Technique for Assessment of Local Effects of Substances Found in Bile upon Opening Pressure of Choledochoduodenal Junction

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Abstract □ An assessment of the localized influences of various chemicals found in bile was made by applying them to the interior of the canine choledochoduodenal junction. Test agents were isolated by air bubbles and introduced into the terminal lumen of the intramural portion of the common bile duct *via* the pressure measurement catheter; they remained in the duct for approximately 1.5 min. The system was flushed and opening pressures were then measured. Responses were measured in terms of alterations in ductal opening pressures generated by a linear pressure ramp. Histamine, serotonin, and bethanechol markedly increased ductal opening pressures, whereas epinephrine and norepinephrine decreased opening pressures. None of the agents affected either cardiovascular or small intestinal motor activity in the vicinity of the sphincter when administered in this manner. The results suggest that the presence in bile of certain neurohumoral transmitters and neurohumoral-like agents may directly affect the canine choledochoduodenal sphincter function.

Keyphrases □ Choledochoduodenal junction, canine—effect of neurohumoral transmitters and neurohumoral-like agents present in bile on opening pressure of choledochoduodenal sphincter □ Bile neurohumoral transmitters and neurohumoral-like agents—technique for determining effect on opening pressure of choledochoduodenal junction, dogs

Experimentally, it has been difficult to evaluate discrete drug-induced effects on the sphincter of Oddi *in situ* without encountering the disturbing influence of the surrounding smooth musculature of the duodenum. For anatomical reasons (1), this difficulty has been found to be particularly true in the dog.

The effects of intravenous and intraarterial injections of drugs on the canine sphincter of Oddi have received much attention, but repeated results have sometimes been inconsistent. Presumably, the reason for injecting drugs into the arterial supply to the terminal bile duct is to affect the sphincter of Oddi selectively. However, because of the close anatomical relation between the terminal bile duct and the duodenum, it is probable that an injected drug would reach the surrounding duodenum even though it reached the terminal bile duct first. Of course, drugs injected intraarterially also may exert their effects on the sphincter of Oddi indirectly by affecting the vasculature.

An alternative way of selectively affecting the sphincter of Oddi would be to introduce drugs into the lumen of the terminal bile duct. This proposal is in conformity with an interesting series of experiments on the action of some antibiotics on the extrahepatic biliary tract (2–5). In one experiment (3), intraductal erythromycin increased the motility and tone of the terminal bile duct in the guinea pig, cat, dog, and monkey. In subsequent experiments (6), intraductal aminosiderin decreased the motility and tone of the normal and hypertonized choledochus in the same species of animals. The consensus was that antibiotics eliminated in the active form in the bile can interfere with both the tone and motility of the extrahepatic biliary tract.

Published work so far has dealt primarily with intraductal antibiotics. However, biliary excretion into the lumen of the intestine is a major pathway of elimination of a whole host of drugs and drug metabolites. An excellent summary of the literature data relating to biliary excretion of drugs in animals and humans is available (7).

It was considered worthwhile to determine whether various normal constituents of bile, as well as pharmacological agents known to be excreted in the bile, can produce a discrete, localized effect upon the choledochoduodenal junction, which may or may not be similar to their action when administered intragastrically or systemically.

EXPERIMENTAL

Animals and General Procedure—Thirty-four adult mongrel dogs were used. All experiments were undertaken after the animals had been fasted overnight with water *ad libitum*. The animals were anesthetized at first with intravenous injections of thiopental sodium[1] (25 mg/kg), followed by maintenance with a mixture of α-chloralose (5%, dissolved in polyethylene glycol 200) and urethan (50% in 0.9% saline solution). Further injections of the chloralose–urethan mixture were given when necessary.

Surgical and Recording Procedure—Along with a tracheotomy, a thoracotomy and laparotomy were performed in each dog through a long midline incision. A tracheal cannula was routinely inserted. Systemic pressure was measured from a cannula inserted into a femoral artery. Through an ileostomy, an open-tip catheter with a balloon attachment was introduced into the upper jejunum. For recording biliary flow, another catheter was inserted into one hepatic duct toward the liver and the other end was connected to a photoelectric cell drop counter.

Measurements of sphincter opening pressures were conducted as follows. A polyethylene catheter was inserted into the common bile duct *via* the fundus of the gallbladder. The end of the catheter was advanced through the duct toward the duodenum until palpation indicated that it was *juxta* the sphincter of Oddi. The catheter was secured by tying it at its insertion into the fundus of the gallbladder. A longitudinal duodenotomy was performed at a point opposite the opening of the choledochoduodenal junction to permit visualization of the sphincter.

Duodenal stabilization was effected in such a manner that the sphincter opening could be completely immersed in a pool of clear saline. The perfusion system was filled with warmed normal saline containing a blue food dye. It was thus possible to confirm an opening of the sphincter by direct visualization of a thin stream of blue fluid in the clear pool of saline covering the aperture. The

[1] Pentothal.

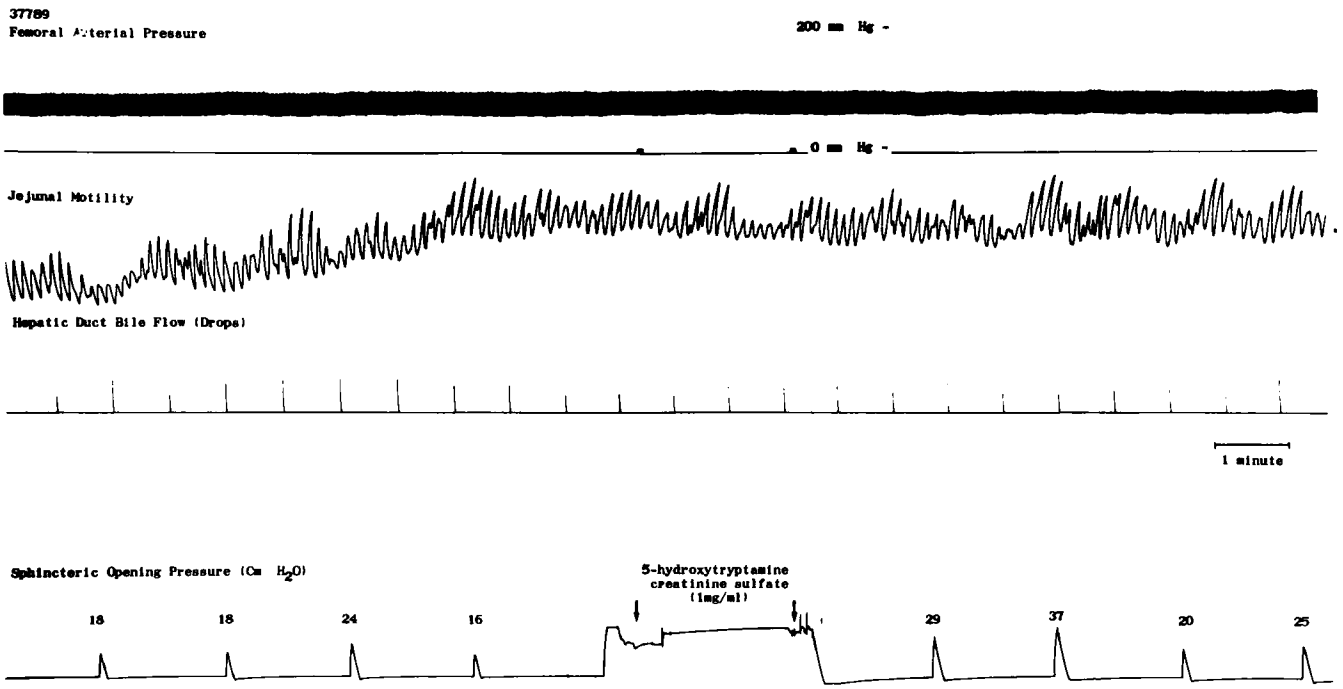

Figure 1—*Polygram depicting the time course of femoral arterial blood pressure, intrajejunal pressure activity, bile flow rate as measured with a drop recorder, and ductal opening pressure in a bilaterally vagotomized dog. Serotonin was introduced into the terminal bile duct during the period indicated by arrows. The extraductal events were not altered during or following drug administration. The pressure limb rise and plateau configuration seen in this figure represent the measurement system pressure during the introduction and subsequent stasis of a test agent. The drop in pressure limb represents the rapid washout of the small fluid volume (0.2 ml) and the reversal of the infusion pump.*

dyed saline flowing through the choledochus was drained outside the duodenum by a large outflow tube inserted into the duodenal lumen.

The perfusing catheter was connected to a constant-flow infusion pump through a "T" tube. The side arm was connected by valves to a fluid reservoir, pressure transducer, and tall manometer tube. The pressure transducer was simply calibrated in terms of the height of the column of saline in the manometer tube. In essence, the compliance represented by the fluid column is so large with respect to the lumped compliances of both the terminal common duct and the polyethylene catheter that the entire system compliance is substantially that of the manometer. Thus, infusion at a constant flow results in a linear rate of pressure rise in the duct.

Initial experiments (8) had shown that a pressure ramp value of 7 cm/sec was satisfactory. This value is in substantial agreement with that utilized previously (9, 10). Femoral blood pressure, upper jejunal pressure, hepatic duct flow, and sphincter opening pressures were recorded with an ink-writing polygraph.

Chemicals—The following were used: histamine dihydrochloride[2], epinephrine hydrochloride[2], norepinephrine hydrochloride[2], 5-hydroxytryptamine creatinine sulfate[2], magnesium sulfate[2], papaverine[2], secretin[2], bethanechol chloride[3], morphine sulfate[3], lidocaine hydrochloride[4], penicillin G potassium[5], cephaloridine[5], chloramphenicol succinate[6], rifampin[7], and cholecystokinin[8].

All drugs were administered intraductally. Drugs were introduced into the region of the sphincter by administration through the pressure measurement catheter and allowed to remain for a definite period. Both ends of the solution column were marked by air bubbles. It was thus possible to determine when the drug arrived at its intended site of action, because the blue marker dye abruptly disappeared. Similarly, removal of the test agent was also clearly marked by reappearance of the dye marker. Drug sequences were randomized among the various experiments. Following intraductal stasis, each drug was flushed from the ductal lumen with copious amounts of saline to ensure that chemical or functional interactions would be minimized.

Each series of experimental opening pressure determinations following drug administration was immediately preceded by a control series of determinations. Mean opening pressures for each posttreatment series were statistically compared to the mean values of the preceding pretreatment values under the null hypothesis that mean posttreatment opening pressures were the same as pretreatment values. The alternative hypothesis stated that posttreatment mean values were different, with rejection of the null hypothesis being made at the 5% level of confidence for a two-tailed test.

RESULTS AND DISCUSSION

Figure 1 is a complete polygram depicting arterial blood pressure, intrajejunal pressure activity, and biliary flow preceding, during, and following the intraductal administration of serotonin. It can be readily seen that these parameters were unaffected. Sphincter opening pressures measured after washout of the drug were increased.

Table I summarizes the mean opening pressures observed prior to and subsequent to the intraductal administration of histamine, norepinephrine, epinephrine, serotonin, and bethanechol. Histamine, serotonin, and bethanechol administrations were subsequently associated with elevated mean opening pressures, while norepinephrine and epinephrine were associated with subsequently lowered mean opening pressures. In all cases, the differences in mean values were significant.

The data shown in Table I were obtained from polygrams that also recorded vascular activities and jejunal pressure activity as depicted in Fig. 1. Although not shown, these polygrams revealed that in no case was the intraductal administration of any of these agents effective in altering either ongoing cardiovascular activity or intestinal motor function in the near vicinity of the choledochoduodenal junction.

Preliminary experiments with other agents revealed that rifampin (12 trials in two dogs) was also associated with apparent in-

[2] Sigma.
[3] Merck.
[4] Astra.
[5] Lilly.
[6] Parke-Davis.
[7] Ciba.
[8] Karolinska Institute.

Table I—Comparison of Mean Forward Ductal Opening Pressures after Intraductal Drug Administration

Dog Number[a]	Pretreatment Opening Pressures[b]	Posttreatment Opening Pressures
Histamine Dihydrochloride (0.65 mg/ml)		
37600	16.8 ± 2.99 (6)	26.6 ± 2.96 (7)
36557	14.9 ± 2.73 (7)	30.0 ± 4.36 (6)
38230	18.8 ± 3.60 (6)	29.2 ± 2.06 (6)
38236	14.9 ± 3.18 (7)	25.0 ± 2.25 (9)
36672	15.7 ± 1.50 (12)	29.3 ± 3.37 (6)
36489	12.2 ± 2.36 (10)	28.4 ± 2.85 (10)
11880	16.1 ± 4.84 (14)	38.2 ± 3.91 (6)
11949	15.3 ± 2.83 (6)	27.5 ± 3.02 (6)
Norepinephrine Hydrochloride (0.45 mg/ml)		
37600	20.3 ± 2.87 (7)	10.3 ± 2.97 (6)
37604	20.3 ± 2.89 (8)	13.4 ± 2.64 (8)
67491	18.4 ± 3.78 (6)	11.8 ± 1.71 (6)
36557	31.7 ± 7.66 (6)	14.7 ± 2.81 (6)
11880	20.6 ± 3.01 (6)	13.8 ± 2.38 (8)
Epinephrine Hydrochloride (0.45 mg/ml)		
39593	19.0 ± 1.26 (6)	11.7 ± 1.51 (6)
37789	21.4 ± 4.90 (8)	13.8 ± 2.40 (6)
37600	17.2 ± 2.56 (6)	10.6 ± 1.26 (8)
11949	19.5 ± 3.39 (6)	12.2 ± 2.36 (8)
11411	24.5 ± 3.99 (6)	12.8 ± 2.10 (6)
5-Hydroxytryptamine Sulfate (1 mg/ml)		
37789	18.2 ± 2.82 (12)	27.7 ± 1.74 (8)
11880	19.0 ± 2.36 (8)	28.3 ± 2.16 (6)
67491	16.3 ± 3.92 (8)	26.5 ± 4.97 (6)
36218	16.0 ± 0.63 (10)	25.5 ± 1.52 (8)
Bethanechol Chloride (5 mg/ml)		
11411	23.8 ± 2.35 (10)	86.8 ± 14.40 (8)
14788	17.8 ± 3.43 (8)	80.7 ± 8.94 (6)
44599	18.6 ± 4.71 (6)	90.8 ± 11.35 (8)
37600	26.3 ± 3.25 (8)	79.1 ± 6.47 (8)

[a] Difference in mean opening pressures are significant in each experiment. [b] Opening pressure measurements are in centimeters of saline; ± indicates 1 *SD*; parentheses contain number of observations.

creases in opening pressures. The following agents did not appear to produce any qualitative changes in sphincter opening pressures when administered intraductally: lidocaine (18 trials in two dogs), magnesium sulfate (18 trials in two dogs), papaverine (18 trials in two dogs), morphine sulfate (22 trials in five dogs), cephaloridine (12 trials in two dogs), penicillin G potassium (12 trials in two dogs), chloramphenicol (12 trials in two dogs), secretin (18 trials in four dogs), and cholecystokinin (15 trials in two dogs).

A histological description of the terminal ductal mucosa probably illuminates the observations made with respect to the marked elevation of mean opening pressures produced by the intraductal administration of histamine and bethanechol. According to Bhatnagar *et al.* (11), the mucosal coat of the interior of the canine common bile duct is continuous with the lining membrane of the hepatic ducts and gallbladder and also with that of the duodenum. The epithelium is of columnar variety.

This description is characteristic of highly vascularized tissue, and the effect noted in the present study was probably due to a parasympathomimetic edematous effect which is similar to the increase in upper airway resistance common in such reactions as allergic rhinitis. The inhibitory effect of intraductal norepinephrine, and to a lesser extent epinephrine, was probably produced by local vasoconstriction, a phenomenon that is analogous to shrinking the edematous nasal membrane with this type of agent.

The intraductal administration of serotonin also significantly elevated mean opening pressures. Although the functional significance of this observation is not at all clear, the observation itself again points directly to the as yet baffling question of just what is the functional significance of the serotonin content of the large enterochromaffin cell population of the intramural portion of the common duct or, for that matter, of the pancreatic duct (12).

A few of the remaining results were generally supportive of data collected previously by other investigators, and only a few points require discussion. The observation that the intraductal administration of lidocaine was ineffective in altering mean opening pressures supports the observations of McCarthy and Picazo (13) in humans. The observation that rifampin tended to increase ductal opening pressures is in agreement with the results of Benzi *et al.* (3), who noted that intraductal administration of rifampin (rifamycin SV) induced a hypertonus of the canine choledochus.

In light of these findings, it is believed that intraductal administration of various test agents may have revealed an interesting facet to the evaluation of various experimental observations relative to ductal control mechanisms. Thus, the observation that intraductal administration of cholecystokinin has no effect upon mean ductal opening pressures is in opposition to the experimental findings of others that intravenous administration of the same substance lowers mean opening pressures. However, intravenous administration of cholecystokinin also causes relaxation of the duodenal musculature in the region of the ampulla (14). Undoubtedly, the relaxing action of cholecystokinin on the duodenum would increase flow through the terminal duct in this species. The difference in effects accompanying the administration of an agent by different routes indicates that a procedure that introduces an agent *via* a route permitting widespread systemic action is dangerous if the purpose of the study is to determine whether a highly discrete and localized effect does occur *in situ*.

The same argument may be advanced with respect to the systemic administration of other agents such as morphine or epinephrine, especially when the *in vivo* and *in vitro* sphincter effects are already known to be different. For example, according to Persson (15), *in vivo* morphine is active locally on the cat choledochoduodenal junction but inactive when added to the bath fluid of the isolated feline sphincter of Oddi. On the other hand, isolated spiral strips cut from the terminal bile duct respond to epinephrine by contracting (16). However, when the terminal bile duct remains surrounded by the wall of the duodenum into which it is inserted, the action of intravenous epinephrine seems to depend on the tone and motility of the duodenum (17).

CONCLUSIONS

It appears that the intraductal technique of administering drugs permits a very localized and quantitative estimation of the effects of various test agents on the tone and motility of the choledochoduodenal junction. This technique may eventually prove of value in the evaluation of the effects of therapeutic agents and/or their metabolites that are secreted in the bile if it is applied systematically, as part of well-defined protocols, so that adequate experience can be obtained to permit a judgment of its merit.

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Binding Study of Sulfonylureas and Phenothiazines to Bovine Serum Albumin Using Difference Spectrophotometry

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Abstract □ 2-(4′-Hydroxybenzeneazo)benzoic acid is a spectrophotometric probe which shows absorption spectrum changes upon binding to protein. Difference absorption spectra of this probe were used as an indirect measurement of the binding of selected sulfonylurea and phenothiazine drugs to bovine serum albumin. The results obtained using the spectrophotometric probe were similar to data obtained from other methods, especially fluorescent methods. Of the four sulfonylureas studied, tolbutamide showed the highest binding affinity, followed by glyburide, glipizide, and acetohexamide, in that order. The data collected for phenothiazine drugs indicated that chlorpromazine has the highest affinity, followed in order by trifluoperazine, perphenazine, fluphenazine, and promazine. Correlation of these results with chemical composition indicated that the interaction of phenothiazine drugs with bovine serum albumin was of a hydrophobic nature.

Keyphrases □ Sulfonylureas and phenothiazines—binding to bovine serum albumin, difference spectrophotometry using 2-(4′-hydroxybenzeneazo)benzoic acid □ Phenothiazines and sulfonylureas—binding to bovine serum albumin, difference spectrophotometry using 2-(4′-hydroxybenzeneazo)benzoic acid □ Spectrophotometry, difference—determination, binding of sulfonylureas and phenothiazines to bovine serum albumin using 2-(4′-hydroxybenzeneazo)benzoic acid □ 2-(4′-Hydroxybenzeneazo)benzoic acid—used to determine binding of sulfonylureas and phenothiazines to bovine serum albumin, difference spectrophotometry

The phenomena of drug–protein binding and of competitive binding of drugs for available protein sites have been the subjects of many investigations and have been reviewed (1). Various experimental procedures and analysis methods have been used to study drug–protein interactions. These include equilibrium dialysis (2), ultrafiltration (3), gel filtration (4), NMR rate measurements (5), and fluorescence techniques (6).

Compared to dialysis or ultrafiltration techniques, spectrophotometric and/or fluorescence probe techniques are capable of providing data similar to what could be obtained through dialysis or ultrafiltration studies but are less time consuming, simpler, and more reproducible. It also may be possible to estimate the nature of binding and binding sites from the chemical structure and spectral properties of the probes used (7).

In spite of their usefulness in protein binding studies, fluorescence probes cannot be used successfully in certain instances such as the lack of a fluorescence change upon binding and/or a change of fluorescence due to a mechanism other than binding (*e.g.*, photoxidation).

Although fluorescence probes have been explored in recent years, little information has been generated concerning spectrophotometric probes. In 1968, Moriguchi and coworkers (8–11) studied the binding of the 2-(4′-hydroxybenzeneazo)benzoic acid probe to bovine serum albumin. Recently, Nazareth *et al.* (12) used this probe as an agent that reflects the binding of L-thyroxine to serum albumin. However, in this study they employed ultrafiltration to separate the free probe from serum albumin to calculate the ratio of bound drug to protein.

The usefulness of spectrophotometric probes in the study of the binding of drugs to protein has not been adequately explored. In addition, certain procedures and methods of data treatment reported for fluorescence probes (13, 14) appear to be useful for the spectrophotometric probe technique. This study was made to explore the usefulness of 2-(4′-hydroxybenzeneazo)benzoic acid as a spectrophotometric probe in studying the interaction of sulfonylurea and phenothiazine drugs with bovine serum albumin and to define, if possible, the binding parameters of some of these drugs.

EXPERIMENTAL

Materials—Bovine serum albumin[1] (crystalline), 2-(4′-hydroxybenzeneazo)benzoic acid[2], tolbutamide[3], acetohexamide[4], glipizide[5], glyburide (glibenclamide)[3], chlorpromazine hydrochloride[6], trifluoperazine hydrochloride[6], promazine hydrochloride[7], perphenazine[8], and fluphenazine hydrochloride[8] were used as obtained without further purification. The solvents used were spec-

[1] Nutritional Biochemical Corp., Cleveland, Ohio.
[2] Aldrich, Milwaukee, Wis.
[3] The Upjohn Co.
[4] Eli Lilly and Co.
[5] Instituto Carlo Erba Per Richerche Therapeutiche, Italy.
[6] Smith Kline and French Corp.
[7] Wyeth Laboratories.
[8] Schering Co.