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Further evidence for the involvement of kinin in anaphylactic shock in the rat

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1. Sodium phenylbutazone, soya bean trypsin inhibitor, or the 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.

2. During anaphylactic shock in rats at 10 days after sensitization, the plasma bradykinin and bradykininogen levels, as well as those in the intestinal lumen and peritoneal cavity, were markedly raised.

3. The results support the hypothesis that there are two phases in anaphylaxis in the rat—an early phase in which bradykinin is a mediator and against which phenylbutazone or soya bean trypsin inhibitor or the mixture of ascorbic acid and mepyramine, give protection, and a late phase which does not involve bradykinin.

During anaphylaxis in the rat bradykinin is formed and released in large quantities (Dawson, Starr & West, 1966). Histamine does not appear to play a major role in this type of shock as the specific antihistamine drug, mepyramine, fails to protect the animal. 5-Hydroxytryptamine also may not be involved, although its toxicity like those of bradykinin and histamine is greatly increased during the period of maximal sensitization (Sanyal & West, 1958).

We have now examined the effects of another group of antagonists on the severity of anaphylactic shock in the rat, and then studied changes in the kinin and kininogen levels in certain body fluids and tissues during anaphylaxis.

Methods

Groups of ten or more male or female Wistar albino rats weighing 120–150 g were obtained from Fison's Ltd (Holmes Chapel). They were allowed free access to food and water.

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Production of anaphylactic shock

The rats were injected intraperitoneally with 0·5 ml. horse serum (recalcified, heated horse serum, Wellcome Laboratories) and 0·25 ml. *Bordetella pertussis* vaccine (10^8 organisms/ml.). At different times after this sensitizing dose the animals were injected intravenously with 1 ml. horse serum, and survival rates were recorded after the next 24 hr. Most of the experiments were carried out in the winter months when the sensitivity to antigen is highest (Ankier, Dawson, Karady & West, 1965). Drugs were tested for their inhibitory effect on anaphylactic shock by recording survival rates of groups of rats injected either intravenously or intraperitoneally with the compound under test at different times before the intravenous dose of antigen.

Kininogen content of plasma

Blood removed by cardiac puncture from rats under light ether anaesthesia at different times after anaphylactic shock induced 10 days after sensitization was centrifuged and the level of plasma kininogen was measured as previously described (Dawson *et al.*, 1966).

Kinin and kininogen contents of abdominal fluids

Antigen was injected intravenously into rats at different times after sensitization. Fifteen minutes after the injection of antigen an incision was made in the abdominal wall under light ether anaesthesia and the peritoneal cavity was washed with 5 ml. isotonic saline. The peritoneal washings were removed for assay and two ligatures were tied 3 cm apart, around an empty loop of ileum. The ligatured loop was then injected with 1 ml. isotonic saline which was quickly recovered and retained for assay. Polythene syringes and iced polycarbonate tubes were used throughout. The washings were assayed for free kinins on the isolated rat uterus and for kininogen, as stated for plasma.

Kininogen content of tissues

Antigen was injected intravenously into rats at different times after sensitization. They were killed 15 min after the injection by cervical dislocation and the thorax and abdomen were opened. The liver, small intestine, heart and lungs were removed and washed free of blood with isotonic saline. Each tissue was dried on filter paper and 100 mg samples were homogenized with 5 ml. ice-cold isotonic saline. After centrifugation, 1 ml. aliquots of the supernatants were heated with 1 ml. 0·2% (w/v) acetic acid in a boiling water bath for 30 min, cooled, and neutralized with 0·1 N sodium hydroxide. They were then buffered to pH 7·8 by the addition of 0·2 ml. of 0·2 M Tris buffer, and incubated at 37° C for 30 min with 0·1 mg crystalline trypsin. The kinins were purified by cation-exchange using 5 cm × 1 cm columns of Amberlite 1RC-50 (Oates, Melmon, Sjoerdsma, Gillespie & Mason, 1964) and assayed on the isolated rat uterus. The kininogen content of the original tissue was expressed as the amount of kinin formed (μg/g tissue).

Chemicals used

We should like to thank Dr H. O. J. Collier (Parke Davis) for the gift of the fenamic acids, and Dr Berde (Sandoz) for the ampoules of synthetic bradykinin and kallidin. We are also grateful to Geigy Pharmaceuticals, who kindly donated the phenylbutazone, and to Bayer Ltd., for the present of the Trasylol. Soya bean trypsin inhibitor was obtained from Koch Light & Co., Colnbrook.

Results

Inhibition of anaphylactic shock

In saline-treated control animals sensitized 10 days previously, shock developed in less than 10 min after intravenous antigen and the survival rate over the next 24 hr was only 15%. High doses of phenylbutazone, soya bean trypsin inhibitor, or a mixture of mepyramine and ascorbic acid afforded a highly significant protection ($P=<0{\cdot}001$) in these animals. The results are shown in Table 1. The fenamates, sodium salicylate, Trasylol and hexadimethrine (the last two are inhibitors of kallikrein, the kinin-forming enzyme) did not increase survival rates although they delayed the onset of symptoms and prolonged survival times (from a mean control value of 2·5 hr to 8–10 hr).

When tests were made 20 days after sensitization, the survival rate in saline-treated control animals was 37% and none of the twelve treatments listed in Table 1 modified the course of anaphylaxis.

At autopsy, gross haemorrhagic lesions were always present in the small intestine of animals sensitized 10 days before challenge.

Plasma kininogen

An increase of 80% in plasma kininogen was found 5 min after the intravenous injection of antigen into rats sensitized 10 days previously (Fig. 1), but the level was markedly decreased 55 min later.

Pretreatment with sodium phenylbutazone or a mixture of mepyramine and ascorbic acid significantly reduced the levels at all the times tested.

TABLE 1. *Effect of intraperitoneal injections of drugs on the survival rates of groups of sensitized rats injected with the specific antigen at* 10 *and* 20 *days after sensitization*

Drug	Dose (mg/kg)	Time of injection before intravenous antigen (min)	Survival rate %	
			10 days	20 days
Saline	—	5	15	37
Sodium phenylbutazone	100	60	72*	25
Sodium flufenamate	50	60	13	40
Sodium meclofenamate	50	60	25	40
Sodium mefenamate	50	60	25	30
Sodium salicylate	200	60	20	40
Calcium acetylsalicylate	500	60	0	50
Hexadimethrine bromide	10	60	20	30
Ascorbic acid	200	15	30	30
Mepyramine	10	30	0	20
Ascorbic acid and mepyramine	200 10	15 30	100*	20
Soya bean trypsin inhibitor†	20	60	20	40
Trasylol†	20 5,000 u.	5 5	70* 0	30 40

* $P=<0{\cdot}001$. †These were given intravenously.

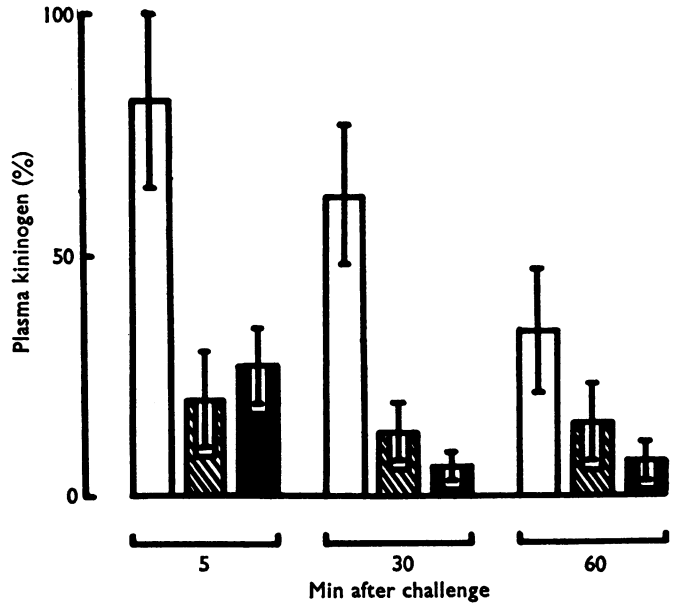

FIG. 1. Percentage increases in the level of plasma kininogen in 10 days sensitized rats at different times after intravenous challenge with antigen. Values shown are the means (±S.E.) of groups of rats pretreated with intraperitoneal doses of saline (open columns), mepyramine (10 mg/kg) and ascorbic acid (200 mg/kg) (hatched columns) or sodium phenylbutazone (100 mg/kg) (closed columns).

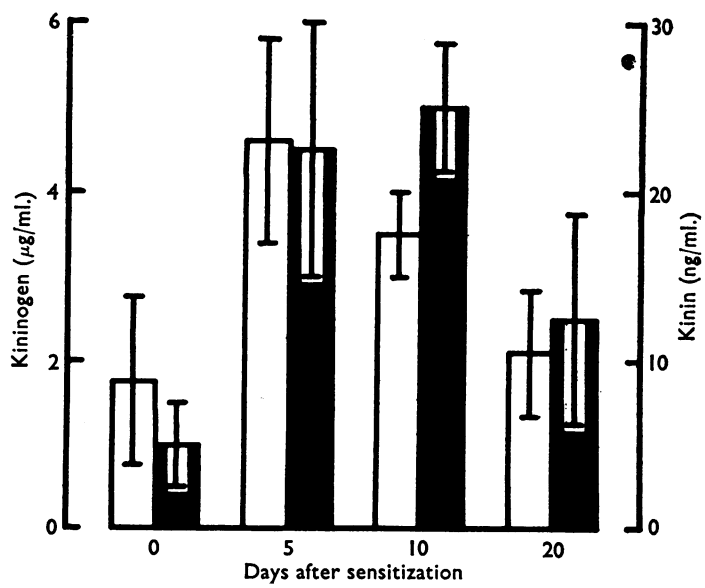

FIG. 2. Levels of kininogen (□) and kinin (■) in the washings collected from the peritoneal cavity of sensitized rats 15 min after intravenous challenge with antigen at different times of sensitization. Each histogram is the mean (±S.E.) of ten determinations.

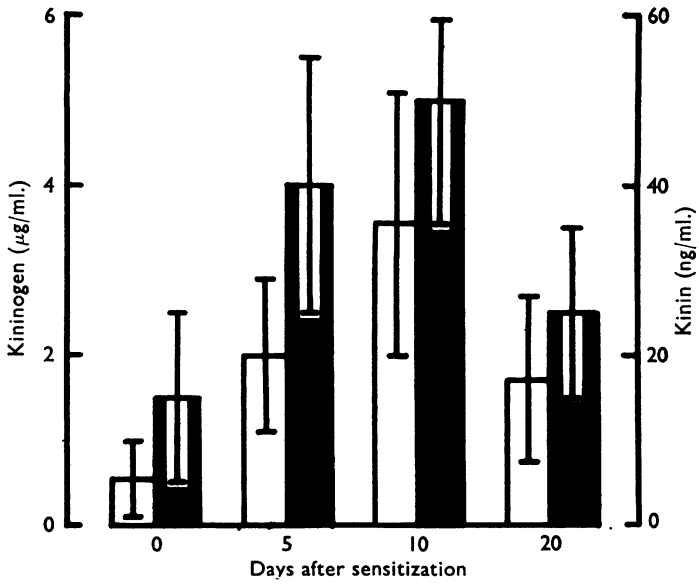

FIG. 3. Levels of kininogen (□) and kinin (■) in the washings collected from the intestinal lumen of sensitized rats 15 min after intravenous challenge with antigen at different times of sensitization. Each histogram is the mean (±S.E.) of ten determinations.

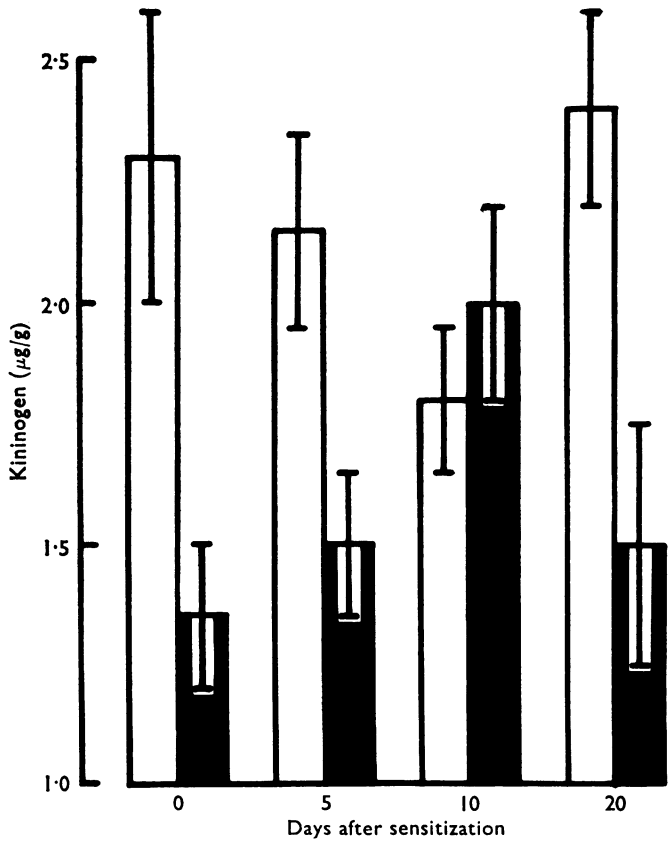

FIG. 4. Levels of kininogen in the liver (□) and intestinal wall (■) of sensitized rats 15 min after intravenous challenge with antigen on different days after sensitization. Each histogram is the mean (±S.E.) of six determinations.

Kinin and kininogen in the abdomen

Intestinal congestion and haemorrhage, two predominant features of anaphylaxis in rats, were accompanied by the passage of significantly increased amounts of both free kinin and kininogen into the peritoneal cavity and intestinal lumen of rats challenged as early as 5 days after sensitization and the increase was still apparent 10 days after sensitization (Figs 2 and 3). However, 20 days after sensitization the values of free kinin and kininogen in the two fluids were not significantly different from those of control animals. The concentrations of histamine and 5-hydroxytryptamine in these fluids were small and variable, the highest amounts being recorded during anaphylaxis induced 10 days after sensitization (about 20 ng/ml. of each).

Tissue kininogen

Figure 4 shows that the kininogen levels in the liver decreased whereas those in the intestine increased when anaphylaxis was induced in rats 10 days after sensitization. No such changes occurred when the estimations were made 20 days after sensitization. Kininogen levels in heart and lung tissue did not alter at any of the times studied.

Discussion

The results of the present experiments confirm those of previous findings that changes occur in the kinin system of the blood and tissues of rats undergoing active anaphylaxis when the intravenous challenge with antigen is administered 10 days (but not 20 days) after sensitization. Plasma kininogen levels as well as those in the peritoneal cavity and intestinal lumen washings are significantly raised. Whereas kininogen levels in the liver are reduced, those in the intestinal wall are increased. The target organ in rat anaphylaxis is the small intestine (Sanyal & West, 1958) where haemorrhagic lesions and sloughing of the mucosal wall are particularly prominent. These results support the hypothesis that there are two phases in anaphylaxis in the rat—an early phase in which a kinin is a mediator and a late phase which involves other mediators.

Sodium phenylbutazone was found to be one of the more active antagonists of anaphylaxis in the present experiments, being effective, however, only when the shock was induced at 10 days after sensitization. This antagonist has already been shown to reduce significantly the increase in capillary permeability produced in rats by intradermal bradykinin and to be effective even when given locally mixed with the kinin (Starr & West, 1967). Hence it is probably antagonizing the endogenously released bradykinin in rat anaphylaxis as well as reducing the amount of kininogen available for kinin formation. Experiments showed that plasma kininogen levels were markedly reduced by phenylbutazone when the intravenous antigen was administered to rats 10 days after sensitization.

Soya bean trypsin inhibitor was only effective in protecting rats against anaphylactic shock when given a short time before the intravenous dose of antigen. Previous workers have shown it to suppress kaolin-induced rat paw oedema when used in high doses intraperitoneally (Hladovec, Mansfeld & Horakova, 1958) and to inhibit plasma kallikrein-mediated kinin release *in vitro* (Werle & Maier, 1952). Trasylol, a kallikrein inactivator, and hexadimethrine, an anti-heparin agent, were

both ineffective against rat anaphylaxis. The fenamates, effective antagonists of bradykinin in guinea-pigs and on isolated arteries of rats, also failed to alter the survival rate of the shocked animals, although they are potent antagonists of the heat reaction in rats (Starr & West, 1967).

The concomitant administration of ascorbic acid and mepyramine protected rats against anaphylactic shock at 10 days after sensitization but gave no protection at 20 days. Such a mixture has already been shown to reduce the elevated blood kinin and kininogen levels found in shock at 10 days (Dawson, Starr & West, 1966) and the results of the present work have also confirmed this finding. Thus, the mechanism by which the mixture affords protection is probably through an alteration in the kinin system although further work is needed to elucidate the exact point of attack.

REFERENCES

ANKIER, S. I., DAWSON, W., KARADY, S. & WEST, G. B. (1965). Seasonal variation in the resistance of rats. *J. Pharm. Pharmac.*, **17**, 187–188.

DAWSON, W., STARR, M. S. & WEST, G. B. (1966). Inhibition of anaphylactic shock in the rat by antihistamines and ascorbic acid. *Br. J. Pharmac. Chemother.*, **27**, 249–255.

HLADOVEC, J., MANSFELD, V. & HORAKOVA, Z. (1958). Inhibitory action of trypsin and trypsin-inhibitors on experimental inflammation in rats. *Experientia*, **14**, 146–147.

OATES, J. A., MELMON, K., SJOERDSMA, A., GILLESPIE, L. & MASON, D. T. (1964). Release of a kinin peptide in the carcinoid syndrome. *Lancet*, **1**, 514–517.

SANYAL, R. K. & WEST, G. B. (1958). Anaphylactic shock in the albino rat. *J. Physiol., Lond.*, **142**, 571–584.

STARR, M. S. & WEST, G. B. (1967). Bradykinin and oedema formation in heated paws of rats. *Br. J. Pharmac. Chemother.*, **31**, 178–187.

WERLE, E. & MAIER, L. (1952). Zur Kenntnis des Kallikrein-Inaktivators aus Kartoffeln. *Biochem. Z.*, **322**, 414–417.

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