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European Journal of Pharmacology 399 (2000) 235–242

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Involvement of kinins, mast cells and sensory neurons in the plasma exudation and paw oedema induced by staphylococcal enterotoxin B in the mouse

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Received 6 December 1999; received in revised form 15 May 2000; accepted 19 May 2000

Abstract

Intraplantar injection of staphylococcal enterotoxin B induces long-lasting oedema mediated by both cyclooxygenase and lipoxygenase products as well as by neuropeptides from sensory nerves. This study was undertaken to further clarify the role of peripheral primary afferent sensory nerves in staphylococcal enterotoxin B (25 μg/paw)-induced plasma extravasation and oedema formation. The tachykinin NK_1 receptor antagonist (*S*)-1-[2-[3-(3,4-dichlorophenyl)-1 (3-isopropoxyphenylacetyl)piperidin-3-yl] ethyl]-4-phenyl-1 azoniabicyclo [2.2.2]octane cloride (SR140333; 120 nmol/kg, s.c. + 120 nmol/kg, i.v.) significantly inhibited plasma exudation and paw oedema evoked by staphylococcal enterotoxin B. The tachykinin NK_2 receptor antagonist (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]-benzamide (SR48968) had no effect on the staphylococcal enterotoxin B-induced responses. The bradykinin B_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin (Hoe 140; 400 nmol/kg, i.v.) significantly reduced staphylococcal enterotoxin B-induced responses. The magnitude of the inhibition observed with Hoe 140 alone was similar to that caused by concomitant treatment of animals with SR140333 and Hoe 140, suggesting that there is a final common pathway. Additionally, SR140333 given alone reduced bradykinin (3 nmol/paw)-induced paw oedema. The vanilloid receptor antagonist *N*-[2-(4-chlorophenyl) ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2*H*-2-benzazepine-2-carbothioamide (capsazepine; 100 μmol/kg) significantly reduced staphylococcal enterotoxin B-induced responses. The 5-HT receptor antagonist methysergide (10 mg/kg, i.v.) and the histamine H_1 receptor antagonist mepyramine (10 mg/kg, i.v.) produced a significant reduction in paw oedema whereas plasma exudation was reduced only by methysergide. In diabetic mice, exudation and oedema evoked by staphylococcal enterotoxin B were markedly reduced. Acute administration of insulin (20 UI/kg, s.c., 30 min before) did not restore the increased permeability induced by staphylococcal enterotoxin B. We conclude that plasma exudation and paw oedema in response to staphylococcal enterotoxin B are a consequence of a complex neurogenic response involving direct activation of vanilloid receptors on sensory nerves, release of kinins and subsequent activation of bradykinin B_2 receptors at a prejunctional level, and direct or indirect degranulation of mast cells.

Keywords: Staphylococcal enterotoxin B; Kinin; Mast cell; Neurogenic inflammation; Tachykinin NK_1 receptor; Vanilloid receptor

1. Introduction

Enterotoxins secreted by *Staphylococcus aureus* have been implicated in several pathological processes including inflammatory skin diseases (eczema, dermatitis, psoriasis) and foodborne debilitating enteric intoxication (Aly et al., 1997; Lever et al., 1988). They consist of at least five different types of single-chain proteins, designated as enterotoxin A, B, C, D and E based on their immunological reactions (Bergdoll, 1979). The administration of *Staphylococcus* enterotoxins to monkeys or human volunteers causes emesis and diarrhea, the classical symptoms of food poisoning (Bergdoll, 1970). Staphylococcal enteropathogenic manifestations are believed to be a consequence of the binding of enterotoxins to intestinal cells via receptors, followed by the release of neuropeptides (substance P) from sensory neurons (Micusan and Thibodeau, 1993). Furthermore, the skin reactions caused by staphylococcal enterotoxin B in monkeys are likely to be triggered by degranulation of cutaneous mast cells in response to the release of substance P from sensory neurons (Gottfried et al., 1989). In the mice hind paw, the intraplantar injection

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00375-7

of staphylococcal enterotoxin B induces long-lasting oedema, which is mediated by both lipoxygenase (early phase) and cyclooxygenase (late phase) products (Desouza et al., 1996). Additionally, the early phase is significantly inhibited by 8-methyl *N*-vanillyl-6-nonenamide (capsaicin; Desouza et al., 1996), a well-known substance used to deplete neuropeptides from sensory nerves (Jancsó et al., 1967).

The chemical mediators involved in the acute inflammatory process evoked by staphylococcal enterotoxin B in monkeys and human volunteers seem to be similar to some of those observed in the mouse (Miethke et al., 1992; DeSouza et al., 1996). Using the mouse paw oedema model, the present study was undertaken to further clarify the role of peripheral primary afferent sensory nerve terminals in staphylococcal enterotoxin B-induced plasma extravasation and oedema formation by using mice pretreated with the pre-junctional vanilloid receptor antagonist *N*-[2-(4-chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2-*H*-2-benzazepine-2-carbothioamide (capsazepine). The possibility that neuropeptides such as substance P, the putative mediator of neurogenic inflammation, are involved in the staphylococcal enterotoxin B inflammatory response was also investigated using both post-junctional tachykinin NK_1 (*S*)-1-[2-[3-(3,4-dichlorophenyl)-1(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl]-4-phenyl-1azoniabicyclo[2.2.2]octane cloride (SR140333; Emonds-Alt et al., 1993) and NK_2 (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]-benzamide (SR48968; Emonds-Alt et al., 1992) receptor antagonists. The participation of mast cells and kinins was investigated by using histamine H_1 and 5-HT receptor antagonists as well as the selective bradykinin B_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin (Hoe 140; Wirth et al., 1991).

2. Materials and methods

2.1. Measurement of paw oedema and vascular permeability assay

All experiments were in accordance with the guidelines for animal care of the State University of Campinas (UNICAMP). The experimental procedures were performed on adult male Swiss mice (25–30 g) obtained from Central Animal House (CEMIB-UNICAMP). The animals received an intraplantar injection of staphylococcal enterotoxin B (0.05 ml) or test agents (0.05 ml) in the left hind paw. The contralateral paw of each mouse was injected with saline (0.05 ml/paw) and served as control. Ninety minutes thereafter, the animals were intravenously injected with Evans blue (0.25% (w/v)/g of body weight). Thirty minutes later, the animals were killed and paw oedema (Levy, 1969) and dye exudate (Gamsé et al., 1980) were measured. Briefly, animal paws were amputed at the tarsocrural joint and weighed on an analytical balance. The oedema (expressed in milligram) was evaluated as the weight difference between the left (treated) and right (untreated) paws. Subsequently, each paw was chopped into small pieces and placed in a test tube with formamide (3 ml) and then incubated in a water bath (57°C) for 24 h. The absorbance of the supernatants was measured at 619 nm and the concentration of Evans blue present in the extracts was determined from a standard curve of the dye prepared in formamide.

2.2. Alloxan diabetes induction

Male mice were fasted for 24 h prior to intravenous injection of alloxan (65 mg/kg) into the tail vein (Ptak et al., 1975). The animals were then allowed access to normal chow and saline (0.9%), and were used 6 days later. The haematocrit values and glucose levels were determined in animals fasted for 12 h. Only animals with a blood glucose level exceeding 250 mg/dl were used. In insulin (20 UI/kg; s.c.)-pretreated animals, the haematocrit and glucose levels were measured 2.5 h after drug administration.

2.3. Drug treatments

The efficacy of treatment with antagonists of the pre-junctional vanilloid receptor (capsazepine; 100 μmol/kg, s.c.; 1 h before; Perkins and Campbell, 1992) and of postjunctional tachykinin NK_1 (SR140333, 120 nmol/kg; s.c. + 120 nmol/kg; i.v., 10 min before; Pinter et al., 1999) and NK_2 (SR48968, 1.7 μmol/kg; i.v., 10 min before; Inoue et al., 1997) receptors was based on previous studies as well as on the use of appropriate agonists including the vanilloid receptor agonist capsaicin (5 μg/paw; Szikszay et al., 1998) and the tachykinin NK_1 receptor agonist D-Ala-[L-Pro^9,Me-Leu^8]substance P-(7-11) (GR73632, 50 pmol/paw; Santos and Calixto, 1997), respectively. The involvement of bradykinin (3 nmol/paw; Tratsk et al., 1997) in the staphylococcal enterotoxin B-induced responses was assessed by using the selective bradykinin B_2 receptor antagonist Hoe 140 (400 nmol/kg; i.v., 10 min before; Costa et al., 1996). The participation of mast cells was evaluated by using both the histamine H_1 receptor antagonist mepyramine (10 mg/kg; i.v., 10 min before; De Bie et al., 1998) and the 5-HT receptor antagonist methysergide (10 mg/kg; i.v., 10 min before; Moura et al., 1998). Both histamine and 5-HT (100 μg/paw each; Sampaio et al., 1995) served as positive controls. In both diabetic and non-diabetic animals, insulin was administered 30 min before staphylococcal enterotoxin B (Ottlecz et al., 1976). Drugs were injected in the tail vein in awake animals using a box restraint.

2.4. Enterotoxin and drugs

Highly purified staphylococcal enterotoxin B (provided by Sigma, St. Louis, MO, USA) was dissolved in saline

(0.9%) and stored at −20°C at a concentration of 1 mg/ml before use.

Bradykinin, Evans blue, histamine, 5-HT, mepyramine and alloxan were purchased from Sigma. GR73632 was a gift from Dr Beattie, Glaxo Group Research (Ware, UK). SR140333 and SR48968 were provided by Dr. Emonds-Alt at Sanofi (Montpellier, France). Capsazepine and Hoe 140 were obtained from Research Biochemical (USA) and Hoechst (Frankfurt, Germany), respectively. Insulin (Monotard MC*) was obtained from Novo Nordisk (Araucária, Brazil). Methysergide and Formamide were obtained from Sandoz (Bale, Swiss) and Merck (Darmstadt, Germany), respectively.

2.5. Statistical analysis

The data are presented as the means ± S.E.M. for five to eight animals. Statistical analyses were done using analysis of variance (ANOVA) followed by the Bonferroni test or Student's unpaired *t*-test where appropriate. Values of $P < 0.05$ were considered as significant.

3. Results

3.1. Effect of SR140333 and SR48968

Intraplantar injection of staphylococcal enterotoxin B (6.25–50 μg/paw) in the mouse hind-paw caused a dose- and time-dependent plasma exudation and paw oedema ($n = 5$; not shown). For further studies, staphylococcal enterotoxin B was administered at the dose of 25 μg/paw. Ninety minutes thereafter, the animals were intravenously injected with Evans blue and 30 min later paw oedema and dye exudate were evaluated.

Fig. 1 shows that treatment of the animals with the tachykinin NK_1 receptor antagonist SR140333 (120 nmol/kg, s.c. + 120 nmol/kg, i.v.; 10 min before) significantly ($P < 0.05$) inhibited both plasma exudation and paw oedema evoked by staphylococcal enterotoxin B. At this dose, SR140333 markedly reduced the plasma exudation (1.2 ± 0.04 and 0.15 ± 0.15 μg/g, Evans blue for control and treated animals, respectively) and paw oedema (20.0 ± 0.01 and 12.0 ± 2.0 mg for control and treated animals, respectively) induced by the tachykinin NK_1 receptor agonist GR73632 (50 pmol/paw).

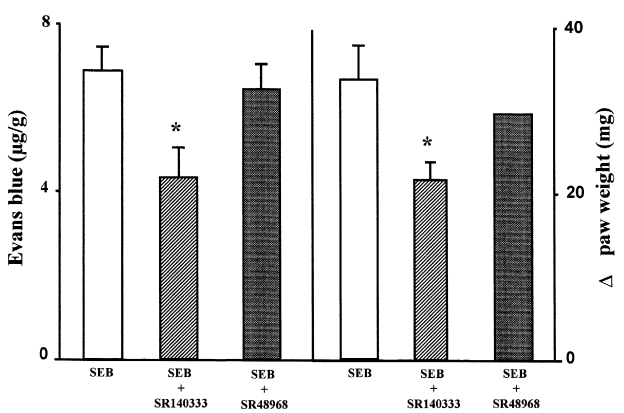

Fig. 1. Effect of tachykinin NK_1 (SR140333) and NK_2 (SR48968) receptor antagonists on plasma exudation and oedema induced by staphylococcal enterotoxin B (SEB). Both SR140333 (120 nmol/kg, i.v. + 120 nmol/kg, s.c.) and SR48968 (1.7 μmol/kg, i.v.) were administered 10 min before intraplantar injection of staphylococcal enterotoxin B (SEB; 25 μg/paw). The bars are the means ± S.E.M. of $n = 5$ for each group. * $P < 0.05$ compared to staphylococcal enterotoxin B (SEB) alone.

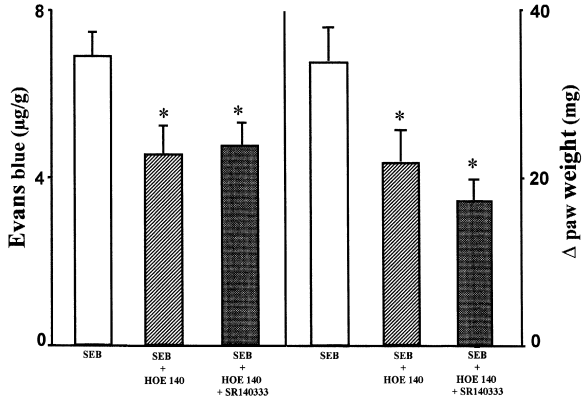

Fig. 2. Effect of bradykinin B_2 receptor antagonist (Hoe 140) given alone or combined with SR140333 on staphylococcal enterotoxin B (SEB)-induced plasma exudation and paw oedema. Both Hoe 140 (400 nmol/kg; i.v.) and SR140333 (120 nmol/kg, i.v. + 120 nmol/kg, s.c.) were administered 10 min before staphylococcal enterotoxin B injection (25 μg/paw). Bars represent the means ± S.E.M. of $n = 5$ for each group. * $P < 0.05$ compared to untreated animals.

The tachykinin NK_2 receptor antagonist SR48968 (1.7 μmol/kg, i.v.; 10 min before) affected neither plasma extravasation nor paw oedema induced by staphylococcal enterotoxin B (Fig. 1). This compound also had no effect on the plasma exudation and paw oedema evoked by GR73632 (50 pmol/paw; not shown).

3.2. Effect of Hoe 140 and SR140333

Fig. 2 shows that both plasma exudation and paw oedema evoked by staphylococcal enterotoxin B were significantly reduced by the bradykinin B_2 receptor antagonist Hoe 140 (400 nmol/kg, i.v.; 10 min before). The simultaneous treatment of the animals with Hoe 140 and SR140333 (same doses as described above) substantially inhibited staphylococcal enterotoxin B-induced plasma exudation and paw oedema (Fig. 2). However, this inhibition was similar to that observed when the antagonists were administered either drug alone. At the dose used, Hoe 140 markedly inhibited bradykinin (3 nmol/paw)-induced plasma exudation and paw oedema (Fig. 3). In addition, SR140333 given alone markedly inhibited bradykinin-induced responses (Fig. 3).

3.3. Effect of capsazepine

The vanilloid receptor antagonist capsazepine (100 μmol/kg, s.c.; 1 h before) significantly reduced plasma

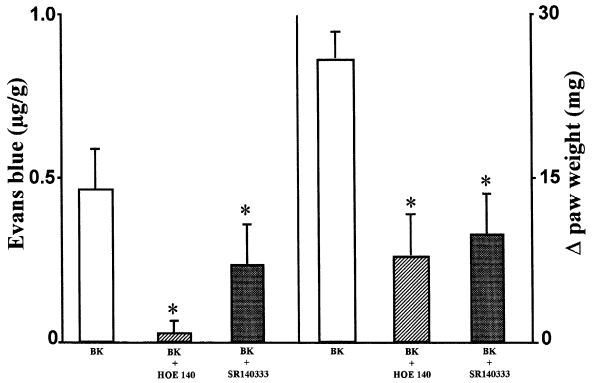

Fig. 3. Effect of treatment with Hoe 140 (400 nmol/kg; i.v.) or SR140333 (120 nmol/kg, i.v. + 120 nmol/kg, s.c.) on bradykinin (BK)-induced plasma exudation and paw oedema. Both oedema and exudation were measured 2 h after bradykinin (BK). The bars are the means ± S.E.M. of $n = 5$ for each group. * $P < 0.05$ compared to bradykinin (BK) alone.

Table 1
Effect of mepyramine (histamine H_1 receptor antagonist) and methysergide (5-hydroxytryptamine receptor antagonist) on plasma exudation and paw oedema evoked by staphylococcal enterotoxin B in the mouse paw. Mepyramine (10 mg/kg; i.v.) and methysergide (10 mg/kg; i.v.) were administered 10 min before staphylococcal enterotoxin B injection (25 μg/paw), histamine or 5-hydroxytryptamine (5-HT; 100 μg/paw). The data represent the means ± S.E.M. of $n = 5$ for each group

Treatment	Exudation (μg/g)	Oedema (mg)
Staphylococcal enterotoxin B	9.4 ± 0.9	40.0 ± 4.0
Staphylococcal enterotoxin B + mepyramine	7.0 ± 1.2	24.2 ± 2.5 *
Staphylococcal enterotoxin B + mepyramine	3.5 ± 0.7 *	16.0 ± 2.4 *
Histamine	0.7 ± 0.2	35.5 ± 1.7
Histamine + mepyramine	0.1 ± 0.1 *	25.0 ± 2.9 *
5-HT	1.5 ± 0.3	40.0 ± 2.9
5-HT + methysergide	0.3 ± 0.2 *	30.0 ± 4.1 *

* $P < 0.05$ compared to their respective controls.

exudation and paw oedema evoked by staphylococcal enterotoxin B (Fig. 4). At this dose, capsazepine produced a significant reduction ($P < 0.05$) in the capsaicin (5 μg/paw)-induced paw oedema and plasma exudation (67% and 72% reduction, respectively).

3.4. Effect of mepyramine and methysergide

The histamine H_1 receptor antagonist mepyramine (10 mg/kg, i.v.; 10 min before) significantly ($P < 0.05$) inhibited plasma exudation and paw oedema formation induced by histamine (100 μg/paw). Similarly, the 5-HT receptor antagonist methysergide (10 mg/kg, i.v.; 10 min before) caused a marked reduction in the 5-HT (100 μg/paw)-induced oedematogenic responses in the mouse hind paw (Table 1). At the doses used, methysergide produced a significant inhibition of the staphylococcal enterotoxin B-induced plasma exudation and oedema formation (Table 1). Mepyramine caused a significant reduction of the staphylococcal enterotoxin B-induced oedema whereas the plasma exudation was not significantly affected (Table 1).

3.5. Effect of staphylococcal enterotoxin B on diabetic animals

In diabetic animals, the plasma exudation and oedema formation induced by either staphylococcal enterotoxin B or capsaicin were markedly reduced ($P < 0.05$) compared to those in non-diabetic animals (Table 2). Pretreatment of the animals with insulin (20 IU/kg, s.c.) prevented the hyperglycaemia in the diabetic animals (85 ± 7 mg/dl; $n = 8$) when compared to untreated diabetic mice (417 ± 9 mg/dl; $n = 8$). However, insulin therapy failed to restore the inflammatory response to staphylococcal enterotoxin B or capsaicin (Table 2).

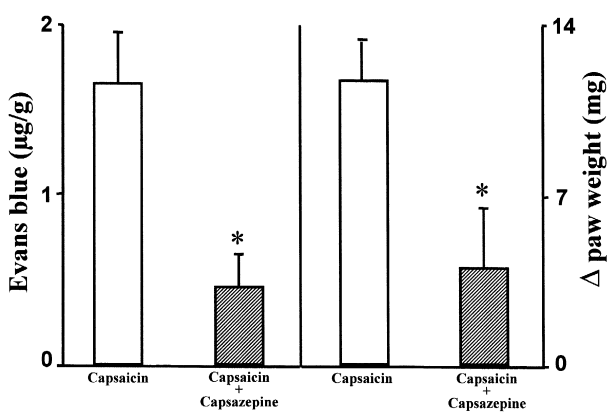

Fig. 4. Effect of pretreatment with the capsaicin receptor antagonist, capsazepine (100 μmol/kg; s.c., 60 min), on staphylococcal enterotoxin B (SEB; 25 μg/paw)-induced plasma exudation and paw oedema. The bars are the means ± S.E.M. of $n = 5$ for each group. * $P < 0.05$ compared to staphylococcal enterotoxin B (SEB) alone.

Table 2
Lack of effect of insulin on staphylococcal enterotoxin B-induced plasma exudation and oedema formation in both diabetic and non-diabetic mice. Staphylococcal enterotoxin B (25 μg/paw)- and capsaicin (5 μg/paw)-induced responses were evaluated 2.5 h after insulin administration (20 IU/kg, s.c.). The data are the means ± S.E.M. of $n = 8$ for each group

Groups	Capsacin		Staphylococcal enterotoxin B	
	Exudation (μg/g)	Oedema (mg)	Exudation (μg/g)	Oedema (mg)
Non-diabetic	6.5 ± 0.6	25.5 ± 1.8	7.4 ± 1.2	44.3 ± 3.7
Diabetic	1.5 ± 0.4 *	11.2 ± 2.9 *	3.7 ± 0.6 *	15.0 ± 2.2 *
Non-diabetic + insulin	5.8 ± 1.3	23.3 ± 4.9	5.5 ± 0.5	35.7 ± 2.9
Diabetic + insulin	1.7 ± 0.3 *	18.3 ± 1.7 *	2.7 ± 0.7 *	12.5 ± 4.9 *

* $P < 0.05$ compared to non-diabetic animals.

4. Discussion

This study shows that the mechanisms underlying plasma exudation and oedema formation in response to intraplantar injection of staphylococcal enterotoxin B in mice are complex and involve different pathways such as (i) local generation of kinins and activation of bradykinin B_2 receptors at the prejunctional level, (ii) activation of vanilloid receptors in sensory nerves and (iii) mast cell degranulation and subsequent release of histamine and 5-HT.

Among the mechanisms and systems that exert their effects rapidly, neuropeptide release (substance P, neurokinin A and calcitonin gene-related peptide) from capsaicin-sensitive primary afferent neurons endings plays a major role in the response to tissue injury (Holzer, 1991, 1998). Once released into the skin, these neuropeptides evoke tissue-specific responses such as vasodilatation, increased vascular permeability, plasma protein leakage and oedema formation, which characterise neurogenic inflammation (Maggi and Meli, 1988; Holzer, 1998). In rodent skin, plasma extravasation evoked by exogenous or endogenous substance P is mainly mediated by tachykinin NK_1 receptors on post-capillary venules (Brain and Williams, 1989; Holzer, 1998). In this study, we used both selective tachykinin NK_1 (SR140333) and NK_2 (SR48968) receptor antagonists to investigate the contribution of endogenous tachykinins (i.e. substance P and neurokinin A) to staphylococcal enterotoxin B-induced plasma exudation and oedema formation. The finding that SR140333 partially inhibited the staphylococcal enterotoxin B-induced responses in the mouse paw is indicative that sensory nerve activation and subsequent release of a tachykinin NK_1 receptor agonist are involved in staphylococcal enterotoxin B-induced plasma exudation and oedema, thus suggesting a neurogenic mechanism. The tachykinin NK_2 receptors do not appear to play a role since SR48968 failed to affect this response. This is expected because tachykinin NK_1 rather than tachykinin NK_2 receptors have been implicated in the pro-inflammatory actions of substance P in the periphery (Quartara and Maggi, 1997, 1998).

An array of endogenous agents, including bradykinin and mast cell contents, are also potentially able to release neuropeptides, amplifying their inflammatory actions (Gamsé et al., 1981; Maggi and Meli, 1988; Geppetti, 1993; Geppetti et al., 1990). Although the mechanisms by which bradykinin activates sensory nerves are controversial, some evidence suggests that it activates peripheral sensory neurons endings via bradykinin B_2-receptors coupled to a second messenger system (Dray and Perkins, 1988, 1993). Kinins, especially bradykinin and Lys-bradykinin, are released from either high-molecular or low-molecular weight kininogen through the enzymatic action of serine proteases, namely, tissue kallikreins or plasma kallikreins (Regoli and Barabé, 1980). The former preferentially forms Lys-bradykinin from low-molecular kininogen whereas the latter forms bradykinin from high-molecular kininogen (Vogel, 1979). To study the involvement of kinin-induced sensory nerve stimulation and the subsequent release of tachykinins in staphylococcal enterotoxin B-induced inflammatory responses, we used the selective bradykinin B_2 receptor antagonist Hoe 140. This compound caused a significant reduction in the plasma exudation and paw oedema induced by staphylococcal enterotoxin B, suggesting that they depend, at least in part, on the local formation of kinins. The observation that the magnitude of the inhibition induced by Hoe 140 alone was similar to that caused by the combination of SR140333 and Hoe 140 indicates that kinins and tachykinins do not act independently to produce plasma exudation and paw oedema in response to staphylococcal enterotoxin B, but rather they act through a final common pathway. This is consistent with the suggestion that, in mice, the local generation of kinins evoked by staphylococcal enterotoxin B stimulates the release of a tachykinin NK_1 receptor agonist (substance P) from nerve endings of capsaicin-sensitive primary afferent neurons. This is reinforced by our findings that bradykinin-induced oedema and plasma exudation were significantly inhibited by SR140333 alone in mice. However, it is important to note that although a number of studies showed that SR140333 is highly selective for tachykinin NK_1 receptors (Emonds-Alt et al., 1993; Amann et al., 1995; Palframan et al., 1996; Ridger et al., 1997), a recent study reported that SR140333 inhibited bradykinin-induced plasma extravasation in the rat by non-specific mechanisms (Cellier et al., 1999).

It has been demonstrated that bradykinin- and substance P-induced plasma extravasation results from a direct action of the peptide on the endothelium of postcapillary venules and on the release of histamine and 5-HT from mast cells (Kowalski et al., 1990; Walsh et al., 1995). This suggests that mast cell degranulation by these peptides may account for the inflammatory actions of staphylococcal enterotoxin B, a proposal corroborated by our findings that treatment of the animals with methysergide and mepyramine significantly inhibited these inflammatory responses. Consistent with this, a previous study showed that staphylococcal enterotoxin B releases 5-HT from rodent mast cell cultures and this is greatly enhanced by bradykinin (Komisar et al., 1992). Whether 5-HT and bradykinin interact in vivo, leading to an amplification of the increased vascular permeability and oedema induced by staphylococcal enterotoxin B, is yet to be elucidated. Cationic peptides including substance P and bradykinin are believed to degranulate mast cells due to the positive charge of their N-terminal arginine residue, an effect independent of receptor activation (Devillier et al., 1989; Holzer, 1992). However, the presence of tachykinin NK_1 and NK_2 receptors in mouse mast cells and their involvement in histamine release has been reported (Krumins and Broomfield, 1992). We can not ascertain from our present study whether in vivo mast cell degranulation by staphylococcal enterotoxin B is due

to direct activation through cationic charge or via selective tachykinin receptors on the mast cell surface. Additionally, we have no evidence to suggest that the remaining responses induced by staphylococcal enterotoxin B in the presence of mepyramine and methysergide reflect activation of sensory fibers.

We finally focused our attention on the role played by vanilloid receptors on the staphylococcal enterotoxin B-induced stimulation of sensory neurons, by using the vanilloid receptor antagonist capsazepine (Bevan et al., 1991). The observation that the oedematogenic response evoked by the vanilloid receptor agonist capsaicin was significantly inhibited by capsazepine corroborates previous findings (Perkins and Campbell, 1992; Fox et al., 1995) and indicates the efficacy of the dose used. Similarly, capsazepine markedly reduced the staphylococcal enterotoxin B-induced plasma exudation and oedema, suggesting that vanilloid receptors are the main route by which staphylococcal enterotoxin B acts to stimulate capsaicin-sensitive primary afferent neurons. There is no evidence from this study to suggest that kinins formed in response to staphylococcal enterotoxin B can per se stimulate vanilloid receptors present on these neurons. Of particular interest in relation to this subject is the finding that capsazepine does not affect the release of neuropeptides induced by bradykinin on rat trachea, which suggests that these responses are unrelated to vanilloid receptors but rather reflect activation of B_2 receptors on peripheral nerve endings (Hua et al., 1995).

It is well established that the peripheral neuropathy observed in diabetes mellitus is an important complication of this state (Dicky, 1992; Levy et al., 1992). In diabetic animals, several studies have demonstrated a reduction of neurogenic inflammatory responses (Gamsé and Jancsò, 1985; Garcia-Leme et al., 1992; Bennett et al., 1998), which is believed to be due to alterations in the microvasculature (Fortes et al., 1984) or to a decrease in the content of inflammatory mediators (Bennett et al., 1998) caused by the insulin deficiency. Since the oedema induced by staphylococcal enterotoxin B was partially neurogenic and animal toxins are poorly studied with respect to activation of sensory fibers, we decided to investigate the staphylococcal enterotoxin B-induced responses in diabetic animals in an attempt to further clarify the mechanisms involved in the neuropeptide release in this disorder. In agreement with previous studies (Gamsé and Jancsò, 1985; Bennett et al., 1998), the neurogenic response elicited by staphylococcal enterotoxin B was reduced in the diabetic mice. However, in contrast to most of the exogenously applied stimuli (Garcia-Leme et al., 1992), the insulin treatment, at a dose sufficient to prevent hyperglycaemia in diabetic mice, failed to restore the exudative responses to staphylococcal enterotoxin B (or capsaicin). This suggests that the release of neuropeptides (or possibly sensory ultrastructural changes) cannot be restored by insulin in the mouse. It is interesting to note that the reduction of neurogenic plasma extravasation in diabetic rats was attenuated by long-term treatment with insulin (Bennett et al., 1998). Whether inhibition of neurogenic exudative responses to staphylococcal enterotoxin B in diabetes can be restored by long-term insulin treatment remains to be further investigated.

In conclusion, our present findings show that staphylococcal enterotoxin B induces a complex neurogenic exudative response in mice involving both the local generation of kinins and the activation of vanilloid receptors, culminating in the release of a tachykinin NK_1 receptor agonist (possibly substance P) from the endings of capsaicin-sensitive primary afferent neurons. This agonist, in turn, acts on tachykinin NK_1 receptors on post-capillary venules. The release of 5-HT and histamine from cutaneous mast cells may account for the amplification of staphylococcal enterotoxin B-induced plasma exudation and oedema.

Acknowledgements

Alessandra Linardi was recipient of a grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Fundação de Assistência a Educação e Pesquisa (FAEP-UNICAMP).

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