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INHIBITION OF ANAPHYLACTIC SHOCK IN THE RAT BY ANTIHISTAMINES AND ASCORBIC ACID

BY

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Beraldo (1950) found that plasma levels of a kinin-like substance were raised during anaphylactic shock in dogs but he concluded that the substance did not play an important role in this type of shock. Later, Brocklehurst & Lahiri (1962) reported that the levels of plasma bradykinin in the rat, rabbit and guinea-pig were elevated after anaphylactic shock; these authors postulated that, in these three species, bradykinin was involved in the anaphylactic syndrome. The results of preliminary experiments (Dawson & West, 1965a) indicated that the intravenous toxicity of bradykinin is markedly increased at the times when the sensitized rat is most sensitive to the specific antigen. Furthermore, recent work (Dawson & West, 1965b) showed that the concomitant administration of ascorbic acid and mepyramine (a specific antihistamine drug) gives marked protection against anaphylactic shock in the rat.

We have first examined the kinin formation and destruction during anaphylactic shock in the rat, and then studied the effect of some antagonists on these kinin systems.

METHODS

Groups of 10 Wistar albino rats (body weight 150–200 g) from Fisons Ltd. (Holmes Chapel) were sensitized with horse serum (0.5 ml. intraperitoneally) with the aid of an adjuvant (*Bordetella pertussis* vaccine, 0.25 ml. containing $10{,}000\times10^6$ organisms). They were then either challenged intravenously at various times after sensitization with 1 ml. of the specific antigen or injected with bradykinin (2 mg/kg), and mortality rates were recorded over the next 24 hr. Other groups of sensitized animals were challenged with the antigen and 5 min later under light ether anaesthesia blood samples were taken by cardiac puncture into heparinized polythene syringes for estimating some of the factors concerned with bradykinin formation and inactivation, as described below. Control experiments were made after injecting other sensitized rats with physiological saline and other groups of untreated rats with horse serum. The effect of pretreating sensitized rats 30 min before challenge either with an intraperitoneal dose of ascorbic acid (200 mg/kg) or mepyramine (10 mg/kg) or both or with an oral dose of acetylsalicylic acid (1,000 mg/kg), was also studied. In a few experiments, crude egg albumin (B.D.H. Ltd.) was used as antigen in place of the horse serum; a dose of 100 mg/kg (together with *B. pertussis* vaccine) was injected intraperitoneally for sensitization and the challenging intravenous dose was also 100 mg/kg. All experiments were made during the months October to March, at a time when sensitivity of Wistar rats to anapyhlactic shock is maximal (Ankier, Dawson, Karady & West, 1965).

Bradykinin content. The method used was that described by Allwood & Lewis (1964). Samples of 1 ml. whole blood were transferred immediately after collection in polythene syringes to polythene tubes containing 4 ml. ice-cold absolute ethanol. After centrifugation, the supernatants were poured off into polythene petri dishes and evaporated to dryness under reduced pressure at 37° C. The residues were reconstituted with physiological saline (usually 1 ml.) and assayed directly for bradykinin activity on the isolated uterus of a rat previously injected subcutaneously with 50 μg hexoestrol. A 5 ml. bath at 30° C was used with Tyrode solution containing atropine (10^{-6}), mepyramine (10^{-7}) and 2-bromo-lysergic acid diethylamide (10^{-7}). The standard solution was a dilution of synthetic peptide secured from Parke, Davis & Co. Ltd. Specificity of the response was confirmed on the rat duodenum preparation (which relaxes to bradykinin) and by incubation with chymotrypsin (which rapidly hydrolyses bradykinin). Using this method, recovery of added bradykinin 10–80 ng) was $89.0 \pm 7.4\%$. The reconstituted residues were found to be devoid of kinin-forming enzyme and kininase activities.

Bradykininogen content. The method used was a modification of that described by Diniz, Carvalho, Ryan & Rocha e Silva (1961). Whole blood was transferred immediately after collection in polythene syringes to polythene tubes and centrifuged. Samples of 0.2 ml. plasma were added to tubes containing 1.8 ml. acetic acid (0.2% w/v) and heated in a boiling water-bath for 30 min to inactivate kinin-forming enzyme, kininase, and trypsin inhibitor contained in the plasma. After cooling, the samples were neutralized with sodium hydroxide (0.6 N) and buffered to *p*H 7.8 with Tris buffer (0.5 ml.) Then 200 μg crystalline trypsin (1 mg/ml.) was added to each sample. After incubation at 37° C for 30 min, the reaction was stopped by placing the tubes in ice and the bradykinin in the incubates was assayed. The bradykininogen content of the original plasma sample was expressed as the amount of bradykinin formed (μg/ml. plasma). When trypsin was omitted from the incubation mixtures no bradykinin was detected on assay.

Kinin-forming enzyme activity. A modification of the method described by Amundsen, Nustad & Waaler (1963) was used to measure the activation of plasma kallikreinogen. Whole blood of rats was transferred immediately after collection in polythene syringes to polythene tubes and centrifuged. Samples of 0.2 ml. plasma were added to polythene tubes containing 0.2 ml. rabbit plasma which had been previously heated in a boiling water-bath for 30 min with 1.8 ml. acetic acid (0.2% w/v), cooled, neutralized, and buffered to *p*H 7.8 with 0.5 ml. Tris buffer. Phenanthroline hydrochloride (100 μg) was then added to each tube to inhibit specifically the kininase (Vogt, personal communication). After incubation at 37° C for 30 min, the reaction was stopped by placing the tubes in ice, and the bradykinin in the incubates was then assayed. The kinin-forming enzyme activity of the original rat plasma was expressed as the amount of bradykinin formed (μg/ml.) plasma). When the prepared rabbit plasma substrate was omitted from the incubation mixture, no bradykinin was detected on assay. Experiments were also carried out using as substrate samples of control rat plasma which had been previously heated either in a boiling water-bath for 30 min with acetic acid or at 60° C for 1 hr without acetic acid. The results using rat plasma as substrate were similar to those obtained using rabbit plasma.

Kininase activity. The method used was similar to that of Edery & Lewis (1962) in which a fixed amount of standard bradykinin is added to a known volume of plasma and the minimal time taken to destroy all the bradykinin is measured. Whole blood was transferred immediately after collection in polythene syringes to polythene tubes and centrifuged. Samples of 0.1 ml. plasma were added to polythene tubes containing 5 μg synthetic bradykinin in 3.9 ml. physiological saline, and incubated at 37° C. At intervals of 5 min, 0.02 ml. aliquots were removed and assayed for bradykinin. Kininase activity of the original rat plasma was expressed in terms of the reciprocal of the time taken to inactivate the added bradykinin. When the synthetic bradykinin was omitted from the incubation mixture no bradykinin was detected on assay. When phenanthroline (100 μg) was included in the incubation mixture the added bradykinin was not destroyed.

RESULTS

Sensitivity to antigen. Maximal sensitivity to horse serum occurred 10 days after sensitization and all rats died when challenged with antigen on that day. At 20 days after sensitization, the mortality rate on challenge was 80% whereas at 40 days none of the animals died when the specific antigen was administered. These results are shown in Table 1 and Fig. 1. In the rats which died within 20 min of challenge, there was always haemoconcentration and haemorrhage in the heart and small intestine, with occasional damage in the lungs; when deaths occurred later, haemorrhage in the small intestine was always excessive. When examined microscopically, the cardiac damage was always found in the right ventricle where separation of the muscle fibres occurred, with gross extravasation and vacuolization of the tissue. This finding is similar to that reported earlier by Malkiel & Hargis (1952). The electrocardiogram showed an increased and prolonged T-wave, probably as a result of impairment of conduction; just before death auricular fibrillation was prominent. The microscopical changes in the small intestine were also indicative of extravasation of the submucosa, as reported by Sanyal & West (1958a). Deaths after sensitization and challenge with egg albumen occurred at similar times to those recorded with horse serum but marked congestion and haemorrhage were always noted in the lungs and small intestine, with only slight damage in the heart.

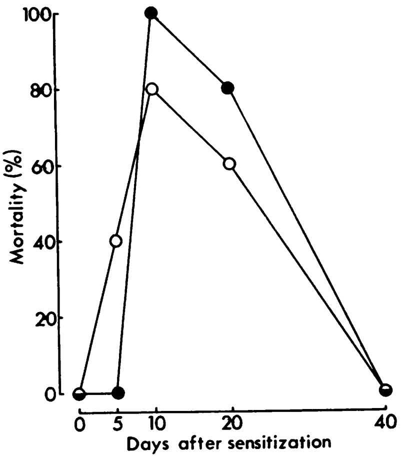

Fig. 1. Mortality rates of Wistar rats given intravenous injections of either specific antigen (●——●) or bradykinin (○——○) on different days after sensitization with horse serum and *B. pertussis* vaccine.

TABLE 1

MORTALITY RATES OF WISTAR RATS GIVEN INTRAVENOUS INJECTIONS OF THE SPECIFIC ANTIGEN OR BRADYKININ (2 MG/KG) AT DIFFERENT TIMES AFTER SENSITISATION WITH HORSE SERUM AND *B. PERTUSSIS* VACCINE

The results of pretreating the animals with ascorbic acid (200 mg/kg), mepyramine (10 mg/kg), or acetylsalicylic acid (1000 mg/kg) are also shown

Time after sensitisation (days)	Pretreatment	Mortality (%)	
		Antigen	Bradykinin
0	None	0	0
5	None	0	40
10	None	100	80
	Mepyramine	100	—
	Ascorbic acid	70	—
	Ascorbic acid and mepyramine	0	60
	Acetylsalicylic acid	100	—
20	None	80	60
	Ascorbic acid and mepyramine	80	—
	Acetylsalicylic acid	80	—
40	None	0	0

Whereas mepyramine failed to protect rats undergoing anaphylactic shock 10 days after sensitization (as found by Sanyal & West, 1958b), ascorbic acid afforded slight protection, only 70% of the animals dying. When ascorbic acid and mepyramine were given together at this time after sensitization, there was complete protection (see Table 1). There was also no haemoconcentration. However, ten days later (at 20 days after sensitization), the combination of ascorbic acid and mepyramine failed to protect rats undergoing anaphylactic shock. Acetylsalicylic acid exerted no protection against anaphylactic shock in rats receiving the specific challenge at 10 days after sensitization, although death was delayed beyond 2 hr ; there was less intestinal and heart damage than in control sensitized rats although the liver was engorged with blood. As with mepyramine and ascorbic acid, acetylsalicyclic acid afforded no protection when the challenge was made at 20 days after sensitization.

Sensitivity to bradykinin. In a group of rats not sensitized with horse serum, the intravenous injection of bradykinin (2 mg/kg) caused no deaths. Five days after sensitization, the mortality rate of rats receiving the standard dose of bradykinin was 40% (see Table 1 and Fig. 1). Deaths usually occurred within 30 min of the injection, slight haemorrhage showing in the lungs, heart and jejunum. Sensitivity of rats to the polypeptide reached high values 10 and 20 days after sensitization to antigen, the cause of death then being characteristic haemorrhage in the right ventricle, with less damage in

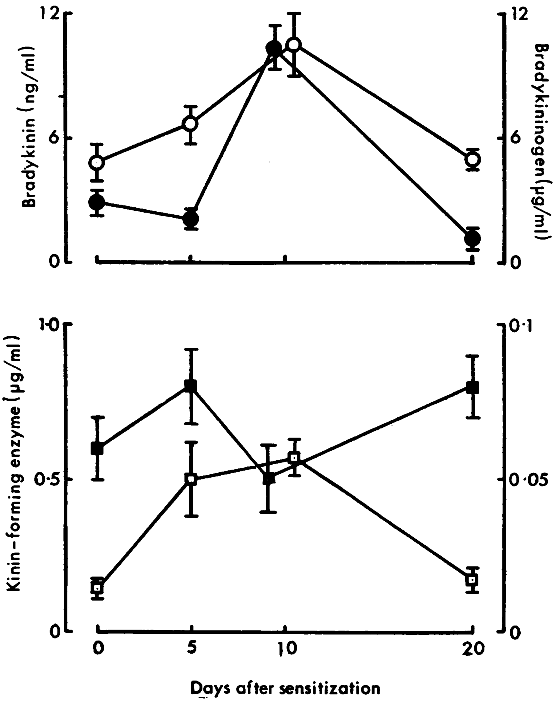

Fig. 2. Bradykinin (●——●), bradykininogen (○——○) kinin-forming enzyme (□——□), and kininase (■——■) levels in plasma of rats 5 minutes after challenge with the specific antigen on different days after sensitization with horse serum and *B. pertussis* vaccine. Standard errors of each value are shown by the vertical lines.

the lungs and jejunum at 10 days and much less haemorrhage in these tissues at 20 days. Microscopically, the cardiac ventricular damage was similar to that found in anaphylactic shock, with extravasation most prominent. Changes in the electrocardiogram were also similar to those obtained during anaphylaxis. The combination of ascorbic acid and mepyramine afforded slight protection against bradykinin shock at 10 days after sensitization, although the reduction in mortality rate was not statistically significant. There were no deaths when the rats were challenged with the standard dose of bradykinin at 40 days after sensitization.

Plasma bradykinin. The bradykinin level in the plasma of control rats was 2.9 ± 0.6 ng/ml. This value in sensitized rats 5 min after challenge with the specific antigen reached high levels when tests were made 10 days after sensitization; at this time, the increase was about 3-fold and was significantly different from the control value Fig. 2). At 20 days after sensitization (when the mortality rate was still as high as 80%), the bradykinin level was lower than that found at the time of sensitization.

Plasma bradykininogen. The bradykininogen level in the plasma of control rats was 4.9 ± 1.1 μg/ml. As with bradykinin, the peak value of the bradykininogen was recorded at 10 days after sensitization, although the increase was only about 50% (Fig. 2). At 20 days, the value was similar to that found before sensitization.

Kinin-forming enzyme. The level of this enzyme activity in the plasma of control rats was 0.14 ± 0.04 μg/ml. This increased about 3-fold when rats were tested at 10 days after sensitization. As with bradykinin and bradykininogen, the level at 20 days was similar to the control value (see Fig. 2).

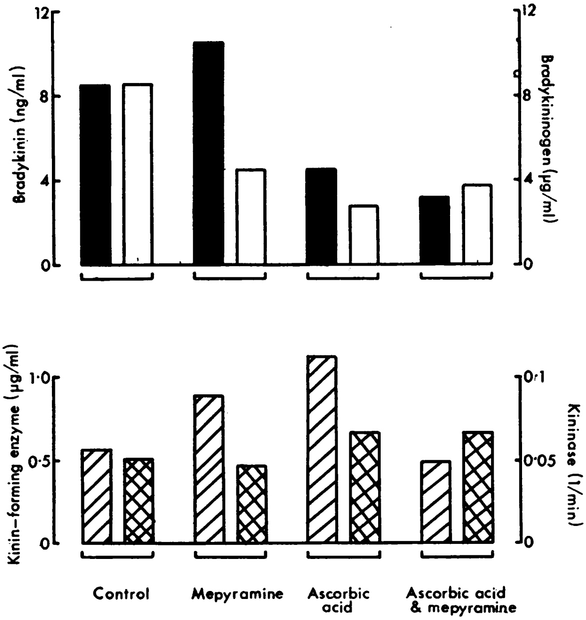

Fig. 3. The effect of various pretreatments on the bradykinin (■), bradykininogen (□), kinin-forming enzyme (▨), and kininase (▩) levels in plasma of rats 5 min after challenge with the specific antigen at 10 days after sensitization with horse serum and *B. pertussis* vaccine.

Plasma kininase. There was much variation in the level of kininase. A small insignificant increase was found at day 5, the value was lower at day 10, whilst the level was again slightly increased at 20 days (see Fig. 2).

Effect of ascorbic acid and mepyramine. The bradykinin and bradykininogen levels in the plasma of sensitized rats 5 min after challenge with the specific antigen at 10 days after sensitization were reduced by pretreatment with ascorbic acid (Fig. 3), but the level of the kinin-forming enzyme was raised. Mepyramine alone also produced a reduction in the bradykininogen and an increase in the kinin-forming enzyme activity. With the combination of ascorbic acid and mepyramine, there was a large reduction in the bradykinin and bradykininogen levels but the kinin-forming enzyme and kininase activities were unchanged. At 20 days after sensitization, pretreatment with the combination did not modify any of the values which were similar to those of control animals. None of the activities of the enzyme systems was modified by including ascorbic acid (10 mg/ml.), mepyramine (1 mg/ml.), a mixture of ascorbic acid and mepyramine, or acetylsalicylic acid (10 mg/ml.) in the *in vitro* incubation mixtures.

DISCUSSION

The results suggest that in anaphylaxis in the rat bradykinin is formed and released in large quantities. Histamine does not appear to play a role in this type of shock as the antihistamine drug, mepyramine, fails to protect the animal, and 5-hydroxytryptamine also may not be involved, although the toxicity of both compounds is greatly increased during this period of maximal sensitization (Sanyal & West, 1958).

In the present work, ten days after sensitization, the rats were completely protected from anaphylactic shock by pre-treating them with a combination of ascorbic acid and mepyramine, but not with either alone. Under ascorbic acid treatment, there was a slight reduction in toxicity, an increase in the activity of the kinin-forming enzyme, but a reduction in bradykinin and bradykininogen content. With mepyramine alone, there was no protection, and the kinin-forming enzyme and bradykinin content were increased although the bradykininogen was reduced. When both ascorbic acid and mepyramine were present, the full protection against anaphylactic shock was associated with reduced bradykinin levels, bradykininogen levels and kinin-forming enzyme activity. Nevertheless, the toxicity of bradykinin in sensitized animals was not markedly reduced by the combination of ascorbic acid and mepyramine. This result suggests that the important step in the protection is the reduction in the activity of the kinin-forming enzyme to below control values, and not the reduction in circulating bradykinin levels.

Ten days later (20 days after sensitization), this combination of ascorbic acid and mepyramine exerted no protection against anaphylactic shock; at this time, the levels of the kinin-forming enzyme and of the kinin and its precursor were not significantly different from those of control unsensitized rats. Thus it is inferred that bradykinin is not so important at this stage in anaphylaxis in the rat as it is at 10 days after sensitization. The post-mortem findings generally are in line with these conclusions. All rats of another strain (Sprague Dawley) also died on challenge at 10 days after sensitization but the mixture of ascorbic acid and mepyramine did not alter the sensitivity to antigen in these animals. Again, it may be that a factor other than bradykinin is involved in this reaction.

Lastly, acetylsalicylic acid, an antagonist of bradykinin bronchoconstriction in the guinea-pig, slightly modified anaphylactic shock in Wistar rats receiving the specific challenge at 10 days after sensitization, but had no effect at 20 days. This result adds further support for the involvement of bradykinin in at least one stage of anaphylaxis in the rat.

SUMMARY

1. The role of bradykinin in anaphylactic shock in rats has been examined.

2. At various times after sensitization to the specific antigen, the toxicities of specific antigen and of synthetic bradykinin are similar.

3. The plasma levels of bradykinin, bradykininogen, kinin-forming enzyme and kininase in sensitized rats after challenge with specific antigen have been followed. A peak in the first three of these parameters occurred at 10 days after sensitization.

4. Concomitant administration of ascorbic acid and mepyramine protected rats against anaphylactic shock at 10 days after sensitization, but gave no protection against anaphylaxis at 20 days ; the combination was also ineffective against bradykinin.

5. Ascorbic acid and mepyramine together reduced the values of bradykinin and bradykininogen but the kinin-forming enzyme activity remained unchanged at 10 days after sensitization.

6. It is proposed that there are two phases in anaphylaxis in the rat—an early phase in which bradykinin is a mediator and against which the mixture of ascorbic acid and mepyramine gives protection, and a late phase which does not involve bradykinin.

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