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Intraocular pressure reduction induced by hornet venom in the normal eye

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Summary

Intravenous injection into rabbits and cats of an aqueous extract of hornet venom sac, produced a significant reduction in the intraocular pressure. The mean reduction of the ocular pressure was between 7 and 15 mm Hg within one hour past injection and it lasted 6–12 h or in several instances even more than 24 h. The effect is produced by some thermolabile high-molecular-weight fraction(s), is dose-dependent and is reproducible by repetitive injections. Side effects may include diarrhea, micturition and lethargy, but usually there is no marked effect on the size of the pupil.

Key words: hornet venom; intraocular pressure.

Introduction

Venoms of insects belonging to the order Hymenoptera are known to harm animals and man. Among their various effects is usually a drop in the arterial blood pressure (Habermann, 1968; Ishay et al., 1974a; Edery et al., 1978), attributable to hypotensive phenomena, vasodilatation induced by biogenic amines (Ishay et al., 1974b), and to certain polypeptides present in these venoms. The venoms of social wasps contain also protein fractions responsible for reducing the blood pressure (Ishay et al., 1973, 1974b). Ordinarily the drop in arterial pressure occurs shortly after the intravenous injection of the venom. It may appear within less than a minute, while the low pressure lasts a relatively brief period – usually not more than 5 to 15 min. Subsequently the blood pressure reverts to normal. On repeated injections of hornet venom, there is a con-

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siderable lessening of the blood pressure reduction, due to the appearance of tachyphylaxis.

In the venom of the Oriental hornet, *Vespa orientalis*, anticholinesterase-like activities have also been detected (Ishay, 1979). Injection of anticholinesterase results, inter alia, in miosis and decreased intraocular pressure (Havener, 1978). In the course of several experimental investigations of the effect of hornet venom on various mammals (Ishay, unpublished observations), it was noted that in cats or rabbits following recovery from the effect of the venom and restoration of normal blood pressure, there was an interesting after-effect, namely, that the eyes became soft to palpation, indicative of reduced intraocular pressure. This observation has prompted the present study wherein the effect of sublethal doses of hornet venom on the intraocular pressure was evaluated quantitatively in cats and rabbits.

Materials and Methods

Venom extraction and processing were as previously described (Ishay, 1975). Dialysis of the aqueous extract was performed in a Visking cellophane tube, size W.D.C. at 4° C for 24 h in two ways: either in mammalian saline in an outer volume of 1:5,000, so as to obtain the high-molecular-weight fractions of the venom (the retained part), or against a 1:1 dilution to obtain the low-molecular-weight fraction (the dialysate). The dialyzed venom was injected into: 1) cats pre-anesthetized with sodium pentobarbital (Nembutal – Abbott) administered i.v. in a dose of 25 mg/kg; 2) 16 albino rabbits similarly anesthetized; 3) 30 unanesthetized rabbits; and 4) 12 rabbits serving as controls.

In the first two instances, the venom solution was injected via the femoral vein and in the third instance via the vena marginalis. The injected venom solution comprised: 1) whole (crude) venom sac extract (VSE); 2) whole VSE that had been boiled for 15 min; 3) the dialyzed VSE; and 4) the dialysate of the venom. In all instances, the VSE was diluted 1:5 in mammalian saline solution. Invariably the rectal temperature of the experimental animals was measured before and after the VSE injection. The effect of the VSE on the reduction in introocular pressure was assessed: a) on single injection to a naive animal, i.e., an animal never previously injected with hornet VSE; b) on sequential injection of a uniform dose of VSE to a naive animal; c) on repeated injections of increasing and decreasing doses of the VSE to a naive animal; d) on injection to immunized rabbits in which the immunization was performed during three months prior to the VSE injection by the method described previously (Ishay et al., 1972); and e) by topical application of two droplets of VSE on the rabbit cornea.

During the ocular examination, test rabbits were restrained within a towel to keep them from moving. Prior to the ocular examination 0.4% Benoximate HCl (Fisher) was dropped into the eye to effect local anesthesia of the cornea. The intraocular pressure (IOP) was measured prior to venom injection and at regular intervals after the injection, by means of Draeger Applanation Tonometer (and sometimes, in addition, by Schiotz Tonometer). Additionally, diameter of the pupil was measured before and after venom injection by use of a ruler. Each experiment also included measurement of the IOP in control rabbits which were either left uninjected or were injected with saline solution only. In no instance was any significant change in the IOP detected in the control rabbits. Since the composition of the VSE solution is variable due to its being a mixture of naturally produced materials (proteins, polypeptides and biogenic amines), it had to be prepared in sufficient quantity to inject into an entire series of test rabbits on any single day. This ensured uniformity of the results.

Results

Topical application of whole VSE droplets did not induce any change in the intraocular pressure in rabbits (6 repetitions) even 6 h after application.

As for the effect of the VSE when injected, here the results were invariably significant, all test animals responding to the venom injection. The effects of VSE injection (2 mg/kg) on IOP in rabbits and cats are summarized in Table 1.

In commenting on the data in Table 1, we note that:

1. In the anesthetized animals, cats as well as rabbits, the initial IOP (at time 0) was lower than in the non-anesthetized animals.
2. In naive as well as in immunized animals the whole VSE or its dialyzed portion produce the same effect, namely, a sharp and significant drop in the IOP which lasts for several hours and in some instances even for 24 h or more.
3. Boiled VSE or VSE dialysate (unboiled) both produce a significant drop (of about 40%) in IOP, but this lasts a relatively short time usually about 60 min.
4. In cases where the IOP reverted to normal shortly after the injection, follow-up at 24 h was deemed pointless; likewise, cats anesthetized prior to injection were not examined again at 24 h because once they awakened from the anesthesia they were too unruly to hold for measurement.
5. At higher dosage, such as 5–10 mg/kg, there is rapid and drastic drop of the IOP, but this is accompanied by toxic side effects, e.g. hematuria, almost total immobility, diarrhea and micturition. The excretions and the lethargy occur also under the regular dosage, but in the higher dosage they increase with time, usually leading to death of the animal within 24 h.
6. Usually the venom injection is accompanied by a rapid change in diameter of the pupil – at first some dilatation and then contraction, but within 2–3 min the original pre-injection diameter is restored.
7. No change in body temperature of more than 1 ° C was noted throughout the test period.

We also tested the effect of repeated injections of relatively small doses of VSE, to ascertain whether they might produce a cumulative depression of the IOP. In Table 2 are summarized the results of 3 sequential injections of VSE, given 90 min apart, in a dose of 0.75 mg/kg, into three different rabbits.

In commenting on Table 2, we note that the numerical values are a mean of two measurements on both eyes. Similar injections to rabbits using boiled VSE or a dialysate of VSE, resulted in a 10–30% drop in the IOP only after the initial injection. On subsequent repeated injections there was either no drop at all or a slight drop, and generally there was reversion to the original IOP within 90–120 min.

Dose dependence. We also tested the dependence of the drop in IOP on the size of the venom dose. Table 3 presents the outcome of the injection of different doses of VSE to three groups of three albino rabbits each. As can be seen in Table 3, there is a clear dose dependence on the drop in IOP.

Table 1. Changes in IOP* following injection of *V. orientalis* VSE

Test animals	Cats	Rabbits					
Status	Anesthetized		Awake				
Material injected	Whole venom sac extract				Inner dialysate	Outer dialysate	Boiled VSE
No. of animals tested	6	6	8**	8	9	6	6
Intraocular pressure							
Time:							
0	18 (±3.0)	21 (±3.5)	26 (±2.4)	27 (±3.1)	25 (±2.3)	26 (±2.4)	27 (±3.1)
10′	12 (±2.4)	11 (±2.6)	14 (±2.0)	16 (±2.0)	13 (±2.1)	15 (±1.1)	16 (±1.8)
30′	12 (±1.8)	11 (±1.9)	11 (±2.2)	13 (±1.4)	9 (±1.9)	21 (±2.5)	21 (±1.9)
60′	8 (±1.1)	16 (±1.3)	12 (±1.8)	12 (±1.2)	9 (±1.4)	23 (±2.6)	24 (±1.4)
90′	6 (±1.3)	16 (±2.1)	10 (±2.1)	9 (±1.3)	7 (±1.1)	25 (±1.4)	25 (±1.1)
120′	6 (±1.8)	16 (±0.8)	11 (±1.9)	11 (±1.2)	9 (±1.2)	26 (±1.0)	26 (±1.0)
240′	7 (±2.2)	16 (±0.9)	11 (±2.3)	11 (±1.1)	7 (±1.3)	26 (±1.2)	27 (±1.3)
360′	7 (±2.5)	19 (±1.6)	13 (±2.0)	11 (±2.3)	7 (±2.1)	27 (±1.1)	27 (±1.4)
24 h	–	21 (±2.9)	26 (±3.0)	16 (±6.0)	13 (±4.0)	–	–

* The results are given as mean mm Hg pressure ±standard deviation.

** These rabbits were previously immunized against VSE.

Table 2. The effect of sequential injections of 0.75 mg/kg VSE into rabbits. The results are the mean of two consecutive measurements on both eyes.

	Rabbit No.		
	1	2	3
Intraocular pressure			
Time:			
0	27	26	27
Injection No. 1:			
10′	19	18	16
30′	16	17	15
60′	17	16	15
90′	16	16	15
Injection No. 2:			
10′	12	13	14
30′	11	11	13
60′	10	10	12
90′	10	11	12
Injection No. 3:			
10′	7	8	7
30′	6	7	7
60′	6	7	6
90′	6	7	5

Table 3. Changes in IOP following injection of various doses of *V. orientalis* VSE. Three groups of 3 rabbits were tested.

	Group I Injected with	Group II	Group III
	2 mg/kg	1 mg/kg	0.5 mg/kg
Intraocular pressure			
Time:			
0	27 (±2.0)	26 (±1.2)	25 (±1.8)
10′	12 (±1.6)	18 (±2.0)	19 (±2.0)
30′	10 (±1.7)	17 (±2.4)	20 (±2.1)
60′	11 (±2.1)	16 (±1.8)	20 (±2.2)
90′	9 (±3.0)	19 (±2.2)	21 (±2.2)
240′	15 (±5.1)	20 (±4.3)	24 (±1.5)

Discussion

Most of the determinations were made on non-anesthetized rabbits, with only a few subjected to anesthesia. In the case of cats, measurements were made only on narcotized ones because you cannot keep alley cats placid during determinations. In anesthetized rabbits and cats, the initial IOP was lower than in non-anesthetized rabbits because the narcotizing agent itself produces a drop in the IOP (Stone and Prijot, 1955; Magora and Collins, 1961). The findings presented in Tables 1–3 stress the fact that whole as well as dialyzed VSE induce, only when injected, within a short interval after injection, into rabbits or cats, a sustained reduction in the IOP which is dose-dependent. It should be emphasized, however, that our rabbits and cats were not a uniform group insofar as the IOP was concerned, so there was need to select among the albino rabbits individuals of similar weight and with a fairly close (albeit not identical) IOP. Neither was the VSE solution used in this study uniform because the amount required for the experiments could only be obtained from the pooled VSE of a large number of hornets. Bearing in mind, also, that the VSE is natively a mixture of natural substances, we prepared all the VSE necessary for a particular experiment in one batch so that all the animals would receive the identical material. In view of the fact that reduced IOP was obtained also in rabbits which had been experimentally immunized to the VSE over a prolonged period, it is reasonable to presume that the active fraction(s) is not antigenic or only weakly so. Again, since in the dosage employed no miosis occurred, assumably the anticholinesterase properties of the VSE (Ishay, 1979) had not come into play.

As a preliminary step towards ascertaining the active component(s) in the VSE, insofar as the effect on the IOP is concerned, we dialyzed or boiled the VSE solution. Both the dialysate and the boiled VSE induced a drop in the IOP but this was of a transient nature as compared to the more lasting effect produced by dialyzed or by whole, unboiled venom. It would seem, that crude VSE contains two groups of substances, each of which is capable of producing reduced IOP. The first group comprises high-molecular-weight substances which are thermolabile (destroyed by boiling) and produces a sustained drop in IOP, only when injected, whereas the second group is comprised of low-molecular-weight substances which are thermostable, but of transient reducing effect on the IOP, probably inducing rapid tachyphylaxis.

In a previous study on the cardiovascular dynamics of Oriental hornet venom (Kaplinsky et al., 1974), it was found that the venom acted within 10 sec and its effect lasted for several minutes, with one of the main effects being a sharp drop in the peripheral vascular resistance. Most of the hemodynamic effects of hornet venom, except for vasodilatation, have been attributed to one of its components, namely, 5-hydroxytryptamine (5-HT) (Ishay et al., 1974b). This substance, together with histamine, acetylcholine, epinephrine, nonepi-

nephrine, and dopamine, which are mostly present in the venom in small amounts (Edery et al., 1972), could possibly be responsible for the short lasting drop in IOP which is obtained by the injection of boiled venom or dialysate of venom. This seems reasonable because the eye is known to contain receptors for both 5-HT and acetylcholine, either of which could affect the IOP (Conti-Tronconi et al., 1979; Thomas and Redburn, 1979). However, the more pronounced and lasting effect is produced by the thermolabile, high-molecular-weight component(s). It is rather unlikely that this substance is hyaluronidase, which occurs in the hornet venom (Allalouf et al., 1972), because this enzyme is antigenic, whereas in the venom-immunized rabbits there was still a drop in the IOP after the VSE injection.

In previous studies as well (Kaplinsky et al., 1974) we detected a vasodilatatory agent whose activity could not be abrogated. This latter compound resembled in its hypotensive activity the β blockers, and it should be remembered that the rabbit has both α and β receptors (Sears, 1975). Hence it seems likely that the drop in IOP is not correlated, from the standpoint of *time of appearance* and *duration*, to the overall reducing effect of the venom or VSE on the blood pressure. The fact that the VSE that has undergone dialysis is still active excludes the possibility that the minute amounts of epinephrine in the VSE are responsible for the drop in IOP.

Thus, it seems that in the final analysis, the sustained effect observed is a summation of a number of discrete activities, each produced by a different protein fraction in the VSE. Of these, those that produce anticholinergic effect reduce the rate of fluid formation within the eye, while those acting like β blockers enhance the fluid clearance rate of the eye (Zimmermann and Boger, 1979). Presumably, then, the final outcome is a combination of two or more such separate activities. Efforts are now being made to identify the active fractions and elucidate their mode of action.

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