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Gallbladder Mechanics in Newborn Piglets

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ABSTRACT. The mechanical properties of the newborn piglet gallbladder were evaluated in both the stimulated and unstimulated states. The pressure-volume relationships, compliance, and the estimated active tension of the gallbladder were determined in 10 newborn piglets (2–7 days of age). Agonist stimulation was achieved by administration of histamine (25 μg/kg/h) and cholecystokinin (CCK) (60 ng/kg/h). Both histamine and CCK increased the intracholecystic pressure at the 50% resting volume from 12.4 cm H_2O to 18.9 and 15.5 cm H_2O, respectively. This resulted in a significant ($p < 0.05$) increase in the active tension. However, no significant changes were observed in the gallbladder compliance after stimulation. These findings characterize the mechanical properties of the normal neonatal gallbladder. The low magnitude of intracholecystic pressure response to agonist stimulation, when compared to adult data, may explain the occurrence of decreased neonatal choledochal bile flow. (*Pediatr Res* 18:1181–1184, 1984)

Abbreviation

CCK, cholecystokinin

Bile flow into the duodenum is dependent on bile acid excretion as determined by hepatic function, and also on the active contraction of the main biliary storage organ: the gallbladder. Following birth, the neonatal gastrointestinal tract undergoes significant functional and morphological adaptation; the rate of such transition has been considered to be inversely related to gestational age of the neonate at birth (2). Inadequate adaptation of the hepatobiliary system has been implicated in the decreased bile excretion (10), inefficient fat digestion (2), and propensity to cholestasis observed in the preterm neonate (7, 13). Several investigators have studied and quantitated the decreased synthesis and secretion of bile acids from hepatic canaliculi and the consequent reduction of the bile acid pool in full term neonates (7, 11, 12, 14). The ultimate excretion of bile into the duodenum also is dependent on the contractile ability of the gallbladder. Sufficient intraluminal pressure needs to be generated during active contraction to overcome the resistances offered by the common bile duct and the sphincter of Oddi. Thus, the mechanical properties of the gallbladder and its response to agonist stimulation play a major role in choledochal bile flow. Presently, limited data are available on the functional behavior of the neonatal gallbladder (5).

The mechanical performance of the gallbladder and its ability to attain adequate intraluminal pressures are best evaluated by quantitating the mechanical properties of this organ in both the unstimulated and stimulated states (8, 9). These aspects of gallbladder physiology are well documented in adult animal models including guinea pigs (5), opossums (7) and baboons (9). The purpose of this paper is to document and characterize the mechanical properties of the term neonatal gallbladder in an *in situ* piglet model. In addition, the sensitivity of responses to agonist stimulation are assessed by applying biomechanical theories. These data will be useful in understanding the physiology of neonatal cholecystic mechanics and factors which govern choledochal bile flow.

MATERIALS AND METHODS

Animal preparation. Experiments were performed in 10 newborn piglets; the postnatal age ranged from 2 to 7 days and the mean weight was 2280 ± 260 g (SEM) at the time of the study. The animals were in a fasting state of 4 to 6 h duration prior to experimentation. Anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (30 mg/kg). The jugular vein was catheterized for drug infusions and the carotid artery was catheterized to monitor arterial blood gas tension and arterial blood pressure. The trachea was exposed by a midline cervical dissection and cannulated to maintain a stable airway and, when necessary, to provide means of ventilatory support. The gallbladder was exposed at laparotomy through an epigastric incision. The total duration of each study was 3 h.

The contents of the gallbladder were evacuated by a microsyringe through a needle puncture in the fundus and the gallbladder volume in the resting state was recorded. A small incision was made in the fundus to introduce the catheter system. A saline-filled size 5 French polyvinyl catheter with additional side holes was utilized and secured by a pursestring suture. The cystic artery was carefully dissected from the cystic duct in order to maintain blood supply, and the duct was then ligated at the ampulla. The viability of the gallbladder smooth muscle was confirmed by the observation of spontaneous contractions and contractile responses to agonist stimulation throughout the experiment.

The catheter system was connected to a calibrated microsyringe (1 ml × 10 μl) for incremental volume changes and a pressure transducer (Statham 23db) was attached to measure intraluminal pressure (Fig. 1). Volume and pressure changes were monitored on a polygraph Grass recorder. All air bubbles were removed and the system was checked for leaks.

Pressure-volume relationships of the gallbladder. The mechanical properties of the gallbladder were assessed from pressure-volume relationships as previously described (3, 8, 9). With

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gallbladders devoid of any volume and the intraluminal pressure at 0 cm H_2O, increments in gallbladder volume were achieved by introducing 0.1 ml of normal saline by the microsyringe. Concomitant changes in intraluminal pressures were recorded (Fig. 2). Step changes in volume were made at intervals of 20–30 s. This time interval was required to reach a steady state intraluminal pressure. The gallbladder was inflated to a maximum of 20–25 cm H_2O and then deflated in similar volume decrements. The changes in volume and the corresponding changes in pressure were plotted to provide individual *in situ* quasistatic pressure-volume relationships. The slope of the *P-V* curve represents the $\Delta V/\Delta P$, or the quasistatic compliance of the gallbladder (3, 8). For purposes of comparison, the compliance was determined at 50% of the fasting gallbladder volume (V_{50}) as previously described (8, 9).

Stimulation of gallbladder smooth muscle tone. To evaluate and demonstrate changes in gallbladder tone, pharmacological stimulation was induced. Two agents were used: (i) histamine and (ii) CCK. Histamine phosphate was infused intravenously at a rate of 2.5 μg/kg/h. Hormonal manipulation was achieved by an intravenous infusion of CCK (the octapeptide of cholecystokinin; Sinclaide, Squibb Institute for Medical Research) at 60 ng/kg/h infusion. The dosages of these agents were based on previous studies with adult opossum (7, 9) and *in vitro* neonatal guinea pig gallbladders (5). We attempted to achieve maximal gallbladder muscle response, yet with minimal cardiovascular effects. Each agent was diluted in normal saline and administered at 5 ml/kg/h through the jugular venous catheter. The drugs were used as separate infusions in two groups of five animals each. An infusion was commenced 30 min prior to the period of data collection and maintained during this period. The total duration of drug infusion was 90–120 min.

Control pressure-volume relationships were determined in the unstimulated state. These data were collected while the animals had been in a fasting state for at least 4–6 h. Hemodynamic measurements and arterial blood gas tensions following anesthesia and surgery were utilized to ensure the stability of the animal preparation. Experimental data were obtained in the stimulated state.

Estimation of gallbladder active tension following stimulation. The active tension of the gallbladder smooth muscle wall was determined by applying LaPlace's law. The shape of the organ is best assumed as a simplified cylindrical model (6). The length of the gallbladder was measured as the distance between the fundus and the ligature on the cystic duct. Since the measured volume (V) of the gallbladder was known, the average radius (R) was estimated by the volume of the cylindrical model:

$$V = \pi R^2 \text{ length} \quad (1)$$

LaPlace's law for a cylinder states

$$P = T/R \quad (2)$$

where P = intraluminal, or intracholecystic pressure, T = tension on the wall or the sum force of active tension developed by muscular contraction plus the passive tension exerted by the elastic properties of the wall, and R = average radius of the cylinder. The change in the intracholecystic pressure at 50% resting volume of the gallbladder taken from *P-V* curves was determined following agonist stimulation. The increase in the pressure was utilized to calculate the individual change in the gallbladder active tension. The values of the active tension were converted to dynes/cm, such that:

$$\Delta T = 9.8 \times 10^2 (R_{50}) (\Delta P_{50})$$

where R_{50} = average radius of gallbladder at 50% resting volume, ΔP_{50} = change in the intracholecystic pressure at 50% resting volume following agonist stimulation, and ΔT = the estimated change in active tension at 50% resting gallbladder volume following agonist stimulation.

Thus, the estimated increases in active tension were determined following histamine and CCK infusions, respectively. In addition, gallbladder pressure-volume curves and compliance were determined before and during stimulation, and these data were compared by Student's t test for paired values.

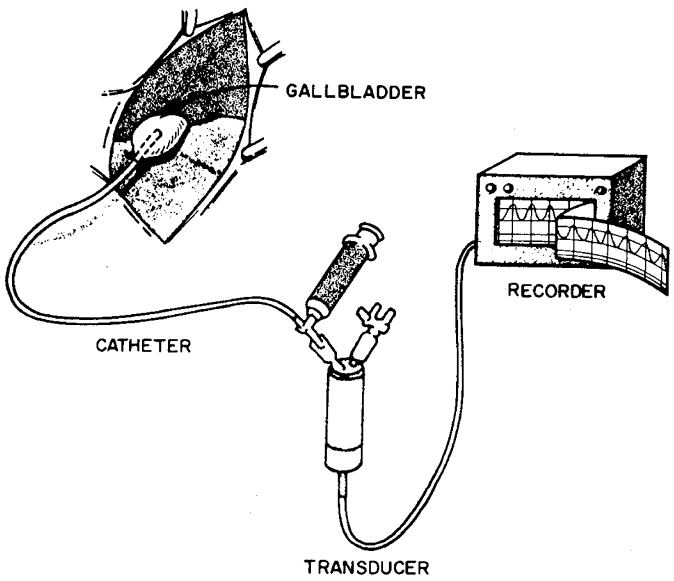

Fig. 1. Schematic of the experimental apparatus.

RESULTS

The neonatal piglet provided a suitable model for *in vivo* experimentation. The animals tolerated anesthesia and surgical procedures with no evidence of metabolic acidosis or hemodynamic instability. Only one piglet required ventilatory support due to respiratory depression from anesthetic narcotization and was included in the study. Mean values of the arterial blood gas tensions were Pa_{O_2} = 69.6 ± 4.8 mm Hg, Pa_{CO_2} = 23.3 ± 1.1 mm Hg, and pH 7.50 ± 0.01 (SEM). These values were obtained while the animals spontaneously breathed room air through a tracheotomy, and thus exhibited an uncompensated respiratory alkalosis.

The mean volume and length of the gallbladder were 1.0 ± 0.06 ml, and 1.3 ± 0.18 cm, respectively. The pressure-volume

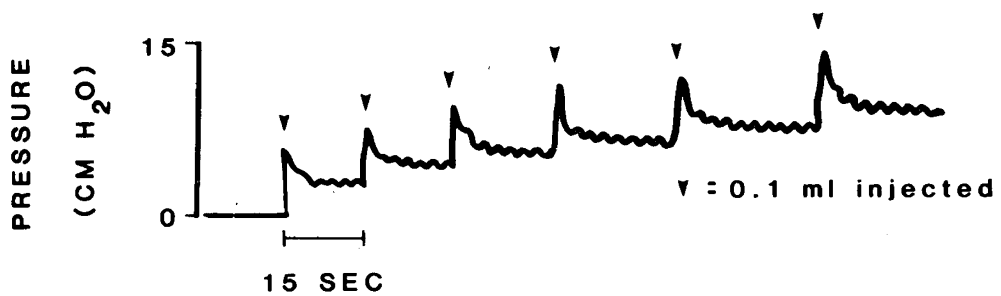

Fig. 2. Typical response to *in situ* pressure change following incremental changes in volume in a 3-day-old newborn piglet gallbladder.

relationships of the porcine gallbladder indicate distinct inflation and deflation limbs with a hysteresis loop. Figure 3 illustrates one such relationship in a 2-day-old piglet. Following an initial surge of intraluminal pressure, the subsequent P-V relationship is relatively linear. Figure 4 illustrates mean ± SEM values of inflation pressure-volume relationships of the gallbladder in both the control and stimulated states for all animals. The gallbladder compliance was determined at 50% resting volume (V_{50}). Mean intracholecystic pressure at V_{50} was 12.4 ± 1.3 cm H_2O and the mean compliance was 0.51 ± 0.006 ml/cm H_2O.

Cholecystic pressure, gallbladder compliance, and increase in active wall tension following agonist stimulation are tabulated in Table 1. Though the changes in smooth muscle tone and subsequent pressure changes are significant ($p < 0.05$), no changes were observed in the gallbladder compliance during either the histamine or CCK infusions.

DISCUSSION

The neonatal biliary system consists of a reservoir and a series of ducts. As such, it is subject to the laws of fluid mechanics. The choledochal bile flow is governed by the intracholecystic pressure and the resistance offered by the bile duct and its sphincter. The ability of the neonatal gallbladder to function as a storage and contractile organ depends on its biomechanical properties. These properties are dependent on the mechanical behavior of all its tissue components. Of these, the active tissue component is smooth muscle and the passive tissue elements are fibroelastic structures.

The properties of the active tissue components have been extensively investigated by *in vitro* experiments. These studies have evaluated the maximal tension in smooth muscle strips and determined their force-generating capacities (4, 5, 15) and dose-response characteristics (5). These data have been invaluable in understanding smooth muscle physiology and in characterizing

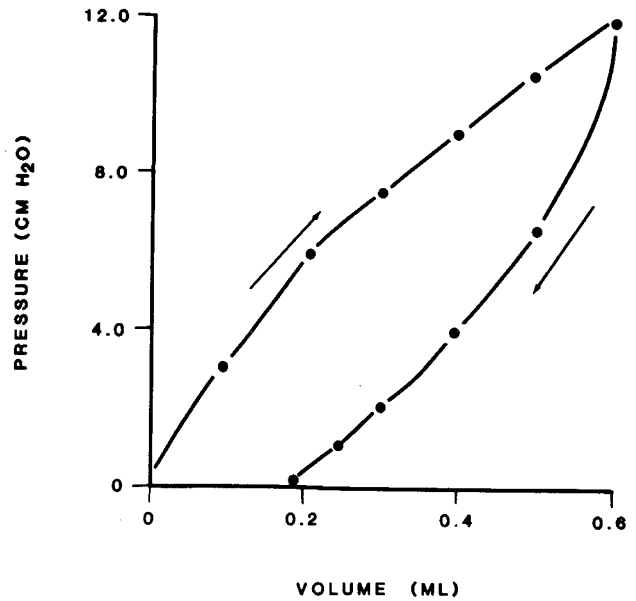

Fig. 3. Representative pressure-volume relationship of a 2-day-old newborn piglet gallbladder in the unstimulated state.

Table 1. *Intracholecystic pressure, gallbladder compliance, and increase in active wall tension after agonist stimulation**

	Histamine (2.5 μg/kg/h)	Cholecystokinin (60 ng/kg/h)
Intracholecystic pressure (cm H_2O)	18.9 ± 2.4†	15.5 ± 2.7†
Compliance (ml/cm H_2O)	0.049 ± 0.005	0.048 ± 0.005
Active tension (dynes/cm)	2.3×10^3†	1.1×10^3†

* Mean ± SEM values; values computed at 50% resting volume.
† Significance of data as compared to control values; $p < 0.05$.

developmental changes in the neonatal gastrointestinal tract function.

The contractile ability of the gallbladder following agonist stimulation can be assessed by the increase in the intracholecystic pressure and the wall active tension. These are the major factors in producing choledochal bile flow and would be influenced by Poiseulle's law.

$$\dot{V} = \Delta P \pi r^4 / 8 L \eta \quad (3)$$

where $\dot{V}$ is the flow of bile per unit time, ΔP is the pressure gradient, π is 3.1416, r is the radius of the bile duct lumen, L is the length of the duct, and η is the viscosity of the bile. Thus, biliary flow is further dependent on the viscosity of the bile and the lumen size of the common bile duct. Alterations in biliary viscosity as a function of developmental age are not known. However, the lumen of the neonatal bile duct is considerably smaller than that of the adult. Thus, a similar change in the intracholecystic pressure would result in a greater choledochal bile flow in the adult as compared to the flow in the neonatal bile duct. The adult opossum gallbladder appears to generate a larger magnitude of intracholecystic pressure; stimulation with CCK (25 ng/kg/h) resulted in an increased intraluminal pressure from 20.5 to 32.5 mm Hg, (58% increase) above the resting intraluminal pressure (8). In comparison, we found that the intracholecystic pressure increased from 12.4 to 15.5 cm H_2O (25%) in the newborn piglet. Thus, the pressure is lower at similar gallbladder volumes and the increase in pressure subsequent to stimulation is less in the neonatal than in the adult gallbladder. These data suggest that adequate intraluminal pressure is probably not generated to overcome the choledochal resistance in the neonate.

The biomechanical behavior of an entire organ has been conventionally assessed by pressure-volume relationships (1, 3). The determination of the compliance and hysteresis of the relationships has been used to characterize the active and passive tissue components in terms of the elastic and viscoelastic behavior, respectively. These determinations have been made in *in situ* adult gallbladders; the mechanical properties of the neonatal gallbladder have not yet been characterized in the literature. Our study determines the neonatal gallbladder compliance to be 0.051 ± 0.006 ml/cm H_2O. The dynamic compliance of the gallbladder of the adult model ranged from 0.66 to 0.79 ml/mm Hg (or, 0.50 to 0.60 ml/cm H_2O). The quasistatic compliance of the adult opossum gallbladder calculated from the data presented by Ryan and Cohen (8) was equivalent to 0.143 ml/cm H_2O. Both studies suggest a 3- to 12-fold more compliant gallbladder in the adult when compared to neonate.

The dose-response characteristics to agonists acetylcholine and cholecystokin have been evaluated in fetal and neonatal guinea pig gallbladder muscle strips (5). These *in vitro* studies indicate that both cholinergic and CCK receptors are present and functioning in the neonate and that the maximal tension or force generating capacity of the gallbladder increases with developmental age. The force generating capacity of the *in vivo* gallbladder can be evaluated by applying LaPlace's law for a cylinder. The adult gallbladder has been previously assumed as a cylinder to calculate its volume during ultrasonic evaluation (6). This assumption documents a significant increase in neonatal cholecystic active tension. The increased active tension of the smooth muscle wall would not only be reflected as an increased intracholecystic pressure but also by altered mechanics. Ryan and Cohen (7) and Schoetz *et al.* (9) have both observed a significant reduction in adult cholecystic compliance following agonist stimulation.

The effect of agonist stimulation was further quantitated by determining the hysteresis in loop area of the pressure-volume relationships by Schoetz *et al.* (9). They documented a 4-fold increase in the loop area and associated this observation with a reduction in cholecystic compliance. In our newborn model, in spite of increased wall tension, a similar decrease in wall com-

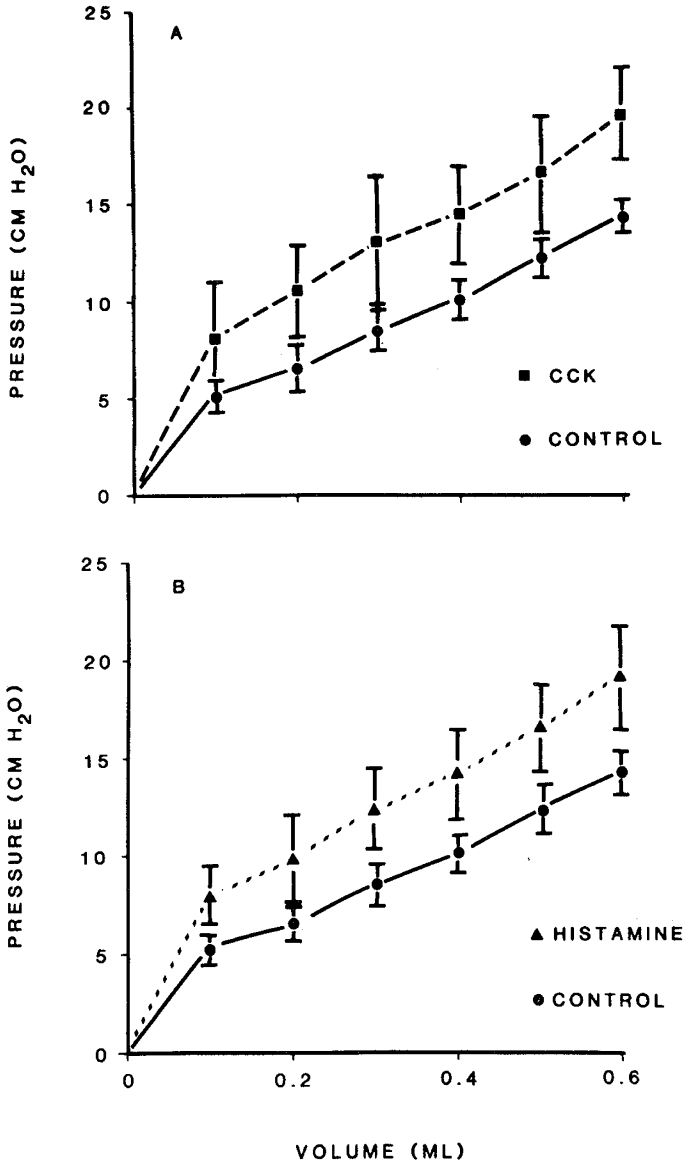

Fig. 4. Mean ± SEM values of inflation limbs of 10 neonatal piglet gallbladders in stimulated states; during (*A*) histamine, (*B*) CCK administration, and as compared to control values.

pliance was not observed. These findings suggest that neonatal gallbladder smooth muscle is responsive to both histamine and CCK; however, sufficient muscle mass may not be present to lead to appreciable changes in gallbladder compliance.

In conclusion, we have characterized the functional behavior of the neonatal gallbladder and its responses to agonist stimulation. Although the mechanical behavior of the neonatal gallbladder is similar to that of adults, it appears to be less compliant. There is also an indication that the lower intracholecystic pressure and lesser response to agonist stimulation are related to its decreased active tissue components. These data offer probable mechanisms for decreased neonatal choledochal bile flow.

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