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THE IMPORTANCE OF PLASMA KININS IN THE ANAPHYLACTOID REACTION IN RATS

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A single injection of dextran into rats produces an acute inflammatory response termed the anaphylactoid reaction, which is characterized by hyperaemia, gross peripheral oedema and pruritus. Earlier workers (Léger, Masson & Prado, 1947; Halpern & Briot, 1954) considered that this reaction was mediated by histamine, but later work has shown that 5-hydroxytryptamine (5-HT) is also important in the mediation of this type of inflammatory response (Rowley & Benditt, 1956; Parratt & West, 1957). There is evidence to suggest that substances other than these two biogenic amines are involved in the anaphylactoid reaction. For example, the intravenous injection of relatively large doses of histamine (Feldberg & Talesnik, 1953) or of 5-HT (Bois & Selye, 1956) never fully reproduces all of the symptoms of a typical anaphylactoid reaction.

The participation of the plasma kinin system in the anaphylactoid reaction has previously been investigated. On the one hand, Lecomte (1964) found that the plasma kininogen content of rats injected intravenously with dextran was not decreased, whereas other workers reported that the plasma level of the kinin precursor after the intraperitoneal administration of dextran was lowered (Scharnagel, Greeff, Lühr & Strobach, 1965; Greeff, Scharnagel, Lühr & Strobach, 1966). In the present study, estimates of the parameters of the plasma kinin system during the anaphylactoid reaction, as well as the effects of anaphylactoid-inducing agents on the plasma kinin system *in vitro* have been studied.

METHODS

Source of animals

Male or female Wistar albino rats (body weight 150–200 g) obtained from the Agricultural Research Council's Field Station (Compton) were injected with single intraperitoneal doses of 240 mg/kg dextran (Intradex, Glaxo) once a week on three occasions. Animals showing peripheral oedema during the 4 hr after an injection were classed as "reactors," and those animals failing to react to all three injections were classed as "non-reactors." Rats tested in this way were used subsequently after a period of time of not less than one week. The anaphylactoid reaction was then induced by injecting dextran (40–240 mg/kg) intravenously or intraperitoneally and tests made as described later.

In some experiments, male or female Wistar albino rats (150–200 g) obtained either from Fison's Ltd. (Holmes Chapel) or from the Wellcome Laboratories (Beckenham) were used. Animals obtained from these sources were always found to be reactors.

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Coaxial hind-paw perfusion

Reactor and non-reactor rats were anaesthetized with pentobarbitone sodium (45 mg/kg) and one hind-paw of each rat was perfused according to the method of Rocha e Silva & Antonio (1960). After washing the subcutaneous spaces of the paw with Tyrode solution at 37° C, the animals were injected intravenously with a dose of 240 mg/kg dextran and the perfusates were collected at a rate of 5–10 drops/min into polyethylene containers placed in ice. Swelling in the non-perfused paws served as a visual control of the anaphylactoid oedema.

Assay and characterization of kinins

The rat uterus was generally used for assaying kinins (Gaddum, Peart & Vogt, 1949). Virgin female rats were injected subcutaneously with 50 μg hexoestrol daily for three days, and then they were killed on the fourth day and both uterine horns were removed and suspended in 5 ml. organ baths containing either aerated Tyrode or de Jalon solutions at 30° C. Atropine sulphate (10^{-6} g base/ml.), mepyramine maleate (10^{-7} g base/ml.) and 2-bromolysergic acid diethylamine (BOL-148, 10^{-7} g base/ml.) were included in the bathing fluids to prevent responses from choline derivatives, histamine and 5-HT respectively. The contractions of the uteri were recorded on a smoked drum using an isotonic lever system, and were compared with those produced by synthetic bradykinin (BRS-640, Sandoz).

Further characterization of the kinin nature of perfusate and plasma samples was made on the isolated terminal guinea-pig ileum (which contracts to kinins) and on the isolated rat duodenum (which relaxes to kinins) set up in a similar manner to the rat uterus; these samples were also incubated with chymotrypsin (which destroys kinins) and with trypsin (which does not).

Estimation of plasma kinins

Blood samples were collected after cardiac puncture. The methods used to estimate the free kinin levels of whole blood and the plasma contents of kininogen, kinin-forming enzyme and kininase have been described in detail elsewhere (Dawson, Starr & West, 1966).

Paw perfusates were assayed for free kinin directly on the isolated rat uterus preparation, while the kininogen, kinin-forming and kininase contents were estimated using the methods described for plasma, using a sample volume of 1 ml.

Intradermal injections

Rats were lightly anaesthetized with ether and their abdominal hair was removed with electric clippers. Azovan blue dye (18 mg/kg) was injected intravenously and each rat then was given six intradermal injections (dose-volume 0.1 ml.), one of which was always isotonic saline (pH 7.4). After 30 min the rats were killed and the skin over the injection sites was carefully removed and pinned, with the outer surface downwards, on to a cork board. The extent of blueing at each lesion was estimated on a relative scale from 0 to +++. All drugs were dissolved in isotonic saline and the solutions adjusted to pH 7.4 before injection.

In vitro *kinin formation*

Whole blood was transferred immediately after collection from reactor rats into polyethylene tubes and centrifuged. Samples of 1 ml. plasma were placed in polyethylene tubes containing 0.1 ml. phenanthroline hydrochloride (10 mg/ml.), an inhibitor of the kininase enzyme. The substance under test, dissolved in 0.1 ml. isotonic saline and adjusted to pH 7, was then added. The mixtures were incubated at 37° C and aliquots were removed every 4 min and assayed for kinin activity. Occasionally, samples of 1 ml. whole blood were used instead of the plasma.

Blood pressure recordings

Reactor rats were anaesthetized with pentobarbitone sodium (45 mg/kg) and 100 u. heparin (Evans Medical) were injected intravenously *via* a tail vein. A carotid artery was cannulated and the arterial blood pressure was recorded on a smoked drum using a Condon mercury manometer. All drugs were dissolved in isotonic saline and were injected *via* a cannula in a femoral vein in a dose-volume not exceeding 0.2 ml. Each injection was washed in with 0.05 ml. isotonic saline.

RESULTS

Effect of dextran on the kinin system in reactor rats

The anaphylactoid oedema was fully developed in reactor rats 30 min after the intravenous injection and 90 min after the intraperitoneal injection of dextran. Estimates of the circulating levels of kininogen, kinin-forming enzyme, kininase and free kinin were made at these times respectively, after the injection of a wide range of doses of dextran. Only small amounts of free kinin were present in the blood of control rats and the level of this peptide was not elevated at the height of the anaphylactoid oedema, produced either by low (40 mg/kg) or high (240 mg/kg) doses of dextran. Of the other kinin parameters measured, the kinin-forming and destroying enzymes were the most variable; however, in none of the experiments were the changes statistically significant.

The same four parameters of the kinin system were also measured at different times during the development of the anaphylactoid reaction, after the intravenous administration of a fixed dose of dextran (240 mg/kg). The kinin-destroying activity of the plasma was somewhat variable, while small increases in plasma kininogen and kinin-forming enzyme were apparent at 15 min after the injection of the dextran (Fig. 1). However, these returned to control levels by 60 min, although a gross oedema persisted for several hours.

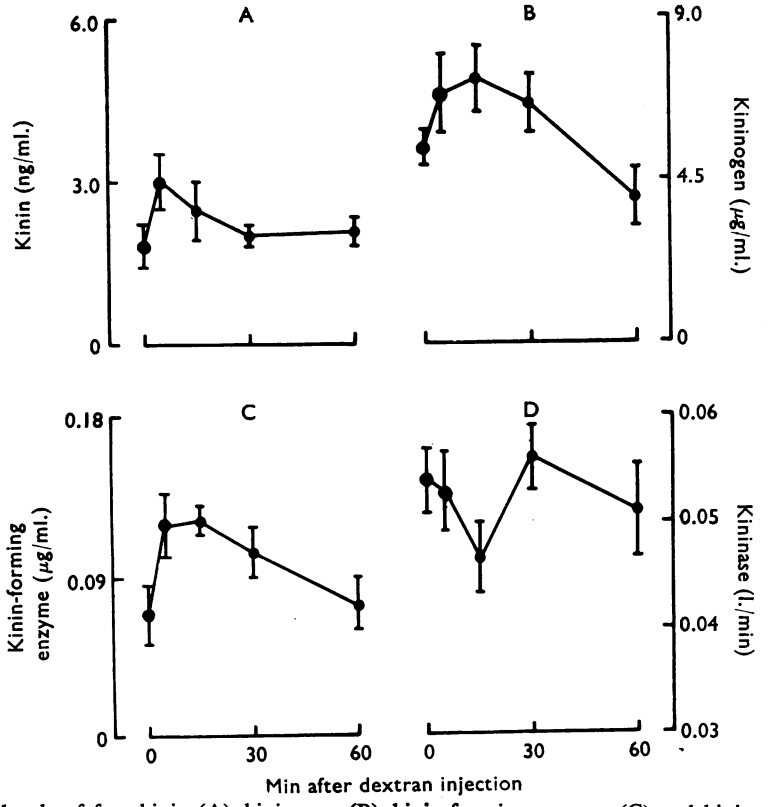

Fig. 1. The levels of free kinin (A), kininogen (B), kinin-forming enzyme (C) and kininase (D) in the blood of reactor rats at different times after receiving an intravenous injection of dextran (240 mg/kg). Each result (±S.E.) represents the mean of six experiments.

Perfusion of hind-paws

The local release of mediators into the subcutaneous tissue spaces of the hind-paws of both reactor and non-reactor rats was measured during a 90 min period after the intravenous injection of 240 mg/kg dextran. After this time the non-perfused paws of reactor rats were oedematous, while those of non-reactor rats were similar to the untreated controls. Initially, there were easily detectable amounts of free kinin in the perfusates collected from both treated and untreated reactor and non-reactor rats, but as the perfusion proceeded these amounts gradually decreased. In a similar manner, the kininogen and the kinin-forming enzyme contents also declined during the period of the paw perfusion. A few determinations of the concentration of kininase in the perfusates showed that this enzyme was present in small quantities throughout the whole of the experimental period. Some of the perfusates were also assayed for histamine and 5-HT; increased amounts of both these amines were released after the injection of dextran, more especially from the perfused hind-paws of reactor rats.

In a few experiments, reactor rats were injected with dextran and the oedema fluid in the swollen paws was collected directly into cooled polyethylene syringes and assayed immediately for kinin-like activity. Small amounts of a bradykinin-like substance were usually found to be present, but they did not correlate with the severity of the oedema.

TABLE 1

THE AMOUNT OF KININ FORMED IN REACTOR RAT PLASMA DURING INCUBATION WITH SOME ANAPHYLACTOID-INDUCING AGENTS IN THE PRESENCE OF A KININASE INHIBITOR (PHENANTHROLINE HYDROCHLORIDE)

Using the rat uterus as the test organ, the delay in the onset of the contraction to synthetic bradykinn and to the incubates was 20–45 sec. Each result is the mean of six determinations

Test substance	Concentration (/ml. plasma)	Incubation time (min)	Maximum kinin activity (ng)
Glass ballotini	0·1 g	1	2040
Dextran sulphate	0·6 mg	32	196
Dextran	6·0 mg	40	0
Dextrin	10·0 mg	40	0
Ovomucoid trypsin inhibitor	6·0 mg	40	0
Saline control	0·1 ml.	40	0

In vitro *activation of plasma kallikreinogen*

Of the substances tested for their ability to activate the intrinsic kinin-forming enzyme system in rat plasma only glass ballotini and dextran sulphate were found to be effective (Table 1). Glass activation was very rapid and usually complete within 1 min, while dextran sulphate worked more slowly, over a period of 32 min, and released only about one-tenth the amount of kinin as did glass. Higher concentrations of dextran sulphate or longer periods of incubation did not cause greater amounts of kinin to be released. An important finding was that, although dextran, dextrin and ovomucoid trypsin inhibitor all induced an anaphylactoid reaction when they were injected into reactor rats, they did not initiate a release of kinins over a period of 40 min when they were incubated with plasma from reactor rats in similar concentrations *in vitro*. When aliquots from these incubates, and from those containing saline, were left in contact with the test organ for longer than one minute, the plasma kinin system was activated

by dilution (Schachter, 1956) and a kinin activity developed which usually corresponded to about 60 ng/ml. None of the substances contracted the isolated test organ by itself and they did not affect the responses of the muscle to added bradykinin. Similar results were obtained when these experiments were repeated using whole blood.

In vivo *studies*

When dextran, dextrin and ovomucoid trypsin inhibitor were injected intravenously into reactor rats, in a dose of 40 mg/kg, they all elicited a generalized anaphylactoid reaction (Table 2). These three compounds also markedly increased the vascular permeability in the skin in these animals, as judged by the extravasation and accumulation of circulating blue dye at the site of their intradermal injection (60 μg). In contrast, intravenously or topically administered dextran sulphate was completely inactive in either respect, even when doses as high as 20 times those of the above agents were employed.

TABLE 2

THE INCREASE IN VASCULAR PERMEABILITY PRODUCED BY THE INTRAVENOUS OR THE INTRADERMAL ADMINISTRATION OF VARIOUS AGENTS INTO REACTOR RATS

The generalized oedema and the local blueing responses are recorded on a relative shock score from 0 to +++

Test substance	Intravenous dose (mg/kg)	Shock score	Intradermal dose (μg)	Shock score
Dextran sulphate	900	0	600	0
Dextran	40	+++	60	++
Dextrin	40	+++	60	++
Ovomucoid trypsin inhibitor	40	+++	60	++

Other experiments showed that the sensitivity of reactor and non-reactor rats to intradermally injected synthetic bradykinin was similar, the threshold for permeability-increasing activity being about 25 ng in each case.

The effect of dextran on rat blood pressure

In reactor rats, intravenous dextran (40 mg/kg) lowered the blood pressure after a short delay lasting about 5 min (Fig. 2A). The effect usually reached a maximum after 15–20 min, when a fall in the initial blood pressure of 40–80% was recorded. At this point, the blood pressure often recovered slightly, although a pronounced hypotension was still maintained for several hours. Sometimes a small hypertensive response preceded the fall in blood pressure, and this was always found to be greater than that expected from the volume effect of the injection alone. Similar injections of dextran into non-reactor rats never produced hypotension, even in a dose as high as 900 mg/kg.

The vasodepressor action of histamine, and to some extent of dextran, was blocked by intraperitoneal pretreatement with mepyramine (10 mg/kg), while the responses to 5-HT and bradykinin were not modified (Fig. 2B). On the other hand, the hypotensive responses obtained in reactor rats to dextran and 5-HT were abolished by similar pretreatment with BOL (3 mg/kg), whereas the responses obtained with bradykinin and histamine were only slightly affected (Fig. 2C). In other experiments, rats rendered refractory to the hypotensive action of bradykinin, by the repeated intravenous injection of large doses of this peptide, still responded to dextran with a typical fall in blood pressure.

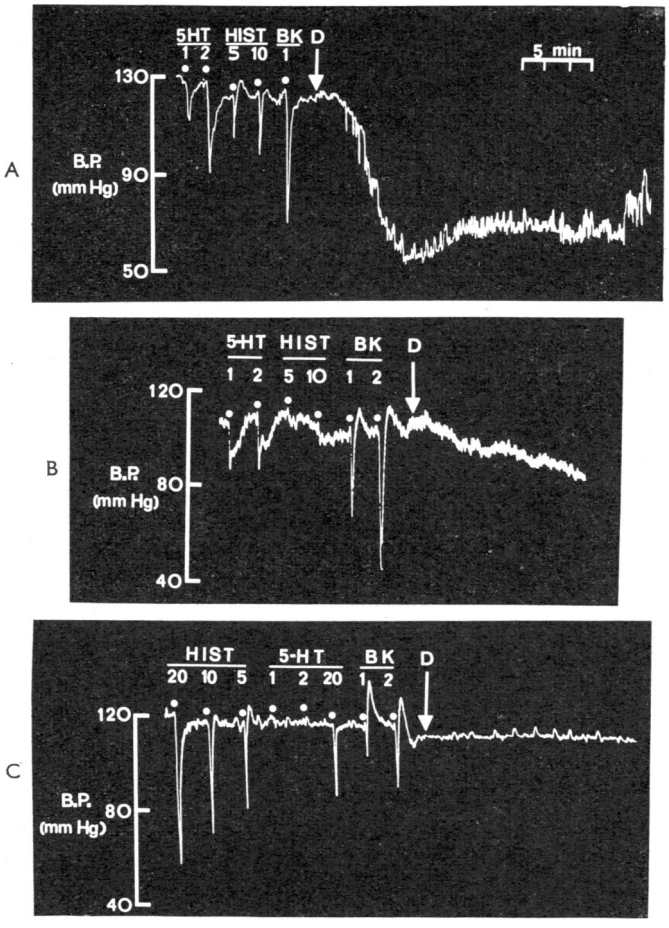

Fig. 2. Blood pressure recordings made from Wistar albino reactor rats anaesthetized with pentobarbitone sodium (45 mg/kg). Responses are shown for the intravenous injection of microgram doses of 5-hydroxytryptamine (5-HT), histamine (HIST), bradykinin (BK) and dextran (D, 40 mg/kg), after intraperitoneal pretreatment with 1 ml. isotonic saline (A), 10 mg/kg mepyramine (B) or 3 mg/kg BOL (C).

These results therefore indicate that bradykinin, or a related compound, is probably not a mediator of the anaphylactoid hypotension, while histamine and more especially 5-HT are more important in this respect.

DISCUSSION

The present findings support the view that kinin-like polypeptides are not of primary importance for the initiation or for the subsequent maintenance of the dextran anaphylactoid reaction in rats. Furthermore, although substances such as dextran, dextrin and ovomucoid trypsin inhibitor increase local vascular permeability and elicit a generalized anaphylactoid reaction in the intact animal, these effects cannot be explained on the grounds of kinin release.

Experiments *in vivo* show that, at the height of the response to dextran, no significant changes occur in the main parameters of the kinin system in reactor rats which are consistent with a formation and release of active kinins into the general circulation. As free kinins are rapidly destroyed in rat plasma (Fasciolo & Halvorsen, 1964), a decrease in the level of the kinin precursor in plasma after dextran is therefore a better indication of kinin release. No such decrease was found, and so kinin participation in the reaction is considered unlikely. The further possibility that kinin release is confined to the swollen areas, without registering any changes in the circulating kinin parameters, may be discounted by the results obtained from the local perfusion experiments.

Dextran is known to release both histamine and 5-HT in reactor rats but not in non-reactor rats (Luscombe & Harris, 1965). Kinin formation may therefore occur secondary to the release of these two biogenic amines, as histamine (Edery & Lewis, 1963) and 5-HT (Bonta & de Vos, 1965) have been reported to promote the release of kinin-forming enzyme and the formation of kinin respectively. Thus the passage of kinin precursors into the inflamed regions may be augmented by the influence of these "primary" mediators, and kinin activation might then occur through the dilution of these precursors in oedema fluid or by their contact with foreign tissue surfaces. This proposition allows for the fact that reactor and non-reactor rats are equally sensitive to systematically and topically administered bradykinin, and that the intrinsic plasma kinin systems in both types of rat are similar (Starr & West, 1967). However, no evidence has been found to support such a role for plasma kinins.

The present results agree with those of Lecomte (1964), who excluded the participation of kinins in the mechanism of dextran hypotension in rats. Although large intravenous doses of bradykinin markedly lower the mean arterial blood pressure of rats the effect is comparatively short-lasting and does not result in the formation of peripheral oedema. Similarly, the fact that the hypotensive responses to intravenous dextran can be modified by pretreatment with BOL or mepyramine without affecting those to injected bradykinin, together with the unaltered efficacy of dextran in rats made refractory to this peptide, is further evidence that kinin involvement is absent.

Additional studies *in vitro* indicate that dextran, as well as dextrin and ovomucoid trypsin inhibitor, has no kinin-releasing activity on crude kininogen substrates prepared from rat plasma, and does not activate rat plasma kallikreinogen or potentiate the kininogenase activity of rat plasma kallikrein. Also, whereas the intradermal and intravenous effects of these three agents can be suppressed by pretreatment with BOL, glucose or 2-deoxyglucose (Ankier, unpublished results), none of these inhibitors affects the release of kinins by active kallikreins from kininogen substrates. Furthermore, an effect of dextran on the plasminogen-plasmin system in blood, and hence a release of kinins through a liberation of active plasma kallikrein from its plasma pro-enzyme (Vogt, 1964), also seems unlikely. Although epsilon-aminocaproic acid inhibits both plasminogen activation (Sherry, Fletcher, Alkjaersig & Sawyer, 1959) and the vasodepressor action of dextran in rats (Lecomte, 1964), other investigators have previously failed to demonstrate an effect of dextran on the fibrinolytic system in blood (Levy & de Vaillancourt, 1960; Lecomte & Salmon, 1962). Finally, it is interesting to note that an allied substance such as dextran sulphate, as well as heparin, accelerates kinin formation *in vitro* (Armstrong & Stewart, 1962), and yet does not induce an increase in vascular permeability *in vivo*.

SUMMARY

1. A detailed investigation into the role of kinins in the dextran anaphylactoid reaction in rats has been carried out.

2. The circulating levels of free kinin, kininogen and kinin-forming and destroying enzymes were measured after the injection of dextran and found to remain unchanged.

3. Subcutaneous perfusions of the paws of rats, pretreated intravenously with dextran, showed that a local formation and release of kinins does not occur during the anaphylactoid reaction.

4. Dextran markedly lowered the blood pressure in rats and this effect was partly modified by pretreatment with mepyramine or BOL, without altering the responses to injected bradykinin. Rats made refractory to intravenously injected bradykinin still responded to dextran with a typical hypotension.

5. Intravenously or intradermally administered dextran, dextrin and ovomucoid trypsin inhibitor elicited in rats a generalized anaphylactoid reaction and an increase in vascular permeability respectively; *in vitro*, none of these agents elicited a release of kinins. In contrast, dextran sulphate did not increase vascular permeability *in vivo* but induced kinin release from plasma *in vitro*.

6. Kinins are therefore not considered to contribute in any way to the reaction elicited by dextran in rats.

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