Pancreas
Vol. 2, No. 6, pp. 664–668

Effect of the Organophosphate iso-OMPA on Amylase Release by Pancreatic Lobules of Dog, Guinea Pig, and Cat

Yoshiro Oguchi, Thomas W. Frick, Thomas L. Satterberg, Thomas D. Dressel, John W. Borner, and Robert L. Goodale

Department of Surgery, University of Minnesota Medical School, Minneapolis, Minnesota, U.S.A.

Summary: Organophosphates (OPs) cause irreversible inhibition of cholinesterases (ChEs) and profound cholinergic stimulation. There are major differences in the response of the dog and cat pancreas to the in vivo administration of Diazinon (*O,O*-diethyl *O*-2-isopropyl-4-methyl-6-pyrimidyl phosphothioate), a butyrylcholinesterase (BuChE) inhibitor. Acute edematous pancreatitis is found in the dog but not in the cat. The present experiments were designed to see what effect OP had in vitro on pancreatic exocrine function of dog, cat, and guinea pig, and whether the effects were consistent with an anti-ChE activity. A water-soluble OP agent, tetraisopropyl pyrophosphoramide (iso-OMPA) at 10^{-3} *M*, which like Diazinon inhibits BuChE, was used. Minced pieces of fresh whole pancreata 3 mm in size were taken from 3 dogs, 4 guinea pigs, and 2 cats. The tissues were placed in flasks containing Eagle's solution and gassed with 100% O_2. Cumulative amylase release was measured by *Phadebas* method up to 3 h. At half-maximal acetylcholine (ACH) concentration (10^{-5} *M*), the canine pancreas pretreated with iso-OMPA (10^{-3} *M*) showed a 42–87% greater release of amylase than tissues receiving ACH alone ($p < 0.001$). The same potentiated response to ACH was seen in guinea pig pancreas pretreated with iso-OMPA ($p < 0.001$), but iso-OMPA pretreatment did not augment the ACH response in the cat. Atropine pretreatment effectively blocked all ACH responses, and there was no effect seen with iso-OMPA alone. In the dog, iso-OMPA in combination with half-maximal carbachol (10^{-6} *M*), or in combination with half-maximal cholecystokinin (CCK-8) stimulation (10^{-9} *M*), provided no potentiated amylase release. BuChE is normally present in dog and guinea pig acinar cells, but is lacking in cat acinar cells. We conclude that OPs such as iso-OMPA cause a potentiated exocrine response to ACH in vitro and this effect can be satisfactorily explained by an anti-BuChE activity. Our results do not identify a mechanism relating the heightened cholinergic exocrine response to pancreatic toxicity. **Key Words:** Organophosphates—Amylase release—Diazinon—Exocrine response.

The best-known mechanism of action of organophosphates (OPs), whereby they exert their toxic effect, is by irreversible inhibition of cholinesterases (ChEs). With accumulation of acetylcholine (ACH) at cholinergic synapses, there are nicotinic, muscarinic, and central nervous system responses according to the site of action. Two classes of enzymes comprise total ChE activity: a red cell- and membrane-bound acetylcholinesterase (AChE) which is present in muscarinic ganglia and neuromuscular junctions, and a soluble butyrylcholinesterase (BuChE) present in the serum and also in au-

Manuscript received January 30, 1987; revised manuscript accepted June 15, 1987.
Address correspondence and reprint requests to Dr. Y. Oguchi at Box 379, UMHC, 420 Delaware Street SE, Minneapolis, MN 55455, U.S.A.

tonomic ganglia, liver, and variously distributed in other tissues. But the correlation between the inhibition rate for ChE and the respective index of lethality such as LD_{50} of OP agents is poor (1). Organophosphates also work through mechanisms other than inhibition of ChE. In the cerebral cortex they cause inhibition of O_2 uptake (2), catecholamine depletion (3), and excitatory effects through noncholinergic mechanisms (4). Fatty degeneration of the liver and kidney have been ascribed to intracellular hypoxia (5).

Pancreatitis in humans has been reported after poisoning with Dyfonate (6), Mestinon (7), and Diazinon (*O,O*-diethyl *O*-2-isopropyl-4-methyl-6-pyrimidyl phosphothioate) (8), and it is not certain whether some noncholinergic mechanisms are responsible. When the OP Diazinon in sublethal doses (75 mg/kg i.v.) is given to dogs, along with a secretin infusion (1 U/kg/h), there is a prompt rise in serum amylase and lipase (6,9) and acute edematous pancreatitis. However, in cats sublethal doses of Diazinon, with or without secretin, cause no increase of serum amylase or lipase, and no pancreatitis (10), although the signs of cholinergic intoxication are just as severe. This anomalous type of response in different mammalian species is not well understood, but has been reported in other organs (1,11,12). Also, the effects of one particular OP may not be similar to other OPs (1,11,12) in comparable dosage; for example, DFP, Diazinon, and iso-OMPA have greater binding affinity for BuChE, while Sarin and Tabun selectively inhibit AChE.

One explanation for the different pancreatic responses to Diazinon may be that indirect or noncholinergic mechanisms are active. There may be tissue (splanchnic) hypoxia, or release of vasoactive substances to which the canine pancreas is more sensitive. There may be central neurotransmitters which possibly cause differing pancreatic responses. Since there are known species differences in the distribution of BuChE (10,13) in tissues, a third hypothesis is that the BuChE accounts for the different effects. Canine and guinea pig acinar cells contain BuChE; however, the enzyme is completely lacking in cat pancreas.

To evaluate the relative contribution of BuChE inhibition on pancreatic exocrine function, we used an in vitro pancreatic tissue preparation. Diazinon has poor water solubility, so we chose the water-soluble OP agent, iso-OMPA, which at 10^{-3} *M* inhibits BuChE activity (14,15).

METHODS

In initial experiments in dogs, guinea pigs, and cats, dose–response curves to ACH (Nutrition Biochemicals Corp., Cleveland, OH, U.S.A.) were established. In dogs, response to cholecystokinin (CCK-8) (Squibb Diagnostics, New Brunswick, NJ, U.S.A.) and carbachol (Sigma Chemical Co., St. Louis, MI, U.S.A.) were also tested. The animals were anesthetized with 40 mg/kg Pentobarbital (Veterinary Laboratories, Inc., Lenexa, KS, U.S.A.) by the intravenous or intraperitoneal route. The whole pancreas was excised, quickly placed in iced Ringer's lactate solution, and the interstitium distended with Ringer's lactate injection via small-gauge needle. The pancreatic tissue was minced by fine scissors into 3-mm pieces after the method of Scheele and Palade (16). The lobules were separated intact in the guinea pig; but in the dog and cat the pancreas was denser and some lobules were minced. Three to five small pieces were then transferred to flasks containing 5 ml Eagle's medium [for reference see (20)] at 25°C, bubbled constantly with 100% O_2. A pH of 7.4 was maintained by the addition of HEPES. The concentration of ACH ranged from 10^{-8} to 10^{-2} *M*, CCK from 10^{-12} to 10^{-7} *M*, and carbachol from 10^{-9} to 10^{-3} *M*. After adding the secretagogue to individual flasks, the amylase activity at 2 h was measured in duplicate flasks from 10 µl samples by the *Phadebas* method (Pharmacia Diagnostics, Piscataway, NJ, U.S.A.), and the tissues weighed (the mean amylase release expressed in IU/L/g wet weight). Half-maximal dose of ACH was 10^{-5} *M* for each species. For CCK-8 it was 10^{-9} *M*, and for carbachol it was 10^{-6} *M*.

The cumulative amylase release at ACH 10^{-5} *M* was then measured in tissues from 3 dogs, 4 guinea pigs, and 2 cats. In the test group there was 1-h preincubation with iso-OMPA (Sigma Chemical Corp.) 10^{-3} *M*. In control groups the tissues were preincubated with atropine 10^{-4} *M*. Also, iso-OMPA alone, without ACH, and the unstimulated or basal release in Eagle's medium alone, were evaluated. Amylase activity at 1, 2, and 3 h was determined from fluid samples from quadruplicate flasks in each group and expressed as the mean percent ± SD of total amylase activity remaining in final tissue homogenate. Statistical analysis was by the two-tailed Student's *t*-test for paired samples. In a similar manner in 4 dogs, CCK-8 at half-max-

imal dose was used instead of ACH, and in 2 dogs the effect of half-maximal carbachol was evaluated.

RESULTS

In dogs, cumulative amylase release at half-maximal stimulation with ACH was 42–87% greater after iso-OMPA pretreatment than after ACH alone ($p < 0.001$) (Fig. 1). The continued steep rise after iso-OMPA combined with ACH suggests increased synthesis as well as release of amylase. Cholinergic receptor blockade with atropine pretreatment reduced the release to basal levels.

In the guinea pig, the cumulative release with ACH stimulation was also greater after iso-OMPA pretreatment, by 41–54% ($p < 0.001$), than the response to ACH itself. These two responses were also significantly greater than the release after atropine blockade or with iso-OMPA alone, or the basal release ($p < 0.001$) (Fig. 2). The guinea pig pancreas did not release as much stored tissue amylase on a percentage basis.

In the cat pancreas (Fig. 3), ACH caused increased amylase release at 2 and 3 h when compared with the atropine-treated or basal release ($p < 0.01$); however, there was no further augmentation of the response to ACH by iso-OMPA pretreatment. Like the guinea pig, the release of amylase after ACH stimulation was less than one half that found in the dog.

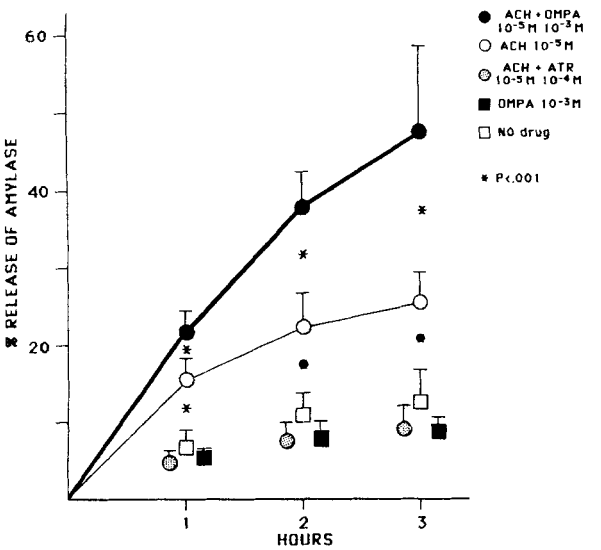

FIG. 1. Minced canine pancreatic tissue. Composite graph of cumulative amylase release up to 3 h after half-maximal ACH stimulation. Symbols represent mean ±1 SD.

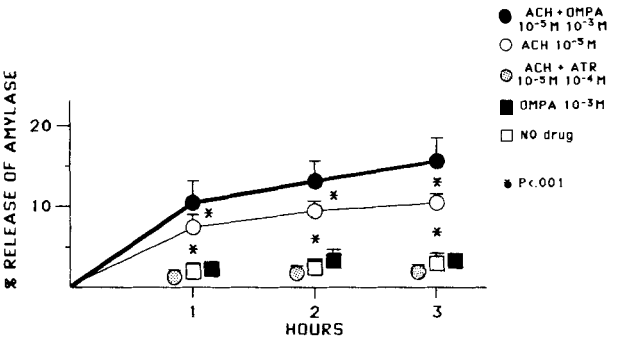

FIG. 2. Guinea pig lobule preparation. Composite graph of cumulative amylase release up to 3 h after half-maximal ACH stimulation.

In dogs the cumulative release after half-maximal CCK-8 stimulation was very similar in magnitude to that of ACH stimulation 10^{-5} *M*. The response to CCK-8, as seen in Fig. 4, was not augmented by iso-OMPA or blocked by atropine.

In dogs, cholinergic stimulation with half-maximal carbachol (Fig. 5) gave a cumulative response that was quite similar in magnitude to that seen with the potentiated response to ACH (as shown in Fig. 1). Carbachol is not hydrolyzed by cholinesterases. The effect of carbachol on amylase release was unaffected by anticholinesterase pretreatment. Atropine reduced to basal levels the amylase release.

DISCUSSION

In keeping with earlier in vivo studies showing different pancreatic responses in dogs and cats after sublethal doses of Diazinon, the present in vitro study shows a different amylase-release pat-

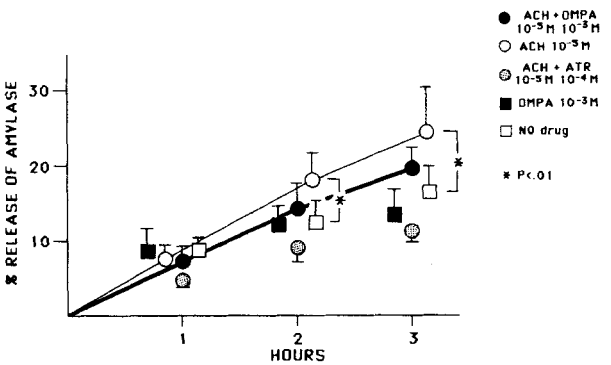

FIG. 3. Minced cat pancreatic tissue. Composite graph of cumulative amylase release up to 3 h after half-maximal ACH stimulation.

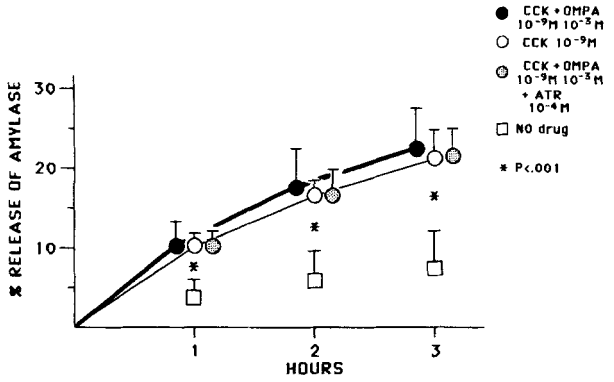

FIG. 4. Minced canine pancreatic tissue composite graph of cumulative amylase release after half-maximal CCK-8 stimulation.

tern in dog and cat—namely a potentiated response to ACH administration when there is inhibition of acinar-cell BuChE. Our data do not explain how cholinergically augmented enzyme release relates to pancreatic toxicity in vivo, but one could speculate that augmented release leads to a similar type of injury that is found with supramaximal caerulein stimulation (17). In vivo studies have reported intracellular vacuolization suggesting supramaximal stimulation in canine acinar cells (9), and mitochondrial damage in guinea pig acinar cells (18) after Diazinon administration, but no apparent effects in the cat (18). Presently, EM studies are planned in the dog, guinea pig, and cat pancreas exposed to ACH and iso-OMPA in vitro.

The peripheral (i.e., nonneuronal) distribution of BuChE can also account for other OP-related effects. BuChE is abundantly present in the canine

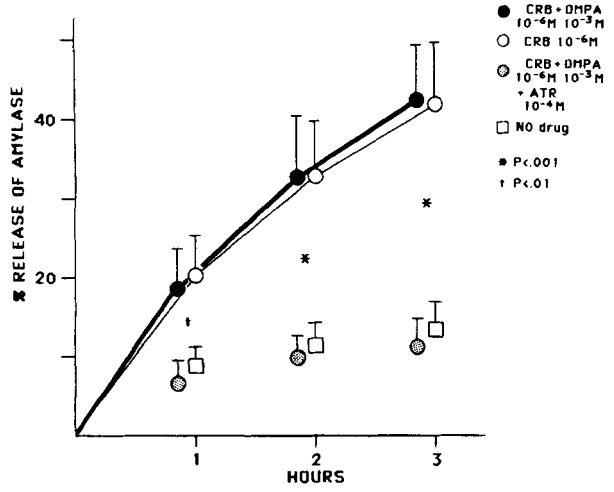

FIG. 5. Minced canine pancreatic tissue composite graph of cumulative amylase release after half-maximal carbachol stimulation.

ampullary sphincter smooth muscle and there are sphincter spasm and pancreatic ductal hypertension reported after Diazinon (14). In the cat ampullary sphincter, however, there is no BuChE, and no ductal hypertension occurs after Diazinon.

The present data do not exclude in vivo noncholinergic effects such as hypoxia, which may contribute to pancreatic injury. However, the potentiated enzyme release to ACH after iso-OMPA treatment can be explained satisfactorily by a specific anti-BuChE effect.

The supporting evidence for this conclusion is that (a) the effect so far as has been shown occurs in species which have BuChE in pancreatic acinar cells; (b) the potentiation of iso-OMPA and the response to ACH are eliminated by cholinergic receptor blockade with atropine; (c) iso-OMPA has no effect on CCK which acts via a noncholinergic receptor; and (d) iso-OMPA has no potentiating effect on carbachol, an acetylcholine analog insensitive to BuChE.

In preparing lobules and small pancreatic fragments, mechanical injury undoubtedly caused some amylase leakage directly into the medium. However, the release under basal conditions was not great enough to mask the response to any of the secretagogue at half-maximal concentration. These differences were maintained over the entire 3-h period of observation, except in the cat where there was overlap in the first hour. In the guinea pig the basal and stimulated rates of discharge of amylase after cholinergic stimulation were quite consistent with the respective figures reported by Scheele and Palade (16) when adjusted for different dosage and temperature. Also, our dose–response curves to carbachol in the dog coincided with those of Scheele and Palade and Jensen and Gardner (19) for dispersed guinea pig acini, while our dose–response curves for CCK were one log-order less sensitive than described by Gardner et al. (20), but were the same as reported by Scheele and Palade (16).

Since ganglia and postganglionic neurons are still present in the pancreatic interstitial tissue, ACH synthesis probably exists. However, it may be small. In the dog and guinea pig, we did not find differences between basal- and atropine-treated groups, but there was a small difference in the cat between basal- and atropine-blocked tissue, consistent with endogenous ACH production.

Previously, in dogs, Dressel et al. showed AChE is still active and present in interstitial nerves and

ganglia after treatment with Diazinon (14) and iso-OMPA. We are now conducting experiments to evaluate an OP compound which inhibits both AChE and BuChE.

Acknowledgment: Funds for this research were supplied in part by a grant from the Graduate School of the University of Minnesota and from the Minnesota Medical Foundation. The authors wish to express their appreciation to Alice Migliazza for expert assistance in preparation of the manuscript.

REFERENCES

1. Ellin RI. Anomalies in theories and therapy of intoxication by potent organophosphorus anticholinesterase compounds. *Gen Pharmacol* 1982;13:457–66.
2. Jovic R, Bachelard HS, Clark AG, Nicholas PC. Effects of soman and DFP in vivo and in vitro on cerebral metabolism in the rat. *Biochem Pharmacol* 1971;20:519–27.
3. Dasheiff RM, Einberg E, Grenell RG. Sarin and adrenergic–cholinergic interaction in rat brain. *Exp Neurol* 1977;57:549–60.
4. Williamson AW, Sarvey JM. Effects of cholinesterase inhibitors on evoked responses in field CA1 of the rat hippocampus. *J Pharmacol Exp Ther* 1985;235:448–55.
5. Hettwer VH. Histochemical investigations on liver and kidney of the rat after intoxication with organophosphates. *Acta Histochem* 1975;52:239–52.
6. Dressel TD, Goodale RL, Arneson MA, Borner JW. Pancreatitis as a complication of anticholinesterase insecticide intoxication. *Ann Surg* 1979;189:199–204.
7. Renner IG, Savage WT, Pantoja JL, Renner VJ. Death due to acute pancreatitis. *Dig Dis Sci* 1985;30:1005–18.
8. Limaye MR. Acute organophosphorus compound poisoning. *J Indian Med Assoc* 1966;47:492–8.
9. Dressel TD, Goodale RL, Zweber B, Borner JW. The effect of atropine and duct decompression on the evolution of Diazinon-induced acute canine pancreatitis. *Ann Surg* 1982;195:424–34.
10. Baraniewski HM, O'Leary JF, Goodale RL. Effect of anticholinesterase on pancreatic flow and pressure in cats. *Fed Proc* 1983;42:507.
11. Hopff WH, Riggio G, Waser PG. Sarin poisoning in guinea pigs compared to reactivation of acetylcholinesterase in vitro as a basis for therapy. *Acta Pharmacol Toxicol* 1984;55:1–5.
12. Coudray-Lucas C, Prioux-Guyonneau M, Sentenac H, Cohen Y, Wepierre J. Effects of physostigmine, paraoxon and soman on brain GABA level and metabolism. *Acta Pharmacol Toxicol* 1984;55:153–7.
13. Hebb C, Hill K. Distribution of cholinesterase in the mammalian pancreas. *Q J Exp Physiol* 1955;40:168–75.
14. Dressel TD, Goodale RL, Borner JW, Etani S. A study of the cholinesterases of the canine pancreatic sphincters and the relationship between reduced butyrylcholinesterase activity and pancreatic ductal hypertension. *Ann Surg* 1980;192:614–9.
15. Karnovsky MJ, Roots LA. A "direct-coloring" thiocholine method for cholinesterases. *J Histochem Cytochem* 1964;12:219–21.
16. Scheele GA, Palade GE. Studies on the guinea pig pancreas. *J Biol Chem* 1975;250:2660–70.
17. Lampel M, Kern HF. Acute interstitial pancreatitis in the rat induced by excessive doses of a pancreatic secretagogue. *Virchows Arch [A]* 1977;373:97–117.
18. Frick TW, Dalo S, O'Leary JF, et al. Effects of insecticide Diazinon on pancreas of dog, cat, and guinea pig. *J Environ Path Toxicol Oncol* 1987;7:1–11.
19. Jensen RT, Gardner JD. Identification and characterization of receptors for secretagogues on pancreatic acinar cells. *Fed Proc* 1981;40:2486–96.
20. Gardner JD, Conlon TP, Klaeveman HL, et al. Action of CCK and cholinergic agents on calcium transport in isolated pancreatic acinar cells. *J Clin Invest* 1975;56:366–75.
21. Eagle H. Amino acid metabolism in mammalian cell cultures. *Science* 1959;130:432–7.