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Effect of Atropine on Rat Basal Pancreatic Secretion during Return or Diversion of Bile–Pancreatic Juice (41723)

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Abstract. The effect of atropine on basal pancreatic exocrine secretion in conscious rats was determined with and without return of bile–pancreatic juice (BPJ) to the intestine. Rats were prepared with cannulas draining bile and pancreatic juice separately and with duodenal cannulas for return of secretions. Experiments were begun 3 days postoperatively. During continuous return of bile–pancreatic secretions, intravenous atropine (100 μg/kg-hr) caused a sustained 80–90% inhibition of pancreatic protein secretion, with a much smaller suppressive effect on pancreatic juice volume. Diversion of bile–pancreatic juice from the intestine markedly increased pancreatic protein and fluid secretion in control and atropine-infused rats, but the response was significantly inhibited by atropine (volume 31% inhibition, protein output 41% inhibition). The results indicate that basal pancreatic protein secretion during return of BPJ is largely maintained by cholinergic influence, possibly due to an enteropancreatic reflex, and that the inhibition of the pancreatic response to acute diversion of BPJ by atropine is probably secondary to inhibition of gastric secretion.

Diversion of bile–pancreatic juice (BPJ) from the intestine or introduction of trypsin inhibitors into the intestine in presence of BPJ stimulates exocrine pancreatic secretion in the rat, and these responses were attributed to a common mechanism, i.e., negative feedback regulation of the pancreas by luminal pancreatic proteases (1). It was proposed that the pancreatic response to trypsin inhibitors was hormonally mediated (1), partly on the basis of the lack of effect of vagotomy or atropine on the pancreatic response (measured indirectly) to trypsin inhibitor in rats (2). However, recent reports indicate that a cholinergic mechanism may be important in feedback regulation of the pancreas by luminal proteases (3, 4). We reexamined this possibility by determining the effect of atropine on pancreatic exocrine secretion during return of BPJ to the intestine and on the pancreatic response to diversion of BPJ to the exterior.

Methods. Male Wistar strain rats (HLA-W), 262–350 g (mean 305 g), obtained from Hilltop Laboratory Animals, Inc. (Chatsworth, Calif.) were maintained *ad libitum* on commercial rat chow. Rats were anesthetized with methoxyflurane (Metafane, Pitman-Moore, Inc.) delivered by a vaporizer. The duodenum was exposed by a midline incision. The bile duct was ligated below the hilum of the liver, and Silastic tubing (Dow-Corning) 0.020 × 0.037 in. (i.d. × o.d.) was inserted above the ligature to collect pure bile. Identical cannulas were inserted into the common duct at the ampulla of Vater to collect pure pancreatic juice, and into the duodenum for returning secretions to the intestine. Details of these operative procedures are described elsewhere (5). Finally, the external jugular vein was cannulated for intravenous (iv) infusion of isotonic saline or atropine.

Experiments were done on the third to seventh day postoperative after an 8- to 12-hr fast. Bile and pancreatic juice were collected separately at 30-min intervals. Volume of pancreatic juice and bile were measured to the nearest 0.01 ml. Ten microliters of pancreatic juice were taken from each collection to measure pancreatic protein concentration by optical density at 280 nm (6) and the remainder returned to the intestine.

To determine the effect of atropine on basal secretion during return of BPJ, secretion was followed for 90 min during iv infusion of isotonic saline at 1 ml/hr, then atropine was infused (100 μg/kg-hr) for an additional 4.5 hr. Controls had BPJ returned throughout. To determine the effect of atropine on hypersecretion induced by BPJ diversion, atropine was infused for 1 hr during return of BPJ and

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for an additional 4.5 hr after BPJ was diverted; 0.05 *M* $NaHCO_3$ was infused intraduodenally at 1 ml/hr to replace bicarbonate. Controls received iv saline throughout. Statistical analyses of results was done by Student's *t* test.

Results. *Effect of atropine on exocrine pancreatic secretion during return of BPJ.* Atropine greatly reduced pancreatic protein output, whereas the inhibitory effect on pancreatic flow was not nearly as pronounced (Figs. 1 and 2). Within 0.5 hr after atropine began, protein output was significantly decreased compared to both pre-atropine infusion values and time-paired control values ($P < 0.001$). Protein output was 15–20% of control values throughout the remainder of the experiment. Pancreatic juice flow rate was not significantly inhibited when compared to pre-atropine infusion values, although it was significantly lower than time-paired control between 2.5 and 3.5 hr.

Effect of atropine on the pancreatic response to diversion of BPJ. Diversion of BPJ from the intestine greatly increased pancreatic protein and fluid output in both groups, but the response was inhibited by atropine (Figs. 3 and 4). Fluid and protein output for control and atropine-infused rats during the entire period of diversion of BPJ were 3.72 ± 0.43 ml vs 2.56 ± 0.18 ml and 189 ± 23 mg vs 111 ± 18.2 mg, respectively (significantly different for both fluid and protein at $P < 0.05$). Two hours postdiversion and thereafter, protein output remained elevated above basal, but was not significantly different between groups. Maximum increase in flow rates occurred between the second and third hours postdiversion and were three- and twofold above basal for control and atropine-treated rats, respectively (significantly different between groups, $P < 0.02$).

The volume of bile was not significantly affected by atropine during BPJ return or diversion.

Discussion. These results are in partial agreement with results reported by Noda *et al.* (4). They reported that atropine (300 μg/kg-hr) inhibited pancreatic fluid and protein output in conscious rats during return of bile and pancreatic juice to the intestine, and that atropine prevented a significant increase in pancreatic secretion in response to diverson

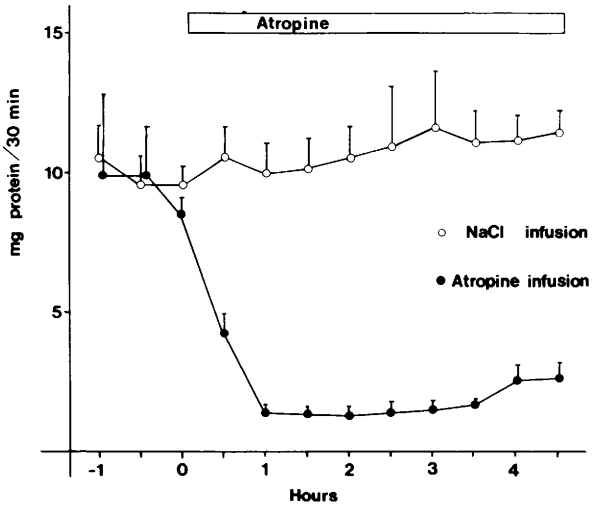

FIG. 1. Effect of atropine on pancreatic protein secretion in conscious rats during recirculation of bile–pancreatic juice (BPJ) into the duodenum. Closed symbols represent iv infusion of 100 μg/kg-hr of atropine, open circles represent iv saline infusion. BPJ was recirculated to the duodenum throughout the experiment. Each point represents the mean of 11 experiments with nine rats in controls (saline-infused) and 5 experiments in five rats with atropine infused. Verticle bars indicate standard error of the mean. All values following atropine infusion are significantly different from pre-atropine values and time-paired control values at $P < 0.001$.

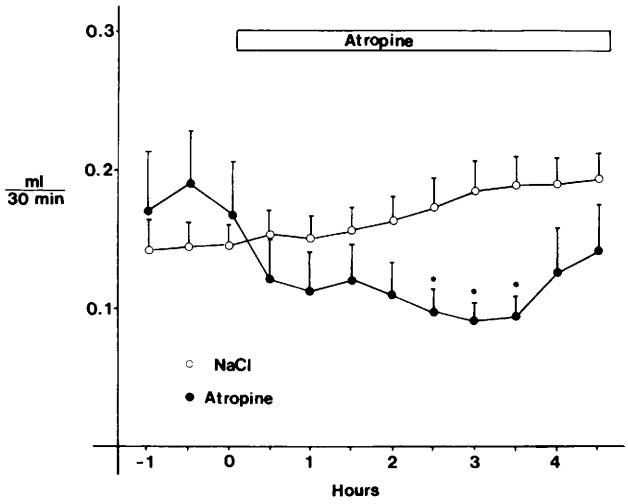

FIG. 2. Effect of atropine on volume of pancreatic juice during recirculation of bile–pancreatic juice (BPJ) to the duodenum in conscious rats. Results are from the same experiment as in Fig. 1. Symbols as in Fig. 1. Statistical significance of values compared to time-paired control values indicated by asterisks: $*P < 0.05$.

of pancreatic juice from the intestine. In contrast to their results, we found that atropine only partially suppressed the pancreatic response to diversion of BPJ. Differences in experimental procedures may have contributed to these apparently conflicting results. In their experiments, bile was continuously returned to the intestine at all times. Green and Nasset (7) showed that returning bile to the intestine blunts the pancreatic secretory response to diversion of BPJ. Thus, the combined effect of returning bile to the intestine plus infusing a threefold higher dose level of atropine than we did may explain the small, statistically nonsignificant increase in pancreatic secretion following diversion of pancreatic juice reported by Noda *et al.* (4).

Atropine may inhibit the pancreatic response to diversion of pancreatic juice or BPJ by inhibiting gastric secretion. This was also the conclusion of Noda *et al.* (4) based on their demonstration that the pancreatic response to diversion of pancreatic juice was greatly reduced by diversion of gastric juice to the exterior, and this suppression was reversed by intraduodenal infusion of HCl. Our recent studies, reported in preliminary form elsewhere (8), support this conclusion. Diversion of gastric juice to the exterior inhibited pancreatic protein secretion stimulated by diversion of BPJ, and the degree of inhibition was approximately the same as atropine caused in the present study. Furthermore, intraduodenal infusion of HCl in pylorus-ligated rats resulted in a normal pancreatic protein secretory response to diversion of BPJ, similar to the response in controls reported here. On the other hand, HCl in the intestine did not influence the pancreatic response to trypsin inhibitors in our study (8), which contradicts their contention (4) that gastric acid in the intestine is necessary for elaboration of the feedback response. However, we agree with Noda *et al.* (4) that the effect of atropine on the pancreatic response to acute diversion of pancreatic juice or BPJ is secondary to inhibition of gastric acid secretion.

Taken together, the results discussed above indicate that cholinergic mechanisms are not directly involved in feedback regulation of the pancreas by luminal proteases. Ihse *et al.* (3) arrived at a different conclusion based on their study of the effects of vagotomy and anticholinergic blockage (propantheline) on the rat pancreatic response to trypsin and trypsin inhibitors. They reported that intraduodenal trypsin did not significantly inhibit protein output in BPJ in either vagotomized or propantheline-treated rats. However, their data show that in both groups protein output tends

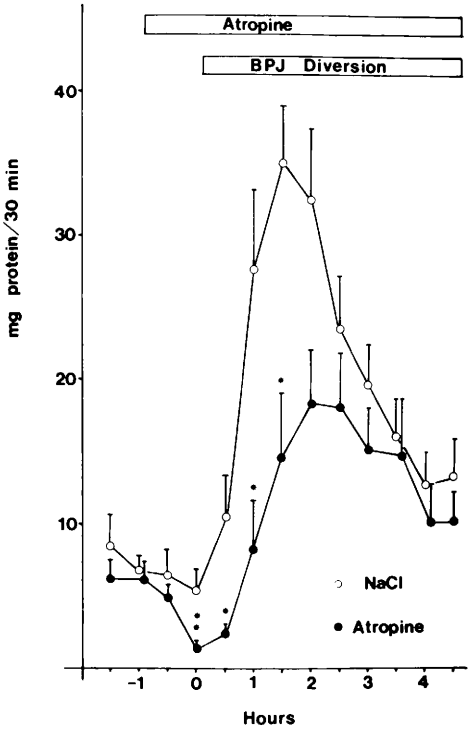

FIG. 3. Effect of atropine on pancreatic protein secretion in response to diversion of bile-pancreatic juice (BPJ) from the intestine. BPJ was diverted from intestine at time indicated and replaced with 0.05 *M* $NaHCO_3$ at 1 ml/hr. Closed symbols indicate atropine infusion, open symbols indicate saline infusion. Each point represents the mean from seven experiments with six rats with atropine, and seven experiments with five rats in controls (saline-infused). Statistical significance between atropine-infused and saline-infused groups indicated by asterisks: $*P < 0.05$, $**P < 0.02$, $***P < 0.001$.

to decrease with trypsin infusion and this tendency is reversed by trypsin inhibitor infusion, markedly so in propantheline-treated rats. Thus, the responses seem more to be truncated than eliminated by vagotomy and propantheline, and this may be a consequence of the short (14–15 hr) postoperative recovery period rather than due to direct effects of the treatments on pancreatic function.

The effect of atropine on pancreatic secretion during *return* of BPJ is not so readily explained. Noda *et al.* (4) showed, as we did here, that atropine inhibited pancreatic secretion during return of BPJ, and that protein secretion was inhibited much more than volume (in contrast to the parallel decreases in protein and volume with atropine in response to diversion of pancreatic juice or BPJ). However, it is not clear that gastric secretion is important in this case. We found no significant effect of HCl (infused intraduodenally at 60–240 μeq/hr) on pancreatic protein or fluid secretion during return of BPJ in pylorus-ligated rats (8), and the values we observed for fluid and protein secretion during return of BPJ in pylorus-ligated rats are nearly identical to values reported here for control rats with BPJ returned. In agreement with this, Noda *et al.* (4) found no significant effect of diversion of gastric juice (open gastric fistula) or replacement of HCl on pancreatic juice *volume* during return of pancreatic juice in any of their experiments. In two out of three experiments during return of pancreatic juice they observed significantly reduced pancreatic protein output

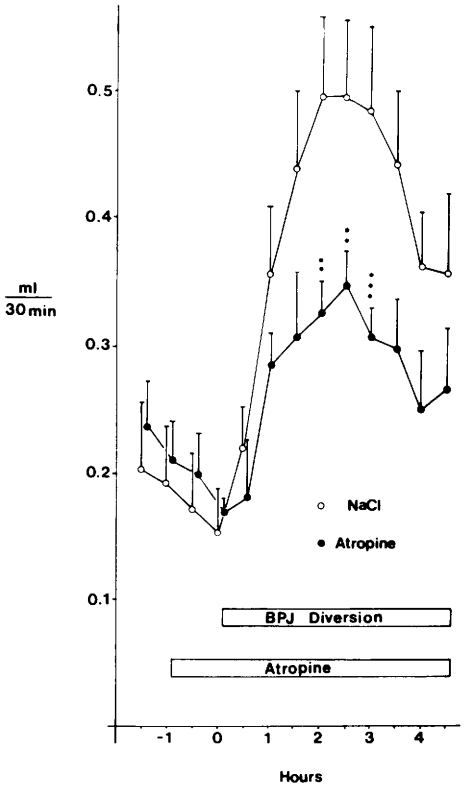

FIG. 4. Effect of atropine on volume of pancreatic juice in response to diversion of bile-pancreatic juice from the intestine. Results are from the same experiment as in Fig. 3. Symbols as in Fig. 3.

in rats with gastric juice diversion, but the reduction was considerably less marked (and significantly different) compared to that caused by atropine. Taken together, the results discussed above suggest that inhibition of pancreatic protein secretion by atropine during return of BPJ to the intestine is not caused by inhibition of gastric secretion, but may reflect inhibition of a local cholinergic enteropancreatic reflex in the rat, such as has been postulated to occur in the dog (9–11).

Our observation of a profound suppression of pancreatic protein secretion by atropine during return of BPJ may help to reconcile some species differences in pancreatic secretion between rat and dog. One of the notable differences in pancreatic physiology between rat and dog is the range between basal and stimulated pancreatic protein output, which is smaller in the rat. This appears to be mainly due to much higher basal secretion (with or without BPJ return) in the rat. From a survey of recent studies (12–19) we estimate that basal vs stimulated pancreatic protein secretion in dogs is approximately 16 mg/kg-hr vs 130 mg/kg-hr, or an eightfold range, while corresponding values for rats were found to be approximately 60 mg/kg-hr vs 210 mg/kg-hr. Basal values for rats were with BPJ returned to the intestine; return of pancreatic juice does not affect basal pancreatic protein secretion in the dog (20). In the study reported here, basal pancreatic protein secretion in atropinized rats with BPJ returned was ~14 mg/kg-hr, very close to basal values for the dog. This suggests that basal pancreatic protein secretion in the rat (with BPJ returned) and dog differ mainly because of the existence of a chronic cholinergic stimulus or tone in the rat, which may act through an enteropancreatic reflex. An alternative view was expressed by Peterson and Grossman (18), who speculated that the high basal pancreatic protein secretion in rats with BPJ returned may mean that CCK release in the rat is incompletely inhibited by BPJ in the intestine. This seems unlikely because recent studies indicate that atropine does not inhibit CCK release (21).

The dose of atropine used in our studies should be considered in interpreting the results. The dose level, 100 μg/kg-hr, is severalfold higher than that used in recent studies of pancreatic function in the dog (10, 11), and may be much greater than necessary to inhibit pancreatic function based on preliminary studies reported by Singer *et al.* (22). They found that 5 μg/kg-hr of atropine inhibited the canine pancreatic bicarbonate response to low doses of secretin, and that higher doses of atropine did not further affect pancreatic function but did cause systemic effects (i.e., increased heart rate). Thus, it is possible that 100 μg/kg-hr may be well in excess of that required for complete blockage of cholinergic mechanisms directly regulating pancreatic secretion in the rat.

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