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LOCAL KALLIKREIN AND TRYPSIN RESPONSES IN THE RAT

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1 Locally administered commercial hog pancreatic kallikrein (Depot-Glumorin) and bovine pancreatic tyrpsin both increased vascular permeability in the skin and paws of rats.

2 By the use of numerous antagonists and enzyme inhibitors, this vascular response was found to be the result not of kinin formation but of a direct action mostly on histamine receptors.

3 Highly purified kallikrein did not increase vascular permeability in rats, suggesting either that the effect was due to an impurity in the commercial preparation or that a structural change in the enzyme occurred on purification.

4 Soya bean trypsin inhibitor prevented the trypsin response when both were injected locally. On intraperitoneal injection, the inhibitor was effective only against local kallikrein.

5 The kallikrein inhibitor, aprotinin (Trasylol), was not effective against local kallikrein but it reduced the trypsin response when both were injected locally.

Introduction

Whilst re-investigating the involvement of kinin in the dextran reaction in rats, enzymes activating kinin formation, as well as bradykinin itself, were injected into the skin and paws of rats and their actions were subjected to modification by a variety of pharmacological agents. One of these enzymes is kallikrein, a kininogenase capable of liberating kinin from α_2-globulin kininogen. Kallikrein can be isolated from plasma γ-globulin, pancreas, salivary glands, intestine and urine. It is a vasodilator substance, with activity expressed in terms of a biological unit based on its vasodilator effect in the dog after intravenous injection. In some countries, it is used therapeutically in the treatment of vascular disorders. Trypsin too releases kinin from kininogen but it also activates kallikrein from precursors (Eisen, 1970).

The anaphylactoid reaction produced in rats by dextran is mediated chiefly by the release of histamine and 5-hydroxytryptamine (5-HT) from mast cells (Parratt & West, 1957). However, large doses of these amines never fully reproduce all the symptoms (oedema and pruritus) and selective antagonists are not always totally effective in preventing the reaction. Other mediators (e.g. kinin) may be involved although Ankier & Starr (1967) found that changes in the levels of kinin and kinin-controlling factors were not related to the extent of oedema resulting from systemic dextran.

Some rats do not respond to dextran with an anaphylactoid reaction (Harris, Kalmus & West, 1963) and these rats also have a higher threshold for kinin release in some forms of shock than do rats which react to dextran (Starr & West, 1970). Locally administered kallikrein and trypsin have, therefore, been tested in both types of rat. A preliminary account of this work has already been published (Bennett & West, 1978a).

Methods

Male Wistar rats (150 to 250 g) obtained from Tuck Ltd., Rayleigh, Essex respond to dextran and are designated R rats. Rats bred in the North East London Polytechnic laboratory do not respond to dextran (NR rats). To assess the effects of compounds on vascular permeability in shaved skin, each rat was injected intravenously with azovan blue dye (30 mg/kg) before the intradermal injections of the compounds dissolved in Tyrode solution (0.1 ml) at pH 6 to 8 (Bonaccorsi & West, 1963). The rats were killed 30 min later and the dye in each weal was extracted and measured spectrophotometrically at 620 nm (Harada, Takeuchi, Fukao & Katagiri, 1971). After injection of the compounds into paws, changes in paw volume were measured on an Ugo Basile volume differential meter at intervals of 15 min for 2 h. Antagonists or inhibitors were administered either intraperitoneally 30 min before the agents or locally mixed with them. Results were analysed by Student's *t* test.

A commercial preparation of hog pancreatic kallikrein (Depot-Glumorin) with esterolytic activity equiv-

0007-1188/80/080625-05 $01.00

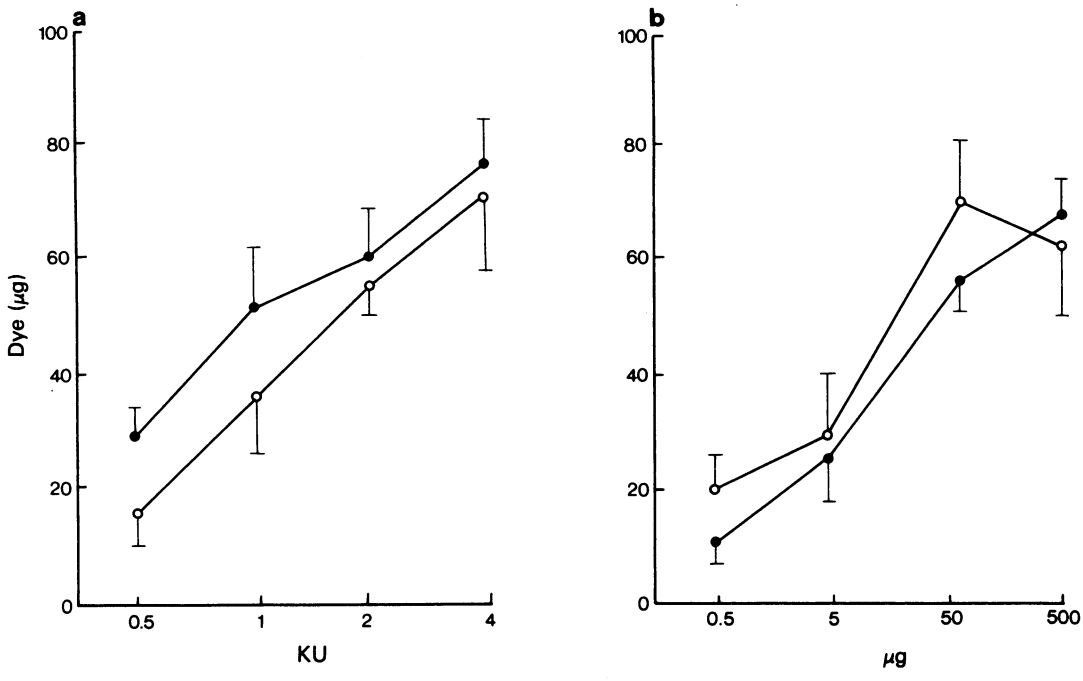

Figure 1 Dose-response curves to commercial kallikrein (a) and trypsin (b) in R (●) and NR (○) rats. Responses after intradermal injection are recorded as dye (μg) extracted from weals. Vertical lines show s.e. mean. Note the different dose scales.

alent to 1 kallikrein unit (KU) per mg and a highly purified hog pancreatic kallikrein equivalent to 1,150 KU per mg were kindly supplied by Bayer U.K. Ltd. Trypsin (ex bovine pancreas, 90 iu per mg, Sigma), dextran (molecular weight 110,000 Pharmacia), bradykinin (Sandoz), histamine and 5-hydroxytryptamine (5-HT) were also used as agonists. The antagonists included mepyramine (to block histamine H_1-receptors), metiamide (to block histamine H_2-receptors), methysergide (to block 5-HT receptors), disodium cromoglycate (DSCG, to stabilize mast cell membranes), soya bean trypsin inhibitor (SBTI), aprotinin (Trasylol, an inhibitor of kallikrein), phenanthroline (to inhibit kininase), and indomethacin (to inhibit some of the actions of kinins and prostaglandin generation).

Results

With kallikrein, only the commercial preparation (Depot-Glumorin) injected intradermally produced a graded dose-response relationship in rat skin, the dose range being 0.5 to 4 KU (Bennett & West, 1978b). Both R and NR rats responded equally (Figure 1), indicating a difference in action between commercial kallikrein and dextran (the latter being only effective in R rats). A similar response was obtained in rat paws, commercial kallikrein having a peak effect in both R and NR rats at 30 min when dextran was also at its peak in R rats (Figure 2). The more highly purified preparation of kallikrein was not effective locally in the skin or paws of both R and NR rats in doses up to 200 KU. Bradykinin was equally effective in both types of rat.

Trypsin also produced a graded dose-response relationship in both R and NR rats (dose-range 0.5 to 500 μg) with a peak effect at 30 min (Figures 1 and 2), and this action, like that of commercial kallikrein, was lost on heating to 56°C for 1 h.

A subcutaneous injection of insulin (10 iu/kg) potentiated, as expected (Harper & West, 1976), the dextran response in R rats (Figure 2). Insulin did not alter responses to kallikrein, trypsin or bradykinin in the skin or paws of R and NR rats. Repeated doses of dextran, either 180 mg/kg intraperitoneally daily for 4 days or 1 mg locally into a paw twice daily for 4 days, rendered R rats refractory to systemic and to local dextran but the kallikrein, trypsin and bradykinin actions remained unchanged.

Mepyramine, in doses sufficient to block the histamine H_1-receptors in the skin and paws, reduced

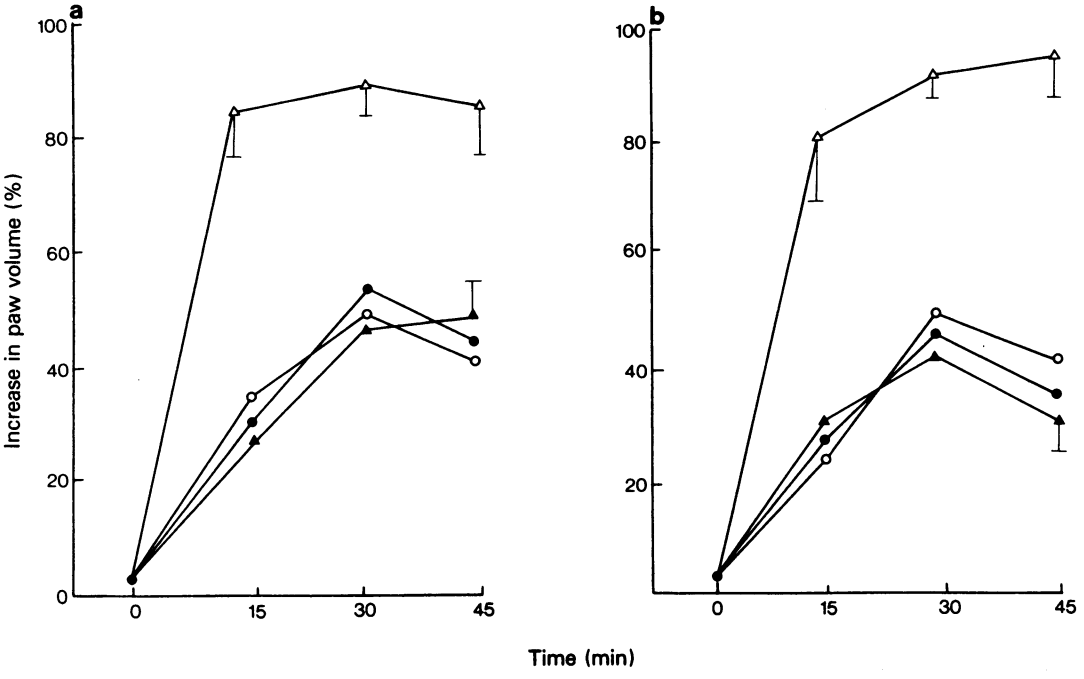

Figure 2 Dose-response curves to commercial kallikrein (2 KU, a) and trypsin (50 μg, b) in R rats before (○) and after (●) insulin. Responses recorded after subcutaneous injection into the paws are increases (%) in paw volume over 45 min. For comparison, the effects of dextran (100 μg) in R rats before (▲) and after (△) insulin are shown.

the dextran response and prevented those to commercial kallikrein and trypsin in R rats (Table 1). Metiamide, a blocker of histamine H_2-receptors, also prevented the kallikrein and trypsin responses in R rats though it was ineffective against dextran. The H_1- and H_2-blockers were also effective antagonists of histamine, kallikrein and trypsin in NR rats. Methysergide, a potent antagonist of 5-HT, blocked the dextran reaction in R rats whilst DSCG also prevented this reaction; both antagonists failed to modify the responses to kallikrein, trypsin, bradykinin and histamine, thereby indicating that commercial kallikrein and trypsin are not mast cell degranulators. SBTI injected intraperitoneally was an effective antagonist

Table 1 Effect of antagonists on the responses in the skin or paws of R rats (n = 6) to local commercial kallikrein (2 KU), trypsin (50 μg), bradykinin (8 μg), dextran (100 μg), histamine (100 μg) and 5-hydroxytryptamine (5-HT, 2 μg)

Antagonist	*Dose* (mg/kg)	*Route*	*Agonist* *Kallikrein*	*Trypsin*	*Bradykinin*	*Dextran*	*Histamine*	*5-HT*
Mepyramine	20	i.p.	++	++	0	+	++	0
Metiamide	20	i.p.	++	++	0	0	+	0
Methysergide	2	i.p.	0	0	0	++	0	++
DSCG	100	i.v.	0	0	0	++	0	0
SBTI	100	i.p.	++	0	0	0	0	0
Aprotinin	100,000 KIU*	i.v.	0	0	0	++	0	0
	50 KIU*	paw	0	++	0	0	0	0
Phenanthroline	100 μg	paw	0	0	P	0	0	0
Indomethacin	20	i.p.	0	+	++	+	0	0

++ = Gross reduction (>60%); + = Moderate reduction (30–60%); 0 = no significant effect: P = potentiation; * = kallikrein inhibitor units; DSCG = disodium cromoglycate; SBTI = soyabean trypsin inhibitor.

of local kallikrein but not of local trypsin. High doses of SBTI injected locally (for example, 5 mg) *per se* increased vascular permeability in the skin and paws of both R and NR rats, but smaller doses (less than 1 mg) abolished the response to a small dose of trypsin (5 μg) without inhibiting the kallikrein, bradykinin and histamine responses. Locally injected aprotinin, a recognised inhibitor of kallikrein, failed to modify the local action of commercial kallikrein but inhibited the response to trypsin. Intravenously administered aprotinin prevented only the dextran reaction suggesting that this inhibitor exerts a protective action on mast cells which degranulate after dextran treatment. Phenanthroline, an inhibitor of kininase, the enzyme inactivating kinin, has been reported to potentiate the kinin phase of carrageenan-induced oedema in rat paws (Capasso, Balestrieri, Di Rosa, Persica & Sorrentica, 1975) and in the present study it potentiated the bradykinin response without modifying those to kallikrein and trypsin. Finally, indomethacin (a prostaglandin synthetase inhibitor) reduced the dextran and trypsin responses and blocked that to bradykinin without affecting that to kallikrein, indicating that bradykinin and prostaglandins are not involved in the kallikrein response.

Discussion

The results show that commercial kallikrein obtained from hog pancreas, like trypsin, probably exerts a direct histamine-like action in the skin and paws of both R and NR rats and does not affect vascular permeability through an effect on the kinin system. This action differs from that of clinical dextran in that (1) the two enzymes are effective in NR rats (when dextran is not), (2) the responses in R rats are not potentiated when the blood sugar level is lowered by insulin (unlike those to dextran), and (3) the actions in R rats made refractory to dextran by repeated doses of dextran are little affected.

Differences between the action of commercial kallikrein and trypsin were found when antagonists were used. For example, SBTI and aprotinin injected locally in the paws greatly reduced the trypsin response without altering that to kallikrein. On the other hand, SBTI injected intraperitoneally only inhibited the kallikrein response. These results are difficult to interpret in the light of the observation that SBTI does not inhibit the enzymatic activity of pancreatic kallikrein *in vitro* (Werle & Maier, 1952). Furthermore, the commercial sample of kallikrein has been found to possess kininase activity *in vitro* and to form only minute amounts of kinin when incubated with rat or rabbit plasma kininogen (Fasciolo & Halvorsen, 1964) although it has esterolytic activity. The highly purified kallikrein preparation also possesses esterolytic activity but does not increase vascular permeability in the rat.

Commercial kallikrein in low doses increased vascular permeability in rat skin and paws, but not mainly through the production of kinin. It may therefore contain heat-labile impurities which produce the increased vascular permeability in rats. The response is not due to histamine (as it is abolished by heating but not reduced by dialysis) or to released histamine from mast cells (as it is unaffected by DSCG). Hence, it is probably the result of a direct action on histamine receptors in the rat, as the selective histamine H_1- and H_2-receptor antagonists, mepyramine and metiamide, prevented it.

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