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Method for evaluating spasmolytic activity of drugs on the bile duct

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A method has been described for examining the spasmolytic activity of drugs on the bile duct of the guinea-pig *in situ*. The terminal bile duct was pulled away from its insertion into the duodenum and perfused with Tyrode solution containing either carbachol or barium chloride to produce spasm. Injected intravenously, atropine methonitrate, oxyphencyclimine hydrochloride, tropenziline bromide, phenetamine, papaverine hydrochloride or diprophylline were spasmolytic.

DRUGS may affect flow through the terminal bile duct by acting on the bile duct itself or by acting on the musculature of the duodenum where it surrounds the orifice of the duct (Crema, Berté, Benzi & Frigo, 1963; Benzi, Berté, Crema & Frigo, 1964). To test a drug for its effect in relieving biliary spasm, the bile duct must be made to contract; morphine, codeine, bethanechol or barium chloride, given parenterally or infused through the duct, have been used to produce this contraction. However, these drugs also cause contraction of the duodenum, particularly in the dog or cat, because of the anatomical arrangement of the terminal portion of the duct within the duodenal wall (Boyden, 1937). In man, the pathological conditions of the biliary tract requiring the use of antispasmodic drugs are mainly restricted to the biliary tree without involving the duodenum. Therefore it is desirable to test drugs with potential therapeutic use in alleviating biliary spasm by a method in which alterations in duodenal tone do not interfere with flow through the bile duct. The guinea-pig provides a suitable preparation of the bile duct, which is described in this paper together with observations on the effects of drugs on flow through the duct.

Experimental

METHODS

Guinea-pigs were anaesthetized with a mixture of urethane (1·0 g/kg) and chloralose (20 mg/kg) injected intraperitoneally, and given artificial respiration. Blood pressure was measured through a cannula inserted into a carotid artery. 80 animals were used.

The anatomy of the terminal bile duct in the guinea-pig is shown in Fig. 1 P. The terminal portion of the duct expands into an oval pouch lying adjacent to the duodenum, and a small duct passes from this through the duodenal wall. The smooth muscle fibres of the bile duct are abundant and are arranged in 3 layers (Higgins, 1927). The experimental arrangement is shown diagrammatically in Fig. 1. The abdomen was opened and an inflow cannula was inserted through the gall bladder into the cystic duct and tied immediately below Lütken's sphincter. The hepatic ducts were ligated caudally and partially excised to allow leakage

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of bile which was removed by suction. The oval pouch of the terminal bile duct was dissected free from the peritoneum and the small duct was gently pulled out from the duodenum. The hole in the duodenum was ligated. The slight bleeding from the free end of the duct sometimes stopped in a few min, but occasionally continued throughout the experiment without affecting the results.

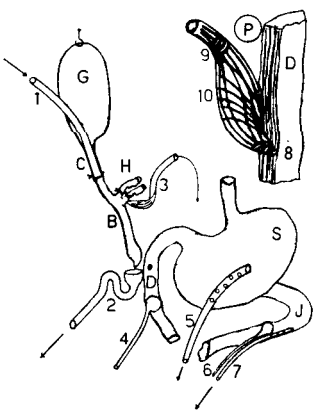

FIG. 1. Diagram of the experimental arrangement. B, bile duct. C, cystic duct. D, duodenum. G, gall bladder. H, hepatic ducts. J, jejunum. S, stomach. 1, inflow-tube. 2, collecting-tube. 3, collecting-tube connected with a suction pump. 4, 6, rubber balloon. 5, 7, drainage tubes. P is a sketch of terminal bile duct in the guinea-pig. 8, sphincter papillae. 9, sphincter choledochus. 10, ampulla's musculature.

Tyrode solution from a Mariotte bottle was perfused through the inflow cannula. The rate of flow was measured with a drop counter inserted between the bottle and the duct. The pressure necessary to produce a flow of about 0·1 ml/min was from 3 to 6 cm of water in the untreated duct and from 6 to 12 cm of water in the presence of drugs producing biliary spasm. The outflow from the duct was collected in a funnel and drained away outside the abdomen.

Movements of the duodenum and the jejunum were recorded by inserting water-filled rubber balloons into the lumen. A drainage tube was inserted into the stomach.

Spasm of the bile duct was produced by adding either barium chloride (30 μg/ml) in the presence of atropine (0·1 μg/ml) or carbachol (0·01 μg/ml) to the Tyrode solution perfusing the duct.

The following drugs were tested for their ability to relive biliary spasm: papaverine hydrochloride, diprophylline and 2-(α-cyclohexylbenzyl)-1,3-di(diethylamino)propane (phenetamine), each in M/100 solution, and atropine methonitrate, oxyphencyclimine hydrochloride, and *O*-benzil-oyl-7-methoxy-*N*-methyltropinium bromide (tropenziline bromide), each in M/1000 solution. The doses are expressed as ml of these solutions injected intravenously.

Results

PRODUCTION OF BILIARY SPASM

Barium chloride or carbachol perfused through the bile duct caused constriction and decreased the flow without changing the blood pressure or the motility of the intestine. Obviously, when these drugs were injected intravenously, they had effects on blood pressure and caused an increase in intestinal motility as well as constricting the bile duct.

In a typical experiment in which the bile duct was constricted by perfusion with 0·01 μg/ml of carbachol, the flow was reduced to about 25% of that occurring in the control period. There were no effects on the motility of the duodenum or the jejunum or on the blood pressure. An intravenous injection of 0·5 μg/kg of carbachol caused a reduction in the flow through the duct to about 30% of that in the control period, and caused increases in pressure and activity in the duodenum and jejunum, and a small fall in blood pressure.

The response of the bile duct to an intravenous injection also showed that the blood supply was not severely impaired, even though it had been pulled away from the duodenum.

RELIEF OF BILIARY SPASM

The effects of intravenous injection of papaverine, diprophylline and phenetamine were examined in relaxing bile ducts which had been put into spasm by perfusing with Tyrode solution containing barium chloride. Fig. 2A shows the log-dose response lines for three doses of each of these drugs. Phenetamine had almost twice the potency of papaverine, and diprophylline had one-tenth the potency of papaverine.

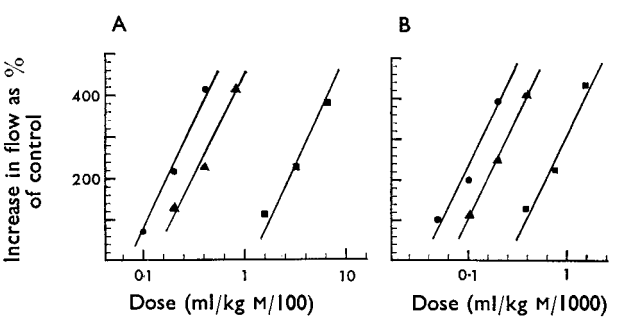

FIG. 2. Log dose-response curves for A, phenetamine (●), papaverine (▲) and diprophylline ■; B, atropine (●), oxyphencyclimine (▲) and tropenziline (■). Estimated relative potencies with 95% confidence limits: papaverine, 1, phenetamine 1·79 (1·49–2·16); diprophylline 0·10 (0·09–0·11); atropine, 1; oxyphencyclimine, 0·57 (0·56–0·58); tropenziline, 0·16 (0·15–0·17).

The effects of intravenous injection of atropine, tropenziline and oxyphencyclimine were examined in relaxing bile ducts which were constricted by perfusing with Tyrode solution containing carbachol.

The results are shown in Fig. 2B. Atropine was the most potent, oxyphencyclimine had slightly more than one-half its potency, and tropenziline had about one-sixth of the potency of atropine. In a typical experiment the injection of 0·1 ml/kg of a M/1000 solution of oxyphencyclimine caused an increase in the rate of flow through the duct to slightly more than double the previous rate. It should be noted that this dose of oxyphencyclimine had no effect on the spontaneous motility of the duodenum or jejunum. However, it was noticed in some experiments that if the fluid from the end of the bile duct was allowed to flow over the duodenum it caused increased motility, and then the antagonistic drugs reduced this in the same doses that relaxed the bile duct.

Discussion

The guinea-pig bile duct *in situ* provides a convenient preparation for the quantitative comparison of drugs useful in relieving biliary spasm. In these experiments, spasm was induced in the duct in two ways. Perfusion with carbachol was used for testing the effects of atropine-like drugs. Perfusion with barium chloride in the presence of atropine was used for testing papaverine-like spasmolytic drugs; atropine was present to prevent the cholinergic component in the response to barium chloride (Feldberg, 1951; Necheles, Scruggs, Kraft & Olson, 1953; Ambache & Lessin, 1955; Della Bella, Gandini & Teotino, 1963). Morphine and other morphine-like drugs have been used by others to induce spasm, but the mechanism of this action is unknown.

Atropine itself was the most potent of the drugs tested against carbachol-induced spasm; tropenziline was less active, as was also reported by Taeschler, Konzett & Cerletti (1960). Phenetamine was the most potent of the other group of spasmolytics, as reported by Levis, Preat & Beersaerts (1960).

It is interesting to note that the bile duct artificially constricted is much more sensitive to antispasmodic drugs than the normal duct and intestinal tract: in fact there is a range of doses for each antispasmodic agent able to relax the hypertonic biliary tract without acting on the intestine. By injecting the spasmogenic agent parenterally and thus producing a spasm in both the organs, previously separated, it is possible to compare the response of the two organs and to study the true selectivity of the drugs.

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