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EFFECT OF PURIFIED SCORPION TOXIN (TITYUSTOXIN) ON THE PANCREATIC SECRETION OF THE RAT

GEOVANA NOVAES,[1] O. L. CATANZARO,[2] W. T. BERALDO[3] and L. FREIRE-MAIA[3]

[1]Departamento de Fisiologia, Universidade Federal da Bahia, Salvador, Brasil, [2]Departamento de Fisiologia Humana, Facultad de Farmacia y Bioquímica, U.B.A., Buenos Aires, Argentina and [3]Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil

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G. NOVAES, O. L. CATANZARO, W. T. BERALDO and L. FREIRE-MAIA. Effect of purified scorpion toxin (tityustoxin) on the pancreatic secretion of the rat. *Toxicon* **20**, 847–853, 1982.— Intravenous injection, in anesthetized rats, of a single dose of purified scorpion toxin (tityustoxin, TsTX), obtained from the venom of the Brazilian scorpion *Tityus serrulatus*, causes a striking increase in flow rate, protein content, kallikrein and amylase activities of the pancreatic juice. The flow rate and protein content of the juice remain significantly higher than in control rats, for at least one hour, whereas the kallikrein activity returns to control values 30 min after tityustoxin injection. Sub-diaphragmatic bilateral vagotomy does not prevent the pancreatic secretion induced by tityustoxin; moreover, vagotomy potentiates the flow rate and kallikrein secretion produced by the toxin. Pre-treatment of the rats with atropine blocks the pancreatic secretion evoked by tityustoxin. It is suggested that the pancreatic secretion induced by tityustoxin is due to actions of acetylcholine, released from postganglionic nerve fibers, on muscarinic receptors. The mechanism by which vagotomy potentiates the pancreatic secretion evoked by tityustoxin is under investigation.

INTRODUCTION

THE VENOM of the Brazilian scorpion, *Tityus serrulatus*, produces, in animals and in men, striking effects on the gastrointestinal system, such as excessive salivation, gastric secretion, vomiting, contraction or relaxation of intestinal smooth muscle and ultrastructural changes in the Auerbach plexus (MAGALHÃES, 1938; DINIZ and VALERI, 1959; FREIRE-MAIA and DINIZ, 1970; TAFURI *et al.*, 1971, 1974; CUNHA-MELO *et al.*, 1973; FREIRE-MAIA *et al.*, 1976*a*, *b*; CATANZARO *et al.*, 1978; CAMPOS *et al.*, 1979, 1980; GONZAGA *et al.*, 1979; ANDRADE *et al.*, 1981). Acute pancreatitis is a common complication of the sting of *Tityus trinitatis* scorpions (WATERMAN, 1938; BARTHOLOMEW, 1970). Experimental data show that *Tityus trinitatis* venom induces exocrine secretion in the pancreas, which is explained by a direct effect of the venom on muscarinic receptors (BARTHOLOMEW *et al.*, 1976; SANKARAN *et al.*, 1977). As the actions of tityustoxin, purified from *Tityus serrulatus* venom, are explained by stimulation of postganglionic nerve fibers, and not by direct effects on adrenergic or cholinergic receptors (DINIZ and TORRES, 1968; TAFURI *et al.*, 1971, 1974; GOMEZ *et al.*, 1973; CUNHA-MELO *et al.*, 1973; LANGER *et al.*, 1975; FREIRE-MAIA *et al.*, 1976*a*, *b*), we decided to investigate the action of tityustoxin on pancreatic secretion in the rat, measuring the flow rate, protein content, kallikrein and amylase activities of the pancreatic juice. Preliminary results were published elsewhere (NOVAES *et al.*, 1978).

MATERIALS AND METHODS

Male Wistar rats (200–250 g) were fasted for 24 hr before the experiments; water was supplied *ad libitum*. The animals were anesthetized with urethane (1.4 g/kg, i.p.) and the trachea was cannulated. All solutions were

injected through a polyethylene cannula into the jugular vein. After a longitudinal incision of the duodenal wall, the minor papilla was found and a polyethylene cannula was inserted into the main pancreatic duct. The accessory duct was ligated to prevent secretion from the liver. After a rest period of 20 min, the pancreatic juice was collected, by a micropipette, from the tip of the polyethylene cannula. Sub-diaphragmatic bilateral vagotomy was performed 20 min before the injection of the tityustoxin. Atropine, calculated as the weight of the salt, was administered 10 min before tityustoxin injection.

Flow rate was measured every 10 min over 1 hr. Kallikrein was determined on the isolated rat uterus, where it causes a contraction without the addition of kininogen (BERALDO *et al.*, 1966). Rat kallikrein contracts the rat uterus but has no effect on other smooth muscle preparations; the contractions show parallelism with those evoked by bradykinin (BERALDO *et al.*, 1972). The oxytocic activity of pancreatic juice is inhibited by Trasylol, a specific kallikrein inhibitor (ARAUJO *et al.*, 1970). The specificity of the direct action of rat kallikrein on the rat uterus was confirmed by NUSTED and PIERCE (1974) and CHAO *et al.* (1981). Amylase activity was determined by the method of MYERS *et al.*, (1944) and protein measured according to the method of LOWRY *et al.*, (1951).

The following drugs were used: atropine sulfate (Sigma Chemical Co., St. Louis, U.S.A.), urethane (Sigma Chemical Co., St. Louis, U.S.A.), bradykinin (Sandoz S.A., Basle, Switzerland). *Tityus serrulatus* venom was obtained from Instituto Butantan (São Paulo, Brazil) and purified toxin fraction (T_1) was prepared using a combination of water extractions and column chromatography on Sephadex G-25 (GOMEZ and DINIZ, 1966) and was kindly provided by Dr. M. V. Gomez, from the Department of Biochemistry, ICB, UFMG, Belo Horizonte, Brasil. Tityustoxin is devoid of phospholipase A and other enzymatic activities. The doses of tityustoxin are expressed as weights of protein. The dose of purified tityustoxin used in this investigation (0.5 mg/kg) was chosen because previous experiments of our group showed that it induced a huge salivary secretion in the rat (CATANZARO *et al.*, 1978). Moreover, a dose two times higher, of a crude venom obtained from scorpions of the same genus (*Tityus trinitatis*), induced pancreatic secretion in anesthetized dogs (BARTHOLOMEW *et al.*, 1976).

The data were subjected to Student's *t*-test, with $P < 0.05$ indicating significance.

RESULTS

Injection i.v. of tityustoxin (0.5 mg/kg) induced abundant flow of pancreatic juice, as shown in Fig. 1A. Ten minutes after the injection, the pancreatic flow increased abruptly reaching a maximum at 20 min ($2.7 \pm 0.5\ \mu$l/min), staying around these values for, at least, 60 min. In control animals, injected with saline, the flow was very small, with a mean of $0.35 \pm 0.04\ \mu$l/min.

As the action of tityustoxin involves release of acetylcholine (DINIZ and TORRES, 1968; GOMEZ *et al.*, 1973; FREIRE-MAIA *et al.*, 1976*a*) we investigated the pancreatic secretion stimulated by the toxin before and after the injection of atropine. The inhibition of pancreatic flow evoked by tityustoxin in rats treated with atropine (Fig. 1A) suggests that acetylcholine released by toxin brings about the increase (around 5 times) of pancreatic flow.

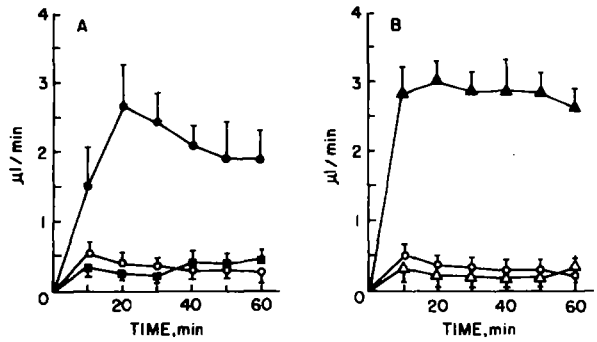

FIG. 1. EFFECTS OF ATROPINE AND VAGOTOMY ON PANCREATIC FLOW INDUCED BY TITYUSTOXIN IN ANESTHETIZED RATS.

(A) Spontaneous flow in rats injected with saline (○——○) and after 0.5 mg/kg, i.v., of tityustoxin (●——●). Atropine (5 mg/kg, i.v.) was injected 10 min before the injection of 0.5 mg/kg, i.v. of tityustoxin (■——■). (B) Spontaneous flow in control (○——○) and vagotomized rats (△——△) injected with saline. Flow rate after injection of 0.5 mg/kg, i.v., of tityustoxin in vagotomized rats (▲——▲). Values are means ± S.E. of 6–8 experiments.

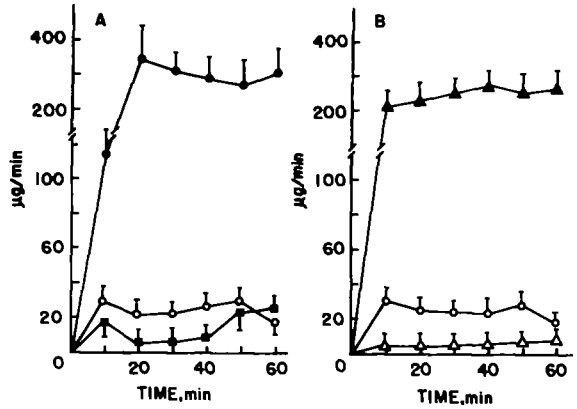

FIG. 2. EFFECTS OF ATROPINE AND VAGOTOMY ON PROTEIN CONTENT OF PANCREATIC JUICE IN ANESTHETIZED RATS, INJECTED WITH TITYUSTOXIN.

(A) Protein content of pancreatic juice of rats injected with saline (○——○), and after 0.5 mg/kg, i.v., of tityustoxin (●——●). Atropine (5 mg/kg, i.v.) was injected 10 min before the injection of 0.5 mg/kg, i.v., of tityustoxin (■——■). (B) Protein content in control (○——○) and vagotomized rats (△——△) injected with saline. Protein content after injection of 0.5 mg/kg, i.v., of tityustoxin in vagotomized rats (▲——▲). Values are means ± S.E. of 6–8 experiments.

The rapid increase of pancreatic flow in vagotomized rats, in the first 10 min after the injection of toxin and its maintenance at a high level of secretion for, at least, 60 min (Fig. 1B) suggests that section of the vagus nerves makes the pancreas more sensitive to the toxin.

In addition to pancreatic flow, the effects of tityustoxin on total proteins, kallikrein and amylase were investigated. The protein content of the pancreatic juice seemed, at least in part, dependent on flow rate, for atropine blocked both the flow and protein content of

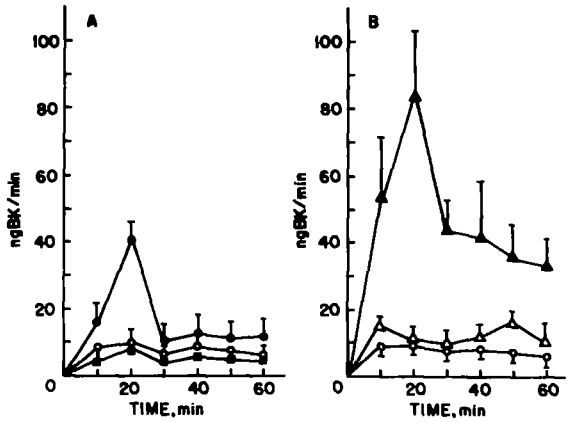

FIG. 3. EFFECTS OF ATROPINE AND VAGOTOMY ON KALLIKREIN SECRETION INDUCED BY TITYUSTOXIN IN ANESTHETIZED RATS.

(A) Kallikrein activity, expressed as ng bradykinin (BK) per min, in pancreatic juice after saline (○——○), and after 0.5 mg/kg, i.v., of tityustoxin (●——●). Atropine (5 mg/kg, i.v.) was injected 10 min before the injection of 0.5 mg/kg, i.v., of tityustoxin (■——■). (B) Kallikrein activity in control (○——○) and vagotomized rats (△——△) injected with saline. Kallikrein activity after i.v. injection of 0.5 mg/kg of tityustoxin in vagotomized rats (▲——▲). Values are means ± S.E. of 6–8 experiments.

TABLE 1. EFFECT OF ATROPINE ON THE PANCREATIC SECRETION INDUCED BY TITYUSTOXIN IN THE RAT

Groups		Flow rate μl/hr	Total proteins mg/hr	Kallikrein†
Control	(6)	21 ± 3	1.5 ± 0.3	502 ± 112
Tityustoxin	(8)	86 ± 10*	13.5 ± 1.1*	1024 ± 286*
Atropine	(6)	24 ± 5	0.7 ± 0.2	443 ± 146
Atropine + Tityustoxin	(6)	26 ± 5§	1.3 ± 0.4§	382 ± 78§

The rats were anesthetized with urethane. Values are means ± S.E. (In parentheses, number of animals). Tityustoxin (0.5 mg/kg, i.v.). Atropine (5 mg/kg, i.v.).

†Kallikrein activity is expressed as ng bradykinin per hour.

*$P < 0.05$ as compared with control.

§$P < 0.05$ as compared with group injected with tityustoxin alone.

pancreatic juice when the pancreas was stimulated by the toxin (Fig. 1A and 2A). Moreover, vagotomy also potentiated the initial effects of tityustoxin (10 min after toxin injection) on the protein content of pancreatic juice, (Fig. 2A and 2B).

The effect of tityustoxin on the secretion of kallikrein was also investigated (Fig. 3A). Twenty minutes after toxin injection the kallikrein secretion reached its maximum, decreasing to control levels 10 min later. Bilateral vagotomy potentiated the magnitude and duration of the kallikrein secretion induced by tityustoxin (Fig. 3B) while atropine inhibited the kallikrein secretion induced by the toxin (Fig. 3A).

Tables 1 and 2 show the accumulated 1 hr effects of atropine, vagotomy and tityustoxin on pancreatic flow, protein content and kallikrein activity of pancreatic juice.

After tityustoxin injection, amylase secretion by the pancreas may reach values up to 12 times greater than those determined in control rats injected with saline (Table 3). Atropine did not significantly change spontaneous amylase secretion, in comparison with control rats injected with saline, but prevented the increased secretion induced by tityustoxin (Table 3). Preliminary experiments showed that bilateral vagotomy did not prevent amylase secretion evoked by the toxin.

TABLE 2. EFFECT OF BILATERAL VAGOTOMY ON THE PANCREATIC SECRETION INDUCED BY TITYUSTOXIN IN THE RAT

Groups		Flow rate μl/hr	Total proteins mg/hr	Kallikrein†
Control	(6)	21 ± 3	1.5 ± 0.3	502 ± 112
Tityustoxin	(8)	85 ± 10*	13.5 ± 1.1*	1024 ± 286*
Vagotomy	(6)	20 ± 3	0.4 ± 0.04*	767 ± 125
Vagotomy + Tityustoxin	(6)	147 ± 43§	11.6 ± 1.2*	2864 ± 531§

The rats were anesthetized with urethane. Values are means ± S.E. (In parentheses, number of animals) Tityustoxin (0.5 mg/kg, i.v.). Bilateral vagotomy was performed 20 min before toxin injections.

†Kallikrein activity is expressed as ng bradykinin per hour.

*$P < 0.05$ as compared with control.

§$P < 0.05$ as compared with tityustoxin alone.

TABLE 3. EFFECT OF ATROPINE ON AMYLASE SECRETION INDUCED BY TITYUSTOXIN IN THE RAT PANCREAS

Groups		Amylase secretion per hr (mg of reduced sugar)
Control	(6)	61 ± 27
Tityustoxin	(8)	731 ± 107*
Atropine	(6)	51 ± 18
Atropine + Tityustoxin	(6)	26 ± 5§

The rats were anesthetized with urethane. Values are means ± S.E. (In parentheses, number of animals).
Tityustoxin (0.5 mg/kg, i.v.). Atropine (5 mg/kg, i.v.).
*$P < 0.05$ as compared with control.
§$P < 0.05$ as compared with tityustoxin alone.

DISCUSSION

The present data show that purified scorpion toxin (tityustoxin, TsTX), obtained from the venom of the Brazilian scorpion *Tityus serrulatus*, induced a dramatic increase in pancreatic secretion in anesthetized rats, confirming previous results with crude venom from *Tityus trinitatis* (BARTHOLOMEW *et al.*, 1976). A single injection of tityustoxin increases the flow rate and the protein content of pancreatic juice for at least 1 hr, which is in accordance with the long acting effects of tityustoxin described previously in other preparations (e.g. FREIRE-MAIA *et al.*, 1974). It seems that tityustoxin binds strongly to its receptors in nerve membrane (FREIRE-MAIA *et al.*, 1975, 1976*a*), which would explain the persistence of biological effects 1 hr after injection. The kallikrein secretion induced by tityustoxin is less prolonged, since 30 min after toxin injection the activity returns to control values. The reason for this briefer effect is not known.

As vagotomy did not prevent the pancreatic secretion induced by tityustoxin, the hypothesis that scorpion toxin produced its effects through stimulation of the central nervous system (MAGALHÃES, 1938; DEL POZO *et al.*, 1945; SAMAAN and IBRAHIM, 1959; EFRATI, 1978) could be ruled out. Moreover, the present results are in accordance with previous publications on other preparations, which showed that section of the vagus nerves did not prevent the scorpion toxin effects (FREIRE-MAIA *et al.*, 1974; GONZAGA *et al.*, 1979).

Ganglionic blockade with hexamethonium does not prevent amylase release induced by scorpion venom (SANKARAN *et al.*, 1977), which is in accordance with experiments on heart, arterial pressure, isolated intestine and spleen strips (DINIZ and GONÇALVES, 1956; CORRADO *et al.*, 1968; CUNHA-MELO *et al.*, 1973; FREIRE-MAIA *et al.*, 1974, 1976*b*). As the pancreatic hypersecretion induced by scorpion toxin is blocked by atropine (Tables 1 and 3), it seems likely that it results from stimulation of postganglionic nerve fibers, with subsequent release of acetylcholine. This hypothesis is supported by experiments which showed that pancreatic secretion induced by cholinergic fibers is due to release of acetylcholine, which in turn acts on muscarinic receptors (WILLIAMS, 1975), and by experiments which showed that tityustoxin releases acetylcholine (DINIZ and TORRES, 1968; GOMEZ *et al.*, 1973; FREIRE-MAIA *et al.*, 1976*a*). Recent data (GALLAGHER *et al.*, 1981) have also shown that amylase secretion, *in vitro*, induced by crude scorpion venom (*Tityus serrulatus*) is due to the release of acetylcholine from pancreatic nerves.

Scorpion toxin induces a very acidic gastric secretion in rats (GONZAGA *et al.*, 1979). It seems likely that under these circumstances the acid chyme reaching the duodenum

releases secretin and cholecystokinin (CCK-PZ), which could explain, at least in part, the pancreatic secretion induced by tityustoxin in rats not treated with atropine.

An interesting observation is that bilateral vagotomy potentiates the flow rate and kallikrein activity of the pancreatic secretion after tityustoxin injection (Table 2). However, this phenomenon is not general, since vagotomy does not increase the protein secretion induced by tityustoxin (Table 2) and does not increase the effect of tityustoxin on gastric secretion (GONZAGA *et al.*, 1979). Additional experiments are necessary to explain the mechanism of the pancreatic supersensitivity evoked by vagotomy.

It has been suggested that pancreatitis induced by scorpion venom could be explained by a greatly increased exocrine output of the pancreas, associated with an outflow obstruction (BARTHOLOMEW *et al.*, 1976). As scorpion toxin induces contraction of intestinal smooth muscle (DINIZ and GONÇALVES, 1956; CUNHA-MELO *et al.*, 1973; FREIRE-MAIA *et al.*, 1976*a*, *b*) it seems likely that pancreatitis could also be explained, at least in part, by an increased intraduodenal pressure leading to reflux of duodenal juice, rich in enterokinase, into the pancreas, with subsequent intrapancreatic formation of trypsin (MCHARDY *et al.*, 1963; MCCUTHEON, 1968). Kallikrein, released by scorpion toxin (Table 1), could also play a role in the pathogenesis of acute pancreatitis, after its activation by trypsin.

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