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Ryptisin—The active principle of the soapfish toxin

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Rypticus saponaceous, or the soapfish, is found in the coral reefs of the North Atlantic. When molested or in danger, it produces a foamy excretion toxic to other fish. In 1967, del Castillo and Maretzki,[1] in a preliminary study postulated that the active principle was either a protein or polypeptide. We are now able to report that the active principle is a polypeptide of high hypotensive activity.

Studies have been performed using, as source of toxin, fish kept in captivity under conditions simulating as much as possible the fish's natural habitat. The toxin, secreted by epidermal glands, can be obtained in crude form by removing the fish from the tank, exposing it to air for 30–45 sec, immersing it in about 300 ml of distilled water and agitating the container. After 3–4 min, the fish is brought back to the tank. The foamy opalescent suspension is filtered under negative pressure and its toxicity in guppies is tested as described by del Castillo and Maretzki.[1] Crude extracts in which guppies survive for longer than 15 min are not considered suitable for further purification. Heating the crude extract to 90° for 30 min does not alter its toxicity. Upon dialysis, the toxic principle is found in the diffusate. The extract, which has a pH of approximately 8, is buffered to pH 4·6 by addition of one-tenth its volume of 0·1 M, pH 4·6, acetate buffer prior to lyophilization. This step has been found to markedly enhance its stability.

The lyophilized extract is dissolved in distilled water and its pH adjusted to 6·8 with 0·1 N NaOH. Purification of the toxin is accomplished by gel filtration using Sephadex G-75 columns. One hundred mg of lyophilized powder is dissolved in 5 ml of distilled water and applied to a 2·5 × 40 cm column. Four-ml samples are collected and read spectrophotometrically at 273 nm. A two-ml aliquot is diluted to 10 ml and bioassay performed as described above. Another aliquot is taken for protein analysis by the method of Lowry *et al.*[2] using bovine serum albumin as standard. The results invariably show that toxicity is directly proportional to protein concentration. Polypeptide in concentration of 2 μg/ml is lethal to guppies.* The approximate molecular weight of the polypeptide calculated from chromatographic columns using Sephadex G-75 is 2300.[4] The toxic polypeptide when concentrated at pH 6·8 in 0·01 M phosphate buffer and chromatographed through DEAE cellulose is not retained, indicating it is positivcly charged at this pH. Even when the polypeptide is positively charged at neutral pH its solubility in water is low. The solubility is increased by addition of organic solvents such as ethanol and *n*-butanol. Preliminary amino acid analyses by the method of Spackman *et al.*[5] indicate that although the basic residues exceed the acid ones (6–1), the non-polar residues (leucine, isoleucine and phenylalanine) make up more than 40 per cent of the total amino acid residues.

TABLE 1. EFFECTS OF THE I.V. INJECTION OF 2 mg/kg OF CRUDE RYPTISIN ON THE MEAN ARTERIAL BLOOD PRESSURE OF PENTOBARBITAL-ANESTHETIZED DOGS

Weight (kg)	Δ BP (mm Hg)*
12·0	−80
9·5	−100
13·0	−105
17·0	−85
13·2	−35
16·8	−100
20·0	−30
14·0	−65
8·0	−115

* Δ BP = 80 ± 30. BP = change in mean arterial blood pressure.

* Incubation of the polypeptide for 1 hr at 37° at pH 7·0 in the presence of the proteolytic enzyme pinguinain[3] leads to abolition of the toxic activity.

The hypotensive effects of the polypeptide have been tested both in dogs and rats. Dogs were anesthetized by the intravenous administration of 25 mg/kg of sodium pentobarbital. Blood pressure recordings in dogs were obtained from the right femoral artery by means of a PE 160 catheter attached to a P 23 AC (Statham) pressure transducer, which in turn was connected to a model P5 grass polygraph. Blood pressure recordings were also obtained from healthy adult male rats of the Sprague–Dawley strain, under Dial–Urethane anesthesia, from the left common carotid artery carefully separated from the vagosympathetic trunk. The procedure was similar to that used in dogs, except that a smaller bore catheter (PE 50) and a P 23 DC (Statham) pressure transducer were utilized. In both species, a dose of 2 mg/kg of the crude extract dissolved in 0·9 per cent saline was injected via catheters implanted in the left femoral vein. The catheters were flushed with equivalent volumes of saline to insure accuracy of dosage.

Table 1 summarizes the results obtained in dogs. Figure 1 illustrates a typical experiment. A marked drop in the mean arterial blood pressure is observed soon after injection, usually accompanied by electrocardiographic changes. The upward deflection recorded immediately after the injection of soapfish toxin (SFT) is an artifact. All animals show recovery to preinjection mean arterial blood pressure and heart rates 30 min thereafter.

TABLE 2. AUTONOMIC INTERACTIONS WITH SOAPFISH TOXIN IN THE ANESTHETIZED RAT*

Pretreatment	Challenge	Response
Soapfish toxin	Acetylcholine	No apparent effect
Soapfish toxin	Epinephrine	No apparent effect
Atropine	Soapfish toxin	No apparent effect
Eserine	Soap fishtoxin	No apparent effect
Dual vagotomy	Soapfish toxin	No apparent effect
Propranolol	Soapfish toxin	Blocked or reversed

* Acetylcholine chloride and epinephrine hydrochloride were used as "challenge" agents. After determination of standardized responses produced by intravenous injections (1-sec duration) of the test challenges, soapfish toxin (SFT) was administered followed by the test challenges 2 min later and at 5- or 10-min intervals. Mean arterial blood pressure was determined prior to and immediately after each drug administration. The effects of SFT on the test challenge responses were estimated by comparisons with the standardized responses evoked by the test challenges before SFT administration.

Table 2 summarizes the results in the rat experiments. We have found that SFT, in doses of 500 μg/kg–1 mg/kg (eight rats, 24 trials), produces an approximate 25–35 mm drop in blood pressure in the anesthetized rat. Higher doses of SFT (2–3 mg/kg) (six rats, 17 trials) produce a depressor response of 40–60 mm Hg. In contrast to SFT responses in the dog, blood pressure in the rat returns to baseline levels in approximately 1 min. SFT (2 mg/kg) did not produce any apparent change in the responses elicited by ACh (0·5 μg/kg) (two rats, six trials) or epinephrine (0·5 μg/kg) (two rats, eight trials). Similar experiments were also performed using SFT itself as the test "challenge". In these studies, treatment with atropine, eserine, propranolol or dual vagotomy followed the determination of standardized responses produced by SFT. The standard dose of SFT was again injected 5 min after pretreatment (and at 10-min intervals) and compared to responses produced by SFT before pretreatment. Our results showed that atropine SO_4 in doses from 1 to 4·0 mg/kg (four rats, five trials), eserine SO_4 (100–200 μg/kg) (two rats, four trials) and dual vagotomy (three rats, six trials) failed to alter the depressor response produced by SFT (1 to 2·0 mg/kg). Propranolol HCl (1 mg/kg) (five rats, five trials) injected 5 min prior to SFT (2–3 mg/kg) caused either a block or reversal of the SFT depressor response.

The hypotensive effects are not blocked by atropine, eserine or bilateral vagotomy but are blocked and reversed by propanolol (Table 2, Fig. 2). These data suggest that the pharmacological actions of the soapfish toxin in the rat and dog are analogous to those produced by beta adrenergic-stimulating agents.

Three preliminary experiments have been performed in isolated electroplax of *Electrophorus electricus*. The experimental procedure has been described by del Castillo *et al.*[6] Addition of soapfish

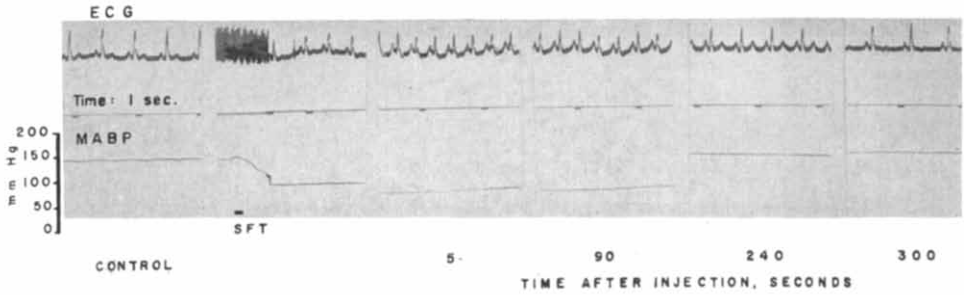

FIG. 1. Effects of soapfish toxin (2 mg/kg) in the anesthetized dog. Notice the marked change in the mean arterial blood pressure and the simultaneous electrocardiographic changes. Of significance is a transient abolition of the T wave, an increase in the amplitude of P accompanied by a decrease of the amplitude of R, suggestive of a transient ischemia.

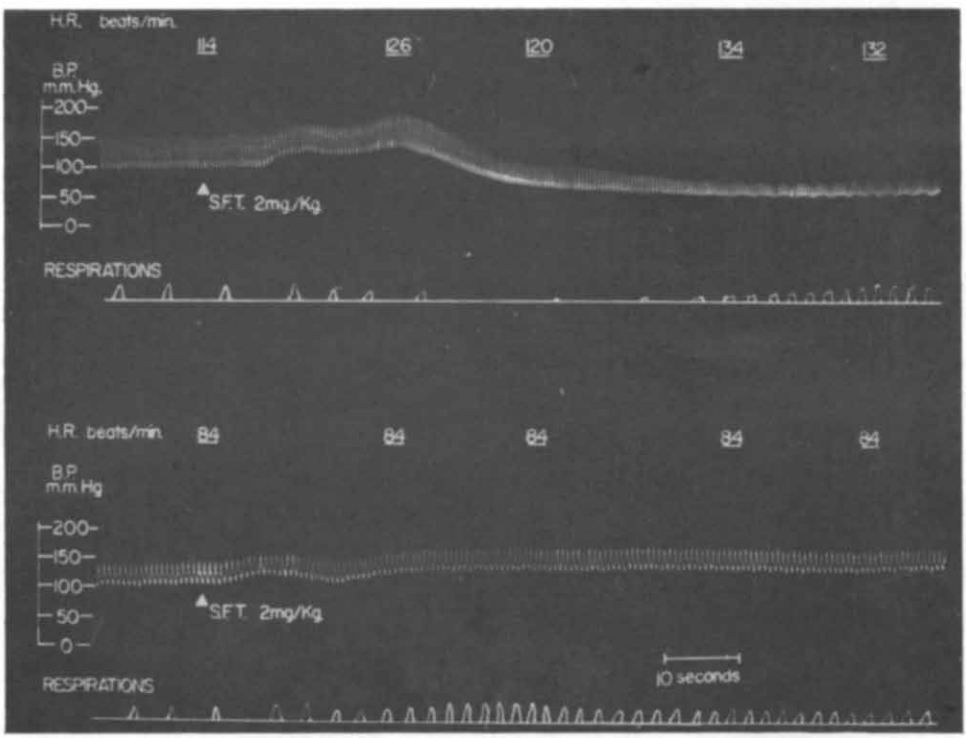

FIG. 2. Typical tracing (one of three experiments) of the effects of the soapfish toxin (SFT) prior to (upper tracing) and after (lower tracing) treatment with propanolol. Notice the blocking of the hypotensive effects as well as the abolition of the transient respiratory arrest observed after treatment with propranolol. This experiment showed that SFT (2 mg/kg) reduced resting blood pressure from 150/96 to 60/35. Recovery back to baseline levels required 30 min, at which time propranolol HCl (1 mg/kg) was administered slowly over a 2-min period. (A transient 10 mm Hg decrease in blood pressure was observed during the administration of propranolol; however, re-establishment of control baseline levels occurred within 1 min after propranolol injection.) SFT (2 mg/kg) was again injected 5 min after propranolol administration. The depressor response was blocked and a slight pressor response occurred (140/100–160/120).

toxin to the buffer solution bathing the isolated cells causes a marked progressive diminution in the resting potential of these cells. Toxin concentrations of 50 μg/ml diminish the resting potential to 10 mV or lower in a period of 30 min. Removal of the toxin from the bath at any time during the experiment ceases the drop in resting potential, but the resting potential does not recover to initial levels.

Powerful hypotensive agents, polypeptide in nature, have been isolated from amphibian species.[7] The structural analogies between these and the polypeptide herein described remains to be elucidated, as well as the evolutionary implications of these findings.

This toxin resembles the pharmacologic effects of eleidosin, an active hypotensive undecapeptide isolated from the posterior salivary glands of the cephalopod *Eledone*.[8] We hereby propose the name ryptisin for the active hypotensive polypeptide isolated from *Rypticus saponaceous*.

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