ABSENCE OF CAPILLARY PERMEABILITY RESPONSE IN RATS TO DEXTRAN AND EGG-WHITE

BY AURORA BONACCORSI AND G. B. WEST

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

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Intradermal dextran and ovomucoid fail to increase capillary permeability in rats resistant to intraperitoneal dextran although intradermal histamine, 5-HT and compound 48/80 are as effective as in control rats. When the skin of control rats is depleted of its histamine, intradermal dextran and ovomucoid are first ineffective but later increase capillary permeability although the skin histamine remains low. Chronic treatment of control rats with intraperitoneal dextran (which only slightly reduces both the skin histamine and 5-HT) prevents the local dextran and ovomucoid responses but does not affect those of compound 48/80, histamine and 5-HT. It is concluded that intradermal dextran and ovomucoid increase capillary permeability in rats by a mechanism involving substances other than histamine and 5-HT, and that this mechanism is absent in rats which do not respond to intraperitoneal dextran.

THE inflammatory anaphylactoid reaction produced in rats by the single intraperitoneal injection of dextran or egg-white has been shown to be mediated chiefly through a release of 5-hydroxytryptamine (5-HT) and histamine (Parratt and West, 1957). Recently, Harris and West (1961) found that not all rats react to this primary injection although both amines are always present in the skin of rats. We have studied this problem further by determining the intensity of colloid-dye accumulation in the abdominal skin of both types of rat after various treatments.

METHODS

Groups of Wistar albino rats obtained from the Agricultural Research Council's Field Station at Compton were used in all experiments. They were injected intraperitoneally with dextran (Intradex, 180 mg./kg.) and subsequently divided into two groups—those which showed pruritus and oedema of the face, tongue and paws (hereinafter called Reactors), and those which failed to show the anaphylactoid reaction (called Non-reactors). The animals initially resistant to dextran (about 25 per cent of the total number) were also resistant to doses of 30, 120 and 480 mg./kg. when tested at weekly intervals: they were also resistant to fresh egg-white (10 ml./kg.).

Tests for capillary permeability. The abdominal skin of the rats (body weight 150–200 g.) was depilated with an electric razor 24 hr. before the test. The animals were injected intravenously on the day of the test with azovan blue dye (7 mg./kg.), and then intradermally at the left and right sides of the midline of the shaved skin with the agents stated below in volumes of 0·1 ml. Thirty min. later, the rats were killed and the shaved skin was removed and firmly pinned to a cork board. 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 blueing. Extent and intensity of blueing were usually of a similar order. The mean response to neutralised saline (NaCl, 0·9 per cent w/v) was 10 ± 1 mm. (100 determinations). In each experiment, groups of at least 3 reactor and 3 non-reactor rats were used, and the results averaged for each type.

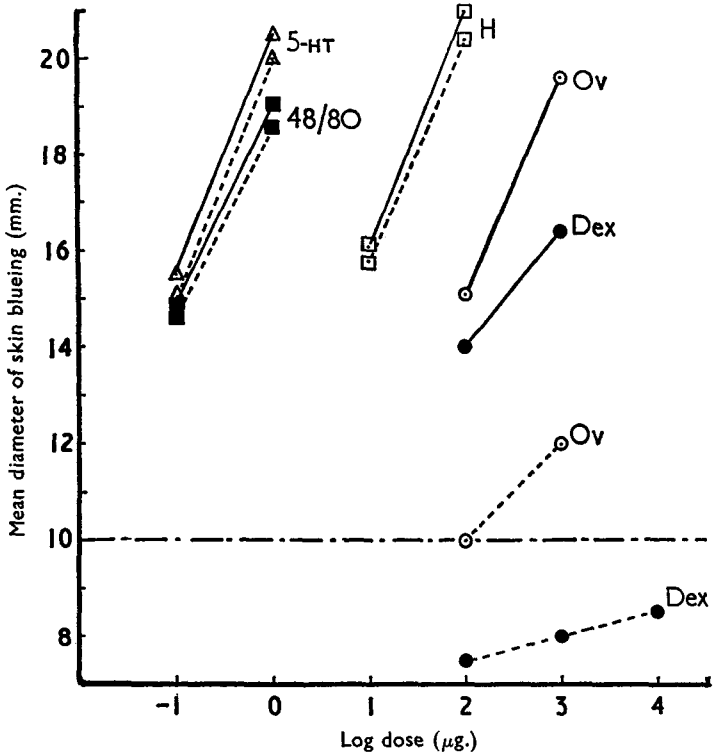

FIG. 1. Dye accumulation in the skin when 5-HT (△), compound 48/80 (■), histamine (□), ovomucoid (○), and dextran (●) are injected intradermally into reactor (continuous lines) and non-reactor (broken lines) rats. Abscissa, dose in μg. (log scale); ordinate, mean diameter of blueing in mm. Mean saline response is 10 ± 1 mm. (shown by dotted line).

Agents used. Six intradermal injections were always given to each rat. These were histamine (10 or 100 μg.), 5-HT (0·1 or 1 μg.), compound 48/80 (0·1 or 1 μg.), ovomucoid (100 or 1000 μg.), dextran (Intradex, approximate molecular weight 145,000, dose 100 or 1000 μg.) and saline (0·1 ml.). In some experiments, dextrans of two other molecular weights (4,000 and 20 million) were used each in doses of 100 or 1000 μg.

Depletion of amines. Polymyxin B was injected intraperitoneally to deplete rats of their skin histamine before the test; the twice daily doses were 2·5 mg./kg. on the first day, 5 mg./kg. on the second, and 7·5 mg./kg.

on the third. Tests of capillary permeability were carried out using the smaller doses of the five agents on the fourth, eighth and tenth days, that is 1, 5 and 7 days after the last dose of polymyxin. 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

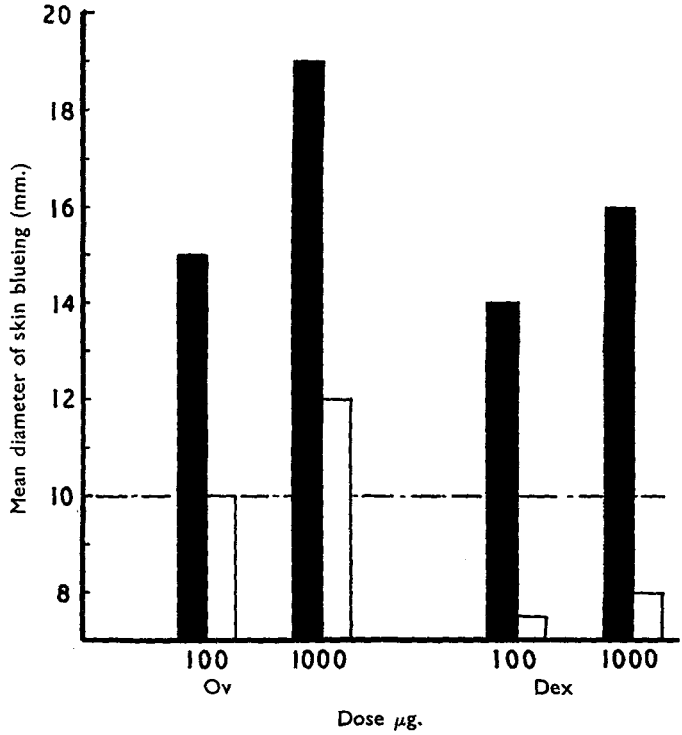

FIG. 2. Dye accumulation in the skin when ovomucoid and dextran (100 and 1,000 μg. doses) are injected intradermally in reactor (shaded columns) and non-reactor (plain columns) rats. Note that the lower dose of ovomucoid and both doses of dextran are ineffective in non-reactor rats.

third. Animals were then tested as previously described. Reserpine was injected intraperitoneally to deplete rats of their skin 5-HT before the test; the doses used were 1 mg./kg. daily for 3 days, the rats being subsequently used as described above.

In other experiments, gradually increasing doses of dextran were given daily to produce resistance to dextran in reactor rats; these intraperitoneal doses started at 600 mg./kg. and finished at 3,000 mg./kg. on the seventh day, the rats being tested on the eighth day.

Antagonists. These were given intravenously 30 min. before the azovan blue dye and active agent. UML 491 (1-methyl-lysergic acid butanolamide), a specific antagonist of 5-HT, was used in doses of 25–250

μg./kg. Mepyramine, a specific antagonist of histamine, was used in doses of 500–2,500 μg./kg. In a few experiments, both antagonists were used simultaneously.

Histamine content of the abdominal skin. This was estimated in control rats and in rats treated with polymyxin B, compound 48/80, reserpine and dextran. The method of Parratt and West (1957) was used.

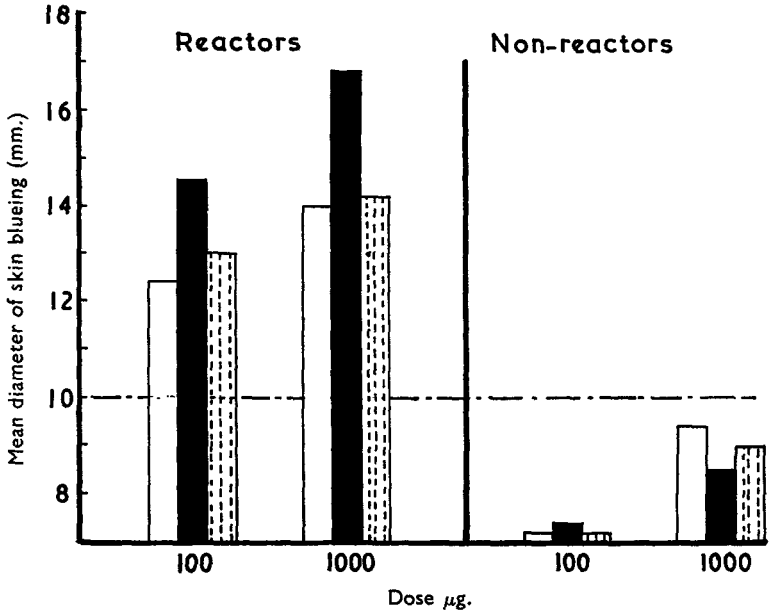

FIG. 3. The effect of intradermal injections of dextrans of different molecular weight. Dye accumulation only occurs in reactor rats. Open column, mol. wt. 4×10^3. Solid column, mol. wt. 145×10^3. Dotted column, mol. wt. 20×10^6.

RESULTS

The relation between dose and response. This is shown in Fig. 1 for the five agents. Compound 48/80 and 5-HT were the most potent and were equally effective in both reactor and non-reactor rats. Histamine was similarly effective in both types of animal but it was about 100 times less potent (Sparrow and Wilhelm, 1957). Ovomucoid and dextran were some 10 times less active than histamine in reactor rats but gave no response in non-reactors. Occasionally, ovomucoid but not dextran produced areas of blueing which exceeded those of saline. Higher doses of dextran, for example 10 mg., were also ineffective in non-reactor rats (see Fig. 1). A comparison of the responses of dextran and ovomucoid in reactor and non-reactor rats is more clearly shown in Fig. 2.

Effect of dextrans of different molecular weight. The three dextrans produced responses in reactor rats but all were ineffective in non-reactors. These results are recorded in Fig. 3. The most active sample had a molecular weight of about 145,000.

Effect of amine depletion. Chronic treatment of reactor rats with polymyxin B prevented the responses of compound 48/80, ovomucoid and dextran but only slightly reduced those of histamine and 5-HT when the test was carried out 1 day after treatment (see Fig. 4). The polymyxin treatment lowered the skin histamine by 81 per cent (control value, 25 μg./g.) and disrupted the mast cells. When tested 4 days later, the

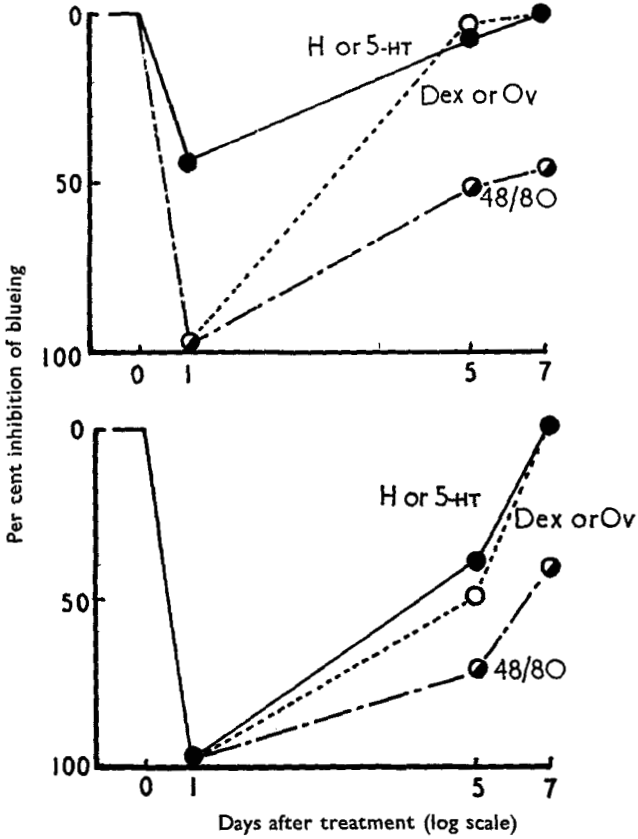

FIG. 4. Inhibition of the dye accumulation in the skin of rats by previous treatment with polymyxin B (upper tracing) or compound 48/80 (lower tracing). Values shown for histamine and 5-HT (●) have been averaged, also those of ovomucoid and dextran (○). Note that the compound 48/80 response (◔) is still reduced after 7 days of recovery.

responses to ovomucoid and dextran had fully recovered although the skin histamine remained low at 20 per cent of the control value; the response of compound 48/80 was still greatly reduced (see Table I). A similar result was obtained with histamine, 5-HT and compound 48/80 when the test was made in non-reactor rats.

Chronic treatment of reactor rats with compound 48/80 prevented the responses of compound 48/80, ovomucoid, dextran, histamine, and 5-HT

(see Fig. 4). This treatment lowered the skin histamine by 83 per cent. All the responses (except that of compound 48/80) had fully recovered when the test was carried out 7 days later, although the skin histamine was still depleted (see Table I). Again, a similar result was obtained with compound 48/80, histamine and 5-HT when the test was carried out in non-reactor rats.

Chronic treatment of reactor rats with reserpine prevented the responses of dextran and ovomucoid but only slightly reduced those of compound 48/80, histamine and 5-HT. However, chronic treatment of reactor rats with dextran, which only slightly reduced both the skin histamine and 5-HT, prevented the responses of dextran and ovomucoid but did not reduce those of compound 48/80, histamine and 5-HT; such reactor rats then are reacting like untreated non-reactor rats.

TABLE I

RESPONSES OF THE 5 AGENTS WHEN INJECTED INTRADERMALLY INTO REACTOR AND NON-REACTOR RATS, ON A RELATIVE SCALE FROM 0 TO ++. THE RESULTS WITH REACTOR RATS CHRONICALLY TREATED WITH DIFFERENT COMPOUNDS ARE ALSO SHOWN; (*a*) TESTS COMPLETED 1 DAY AFTER TREATMENT, AND (*b*) TESTS COMPLETED 7 DAYS LATER. THE SKIN HISTAMINE VALUES ARE GIVEN AS PERCENTAGES OF THE CONTROL VALUES (25 μG./G.)

Agent	Dose (μg.)	Reactor rats	Non-reactor rats	Chronic treatment of reactor rats					
				Dextran	Reserpine	Polymyxin B		Compound 48/80	
						(*a*)	(*b*)	(*a*)	(*b*)
Ovomucoid	100	++	0	0	0	0	++	0	++
Dextran	100	++	0	0	0	0	++	0	++
Histamine	10	++	++	++	++	+	++	0	++
Compound 48/80	0·1	++	++	++	++	0	+	0	+
5-HT	0·1	++	++	++	++	+	++	0	++
Skin histamine per cent		100	100	78	80	19	20	17	15

Effect of antagonists. UML 491 was ineffective at doses of 25 μg./kg. but it prevented the 5-HT response in both reactor and non-reactor rats when the dose was doubled. At this dose level, it also reduced the responses of dextran, ovomucoid and compound 48/80 in reactor rats but had no effect on the histamine response. Increasing the dose to 250 μg./g. prevented all responses except that of histamine.

Mepyramine in doses of 1 mg./kg. reduced the histamine response in both reactor and non-reactor rats but failed to alter the responses to 5-HT, compound 48/80, ovomucoid and dextran in reactor rats. Increasing the dose to 2·5 mg./kg. completely prevented the histamine reaction but still did not affect the other four responses.

When UML 491 (25 μg./kg.) and mepyramine (1 mg./kg.) were given together, the histamine response was reduced but the other four were prevented. The small dose of mepyramine potentiated the action of a small dose of UML 491 producing a result which is similar to that of a larger dose of UML 491.

Discussion

The results show that rats which are resistant to intraperitoneal doses of dextran (the non-reactors) are also resistant to intradermal dextran and ovomucoid although the reactions to intradermal compound 48/80, histamine and 5-HT are similar to those in reactor rats. Such a result suggests that non-reactors either possess an antagonist to dextran and ovomucoid or are deficient in one or more of the components necessary to effect the local capillary response. Non-reactor rats contain as much histamine and 5-HT as do reactors and so it may be that an intermediate stage in the reaction is unable to occur in non-reactor animals. Further, when reactor rats had received many doses of intraperitoneal dextran so that they failed to show a reaction to intraperitoneal or intradermal dextran or ovomucoid, they still reacted to compound 48/80, histamine and 5-HT.

Reactor rats treated chronically with intraperitoneal polymyxin B became refractory to intradermal compound 48/80, dextran and ovomucoid but not to histamine and 5-HT, whereas treatment with compound 48/80 completely prevented all reactions. In the days after treatment, the responses of dextran, ovomucoid, histamine and 5-HT were quickly regained, although the skin histamine was still at low levels, but that of compound 48/80 remained suppressed. Thus released histamine and 5-HT may respond in a different manner from exogenous histamine and 5-HT, and other factors are probably involved in the intradermal reaction, even in reactor rats. A direct effect on the capillary wall may be important, as suggested by Gözsy and Kato (1957).

Further work is needed to study the action of antagonists of dextran and ovomucoid in reactor rats when the skin histamine is depleted, and it appears that through this type of experiment, progress will be made in deciding why non-reactor rats fail to react to dextran or ovomucoid. Non-reactivity to dextran in rats has recently been shown to be a genetically controlled character (Harris, Kalmus and West, 1963) so the problem may be of much wider application in the future.

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