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Insulin or Glucagon Choleresis in the Isolated Perfused Guinea Pig Liver (41609)

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Abstract. Insulin and glucagon choleresis was studied in an *in situ*, isolated perfused guinea pig liver system. Glucagon caused a small, significant increase in bile salt independent flow (1.83 ± 0.19 to 2.02 ± 0.23 $\mu l\ g^{-1}\ min^{-1}$), and dose-related increments over 2–16 μg were observed. Insulin alone had no choleretic effect. However, the combination of insulin and glucagon caused a response (1.89 ± 0.15 to 2.42 ± 0.19) greater than glucagon alone, and insulin stimulated choleresis when glucagon was present in substimulatory amounts. These observations demonstrate direct effects of glucagon and insulin upon the bile secretory apparatus. Glucagon directly stimulates choleresis, while insulin acts more subtly by potentiation with glucagon.

A number of exogenously administered hormones such as secretin, cholecystokinin, cortisone, thyroxine, insulin, and glucagon stimulate bile salt independent secretion at the canalicular or ductular level (1–9). The physiological significance of many of these actions is unclear, but feeding causes both the release of many of those hormones and a bile salt independent choleresis (10–13).

The effects of insulin and glucagon on the bile salt independent fraction of choleresis in animals and humans (2, 3, 5, 14–16) may be explained either by direct stimulation of bile secretion or by an indirect effect, for example release of other choleretic hormones. Insulin choleresis was first observed in 1963 by Fritz and Brooks, who suggested a vagal mechanism activated by hypoglycemia (15). More recent studies, however, revealed a substantial nonvagal component of insulin choleresis likely to represent a humoral effect (2). Subsequent definition of the humoral mechanism has not been possible, although recent investigations suggested that insulin has a direct choleretic effect on the liver (16). Insulin-stimulated bile flow was shown not to be caused by release of other hormones from the gastrointestinal organs or adrenal glands, but whether or not insulin produces its choleretic effects by hormone release from other organs or by hormone interaction was not proved. Investigations of mechanisms of insulin's actions *in vivo* are especially difficult because of a known associated release of other hormones, notably glucagon which itself produces a choleresis similar to insulin's (5). For those reasons the actions of insulin and glucagon on bile salt independent choleresis were studied in an isolated perfused liver preparation.

Materials and Methods. *Apparatus.* The *in situ* liver perfusion method of Mortimore (17) was used. In this preparation, experimental and control livers were perfused side by side within a heated, wooden box (built by Duke Surgical Instruments) with enclosed oxygenator-reservoir and pump systems. A mixture of 95% O_2 and 5% CO_2 was delivered at a rate of 0.5 liters min^{-1} into 500 ml spherical flask reservoirs which rotate at 60 revolutions min^{-1}. Gas exchange occurred within a thin layer of perfusate spread over the inner surface of the flask. Temperature was maintained at 37°C and the wooden box was opened through small Plexiglass windows only for collection of samples. A Holter-type pulsatile pump (Extracorporeal Model 911) delivered perfusate from the reservoir through silicone tubing at a rate of 1.2 ml min^{-1} g^{-1} guinea pig liver.

Perfusion medium. The perfusate consisted of Krebs–Ringer bicarbonate buffer, 3% crystalline bovine plasma (Fraction V, Sigma Chemical, St. Louis, Mo.), 175–200 mg% anhydrous glucose, and 15% washed, buffycoat-poor red blood cells. Bovine, guinea pig, rat, rabbit, human, and canine red blood cells all produced negligible hemolysis in preliminary experiments, so canine blood was selected for subsequent studies because of its ready avail-

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ability. On the morning of experiments, well-oxygenated blood was collected in heparinized syringes from carotid arteries of female, mongrel dogs lightly anesthetized with sodium pentobarbitol. Saline was infused into the donor dogs during collection to avoid hypovolemia. Red blood cells were immediately prepared, added to the perfusion medium in the spherical flasks, and equilibrated with the oxygen mixture. The initial recirculating volume of perfusate was 80 ml.

Operative procedure. Three- to five-hundred-gram Camm–Hartley guinea pigs, fasted for 16 hr, were anesthetized lightly with sodium pentobarbitol. The portal vein was cannulated by direct puncture with an 18-gauge Teflon catheter, and inflow from the reservoir was established within 5 sec. Effluent was collected through Intramedic 240 polyethylene tubing secured in the inferior vena cava above the diaphragm. The cystic duct, hepatic artery, and inferior vena cava between the liver and renal vein were ligated and bile was collected in graduated vials through Intramedic PE 90 tubing in the distal common bile duct. The first 10 ml of perfusate collected after cessation of cardiac contracture was discarded prior to recirculation.

Experimental procedure. After recirculation was established, bile was collected continuously in 15-min samples. Throughout each experiment, sodium taurocholate (Maybridge Co., North Cornwall, United Kingdom) 10 mg in 5 ml of 0.9% sodium chloride/hr was infused directly into the portal vein to avoid depletion of bile salts by the interrupted enterohepatic circulation. In the initial experiments after two basal 15-min periods, livers were randomized to receive an injection of either 100 mU glucagon-free insulin, 4 μg glucagon, (both from Eli Lilly Co., Indianapolis, Ind.; containing less than 0.05% peptide impurities) a combination of insulin and glucagon, or 0.9% sodium chloride. Insulin and glucagon dose-response tests were performed using dosages of 0.025 to 0.8 units and 1 to 32 μg insulin and glucagon, respectively. In additional experiments, insulin choleresis was studied during background infusion of 4 μg/hr of glucagon, and glucagon choleresis was studied during background infusion of 0.2 units/hr insulin.

Each liver underwent one 75-min experimental procedure. One hundred-forty-one livers in total were perfused. All appeared grossly normal at the end of the procedures and when histology was examined normal hepatic architecture was preserved. When known amounts of bromsulphthalein were injected into the reservoirs, uptake was measured to be 92% and recovery in bile was 70% after 30 min of perfusion.

Analysis of bile and perfusate. Bile volumes were measured to the nearest 0.01 ml in capillary tubes or syringes. Bile salt concentrations were determined by the steroid dehydrogenase method (18) as follows: 1 g of hydroxysteroid dehydrogenase was placed in 20 ml of 0.03 *M* Tris buffer and 0.001 *M* ethylenediaminetetraacetate and sonicated. The resultant material was centrifuged in a refrigerated chamber and the supernatant was employed in the assay. Fifty microliters of 1:10 dilution of bile specimen was added to 1 ml of 0.1 *M* sodium pyrophosphate, 1 ml of 1.0 hydrazine, 0.05 ml hydroxysteroid dehydrogenase, 0.1 ml of 0.005 *M* DPN, 0.8 ml water, and incubated 30 min at room temperature. Absorbance of the reaction mixture and blanks were determined in a Gilford 240 spectrophotometer at 340 nm. Bile salt outputs were calculated by multiplying concentration times volume. Sugar concentrations were measured on an Ames Blood Analyzer. Statistical comparisons between experimental and control

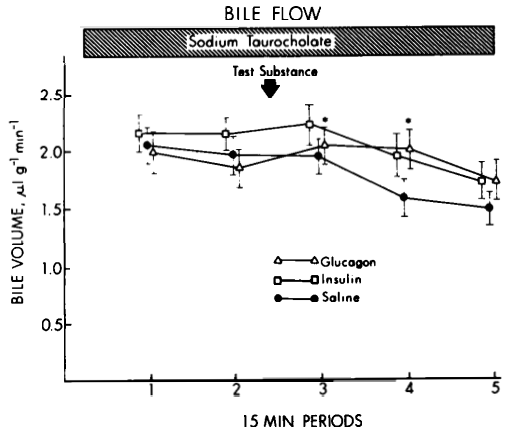

FIG. 1. Bile volume vs time during randomized injection of saline, insulin, or glucagon. *Points* represent means ± SEM of 10 livers. *Asterisks* denote $P < 0.05$ compared to preinjection flow.

periods and between corresponding periods of experimental and saline livers were made using *t* tests for paired and unpaired values (19). Values were considered significantly different if P was less than 0.05. A multivariate analysis of variance produced no new statistical changes between groups.

Results. *Bile flow.* Bile flow did not change significantly after saline administration, comparing the 15-min periods before and after injection (Figs. 1, 3). Similarly, there was no significant change after insulin (Figs. 1, 3). After glucagon injection bile flow increased significantly from 1.83 ± 0.19 $\mu l\ g^{-1}\ min^{-1}$ in the 15-min period before injection to 2.02 ± 0.23 $\mu l\ g^{-1}\ min^{-1}$ in the 15-min period after glucagon (Figs. 1, 3) although bile flow after glucagon injection was not significantly different from saline until the fourth 15-min period. Bile flow also increased significantly after administration of the combination of insulin and glucagon from 1.89 ± 0.15 $\mu l\ g^{-1}\ min^{-1}$ in the 15-min period before injection to 2.42 ± 0.19 $\mu g^{-1}\ min^{-1}$ in the 15-min period after injection (Figs. 2, 3). The change in bile flow in the periods before and after injection of insulin plus glucagon was significantly greater than after glucagon alone. At 30 and 45 min after injection, there were significant decreases in bile flow in the saline and insulin groups compared to the preinjection

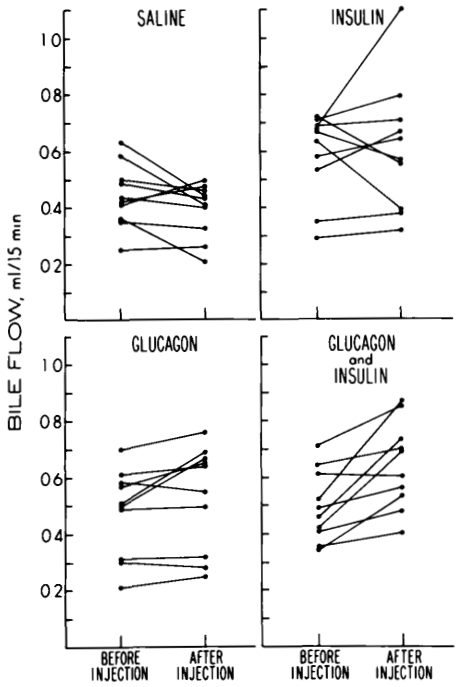

FIG. 3. Bile flow of the individual guinea pig livers during 15-min periods before and after randomized injection of the four test substances: saline, insulin, glucagon, combination of insulin and glucagon. Bile flow increased significantly ($P < 0.05$) after injection of either glucagon or the combination of insulin and glucagon, compared to before injection.

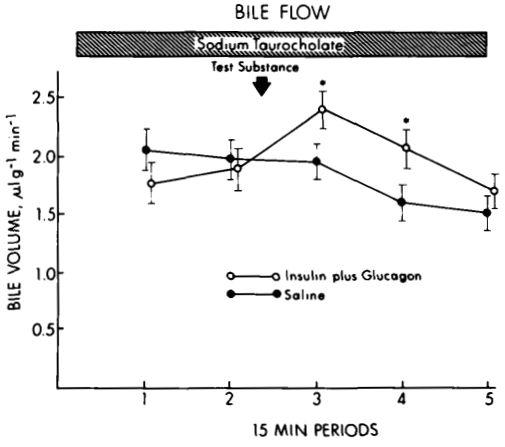

FIG. 2. Bile volume vs time during randomized injection of saline or the combination of insulin and glucagon. *Points* represent means ± SEM of 10 livers. *Asterisks* denote $P < 0.05$ compared to preinjection flow and to saline.

15-min period, but no change in the glucagon or insulin plus glucagon groups. There were no significant changes in bile flow between insulin and saline in those time periods.

When bile flow after each test substance injection was expressed as a percentage of the preinjection flow (15-min period before injection), insulin did not stimulate bile flow (Fig. 4). There were significant decreases in bile flow 30 and 45 min after saline and after insulin when compared to preinjection flows and there were no differences between the insulin and the saline groups. Glucagon increased bile flow significantly to 111.6 ± 4.9% of preinjection values, and the combination of insulin plus glucagon increased bile flow significantly to 131.5 ± 6.8% of preinjection values (Fig. 4). Bile flow after glucagon and after insulin plus glucagon remained significantly higher than saline for 45 min after injection. The increase in bile flow 15 min after

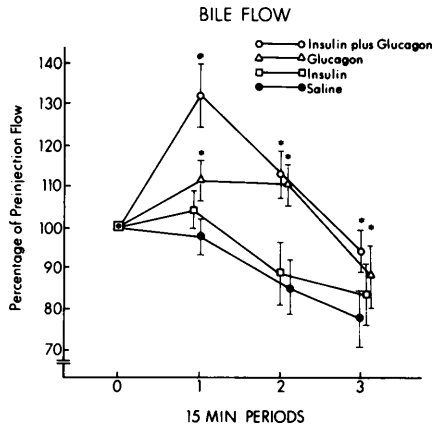

FIG. 4. Bile flow response to injection of test substances expressed as percentage of the 15-min period preceding injection (Period 0). *Points* represent means ± SEM of 10 livers. *Asterisks* denote $P < 0.05$ compared to saline.

the combination was significantly greater than after glucagon alone.

Bile salts (Table I). Bile salt concentrations increased significantly after saline or insulin injection, as bile volume decreased during the fourth and fifth 15-min periods. There were no other significant changes in bile salt concentration. Shown in the table is the data from Period 5, 45 min after injection, when there was the largest change in bile salt concentration compared to Period 2. Bile salt concentrations did not change significantly after glucagon or the combination of glucagon and insulin. Bile salt outputs did not change significantly after saline, insulin, or glucagon injection during all 15-min periods of the experiments.

Perfusate sugar concentrations (Table II). Hyperglycemia was maintained throughout the perfusions in all four experimental groups. There were no significant differences in the perfusate sugar concentrations in the four experimental groups before or after the experiments.

Background glucagon or insulin infusions. Bile flow increased significantly after insulin administration from 3.68 ± 0.31 $\mu l\ g^{-1}\ min^{-1}$ before to 3.99 ± 0.37 $\mu l\ g^{-1}\ min^{-1}$ after injection when glucagon 4 $\mu g\ hr^{-1}$ was being infused at the same time as taurocholate (10 livers). Over the same time periods during background glucagon infusion, bile flow decreased significantly after saline injection from 3.99 ± 0.37 to 3.61 ± 0.28 $\mu l\ g^{-1}\ min^{-1}$ (10 livers). When glucagon was injected during a background of 0.4 units/hr insulin (six livers), bile flow increased significantly and the percentage increase (120.7 ± 3.6) was significantly greater than the percentage increase after glucagon injection without a background infusion of insulin (111.2 ± 4.8).

Dose-response test. In additional experiments, stimulation of bile flow by glucagon during taurocholate infusion appeared to increase linearly over the dose range between 2 and 16 μg (Fig. 5). Insulin did not stimulate bile flow over the dose ranges of 0.025 to 0.8 units without a background infusion of glucagon. However, during background glucagon infusion, a dose-related choleretic response to insulin injection became apparent between 0.05 and 0.2 units (Fig. 6).

Discussion. The purpose of the present experiments was to study possible direct choleretic effects of insulin and glucagon. The *in situ* liver perfusion method of Mortimore (17) was selected because this preparation minimizes trauma, and virtually eliminates a period of hypoxia, which may have profound effects upon insulin's actions. An additional

TABLE I. BILE SALT SECRETION

	Bile salt concentration (μM/ml)		Bile salt output (μM/15 min)		Liver wt. (g)
	Before	After	Before	After	
Saline	4.89 ± 0.84	*7.21 ± 1.31	2.03 ± 0.24	2.10 ± 0.26	17.4 ± 2.0
Insulin	4.81 ± 0.41	*5.86 ± 0.76	2.70 ± 0.47	2.81 ± 0.49	19.2 ± 1.5
Glucagon	4.75 ± 0.62	5.49 ± 0.73	2.19 ± 0.34	2.21 ± 0.35	17.9 ± 1.0
Insulin plus glucagon	6.15 ± 1.13	6.62 ± 1.29	2.96 ± 0.38	2.83 ± 0.52	19.5 ± 1.8

Note. Bile salt analysis of 15 min periods before (Period 2) and 45 min after (Period 5) injection of test substances. Values are means ± SEM for 10 guinea pig livers. *Asterisks* denote $P < 0.05$ compared to preinjection values.

TABLE II. PERFUSATE SUGAR

	Before	After
Saline	202 ± 16	223 ± 29
Insulin	195 ± 12	202 ± 24
Glucagon	187 ± 10	224 ± 19
Insulin plus glucagon	194 ± 7	197 ± 43

Note. Perfusate sugar concentrations in mg/100 ml before and after experiments. Values are means ± SEM of 10 guinea pig livers.

advantage of this system is that experimental and control livers are perfused concurrently within the same, closed environment, so that undetected perturbations in the system affect both sets of livers equally. The guinea pig liver was chosen because of the large quantity of bile that this species makes relative to liver weight, thereby minimizing potential mechanical errors of bile volume measurement which is a problem with rodents that produce small volumes. Much of the volume is ductular secretion (20), and not canalicular which is the fraction influenced primarily by insulin or glucagon.

In these experiments, glucagon was found to stimulate directly choleresis in the guinea pig species whereas insulin acted in a more subtle fashion. Glucagon caused a significant increase in bile formation in comparison with saline control values. The increased bile flow during glucagon administration was associated with no change in bile salt output indicating that glucagon stimulated a bile salt-independent process. Also, glucagon caused dose-related increments in bile flow with maximal response occurring with 16 μg. The data indicate that glucagon produced choleresis by acting directly upon the bile secretory apparatus. Since the livers were totally isolated and perfused, release of other hormones was not possible and therefore is not necessary to explain glucagon's choleretic action.

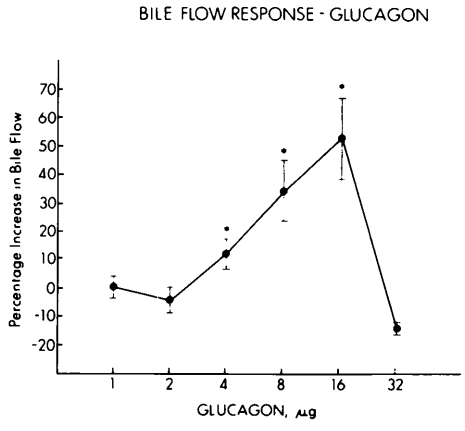

FIG. 5. Bile flow response to injection of varying dosages of glucagon. *Points* represent means ± SEM from four or five livers. *Asterisks* denote $P < 0.05$ compared to saline controls.

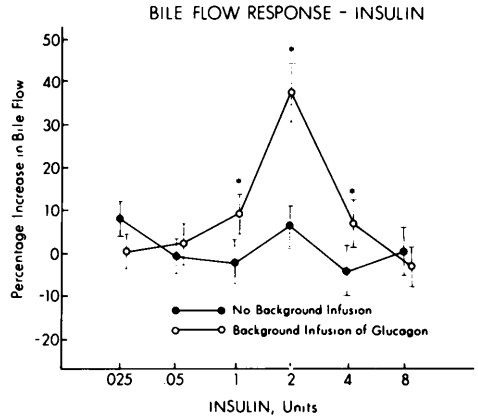

FIG. 6. Bile flow response to injection of varying amounts of insulin with and without a background infusion of glucagon 4 μg/hr. *Points* represent means ± SEM of four or five livers. *Asterisks* denote $P < 0.05$ compared to saline controls.

When insulin was administered to the isolated perfused guinea pig liver, no change in bile flow occurred. Subsequent dose-response studies using dosages from 0.025 through 0.8 units also failed to disclose any significant change in bile flow during insulin administration. However, when insulin was given with glucagon, choleresis occurred. Insulin augmented glucagon's action and insulin caused choleresis when glucagon was present in substimulatory amounts. The fact that the two hormones acting together caused a response greater than the sum of the individual responses and that glucagon was necessary for insulin's choleretic action suggests an interaction or potentiation between the agents. It is not known whether or not the negative dose-response relationships of glucagon at 32 μg or insulin above 0.2 units during background infusion of glucagon represent toxic effects of the drugs at these dosages.

The role of glucose concentrations in the choleretic actions of glucagon and insulin was not investigated. Perfusate sugar concentrations were maintained between 185 and 225 mg%. Therefore, it is unlikely that either the direct choleretic effect of glucagon or the interaction of insulin and glucagon depended upon large changes in blood sugar.

The complex feedback relationships between glucose, insulin, glucagon, and perhaps other hormones makes it difficult to study direct actions of insulin or glucagon *in vivo* (21, 22). When the choleretic action of insulin was first observed in conscious dogs, it was believed due to vagal stimulation by the hypoglycemia (15). Subsequent studies demonstrated that insulin caused choleresis in dogs following vagotomy indicating that the vagus is not a necessary component of insulin choleresis (2). In addition, it was suggested that insulin's choleretic action may be mediated by secretion of other hormones such as glucagon, cortisone, gastrin, etc. Recent studies in anesthetized dogs revealed that either evisceration or adrenalectomy failed to prevent insulin choleresis suggesting a direct action on the liver (16). The present studies suggest that the direct effect of insulin may be more subtle than was suggested in those studies. There are of course rather marked species and methodological differences between the studies. As is common in studies of isolated organ systems, the doses of the substances administered were quite large. The doses of insulin and glucagon used in these studies were on the order of 10 to 1000 times greater than might be expected to be secreted from the intact pancreas (21, 22). Therefore, although the present dosages were appropriate to study mechanisms of pharmacologic actions, the question whether or not insulin or glucagon participate physiologically in the regulation of bile secretion awaits further studies *in vivo.*

The importance of studying the mechanisms of insulin or glucagon choleresis is in part related to its potential physiological significance. *In vivo,* both hormones caused marked increases in the bile salt-independent canalicular fraction of bile secretion (5, 9). This fraction is stimulated by feeding, which also causes the release of these and other choleretic hormones (10–13). Somatostatin, a 14-residue polypeptide found in the gastrointestinal tract and other tissues, inhibits choleresis probably via suppression of choleretic hormones (23). Recently, the stimulation of the bile salt-independent canalicular fraction by insulin and glucagon was found to be more complex than simple dilution by the addition of water and electrolytes; biliary lipid secretion was found to be significantly influenced by these hormones (14).

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