A Study of the Cholinesterases of the Canine Pancreatic Sphincters and the Relationship Between Reduced Butyrylcholinesterase Activity and Pancreatic Ductal Hypertension

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Previous work from this laboratory revealed an increased canine pancreatic intraductal pressure following cholinesterase inhibitor intoxication. The pressure was negatively correlated with serum butyrylcholinesterase (BChE) activity, suggesting that BChE activity mediated the pressure rise. This study uses a histochemical technique to investigate the tissue cholinesterase activity of the canine pancreatic sphincters and the effect of a cholinesterase inhibitor (ChEI) on tissue cholinesterase activity. In five control dogs, serial sections of the major and minor spincters were stained for acetylcholinesterase (AChE) and BChE activity. Four treated dogs were given the ChEI, O,O-diethyl -O- (2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate, 25 mg/kg, one hour prior to excising the ampullae. In the control dogs, BChE activity is present in the periampullary nerves and the pancreatic smooth muscle sphincters. AChE activity is present in nerves but not in smooth muscle. In the treated group, following a dose of ChEI known to cause ductal hypertension, BChE activity was absent in the pancreatic sphincters but AChE activity was preserved in the periampullary nerves. These data suggest that the pancreatic ductal hypertension that occurs following ChEI administration is due to a selective reduction in pancreatic smooth muscle BChE activity.

OBSTRUCTION AT THE AMPULLA of Vater and subsequent ductal hypertension are important triggering mechanisms in the pathogenesis of acute and chronic pancreatitis.[1-3] Factors that normally affect the caliber of the ductal sphincter of the pancreas are, thus, of interest because an increased resistance to pancreatic exocrine secretion in this location can cause ductal hypertension. Anatomic studies by Boyden[4-8] and others have demonstrated the presence of circular and longitudinal smooth muscle fibers in the pancreatic sphincter in all mammalian species studied (cat, dog, guinea pig, opossum, chimpanzee, and humans). Although extensive autonomic nerve fibers can be demonstrated in the papilla of Vater and ampullary region in humans and dogs,[9] considerable uncertainty exists whether autonomic innervation of this region is of significance in the pathogenesis of pancreatitis. Experimental studies[10-16] have shown that changes in vagal or sympathetic tone play a minor role in modifying pancreatic intraductal pressures, and do not cause pancreatitis.

We recently reported, using a canine model, a prompt increase in pancreatic intraductal pressure to 40 cm saline, and the formation of acute interstitial pancreatitis, following cholinergic stimulation by the systemic injection of a sublethal dose of an irreversible cholinesterase inhibitor, Diazinon*.[17,18] Cholinesterase inhibitors depress cholinesterase activity and cause accumulation of acetylcholine on postsynaptic receptors. Two enzymes are responsible for the total cholinesterase activity of whole blood—a membrane-bound cholinesterase, acetylcholinesterase (AChE) associated with the erythrocyte membrane, and a soluble enzyme, pseudocholinesterase or butyrylcholinesterase (BChE) in the serum.[19] In tissues, which also contain the two forms of cholinesterase, AChE is important in the regulation of neuromuscular activity and parasympathetic ganglion transmission,[20] while the role of BChE is unknown. The pancreatic pressure increase we previously observed was related to the dose of cholinesterase inhibitor, was correlated with a decrease in serum BChE activity, and was blocked by atropine.[18] Those data

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* Ciba Geigy Corporation, Greensboro, NC.

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TABLE 1. *Cholenesterase Histochemistry*

Substrate	Inhibitor	Enzyme Staining AChE	Enzyme Staining BChE
Acetylthiocholine	None	+	+
Acetylthiocholine	iso-OMPA	+	0
Acetylthiocholine	Physostigmine	0	0
Butyrylthiocholine	None	0	+
Butyrylthiocholine	iso-OMPA	0	0
Butyrylthiocholine	Physostigmine	0	0
None	None	0	0

suggested the possibility that reduced BChE activity is important in mediating strong spasm of the pancreatic sphincter.

This is a report of our further study of the relationship between cholinesterase activity and ductal hypertension. The canine ampullary region was examined histochemically for AChE and BChE activity, and the effect of the intravenous administration of the cholinesterase inhibitor, Diazinon, on the activity of these tissue enzymes was observed.

Materials and Methods

In five anesthetized dogs (control group), the major and minor pancreatic ampulla were excised. The orifice was cannulated with PE 50 tubing to orient the specimen for sectioning. The specimen was then frozen at −70 C for storage. In four other dogs (treatment group), a sublethal dose (25 mg/kg) of the cholinesterase inhibitor O,O-diethyl -O- (2-isopropyl - 6 - methyl - 4 - pyrimidinyl) phosphorathioate (Diazinon) was given intravenously one hour before the major and minor ampulla were similarly excised, oriented and frozen. In both groups, serial cryostat sections, 10 μm thick, were stained for acetylcholinesterase and butyrylcholinesterase activity, using the technique of Karnovsky.[21] Briefly, the cut sections were preincubated for one hour at room temperature in a solution containing a 0.065 M sodium hydrogen maleate buffer, 5 mM sodium citrate, 3 mM copper sulfate, 0.5 mM potassium ferricyanide. A substrate, either acetylthiocholine or butyrylthiocholine, was then added and the sections were incubated for an additional hour. A brown precipitate indicated cholinesterase activity. Table 1 summarizes the histochemical technique. With acetylthiocholine as a substrate, both AChE and BChE activity were identified. In the presence of the *in vitro* inhibitor of BChE, iso-OMPA† (0.1 mM), only AChE activity was identified. With butyrylthiocholine as a substrate with no *in vitro* inhibitor, only BChE activity was localized. Control sections were incubated with acetylthiocholine of butyrylthiocholine in combination with physostigmine‡ (0.01 mM), or butyrylthiocholine in the presence of iso-OMPA, or with substrate absent, and showed no precipitate. The mucosa and nuclei were counterstained in hematoxylin prior to dehydration in graded concentrations of alcohol.

† Tetraisopropylpyrophosphoramide, Sigma Chemical Company, St. Louis, MO.

Results

Histologic examinations of the canine major and minor ampulla in longitudinal and cross sections confirmed the earlier anatomic finding of a smooth muscle sphincter surrounding both the biliary and pancreatic ducts. The sphincter is comprised primarily of circular muscle elements that arise near the inner circular muscle layer of the duodenum. The sphincteric muscle accompanies the distal pancreatic duct through the submucosa and fuses with the duodenal muscularis mucosae at the duct orifice.

In the control group, BChE activity was abundantly present in the ductal spincter, the muscularis mucosae of the duodenum, the inner one-fifth of the duodenal circular muscle, and all of the duodenal longitudinal muscle (Fig. 1A and B). BChE activity was present in the nerves of the submucosal plexus (Meissner's plexus), the myenteric plexus of Auerbach, located between the duodenal circular and longitudinal muscles, and within the nerve twigs coursing within the interstices of the smooth muscles. In the juxta-ampullary area, there was an additional nerve plexus within the duodenal smooth muscle layer which also contained BChE activity. AChE was present in the nerve plexus of the duodenum and of the submucosa surrounding the pancreatic duct and it was also present in the nerve twigs coursing between smooth muscle cells. In distinction to BChE, AChE activity was not present in sphincteric or duodenal muscle tissue (Fig. 2A and B).

In all dogs treated with the cholinesterase inhibitor, Diazinon, at a dose known to cause ductal hypertension, no histochemically demonstrable BChE activity could be seen in either smooth muscle or the nerves. AChE activity was still present, although reduced, in the nerves of the duodenum, pancreatic duct, and the nerve twigs innervating the smooth muscle (Fig. 3). The presence of smooth muscle in the ampullary region was confirmed by immunoperoxidase staining for actin.

Discussion

We previously demonstrated a negative correlation between serum BChE activity and pancretic intraductal pressure.[18] The present study confirms the presence

‡ Sigma Chemical Company.

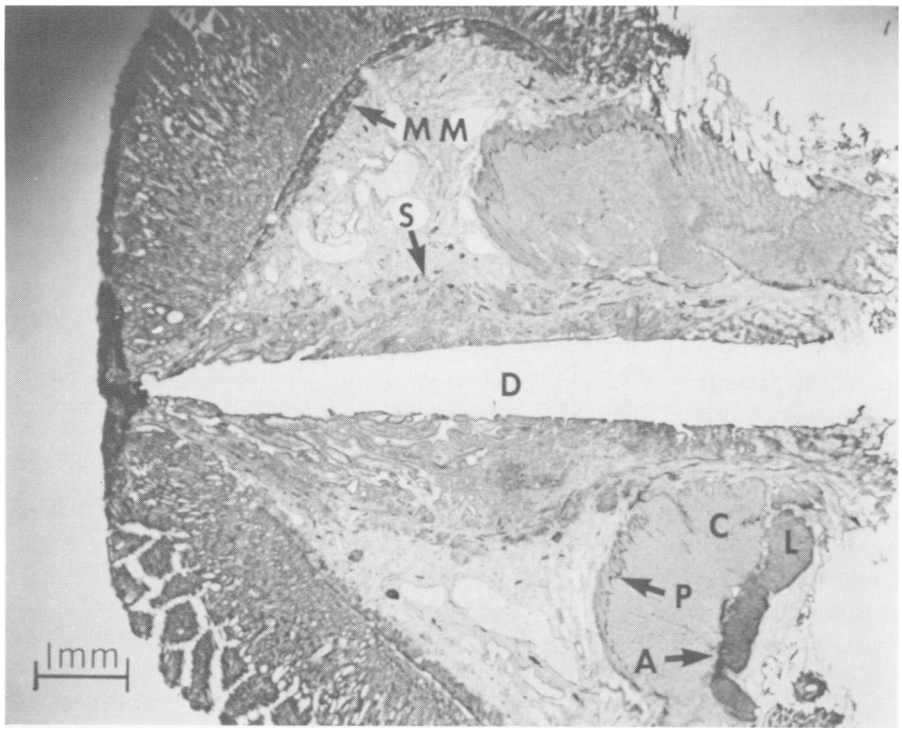

FIG. 1A. Section from major pancreatic ampulla. Dark precipitate indicates BChE activity in smooth muscle sphincter (S) surrounding pancreatic duct (D), muscularis mucosae (MM), duodenal longitudinal muscle (L), and inner layer of duodenal circular muscle (C). BChE activity is also present in Meissner's submucosal plexus (M), Auerbach's plexus (A), and unnamed plexus (P), located in the circular muscle. Substrate; Butyrylthiocholine; in vitro inhibitor: none. The nuclei and mucosa are counterstained with hematoxylin in all figures.

of BChE in the canine sphincters at the pancreatic orifice. Complete inhibition of histochemically demonstrable sphincter smooth muscle BChE occurred at a dose of cholinesterase inhibitor which causes reduction of serum BChE activity, and ductal hypertension. AChE activity in nerves was preserved, indicating that the Diazinon is a relatively selective inhibitor of BChE. These results are similar to those of Koelle,[22] who demonstrated that selective inhibition of BChE (with preservation of AChE activity) produced increased tone

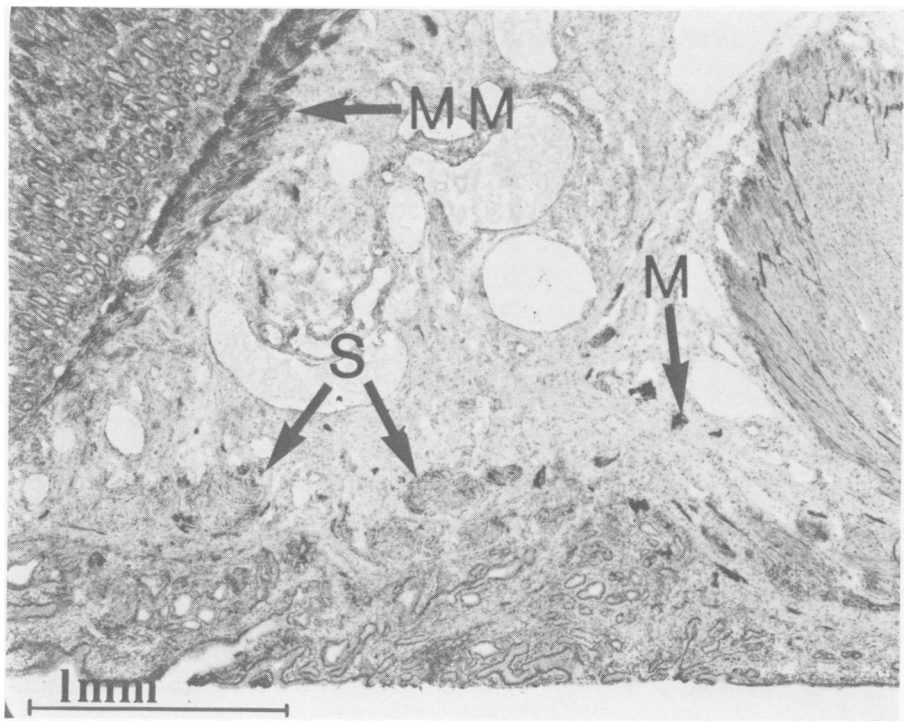

FIG. 1B. High power view of Figure 1A showing BChE activity in smooth muscle sphincter.

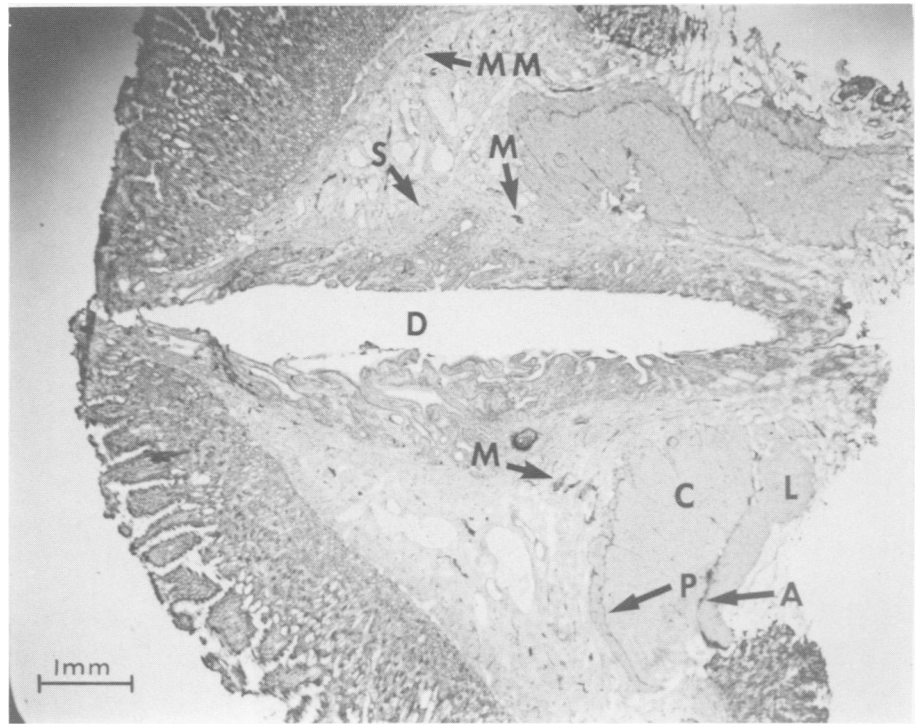

FIG. 2A. Section from same ampulla shown in Figure 1 showing AChE activity in submucosal plexus (M), Auerbach's plexus (A), and plexus (P). The sphincter (S), muscularis mucosae (MM), and duodenal muscle contain no AChE activity. Substrate: Acetylthiochonine; *in vitro* inhibitor: iso-OMPA (0.1 mM).

and amplitude of contraction of cat ileum. These data suggest that, in the dog, BChE of smooth muscle plays an important role in modulation ampullary tone.

The relatively minor effect of cholinergic stimulation on pancreatic ductal pressures reported by other investigators contrast sharply with our previous results.[18] Anrep[10] noted that peripheral vagal stimulation in the dog caused transient pancreatic sphincter contraction

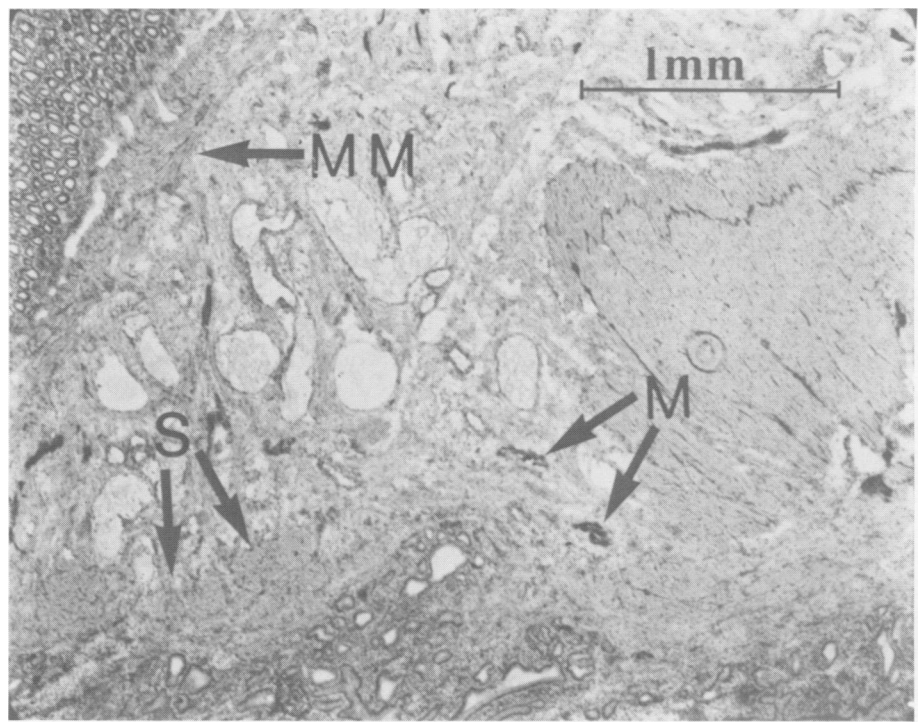

FIG. 2B. High power view of Figure 2A.

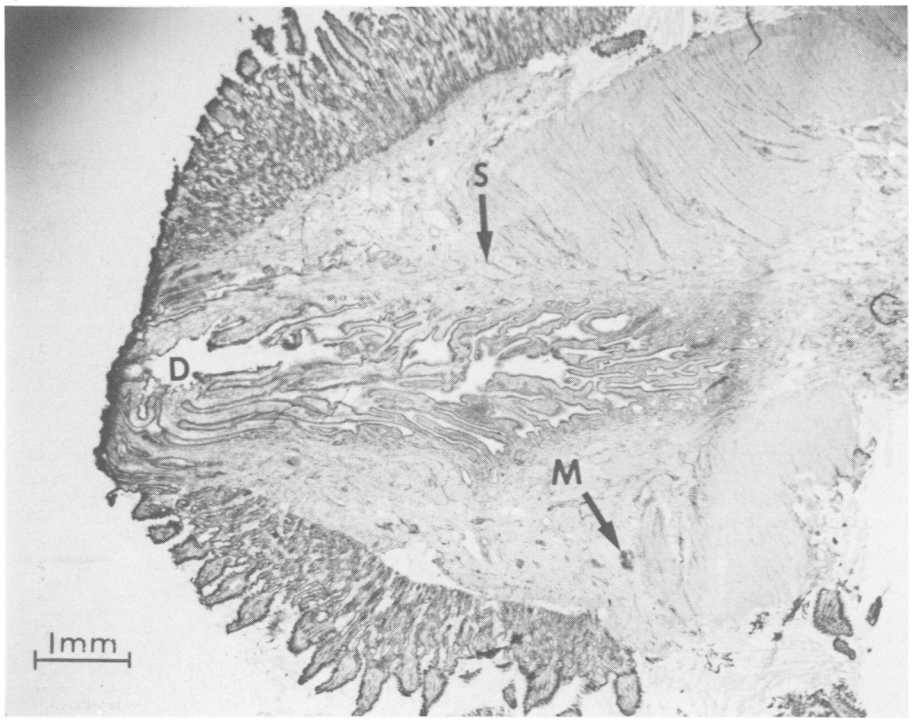

FIG. 3. One hour following IV cholinesterase inhibitor, Diazinon, 25 mg/kg, BChE activity associated with sphincter muscle (S) and muscularis mucosae is absent. However, AChE activity is present in submucosal plexus (M). Substrate: Acetylthiocholine; *in vitro* inhibitor: none.

with resultant intrapancreatic retention of secretions. Korovitsky[11] and Lenninger[12,13] demonstrated that pilocarpine, methacholine, and vagal stimulation caused mild increased ductal resistance in cats. In dogs, a transient increase in pressure was noted by Menguy[14] after intravenous pilocarpine, however, a decreased pressure during parasympathetic stimulation was observed by Gilsdorf.[15] Tansy[16] reported that both peripheral vagal stimulation and ductal infusions of bethanechol chloride produced slight pressure increase, while ductal infusion of norepinephrine bitartrate resulted in lower pressures. These results were attributed to changes in local vascular dynamics.

In none of these studies were ductal hypertensive changes ever reported to the degree that we have previously observed.[18] It must be recognized however, that the net effect of cholinergic stimulation is determined by the number of acetylcholine receptors occupied at any time. This is in turn determined by the rate of release of acetylcholine by the terminal axon minus the rate of hydrolysis of acetylcholine by AChE or BChE in the juxta-receptor area. Cholinesterase activity thus determines the response to cholinergic stimulation. Other investigators who failed to alter or depress this variable observed relatively minor changes in pancreatic intraductal pressure following cholinergic stimulation. We, however, observed major pressure changes when tissue BChE activity was decreased.

A number of patients have been described with abnormalities of serum BChE which result in a reduction in total serum BChE activity and sensitivity to succinylcholine, yet no increased incidence of pancreatitis has been reported in this patient population. As many as 12 isoenzymes of BChE have been described in human plasma. Ninety to 95% of the serum BChE activity is found in the so-called C_4 band found using starch gel or acrylamide electrophoresis.[24] The principal source of this enzyme in the serum is considered to be the liver.[23] The source of the other isoenzymes has, however, not been determined. Unpublished data from this laboratory indicate that the canine pancreatic acinar cells are a source of an isoenzyme of BChE; smooth muscle may also be a source. Patients with genetic abnormalities of serum BChE of hepatic origin have reduced serum BChE activity but may have completely normal smooth muscle BChE activity and therefore normal ampullary tone. Intoxication with the lipid soluble cholinesterase inhibitors however, results in reduced serum and tissue BChE and results in pancreatic ductal hypertension.

These data suggest that BChE may be the only juxtareceptor cholinesterase present at canine smooth muscle neuromuscular junction. The ampullary smooth muscle would seem to be absolutely dependent upon its ability to synthesize active BChE in order to maintain ductal patency. Drugs or toxins that interfere with activity of smooth muscle BChE cause ampullary spasm and ductal hypertension. These data support the hy-

pothesis that the pancreatic ductal hypertension which occurs following cholinesterase inhibitor intoxication is the result of a reduction in sphincter BChE activity.

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