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Comparative biochemistry and pharmacology of salivary gland secretions*

I. Electrophoretic analysis of the proteins in the secretions from human parotid and reptilian parotid** (Duvernoy's) glands

Electrophoretic methods have been widely used in our laboratory for the study of the protein composition of human salivary and venom gland secretions. In a previous communication[1] a modified method for high resolution polyacrylamide electrophoresis of Crotalus and Agkistrodon venoms from North American snakes was described. Some modifications and an extension of the same method to include twenty-four additional Crotalid venoms are reported in this paper. Special reference to the small molecular basic proteins in these venoms is made and some significant aspects of the comparative biochemistry of salivary gland secretions are, hereby, reported for the first time.

Materials and methods

Lyophilized venoms were obtained from commercial sources (Sigma Chemical) or freshly collected in our serpentarium***. Venoms were prepared for electrophoresis by dissolving 14–28 mg in 1.0 ml of 0.1 *M* Tris–0.1 *M* citric acid buffer, pH 2.9 containing 6 *M* urea and 10^{-4} *M* pyronine-Y dye as a marker. Insoluble material was removed by centrifugation at 27,500 *g* (30 min) in a refrigerated (2°) RC2-B Sorvall centrifuge. Human parotid fluid was collected from healthy volunteers using Carlson-Crittenden vacuum cups, at a flow rate of 1.09 ml/min. All other details for mixed and parotid saliva collection and sample preparation have been previously described[2,3].

Electrophoresis in 6 *M* urea, polyacrylamide gels (12%) was carried out essentially as described in an earlier paper[1]. The method, including minor modifications which yield increased resolution of the small molecular weight basic proteins can be briefly summarized as follows: voltage, 250 V (constant); current, 100 mA; pre-run, 6.0 h; electrolyte, 0.37 *M* glycine–citric acid buffer, pH 3.0; load, 20–40 μl; stain, Amido Black (24 h); destaining solution, methanol–acetic acid–water (5:1:5), or 7% acetic acid. Separations were carried out for 5 h at room temperature but coolant (2°) was circulated through the cooling plates at all times.

Results

In an earlier study[1] the electrophoretic patterns of several Crotalid venoms were compared. The similarities between *C. adamanteus* and *C. horridus atricaudatus* venoms and the presence of a small molecular weight basic protein in both secretions were noted. An extension of comparative electrophoresis to other members of the Crotalid family, human parotid fluid and Gila Monster (*Heloderma suspectum*) salivary

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** The term "reptilian parotid" is used here, solely to emphasize the reasons for comparing the gland secretions (see *Discussion*). It is not used as a histological definition.

*** A gift of *Heloderma suspectrum* venom from Dr. HERBERT STANKE is gratefully acknowledged.

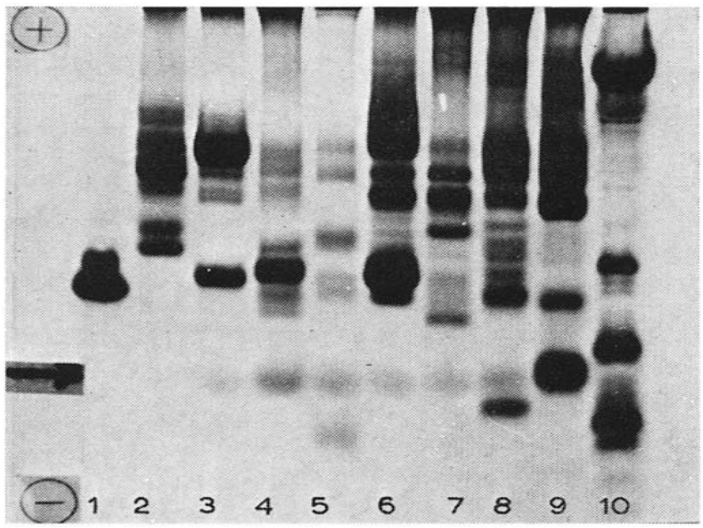

Fig. 1. Comparative electropherogram of: 1, lysozyme; 2, *Heloderma suspectum*; 3, *C. scutulatus*; 4, *C. m. mollosus*; 5, *C. basiliscus*; 6, *C. v. helleri*; 7, *C. v. cerberus*; 8, *C. v. viridis*; 9, *C. h. horridus*; 10, human parotid fluid. In slots 2, 3, 6 and 7 the fastest migrating component (shown by arrow) is the dye marker pyronine-Y.

secretion, is shown in Figs. 1 and 2. Lysozyme and the venom from one member of the Elapid family (*Naja naja*), known to contain small molecular weight basic proteins were used (Fig. 2) as reference markers.

A total of 27 venoms and human parotid fluid have been tested under identical

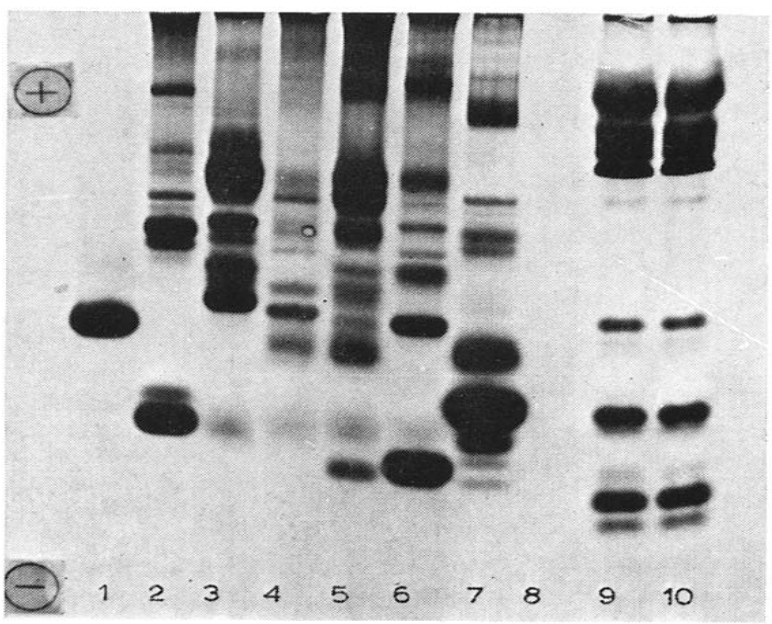

Fig. 2. Comparative electropherogram of: 1, lysozyme (14,500); 2, *C. adamanteus* (10,900); 3, *C. scutulatus*; 4, *C. h. atricaudatus* (6300); 5, *C. v. viridis* (6300); 6, *C. d. terrificus* (10,000–15,000); 7, *Naja naja*; 8, blank; 9, 10, human parotid fluid. The fastest component in sample 3 is the dye marker pyronine-Y. Slot 8 was left blank in order to avoid protein–protein interactions between the human parotid glycoproteins and the two fastest migrating components in *Naja naja* venom (slot 7) which are the cobramines A and B (5840). The numbers in parentheses represent the molecular weights of the pure, highly basic proteins so far isolated. In each case the most basic proteins are also the fastest migrating component.

TABLE I

PRESENCE OR ABSENCE OF SMALL MOLECULAR WEIGHT BASIC PROTEINS (SMBP) IN SOME SNAKE VENOMS AND SALIVARY SECRETIONS

SMBP may constitute: over 20% of total venom protein (+++++); up to 12% (++++); up to 8% (+++); 2 to 4% (++); less than 1% (+); 0% (—). These percentages are based on the recovery of purified basic proteins following isolation by gel filtration, ion exchange and adsorption chromatography[2,24].

Venoms	*Presence*
Crotalus adamanteus (Eastern Diamondback Rattler)	+++
C. atrox (Western Diamondback Rattler)	—
C. basiliscus[26] (Mexican West-coast Rattler)	+
C. cerastes (Sidewinder)	—
C. terrificus (Tropical Rattler)	++++
C. horridus horridus (Timber Rattler)	+++
C. horridus atricaudatus (Canebrake Rattler)	++
C. viridis viridis (Prairie Rattler)	+++
C. scutulatus (Texas; Mohave Rattler)	—
C. scutulatus (Arizona; Mohave Rattler)	+
C. v. helleri (Southern Pacific Rattler)	+
C. v. cerberus (Prairie Rattler)	—
C. molossus molossus (Black Tail Rattler)	+
C. lepidus lepidus (Rock Rattler)	—
Sistrurus miliarius barbouri[26] (Pigmy Rattler)	—
S. catenatus tergeminus[26] (Eastern Massagauga)	—
S. c. catenatus[26] (Western Massasauga)	—
Agkistrodon contortrix contortrix[1] (American Copperhead)	—
A. c. laticinctus[1] (Broad-banded Copperhead)	—
A. piscivorus leukostoma[1] (Western-cottonmouth Moccasin)	—
A. p. piscivorus[1] (Eastern Cottonmouth Moccasin)	—
A. rhodostoma[27] (Malayan Pit Viper)	—
Naja naja (Indian Cobra)	+++++
Vipera russelli[27] (Russell's Viper)	—
V. ammodytes[27] (Long-nosed Viper)	—
Bothrops atrox[27] (Fer-de-lance)	—
Heloderma suspectum (Gila Monster)	—
Human parotid fluid[4,25]	++
Human mixed saliva[4,25]	+

conditions in our laboratory for the presence of small molecular basic proteins (SMBP). Only seven (all Crotalidae), excluding *Naja naja*, of the twenty-five venoms studied contained SMBP and of these only five had appreciable amounts (range: 1–8% of total protein). Basic proteins of low molecular weight (below 14,500) were absent in the *H. suspectum* secretion. It is of great interest to note that proteins similar to those found in the seven Crotalidae venoms have been isolated from human parotid fluid[4].

A summary of the results obtained from the gel electrophoretic patterns of all

the venoms so far investigated in this laboratory, is presented in Table I. In this respect, it should be noted that at least twelve different samples of each venom and three to five electrophoretic separations of each sample were carried out until reproducibility of each pattern could be insured. However, only eight individual human parotid fluid and two *Heloderma suspectum* samples were studied. The reproducibility of this technique with regard to the human salivary secretion has previously been reported[3].

Discussion

The snake venom gland has been termed both homologous[5,6], and analogous[7,8] with the mammalian salivary parotids. As pointed out by GANS AND ELLIOTT[9] however, the similarity is analogous only. Homology or analogy, notwithstanding, it is of interest to note that: (a) the nerve growth factor (NGF), a protein endowed with growth promoting activity on specific nerve cell types has been isolated from mouse sarcoma 180 (see ref. 10), the submandibular gland of male Swiss albino mice[11], mouse saliva[12] and the venoms from various species of the three families of venomous snakes: *Elapidae*, *Viperidae* and *Crotalidae*[13,14]; (b) some of the small molecular weight basic proteins (SMBP) in human parotid fluid closely resemble those of some Crotalid venoms from North American rattlesnakes, in their physical-chemical and biological[4] properties; (c) partially purified protein fractions from mouse submandibular gland homogenates[15] as well as mixed human saliva[16] exhibit marked toxicity giving lethal dose values well within the realm of those of some partially purified protein toxins from snake venoms. The relationship between the salivary gland[17], human saliva[16] and mineral metabolism has been studied in this laboratory and recently a polypeptide with hypocalcemic activity in mice was isolated from whole and submandibular human saliva[18,19]. In an attempt to find a more plentiful source for the hypocalcemic peptide we have begun a detailed investigation of the comparative biochemistry of the basic proteins in salivary gland secretions.

The present paper has described the results of a comparative study in which the protein composition of human parotid fluid, with special reference to its basic polypeptides and proteins, could be determined (by high resolution electrophoresis) in relation to that of several reptilian venoms.

With the exception of Crotamine, the basic protein neurotoxin from *Crotalus durrisus terrificus*, nothing is known about the basic proteins from American rattlesnakes. The absence of highly basic proteins in said venoms has, in fact, been reported by several authors[20,21]. It is of interest to note, however, that small molecular weight basic proteins having neurotoxic activity have already been obtained in a high degree of purity from *C. viridis helleri*[22], *C. adamanteus*[23], *C. horridus horridus*, *C. h. atricaudatus* and *C. v. viridis*[24]. The isolation of similar basic proteins from human parotid fluid has also been reported[4,25]. Preliminary investigations on the highly purified basic proteins have shown their pharmacological actions (*in vivo*) to mimic those of Crotamine, the neurotoxin from *C. d. terrificus*, the South American tropical rattlesnake. More striking, however, is the fact that one of SMBP isolated from human parotid secretion has similar biochemical properties to those of *C. v. viridis* (Prairie rattlesnake) basic protein. Further characterization of the small molecular weight basic proteins should shed some light on the developmental and comparative biochemical–pharmacological aspects of salivary gland secretions.

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Department of Physiology and Biophysics,
School of Veterinary Medicine and Biomedical Sciences,
Fort Collins, Colo. 80521 (U.S.A.)
CARLOS A. BONILLA

Department of Biology,
Midwestern University,
Wichita Falls, Texas 76308 (U.S.A.)
M. KATIE FIERO*
WAYNE SEIFERT

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* Present address: Department of Biochemistry, University of Texas, (Southwestern) Medical School, Dallas, Texas 75235, U.S.A.