Inhibition of Insulin Release by Scorpion Toxin in Rat Pancreatic Islets

David G. Johnson, M.D., David P. Henry, M.D., Jonathan Moss, M.D., and Robert H. Williams, M.D., Seattle

SUMMARY

Toxin purified from venom of the scorpion Leiurus quinquestriatus was used to release norepinephrine from adrenergic nerve terminals in isolated pancreatic islets perifused in vitro. Addition of toxin (10 μg./ml.) to the perifusion medium caused a sixfold increase in release of norepinephrine in the presence or absence of 3×10^{-5}M phenoxybenzamine. During 20 minutes of stimulation with toxin, the pancreatic islets released an average of 15 pg. of norepinephrine per islet, which represented 20 per cent of the normal content of norepinephrine in islets. Insulin secretory rates in response to either 1.0 or 3.0 mg./ml. glucose were inhibited similarly by scorpion toxin. Addition of phenoxybenzamine abolished the inhibition of insulin release caused by scorpion toxin. Phenoxybenzamine alone did not affect release of insulin. Neither the enhanced release of norepinephrine nor the decreased release of insulin was reversed by a 20-minute wash-out period after infusion of toxin.

These results indicate that the sympathetic nerve terminals in the rat pancreatic islet contain considerable amounts of norepinephrine that can be released by scorpion toxin. The norepinephrine released from sympathetic nerve endings in the pancreatic islet can inhibit release of insulin through an alpha-adrenergic action that is blocked by phenoxybenzamine. DIABETES *25*:198-201, March, 1976.

Infusion of epinephrine can affect release of insulin in man [1-3] and several species of animals.[4-6] Studies with isolated pancreatic tissue incubated in vitro[7,8] demonstrated that epinephrine acts directly on adrenergic receptors in pancreatic endocrine cells to cause changes in the rate of hormonal release. Intravenous infusion of epinephrine produces first a decrease in insulin release, followed by a later increase.[3] The inhibition of insulin release appears to be mediated by an alpha adrenergic action, whereas the later augmentation of insulin release is due to beta-adrenergic activation. In general, the effects of norepinephrine infusion on insulin release appear to be less striking than those produced by equivalent amounts of epinephrine and are not seen at the concentrations of norepinephrine usually present in circulating plasma.[9]

It is not known if norepinephrine released from sympathetic nerves within the pancreatic islet can influence insulin release. Electrical stimulation of the autonomic nerves to the pancreas of the atropinized dog has been reported to change the rate of release of insulin,[10] but this could have been caused by effects on pancreatic exocrine tissue or the pattern of perfusion in the pancreatic vascular bed.

Recently, Moss et al.[11] have demonstrated that a toxin purified from the venom of the scorpion Leiurus quinquestriatus can release norepinephrine from sympathetic nerve endings in several body organs of the rat. The neurotransmitter appears to be released by a process that resembles normal neurosecretion. This action of scorpion toxin makes it a particularly useful tool to study the effects of sympathetic nerves in organs, such as the pancreatic islet, where electrical stimulation of the nerve axons is difficult or impossible. In the present study scorpion toxin was added to medium perifusing isolated pancreatic islets of the rat to examine the effects on release of norepinephrine and insulin.

From the Divisions of Endocrinology and Clinical Pharmacology, Department of Medicine, University of Washington, Seattle, Washington 98195.

Accepted for publication December 8, 1975.

MATERIALS AND METHODS

Isolation of pancreatic islets. Pancreatic islets were

isolated by the collagenase method as modified by Aleyassine and Gardiner.[12] For each perifusion experiment 80-100 islets from one animal were placed in a Millipore filter chamber and perifused at a constant rate of 0.7 ml. per minute with synthetic interstitial fluid[13] containing 0.3 per cent bovine serum albumin.[14] The medium was aerated with 95 per cent oxygen:5 per cent carbon dioxide and maintained at 37° C.

Norepinephrine assay. Samples were collected over a 10-15-minute period and immediately acidified to approximately pH 3.0 with 0.6 ml. of 1.0 N hydrochloric acid. After addition of 10 mg. of sodium metabisulfite to each sample, the samples were frozen until assayed. Norepinephrine was measured by the radioenzymatic method of Henry et al.[15] The pH of 5-ml. aliquots was raised to 8.5 with 2.0 M Tris buffer and the samples were treated as described for plasma. The standard deviation of 10 samples of pooled perifusion fluid assayed for norepinephrine was ± 4.8 per cent of the mean value. To measure the norepinephrine content of pancreatic islets, groups of 10 islets were homogenized in 0.3 ml. of 0.1 N perchloric acid and centrifuged for five minutes at 2,000 × g. Aliquots (50 μl.) were taken for norepinephrine assay as described for tissues.

Insulin assay. The immunoactive insulin (IRI) concentration of the medium was collected as one-minute samples and assayed by radioimmunoassay, cellulose being used to absorb the free insulin.[16] Results were compared to a standard curve obtained with rat insulin.

Materials. Scorpion toxin was purified from venom of the North African scorpion L. quinquestriatus (Sigma, St. Louis), as described by Moss et al.[11] 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. Phenoxybenzamine was kindly supplied by Smith, Kline, and French Laboratories, Philadelphia.

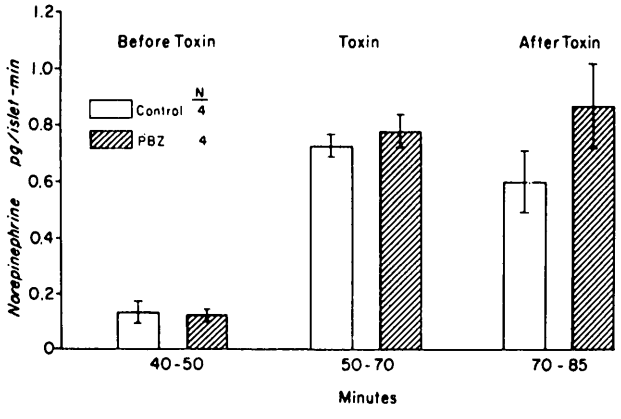

FIG. 1. Release of norepinephrine by perifused pancreatic islets exposed to scorpion toxin. Islets were preincubated for 40 minutes. Bars represent the mean (± S.E.M.). Lined bars represent experiments conducted in 3 × 10^{-5} M phenoxybenzamine (PBZ). The glucose concentration was 1.0 mg. per milliliter.

TABLE 1

Release of norepinephrine from perifused pancreatic islets. Islets were preincubated for 29 minutes.

Time Interval (minutes)	Norepinephrine (pg./islet-minutes)	
	Experiment 1	Experiment 2
30 - 39	.15	.23
40 - 49	.18	.25
50 - 59	.13	.18
60 - 69	N.D.*	.10
70 - 79	.11	.18
80 - 89	.15	.20

*Not detectable.

RESULTS

Effect of scorpion toxin on norepinephrine release. Only small amounts of norepinephrine were released from pancreatic islets (table 1 and figure 1), during perifusion with medium containing 1.0 mg./ml. glucose. Addition of scorpion toxin, 10 μg./ml., caused a six-fold increase in release of norepinephrine (figure 1). After 20 minutes the pancreatic islets were perifused again with regular medium, but norepinephrine continued to be released in high amounts similar to those found during perifusion with toxin. During the 20-minute perifusion with scorpion toxin, the pancreatic islets released 15 ± 0.8 pg. norepinephrine per islet. The average content of norepinephrine in rat pancreatic islets was 74 ± 11 pg. per islet. Therefore, the islets released 20 per cent of their total norepinephrine content during the stimulation period. Pancreatic islets weigh approximately 8 μg. each. Thus, the norepinephrine concentration of pancreatic islets is almost 10 μg. per gram., which is one of the highest of any organ in the body.

Insulin release during perifusion with scorpion toxin. Addition of scorpion toxin caused a rapid fall in release of insulin to rates that were only half-normal (figure 2). The inhibition of insulin release persisted during 40 minutes of perifusion with toxin and did not return toward normal values following administration of toxin. Insulin release in response to 3.0 mg. per milliliter glucose continued to increase for 40

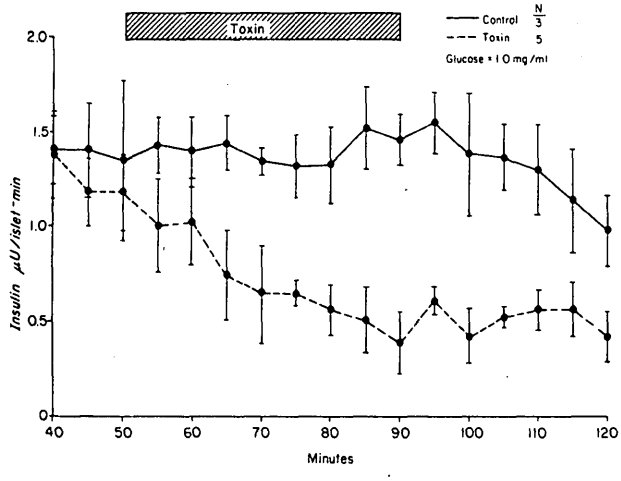

FIG. 2. Release of insulin by perifused pancreatic islets exposed to scorpion toxin. Plotted values represent the mean (± S.E.M.).

minutes and then became constant at approximately 4-5 μg. per islet per minute (figure 3). Addition of scorpion toxin after the 45 minutes of stimulation with glucose produced a rapid decline in insulin secretory rates to 3.0 μg. per islet per minute. The inhibition of glucose-stimulated insulin release caused by scorpion toxin persisted throughout the 15-minute perfusion of toxin.

Phenoxybenzamine reversal of scorpion toxin effects. Phenoxybenzamine (3×10^{-5}M) had no effect on either the release of insulin into medium containing 1.0 mg. per milliliter glucose (figure 4, 40-50-minute period) or the release of norepinephrine (figure 1). However, the inhibition of insulin release caused by scorpion toxin was entirely prevented in the presence of phenoxybenzamine (figure 4). Similarly, there was no change in the rate of insulin release after stopping the perifusion with toxin.

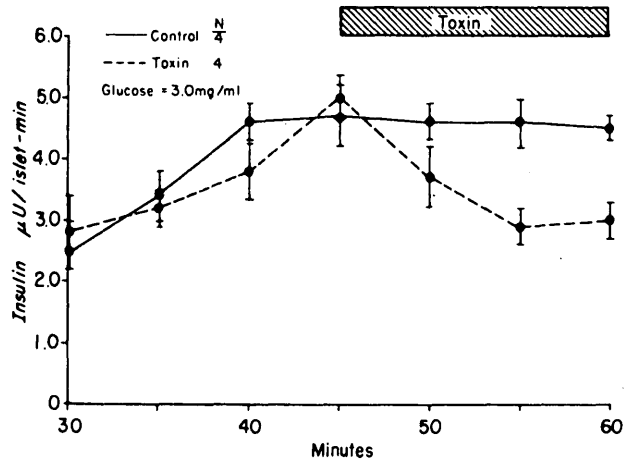

FIG. 3. Effect of scorpion toxin on glucose-induced insulin release. Islets were incubated for 45 minutes in medium containing 3.0 mg./ml. glucose before addition of toxin. Plotted values represent the mean (± S.E.M.).

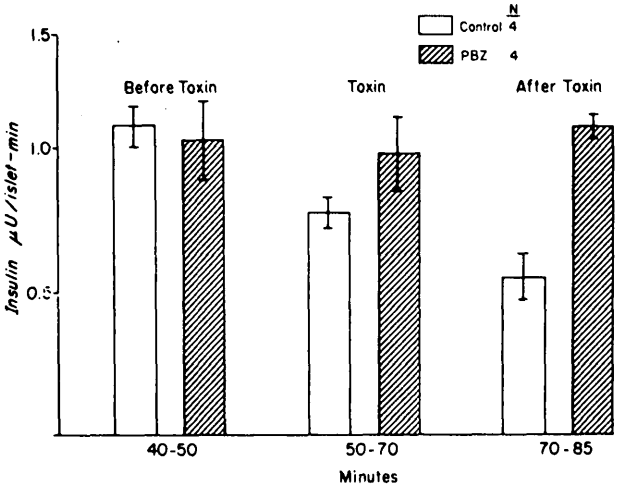

FIG. 4. Effect of 3×10^{-5} M phenoxybenzamine (PBZ) on inhibition of insulin secretion caused by scorpion toxin, 10 μg./ml. Bars represent the mean (± S.E.M.). The glucose concentration was 1.0 mg. per milliliter.

DISCUSSION

Pancreatic islets isolated by the collagenase technic contain relatively high amounts of norepinephrine compared with most other body tissues. Presumably, most of this norepinephrine is stored within the sympathetic nerve terminals scattered throughout the organ.[17,18] Scorpion toxin released norepinephrine from the sympathetic nerves in a manner very similar to its action on sympathetic nerve endings in other tissues.[11] The failure of norepinephrine release to be reversed in the wash-out period after toxin suggests that the small amounts of toxin that remain bound to the nerves continue to exert their effects.

The inhibition of insulin release by scorpion toxin at either normal or high glucose concentrations was apparently due to the action of released norepinephrine on alpha-adrenergic receptors, since it could be blocked by phenoxybenzamine. The effect of released norepinephrine on beta-adrenergic receptors in the pancreatic beta cell did not influence the rate of insulin release, even in the presence of phenoxybenzamine. This agrees with the lack of any reversal of the inhibition of insulin release caused by norepinephrine in isolated islets, in contrast with the later rise of insulin release that occurs in vivo.[3]

Previous morphologic studies have demonstrated

that the pancreatic islets of many species, including the rat, contain sympathetic nerve terminals.[17,18] Precise quantitation of the amount of norepinephrine present in tissues has not been possible with morphologic methods, but the amounts of norepinephrine measured in the present study are even greater than anticipated on the basis of morphologic findings.

Although terminal varicosities of sympathetic nerves have been seen lying adjacent to pancreatic beta cells, their possible effects on insulin release could be inferred only on the basis of studies with exogenous norepinephrine. The results with scorpion toxin suggest that norepinephrine released from sympathetic nerve terminals in the pancreatic islet can inhibit insulin release. However, the amounts of neurotransmitter released by toxin are very great compared with normal stimulation. Furthermore, it is not known what physiologic conditions result in stimulation of the sympathetic nerves in the pancreatic islet. The use of sensitive methods of assaying norepinephrine in small amounts of tissue, as described here for pancreatic islets, may prove useful in future research directed at this problem.

ACKNOWLEDGMENTS

We thank Barbara Starman and Sandra Musgrove for expert technical assistance.

These investigations were supported by U.S.P.H.S. Grant AM17698 from the National Institute of Arthritis, Metabolism, and Digestive Diseases. Dr. Johnson was supported by Research Career Development Award 1 K04 AM70727. Dr. Henry was a NIH Special Research Fellow (F03 AM53427).

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