Biochemical Pharmacology, Vol. 28, pp. 3149–3150

0006-2952/79/1015-3149 $02.00/0

SHORT COMMUNICATION

Dextran-induced paw edema and 5-hydroxytryptamine release

(*Received* 13 *February* 1979; *accepted* 27 *April* 1979)

A single intraperitoneal or intravenous injection of dextran into rats produces an anaphylactoid reaction, including serious hyperemia and edema of the hind paws. An anaphylactoid reaction limited to the site of injection is also observed with a local injection of dextran into the paw or the abdominal skin of rats. The mechanism of the anaphylactoid reaction has been analyzed with the use of pharmacological agents. Both histamine and 5-hydroxytryptamine (5-HT) have been suggested as possible mediators of the increased vascular permeability of skin [1, 2] and of the paw edema [3–5] produced by local injection of dextran in rats.

Investigating the time course of release of biologically active substances during inflammation and edema formation could give useful information, by which the roles of these substances in inflammation might be clarified. Little is known about the time course of release of putative mediators in relation to the paw edema formation after local injection of dextran in rats. In our previous work [6], the highest concentration of histamine in the exudate collected from the swollen rat paw after injection of dextran was obtained in the initial stage before the peak of edema, and the concentration of histamine correlated with the severity of the edema. These findings suggested that histamine release might play a role in initiating paw edema by dextran. This short communication deals with the time course of 5-HT release in relation to the formation of paw edema by dextran.

Male Sprague–Dawley rats (Charles River Japan Inc., Atsugi, Japan), weighing 140–160 g, were used. Dextran was supplied by the Meito Sangyo Co., Nagoya, Japan. Histamine dihydrochloride and 5-hydroxytryptamine creatinine sulfate were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

In our previous experiment [6], three dextrans with average molecular weights of 28,000, 65,000 and 207,000 had about the same potency in producing paw edema after local injection. Therefore, dextran with an average molecular weight of 65,000 was employed in the present experiment. It was dissolved in 0.9% saline in concentrations of 0.25, 1.0 and 4.0% (w/v), and 0.05 ml of the solution was injected into the subplantar region of one hind paw. The contralateral paw was injected with physiological saline alone as a control. Before any injections, the paw volume was measured by a volume differential method. At various periods after the injection, changes in paw volume were determined and the animals were decapitated. Incisions about 1 cm long were made immediately on the dorsal and ventral skin of both hind paws. Without squeezing, the exudate was collected with hematocrit capillary tubes (Propper Manufacturing Co., Inc., Long Island, NY). The subcutaneous space of the contralateral paw was washed with saline of nearly the same volume as that of the exudate collected. The degree of paw edema is expressed as the percentage increase relative to the initial paw volume. Samples of the exudate or washings from three rats were pooled. 5-HT was determined by the extraction procedure of Snyder *et al.* [7] and the fluorometric procedure of Maickel and Miller [8]. In some experiments, the contents of 5-HT and histamine in the paw skin of the intact animals were measured by the method described above and by that of Shore *et al.* [9] respectively. The concentrations of 5-HT in the exudate is expressed as ng/ml. The total amount of 5-HT in the exudate (ng) was calculated by multiplying the concentration of 5-HT in the exudate (ng/ml) by the increase in paw volume (ml). The contents of 5-HT and histamine in the paw skin are expressed as μg/g of wet tissue. Values of the amines are expressed in terms of the free base.

The time course of edema formation and the 5-HT levels in the exudate are summarized in Fig. 1. Dextran of molecular weight 65,000 produced paw edema, the degree of which depended on the concentration [between 0.25 and 4.0% (w/v)]. The maximal response to the dextran was obtained 30–60 min after injection. The highest concentrations of 5-HT in the exudate of the paw treated with dextran were 109 ng/ml for 0.25%, 149 ng/ml for 1.0% and 207 ng/ml for 4.0% dextran; these values were obtained 15 min after injection, among the four time points tested, namely, 15, 30, 60 and 120 min. At this time, the edema had not reached its peak.

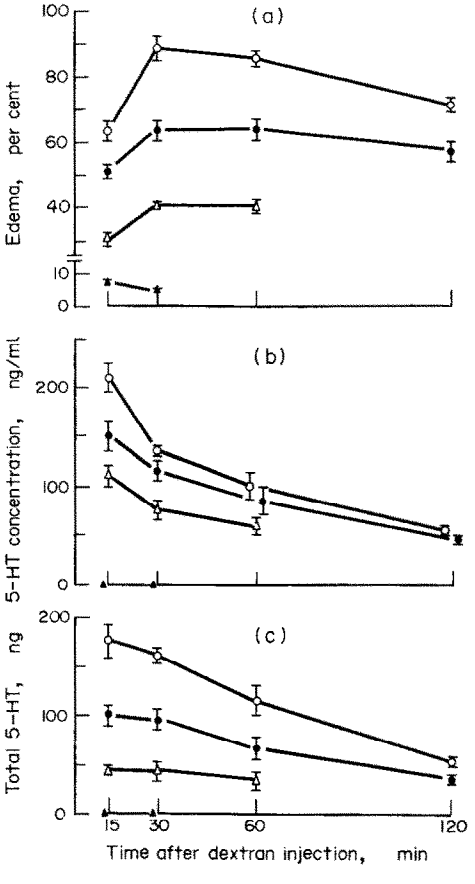

Fig. 1. Time course of paw edema (A), concentration of 5-HT (B) and total amount of 5-HT (C) in the exudate collected from the rat paw after local injection of 0.25 (△), 1.0 (●) and 4.0% (○) dextran, and in the washings from the saline-injected paw (▲). Each point is the mean value of five to seven samples, and the vertical bars indicate S.E.M. Paw volume before injection was 1.33 ± 0.01 ml.

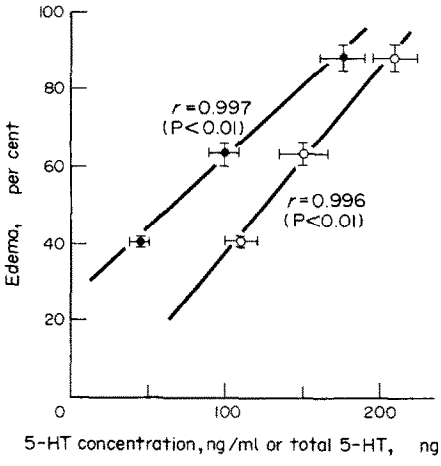

Fig. 2. Correlation between the concentration (○) or total amount (●) of 5-HT in the exudate collected from the paw 15 min after injection and the degree of paw edema 30 min after local injection of dextran dissolved in concentrations of 0.25, 1.0 and 4.0%. The lines shown were obtained by the method of least squares. Each point is the mean value of five to seven samples, and the bars indicate S.E.M.; r indicates correlation coefficient.

When the edema was at its peak, at 30–60 min, no further increases of concentration and total amount of 5-HT in the exudate were observed. Only a trace of 5-HT was detected in the washings of the contralateral paw. Figure 2 shows a significant correlation between the concentration or total amount of 5-HT in the 15-min exudate and the degree of the 30-min edema, after injection of dextran. The time course of 5-HT release was similar to that of histamine release reported previously [6].

It is improbable that the amount of 5-HT recovered from the inflamed paw in this experiment is the total amount of 5-HT released into the inflammatory locus, because of the disappearance of the 5-HT from the inflammatory site by enzymatic inactivation [10] or by diffusion into the circulation. Since we do not know what factor is primarily involved in the disappearance, it is impossible to determine what proportion of the released 5-HT was recovered from the inflammatory site in this study.

In our experiment, the contents of 5-HT and histamine in the paw skin of the intact rat were 1.2 ± 0.1 μg/g ($n = 7$) and 45.8 ± 1.4 μg/g ($n = 6$) respectively. These values roughly agree with those reported by other authors [11–14]. In our previous work [6], the highest concentration of histamine detected in the exudate of the paw treated with 4.0% dextran of molecular weight 65,000 was about 6 μg/ml. Thus, the concentration of 5-HT in the inflammatory exudate was about $\frac{1}{30}$ that of histamine, and this concentration ratio of 5-HT to histamine was similar to that in the paw skin of the intact rat.

It has been shown that, when given exogenously, 5-HT is an exceedingly potent substance in increasing vascular permeability of the rat skin [15, 16] and in producing rat paw edema [3, 6, 16]. However, it cannot be concluded that 5-HT alone is responsible for the development of edema produced by dextran, since it has been recognized that one substance alone does not account for all the changes observed in inflammation but that several mediators may be involved [17–19]. Histamine has already been suggested as a possible mediator of the acute inflammation produced by dextran [1–6], whereas controversy continues as to whether kinins are involved in edema formation by dextran [20] or not involved [21, 22]. In addition, the possibility must be considered that prostaglandins may be involved in edema formation, since some of them may interact with other putative mediators of inflammation [23–26].

In summary, local injection of dextran produced paw edema, the degree of which depended on the dose of dextran. The highest concentration of 5-HT in the exudate after injection was obtained in the initial stage, before the peak of edema. Once edema was fully developed, no further increase of 5-HT in the exudate was observed. The concentration of 5-HT in the exudate did correlate with the severity of the edema. The peak of the amine concentration occurring prior to that of edema suggests that release of 5-HT, as well as histamine, is one of the causes, rather than a result, of the edema formation. Both amines may play a role in initiating paw edema by dextran.

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