J. Pharm. Pharmacol., 1964, **16**, 79–84. Received September 30, 1963

Capillary permeability responses to snake venoms

H. J. FEARN, CAROLINE SMITH AND G. B. WEST

Russell's viper venom is many times more toxic to mice on intravenous administration than is saw-scaled viper venom, and yet on local intradermal injection the two venoms have equal capillary permeability effects in mice, rats, guinea-pigs and rabbits. Their effects are completely prevented by drugs possessing both anti-histamine and anti-5-HT properties. It is concluded that the increased capillary permeability induced by the venoms is largely mediated through the release of histamine and 5-HT.

THE clinical symptoms after snake bites often resemble the acute effects of histamine. There is, for example, widespread capillary endothelial damage and this often leads to acute thrombosis and death. Some viper venoms have also been shown to degranulate mast cells thereby releasing heparin and histamine (Higginbotham, 1959). We have now tested the toxicity to mice of two snake venoms and then studied their effects on capillary permeability in four mammalian species.

Methods

TOXICITY OF SNAKE VENOMS

The acute intravenous toxicities of Russell's viper venom (*Vipera russellii*) and of saw-scaled viper venom (*Echis carinatus*) were determined in groups of 10 adult albino mice (weight 18–22 g) using the method of Litchfield & Wilcoxon (1949). Mortality rates were measured 24 hr after dosage.

TESTS ON CAPILLARY PERMEABILITY

The abdominal skin of adult albino rats, guinea-pigs and rabbits was shaved with an electric razor and 24 hr later the animals were anaesthetised and injected intravenously with azovan blue dye (7 mg/kg). Injections of the agents stated below were then made intradermally on both sides of the midline of the shaved area; 20 min later, the animals were killed, the shaved skin was removed and firmly pinned to a cork board, and the extent of the colloid dye accumulation was estimated on the inner side of the skin by measuring the average diameter of the extent of dye spread. The mean response to neutralised saline (NaCl, 0·9% w/v) was $10 \pm 1{\cdot}5$ mm diameter (60 determinations).

In the experiments with mice, the skin was similarly shaved and 24 hr later the animals were injected intravenously with congo red (50 mg/kg). One intradermal injection was made into each animal and later the shaved skin was extracted with acetone (6 ml/g). The extent of the colloid dye accumulation was then estimated absorptiometrically (Hilger absorptiometer: green filter, OG 1). In each experiment, groups of at least 3 animals were used and the results were averaged.

From the Department of Pharmacology, School of Pharmacy, University of London, Brunswick Square, London, W.C.1

AGENTS USED

The agents were histamine, 5-hydroxytryptamine (5-HT), compound 48/80, ovomucoid, dextran (Intradex), bradykinin, Russell's viper venom (*Vipera russelli*), saw-scaled viper venom (*Echis carinatus*) and saline. A few experiments were made with hooded cobra venom (*Naja naja*) and scorpion venom (*Leinrus quinquestriatus*). Each was used in a volume of 0·1 ml neutralised saline.

ANTAGONISTS

These were given either intravenously 30 min before the dye and active agent or intradermally together with the active agent but after the colloid dye.

DEPLETION OF AMINES

Compound 48/80 was injected intraperitoneally to deplete rats of their skin histamine and part of their skin 5-HT before the test; the twice daily doses were 1 mg/kg on the first day, 2 mg/kg on the second and 3 mg/kg on the third. Animals were then tested on the fourth and eighth days, that is, 1 and 5 days after the last dose of compound 48/80.

Results

TOXICITY OF VENOMS

The acute intravenous LD50 of Russell's viper venom in mice was 35·0 μg/kg (limits of error at P = 0·95, 30·2–40·6) whereas that of the saw-scaled viper venom was 620 μg/kg (limits, 470–818). Thus Russell's venom is more than 17 times more toxic than that of the saw-scaled viper. The complete results from which these LD50 values were calculated are shown diagrammatically in Fig. 1. With Russell's venom, deaths usually occurred within the first 30 min, the cause being pulmonary oedema; with the saw-scaled viper venom, deaths were much delayed, many dying at about 18 hr after injection and showing gross haemorrhage in the lungs and blood in the urine and faeces.

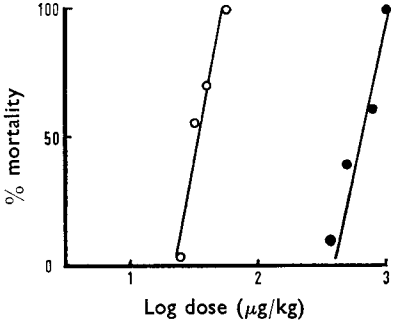

FIG. 1. The acute intravenous toxicities of Russell's viper venom (○—○) and of saw-scaled viper venom (●—●) using groups of 10 mice. Note that Russell's viper venom is many times more toxic.

THE RELATION BETWEEN INTRADERMAL DOSE AND RESPONSE

This is shown in Fig. 2 for most of the agents studied. By contrast with their relative activities on intravenous administration into mice, Russell's viper venom and saw-scaled viper venom were equally effective intradermally in the three species, doses of 0·1 to 4 μg being sufficient to give dose-response relationships. The local inflammatory reaction of the saw-scaled viper venom was always accompanied by minute petechial haemorrhages. Similar intradermal doses of cobra venom and of scorpion

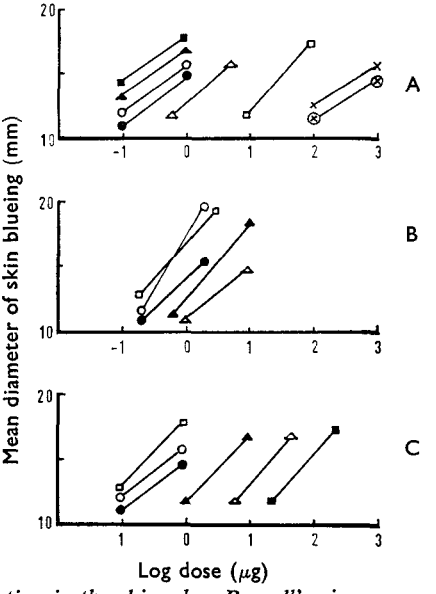

FIG. 2. Dye accumulation in the skin when Russell's viper venom (○), saw-scaled viper venom (●), histamine (□), 5-HT (■), bradykinin (△), compound 48/80 (▲), ovomucoid (×), and dextran (⊗) are injected intradermally into rats (A), rabbits (B) and guinea-pigs (C). Note that Russell's viper venom and saw-scaled viper venom are equi-active in all three species.

venom were also effective in rats. Of the other agents, histamine, ovomucoid and dextran were weakly active in rats, and so were 5-HT and bradykinin in guinea-pigs; furthermore, 5-HT was inactive in rabbits and ovomucoid and dextran were inactive in rabbits and guinea-pigs. These results are also shown in Table 1.

TABLE 1. THE RELATIVE EFFECTIVENESS OF DIFFERENT AGENTS ON INTRADERMAL INJECTIONS IN THE FOUR SPECIES. RESPONSES MEASURED ON A RELATIVE SCALE FROM 0 TO +++, EACH + REPRESENTING A 10-FOLD DECREASE IN EFFECTIVE DOSE

Agent	Rats	Mice	Rabbits	Guinea-pigs
Venoms	+++	++	+++	+++
Bradykinin	++	++	++	++
Compound 48/80 ..	+++	+	+++	++
Histamine	+	++	+++	+++
5-HT	+++	+++	0	+
Ovomucoid, dextran ..	+	0	0	0

EFFECT OF THE AGENTS IN MICE

With compound 48/80, histamine and 5-HT, the response decreased as the dose was increased, but the increase in capillary permeability produced by the venoms intensified as higher doses were used. This is shown in Fig. 3. The two venoms were equally effective intradermally in mice and were about 10 times less active than in the other three species.

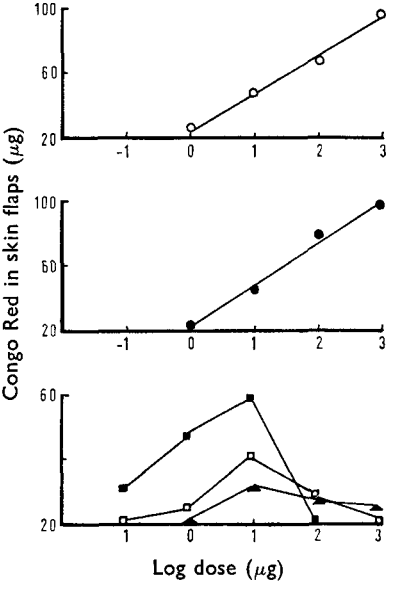

FIG. 3. Dye accumulation in the skin when Russell's viper venom (○), saw-scaled viper venom (●), histamine (□), 5-HT (■) and compound 48/80 (▲) are injected intradermally into mice. Note the different scales. Whereas the venom effects increase with higher doses, those of the standard substances decrease.

EFFECT OF AMINE DEPLETION

Chronic treatment of rats with compound 48/80 prevented the responses to compound 48/80, ovomucoid, dextran, histamine and 5-HT and reduced the venom effects. When tested 4 days later, the responses to all the agents had fully recovered although the skin histamine was still low at 20% of the control value (Bonaccorsi & West, 1963).

EFFECT OF ANTAGONISTS

Doses of 10 mg/kg of mepyramine, an antihistamine drug, completely inhibited the responses of histamine in rats and rabbits and markedly reduced those of the venoms and of compound 48/80. Doses of 2 mg/kg of 1-methyl-lysergic acid butanolamide (UML 491), an anti-5-HT drug, were effective in preventing the responses to 5-HT in rats and mice but were very weak against the venoms. Doses of 8 mg/kg of promethazine, an antihistamine and anti-5-HT drug, effectively prevented the responses

of the venoms, compound 48/80, histamine and 5-HT in guinea-pigs, rats and mice, although they were only weakly active against bradykinin. These results are shown in Table 2.

TABLE 2. THE RELATIVE EFFECTIVENESS OF DIFFERENT ANTAGONISTS ON INTRAVENOUS INJECTION IN THE FOUR SPECIES. REDUCTION OF THE LOCAL RESPONSE OF EACH AGENT IS MEASURED ON A RELATIVE SCALE FROM 0 TO +++

Agent	Mepyramine		UML 491		Promethazine		
	Rat	Rabbit	Rat	Mouse	Guinea-pig	Rat	Mouse
Venoms	++	++	+	+	+++	+++	+++
Bradykinin	+	+	+	+	+	+	+
Compound 48/80	++	++	+	0	+++	+++	+++
Histamine	+++	++	0	0	+++	+++	+++
5-HT	0	—	+++	+++	+++	+++	+++
Ovomucoid, dextran	+	—	+++	—	—	+++	—

When the antagonists were given together with the active agents in rats, promethazine in doses of 2 μg was again the most effective compound. The venom responses were also completely prevented by similar doses of cyproheptadine, an antagonist with both antihistamine and anti-5-HT activities. The results of these local effects are shown in Table 3. Note that mepyramine exerted a slight inhibitory effect on the 5-HT response when given by this route, and UML 491 slightly reduced the histamine effect.

TABLE 3. THE RELATIVE EFFECTIVENESS OF ANTAGONISTS (2 μg) ON INTRADERMAL INJECTION IN RATS. REDUCTION OF RESPONSES IS MEASURED ON A RELATIVE SCALE FROM 0 TO +++

Agent	Dose (μg)	Mepyramine	UML 491	Promethazine	Cyproheptadine
Venoms	1	+	++	+++	+++
Bradykinin	10	+	+	+	+
Compound 48/80	1	+	++	++	++
Histamine	100	+++	+	+++	+++
5-HT	1	+	+++	+++	+++
Ovomucoid, dextran	100	+	+++	+++	+++

Discussion

The results show that Russell's viper venom, a thromboplastic agent, is many times more toxic to mice than is saw-scaled viper venom, a haemolytic agent, when given intravenously, and yet it is only equally active when given intradermally to mice, rats, guinea-pigs and rabbits and when the changes in capillary permeability are measured. The treatment of viper snake poisoning continues to be a therapeutic problem in many parts of the world in spite of available antivenene and it is of fundamental importance to elucidate the exact mechanism producing the increased capillary permeability as the extent of the capillary damage determines to a large extent the ultimate outcome of the snake bite.

The local responses of the venoms are effectively antagonised by promethazine or cyproheptadine and anti-5-HT compounds, and it seems

that the snake venoms exert their effects through the release of at least both 5-HT and histamine. This is, however, not the complete mechanism as the venoms, for example, are effective local inflammatory agents even when the skin histamine content is much reduced by chronic treatment with compound 48/80. They are also effective in mice at high doses when histamine and 5-HT are exhibiting tachyphylaxis. Bradykinin is also an effective initiator of the local inflammatory response but its actions in the four species studied are only feebly reduced by the more specific antagonists.

Sparrow & Wilhelm (1957) reported the species differences in susceptibility to capillary permeability factors such as histamine, 5-HT and compound 48/80, and the present results confirm that histamine is most active and 5-HT is least active in guinea-pigs and rabbits, whilst compound 48/80 is the least active in mice. Ovomucoid and dextran have again been shown to be ineffective by this test in mice, rabbits and guinea-pigs.

The administration of an antihistamine and anti-5-HT drug, together with the antivenene, is suggested in the management of viperine snake poisoning.

Acknowledgements. We wish to acknowledge the gifts of compound 48/80 from Burroughs, Wellcome and Co., London; bradykinin and UML 491 from Sandoz Products Ltd., London; and scorpion venom from Dr. K. R. Adam, Khartoum.

References

Bonaccorsi, A. & West, G. B. (1963). *J. Pharm. Pharmacol.*, **15**, 372–378.
Higginbotham, R. D. (1959). *Int. Arch. Allergy*, **15**, 195–210.
Litchfield, J. T. & Wilcoxon, F. (1949). *J. Pharmacol.*, **96**, 99–113.
Sparrow, E. M. & Wilhelm, D. L. (1957). *J. Physiol.*, **137**, 51–65.