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An *In Vitro* Evaluation of Possible Cholinergic and Adrenergic Receptors Affecting Pancreatic Amylase Secretion (39067)

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Secretion of pancreatic enzymes is under control of both neural and hormonal factors with neural control exerted by cholinergic fibers running in both the vagus and splanchnic nerves (1). Cholinergic stimulation is blocked by atropine which, however, does not affect stimulation by the gastrointestinal hormone cholecystokinin–pancreozymin (2). Acetylcholine and various nonmetabolized analogs have also been shown to induce enzyme release *in vitro* with the stimulation blocked by atropine (3–7).

Adrenergic agents cause profound inhibition of pancreatic function *in vivo* associated with vasoconstriction (8, 9). In a recent *in vitro* study, isoproterenol was found to stimulate amylase release from perfused cat pancreas but the effect was blocked by atropine and was felt mediated by acetylcholine in a nonphysiological manner (10). Both cholinergic and adrenergic nerve endings are present in the exocrine pancreas but essentially all fibers directly innervating acinar cells are cholinergic (11, 12).

In the present work we have examined the effects of a number of cholinergic and adrenergic agonists on amylase release from mouse pancreas *in vitro*. We have specifically looked for the existence of nicotinic or adrenergic receptors affecting amylase release and compared the secretory response to commonly used cholinergic analogs in the same preparation.

Materials and methods. All studies were carried out using male White Swiss mice (18–22 g) which were fasted overnight prior to use. The pancreases were removed and incubated in Krebs-Henseleit bicarbonate (KHB) solution (at 37°) either in 25 ml Erlenmeyer flasks or by means of a superfusion apparatus (13, 14). In experiments involving addition of catecholamines, ascorbic acid was added at a concentration of 0.5 mM to retard oxidation. In flask experiments each pancreas was divided into four or five 20–25 mg fragments while in superfusion experiments the fragments were split between two chambers. A 30-min preincubation period was used to equilibrate the tissue and wash out amylase from damaged cells followed by a 30-min incubation in fresh medium. Amylase activity released into the medium was measured by the method of Rinderknecht (15) using the dye-coupled substrate amylose azure (Calbiochem). Amylase release is reported in international units based on the reported activity of the α-amylase used as a standard (Sigma Type VI). Acetylcholine chloride, carbamylcholine, atropine sulfate, L-norepinephrine, L-isoproterenol and hexamethonium bromide were obtained from Sigma; D-tubocurarine from Calbiochem, acetyl-β-methylcholine from RCA corporation and carbamyl-β-methylcholine from Koch-Light and Merck, Sharp and Dohme.

Results. Release of amylase from mouse pancreas *in vitro* as a function of the concentration of acetylcholine and a number of analogues is shown in Fig. 1. Acetylcholine, carbamylcholine and methylcholine (acetyl-β-methylcholine) all induced a similar pattern of amylase release with peak response between 3×10^{-7} and 3×10^{-6} M with a much smaller response at higher concentrations. Bethanechol (carbamyl-β-methylcholine) also induced amylase release but required about 10–30 times the concentration of the others to produce a similar response. The phenomenon of less stimulation at supramaximal agonist concentrations was further studied by means of continuous superfusion to analyze the time course of amylase release. As shown in Fig. 2 the initial response to carbamylcholine at concentration of 10^{-6} and 10^{-4} M was similar

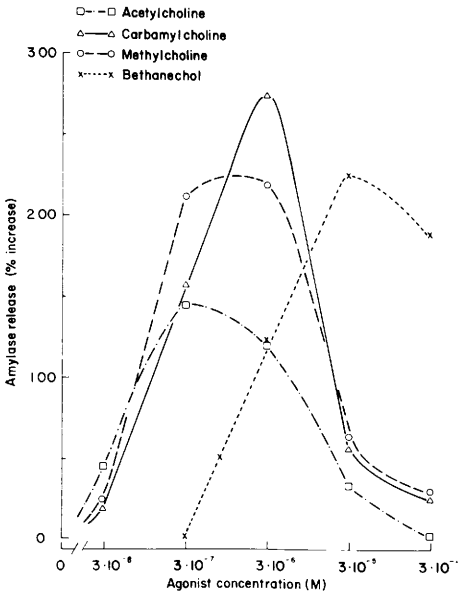

FIG. 1. Concentration dependence of amylase release from mouse pancreas *in vitro* stimulated by cholinergic agonists. Amylase release is plotted as the percent increase over unstimulated release measured during a 30-min incubation period. The data is derived from concentration-response curves where the effect of each concentration was determined on 5–6 pancreases.

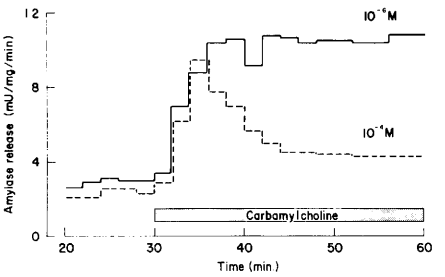

FIG. 2. Effect of carbamylcholine on amylase release from mouse pancreatic fragments. Fragments from a single pancreas were divided in half and superfused in parallel in a 1 ml chamber through which KHB was passed at a rate of 3 ml/min. Two min fractions were collected and assayed for amylase. Carbamylcholine at a concentration of 10^{-6} or 10^{-4} *M* was added as indicated by the bar.

but in the presence of the supramaximal concentration of agonist, the amylase release rapidly fell to near the unstimulated release rate.

A possible role for nicotinic cholinergic or adrenergic receptors in the control of pancreatic protein secretion was also studied. Table I shows that nicotine, L-norepinephrine and L-isoproterenol had no effect on either unstimulated or bethanechol stimulated amylase release. Table II shows the effect of several different types of cholinergic blocking agents. Amylase release in response to acetylcholine was almost completely abolished by atropine while *d*-tubocurarine and hexamethonium-bromide were without effect.

Discussion. In vitro studies of enzyme release by pancreatic fragments readily allow comparison of stimulation or inhibition by a large number of agents. *In vitro* studies also allow secretion to be studied in the absence of neural control and the effects of circulatory changes. In the present study, we have confirmed the muscarinic nature of the cholinergic receptor mediating amylase release while finding no evidence in support of a role for nicotinic or adrenergic receptors. While adrenergic fibers are present in the exocrine pancreas, they terminate on blood vessels and not acinar cells (11, 12). Thus, the effects of catecholamines to inhibit enzyme secretion *in vivo* (8, 9) with little or no catecholamine effect *in vitro* (6, present work) would appear likely due to a change in pancreatic blood flow rather than a direct effect on the pancreatic acinar cell. In contrast to these studies on pancreatic protein secretion, evidence has been presented for direct α-adrenergic inhibition of pancreatic fluid secretion (16).

Cholinergic fibers do terminate directly on pancreatic acinar cells (11) and a number of *in vitro* studies have demonstrated stimulation of pancreatic enzyme release by cholinergic agonists (3–7). In the present work it has been found that the actions of acetylcholine on pancreatic amylase release involve only a muscarinic type receptor with no role for a nicotinic receptor. *In vivo* nicotine inhibits pancreatic fluid secretion indirectly by release of catecholamines (17). *In vitro*, however, nicotine did not inhibit fluid secretion until toxic concentrations were reached (17). Thus pancreatic cholinergic receptors affecting both protein and fluid secretion appear muscarinic in nature. By contrast, in the parotid gland catecholamines

TABLE I. EFFECT OF NICOTINE AND CATECHOLAMINES ON UNSTIMULATED AND BETHANECHOL STIMULATED PANCREATIC AMYLASE RELEASE.[a]

Addition	Amylase release (units/mg/30 min)	
	Unstimulated	Bethanechol (3×10^{-5} *M*)
None (KHB)	0.51 ± 0.05	1.91 ± 0.18
Nicotine (3×10^{-5} *M*)	0.60 ± 0.11	1.86 ± 0.20
L-Norepinephrine (10^{-4} *M*)	0.48 ± 0.08	1.84 ± 0.13
L-Isoproterenol (10^{-4} *M*)	0.59 ± 0.04	1.67 ± 0.11

[a] All values are the mean ± SE for six (unstimulated) or nine (bethanechol stimulated) pancreases.

TABLE II. EFFECT OF CHOLINERGIC BLOCKING AGENTS ON UNSTIMULATED AND ACETYLCHOLINE STIMULATED PANCREATIC AMYLASE RELEASE.[a]

Addition	Amylase release (units/mg/30 min)	
	Unstimulated	Acetylcholine (5×10^{-7} *M*)
None (KHB)	0.48 ± 0.08	1.51 ± 0.11
Atropine (3×10^{-6} *M*)	0.52 ± 0.08	0.48 ± 0.05
Hexamethonium bromide (10^{-4} *M*)	0.52 ± 0.06	1.60 ± 0.15
D-tubocurarine (3×10^{-5} *M*)	0.54 ± 0.05	1.46 ± 0.07

[a] All values are the mean ± SE for six (unstimulated) or nine (acetylcholine stimulated pancreases. Eserine (10^{-5} *M*) was added to all solutions containing acetylcholine to prevent metabolism by endogenous acetylcholinesterase.

activate two types of receptors which mediate different secretory effects (18).

The effects of several nonmetabolized acetylcholine analogues on amylase release have also been studied. While acetylcholine itself is frequently used in single pass perfusion or superfusion experiments (13, 19), when pancreatic fragments are incubated in flasks, small amounts of acetylcholine will be inactivated by acetylcholinesterase. Of the nonmetabolized acetylcholine analogs, carbamylcholine and bethanechol have most frequently been used for *in vitro* studies. Since carbamylcholine has a stimulant action on nicotinic receptors (20) and has been noted to be less active at high concentration (5, 6) we compared the effects of a number of acetylcholine analogs for their ability to release amylase from mouse pancreatic fragments. All agonists tested showed a reduced response to high concentrations although the effect was not as pronounced with bethanechol (Fig. 1). This phenomenon is apparently due to tachyphylaxis as when the time dependence of carbamylcholine stimulated secretion was analyzed the initial secretory rate was similar when both maximal and supramaximal concentrations of agonist were used. There also may well be species differences in response to cholinergic agonists. Guinea-pig pancreas, for example, is less sensitive to both carbamylcholine (21) and bethanechol (7) than is the mouse pancreas as reported here. These results suggest that the stimulating concentration of cholinergic agonists should be chosen with care in studying pancreatic enzyme secretion.

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