HEMORRHAGIC ACTION OF THE VENOMS OF CENTRAL ASIAN SNAKES

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Collective characteristics of the venoms belonging to various taxons of Central Asian snakes, demonstrating levels of toxicity and efficiency of hemorrhagic action, together with proteolytic, esterase, and phospholipase activities, have been obtained. A comparative analysis of the collective characteristics ("portraits") of the venoms and their changes on degradative treatment has shown that the hemorrhagic action of the venoms of viperine and crotalid snakes is connected with metalloproteins. In contrast to the venoms of the elapids (cobras) the toxicity of the venoms of the viperine and crotalid snakes exhibits a dependence on the hemmorhagic effect and the activity of the metalloproteins causing it.

A snake venom is a complex mixture of biologically active proteins and peptides [1, 2]. The complex composition of the venom corresponds to the multiplicity and diversity of its biological effects, including those that are unfavorable for man and animals [3]. The latter include hemorrhage. It is considered that, as the most characteristic manifestation of the local action of a venom, hemorrhage plays an important role in the pathogenesis of intoxication, particularly in the case of the venoms of the viperine and crotalid snakes [4]. The most important active factors of these venoms probably differ with respect to their main structural–functional principles from those that are present in elapoid venoms. This is confirmed by our own results (Table 1), according to which the venoms of viperine and crota-

TABLE 1. Toxicities (LD_{50}) and Hemorrhagic Activities (MHD) of the Venoms of Central Asian Snakes

Venom	Family	Protein content, %	Toxicity, mg of protein per 1 kg body wt. on injec.			Hemorrhagic activity, MHD μg/g body weight	
			i.v.	i.p.	i.d.	abs. μg of venom	spec., μg of protein
Vipera labetina turanica	Viperines	91	0,68	1,54	4,82	0,30	0,27
Vipera ursini renardi		79	0,98	2,81	6,91	0,10	0,07
Vipera berus berus		87	0,73	1,69	5,11	0,20	0,16
Echis multisquamata		90	1,08	2,88	7,56	0,30	0,27
Agkistrodon halys halys	Crotalids	80	0,84	2,40	4,73	0,40	0,32
Naja oxiana	Elapids	74	0,70	0,81	1,03	—	—

TABLE 2. Activities of Proteinases, BAEE Esterases, and Phospholipases A_2 in the Venoms of Central Asian Snakes

Venom	Family	Enzymatic activity, units/mg of protein in the venom				
		proteinase in the hydrolysis of			BAEE esterase	phospholipase A_2
		hemoglobin	casein	dimethylcasein		
Vipera lebetina turanica	Viperines	1,20	6,70	39,34	2,13	5165
Vipera ursini renardi		1,20	4,62	26,33	1,63	1519
Vipera berus berus		1,49	2,69	15,75	2,64	2529
Echis multisquamata		0,67	4,89	31,11	4,11	1222
Agkistradon halys halys	Crotalids	6,50	11,50	67,50	1,88	7255
Naja oxiana	Elapids	—	—	—		9189

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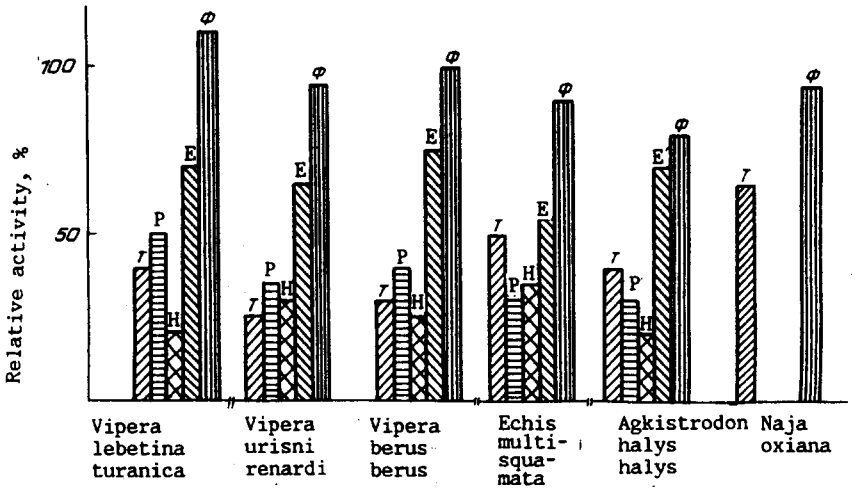

Fig. 1. Influence of heating (60°C, 20 min, pH 7.2) on the toxicities and enzymatic and hemorrhagic activities of the venoms of Central Asian snakes: T) toxicity ($1/LD_{50}$); H) hemorrhagic activity (1/MHD); P) proteolytic activity; E) BAEE esterase activity; Φ) phospholipase (A_2) activity.

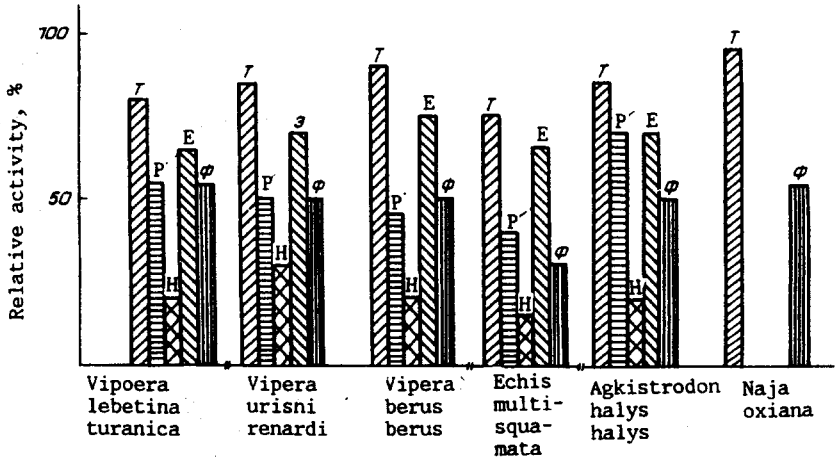

Fig. 2. Influence of EDTA (2 mM) on the toxicities and enzymatic and hemorrhagic activities of the venoms of Central Asian snakes. The venoms in a concentration of 1 mg/ml were incubated with PMSF at 37°C for 1 h (for symbols, see Fig. 1).

lid snakes differ from those of the elapids (cobras) by a pronounced hemorrhagic activity and by a dependence of the lethal effect (LD_{50}) on the method of administration. The local action of cobra venom is weak, and a generalized neurotropic effect and the corresponding symptoms are more characteristic of it.

The literature [5] indicates that the hemorrhagic action of snake venoms is connected with their protein components, but different authors suggest proteinases, phospholipases A_2, and independent hemorrhagic proteins or peptides possessing no enzymatic activity in the role of hemorrhagins. It appeared of interest to investigate this question in relation to each of the venoms of Central Asian snakes that we are studying.

We employed the usual approach, consisting in the parallel biological testing of materials that had been subjected to the degradative or inactivating action of heating and storage and of some specific proteinase inhibitors. As a preliminary, we first investigated the proteolytic, BAEE esterase, and phospholipase activities of these venoms (Table 2) in order, from these characteristics, supplemented by characteristic toxicity (LD_{50}) and hemorrhagic activity (MHD), to compare the generalized "portraits" of each of the venoms isolated. In

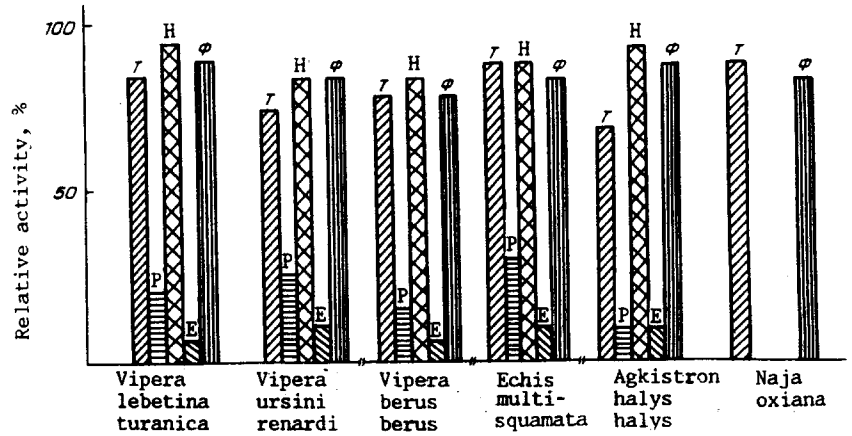

Fig. 3. Influence of PMSF (20.0 mM) on the toxicities and enzymatic and hemorrhagic activities of the venoms of Central Asian snakes. The venoms in a concentration of 1 mg/ml were incubated with PMSF at 37°C for 1 h (for symbols, see Fig. 1).

the comparison of these "portraits," the cobra [Naja oxiana] venom immediately stood out: Unlike the others it does not contain proteinases and BAEE esterases and does not possess the hemorrhagic action typical for the "cutaneous reaction;" its local effect appears only as an irritation at the injection site. We subsequently observed how such "portraits" change under the influence of various types of degradative treatment of the venoms. The results obtained are shown in Figs. 1-3. The change in the "portraits" of the venoms under the action of one of the above-mentioned physicochemical factors on them permitted not only an elucidation of the nature of the hemorrhagic principle but also a determination of the probable functional group of biologically active proteins found as components of the venoms to which it belonged (enzymes, toxins, enzyme inhibitors, etc.). The proteinase-rich venoms of the viperine and crotalid snakes were extremely effective hemorrhagically; it was possible to determine more accurately the role of the proteinases in the hemorrhagic action of these venoms that had been established previously [6] and to show that the hemorrhagic proteinases were more probably metal-dependent, and not "serine," enzymes.

In the presence of EDTA the hemorrhagic action activity fell, together with the proteolytic activity, while PMSF acted as a powerful inhibitor of the snake venom proteinases and esterases but, at the same time, scarcely blocked their hemorrhagic action. All these studies permitted, with a high degree of probability, the hemorrhagin to be identified with the protein components of the venoms and, more concretely, with the metal-dependent proteinases. According to our results the participation of the phospholipases A_2 in the hemorrhagic effect of the venoms is doubtful, although there are statements in the literature [7] that phospholipase A_2 potentiates the hemorrhagic action of factors represented by metalloproteinases.

In conclusion, it must be mentioned that the contribution of the hemorrhagic action to the total toxic effect of the venoms has no decisive significance, although it seems fairly probable. This may be indicated by the unidirectional changes in toxicity, hemorrhagic activity, and the activity of the metalloproteinases, while the suppression of the "serine" enzymes had little influence on either the hemorrhagic or the toxic (LD_{50}) effects of the venoms of the viperine and crotalid snakes. In the case of the cobra venom the lack of dependence of the toxic effect on the activity of the enzymes is clearly seen.

EXPERIMENTAL

The venoms of Vipera lebetina turanica C., Vipera ursini renardi Ch., Echis multisquamatus Ch., Agkistron halys halys Schn., Naja oxiana Eich., and, taken for comparison, the venom of Vipra berus berus, that had been dried in a desiccator over $CaCl_2$ were obtained in the Central Asian Zonal Zoological Combine and the Institute of Zoology and Parasitology of the Academy of Sciences of the Uzbek SSR (Tashkent). Batches of venoms collected in 1984-1989 were studied. As substrates we used commercially available hemoglobin and BAEE from Reanal (Hungary), dimethylcasein [8], and an emulsion of egg yolk phospholipids [9], and for inhibitor analysis EDTA and PMSF from Serva (FRG); the other reactants were of KhCh ["chemi-

cally pure"] or ChDA ["pure for analysis"] grades. Random-bred white mice weighing 18-20 g were used.

Proteolytic activity was determined by known methods using various protein substrates [10]. As the unit of activity we took the amount of enzyme that caused an increase in optical density by 0.1 under the conditions used. In the case of dimethylcasein the unit of activity was calculated from a formula given in the literature [8]. The BAEE esterase activity was determined spectrophotometrically [11], and the phospholipase A_2 activity titrimetrically [12]. Toxicity was studied by calculating LD_{50} values (mg of protein or venom per 1 kg body weight of a mouse under various conditions of injecting the venoms [13]), and hemorrhagic activity by Kondo's method [14] with calculation of the MHD (μg of protein or venom per 1 g body weight of a mouse). The results were treated statistically and represent the mean values of not less than six measurements [13].

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