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Scorpion Toxin-Induced Amylase Secretion in Guinea Pig Pancreas: Evidence for a New Neurotransmitter (41447)

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Abstract. Scorpion toxin, a substance that induces neurotransmitter release by depolarizing neurons, was used to stimulate amylase release from guinea pig pancreatic lobules. Scorpion toxin was found to act selectively on acinar cell neurons but not directly on acinar cells. The toxin's action on lobules was blocked by tetrodotoxin, whereas scorpion toxin itself had no effect on isolated pancreatic acini. Toxin-induced amylase secretion from pancreatic lobules included two components, one that was atropine sensitive and one that was atropine resistant. This finding is consistent with the notion that scorpion toxin releases cholinergic and noncholinergic transmitters. Both cholinergic and noncholinergic effects showed a similar dose dependence, being maximal at a toxin concentration of 3 μg/ml. The noncholinergic pathway was not mediated through the CCK receptor since it was not blocked by dibutyryl cyclic GMP, a CCK antagonist. Moreover, the noncholinergic mechanism appeared to have a different biochemical mechanism of action than CCK in that scorpion toxin in the presence of atropine did not release rapidly intracellular Ca^{2+} prelabeled with $^{45}Ca^{2+}$. The results suggest, therefore, the existence of a second neurotransmitter present in pancreatic nerve endings capable of stimulating amylase release in the guinea pig.

Exocrine pancreatic zymogen secretion is regulated by both gut hormones and neural mechanisms. Neural control is mediated by fibers from the vagus with acetylcholine as the major neurotransmitter (1). Other putative regulatory peptides that have also been histochemically localized in pancreatic nerves are vasoactive intestinal peptide (VIP) and cholecystokinin (CCK) (2, 3). It is unknown, however, whether these or other peptides are released physiologically and, if released, whether they act directly on secretory cells or only on blood vessels to regulate blood flow, as suggested for VIP in the cat salivary gland (4).

We have recently reported that scorpion toxin and veratridine, agents that depolarize neurons by holding voltage-sensitive Na^+ channels in the open position (5, 6), will indirectly stimulate pancreatic amylase release from rat pancreas by release of acetylcholine (7). These agents had no direct effect on isolated rat pancreatic acinar cells because these cells do not possess voltage-sensitive Na^+ channels (8). In the present study, we evaluated the effect of scorpion toxin on amylase release by guinea pig pancreas. Guinea pigs were studied because their acinar cells are known to respond to a variety of potential neuropeptides, including VIP, substance P, and bombesin (9). We now find evidence for two (or more) neurotransmitters in guinea pig pancreas that could be released by scorpion toxin and are capable of eliciting amylase release; one of these transmitters is cholinergic in nature because its effects can be blocked by atropine. The other transmitter is noncholinergic in nature because it is not blocked by atropine and appears to act via a different biochemical mechanism.

Materials and Methods. Male Hartley guinea pigs, 300–500 g, were fasted overnight prior to decapitation and the pancreas removed and trimmed of adherent fat and connective tissues. Pancreatic lobules were

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prepared by the method of Scheele and Palade (10). Isolated pancreatic acini were prepared by the method of Williams *et al.* (11), modified in that the amount of purified collagenase was increased to 0.15 mg/ml as recommended for preparation of guinea pig acini (12). Lobules and acini were incubated at 37° in Hepes-buffered Ringer (pH 7.35) which was enriched with Eagle's medium essential amino acid supplement, 5 mg/ml bovine serum albumin, and 0.1 mg/ml purified soybean trypsin inhibitor (7, 11).

Amylase release from lobules and acini was measured as previously described and expressed relative to the total tissue content of amylase at the beginning of the incubation (7). Amylase was assayed using either amylose azure (13) or procion yellow starch (14) as substrate.

$^{45}Ca^{2+}$ efflux from pancreatic lobules was determined by the following procedure: 30–35 lobules were incubated 1 hr in 5 ml Hepes–Ringer containing 5 μCi/ml $^{45}Ca^{2+}$ (New England Nuclear, Boston, Mass.). Lobules were then washed with nonradioactive medium and five or six lobules placed in separate 25-ml flasks containing 2 ml Hepes–Ringer and a 2-cm disk of 250-μm mesh nylon cloth. The lobules adhered to the nylon cloth, facilitating rapid removal of the incubation medium during incubation at 37° in a shaking water bath. At 10-min intervals for 30 min, and thereafter at 5-min intervals, the medium was withdrawn as completely as possible, saved for liquid scintillation counting, and replaced with 2 ml fresh medium that had been prewarmed to 37° and equilibrated with 100% oxygen. Secretagogues were added after 60 min of $^{45}Ca^{2+}$ efflux; at the end of the efflux period, the lobules were homogenized in 2 ml distilled water. The fractional efflux of $^{45}Ca^{2+}$ for each period was then calculated (15).

The scorpion toxin used was a crude venom preparation from *Tityus serrulatus* (Sigma Chemical Co., St. Louis, Mo.). The following were obtained as gifts: veratridine from J. Putney, Medical College of Virginia, Richmond, Virginia; and synthetic CCK_8 (C-terminal octapeptide of CCK) from M. Ondetti, Squibb Institute for Medical Research, Princeton, New Jersey.

Results. *Effect of neurotoxins on pancreatic lobules.* Scorpion toxin stimulated amylase release from guinea pig pancreatic lobules in a dose-dependent manner; the minimal effect occurred at a toxin concentration of 0.5 μg/ml and the maximal effect at 3 μg/ml (Fig. 1). The effect of scorpion toxin was inhibited by tetrodotoxin, a known inhibitor of voltage-dependent Na^+ channels (Fig. 2). Amylase release was also

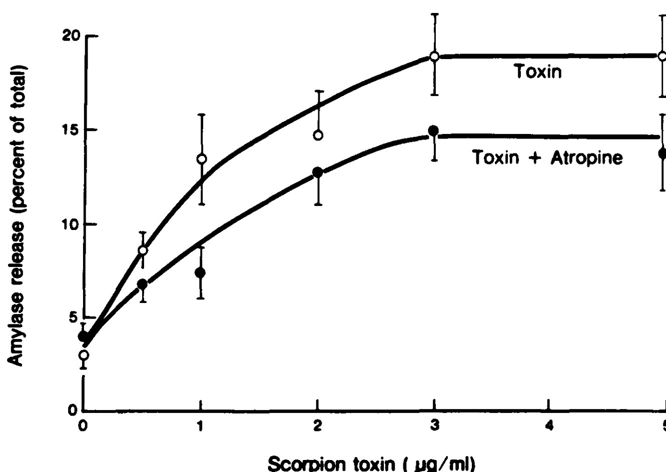

FIG. 1. Concentration dependence of scorpion toxin-induced amylase release from guinea pig pancreatic lobules in the presence and absence of atropine (5 μM). All values are the mean ± SE from four experiments in which amylase release was measured over a 30-min period.

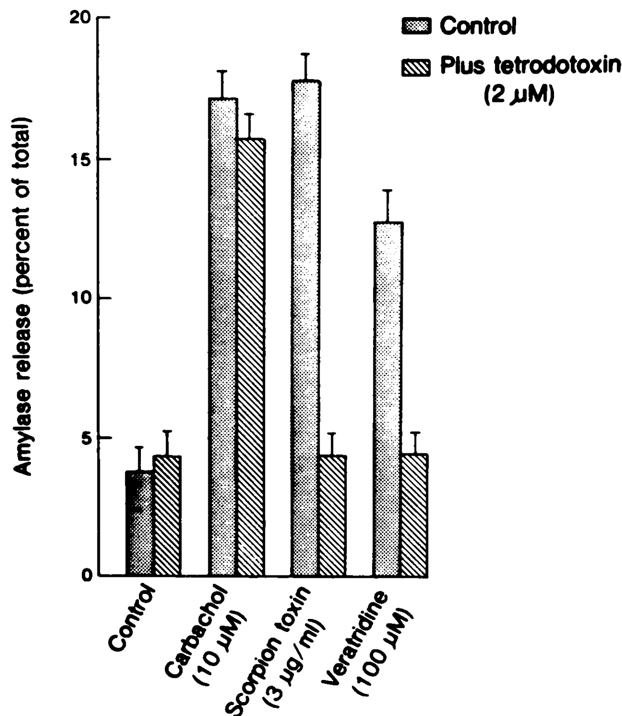

FIG. 2. Effect of tetrodotoxin on control, carbachol, scorpion toxin, and veratridine-induced amylase release from guinea pig pancreatic lobules during a 30-min incubation. Values are the mean ± SE of four incubation flasks from a representative experiment.

stimulated by veratridine, a plant alkaloid neurotoxin, and this release was also blocked by tetrodotoxin (Fig. 2). Tetrodotoxin had no effect on carbachol-stimulated amylase release, confirming that its action was on neurons and not on pancreatic acinar cells themselves. An elevated concentration of K^+ in medium, known to depolarize both neuronal and pancreatic cell membranes, also stimulated amylase release (data not shown).

Effect of neurotoxins on isolated pancreatic acini. Scorpion toxin, veratridine, and K^+ all had no effect on isolated pancreatic acini, whereas carbachol had an effect on acini comparable to that seen with pancreatic lobules (Fig. 3). Thus the neurotoxins stimulated amylase secretion by the release of neurotransmitters and had no direct effect on guinea pig acinar cells. Because of its consistently large effect and ease of use, subsequent studies were carried out with scorpion toxin.

Effect of atropine and dibutyryl cyclic GMP. Atropine at 5 μM partially blocked scorpion toxin-induced amylase release from guinea pig pancreatic lobules (Fig. 1); higher concentrations of atropine produced no further inhibition. Scorpion toxin-induced amylase release, in both the absence and presence of atropine, showed a comparable dose dependence. Atropine also only partially blocked amylase release induced by veratridine (data not shown). To determine whether the atropine-resistant component was acting via the CCK receptor, dibutyryl cyclic GMP, a competitive inhibitor of CCK was used (16). Dibutyryl cyclic GMP blocked the action of CCK_8 on lobules but had no effect on the atropine-resistant component of scorpion toxin-induced amylase release (Fig. 4).

Effect of scorpion toxin on $^{45}Ca^{2+}$ efflux. Acetylcholine and CCK are believed to bring about amylase release by mobilizing intracellular Ca^{2+}. As shown in Fig. 5, car-

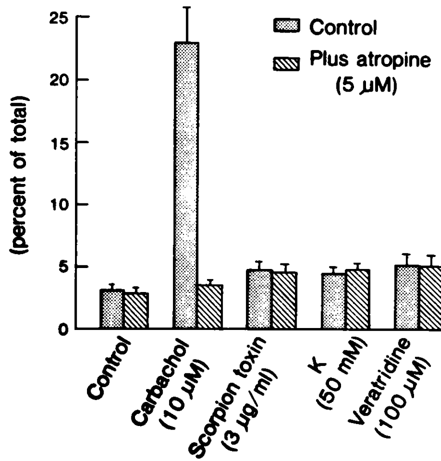

FIG. 3. Effect of atropine on control, carbachol, scorpion toxin, K^+, and veratridine-induced amylase release from guinea pig isolated pancreatic acini measured over a 30-min period. All values are the mean ± SE from four experiments.

bachol caused a rapid, transient increase in the efflux of $^{45}Ca^{2+}$ from prelabeled pancreatic lobules. Scorpion toxin brought about a similar, although lesser, increase in $^{45}Ca^{2+}$ efflux. Atropine blocked 80% of the toxin-induced release and the residual increase in $^{45}Ca^{2+}$ efflux was delayed. Thus, the atropine-sensitive component of scorpion toxin-induced amylase release was accompanied by rapidly accelerated $^{45}Ca^{2+}$ efflux, whereas the atropine-resistant component of scorpion toxin-induced amylase release was not.

Discussion. The neurotoxins scorpion toxin and veratridine, that act by holding open voltage sensitive Na^+ channels, are potent stimulators of zymogen release from pancreatic lobules in both rat (7) and guinea pig (present work). The observations that the action of these agents is blocked by tetrodotoxin, an inhibitor of voltage-sensitive Na^+ channels in nerves, and that they have no effect on isolated pancreatic acini, indicate that the toxins do not act directly on acinar cells or bring about amylase release by damaging acinar cells. That scorpion toxin acts through release of endogenous neurotransmitters in pancreas is also consistent with its known ability to release neurotransmitters from brain slices (17) and to elicit muscular contraction by releasing acetylcholine at the neuromuscular junction (18). The lack of a direct effect on acinar cell is also consistent with the previous demonstration that acinar cells do not show action potentials in response to electrical depolarization (19).

Although the scorpion toxin used is not purified it can be used as a tool to study the action of endogenous transmitters present in pancreatic nerve endings. In the present study, atropine only blocked scorpion toxin action partially. Thus, assuming that scorpion toxin acts indirectly by releasing neurotransmitters, the present results provide evidence in the guinea pig for the presence of at least two releasable neurotransmitters. The action of one neurotransmitter is blocked by atropine and is presumably acetylcholine; the other neurotransmitter is

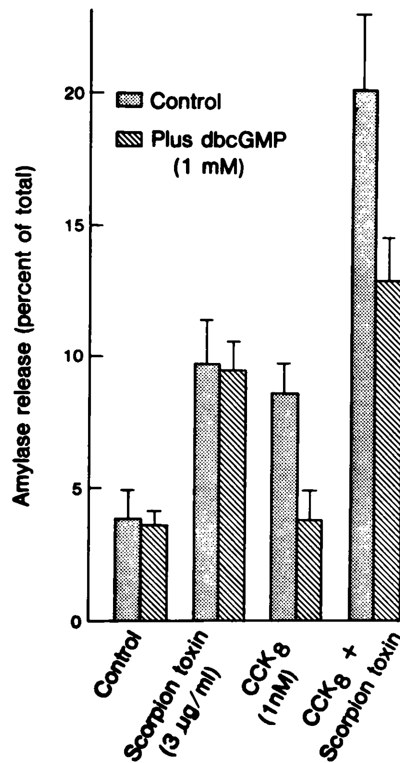

FIG. 4. Effect of dibutyryl cyclic GMP (dbcGMP) on amylase release induced by scorpion toxin and CCK_8 from guinea pig pancreatic lobules during a 30-min incubation. All values are the mean ± SE from three experiments.

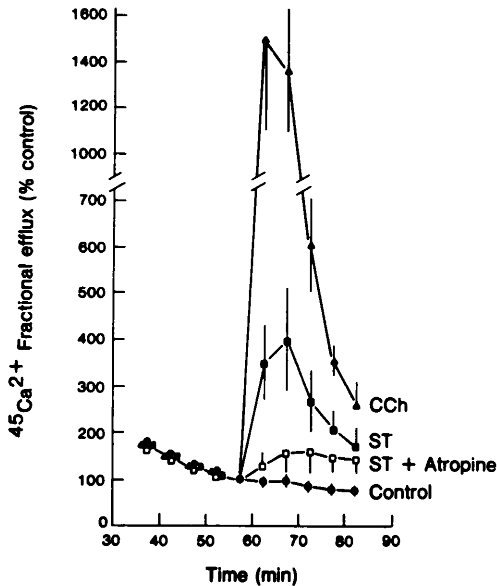

FIG. 5. Effect of carbachol (CCh), scorpion toxin (ST), and atropine on $^{45}Ca^{2+}$ efflux from guinea pig pancreatic lobules. The $^{45}Ca^{2+}$ release for each experiment was normalized relative to the last value prior to addition of secretagogues at 60 min. Points are shown at the midpoint of the collection period; the first four time points are slightly off set for clarity but were all taken at the same time. Values are the mean ± SE from four experiments.

noncholinergic in that it is not blocked by atropine. The existence of a noncholinergic transmitter stimulating amylase release may explain the recent report of Scheele and Haymovits (20) that K^+-stimulated amylase from guinea pig lobules is also incompletely blocked by atropine. In addition, Pearson *et al.* (21) have recently reported that electrical field stimulation of fragments of guinea pig pancreas also stimulates nerves and brings about amylase release by an atropine-insensitive mechanism.

The nature of the noncholinergic neurotransmitter released by nerves in guinea pig pancreatic lobules is at present unknown. It is unlikely that the transmitter is adrenergic in nature, since adrenergic agonists do not elicit pancreatic amylase release (22, 23). Isolated guinea pig pancreatic acini do respond to a number of known neuropeptides, including CCK, VIP, bombesin, and substance P (9). Immunohistochemical studies have provided evidence for nerves containing CCK and VIP in pancreas (2, 3). However, the potential involvement of CCK in scorpion toxin action can be eliminated since the action of the noncholinergic transmitter was not blocked by dibutyryl cyclic GMP, a competitive antagonist of the acinar cell CCK receptor (16).

All known transmitters and hormones that stimulate pancreatic amylase release act either via Ca^{2+} or cyclic AMP as intracellular messengers (9). Acetylcholine, CCK, bombesin, substance P, and their analogs act via Ca^{2+}, whereas VIP, secretin, and their analogs act via cyclic AMP. Maximal concentrations of secretagogues within each group are not additive to each other, whereas two secretagogues from the different groups are additive and frequently show synergism. In constrast to acetylcholine, the toxin-induced noncholinergic transmitter does not appear to be similarly mediated by Ca^{2+} in that the toxin did not increase the rapid efflux of $^{45}Ca^{2+}$ although a delayed efflux was observed. Furthermore, the action of the toxin was at least additive when combined with a maximal dose of CCK. We were unable to demonstrate that scorpion toxin, either in the presence or absence of atropine, acted to increase cyclic AMP content (data not shown). Thus, studies of possible second messengers, Ca^{2+} and cyclic AMP, do not provide clear evidence for the nature of the noncholinergic neurotransmitter.

While pancreatic exocrine function appears to be regulated by multiple neurotransmitters, the roles and relative importance of these transmitters also show considerable variation. In the rat, amylase release is primarily under cholinergic control (7), whereas in the guinea pig both cholinergic and noncholinergic components are of equal importance. In the pig, the cholinergic component is primarily concerned with enzyme release, whereas a VIP-sensitive component regulates the secretion of fluid and electrolytes (24). Both neural components interact with gut hormones, such as CCK and secretin (1, 9), and islet hormones, such as insulin (25), to

provide a complex and integrated control of pancreatic exocrine function.

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