1} liver, bile flow decreases in a near linear relationship with the reduction in perfusate flow. During the

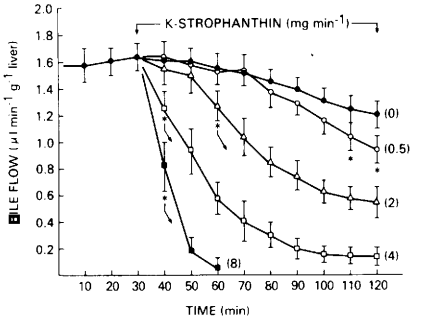

FIG. 1. Effect of portal infusion of *k*-strophanthin (0.5–8 mg min^{-1}) on bile flow in the isolated perfused rat liver. Values are mean ± SD of 5 to 6 experiments for each group. *Significantly different from control value ($P < 0.05$–0.001); arrow refers to all values thereafter.

TABLE I. BILIARY CLEARANCE OF [^{14}C]ERYTHRITOL BY THE ISOLATED PERFUSED RAT LIVER.[a]

	Controls		*K*-Strophanthin 2 mg min^{-1}	
min[c]	B/P[b]	μl min^{-1} g^{-1}	B/P	μl min^{-1} g^{-1}
40	1.10 ± 0.11	1.75 ± 0.19	1.12 ± 0.13	1.68 ± 0.20
60	1.05 ± 0.14	1.59 ± 0.18	1.01 ± 0.17	1.26 ± 0.18
100	1.00 ± 0.10	1.33 ± 0.13	1.07 ± 0.15	0.68 ± 0.16

[a] Values are means ± SD of 4 experiments for each group.
[b] Bile-to-plasma ratios of ^{14}C activity.
[c] Min after the perfusion started. In the case of *k*-strophanthin experiments, values correspond to 10, 30 and 70 min after the glycoside infusion was initiated.

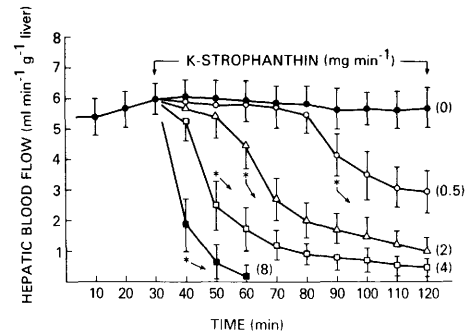

FIG. 2. Effect of portal infusion of *k*-strophanthin on hepatic perfusate flow in the isolated perfused rat liver. Values are mean ± SD of 5 to 6 experiments for each group. *Significantly different from control value ($P < 0.05$–0.001); arrow refers to all values thereafter.

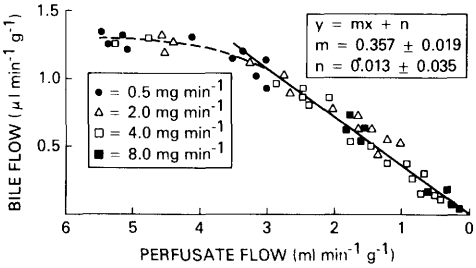

FIG. 3. Relationship between bile flow and hepatic perfusate flow during *k*-strophanthin infusion (doses are shown in the insert). Pairs are obtained from values of bile flow and perfusate flow in the time range 10–90 min after the glycoside infusion was started. The regression line of the relationship is obtained with perfusate flow rates ranging from 3.5 to 0.15 ml min^{-1} g^{-1} liver and calculated from the equation $Y = mx + n$, where Y = bile flow, x = perfusate flow, m = slope and n = y-intercept. The regression line is highly significant ($r = 0.928$, $P < 0.001$). The y-intercept is not significantly different from zero ($P < 0.5$).

vasoconstrictive effect of *k*-strophanthin the liver appeared ischemic and irregularly perfused. Darkened areas whose extension correlated with the degree of reduction in perfusate flow, could be noted all over the surface of the organ. No significant differences were observed in the wet liver weight-body weight ratio between control and *k*-strophanthin-treated livers obtained at the end of the experiments (Table II).

Discussion. The results of the present studies have demonstrated that *k*-strophanthin profoundly depresses the bile secretory function and hepatic perfusion in the isolated rat liver. Basically, the present findings resemble

TABLE II. LIVER WEIGHTS AND LIVER/BODY WEIGHT RATIOS OF CONTROL AND *K*-STROPHANTHIN-TREATED LIVERS.[a]

Treatment	Wet liver weight (g)	Liver/body weight (%)
Controls (0)	9.8 ± 1.1	3.5 ± 0.3
k-strophanthin (0.5)	10.4 ± 1.3	3.6 ± 0.4
k-strophanthin (2.0)	9.6 ± 0.9	3.3 ± 0.3
k-strophanthin (4.0)	10.7 ± 1.4	3.5 ± 0.4
k-strophanthin (8.0)	10.3 ± 1.1	3.7 ± 0.5

[a] Values are means ± SD of 5 to 6 livers for each group. *K*-strophanthin dose (mg min^{-1}) is shown in parenthesis.

those reported by Boyer (2) in that ouabain, delivered as a bolus, was found to diminish canalicular bile flow and hepatic perfusate flow in the same experimental model. In the present experiments, canalicular bile flow, as estimated by [^{14}C]erythritol clearance, closely paralleled total bile flow both in control and *k*-strophanthin-treated livers. This finding supports previous observations that bile flow, in the isolated perfused rat liver and in the intact animal as well, is essentially of canalicular origin under a variety of experimental conditions producing either choleresis (8) or cholestasis (2).

Primarily, the objective of the present investigation was to determine whether any direct effect of *k*-strophanthin on bile secretion could be demonstrated with doses of the drug which did not alter hepatic hemodynamics. The results obtained, however, failed to clarify this question. For each dose of *k*-strophanthin tested, the inhibition of bile flow was invariably associated with a dose-related diminution of perfusate flow. When the glycoside was infused at the lowest dose (0.5 mg min^{-1}), the decline in perfusate flow (Fig. 2) was detected even before any effect on bile secretion (Fig. 1) could be observed.

Brauer and coworkers (9) observed that in the isolated perfused rat liver the rate of bile secretion was practically unaffected by mechanically induced changes in perfusate flow beyond 20–30 ml min^{-1}. Consequently, they concluded that bile production is relatively independent of blood flow and pressure perfusing the hepatic sinusoids, once critical levels are provided to maintain hepatocellular function. In the present experiments, where hepatic blood flow is altered pharmacologically, the rate of bile secretion is only slightly

affected when the glycoside decreases perfusate flow from 5.5 to 3.0 ml min^{-1} g^{-1} liver; below 3.0 ml min^{-1} g^{-1} liver, bile flow diminishes in a fashion linearly related to the reduction in perfusate flow (Fig. 3). Relating the present results to Brauer and coworkers' observation, one conclusion could be that the decline in perfusate flow observed with *k*-strophanthin, at least in the region where the bile flow-perfusate flow curve deviates from linearity, is of secondary importance to the impaired secretory activity. This interpretation, however, is limited by the following observations. First, it has recently been demonstrated that in the isolated perfused rat liver a mechanical reduction of portal perfusate flow from 6 to 2 ml min^{-1} g^{-1} liver causes an altered regional distribution of blood flow within the hepatic lobes and produces a significant decline in bile acid independent bile flow without manifestations of hepatic injury (10). Second, considerable reserve exists in comparing hemodynamic changes mechanically induced to those obtained with vasoactive agents which may more likely result in circulatory derangements in localized regions where cell function may be impaired. Visual evaluation of the liver surface during the vasoconstrictive effect of *k*-strophanthin well supports this possibility. The organ, in fact, even when given the lowest dose of the glycoside, appeared ischemic and revealed darkened areas where blood stasis occurred. Thus, it is likely that in these localized areas functional units are inadequately oxygenated. Therefore, it is assumed that more general factors, responsible for cellular activity, are compromised and ultimately influence the bile secretory function.

How the present findings relate to those reported by Graf and Peterlik (3) in that ouabain resulted in choleresis and did not affect the hepatic circulation in the isolated perfused rat liver is difficult to establish. Several differences, however, exist between their and the present studies. In their experiments perfusions were carried out with a red cell-free medium whereas a 25% hematocrit with rat erythrocytes was employed here. Bile flow and perfusate flow rates obtained in the present experiments are about two- and fivefold higher respectively than those reported by them. *K*-strophanthin was used here and infused through the portal vein inflow tubing whereas ouabain was employed in their studies and delivered in a bolus form. Whether some or all of these differences and/or other factors may explain the disparate results requires further investigation.

Presently, no plausible explanation can be offered on the mechanism by which *k*-strophanthin affects the hepatic vascular bed. Regulation of blood flow in the hepatic microvessels is still a controversial issue. Although several other substances have been shown to induce vasocontriction in the isolated perfused rat liver (11), presence of smooth muscle has not been reported in the rat portal vasculature (12). The vasoconstrictive effect of *k*-strophanthin may be unspecific and, as observed with some other compounds in the same experimental conditions (13, 14), unexplainable on a pharmacologic basis. Irrespective of the mechanism, however, the adverse effect of the glycoside on hepatic perfusion seems to result in major disturbance which deranges the normal circulatory events of the isolated rat liver. Although the bearing of these circulatory alterations on the diminished bile flow has to be proven, it is felt that they at least contribute to, if not determine, the impaired secretory activity.

Summary. *K*-strophanthin, infused through the portal vein inflow tubing of the isolated perfused rat liver at the doses of 0.5, 2, 4 or 8 mg min^{-1}, diminishes canalicular bile flow and hepatic perfusate flow in a dose-related fashion. For values of perfusate flow declining in the range 3.0–0.15 ml min^{-1} g^{-1} liver the diminution of bile flow is linearly related to the reduction of hepatic perfusate flow. The results suggest that the alterations in hepatic hemodynamics induced by the glycoside most likely contribute to, if not determine, the impaired secretory activity of the liver.

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