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Vespula vulgaris venom: role of kinins and release of 5-hydroxytryptamine from skin mast cells

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Abstract

Wasp venoms contain several active components, among them kinin-related peptides. Like bradykinin and [Thr^6]bradykinin, *Vespula vulgaris* venom caused paw oedema following subplantar injection in anaesthetized rats. The oedema was partly inhibited by the bradykinin B_2 receptor antagonist icatibant (Hoe 140); the remaining part was abolished by additional pretreatment with 5-hydroxytryptamine (5-HT) receptor antagonists or mast cell depletion. Histamine receptor antagonists were ineffective. Capsaicin pretreatment attenuated oedema formation indicating a neurogenic sensory component. Nociceptive behavioural responses induced by the venom in unanaesthetized rats were abolished by icatibant. It is concluded that kinins, either contained in the venom or released from the tissue, play the predominant role in the inflammatory and algesic effects. The inflammatory effects only partly rely on direct, bradykinin receptor-mediated mechanisms while the remaining part depends on the release of 5-HT from skin mast cells. The algesic effects of the venom are entirely due to direct B_2 receptor activation.

Keywords: Inflammation; Bradykinin receptor antagonist; Icatibant (Hoe 140); 5-HT (5-Hydroxytryptamine serotonin) release; Pain; Capsaicin

1. Introduction

Already in 1954, Jaques and Schachter (1954) have determined that the wasp venom contains high amounts of a component that exhibited physicochemical properties similar to those of purified bradykinin. Several different bradykinin-related peptides have been identified in the venoms of different wasp species (Schachter, 1968). Due to their prominent actions on vascular permeability and nociceptive afferent neurones, these kinins could be anticipated to be responsible for the inflammatory and algesic effects of the venom. However, besides kinins, a number of other factors are also present in wasp venoms which could contribute to their effects either on their own or by releasing secondary mediators from the tissue. Among these factors are histamine, 5-hydroxytryptamine (5-HT) or acetylcholine (e.g., Piek et al., 1983a,b; Sarangi et al., 1990), hydrophobic peptides, mastoparans and chemotactic peptides (Nakajima et al., 1985), or enzymes such as hyaluronidase (Habermann, 1972; Schmidt et al., 1986) and phospholipases (Watala and Kowalczyk, 1990).

The most prominent acute symptoms caused by wasp venoms are the formation of a localized cutaneous oedema and pain. In order to investigate the importance of the kinins contained in the venom of the wasp *Vespula vulgaris*, the bradykinin B_2 receptor antagonist, icatibant (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin, formerly named Hoe 140; Lembeck et al., 1991; Hock et al., 1991; Wirth et al., 1991) was used. The effects of the venom were compared with those of synthetic bradykinin and [Thr^6]bradykinin, another kinin found in wasp venoms (Yasuhara et al., 1987).

2. Methods

2.1. Determination of histamine, 5-HT and kininlike material

The content of histamine and 5-HT was determined in aqueous solutions of *Vespula vulgaris* venom (1 mg/ml

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in 154 mM phosphate-buffered saline, diluted 1:100 with 0.2 N perchloric acid for 5-HT) by reversed phase HPLC (LiChrospher 100 RP18, 5 μm column). For histamine the eluent was 0.1 M acetic acid, 0.1% pentanesulfonic acid and 20% acetonitrile; for 5-HT the eluent was phosphate buffer pH 5.0 containing 4 mM heptanesulfonic acid and 14% methanol. Histamine was analysed by fluorescence detection according to the method of Skofitsch et al. (1981), 5-HT was determined by electrochemical detection according to Sperk (1982).

Measurement of kininlike material was performed on the isolated guinea-pig ileum in vitro. Pieces of ileum (1.5 cm) were taken starting approximately 5 cm proximal to the ileocoecal valve. The preparations were suspended at 37°C in organ baths in Tyrode solution containing atropine (0.35 μM), mepyramin (0.35 μM) and ketanserin (1.0 μM) and oxygenated with 95% O_2 and 5% CO_2. Isotonic contractions were recorded under a resting tension of 2 g. A maximum concentration of the ileum was induced by 10 μM bradykinin at the beginning of the experiment. After an equilibrium period of 45 min which included regular washing procedures, a contraction–response curve was established for *Vespula vulgaris* venom (1–10 μg/ml). The contact time for all concentrations was 1 min. In order to identify the contractile agent in the venom, the concentration–response curve was repeated in the presence of the B_2 receptor antagonist icatibant (3–300 nM). Similar concentration–response curves were established for bradykinin (3–300 nM) and [Thr^6]bradykinin (10–1000 nM). The concentration–response curves were subjected to logit–log transformation to linearize the sigmoid curves. Linear regression analysis was used to calculate pEC_{50} values for each agonist which served for the quantification of the kininlike material in the *Vespula vulgaris* venom.

2.2. Rat paw oedema

Female Sprague–Dawley rats (200–250 g, Forschungsanstalt für Versuchstierzucht, Himberg, Austria) were anaesthetized with pentobarbitone sodium (40 mg/kg, i.p.). At the same time, receptor antagonists were injected s.c. as given below. Thirty minutes later, a subplantar injection of *Vespula vulgaris* venom, bradykinin or [Thr^6]bradykinin was given into one hind paw, whereas the contralateral paw was injected with 50 μl of phosphate-buffered saline. The volume of the paws was determined prior to these injections with a plethysmometer (Ugo Basile, Italy). The measurements were repeated at 5-min intervals during a period of 30 min. The magnitude of the paw oedema was quantified as vol% increase of the agonist-injected paw minus vol% increase of the control paw.

In order to establish the dose–response relationship of the agonists, 5 doses of the agonists were used. The venom of *Vespula vulgaris* was injected at 0.3–30 μg per paw, synthetic bradykinin and [Thr^6]bradykinin were given at 0.3–30 nmol per paw. For the subsequent study using receptor antagonists for inflammatory mediators, only one dose of the venom (10 μg) and of bradykinin and [Thr^6]bradykinin (30 nmol each) was used (see below).

2.3. Treatments

For the exclusion of a heat-sensitive component of the venom, the venom was incubated either for 5 min at 90°C or for 30 min at 70°C. The heated samples were allowed to cool to room temperature before being injected into the rat paws; control animals received subplantar injections of unheated venom instead. Inhibition of a possible release of kinins from the tissue was attempted by pretreatment of rats with soybean trypsin inhibitor (20 mg/kg, i.v.) 20 min prior to the injection of the venom; control animals received an appropriate volume (1 ml/kg) of the solvent, 154 mM NaCl solution, instead. In a further series of experiments, the venom was injected either alone or together with aprotinin (800 KIU).

All receptor antagonists were injected 20 min before the subplantar injections of the venom or the synthetic kinins. Icatibant was injected s.c. at a dose of 30, 100 or 300 nmol/kg, while control rats were received 1 ml/kg of a 154 mM solution of NaCl. Further experimental groups were pretreated i.p. with the H_1 receptor antagonist, mepyramine (20 μmol/kg), the H_2 receptor antagonist, cimetidine (20 μmol/kg), or with the 5-HT_1/5-HT_2 receptor antagonists, ketanserin or methysergide (each at 20 μmol/kg). In order to exclude effects of lowered blood pressure, a separate group of rats was used to monitor systemic blood pressure in a carotid artery by means of a Statham pressure transducer. The α_1-adrenoceptor antagonist phentolamine (7.5 μmol/kg) was injected i.p. to induce a reduction in blood pressure similar to that induced by methysergide.

In order to investigate the involvement of mast cell degranulation, rats were pretreated with compound 48/80. The agent was injected i.p. on 4 consecutive days. During this period one injection of 5 mg/kg was given in the morning of each day, whereas on day 4 a second injection was given in the afternoon. Control rats were injected with equal volumes of a 154 mM NaCl solution. In order to reduce the distress of the animals caused by this treatment, each injection was preceded by pentobarbitone sodium anaesthesia (40 mg/kg, i.p.) and a protective treatment with a combination of mepyramine, cimetidine and methysergide (20 μmol/kg of each, i.p.). The skin content of histamine in the plantar skin of the hind paws was determined by HPLC determination (Skofitsch et al., 1981) in separate groups of rats after homogenisation of the skin (Holzer et al., 1981).

The contribution of primary afferent neurones to the inflammatory effects of the venom and synthetic bradykinin was investigated in rats which were pretreated as neonates (second day) with capsaicin (50 mg/kg, s.c.) for the

irreversible ablation of afferent sensory C-fibres (Holzer, 1981); a control group of rats had been injected with the solvent, 10% (v/v) Tween 80 and 10% (v/v) ethanol in 154 mM NaCl solution, instead.

2.4. Nociceptive behavioural responses

In order to determine the effect of icatibant on nociceptive responses, rats were injected s.c. with icatibant (300 nmol/kg) or with saline (1 ml/kg) and placed in an observation box (30 cm × 30 cm) with nontransparent walls. The behaviour of the animals was observed from below using a mirror. After a period of 10 min to accustom the animals to the new environment, the rats received a subplantar injection of *Vespula vulgaris* venom (50 μg), bradykinin (50 nmol) or [Thr^6]bradykinin (50 nmol) in 50 μl phosphate-buffered saline. The subplantar injections were carried out under brief nitrous oxide analgesia to minimize stress induced by the injections. Then, they were returned to the observation box and behaviour was monitored for a period of 60 min by a person not knowing the nature of the pretreatment of the animals. The behavioural responses were rated with scores (0 = no reaction, 1 = favouring the noninjected contralateral paw, 2 = elevating the injected paw from the ground, 3 = licking or biting the injected paw; Legat et al., 1994) and recorded using a personal computer. For each 1-min period a mean score was calculated. The responses were recorded either in control animals or in rats pretreated with icatibant (300 nmol/kg, s.c.) or with the 5-HT$_3$ receptor antagonist, ondansetron (300 nmol/kg, s.c.).

All animal experiments followed the Principles of Laboratory Animal Care (NIH publication No. 85-23, rev. 1985) and the Austrian Law on Experiments in Living Animals (BGBl. 501/1989), and were granted permission by the Commission for Animal Experiments of the Austrian Ministry for Science.

2.5. Statistical analysis

All values are presented as mean values ± S.E.M. Since in most cases the data did not meet the criteria for parametric evaluation (normal distribution, homoskedasticity), comparisons between different treatment groups were made using rank analysis of variance and multiple nonparametric comparisons (Zar, 1984). Probability values of < 0.05 were considered significant. The dose-dependency of the effects of icatibant on the paw oedema induced by the venom or the synthetic kinins was tested using the Jonckheere test for ordered alternatives (Siegel and Castellan, 1988).

2.6. Drugs

Icatibant (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin; Hyp = L-4-hydroxyproline, Thi = β-(2-thienyl)-L-alanine, D-Tic = D-(1,2,3,4-tetrahydroisoquinoline-2-yl-carboxylic acid), Oic = L-[(3aS,7aS)-octahydroindol-2-yl-carboxylic acid) was provided by Hoechst (Frankfurt/ Main, Germany). Bradykinin, [Thr^6]bradykinin and lyophilized whole venom from *Vespula vulgaris* were obtained from Sigma (St. Louis, MO, USA), Further substances were: 5-hydroxytryptamine (Glaxo, Greenford, UK), histamine, ondansetron and soybean trypsin inhibitor (Sigma), mepyramine and cimetidine (Smith Kline and French, Welwyn Garden City, UK), ketanserin (Janssen, Beerse, Belgium), methysergide (Sandoz, Basel, Switzerland), aprotinin (Trasylol, Bayer, Leverkusen, Germany), capsaicin (Fluka, Buchs, Switzerland), pentobarbitone sodium (Nembutal, Sanofi Santé Nutrition Animale, Libourne, France). The composition of the Tyrode solution was (in mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.15, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, D-glucose 5.6; pH 7.4 when aerated with 5% CO_2 in O_2 at 37°C. Phosphate-buffered saline (mM): NaCl 136.9, KCl 2.7, KH_2PO_4 1.5, Na_2HPO_4 7.7; pH 7.3 at 20°C.

3. Results

3.1. Venom content of histamine, 5-HT and kininlike material

The contents of histamine and 5-HT in the venom of *Vespula vulgaris* were measured by h.p.l.c. analysis and fluorescence or electrochemical detection, respectively. The venom was thus found to contain 10 μg/mg (90 nmol/mg) histamine and 5.4 μg/mg (30 nmol/mg) 5-HT.

The quantification of kininlike material in the venom was performed in an in vitro preparation of the isolated guinea-pig ileum. In the presence of atropine, mepyramine and methysergide, the venom produced concentration-dependent contractions of the ileum when tested in concentrations of 4–10 μg/ml. The contractions elicited by the highest venom concentration amounted to 70% of the bradykinin-induced maximum contractions. Higher concentrations of the venom could not be used due to lack of sufficient amounts of the material. The kininlike nature of the venom component responsible for the contractile activity was confirmed by the concentration-dependent inhibition by the bradykinin B$_2$ receptor antagonist, icatibant. The highest icatibant concentration (300 nM) completely abolished the venom-induced contractions so that the comparison of the concentration–response curve to the wasp venom with those of synthetic bradykinin and [Thr^6]bradykinin could be used for the quantification of kinins in the venom. EC$_{50}$ values for the venom, bradykinin and [Thr^6]bradykinin were 4.4 μg/ml (95% confidence interval 0.4–79 μg/ml), 16.2 nM (0.9–300 nM) and 97.2 nM (9.4–990 nM), respectively. Hence, the content of kininlike material of 1 μg of the venom corresponds to 3.7 pmol bradykinin or 22.1 pmol [Thr^6]bradykinin.

3.2. Rat paw oedema

Following its subplantar injection the whole venom of *Vespula vulgaris* produced a swelling of the rat hind paw which reached its maximum 15–20 min after the injection and remained constant thereafter. Similarly, bradykinin and [Thr^6]bradykinin elicited a paw oedema of the same time course. In previous dose-finding studies, a dose of 10 μg of the venom produced effects of a size similar to that of 30 nmol bradykinin so that these doses were chosen in the subsequent experiments described here. However, during the further course of the study, the rats responded somewhat better to bradykinin. [Thr^6]bradykinin, despite its apparently higher potency compared to bradykinin itself (compare Figs. 1–3), was deliberately used at the same dose as bradykinin (30 nmol).

3.3. Heat inactivation of the venom and effects of protease inhibitors

The paw oedema induced by the venom (10 μg) was not effected by incubation of the venom for 5 min at 90°C ($57 \pm 4\%$ net increase in paw volume, $n = 5$) or for 30 min at 70°C ($58 \pm 4\%$, $n = 5$). Both values were not different from that induced by an unheated sample of the venom ($48 \pm 4\%$, $n = 9$).

Pretreatment with the serine protease inhibitor, soybean trypsin inhibitor (20 mg/kg, i.v.), 15 min prior to the subplantar injection of the venom was ineffective since the ensuing paw oedema ($58 \pm 8\%$, $n = 6$) was equivalent to that observed in control animals injected i.v. with 154 mM NaCl solution ($59 \pm 5\%$, $n = 6$). Similarly, the coinjection of the venom together with aprotinin (800 KIU) resulted in an oedema formation ($57 \pm 6\%$, $n = 5$) which was not significantly different from the paw oedema induced by the venom alone ($42 \pm 4\%$, $n = 5$).

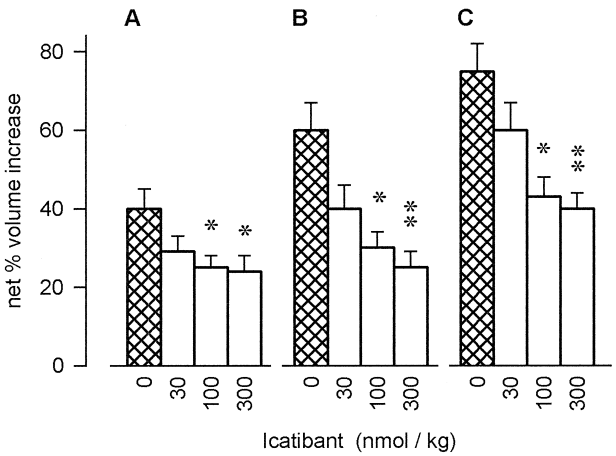

Fig. 1. Effect of icatibant on paw oedema in anaesthetized rats: (A) wasp venom (10 μg), (B) bradykinin (30 nmol) or (C) [Thr^6]bradykinin (30 nmol) was injected s.c. into one hind paw while the contralateral paw was treated with 50 μl phosphate-buffered saline. Icatibant (open columns) was administered s.c. 20 min prior to the experiment in doses given below the columns (in nmol/kg), control animals were injected s.c. with 154 mM NaCl solution (1 ml/kg; cross-hatched columns). Data are given as net percent increase in paw volume 30 min after the subplantar injection of the agonists. Column height represents mean values, vertical lines show S.E.M. Significance of difference from control groups: $*\ P < 0.05$, $**\ P < 0.01$; $n = 6$–9 per group.

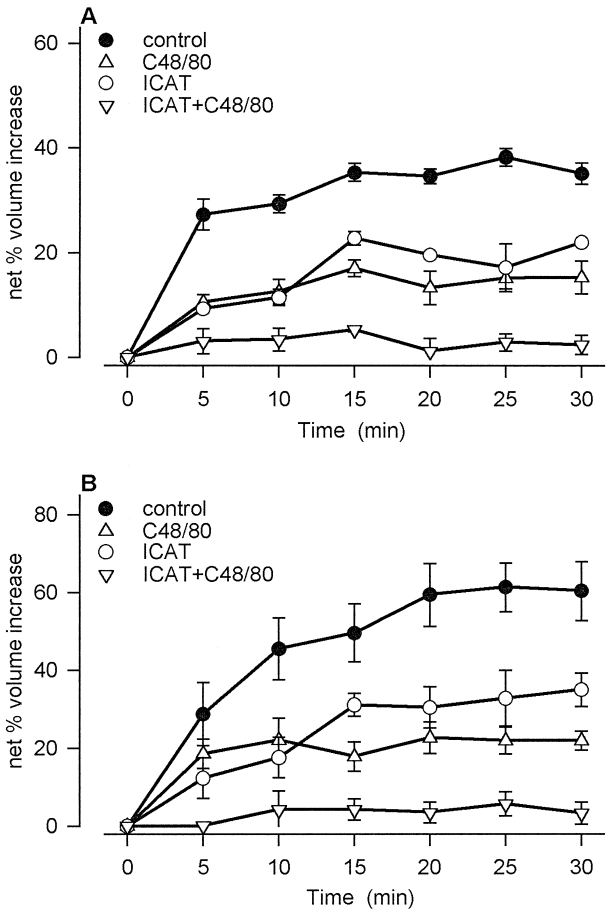

Fig. 2. Effect of compound 48/80 on rat paw oedema induced by subplantar injections of (A) *Vespula vulgaris* venom (10 μg) or (B) bradykinin (30 nmol): compound 48/80 (C48/80) was administered i.p. over 4 days at a total dose of 25 mg/kg while control animals were pretreated with 154 mm NaCl solution (saline). Icatibant (ICAT; 300 nmol/kg), or its vehicle (saline, 1 ml/kg), were injected s.c. 20 min prior to the induction of the paw oedema. Increase in hind-paw volume is given as percent increase in volume of the agonist-injected paw minus contralateral paw injected with 50 μl phosphate-buffered saline. Means ± S.E.M.; $n = 6$–8 per group.

3.4. Effects of B_2 receptor antagonism on paw oedema

The bradykinin B_2 receptor antagonist, icatibant, given s.c. 20 min prior to the experiment at doses of 30–300 nmol/kg, reduced the paw oedema induced by the wasp venom (10 μg), by synthetic bradykinin (30 nmol) or [Thr^6]bradykinin (30 nmol) significantly (Fig. 1). The dose-dependency of the effect of icatibant was confirmed by the Jonckheere test for ordered alternatives ($P < 0.01$). However, the inhibition of the increase in paw volume was incomplete and could not be improved by even higher

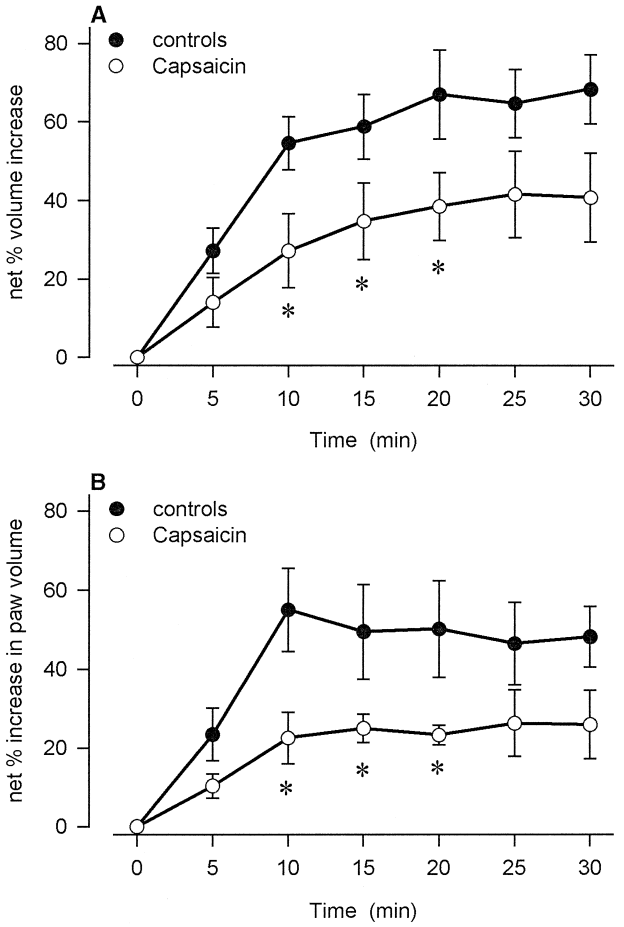

Fig. 3. Effect of neonatal capsaicin treatment on rat paw oedema induced by subplantar injections of (A) *Vespula vulgaris* venom (10 μg) or (B) bradykinin (30 nmol): capsaicin was administered s.c. on the second postpartal day at a dose of 50 mg/kg while control animals were given the vehicle, 10% (v/v) Tween 80 and 10% (v/v) ethanol in a 154-mM NaCl solution. Increase in hind-paw volume is given as percent increase in volume of the agonist-injected paw minus contralateral paw injected with 50 μl phosphate-buffered saline. Means ± S.E.M. Significance of difference from the vehicle control group: $* P < 0.05$; $n = 5$ per group.

doses of icatibant. The maximum inhibition seen with 300 nmol/kg of the B_2 receptor antagonist was 43%, 58% and 46% for the venom, bradykinin and [Thr^6]bradykinin, respectively. Since this dose of icatibant thus can be concluded to produce a complete inhibition of those effects that are mediated by B_2 receptors, this dose was used in the further experiments.

3.5. Effects of histamine receptor antagonists

Pretreatment with the histamine H_1 receptor antagonist, mepyramine (20 μmol/kg, i.p.), given 20 min before the subplantar injections of *Vespula vulgaris* venom, bradykinin or [Thr^6]bradykinin, had no effect on the paw oedema. This lack of effect was observed both in normal rats and in rats pretreated with icatibant (300 nmol/kg, s.c.) (Table 1). The effectivity of histamine H_1 receptor

Table 1
Effect of histamine receptor antagonists on rat paw oedema induced by subplantar injections of *Vespula vulgaris* venom, bradykinin and [Thr^6]bradykinin

Agonist	Paw oedema (net vol% increase)		
	Pretreatment i.p.	Pretreatment s.c.	
		Control	Icatibant
Venom	Control	40 ± 4	18 ± 4
	Mepyramine	38 ± 5	17 ± 5
	Cimetidine	41 ± 3	21 ± 5
Bradykinin	Control	64 ± 7	28 ± 5
	Mepyramine	68 ± 6	30 ± 7
	Cimetidine	66 ± 8	28 ± 8
[Thr^6]bradykinin	Control	79 ± 8	35 ± 4
	Mepyramine	74 ± 7	41 ± 6

Data are presented as means ± S.E.M.; $n = 5$–8 per group.

blockade with mepyramine was tested by investigating the effects of the chosen dose of mepyramine (20 μmol/kg) on rat paw oedema induced by a subplantar injection of histamine (500 nmol). While this oedema amounted to 35 ± 2% ($n = 6$)in control animals, it was completely abolished (1 ± 3%, $n = 6$) by pretreatment with mepyramine. On the other hand, the paw oedema induced by 5 nmol 5-HT (42 ± 6%, $n = 4$) was unaffected by the H_1 receptor antagonist (47 ± 9%, $n = 4$). Since there are some reports of a possible involvement of H_2 receptors in the oedematogenic effects of histamine in the skin of some species (see Section 4), the H_2 receptor antagonist cimetidine (20 μmol/kg, i.p.) was also used. However, cimetidine did not have any effect on the actions of the venom or bradykinin (see Table 1), nor did it affect histamine-induced paw oedema (42 ± 4% paw volume increase, $n = 4$ vs 38 ± 5% in controls, $n = 4$).

3.6. Effects of 5-HT receptor antagonists

Similar to the bradykinin B_2 receptor antagonist, icatibant, the 5-HT receptor antagonists ketanserin and methysergide, each injected at a dose of 20 μmol/kg i.p., reduced the paw oedema determined 30 min after response to subplantar injections of the venom, bradykinin and [Thr^6]bradykinin 40–60% (Table 2). The inhibition by ketanserin and methysergide was significant ($P < 0.05$) from 10 min until the end of the experiment at 30 min. In animals that had been pretreated with the bradykinin receptor antagonist icatibant, the icatibant-resistant part of the oedema was almost abolished by additional pretreatment with ketanserin or methysergide.

Both ketanserin and methysergide have effects on systemic blood pressure. In order to exclude possible unspecific effects of changes in systemic haemodynamics, the hypotensive effects of the 5-HT receptor antagonists were determined. Only icatibant-pretreated rats were used for

Table 2
Effect of 5-hydroxytryptamine receptor antagonists on rat paw oedema induced by subplantar injections of *Vespula vulgaris* venom, bradykinin and [Thr^6]bradykinin

Agonist	Paw oedema (net vol% increase)		
	Pretreatment i.p	Pretreatment s.c.	
		Control	Icatibant
Venom	Control	46 ± 5	25 ± 4
	Ketanserin	22 ± 2^a	3 ± 1^b
	Methysergide	23 ± 3^a	3 ± 2^a
Bradykinin	Control	59 ± 5	29 ± 4
	Ketanserin	24 ± 5^b	5 ± 3^a
	Methysergide	26 ± 7^a	4 ± 2^b
[Thr^6]bradykinin	Control	75 ± 8	30 ± 5
	Ketanserin	35 ± 4^a	8 ± 3^a

Data are presented as means ± S.E.M.; $n = 5–10$ per group.
Significance of difference from animals without 5-HT receptor antagonists: $^aP < 0.05$, $^bP < 0.01$.

this part of the study. While the lowering in blood pressure was more prominent with ketanserin (-59 ± 6 mmHg, $n = 6$) than with methysergide (-35 ± 1, $n = 6$), the fall in blood pressure induced by the latter 5-HT receptor antagonist was still significantly different from that which occurred in rats that had received only icatibant (-10 ± 2 mmHg, $n = 8$). A fall in blood pressure (-32 ± 4 mmHg, $n = 6$) comparable to that observed after methysergide could be mimicked with the α_1-adrenoceptor antagonist phentolamine (7.5 μmol/kg, i.p.). However, the icatibant-resistant part of the oedema formation in response to bradykinin (18 ± 3% paw volume increase, $n = 7$) remained unaffected (16 ± 6%, $n = 5$) by phentolamine. As a further test for possible unspecific inhibitory effects, methysergide was tested against histamine. The paw oedema induced by subplantar injection of 500 nmol histamine (35 ± 2%, $n = 4$) was completely unaffected by methysergide (31 ± 3%, $n = 4$), whereas the oedema caused by 5-HT (42 ± 6%, $n = 5$) was completely abolished (2 ± 3%, $n = 4$).

3.7. Effects of compound 48/80

In order to determine the possible contribution of mediator release from skin mast cells by the venom of *Vespula vulgaris* and by synthetic bradykinin, rats were pretreated with compound 48/80. The i.p. administration of 25 mg/kg compound 48/80, divided into 5 doses administered over 4 days (see Section 2), reduced the histamine levels of the plantar skin of the hindpaws to 1.3 ± 0.1 μg/g wet weight ($n = 6$), while the skin content of histamine in rats pretreated with the vehicle for compound 48/80 (154 mM NaCl solution, 1 ml/kg per injection) was 10.6 ± 0.6 g/g wet weight ($n = 6$) ($P < 0.01$).

The paw oedema caused by the venom of *Vespula vulgaris* was reduced by the pretreatment with compound 48/80 to values of about half of those observed in control animals (Fig. 2A). The differences were significant ($P < 0.05$) throughout the experiment. When the pretreatment with compound 48/80 was combined with an additional treatment with the bradykinin receptor antagonist, icatibant (300 nmol/kg, s.c.), immediately before the subplantar injection of the venom, the remaining part of the oedema was almost completely abolished ($P < 0.05$). The same pattern of inhibition was observed when synthetic bradykinin was used to induce the oedema of the hindpaws: the pretreatments with compound 48/80 and icatibant each caused a reduction of about 40–60% ($P < 0.05$) when given alone, while a combination of the two pretreatments caused an almost complete inhibition of the effect of bradykinin (Fig. 2B).

3.8. Effects of ablation of afferent C-fibres by capsaicin

The increase in vascular permeability measured as increase in paw volume following subplantar injection of the wasp venom was reduced by ablation of primary afferent C-fibres by neonatal capsaicin treatment of the experimental animals (Fig. 3A). However, the difference from values obtained in the control group achieved statistical significance only for the measurements taken at 10, 15 and 20 min. The same effect of capsaicin could be demonstrated for the bradykinin-induced oedema formation (Fig. 3B) where a significant attenuation of the effect could be found between 10 and 20 min.

3.9. Nociceptive behaviour

The doses of *Vespula vulgaris* venom (10 μg), bradykinin (30 nmol) and [Thr^6]bradykinin (30 nmol) which were used in the experiments described above did not consistently produce nociceptive behavioural responses following subplantar injection in unanaesthetized rats. Only when the doses were increased (50 μg of the venom, 50 nmol of bradykinin and [Thr^6]bradykinin) such behaviour (favouring the uninjected paw, elevating or licking the injected paw) could be observed. Following the subplantar injections, nociceptive responses became apparent within the first 2 min (Fig. 4). For all three agents, the behavioural responses lasted for 15–20 min, frequently interrupted by short phases of normal behaviour. Later than 20 min after the injection no behaviour indicative of nociception could be observed. This pattern of behavioural responses was completely prevented when the rats had been pretreated with icatibant (300 nmol/kg, s.c.) 20 min prior to the subplantar injections, i.e., the rats showed normal behaviour (sitting, grooming, or exploring the observation chamber) throughout the observation period of 1 h (only 30 min shown in Fig. 4).

Following the subplantar injection of 5-HT (10 nmol) nociceptive behavioural responses developed more slowly, i.e., within about 5–10 min, and lasted throughout the

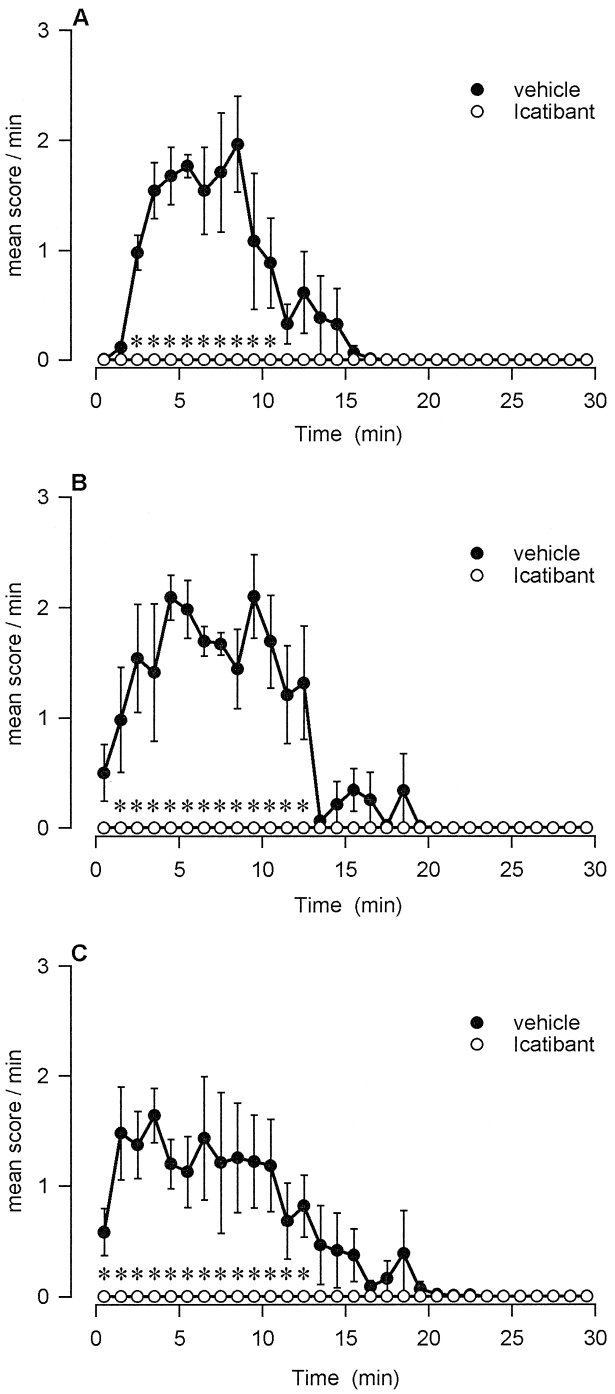

Fig. 4. Nociceptive behavioural responses following subplantar injections of (A) *Vespula vulgaris* venom (50 μg), (B) bradykinin (50 nmol) or (C) [Thr^6]bradykinin (50 nmol) in unanaesthetized rats: The subplantar injections were given under brief nitrous oxide analgesia. Icatibant (300 nmol/kg) was injected s.c. 20 min prior to the injections of the venom or of the kinins; control animals received a 154-mM solution of NaCl (1 ml/kg) instead. The behaviour was rated with scores and mean score values were calculated for each 1-min period (see methods). Mean values ± S.E.M.; where no S.E.M. is given it was smaller than the symbol. Significance of difference to vehicle controls: $* P < 0.05$.; $n =$ 4–6 per group.

Table 3
Effect of the 5-HT$_3$ receptor antagonist, ondansetron, on nociceptive behaviour induced by: subplantar injections of *Vespula vulgaris* venom, bradykinin or 5-HT

Agonist	Pretreatment	Time spent in score (s)		
		1	2	3
Venom	Saline	220 ± 9	4357 ± 45	77 ± 23
	Ondansetron	184 ± 46	305 ± 36	86 ± 26
Bradykinin	Saline	346 ± 88	500 ± 37	30 ± 12
	Ondansetron	266 ± 69	495 ± 42	39 ± 39
5-HT	Saline	292 ± 53	36 ± 9	10 ± 6
	Ondansetron	4 ± 2[a]	8 ± 6[b]	0 ± 0[b]

Data are presented as means ± S.E.M.; $n = 5–6$ per group.
Significance of difference from saline controls: [a]$P < 0.01$, [b]$P < 0.05$.

observation period of 30 min. The responses consisted mainly of signs of slight mechanical hyperalgesia (score 1), whereas signs of more severe nociceptive activation were rare. The 5-HT$_3$ receptor antagonist, ondansetron (300 nmol/kg, s.c.) completely abolished these responses, while those induced by the wasp venom or by bradykinin remained completely unaffected (Table 3).

4. Discussion

Out of the number of pharmacologically active factors that have been described in the literature as components of wasp venoms (see Section 1), we were most interested in the evaluation of the importance of venom kinins for the effects of the venom both in vitro and in vivo. Although an analysis of the exact chemical nature of the kinins contained in the venom of *Vespula vulgaris* clearly is beyond the scope of this investigation, the presence of kininlike material could be verified by bioassay on the guinea-pig ileum. Due to the different activities of different kinin peptides on the preparation, the content of kinins in the venom can only be given as an amount equivalent to those kinins used as reference peptides in the present investigation. The content of 1 μg of *Vespula vulgaris* venom thus was found to be equivalent to 3.7 pmol bradykinin or 22.1 pmol [Thr^6]bradykinin. HPLC analysis of the venom also showed the additional presence of at least histamine (90 nmol/mg) and 5-HT (30 nmol/mg).

In order to investigate the development of the acute inflammatory oedema in response to the venom it was injected subcutaneously into the plantar skin of the hind paws of anaesthetized rats. In this setup, increases in vascular permeability can be induced by kinins, histamine and 5-HT, but also by hyaluronidase (Kalbhen and Smalla, 1977) and phospholipases (Cirino et al., 1989).

The paw oedema induced by the venom clearly involves the action of kinins since it is inhibited by the B$_2$ receptor antagonist, icatibant. However, the kinin content of the

venom (see above) seems to be insufficient to explain the oedema formation. Therefore, a release of kinins from the tissue itself is conceivable, acting either alone or in addition to the kinins contained in the venom. In a first approach, heating of the venom was used to inactivate enzymes in the venom possibly responsible for kinin release such as hyaluronidase or phospholipases. Both types of enzymes are heat-labile (Knepper et al., 1984; Nair et al., 1993). However, neither boiling for 5 min at 90°C nor heating for 30 min at 70°C could reduce the venom-induced paw oedema so that only a heat-insensitive factor in the venom could induce kinin release if it were present. In a second approach, pretreatment of the animals with soybean trypsin inhibitor or coinjection of the venom with aprotinin were used to inhibit kinin release in the tissue. Both regimens have been reported previously to effectively reduce oedema formation by collagenase (Souza-Pinto et al., 1995), trimucase II or cardiotoxin (Wang and Teng, 1988, 1989). However, both treatments were ineffective in the present investigation so that the exact source of the kinins responsible for the icatibant-sensitive part of the paw oedema in response to *Vespula vulgaris* venom must be left open at present.

The observation that the maximum inhibition of the venom-induced oedema achieved by icatibant was only about 50% could at first glance lead to the assumption that the remaining part of the oedema might be due to components of the venom other than kinins. However, a similar limited inhibitory effect of the bradykinin receptor antagonist is also seen when pure synthetic bradykinin or [Thr^6]bradykinin are used to induce the increases in the vascular permeability of the vasculature in the paw skin. Since the extent of inhibition caused by pretreatment with icatibant is identical when the venom or synthetic kinins are used to induce the oedema, the other components of the wasp venom are unlikely to be of relevance because in this case the inhibition caused by the bradykinin receptor antagonist should have been significantly smaller in case of the venom as compared to the synthetic kinins. The lack of full inhibition cannot also be attributed to the chemical nature of icatibant as a peptide since the maximum inhibition of the bradykinin-induced oedema is identical when a new, nonpeptide bradykinin B_2 receptor antagonist, FR173657 ((E)-3-(6-acetamido-3-pyridyl)-*N*-[*N*-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-*N*-methylaminocarbonylmethyl] acrylamide), is used in this model (Griesbacher and Legat, 1997). Therefore, the icatibant-insensitive part of the venom-induced oedema is likely to be due to kinin actions which, however, are not due to bradykinin B_2 receptor activation. The involvement of B_1 receptors, however, is unlikely, since it has been demonstrated that this bradykinin receptor subtype is not involved in the increase in vascular permeability in the rat paw in response to either bradykinin (Whalley, 1987; Campos and Calixto, 1995; Campos et al., 1996) or other agents that induce the release of endogenous kinins in the rat paw (Legat et al., 1994; Décarie et al., 1996) under basal conditions.

If the icatibant-resistant part of the oedema cannot be attributed to any of the accepted bradykinin receptor subtypes, a nonreceptor-mediated action of the kinins is a possible explanation. Such a mechanism has long been suggested for the release of histamine from mast cells in response to various peptides which, like bradykinin, contain basic, especially aromatic, amino acid residues (Devillier et al., 1985; Lawrence et al., 1989) which seem to induce a noncytotoxic activation of G proteins and second messenger mechanisms within the cells (see Hall, 1992). Nevertheless, pretreatment of the rats with the histamine H_1 receptor antagonist, mepyramine, was without any effect. However, it has been suggested that, in some species including the rat, histamine may have effects on the permeability of blood vessels also via H_2 receptors (e.g., Ezeamuzie and Umezurike, 1989). The lack of inhibitory effect of cimetidine as determined here thus finally excludes an involvement of histamine in the oedema-provoking effects of both the wasp venom and also from synthetic kinins, at least in the rat paw skin.

In the rat, mast cells also contain significant amounts of 5-HT (Erspamer, 1966), so that this mediator could serve as explanation for the icatibant-resistant part of the venom- or kinin-induced paw oedema even if histamine is ruled out. Indeed, both ketanserin, an antagonist which is relatively selective for 5-HT_2 receptors (Brogden and Sorkin, 1990), and methysergide, a mixed 5-HT_1/5-HT_2 receptor antagonist (Zifa and Fillion, 1992), almost completely abolished venom-and kinin-induced plasma protein extravasation in icatibant-pretreated animals. Care has to be taken to exclude possible unspecific side effects of both antagonists. Both antagonists have pronounced effects on systemic blood pressure, which may be either a direct effect on vascular 5-HT receptors or, in the case of ketanserin, also an unspecific inhibitory effect at α-receptors (Brogden and Sorkin, 1990). Since methysergide did not affect the histamine-induced paw oedema and reduction of systemic blood pressure by phentolamine also did not change the effects of bradykinin, the present results can confidently be interpreted as evidence for the involvement of 5-HT release.

In order to elucidate the source of the 5-HT apparently released from the tissue, a pretreatment with compound 48/80 was carried out. Compound 48/80 releases both histamine and 5-HT from mast cells (Thon and Uvnäs, 1967; Carlsson and Ritzén, 1969) but apparently has no actions on platelets (Humphrey and Jaques, 1955) which also contain high amounts of 5-HT. The inhibitory effect of compound 48/80 both in normal rats or in rats that were treated with icatibant on the day of the test with the wasp venom or bradykinin confirms that skin mast cells are the most likely source for the 5-HT. In addition the additivity of the effects of compound 48/80 and icatibant further strengthen the view that the release is not mediated

by bradykinin receptors. Whether or not components of the venom other than kinins contribute to the 5-HT release cannot be answered definitely by the present experiments. At least phospholipase A (Cirino et al., 1989) and mastoparan (Nakajima et al., 1985) are known to lead to mast cell degranulation. However, if these factors contributed significantly to the observed effect of the venom, the inhibitory effect of bradykinin receptor blockade with icatibant should be markedly smaller with the venom as compared to the synthetic kinins. Since this is not the case, we conclude that the release of 5-HT is due to a nonreceptor-mediated action of the venom kinins rather than of the other components of the venom. The finding that antihistaminics have no effect while 5-HT receptor antagonists are effective is not an argument against the involvement of mast cells, because despite the likelihood of a release of both amines 5-HT is 30–200 times more potent than histamine in producing oedema when applied subcutaneously (Rowley and Benditt, 1956).

A contribution of primary afferent, capsaicin-sensitive neurones in the mediation of increased vascular permeability have not only been reported for synthetic bradykinin (Arvier et al., 1977) but also for agents that release kinins from the tissue such as collagenase (Legat et al., 1994). In the present investigation a similar mechanism could be demonstrated for the action of the wasp venom. However, as for bradykinin itself, the involvement of afferent neurones seems to be limited to the very early stages of oedema formation. At time points later than 20 min after subplantar injection this mechanism could not be confirmed both for the venom and for synthetic bradykinin.

In contrast to the limited inhibitory action of icatibant on the oedema formation, the bradykinin receptor antagonist completely abolished the nociceptive behavioural effects of the wasp venom. Therefore, this effect of the venom is exclusively due to B_2 receptor activation whereas other components of the venom or nonreceptor-mediated actions of the venom kinins are unlikely to be the cause. However, 5-HT release by any of the components of the venom still could augment the nociceptive effects of the venom kinins since 5-HT has sensitizing properties on afferent neurones (Hong and Abbott, 1994). The effects of 5-HT on afferent neurones should be sensitive to inhibition by 5-HT_3 receptor antagonists. Indeed, collagenase-induced nociceptive responses which are due to endogenous release of kinins from the tissue (Legat et al., 1994) were reported to be largely suppressed by ondansetron (Damas et al., 1997). Nevertheless, since ondansetron could not modify the behavioural responses to the venom an involvement of 5-HT can be ruled out for this symptom of the wasp venom.

The present results show that kinins, either contained in the venom from the wasp *Vespula vulgaris* or released from the tissue, are responsible for both the acute inflammatory and the nociceptive symptoms following s.c. administration in the rat. In contrast, the other components of the venom do not seem to play a significant role in the acute effects of the venom. The inflammatory vascular effects of the whole venom comprise a (B_2) receptor-mediated action on the cutaneous blood vessels and possibly also involving a neurogenic component mediated by primary afferent neurones, as well as a nonreceptor-mediated release of 5-HT from skin mast cells. The pain-related effects of the venom are solely due to direct B_2 receptor activation. Bradykinin receptor antagonists such as icatibant may thus offer a possibility to alleviate pain following wasp stings.

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References

Arvier, P.T., Chahl, L.A., Ladd, R.J., 1977. Modification by capsaicin and compound 48/80 of dye leakage induced by irritants in the rat. Br. J. Pharmacol. 59, 61–68.

Brogden, R.N., Sorkin, E.M., 1990. Ketanserin: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in hypertension and peripheral vascular tissue. Drugs 40, 903–949.

Campos, M.M., Calixto, J.B., 1995. Involvement of B_1 and B_2 receptors in bradykinin-induced rat paw oedema. Br. J. Pharmacol. 114, 1005–1013.

Campos, M.M., Souza, G.E.P., Calixto, J.B., 1996. Upregulation of B_1 receptor mediating des-Arg^9-BK-induced rat paw oedema by systemic treatment with bacterial endotoxin. Br. J. Pharmacol. 117, 793–798.

Carlsson, C., Ritzén, M., 1969. Mast cells and 5-HT. Intracellular release of 5-hydroxytryptamine (5-HT) from storage granules during anaphylaxis or treatment with compound 48/80. Acta Physiol. Scand. 77, 449–464.

Cirino, G., Peers, S.H., Wallace, J.L., Flower, R.J., 1989. A study of phospholipase A_2-induced oedema in rat paw. Eur. J. Pharmacol. 166, 505–510.

Damas, J., Liégeois, J.-F., Bourdon, V., 1997. Involvement of 5-hydroxytryptamine and bradykinin in the hyperalgesia induced in rats by collagenase from clostridium histolyticum. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 566–570.

Décarie, A., Adam, A., Couture, R., 1996. Effects of captopril and icatibant on bradykinin (BK) and des[Arg^9]BK in carrageenan-induced edema. Peptides 17, 1009–1015.

Devillier, P., Drapeau, G., Renoux, M., Regoli, D., 1985. Role of N-terminal arginine in the histamine-releasing activity of substance P, bradykinin and related peptides. Eur. J. Pharmacol. 168, 53–60.

Erspamer, V., 1966. Occurrence of indolealkylamines in nature. In: Erspamer, V. (Ed.), 5-Hydroxytryptamine and Related Indolealkylamines (Handb. Exp. Pharmacol., Vol. 19). Springer, Berlin, pp. 132–181.

Ezeamuzie, C.I., Umezurike, C.C., 1989. Effect of histamine H_2-receptor antagonists on acute inflammatory of the rat paw oedema. J. Pharm. Pharmacol. 41, 261–265.

Griesbacher, T., Legat, F.J., 1997. Effects of FR173657, a non-peptide B_2 antagonist, on kinin-induced hypotension, visceral and peripheral oedema formation and bronchoconstriction. Br. J. Pharmacol. 120, 933–939.

Habermann, E., 1972. Bee and wasp venoms. Science 177, 314–322.

Hall, J.M., 1992. Bradykinin receptors: pharmacological properties and biological roles. Pharmacol. Ther. 56, 131–190.

Hock, F.J., Wirth, K., Albus, U., Linz, W., Gerhards, H.J., Wiemer, G., Henke, S., Breipohl, G., König, W., Knolle, J., Schölkens, B.A., 1991. Hoe 140, a new potent and long acting bradykinin-antagonist: in vitro studies. Br. J. Pharmacol. 102, 769–773.

Holzer, P., 1981. Capsaicin: cellular targets, mechanism of action, and selectivity for thin sensory neurons. Pharmacol. Rev. 43, 143–201.

Holzer, P., Saria, A., Skofitsch, G., Lembeck, F., 1981. Increase in tissue concentrations of histamine and 5-hydroxytryptamine following capsaicin treatment of newborn rats. Life Sci. 29, 1099–1105.

Hong, Y., Abbott, F.V., 1994. Behavioural effects of intraplantar injection of inflammatory mediators in the rat. Neuroscience 63, 827–836.

Humphrey, J.H., Jaques, R., 1955. The release of histamine and 5-HT (serotonin) from platelets by antigen–antibody reactions (in vitro). J. Physiol. 128, 9–27.

Jaques, R., Schachter, M., 1954. The presence of histamine, 5-hydroxytryptamine and a potent, slow contracting substance in wasp venom. Br. J. Pharmacol. Chemother. 9, 53–58.

Kalbhen, D.A., Smalla, H.D., 1977. Pharmakologische Studien zur antiphlogistischen Wirkung von Pentosanpolysulfat in Kombination mit Metamizol. Arzneimittelforschung 27, 1050–1057.

Knepper, X., Farbman, A.I., Telser, A.G., 1984. Exogenous hyaluronidases and degradation of hyaluronic acid in the rabbit eye. Invest. Ophthalmol. Vis. Sci. 25, 286–293.

Lawrence, I.D., Warner, J.A., Cohan, V.L., Lichtenstein, L.M., Kagey-Sobotka, A., Vavrek, R.J., Stewart, J.M., Proud, D., 1989. Induction of histamine release from human skin mast cells by bradykinin analogs. Biochem. Pharmacol. 38, 227–233.

Legat, F.J., Griesbacher, T., Lembeck, F., 1994. Mediation by bradykinin of rat paw oedema induced by collagenase from *Clostridium histolyticum*. Br. J. Pharmacol. 112, 453–460.

Lembeck, F., Griesbacher, T., Eckhardt, M., Henke, S., Breipohl, G., Knolle, J., 1991. New, long-acting, potent bradykinin antagonists. Br. J. Pharmacol. 102, 297–304.

Nair, X., Nettleton, D., Clever, D., Tramposch, K.M., Ghosh, S., Franson, R.C., 1993. Swine as a model of skin inflammation. Phospholipase A_2-induced inflammation. Inflammation 17, 205–215.

Nakajima, T., Yasuhara, T., Uzu, S., Wakamatsu, K., Miyazawa, T., Fukuda, K., Tsukamoto, Y., 1985. Wasp venom peptides; wasp kinins, new cytotrophic peptide families and their physicochemical properties. Peptides 6 (Suppl. 3), 425–430.

Piek, T., Buitenhuis, A., Simonthomas, R.T., Ufkes, J.G.R., Mantel, P., 1983a. Smooth muscle contracting compounds in the venom of *Megascolia flavifrons* (Hym: scoliidae) with notes on the stinging behaviour. Comp. Biochem. Physiol. 75C, 145–152.

Piek, T., Buitenhuis, A., Veldsema-Currie, R.D., Mantel, P., 1983b. Smooth muscle contracting compounds in venoms of sphecid wasps (Hym: sphecidae). Comp. Biochem. Physiol. 75C, 153–159.

Rowley, D.A., Benditt, E.P., 1956. 5-Hydroxytryptamine and histamine as mediators of vascular injury by agents which damage mast cells in rats. J. Exp. Med. 103, 399–411.

Sarangi, B., Kar, P.K., Datta, A., Gomes, A., Lahiri, S.C., 1990. Isolation, partial purification and pharmacodynamics of a slow contractile substance in the venom sac extract of the wasp *Vespa cincta* Fabr. Indian J. Exp. Biol. 28, 557–561.

Schachter, M., 1968. Introduction to kinins: a group of vasoactive kinins. Fed. Proc. 27, 49–51.

Schmidt, J.O., Blum, M.S., Overal, W.L., 1986. Comparative enzymology of venoms from stinging Hymenoptera. Toxicon 24, 907–921.

Siegel, S., Castellan, N.J., Jr., 1988. Nonparametric Statistics for the Behavioural Sciences, 2nd edn. McGraw-Hill, New York.

Skofitsch, G., Saria, A., Holzer, P., Lembeck, F., 1981. Histamine in tissue: determination by high-performance liquid chromatography after condensation with *o*-phthaldialdehyde. J. Chromatogr. 226, 53–59.

Souza-Pinto, J.C., Remacle-Volon, G., Sampaio, C.A.M., Damas, J., 1995. Collagenase-induced oedema in the rat paw and the kinin system. Eur. J. Pharmacol. 274, 101–107.

Sperk, G., 1982. Simultaneous determination of serotonin, 5-hydroxyindoleacetic acid, 3,4-dihydroxyphenylacetic acid and homovanillic acid by high performance liquid chromatography with electrochemical detection. J. Neurochem. 38, 840–843.

Thon, I.L., Uvnäs, B., 1967. Degranulation and histamine release: two consecutive steps in the response of rat mast cells to compound 48-80. Acta Physiol. Scand. 71, 303–315.

Wang, J.P., Teng, C.M., 1988. Rat paw edema induced by trimucase II, a kinin-releasing enzyme from *Trimeresurus mucrosquamatus* venom. Eur. J. Pharmacol. 157, 61–66.

Wang, J.P., Teng, C.M., 1989. Roles of mast cells and PMN leukocytes in cardiotoxin-induced rat paw edema. Eur. J. Pharmacol. 161, 9–18.

Watala, C., Kowalczyk, J.K., 1990. Hemolytic potency and phospholipase activity of some bee and wasp venoms. Comp. Biochem. Physiol. C 97, 187–194.

Whalley, E.T., 1987. Receptors mediating the increase in vascular permeability to kinins: comparative studies in rat, guinea-pig and rabbit. Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 99–104.

Wirth, K., Hock, F.J., Albus, U., Linz, W., Alpermann, H.G., Anagnostopoulos, H., Henke, S., Breipohl, G., Knolle, J., Schölkens, B.A., 1991. Hoe 140, a new potent and long acting bradykinin-antagonist: in vivo studies. Br. J. Pharmacol. 102, 774–777.

Yasuhara, T., Mantel, P., Nakajima, T., Piek, T., 1987. Two kinins isolated from an extract of the venom reservoirs of the solitary wasp *Megascolia flavifrons*. Toxicon 25, 527–535.

Zar, J.H., 1984. Biostatistical Analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ.

Zifa, E., Fillion, G., 1992. 5-Hydroxytryptamine receptors. Pharmacol. Rev. 44, 401–458.