Br. J. Pharmac. (1974), **50**, 243-251

MECHANISM OF THE PRO-INFLAMMATORY ACTIVITY OF SYMPATHOMIMETIC AMINES IN THERMIC OEDEMA OF THE RAT PAW

K.L. GREEN
School of Pharmacy, City of Leicester Polytechnic, P.O. Box 143, Leicester, LE1 9BH

1 Thermic oedema induced by heating rat paws at 46.5°C was potentiated by local injection of adrenaline, noradrenaline or high doses of isoprenaline. The pro-inflammatory effect of sympathomimetic amines was antagonized by phenoxybenzamine or phentolamine but not by propranolol.

2 The subcutaneous space of heated rat paws was perfused with Tyrode solution and the perfusate collected and assayed for bradykinin, bradykininogen, kinin-forming activity and kininase activity. When adrenaline (0.5 μg/ml) was included in the perfusion fluid, kininase activity of the perfusate was increased by 76% and free bradykinin reduced by 46%.

3 Increased vascular permeability induced by injection of bradykinin or kallikrein was reduced by adrenaline or noradrenaline, but isoprenaline had no significant effect.

4 Pretreatment with soya bean trypsin inhibitor (SBTI) or heparin did not antagonize the pro-inflammatory effect of adrenaline or thermic oedema *per se*.

5 Potentiation of thermic oedema similar to that induced by sympathomimetic amines was obtained by injecting paws with vasopressin prior to heating, or by applying a ligature to stop blood flow to the paw for the first 15 min of heating.

6 Thermistor probes inserted beneath the paw skin showed that sympathomimetic amines increased the internal temperature of heated paws. This was significant, as small changes in temperature had a marked effect on the development of thermic oedema.

7 It is suggested that sympathomimetic amines potentiate thermic oedema of rat paws heated at 46.5°C by reducing blood flow to the paw, thereby causing a greater rise in paw temperature and consequently greater injury.

Introduction

If the paws of anaesthetized rats are immersed in water at 46.5°C for 30 min or more the paws develop an inflammatory oedema. Bradykinin is present in perfusates collected from the subcutaneous space of heated paws perfused with Tyrode solution and has been implicated as the inflammatory mediator in thermic oedema (Rocha e Silva & Antonio, 1960; Garcia Leme, Hamamura & Rocha e Silva, 1970). Rocha e Silva (1962) reported that α-adrenoceptor blocking agents or reserpine pretreatment suppressed thermic oedema and suggested that catecholamines may activate kinin-forming enzymes. Starr & West (1967), however, found that injected adrenaline or noradrenaline antagonized thermic oedema and speculated that α-adrenoceptor blocking agents suppress thermic oedema by increasing plasma levels of catecholamines.

The aim of the present study was to examine the effects of sympathomimetic amines on parameters affecting the recovery of bradykinin in perfusates from paws heated at 46.5°C. In the course of this study, it was observed that sympathomimetic amines potentiated thermic oedema and the mechanism of this effect was investigated. A preliminary account of this work was presented to the British Pharmacological Society (Green, 1973).

Methods

Rats which are resistant to the anaphylactoid reaction to dextran are reported to be resistant to thermic oedema (Gecse, Karady, Starr & West, 1965) and hence only dextran reactors were used in these experiments. Wistar (Tuck) rats (160-180 g) were anaesthetized with pentobarbitone sodium (45 mg/kg i.p.), injected i.v. with 0.4 ml of a 1% solution of Evans blue in

saline and both paws coaxially perfused, as described by Garcia Leme *et al.* (1970). The hind paws were immersed in water at 46.5°C, perfused with Tyrode solution (pH 7) at 0.2 ml/min and the perfusate draining from the subcutaneous space of the paws collected over the first 30 min of heating. The effect of adrenaline or isoprenaline was examined by perfusing one of the hind paws with Tyrode solution containing sympathomimetic amine and by comparing the perfusate with that from the other paw perfused with Tyrode solution alone. The permeability of the paw blood vessels to Evans blue-labelled albumin was assessed by measuring the optical extinction of the perfusate at 615 nm.

In some experiments the development of thermic oedema was followed by heating the paw without perfusion. Anaesthetized rats were injected i.v. with 0.1 ml of 0.5% Evans blue solution in saline and paw volume was measured every 15 min by the plethysmographic method of Buttle, D'Arcy, Howard & Kellet (1957). Sympathomimetic amines were dissolved in 0.9% w/v NaCl solution (saline), adjusted to pH 7, and 0.05 ml injected beneath the plantar surface of the hind paw 15 min prior to heating.

Assay of perfusate

The perfusates were assayed directly for bradykinin activity on the isolated uterus of a rat injected with stilboestrol (50 μg s.c.) 24 and 48 h previously. A 2 ml bath containing modified Krebs solution, (10% of the usual concentration of calcium) gassed with 95% oxygen and 5% carbon dioxide at 28°C, was used; the Krebs solution contained atropine (1 μg/ml), mepyramine (0.25 μg/ml), methysergide (0.25 μg/ml), phentolamine (2 μg/ml), sotalol (100 μg/ml) and chymotrypsin (2 μg/ml). Chymotrypsin in the bathing fluid increased the sensitivity of the uterus to bradykinin up to five-fold over several hours, enabling responses to be obtained with 0.1-0.4 ng of bradykinin. Each assay preparation was tested to ensure that the concentration of adrenoceptor blocking agents in the bathing fluid was sufficient to antagonize completely the effects of sympathomimetic amines present in the perfusates.

When estimating bradykininogen, kinin-forming activity and kininase activity of the perfusates, the relatively high concentrations of bradykinin present were assayed on the terminal guinea-pig ileum bathed in Tyrode solution at 32°C, containing the same antagonist drugs as described for the uterus. Contact with chymotrypsin (100 μg) for 5 min sensitized the ileum to bradykinin for several hours and hence it was not necessary to include chymotrypsin in the bathing solution. Rat duodenum was sometimes used to assay and characterize the perfusate (Gaddum & Horton, 1959), the antagonist drugs previously described being included in the de Jalon bathing fluid. Further characterization of the kinin-like nature of the perfusates was made by incubation of samples with chymotrypsin, which destroys bradykinin.

Estimation of bradykininogen

The method used was that described by Dawson, Starr & West (1966); 0.5 ml samples of perfusate were used. The bradykininogen content of the perfusates was expressed as the amount of bradykinin which would have been formed from the whole of the 30 min sample.

Kinin-forming activity

Citrated rat plasma, previously heated at 61°C for 1 h, was used as substrate, 3-4 μg of bradykinin being formed when 1 ml of heated plasma was incubated with trypsin. One ml of perfusate was added to polythene tubes containing 0.4 ml heated plasma, 0.1 mg phenanthroline and 0.5 ml Tris (tris(hydroxymethyl)-aminomethane) buffer (0.1 M) pH 7. This mixture was incubated at 37°C for 1 h, the tubes were placed on ice and the kinin formed was assayed. Kinin-forming activity of the perfusate was expressed as the amount of bradykinin which would have been formed by the whole of the 30 min sample in 1 hour.

Kininase activity

The method used was that described by Edery & Lewis (1962). Kininase activity was expressed as the amount of bradykinin which would have been inactivated by the whole of the 30 min perfusate in 1 hour.

Drugs

The following drugs were used: (−)-adrenaline hydrogen tartrate (B.D.H.); (−)-noradrenaline bitartrate (Koch-Light); (±)-isoprenaline sulphate (Abbot); bradykinin triacetate (Sigma); (±)-sotalol hydrochloride (Mead Johnson); (±)-propranolol hydrochloride (I.C.I.); phentolamine mesylate (CIBA); phenoxybenzamine hydrochloride (S.K.F.); pancreatic kallikrein (Bayer); trypsin (Sigma); soya bean trypsin inhibitor (SBTI) (Sigma); α-chymotrypsin (Sigma); heparin (Evans); atropine sulphate (B.D.H.); mepyramine maleate (May & Baker); methysergide bimaleate (Sandoz). All doses and concentrations mentioned in the text refer to the salts, except for adrenaline,

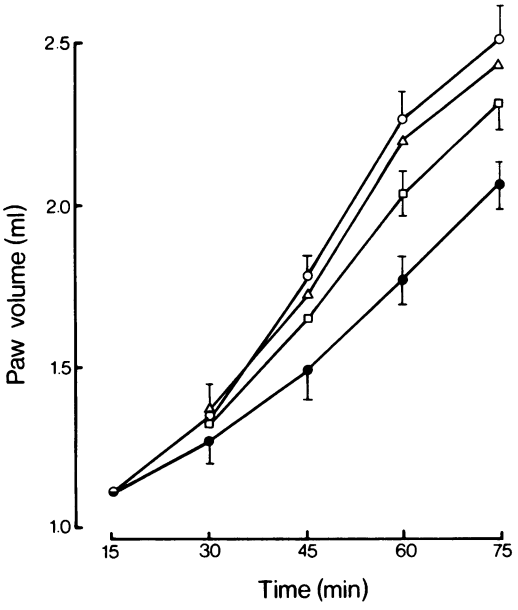

Fig. 1 Effect of adrenaline (1 μg), noradrenaline (1 μg) or isoprenaline (20 μg) on the time course of thermic oedema in the rat hind paw. A subplantar injection of adrenaline (○), noradrenaline (△) or isoprenaline (□) in 0.05 ml saline was made 15 min before heating at 46.5° C. Control paws (●) were injected with 0.05 ml saline. Each point is the mean of at least 10 determinations ±s.e. mean.

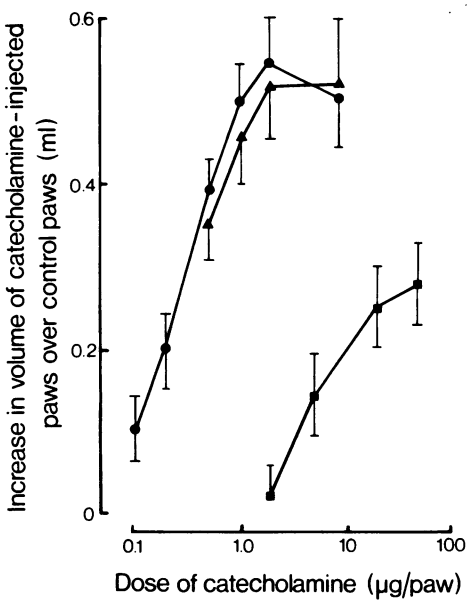

Fig. 2 Effect of sympathomimetic amines on thermic oedema of the rat hind paw. Adrenaline (●), noradrenaline (▲) or isoprenaline (■) were injected into the plantar surface of the hind paw 15 min before heating the paw at 46.5° C. Control paws were injected with 0.05 ml saline. Each point is the mean of at least 10 determinations ±s.e. mean.

noradrenaline and isoprenaline which are expressed in terms of the base.

Results

Local injection of adrenaline, noradrenaline or, in high doses, isoprenaline, increased oedema, petechial haemorrhage and the exudation of dye in paws heated at 46.5°C (Figures 1 & 2). The pro-inflammatory effect was most noticeable 30-60 min after heating had commenced. Pretreatment with phenoxybenzamine or phentolamine reduced the pro-inflammatory effect of sympathomimetic amines, but pretreatment with propranolol had no significant effect (Table 1). These results contrast with those of Starr & West (1967) who found that adrenaline or noradrenaline suppressed thermic oedema when measurements were taken after the paws had been heated at 46.5°C for 30 minutes. Attempts were made to reproduce the results of Starr & West (1967) by heating the paws for only 30 min, but a pro-inflammatory effect of adrenaline and noradrenaline was always observed.

The perfusates obtained from heated, coaxially perfused paws sometimes became contaminated with blood during the period of collection, which greatly increased the kinin-like activity, and the samples were rejected. Changes in the concentration of Evans blue in the perfusates was found to parallel changes in the bradykininogen content. The amount of free bradykinin, kinin-forming activity and kininase activity in the perfusates from heated paws was considerably lower than reported by Starr & West (1967) and Arrigoni Martelli, Corsico & Fagagnola (1969). However, Salmon & West (1973) have recently shown that Tuck Wistar rats release significantly less bradykinin in thermic oedema than rats from other colonies.

When adrenaline (0.5 μg/ml) was included in the perfusion fluid, the kininase activity of the perfusate was significantly increased ($P < 0.05$) and the recovery of bradykinin in the perfusate was correspondingly reduced (Figure 3). Adrenaline appeared to have no effect on bradykininogen or kinin-forming activity of the perfusate and isoprenaline in the perfusion fluid had no significant effect on any of the parameters affecting the kinin system.

Since bradykinin has been implicated as the mediator in thermic oedema, it was of interest to investigate the effect of sympathomimetic amines on the vascular permeability response to injected bradykinin. Adrenaline or noradrenaline suppressed the increase in vascular permeability induced by injected bradykinin or kallikrein but isoprenaline was inactive (Table 2). These results suggest that in the rat hind paw, sympathomimetic amines antagonize the permeability effects of

Table 1 Effect of α- or β-adrenoceptor blocking agents on the pro-inflammatory effect of sympathomimetic amines in thermic oedema.

		Increase in paw volume			
		45 min heating		*60 min heating*	
Pretreatment	*Treatment of paw*	*cc ± s.e. mean*	*Difference*	*cc ± s.e. mean*	*Difference*
None	Control	0.48 ± 0.03	0.25	0.76 ± 0.04	0.50
	Adrenaline	0.73 ± 0.05		1.26 ± 0.06	
Propranolol	Control	0.60 ± 0.04	0.24	0.96 ± 0.06	0.43
	Adrenaline	0.84 ± 0.06		1.39 ± 0.08	
Phenoxybenzamine	Control	0.53 ± 0.05	0.07	0.85 ± 0.06	0.07
	Adrenaline	0.60 ± 0.06*		0.93 ± 0.06**	
Phentolamine	Control	0.38 ± 0.04	0.10	0.63 ± 0.05	0.12
	Adrenaline	0.48 ± 0.06**		0.75 ± 0.06**	
None	Control	0.54 ± 0.04	0.22	0.89 ± 0.07	0.26
	Isoprenaline	0.76 ± 0.06		1.15 ± 0.06	
Propranolol	Control	0.64 ± 0.05	0.26	0.97 ± 0.05	0.23
	Isoprenaline	0.90 ± 0.07		1.19 ± 0.08	
Phentolamine	Control	0.36 ± 0.05	0.10	0.70 ± 0.04	0.09
	Isoprenaline	0.46 ± 0.06**		0.79 ± 0.05**	

Adrenaline (1 μg) or isoprenaline (20 μg) was injected into one of the hind paws and saline into the other, 15 min before the paws were heated at 46.5°C. Propranolol (10 mg/kg) or phentolamine (10 mg/kg) was administered i.p. 30 min before heating and phenoxybenzamine (10 mg/kg) i.v. 1 h before heating. Paw volume was measured after 45 min and 60 min of heating. Pretreatment with phentolamine or phenoxybenzamine had a significant effect at $P < 0.05$ (*) or $P < 0.001$ (**). Each value is the mean of at least 10 determinations.

Table 2 Effect of adrenaline or isoprenaline on paw oedema induced by local injection of bradykinin or kallikrein.

Pretreatment	*Treatment of paw*	*Increase in paw weight (mg)*	*P compared with*
(a) None	Bradykinin	135 ± 28	–
(b) None	Bradykinin + adrenaline	29 ± 10	(a) <0.01
(c) Phenoxybenzamine	Bradykinin	128 ± 24	–
(d) Phenoxybenzamine	Bradykinin + adrenaline	107 ± 22	(c) <0.01
(e) Propranolol	Bradykinin	133 ± 32	–
(f) Propranolol	Bradykinin + adrenaline	19 ± 6	(e) N.S.
(g) None	Bradykinin + isoprenaline	121 ± 34	(a) N.S.
(h) None	Kallikrein	128 ± 29	–
(i) None	Kallikrein + adrenaline	38 ± 16	(h) <0.02

Adrenaline (1 μg) or isoprenaline (20 μg) was injected into the plantar surface of the hind paw 10 min before injecting the paw with bradykinin (1 μg) or kallikrein (1 unit). Control paws were injected with an equal volume of saline (0.05 ml or 0.1 ml). The rats were killed 30 min after injection of bradykinin or kallikrein and the paws amputated at the tarsocrural joint and weighed. The difference in weight between control and treated paws was recorded. Phenoxybenzamine (10 mg/kg i.v.) and propranolol (10 mg/kg i.p.) were administered 30 min before injection of adrenaline. Each value is the mean of at least 10 determinations ± s.e. mean. N.S.—Not significant.

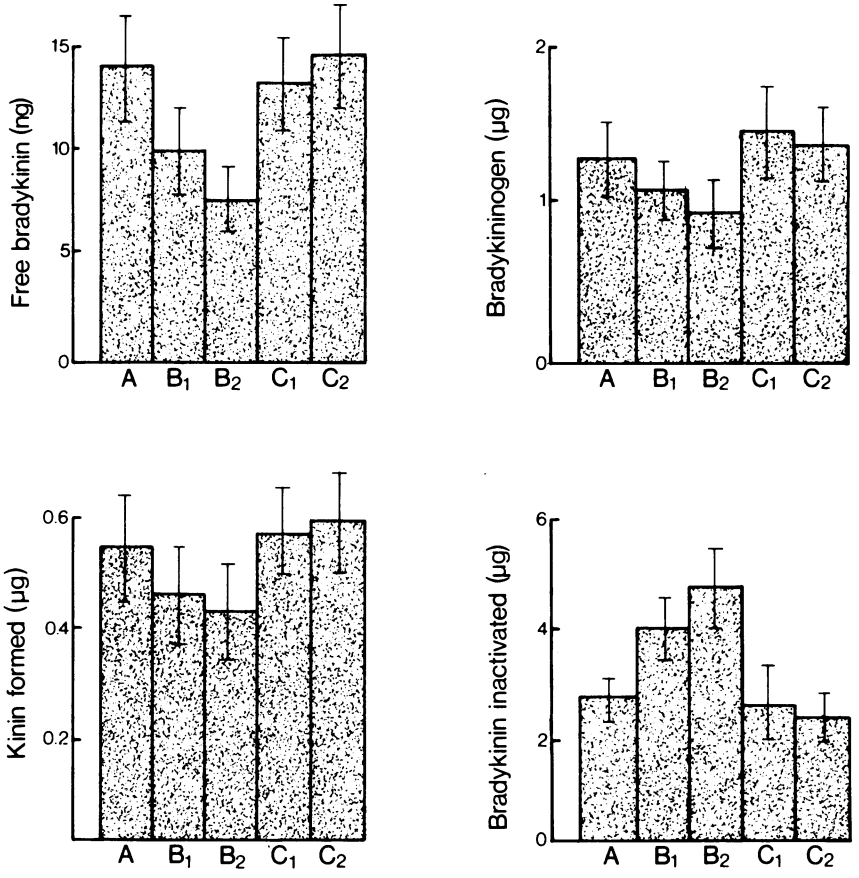

Fig. 3 Effect of adrenaline or isoprenaline on the amount of bradykinin, bradykininogen, kinin-forming activity and kininase activity in perfusate from paws heated at 46.5° C. Paws were perfused with Tyrode solution alone (A), or Tyrode solution containing adrenaline 0.1 µg/ml (B_1), adrenaline 0.5 µg/ml (B_2), isoprenaline 0.1 µg/ml (C_1), isoprenaline 0.5 µg/ml (C_2). Perfusate was collected over the first 30 min of heating. Each value is the mean of at least eight determinations ±s.e. mean.

bradykinin by activation of α-adrenoceptors, in contrast to the mouse paw, where both α- and β-adrenoceptors appear to be involved in the anti-inflammatory effect of sympathomimetic amines (Green, 1972).

As evidence supporting the role of bradykinin as a mediator of thermic oedema, Garcia Leme *et al.* (1970) reported that SBTI and other anti-proteases prevented the appearance of bradykinin in the perfusate from heated paws. However, in experiments described here, local injection of SBTI failed to antagonize the pro-inflammatory effect of adrenaline or thermic oedema *per se* (Table 3).

Heparin did not antagonize the pro-inflammatory effect of adrenaline in thermic oedema (Table 3), suggesting that the effect is not due to altered coagulation mechanisms, as in the Schwartzman or Arthus reactions (Thomas, 1956; Henson, Hartness & Brunson, 1969) or the pro-inflammatory effect of adrenaline described by Selye, Somogyi & Vegh (1968). Pretreatment with heparin greatly increased the severity of petechial haemorrhage in both control and adrenaline-injected paws.

Local injection of vasopressin enhanced the formation of thermic oedema (Table 4), the time course of the response being similar to that obtained with sympathomimetic amines, but the effect was not antagonized by phenoxybenzamine. To ascertain whether the pro-inflammatory effect of sympathomimetic amines in thermic oedema could be reproduced by mechanically occluding blood flow to the paw, a ligature was applied above the tarsocrural joint for the first 15 min of heating. When the ligature was removed, an

increased inflammatory response rapidly ensued (Fig. 4), suggesting that vasoconstrictor drugs might potentiate thermic oedema by reducing blood flow to the paw and thereby effectively increase the temperature at which the paw is heated. To test this hypothesis, thermistor probes were inserted beneath the paw skin and temperature changes monitored while the paws were heated. For the first 15 min of heating at 46.5°C, the internal temperature of control paws was approx 1°C lower than paws injected with adrenaline (1 μg) and 0.5°C lower than paws injected with isoprenaline (20 μg) (Figure 5). Adrenaline or isoprenaline did not significantly reduce the temperature gradient across the skin in rats pretreated with phenoxybenzamine (10 mg/kg i.v.) 1 h previously.

To establish whether the increase in internal temperature of paws injected with sympathomimetic amines was of importance in the pro-inflammatory effect, experiments were performed in which one of the hind paws was heated at a temperature 1°C higher than the other for the first 15 min of heating. Paws heated at 47.5°C for 15 min and then at 46.5°C were significantly more oedematous than paws heated at 46.5°C through-

Table 3 Effect of heparin or soya bean trypsin inhibitor (SBTI) on the pro-inflammatory activity of adrenaline in thermic oedema.

		Increase in paw volume			
		45 min heating		*60 min heating*	
Pretreatment	*Treatment of paw*	*cc ± s.e. mean*	*Difference*	*cc ± s.e. mean*	*Difference*
None	Control	0.55 ± 0.03	0.33	0.85 ± 0.05	0.47
	Adrenaline	0.88 ± 0.05		1.32 ± 0.06	
Heparin	Control	0.52 ± 0.04	0.39	0.81 ± 0.06	0.47
	Adrenaline	0.91 ± 0.08		1.28 ± 0.09	
None	Control	0.52 ± 0.05	0.05	0.83 ± 0.07	0.08
	SBTI	0.57 ± 0.05		0.91 ± 0.09	
None	SBTI	0.56 ± 0.04	0.34	0.89 ± 0.08	0.52
	SBTI + adrenaline	0.90 ± 0.07		1.41 ± 0.10	

Heparin (3,000 units) was injected i.v. 15 min before heating. A subplantar injection of adrenaline (1 μg) and/or SBTI (2 mg) was made 15 min before heating the paws at 46.5°C. Control paws were injected with 0.05 ml saline. Paw volume was measured after 45 min and 60 min of heating. Each value is the mean of at least 10 determinations.

Table 4 Effect of local injection of vasopressin on thermic oedema of rat paws.

		Increase in paw volume			
		45 min heating		*60 min heating*	
Pretreatment	*Treatment of paw*	*cc ± s.e. mean*	*Difference*	*cc ± s.e. mean*	*Difference*
None	None	0.53 ± 0.03	0.26	0.83 ± 0.07	0.30
	Vasopressin 25 mu	0.76 ± 0.04		1.13 ± 0.09	
None	None	0.55 ± 0.04	0.49	0.87 ± 0.08	0.55
	Vasopressin 75 mu	1.04 ± 0.08		1.42 ± 0.13	
Phenoxy-benzamine	None	0.50 ± 0.04	0.52	0.79 ± 0.10	0.50
	Vasopressin 75 mu	1.02 ± 0.09		1.29 ± 0.12	

Vasopressin was injected into one of the hind paws and saline into the other, 15 min before the paws were heated at 46.5°C. Phenoxybenzamine (10 mg/kg) was administered i.v. 1 h before heating. Paw volume was measured after 45 min and 60 min of heating. Each value is the mean of at least 10 determinations.

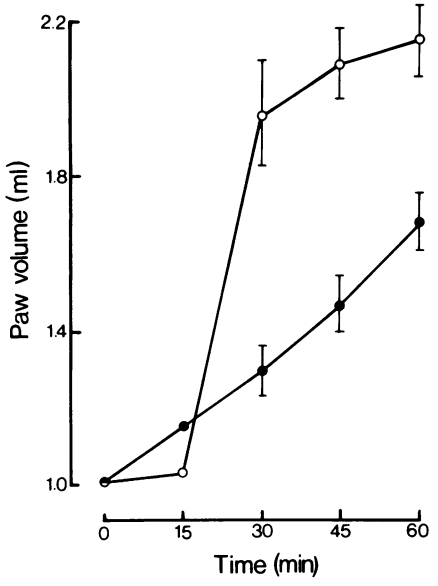

Fig. 4 Effect of temporarily occluding blood flow to the paw on the development of thermic oedema. (○) Blood flow to the paw occluded for the first 15 min of heating at 46.5° C. (●) Control paw heated at 46.5° C. Each point is the mean of at least 10 determinations ±s.e. mean.

out (Fig. 6), showing that small changes in temperature have a large effect on the development of thermic oedema.

Discussion

The low concentrations of free bradykinin, recovered in the perfusates from heated rat paws, would appear to be insufficient to account for the severity of the oedema. Garcia Leme *et al.* (1970) found that SBTI inhibited the release of bradykinin in the perfusate from heated paws, but in experiments reported here, local injection of large doses of SBTI failed to antagonize the pro-inflammatory effect of adrenaline or thermic oedema *per se*. Starr & West (1967) have expressed similar doubts as to the importance of bradykinin in thermic oedema and Arrigoni Martelli *et al.* (1969) detected an increase in bradykinin output only after the heated paws had increased in volume by about 40%. This would suggest that activation of the kinin system is not the initial cause of thermic oedema, but at most serves to potentiate the response.

Adrenaline increased the kininase activity of the perfusates from heated paws, and this effect

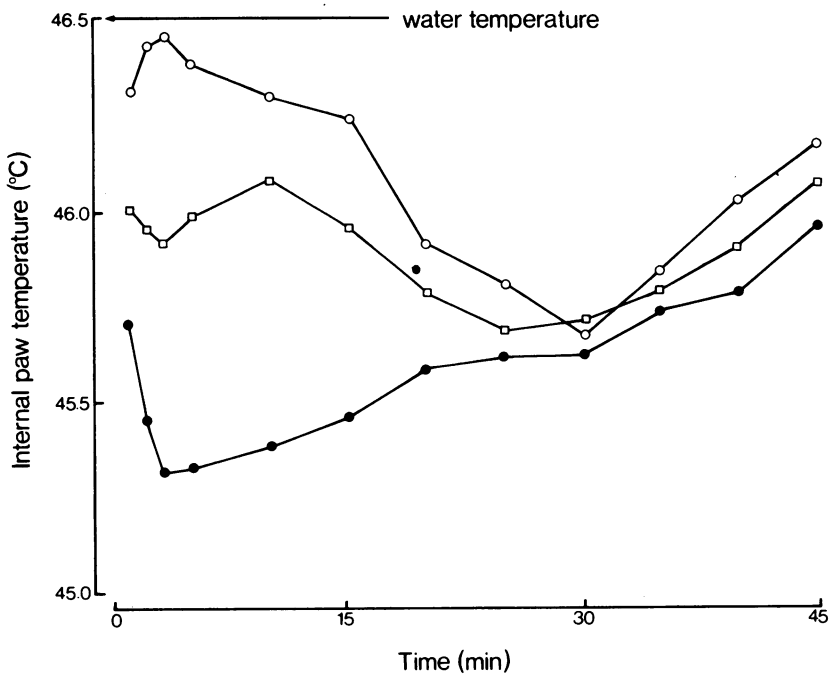

Fig. 5 Effect of adrenaline (○) or isoprenaline (□) on the internal temperature of hind paws immersed in water at 46.5° C. A subplantar injection of adrenaline (1 μg) or isoprenaline (20 μg) was made 15 min before heating. Control paws (●) were injected with 0.05 ml of saline. Each point is the mean of at least 10 determinations.

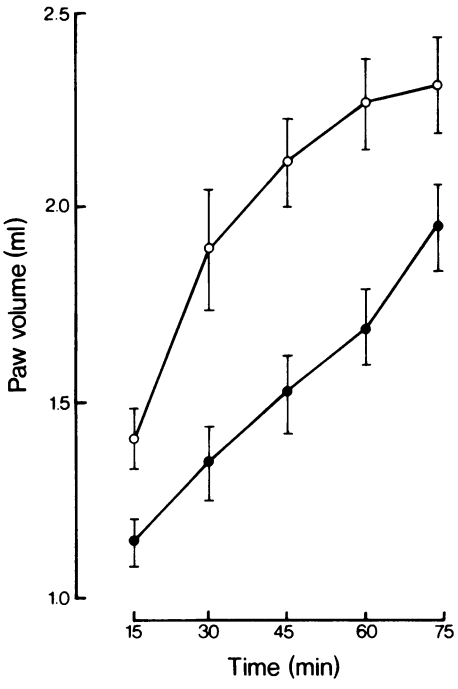

Fig. 6 Effect of temperature on the development of thermic oedema. (•) paws heated at 46.5° C throughout. (○) paws heated at 47.5° C for 15 min and then at 46.5° C. Each point is the mean of at least 10 determinations ±s.e. mean.

was probably responsible for the reduction in the amount of free bradykinin in the perfusate from paws perfused with adrenaline. Isoprenaline had no effect on kininase activity, suggesting that adrenaline mediates its effect on kininase activity via α-adrenoceptors. These results complement the findings of Mashford & Zacest (1967) that adrenaline increases plasma kininase activity in man, an effect which is antagonized by phenoxybenzamine *in vitro*.

Mechanism of the pro-inflammatory effect of sympathomimetic amines

The ability of sympathomimetic amines to potentiate thermic oedema does not appear to be due to activation of the kinin system, since adrenaline reduced the amount of bradykinin in perfusates from heated paws and antagonized the vascular permeability effects of injected bradykinin or kallikrein. Furthermore, local injection of the anti-protease SBTI failed to antagonize the pro-inflammatory effect of adrenaline. It seems more likely that sympathomimetic amines potentiate thermic oedema by causing vasoconstriction, which reduces blood flow to the injected paw so that heat is less readily dissipated. Consequently, the internal temperature of paws injected with sympathomimetic amines is higher than control paws and therefore they sustain greater injury. This hypothesis is supported by the following observations:

(a) The pro-inflammatory effect of sympathomimetic amines appeared to be mediated via α-adrenoceptors.

(b) Injection of vasopressin in doses causing vasoconstriction induced a pro-inflammatory response similar to that produced by sympathomimetic amines.

(c) Temporary occlusion of blood flow to the paw while heating caused a pronounced inflammatory response when blood flow was restored.

(d) The internal temperature of paws injected with adrenaline or isoprenaline was significantly higher than control paws over the first 15 min of heating and was sufficient to account for the pro-inflammatory effect.

Several points deserve comment. Isoprenaline usually causes vasodilatation, but in the heated rat paw it increased paw temperature, and this effect, like its pro-inflammatory effect, was antagonized by phenoxybenzamine. This suggests that the blood vessels of the paw are maximally dilated in thermic oedema and that in these circumstances isoprenaline in high doses causes vasoconstriction mediated via α-adrenoceptors. Although long periods of anoxia increase vascular permeability it is unlikely that anoxia was responsible for the increased inflammatory response following injection of sympathomimetic amines or temporary occlusion of blood flow to the paw. Occlusion of blood flow to the paw for up to 2 h immediately prior to heating did not potentiate the development of thermic oedema and similarly, Willms-Kretschmer & Majno (1969) found no oedema in rat skin after 2 h of ischaemia.

It must be stressed that sympathomimetic amines are only likely to exert a pro-inflammatory effect in thermal injury over a limited temperature range. Most models of experimental thermal injury have employed temperatures of 52-60° C applied for up to 20 seconds. Under these conditions, blood flow to the affected area is unlikely to be substantially increased during the short period of heating and hence, vasoconstrictor agents will have little effect on the internal temperature attained. The results reported here can thus be reconciled with observations that adrenaline suppresses increased vascular permeability in some forms of thermal injury (Spector & Willoughby, 1960).

Since the development of thermic oedema in rat paws, heated at 46.5° C, is so susceptible to

changes in blood flow it would appear to be a poor experimental model for assessing anti-inflammatory activity. This view is supported by Trottier & Malone (1971) who found the test to be non-specific and none of the clinically effective anti-inflammatory agents which they tested significantly inhibited thermic oedema.

I wish to thank Dr B.J. Northover and Prof M. Ginsburg for advice and encouragement throughout this work. I am grateful to Dr A. Blackham and Dr H. Radziwonik for advice on the coaxial perfusion technique and to the following for generous gifts of drugs: Bayer for pancreatic kallikrein; I.C.I. for propranolol; Mead Johnson for sotalol, and Sandoz for methysergide.

References

ARRIGONI MARTELLI, E., CORSICO, N. & FAGAGNOLA, E. (1969). Significance of the release of bradykinin in local inflammatory reactions and related effect of antiphlogistic drugs. In: *Inflammation biochemistry and drug interaction* ed. A. Bertelli & J.C. Houck, pp. 185-196. Milan: Excerpta Medica Foundation.

BUTTLE, G.A.H., D'ARCY, P.F., HOWARD, E.M. & KELLET, D.N. (1957). Plethysmographic measurement of swelling in the feet of small laboratory animals. *Nature (Lond.)*, **179**, 629.

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

EDERY, H. & LEWIS, G.P. (1962). Inhibition of plasma kininase at slightly acid pH. *Br. J. Pharmac. Chemother.*, **19**, 299-305.

GADDUM, J.H. & HORTON, E.W. (1959). The extraction of human urinary kinin (substance Z) and its relation to plasma kinins. *Br. J. Pharmac. Chemother.*, **14**, 117-124.

GARCIA LEME, J., HAMAMURA, L. & ROCHA E SILVA, M. (1970). Effect of anti-proteases and hexadimethrine bromide on the release of a bradykinin-like substance during heating (46° C) of rat paws. *Br. J. Pharmac.*, **40**, 294-309.

GECSE, A., KARADY, S., STARR, M.S. & WEST, G.B. (1965). Release of bradykinin in shock. *J. Physiol., Lond.*, **178**,8-9P.

GREEN, K.L. (1972). The anti-inflammatory effect of catecholamines in the peritoneal cavity and hind paw of the mouse. *Br. J. Pharmac.*, **45**, 322-332.

GREEN, K.L. (1973). A pro-inflammatory effect of adrenaline in thermal injury. *Br. J. Pharmac.*, **48**, 335-336P.

HENSON, E.C., HARTNESS, S. & BRUNSON, J.G. (1969). Adrenal medullary hormones and phenothiazine effects on the Arthus reaction in adult rabbits. *Arch. Path.*, **88**, 249-258.

MASHFORD, M.L. & ZACEST, R. (1967). Effect of adrenaline on blood kinin levels and blood kininase activity in man. *Circulation Res.*, **20**, Suppl. 3, 183-186.

ROCHA E SILVA, M. (1962). Definition of bradykinin and other kinins. *Biochem. Pharmac.*, **10**,3-21.

ROCHA E SILVA, M. & ANTONIO, A. (1960). Release of bradykinin and the mechanism of production of a 'thermic oedema (45° C)' in the rat's paw. *Med. exp.*, **3**, 371-382.

SALMON, G.K. & WEST, G.B. (1973). Variation in the response of rats to chemical and thermal injury. *Br. J. Pharmac.*, **47**, 625-626P.

SELYE, H., SOMOGYI, A. & VEGH, P. (1968). Inflammation, topical stress and the concept of pluricausal diseases. In: *Chemical biology of inflammation*, ed. J.C. Houck & B.K. Forscher, pp. 107-122. Oxford: Pergamon Press.

SPECTOR, W.G. & WILLOUGHBY, D.A. (1960). The enzymic inactivation of an adrenaline-like substance in inflammation. *J. Path. Bact.*, **80**, 271-280.

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

THOMAS, L. (1956). The role of epinephrine in the reactions produced by the endotoxins of Gram negative bacteria. I. Hemorrhagic necrosis produced by epinephrine in the skin of endotoxin treated rabbits. *J. exp. Med.*, **104**, 865-880.

TROTTIER, R.W. & MALONE, H.M. (1971). Re-evaluation of thermal oedema in the rat as a model for studying anti-inflammatory activity. *Proc. West. Pharmacol. Soc.*, **14**, 50-54.

WILLMS-KRETSCHMER, K. & MAJNO, G. (1969). Ischemia of the skin: electron microscopic study of vascular injury. *Am. J. Path.*, **54**, 327-353.

(Received July 5, 1973)