Stimulation of Glucagon Secretion by Scorpion Toxin in the Perfused Rat Pancreas

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SUMMARY

Toxin from the scorpion Leiurus quinquestriatus was used to release norepinephrine from sympathetic nerve endings in the perfused rat pancreas. Addition of toxin, 10 μg./ml., to perfusate containing 0.3 mg./ml. glucose caused a large increase in release of norepinephrine and glucagon. Glucagon secretion was suppressed by perfusate containing 3.0 mg./ml. glucose but still responded to stimulation with scorpion toxin. Atropine, 10 μM, had no effect on either norepinephrine or glucagon release in response to scorpion toxin. The release of glucagon was blocked by 100 μM propranolol, 10 μM phentolamine, or 30 μM phenoxybenzamine. Somatostatin, 55 nM, did not affect the release of norepinephrine by scorpion toxin but totally inhibited the glucagon response. These results suggest that pharmacologic stimulation of the adrenergic nerve endings in the rat pancreas can elicit a rapid release of glucagon. This response can be prevented by appropriate concentrations of either alpha or beta adrenergic blocking agents or somatostatin. DIABETES *25*:645-49, August, 1976.

In recent years, considerable evidence has accumulated that the catecholamines norepinephrine and epinephrine can stimulate secretion of glucagon in man[1] and several species of animals.[2-4] This stimulation can be demonstrated by perfusing the catecholamines into the isolated pancreas, indicating that these agents can act directly on adrenergic receptors within the organ. Electrical stimulation of the autonomic nerves to the pancreas of the cat,[5] calf,[6] or dog[7,8] has also been shown to increase glucagon release. This suggested that the sympathetic nerves in the pancreas might mediate some of the increases in glucagon secretion evoked by physiologic stimuli. However, direct stimulation of the sympathetic nerve endings within the pancreas has not been possible.

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Recently, Moss et al.[9] have reported that a toxin purified from the venom of the scorpion Leiurus quinquestriatus can release norepinephrine from sympathetic nerve endings in several tissues of the rat. Studies in our laboratory[10] showed that low concentrations of this toxin could release norepinephrine from the sympathetic nerve endings in the isolated rat pancreatic islet. Therefore, we have studied the effect of scorpion toxin on release of norepinephrine and glucagon in the perfused rat pancreas.

METHODS

Wistar male rats weighing 350-400 gm. were used in all studies. The pancreases were perfused by a modification of the method described by Penhos et al.[11,12] Perfusion fluid consisting of synthetic interstitial fluid[13] with 0.3 per cent bovine serum albumin (Pentex) and 4 per cent dextran (Pharmacia, T-70) was infused at a constant rate of 4 ml. per minute in each experiment. The perfusion pressure was monitored continuously. The effluent was collected in iced glass test tubes representing one-minute intervals. A 1-ml. aliquot was taken from each sample and treated with 50 μl. of 1.0 M benzamidine to prevent destruction of glucagon. The samples were frozen at −20° C. until assayed for glucagon by radioimmunoassay using standard curves obtained with porcine glucagon.[14]

For measurement of norepinephrine release, samples representing five-minute intervals were collected. A 5-ml. aliquot of each sample was acidified with 200 μl. of 1.0 N. hydrochloric acid and stored frozen until assayed. Norepinephrine was measured by the radioenzymatic method of Henry et al.[15]

MATERIALS

Scorpion toxin was purified from venom of the North African scorpion, Leiurus quinquestriatus

(Sigma, St. Louis, Missouri), as described by Moss et al.[9] Bovine serum albumin (Sigma) was added to the purified toxin in an amount equivalent to the protein concentration of the crude venom. The toxin was lyophilized and kept frozen (−20° C.) until used. After initial experiments showed that the effects of purified toxin were the same as those observed with similar dilutions of crude venom, the subsequent studies reported here were done using scorpion venom at the indicated concentrations. Atropine sulfate was obtained from Sigma. The following chemicals were kindly supplied by the indicated sources: propranolol·HCl (Ayerst Laboratories, New York, N.Y.), phentolamine (Ciba Pharmaceutical Company, Summit, N.J.), phenoxybenzamine·HCl (Smith, Kline, and French Co., Philadelphia, Pa.), and dihydrosomatostatin (Drs. Roger Guillemin and Jean Rivier, Salk Institute, La Jolla, Calif.).

RESULTS

Effect of Scorpion Toxin on Norepinephrine Release and Perfusion Pressure

Addition of scorpion toxin (10.0 μg./ml.) to perfusion medium containing 0.3 mg./ml. glucose caused a large increase in norepinephrine release that became maximal during the first five-minute period following toxin infusion (figure 1). Neither 10 μM atropine nor 55 nM somatostatin had any effect on either the low basal release of norepinephrine or the large increase produced by toxin. In the presence of 100 μM propranolol, scorpion toxin released considerably less norepinephrine than in control preparations (table 1). Release of norepinephrine by toxin was enhanced by both 10 μM phentolamine and 30 μM phenoxybenzamine (table 1). In contrast with the rapid increase in norepinephrine release produced by scorpion toxin, there was a more gradual increase in perfusion pressure (figure 2) that became maximal approximately 10 minutes after beginning perfusion with toxin.

TABLE 1

Effect of adrenergic blocking agents on norepinephrine release in rat pancreas perfused with scorpion toxin

	Perfusion time (min.)	Norepinephrine in perfusate (ng./ml.) Control	Propranolol	Phentolamine	Phenoxybenzamine
Baseline:	30	0.25±0.06	0.33±0.07	0.40±0.06	0.35±0.04
	35	0.24±0.06	0.36±0.07	0.31±0.01	0.31±0.03
	40	0.26±0.08	0.42±0.06	0.37±0.05	0.30±0.03
Toxin:	45	23.9± 3.1	1.12±0.24	37.1± 5.1	66.8± 3.5
	50	23.4± 5.8	2.21±0.23	44.8± 0.6	47.9± 7.3
	55	14.1± 1.3	3.27±0.56	32.7± 0.9	31.4± 3.5
	60	10.2± 1.1	3.73±0.46	25.3± 0.8	21.5± 1.3
After toxin:	65	8.76±0.50	3.61±0.43	16.6± 0.3	16.1± 1.1
	70	8.08±1.25	3.06±0.40	13.1± 0.4	10.7± 0.1
	75	5.63±0.23	2.54±0.42	8.19± 1.8	10.1± 0.9

Values refer to the norepinephrine concentration of perfusate collected during the previous five-minute interval and represent the mean (± S.E.M.) of three experiments in each group. At 45 minutes the propranolol group is significantly different from each of the other groups ($P < 0.005$). The phentolamine and phenoxybenzamine groups differ from control ($P < 0.05$ and $P < 0.001$, respectively) and from each other ($P < 0.005$).

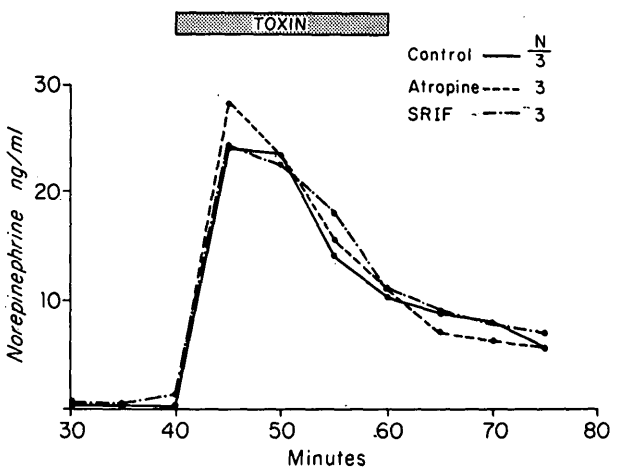

FIG. 1. Norepinephrine release in perfusate of rat pancreas perfused with scorpion toxin, 10 μg./ml. The concentration of atropine used was 10 μM. Somatostatin (SRIF) was used in 55-nM concentration. Plotted values are the norepinephrine concentration of perfusate collected during the previous five-minute interval and represent the mean of three experiments in each group.

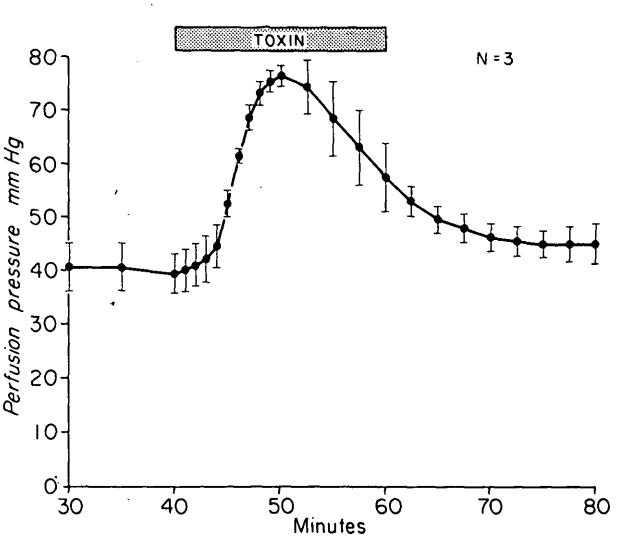

FIG. 2. Perfusion pressure of rat pancreas perfused with scorpion toxin. Medium was perfused at a constant rate of 4 ml. per minute. Plotted values represent the mean (± S.E.M.) of three experiments.

Glucagon Release During Perfusion with Scorpion Toxin

Perfusion with medium containing 0.3 mg./ml. glucose and scorpion toxin (10 μg./ml.) caused a large increase in release of glucagon (figure 3) that became maximal during the five-minute period after starting the toxin perfusion and gradually declined over the next 10 minutes despite the continued release of large amounts of norepinephrine into the perfusate. Glucagon release was inhibited by a high glucose concentration in the medium (figure 4), but scorpion toxin nevertheless stimulated glucagon release in a way similar to the response seen at a lower glucose concentration. Addition of atropine to the perfusate had little effect on the release of glucagon produced by scorpion toxin (figure 5).

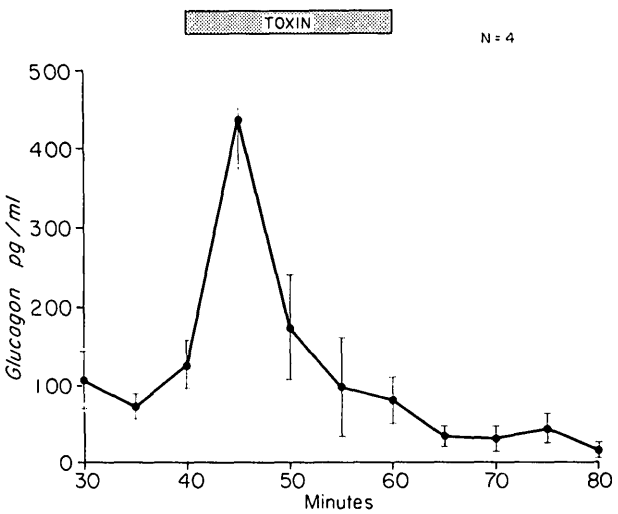

FIG. 3. Glucagon release from rat pancreas perfused with scorpion toxin. Glucose concentration was 0.3 mg./ml. Plotted values are the glucagon concentration of perfusate collected during the previous one-minute interval and represent the mean (± S.E.M.) of four experiments.

Effect of Adrenergic Blocking Agents on Release of Glucagon by Scorpion Toxin

Addition of 100 μM propranolol to the perfusate totally blocked the release of glucagon produced by scorpion toxin, whereas 10 μM propranolol did not prevent the increase in glucagon release (figure 6). The alpha adrenergic blocking agents phentolamine and phenoxybenzamine both blocked the glucagon response to scorpion toxin (figure 7). This blockade of glucagon release occurred despite the release of increased amounts of norepinephrine by toxin in the presence of phentolamine and phenoxybenzamine (table 1).

Effect of Somatostatin on Release of Glucagon

At a concentration that has been shown to block

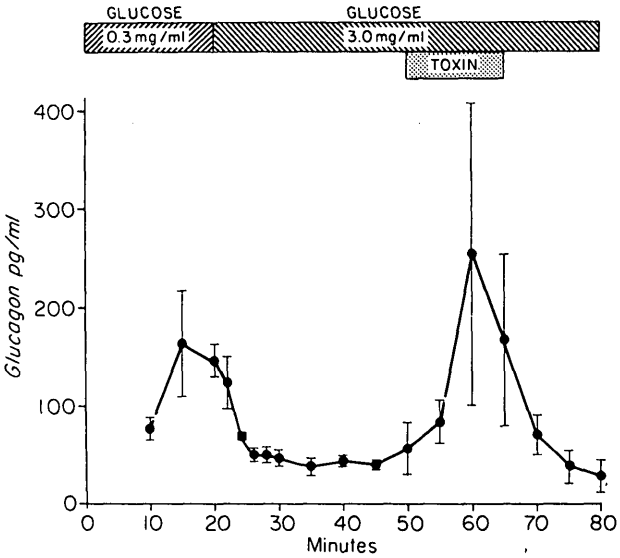

FIG. 4. Glucagon release from rat pancreas perfused with low and high glucose concentrations and scorpion toxin, 10 μg./ml. Plotted values are the glucagon concentration of perfusate collected during the previous one-minute interval and represent the mean (± S.E.M.) of three experiments.

basal and arginine-stimulated release of glucagon,[12] somatostatin blocked the glucagon response to scorpion toxin (figure 7).

DISCUSSION

Scorpion toxin appears to release norepinephrine from sympathetic nerve endings in the pancreas in a manner similar to its action on sympathetic nerves in other tissues[9,10] (figure 1). The rapidity with which both norepinephrine and glucagon release increased after perfusion with toxin contrasts with the slower rise in perfusion pressure and suggests that the hor-

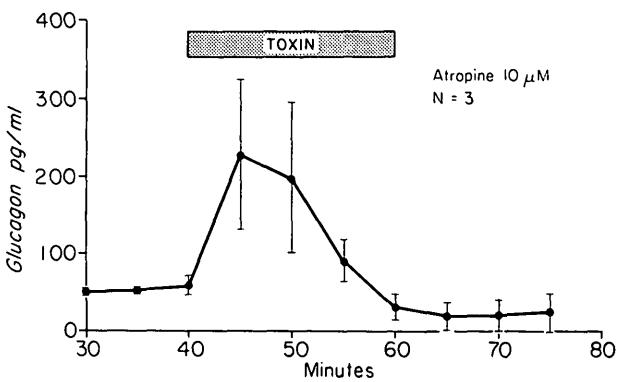

FIG. 5. Glucagon release from rat pancreas perfused with scorpion toxin. Atropine was present throughout the perfusion. Glucose concentration was 0.3 mg./ml. Plotted values are the glucagon concentration of perfusate collected during the previous one-minute interval and represent the mean (± S.E.M.) of three experiments.

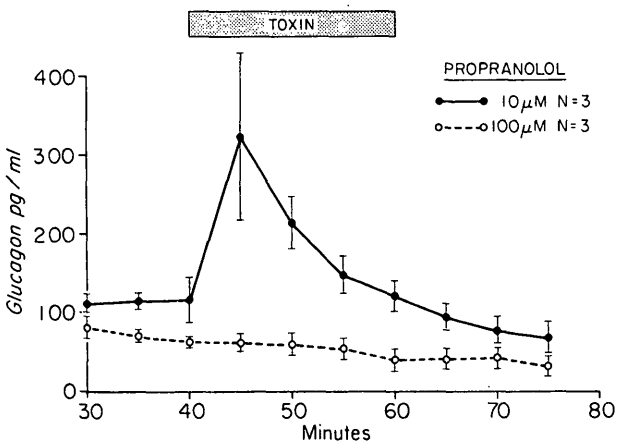

FIG. 6. Glucagon release from rat pancreas perfused with scorpion toxin, 10 μg./ml., in the presence of either 10 or 100 μM propranolol. Glucose concentration was 0.3 mg./ml. Plotted values are the glucagon concentration of perfusate collected during the previous one-minute interval and represent the mean (± S.E.M.) of three experiments.

monal changes observed were not due to alterations in the pattern of perfusion within the pancreas. The increase in release of norepinephrine produced by toxin in the presence of the alpha adrenergic blocking agents phentolamine and phenoxybenzamine agrees with previous studies that have demonstrated a similar increase in norepinephrine release during electrical stimulation of sympathetic nerves in the presence of these agents.[16-18]

The lack of stimulation of glucagon release produced by scorpion toxin in the presence of adrenergic blocking agents (figures 6 and 7) suggests that the effect of scorpion toxin on glucagon release was mediated through its release of norepinephrine at sites adjacent to pancreatic alpha cells. The ability of either alpha adrenergic blocking agents (phentolamine and phenoxybenzamine) or a beta adrenergic blocking agent (propranolol) to inhibit glucagon secretion might be explained by the presence of both alpha and beta adrenergic receptors on the pancreatic alpha cells. Alternatively, the presumed adrenergic receptor on the alpha cell may represent a different type of receptor that can be blocked by both alpha and beta receptor blocking agents. In actual potency, the alpha blocking agents were more effective than propranolol. Furthermore, some of the lack of stimulation of glucagon release by toxin in the presence of propranolol may have been due to the lesser amounts of norepinephrine released (table 1). This differs from the results obtained in the isolated dog pancreas by Iversen,[3] in which propranolol blocked glucagon secretion produced by infusions of catecholamines but phentolamine and phenoxybenzamine had no effect. Luyckx et al.[19] noted that both alpha and beta adrenergic blockers prevented the increase in glucagon release caused by forced swimming in rats. These various results may be explained by species differences, noncomparability of results obtained by norepinephrine infusion with those obtained by norepinephrine released within the pancreas, and differences in the concentrations of blocking agents used.

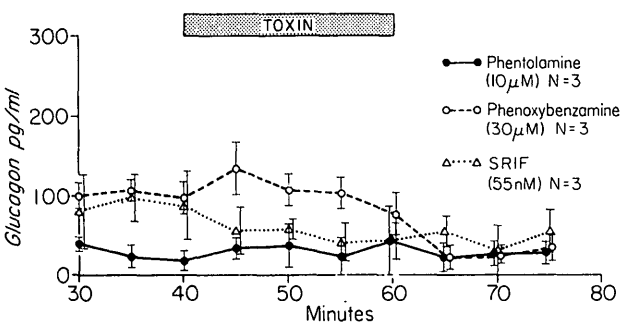

FIG. 7. Glucagon release from rat pancreas perfused with scorpion toxin, 10 μg./ml., in the presence of phentolamine, phenoxybenzamine, or somatostatin. Glucose concentration was 0.3 mg./ml. Plotted values represent the mean (± S.E.M.) of three experiments.

The release of glucagon by scorpion toxin is quantitatively greater than that observed in most previous studies using either infusion of norepinephrine or epinephrine[1-4] or stimulation of the pancreatic nerve.[5-7] This might be due to the greater effectiveness of the toxin in releasing norepinephrine in proximity to the pancreatic alpha cells. The relatively short duration of the glucagon peak did not parallel the slower decline in release of norepinephrine (figures 3 and 1), suggesting that either the stores of releasable glucagon were temporarily depleted or that the stimulation of pancreatic alpha cells by released norepinephrine affects mainly the acute release of glucagon. Perfusion of rat pancreas with epinephrine has been shown to produce both an acute and sustained release of glucagon.[4]

Perfusion of the rat pancreas with medium containing only 0.3 mg./ml. glucose did not produce a very large release of norepinephrine into the perfusate (figure 1, 30- to 40-minute period). Christensen and Iversen[20] have reported that perfusing dog pancreas with medium containing no glucose causes an increase in the release of catecholamines. However, they reported no difference in catecholamine secretion when perfusing with a glucose concentration of 0.25 mg./ml. from perfusion with 1.5 mg./ml. Likewise, we have not found any large differences in the release of norepinephrine from the perfused rat pancreas be-

tween 0.3 or 3.0 mg./ml. glucose concentrations.

Scorpion toxin has been shown to release acetylcholine from certain cholinergic nerves. The inability of atropine to prevent the release of glucagon by scorpion toxin (figure 5) suggests that release of acetylcholine from parasympathetic nerve endings within the pancreas did not produce the glucagon response. Similarly, the release of norepinephrine by scorpion toxin was not affected by atropine.

Somatostatin had no effect on the release of norepinephrine by scorpion toxin (figure 1) or on the subsequent increase in perfusion pressure (not shown). This suggests that somatostatin does not interfere with either the release of norepinephrine or its action on the adrenergic receptors of the vascular bed. Nevertheless, somatostatin totally blocked the release of glucagon by scorpion toxin (figure 7). Thus, it would appear that somatostatin blocked the glucagon response to released norepinephrine by interfering with some step in the release process subsequent to the interaction of norepinephrine with its presumed receptor.

The results with scorpion toxin indicate that norepinephrine released from sympathetic nerve terminals within the pancreas can stimulate glucagon release. It should be emphasized that the amounts of norepinephrine released by toxin are probably much greater than those released by physiologic stimulation. Furthermore, it is not known what physiologic conditions can stimulate the sympathetic nerves in the pancreas in proximity to the alpha cells. However, the large release of glucagon produced by scorpion toxin suggests that norepinephrine released from the adrenergic nerve terminals within the pancreas may be a more effective stimulus to glucagon secretion than norepinephrine reaching the pancreas through the general circulation.

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