*Biochemical Pharmacology*, Vol. 32, No. 6, pp. 1101–1104, 1983.
Printed in Great Britain.

0006-2952/83/061101-04 $03.00/0


# ACTION OF THE VENOM OF THE SCORPION *TITYUS TRINITATIS* ON PANCREATIC INSULIN SECRETION

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(*Received* 7 *May* 1982; *accepted* 8 *September* 1982)

**Abstract**—*In vivo*, canine pancreas was stimulated to secrete insulin by the venom of the scorpion *Tityus trinitatis;* the venom also caused a rise in plasma glucose level as well. The venom-induced insulin secretion was also observed under *in vitro* conditions in rat pancreatic slices, and this stimulation was dose-related. Maximal effect was observed at 20 μg/ml. Atropine ($3 \times 10^{-6}$ M) completely abolished both venom (20 μg/ml)- and acetylcholine ($3 \times 10^{-7}$ M)-induced insulin secretion. It is suggested that the venom-induced insulin secretion is mediated through muscarinic cholinergic mechanisms.

The neurogenic control of hormone secretion by the endocrine pancreas has been well documented. Catecholamines stimulate secretion of glucagon in man [1] and in several animals [2] and affect insulin release [3, 4]. The insulin-secretory response to acetylcholine in perfused dog pancreas has been characterized as a muscarinic action [5].

Venom of the scorpion, *Tityus trinitatis*, causes gastric acid secretion [6] and exocrine pancreatic secretion [7, 8]. The venom-induced exocrine secretion is mediated through muscarinic cholinergic receptors [8]. Although many pharmacological effects, including hyperglycemia induced by the venom [9], have been studied in detail, only a few observations have been reported in regard to the action of venoms on endocrine pancreatic tissue [10, 11]. The importance of muscarinic cholinergic receptors in insulin secretion and the muscarinic secretory action of venom of *T. trinitatis* in exocrine pancreas prompted the present investigation employing anesthetized dogs and rat pancreatic slices.

## MATERIALS AND METHODS

Adult mongrel dogs, weighing 10–15 kg body weight and fasted 18 hr, were anesthetized with pentobarbitone (40 mg/kg). Laparotomy was performed, and the pancreas and the inferior pancreaticoduodenal artery were identified. Scorpion venom at 100 μg/kg body weight (in a volume not exceeding 1 ml) or 1 ml of physiological saline (control) was slowly injected into the interior pancreaticoduodenal artery with minimum handling of the pancreas. The duodenal part of the artery was clamped during injection period to prevent the injected material escaping into the duodenum. The abdomen was then closed (the entire procedure lasted 30–40 min); the animal was allowed to recover from anesthesia and was observed for 24 hr with collection of peripheral venous blood at various time intervals. Blood samples were collected before, and 2 min, 30 min, 60 min, 2.5 hr, 6 hr, and 24 hr after, injection of venom/saline. Plasma separated from blood was stored at 4° until the following day when glucose, amylase and insulin measurements were carried out. After 24 hr the animal was killed, and the abdominal organs were examined.

The preparation of pancreatic slices was performed as previously described [8]. Sprague–Dawley rats (150–200 g) fasted for 18 hr (with water *ad lib.*) were killed by a blow on the head, and the pancreas was quickly removed and trimmed. The adhering fatty material was removed by blunt dissection. The tissue (roughly 400 mg/gland) was cut into four slices of 100 mg each and suspended in physiological salt solution (pH 7.4) of the following compositions (mM): NaCl, 103; KCl, 4.7; $CaCl_2$, 2.56; $MgCl_2$, 1.13; $NaHCO_3$, 25; $NaH_2PO_4$, 1.15; D-glucose, 2.8; Na pyruate, 4.9; Na fumarate, 2.7; and Na glutamate, 4.9; and equilibrated with 95% $O_2$ and 5% $CO_2$ [12]. Trasylol [13], 500 I.U./ml of buffer, was added to prevent protein breakdown in the medium and tissue homogenate. Following three washings with buffer, the pancreatic slices were incubated for 10 min to remove blood adhering to the tissue and to eliminate amylase which had leaked from damaged tissue. At 10 min, each 100 mg piece of tissue was sliced into 25 mg slices and the tissue slices were pooled. Tissue slices (100 mg) in 3 ml of buffer were gassed with 95% $O_2$–5% $CO_2$ and preincubated for 30 min at 37° in a metabolic shaker at 90 oscillations/min. The slices were then washed twice with 3 ml of fresh buffer (at 37°), blotted, and transferred to incubation flasks containing 3 ml buffer (control) or 3 ml medium plus additive (agonist, venom or acetylcholine; antagonist, atropine or agonist + antagonist). The slices were reequilibrated

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with $O_2$–$CO_2$ and incubated as above for 30 min. At the end of incubation the tissue slices were removed by filtration on a nylon-mesh filter. Buffer (2.0 ml) was used to rinse the incubation flask and to wash the tissue on the filter. The 5 ml filtrate (medium) was stored at 4° for assays of insulin and amylase. The tissue was resuspended in 2 ml of buffer and homogenized with an ultra-turrax. The homogenization tube was further rinsed with 3 ml of buffer and added to the homogenate. The homogenate volume was made up to 10 ml with buffer and centrifuged at 1500 *g* at 4° for 10 min, and the supernatant fraction was stored at 4° for the assays of insulin and amylase.

Amylase in the incubation medium and its corresponding tissue homogenate was determined by the Phadebas tablet method of Ceska *et al.* [14] (Phadebas tablets were supplied by Pharmacia, Uppsala, Sweden). Similar measurements for insulin were carried out by the radioimmunossay method of Hales and Randle [15] employing the kit from the Radiochemical Centre, Amersham, England. The sum of amylase/insulin in the incubation medium and the corresponding tissue homogenate yielded total amylase/insulin content. The secretory response of the tissue was determined by the ratio of amylase/insulin level in the incubation medium to that of total amylase/insulin. The response to a stimulant was indicated by the increase in this ratio. Glucose was determined by the glucose-oxidase method using a blood glucose kit supplied by Boehringer.

All chemicals used in this study were of analytical grade. Trasylol was obtained from the Bayer Co., Munich, West Germany. Acetylcholine chloride, atropine, and *Tityus serrulatus* venom were obtained from Sigma Chemicals, London, U.K. Pentobarbitone sodium was supplied by Abbott Laboratories, Kent, U.K. Venom obtained from the scorpion, *Tityus trinitatis*, by electrical stimulation was stored desiccated at −20°. Statistical analysis of results was carried out using Student's *t*-test.

## RESULTS

*Venom-induced insulin secretion from canine pancreas.* Administration of venom (100 μg/kg body weight) through the inferior pancreaticoduodenal artery in dogs produced (a) an increase in peripheral venous blood glucose, and (b) an increase in insulin levels. The stimulated insulin secretion was seen at 2 min after venom injection, coinciding with elevated blood glucose. Immunoreactive insulin level returned to pre-venom level at 60 min, whereas blood glucose remained elevated. Plasma amylase remained unaltered throughout. Control dogs injected with saline did not show significant changes in these variables (Table 1).

Removal of the clamp soon after intra-arterial injection of venom caused contraction of the duodenum, while saline injection did not. This effect was due to the small amount of venom trapped in between the clamp and branching point of the duodenal part of the artery. Autopsy findings of 24 hr post-venom dogs revealed an edematous pancreas, fat necrosis in the pancreas, hemorrhagic spots in both pancreas and the lumen of the duodenum, and inflammation of both the ampulla of Vater and the sphincter opening of the main duct of the pancreas. Stomach, kidneys and spleen were normal. Control animals showed no microscopic changes. In three dogs, examination of pancreas at 60 min after venom treatment showed no gross microscopic changes.

*Venom-induced insulin and amylase secretion.* Rat pancreatic slices were incubated with medium containing crude venom of *T. trinitatis* at concentrations ranging from 5 to 100 μg/ml of incubation medium. The venom-stimulated release of insulin from the slices was linear up to 20 μg/ml. Fifty and 100 μg venom stimulated secretion of insulin, but the effect was significantly below the maximal effect (Fig. 1). This stimulated secretion of insulin from slices closely paralleled amylase output already reported [8] (Fig. 1).

The action of the venom of the scorpion *T. serrulatus*, indigenous to South America, was investigated using rat slices. This venom is available in relatively pure form and hence the concentrations employed were much lower than those of crude venom and ranged from 0.5 to 10 μg/ml. This venom also stimulated the secretion of both insulin and amylase; maximal effect on amylase secretion was at 2 μg/ml. The lower dose of venom (0.5 μg/ml)

Table 1. Effect of intra-arterial injection of *T. trinitatis* on peripheral blood plasma amylase, insulin and glucose levels in dogs*

| Time | Amylase (I.U./liter) | | Insulin (μU/ml) | | Glucose (mg/dl) | |
|---|---|---|---|---|---|---|
| | Venom | Control | Venom | Control | Venom | Control |
| Fasting | 2100 ± 282 | 2250 ± 210 | 13.5 ± 2.2 | 13.5 ± 1.0 | 98.1 ± 2.3 | 96.2 ± 3.3 |
| Pre-venom | 1950 ± 248 | 2175 ± 192 | 13.8 ± 2.6 | 15.6 ± 0.9 | 104.0 ± 4.8 | 100.2 ± 4.2 |
| 2 min P.V. | 2075 ± 179 | 2400 ± 282 | 37.6 ± 4.0† | 15.0 ± 1.1 | 132.0 ± 8.0† | 103.4 ± 1.4 |
| 30 min P.V. | 2632 ± 312 | 2175 ± 313 | 32.8 ± 2.4† | 14.8 ± 1.8 | 126.2 ± 9.4† | 96.2 ± 3.8 |
| 60 min P.V. | 2400 ± 262 | 2160 ± 243 | 16.2 ± 2.6 | 16.2 ± 0.9 | 128.7 ± 8.3† | 103.2 ± 5.2 |
| 2.5 hr P.V. | 2400 ± 312 | 2200 ± 192 | 14.1 ± 2.0 | 15.2 ± 0.5 | 95.7 ± 6.5 | 102.2 ± 5.8 |
| 6 hr P.V. | 2300 ± 263 | 2360 ± 228 | 16.0 ± 2.8 | 17.0 ± 0.6 | 101.0 ± 5.2 | 95.4 ± 3.2 |
| 24 hr P.V. | 2175 ± 208 | 2400 ± 272 | 13.8 ± 1.9 | 14.6 ± 1.0 | 100.9 ± 4.2 | 97.8 ± 4.9 |

* Venom (100 μg/kg body wt) in a volume not exceeding 1 ml was injected through the inferior pancreaticoduodenal artery. Values are means ± S.E.M. Number of experiments: nine for venom and five for saline control. P.V.: post-venom.

† Significantly different from fasting/pre-venom values ($P < 0.02$).

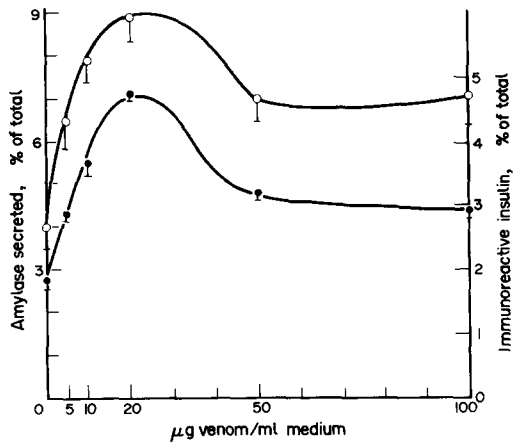


Fig. 1. *Tityus trinitatis* venom-induced amylase and insulin release. Amylase and insulin release (as percentage of total) by 100 mg wet weight of rat pancreatic slices incubated in modified Krebs-bicarbonate buffer containing different concentrations of venom is shown. Each point is the mean ± S.E.M. of seven experiments in triplicate determinations. Key: (○—○) amylase; and (●—●) insulin.

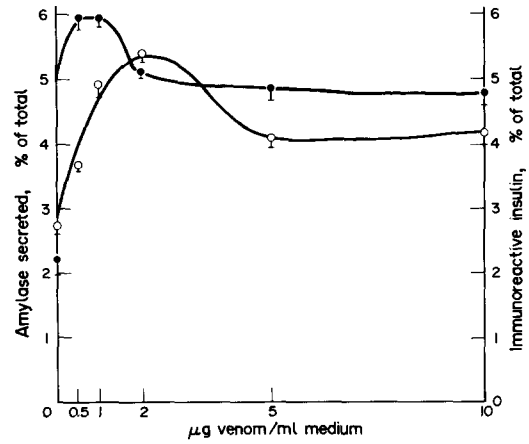


Fig. 2. *Tityus serrulatus* venom-induced amylase and insulin release. Amylase and insulin release (as percentage of total) by 100 mg wet weight of rat pancreatic slices incubated in modified Krebs-bicarbonate buffer containing different concentrations of venom is shown. Each point is the mean ± S.E.M. of six experiments in triplicate determinations. Key: (○—○) amylase; and (●—●) insulin.

produced the maximal insulin secretion; this maximal effect was observed at the next higher concentration (1 μg/ml). Further increases in venom concentration produced an inhibition of secretion (Fig. 2). The quantitative effects of the two venoms on amylase and insulin secretion are not strictly comparable, perhaps due to differences in the purity of the venoms.

*Effect of atropine on venom-induced insulin secretion.* The effect of atropine on venom-induced insulin secretion was investigated in an attempt to study the mechanism of action of the venom of *T. trinitatis*. In this study, the effect of acetylcholine (Ach), a muscarinic cholinergic agonist, was studied for comparison. Venom concentration (20 μg/ml) eliciting maximal insulin secretion from the slices was chosen. Ach was employed at $3 \times 10^{-7}$ M, a concentration which induced maximal amylase secretion from the slices [16]. Both venom and Ach stimulated insulin secretion from the slices. The percent stimulations were 181 and 95 respectively. Atropine at a concentration of $3 \times 10^{-6}$ M completely abolished the secretion stimulated by both venom and Ach; at this concentration atropine did not affect the basal secretion from the slices (Table 2).

## DISCUSSION

Venom of the scorpion, *T. trinitatis*, has been shown to stimulate pancreatic exocrine secretion [7, 8] and to cause contraction of the sphincter of Oddi [17] through a cholinoceptive action involving muscarinic receptors. In the present study, intra-arterially injected venom stimulated canine pancreas to secrete insulin; however, no significant change in amylase level was observed over 24 hr. In an earlier study with cannulated canine pancreatic duct [7], the stimulation caused by a second dose of venom was

Table 2. Effect of atropine on *T. trinitatis* venom-induced insulin secretion from rat pancreatic slices*

| | Insulin secretion (% of total by rat pancreatic slices)† | | | |
|---|---|---|---|---|
| Atropine | Control medium | Ach ($3 \times 10^{-7}$ M) | Control medium | Venom (20 μg/ml) |
| 0 | 2.1 ± 0.3 | 4.1 ± 0.4‡ | 1.6 ± 0.2 | 4.5 ± 0.3‡ |
| $3 \times 10^{-6}$ M | 1.9 ± 0.2 | 1.5 ± 0.4§ | 1.8 ± 0.1 | 1.8 ± 0.2‖ |

* Values are means ± S.E.M. of five experiments.

† Insulin secreted (by 100 mg of rat pancreatic slices at 37° for 30 min) in the incubation buffer is expressed as a percentage of total insulin concentration (incubation medium + tissue).

‡ Significance of difference from corresponding basal values ($P < 0.01$).

§ and ‖ Significant decrease in values from the corresponding values with stimulants ($P < 0.02$).

found to be highly significant compared to the stimulation produced by the first dose (1 mg/kg) of venom. Although the venom was given intra-arterially in the present study, the dose of 100 μg/kg body weight probably was not sufficient to stimulate enzyme secretion from the pancreas enough to significantly increase the blood amylase level. The venom-induced insulin secretion returned to pre-venom level by 60 min in contrast to venom-induced hyperglycemia which remained elevated. In rat pancreatic slices, however, the venom-induced insulin secretion was dose–response related. Atropine, $3 \times 10^{-6}$ M, a muscarinic cholinergic antagonist, completely abolished both venom (20 μg/ml)- and Ach ($3 \times 10^{-7}$ M)-stimulated insulin secretion. This observation is in agreement with the report of Conaway *et al.* [5].

The venom-induced pancreatic tissue damage and hyperglycemia in dogs may initiate a secretory response from the pancreas. These are, however, not the factors in the present study for the following reasons: (1) 60 min post-venom pancreas of dogs, the time by which insulin response returns to normal, showed no microscopic changes; (2) *in vitro* slices, exposed to increasing concentrations of venom, responded in a dose-dependent manner in secreting both insulin and amylase. Furthermore, the secretory response of both insulin and amylase to supramaximal doses of venom declined, indicating thereby the similarities between the actions of the venom and other pancreatic secretagogues, such as cholecystokinin and carbachol [18, 19], which also produce this biphasic amylase response; (3) pancreatic slice incubations were carried out in buffer containg 2.8 mM glucose; the increase in stimulatory response was significantly higher than that of the control, and (4) both the venom and Ach effects on insulin secretion from the slices were abolished by atropine. These considerations lead us to suggest that the stimulated insulin secretion is a direct and specific effect involving a muscarinic cholinergic mechanism.

It is of interest to note that the venom of *T. serrulatus*, a different scorpion from South America, has also been shown to possess Ach-releasing properties in brain slices [20]. Rhoads *et al.* [21] reported that the inhibition by *T. serrulatus* venom of $Na^+$-dependent uptake of neurotransmitter amino acids in rat brain synaptosomal preparation may be due to the action of the venom on the transmembrane $Na^+$-gradient. This venom, in the present study, stimulated both amylase and insulin secretion from rat pancreatic slices. The stimulatory effect was comparable to that produced by *T. trinitatis*. Gallagher *et al.* [17] have reported that, in rat pancreas, the venom of *T. serrulatus* stimulated amylase release by inducing the release of Ach from pancreatic nerves. Furthermore, Williams *et al.* [22] employed this venom as a tool to show the existence of a second neurotransmitter in pancreatic nerve endings capable of stimulating amylase release in the guinea pig. These findings, in combination with the reported inhibition of insulin release in isolated perfused rat pancreatic islets by toxin of a North African scorpion, *Leiurus quinquestriatus* [10], indicate that these venoms could be employed to investigate the physiologic mechanism of insulin and amylase release from the pancreas.

*Acknowledgements*—We thank A. Cullen for technical assistance. This work was supported by a grant from the Wellcome Trust, London; National Institutes of Health Grant NS 15659; and a Biomedical Research Support Grant RR05755 from the Langley Porter Psychiatric Institute, University of California, San Francisco.

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