Heparin and the Dextran Anaphylactoid Reaction in Rats

G.B.

West

Department of Paramedical Sciences, North East London Polytechnic, England

Abstract

Injections of heparin had no effect on the dextran anaphylactoid reaction in rats but neutralised the inhibitory action both of protamine and of borate. Heparin, protamine, and borate did not modify the histamine or 5-hydroxytryptamine responses in rat paws and so their effects on dextran may be due to modification of the release mechanisms of these two amines from tissue mast cells.

Correspondence to: Dr. G.B. West, Department of Paramedical Sciences, North East London Polytechnic, Romford Road, London E15 4LZ (England)

Almost 20 years ago, heparin was reported [2] to increase the rate of wound healing in rat skin, with even better results when histamine was administered just before the heparin. Protamine, on the other hand, had no effect on wound healing by itself but it neutralised the increase in tensile strength of skin wounds produced both by heparin and by heparin and histamine. Protamine is considered to be a selective antagonist of heparin in many situations. Heparin also stimulates the formation of cartilage and antagonises some of the actions of glucocorticoids. It was of interest, therefore, to test heparin and protamine in an acute inflammatory reaction such as the dextran anaphylactoid oedema in rats, in which histamine and 5-hydroxytryptamine are first released from mast cells and heparin release follows.

Recently, we have shown [4] that sodium salicylate enhances the dextran anaphylactoid response in rats administered with the dextran. This followed the report [3] that salicylates are effective adjuvants for the rectal absorption of insulin (a polypeptide) and heparin (a mucopolysaccharide), compounds which normally are either degraded or poorly absorbed after oral administration. Sodium borate has also been shown [1] to enhance histamine release from isolated rat peritoneal mast cells induced by palytoxin, a long aliphatic chain with interspersed cyclic ether, hydrox-yl and carboxyl groups, isolated from marine coelen-terates. Salicylate and borate were therefore also tested on the dextran anaphylactoid reaction in rats in the presence and absence of heparin. In an attempt to

identify their mode of action, the effects of heparin, protamine, borate and salicylate on local actions of histamine and 5-hydroxytryptamine in rat paws were also studied.

Groups of 5 Wistar rats (200–300 g) were obtained from the Tuck colony and injected with clinical dextran (Intradex, molecular weight 110,000) to produce the anaphylactoid reaction after intraperitoneal injection (200 mg·kg⁻¹) or intrapedial injection (100 µg). The responses were measured as increases in hind paw volume as determined on a volume differential meter over 5 h. 7 days later, heparin, protamine, sodium borate or sodium salicylate were included with the dextran or injected 30 min before or after it. The results shown in the figures are the means ± SEM, and statistical analysis of the results was by the Student's t test.

The simultaneous administration of doses of heparin up to 2,000 IU·kg⁻¹ by the intraperitoneal route did not modify the increase in hind paw volume produced by dextran. However, protamine

significantly reduced the reaction in a dose-dependent fashion, as shown in figure 1. When heparin was included in the dextran-protamine mixture, the inhibitory action of protamine was prevented. This result was expected as protamine is usually a selective antagonist of heparin. When sodium borate was tested in a similar manner, it too significantly reduced the dextran anaphylactoid reaction when used in doses of 0.5–2 mg·kg⁻¹ and again heparin significantly antagonised the inhibitory action (fig. 2). This result was unexpected as

374

West

Fig. 1. Effect of protamine (O, 0.5 mg·kg⁻¹; •, 2 mg·kg⁻¹) on the increase in hind paw volume of rats produced by clinical dextran (200 mg·kg⁻¹). All doses injected intraperitoneally as mixtures. Results shown as mean percent increases ($\pm$ SEM) in groups of 5 rats. Control responses without protamine (Δ) and the effect of heparin (A, 2,000 IU·kg⁻¹) on the protamine inhibition (2 mg·kg⁻¹) are also shown. Note the dose-dependent inhibition of the dextran response by protamine and its antagonism by heparin.

Fig. 2. Effect of sodium borate ($\cdot$, $2 \text{ mg} \cdot \text{kg}^{-1}$) on the increase in hind paw volume of rats produced by clinical dextran ($200 \text{ mg} \cdot \text{kg}^{-1}$). All doses injected intraperitoneally as mixtures. Results shown as mean percent increases ($\pm \text{SEM}$). Control responses without borate (Δ) and the effect of heparin (A, $2,000 \text{ IU} \cdot \text{kg}^{-1}$) on the borate inhibition are also shown. Again, note the inhibition of the dextran response by borate and its antagonism by heparin.

Fig. 3. Effect of protamine ($\cdot$, $4 \mu\text{g}$) on the increase in hind paw volume of rats produced by clinical dextran ($100 \mu\text{g}$). All doses injected intrapedially as mixtures. Results shown as mean percent increases ($\pm \text{SEM}$) of groups of 5 rats. Control responses without protamine (Δ) and the effect of heparin (A, 20 IU) on the protamine inhibition are also shown. Note the inhibition by protamine of the dextran response and its antagonism by heparin.

borate enhances the action in vitro of palytoxin, one of the most potent animal toxins known, but does not enhance histamine release by compound 48/80 or concanavalin A [1]. When injected 30 min after (but not 30 min before) the dextran, both protamine and borate exerted their inhibitory effects. Sodium salicylate, as expected, enhanced the dextran anaphylactoid oedema in rats when used in doses of $30 \text{ mg} \cdot \text{kg}^{-1}$, but this enhancement was not modified by heparin.

When the drugs were administered locally into rat paws, protamine ($4 \mu\text{g}$) and borate ($4 \mu\text{g}$) reduced the dextran response ($100 \mu\text{g}$), and heparin (20 IU) antagonised these effects. The result using protamine is shown in figure 3. Salicylate enhanced the local dextran reaction but heparin did not change this enhancement. The local oedema reactions produced in rat paws by histamine ($50 \mu\text{g}$) and 5-hydroxytryptamine ($1 \mu\text{g}$) were not modified by heparin, protamine, borate or salicylate. It is possible, therefore, that most of these effects on dextran are due to a modification of the release mechanisms of histamine and 5-hydroxytryptamine from tissue mast cells. The importance of the plasma membranes of these cells is again stressed.

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375

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