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European Journal of Pharmacology 369 (1999) 91–98

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Lack of evidence for tachykinin NK_1 receptor-mediated neutrophil accumulation in the rat cutaneous microvasculature by thermal injury

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Received 18 January 1999; accepted 22 January 1999

Abstract

The effect of the non-peptide selective tachykinin NK_1 receptor antagonist SR140333 has been investigated on oedema formation and neutrophil accumulation induced by thermal injury (50°C for 5 min), mustard oil, substance P, the tachykinin NK_1 agonist GR73632, and interleukin-1β in the abdominal skin of the anaesthetised rat. SR140333 significantly inhibited (120 nmol/kg i.v.) or prevented (240 nmol/kg i.v.) the early oedema formation (0–10 min) induced by thermal injury. However, a dosing strategy which blocked NK_1 receptors for 5 h (SR140333, 240 nmol/kg i.v. + 240 nmol/kg s.c.) failed to influence neutrophil accumulation measured 5 h after thermal injury. Thus, the neurogenic component mediated by NK_1 receptors is important to elicit the early oedema formation, but does not influence subsequent neutrophil accumulation. Topical application of mustard oil (2%), a neurogenic inflammation stimulant, caused NK_1 receptor-mediated early neurogenic plasma extravasation, but did not induce cutaneous neutrophil accumulation over 5 h. Substance P and GR73632 at high doses (1 nmol/site) also failed to elicit neutrophil accumulation. Neutrophil accumulation induced by interleukin-1β (0.03–3 pmol i.d.) was not affected by SR140333 pretreatment. In conclusion, despite an early pronounced tachykinin NK_1 receptor-dependent oedema response after thermal injury, the results suggest that subsequent neutrophil accumulation is not mediated by NK_1 receptors. Furthermore, we have not obtained any evidence to suggest that either endogenous or exogenous tachykinins can directly induce neutrophil accumulation in the rat cutaneous microvasculature.

Keywords: Thermal injury; Plasma extravasation; Neutrophil accumulation; Mustard oil; Interleukin-1β; SR140333

1. Introduction

The tachykinin neuropeptide substance P is contained in and released from sensory nerve endings to mediate neurogenic inflammation (Lembeck and Holzer, 1979). Substance P is a potent mediator of increased microvascular permeability, leading to oedema formation. It is well-established that neurogenic oedema formation can be blocked by tachykinin NK_1 receptor antagonists (as reviewed by Brain, 1997). A role for substance P in the immediate oedema formation observed after thermal injury was suggested more than 15 years ago (Lundberg et al., 1984; Saria, 1984) and immunoreactive substance P has been detected in rat paw perfusate after thermal injury (Yonehara et al., 1987). These findings have been extended by recent studies in our laboratory where a selective non-peptide antagonist SR140333 (Emonds-Alt et al., 1993) acted to attenuate early, but not ongoing oedema formation (Siney and Brain, 1996), after thermal injury in the anaesthetised rat.

There are studies which indicate that substance P plays a pivotal role in mediating neutrophil accumulation, especially in the rat airways (Baluk et al., 1995). Evidence indicates that substance P causes leukocyte accumulation in vivo secondary to a non-NK_1 receptor mast cell activation (Matsuda et al., 1989; Yano et al., 1989) and release of mediators which induce neutrophil accumulation (Iwamoto et al., 1992, 1993; Suzuki et al., 1995; Walsh et al., 1995). In this study we have developed a treatment regime using the NK_1 receptor antagonist, such that NK_1 receptors remain blocked for the duration of the 5-h experiment. Under these conditions neutrophil accumulation induced by thermal injury continued unaltered, indicating a lack of involvement of the endogenous tachykinin NK_1 receptor agonists which are released to mediate the neuro-

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00054-0

genic oedema formation in the subsequent neutrophil accumulation. This finding prompted us to investigate links between endogenous neuropeptides, NK_1 receptors and neutrophil accumulation. We examined the pure neurogenic inflammation induced by 2% mustard oil which releases neuropeptides via a capsaicin-sensitive mechanism (Szolcsányi et al., 1998) and compared responses with those elicited by the exogenous tachykinins, substance P (1 nmol/site i.d.) and GR73632 (δAva[L-Pro^9,*N*-Me-Leu^{10}]substance P-(7–11)) (1 nmol/site) in the rat skin. There is also evidence suggesting that endogenous tachykinins play a role in interleukin-1-induced neutrophil accumulation acting on NK_1 receptors (Perretti et al., 1993a,b; Ahluwalia and Perretti, 1996; Ahluwalia et al., 1998). Thus, we also tested the effect of an NK_1 antagonist on interleukin-1β (0.03–3.0 pmol/site i.d.) induced neutrophil accumulation.

2. Materials and methods

2.1. Animals

The experiments were performed on male Wistar rats (230–280 g). All procedures were carried out according to the Animals (Scientific Procedures) Act 1986. The animals were anaesthetised with thiopentone sodium (Thiovet; 100 mg/kg i.p.) with maintenance doses administered as required. Their abdominal skin was shaved and depilated with cream (Immac). All rats were left for at least 30 min after removal of their hair before application of heat or intradermal injections. Body temperature was maintained at 37°C, using a heating pad controlled by a rectal thermistor probe (Harvard, UK). The tail vein was cannulated for administration of agents under study. The trachea was cannulated for ease of breathing during long-lasting experiments.

2.2. Experimental procedures

2.2.1. Oedema response and neutrophil accumulation after local thermal injury

Oedema formation was measured as detailed before (Siney and Brain, 1996) by the extravasation of intravenously-injected labelled albumin. For this, ^{125}I-bovine serum albumin (92.5 kBq per rat) mixed with Evan's blue dye (as visual aid; 2.5% w/v in saline) were injected (300 μl, i.v.) 5 min prior to the induction of thermal injury. Local cutaneous thermal injury (1 cm diameter) was induced with a temperature-controlled skin heating probe (Moor Instruments, UK). The probe was applied to the midline area of the abdominal skin for 5 min at 50°C. The effect of the NK_1 receptor agonist GR73632 (30 pmol), co-administered with the vasodilator calcitonin gene-related peptide (CGRP) (30 pmol) in order to potentiate oedema formation (Brain and Williams, 1985), was also investigated after intradermal (i.d.) injection. The tachykinin NK_1 receptor antagonist SR140333 (120 nmol/kg or 240 nmol/kg, i.v.) or vehicle was injected with the ^{125}I-bovine serum albumin when required. SR140333 was dissolved in ethanol and made up to the final volume with 0.9% sodium chloride. Plasma accumulation was measured for 10 min. At the end of the accumulation period, a sample of blood was taken via cardiac puncture and rats were killed by anaesthetic overdose followed by cervical dislocation. The blood samples were centrifuged at $10{,}000 \times g$ for 5 min to obtain plasma. The heated and control, unheated, skin sites were punched out (16 mm diameter) and their radioactivity counted together with a 100 μl sample of plasma. Plasma extravasation was expressed as the volume of plasma accumulated in skin sites compared to total radioactivity present in 1 ml of plasma.

In some experiments neutrophil accumulation was measured after thermal injury. These experiments were carried out as above, except that rats did not receive ^{125}I-bovine serum albumin and Evan's blue and a dose of SR140333 (240 nmol/kg i.v. and 240 nmol/kg s.c.) was used (see Section 2.2.2 for explanation). At the end of the accumulation period (usually 300 min), the rats were killed by anaesthetic overdose followed by cervical dislocation. The thermal injury sites and unheated control sites were punched out (16 mm diameter). The skin was frozen and kept at −18°C until required. In other experiments, the effect of exogenous agents (substance P [1 nmol]; GR73632 [1 nmol] and interleukin-1β [0.03; 0.1; 0.3; 3 pmol], made up in Tyrode solution) were investigated for their ability to stimulate neutrophil accumulation.

2.2.2. Testing the selectivity and duration of action of SR140333

A treatment regime with SR140333 was designed to enable effective and selective blockade of NK_1 receptors over the 5 h duration of the experiment. This effective dosing was found to be SR140333 (240 nmol/kg i.v. and 240 nmol/kg s.c.) based on earlier studies (Jung et al., 1994; Amann et al., 1995a,b). The selectivity of this regime for NK_1 receptors was investigated by comparing oedema formation in SR140333 and control-treated rats to a range of mediators which included the NK_1 agonist GR73632 (30 pmol), histamine (10 nmol), and bradykinin (500 pmol), all of which were co-administered with the vasodilator CGRP (30 pmol) in order to potentiate oedema formation (Brain and Williams, 1985). Test agents were made up in Tyrode solution and 100 μl volumes were injected i.d. into rat abdominal skin. Plasma extravasation was measured as described above. Antagonist or vehicle was added 5 min i.v. and 30 min s.c. prior to ^{125}I-bovine serum albumin and i.d. agents at 5, 60, 180, and 270 min, in order to test effectiveness of the NK_1 receptor antagonist at these times after administration.

2.2.3. Oedema and neutrophil accumulation in response to topically applied 2% mustard oil

Two percent mustard oil in paraffin oil was painted onto one half of the abdominal skin whilst the opposite side, which served as control, was painted with paraffin oil. Oedema formation was measured for 30 min as described above. SR140333 (240 nmol/kg, i.v.) or vehicle were injected i.v. 5 min prior to application of the oils.

In some experiments neutrophil accumulation was assessed. The protocol was as above, except that the painting was repeated every hour of the 5 h accumulation period to maintain the appropriate concentration of the mustard oil on the skin for long-lasting stimulation of the nerve endings. Separate experiments were also carried out where mustard oil was painted onto the hind paw. Rats were killed by anaesthetic overdose followed by cervical dislocation. Pieces were cut from mustard oil-treated and control skin. Determination of neutrophil accumulation in 1 g of each skin sample was performed as described below.

2.2.4. Measurement of neutrophil accumulation

The samples were then thawed and chopped into small pieces. These pieces were homogenized in a phosphate buffer containing 0.5% hexadecyl trimethylammonium bromide (HTAB) detergent. The homogenate was centrifuged twice, once at $1000 \times g$ at 4°C for 30 min in a Coolspin (MSE, UK) centrifuge, then 1.5 ml supernatant placed in Eppendorf tubes and centrifuged at $10{,}000 \times g$ at 4°C for 5 min in a microcentrifuge (Micro Centaur, MSE, UK) to remove solid debris and lipid layers. The resulting homogenate was frozen and kept −18°C.

Neutrophil accumulation was assessed by comparing myeloperoxidase levels in extracts from rat neutrophils and skin sites according to a method of Schierwagen et al. (1990) as modified by Waller et al. (1997). Myeloperoxidase activity was assayed using the H_2O_2-oxidation of 3,3′,5,5′-tetramethyl benzidine (TMB/H_2O_2). Reactions were performed in a 96-well microtitre plate at room temperature. Known quantities of rat neutrophils, purified from peritoneal lavage samples following injection of oyster glycogen solution, as described by Moroney et al. (1988), were used as a standard. Optical density (O.D.) readings at 620 nm were taken at 5-min intervals for 30 min, using an Anthos HTIII microplates reader. These O.D. values were plotted and a reaction rate (ΔO.D./time) measured from the initial slope of the curve. A calibration curve was then produced, with the rate of reaction plotted against the number of neutrophils in the standard samples. This was used to convert reaction rates to numbers of neutrophils for the skin sample homogenates.

2.3. Drugs

The animals were anaesthetised with thiopentone sodium (Thiovet) from C-Vet, Leyland, UK SR140333 ((*S*)1-2-[3,4-dichloro-phenyl)-1-(3-*iso*-propoxyphenylacetyl)piperidine-3-yl]ethyl}-4phenyl-1-azoniabicyclo[2.2.2]octane chloride) was a gift from Sanofi, Toulouse, France. GR73632 (δAva[L-Pro^9,*N*-Me-Leu^{10}]substance P-(7–11)) was gift from Glaxo Wellcome, UK. Bradykinin, Evan's blue dye, HTAB, histamine, substance P, interleukin-1β (human recombinant), mustard oil (allyl isothiocyanate) were purchased from Sigma, UK, CGRP from Bachem, saline (0.9% sodium chloride, pyrogen free) from Baxter Healthcare, UK, K-Blue (TMB/H_2O_2 reagent) from Boinostic, UK, Immac depilatory foam spray from Reckitt & Colman, UK ^{125}I-labelled bovine serum albumin was obtained from ICN, Belgium. The composition of Tyrode's solution was as follows (in mmol/l): NaCl, 137; KCl, 2.7; $MgCl_2$, 0.5; NaH_2PO_4, 0.4; $NaHCO_3$, 11.9; glucose, 5.6.

2.4. Data analysis

Results are shown as the mean ± S.E.M. with units as indicated. The number (n) of rats per group is in figure legends. Data were analysed for statistical significance using one-way analysis of variance (ANOVA) followed by Tukey–Kramer's test. A value of $P < 0.05$ was taken as being significant.

3. Results

3.1. Effect of SR140333 on oedema formation induced by thermal injury over 10 min

Local heating (50°C for 5 min) of the rat abdominal skin caused intensive plasma extravasation compared to the unheated skin samples over the 10 min accumulation period, as shown in Fig. 1. The amount of extravasated

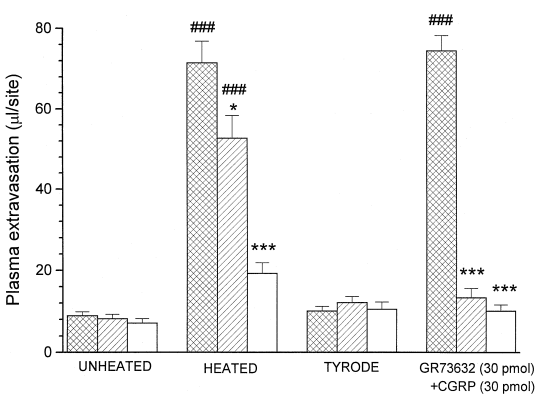

Fig. 1. Effect of two doses of SR140333 on thermal injury and tachykinin NK_1 receptor agonist-induced oedema formation in rat skin. Results are expressed as μl of extravasated plasma in the skin sites 10 min after the treatments. Four abdominal skin pieces were punched out from every rat: unheated, heated (50°C, for 5 min), Tyrode, and tachykinin NK_1 receptor agonist GR73632 (30 pmol) + CGRP (30 pmol) i.d.-injected. The responses are shown in the presence of solvent (cross-hatched columns), SR140333 120 nmol/kg i.v., (hatched columns) and SR140333 240 nmol/kg i.v. (open columns). All data are means ± S.E.M. for $n = 5$ experiments; $^{*}P < 0.05$, $^{***}P < 0.001$ as compared to solvent-treated control group; $^{###}P < 0.001$ as compared to unheated or Tyrode-treated corresponding controls.

plasma was similar to plasma leakage stimulated by the NK_1 receptor agonist GR73632 (30 pmol) when injected intradermally with a potentiating dose of CGRP (30 pmol). CGRP does not increase microvascular permeability when injected alone at this dose, but acts as a consequence of its vasodilator activity to potentiate oedema induced by co-injected mediators of increased microvascular permeability (Brain and Williams, 1985). The non-peptide selective tachykinin NK_1 receptor antagonist, SR140333 (120 nmol/kg i.v.) significantly inhibited the thermal oedema and almost completely suppressed the effect of GR73632. A higher dose of SR140333 (240 nmol/kg i.v.) substantially reduced the early thermal oedema and abolished the effect of GR73632. The results confirm those of studies outlined in the introduction that NK_1 receptors are critically involved in the early oedema formation observed after thermal injury in the rat.

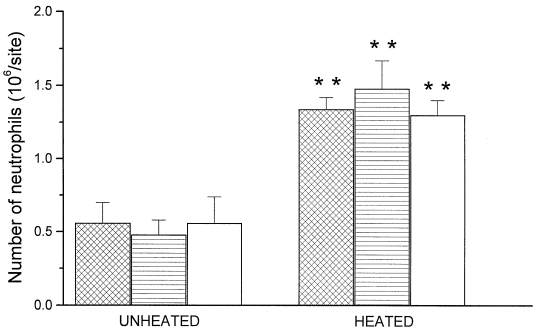

Fig. 3. The effect of SR140333 pretreatment on neutrophil accumulation measured over 300 min in rat abdominal skin after thermal injury (50°C, 5 min). The effect of solvent pretreatment i.v. (cross-hatched columns) and SR140333 pretreatment 240 nmol/kg i.v., (horizontal-hatched columns) and 240 nmol/kg s.c. + 240 nmol/kg i.v. (open columns) are shown. Each pair of bars represents the means ± S.E.M. for $n = 5–10$ rats. $^{**}P < 0.01$ vs. unheated controls.

3.2. Effect of combined doses of SR140333 on inhibition of oedema formation of intradermally-injected inflammatory mediators and on neutrophil accumulation in thermal injury

Experiments were carried out in order to test that SR140333 could be administered at doses that would enable effective NK_1 receptor antagonism over a 5 h experimental period. SR140333 was given as a combination treatment, with 240 nmol/kg injected s.c. 30 min before and 240 nmol/kg i.v. 5 min prior to ^{125}I-BSA. The effect of GR73632 (30 pmol), histamine (10 nmol) and bradykinin (500 pmol) with CGRP (30 pmol) was investigated. The intradermally-injected agents were given at 5 min and GR73632 + CGRP injection was repeated 60, 180 and 270 min afterwards. Results shown in Fig. 2 illustrate that no significant oedema formation was observed at any time point in SR140333-pretreated rats while significant oedema formation was observed in vehicle-treated rats. There was a slight time-dependent decrease in the ability of GR73632 to induce oedema formation as time went on, but this was probably related to the increasing time rats were under anaesthesia. Oedema stimulated by i.d. injected non-neuropeptide agents histamine (10 nmol) and bradykinin (500 pmol), both injected with CGRP (30 pmol) was not affected by the NK_1 receptor antagonist treatment regime, thus demonstrating selectivity.

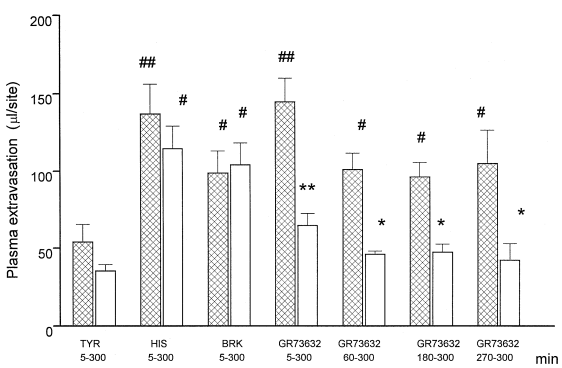

Fig. 2. The selectivity and effectiveness of SR140333 (240 nmol/kg s.c. + 240 nmol/kg i.v.) over 0–300 min in rat skin. The results for vehicle control (cross-hatched colums) and for SR140333, 240 nmol/kg s.c., −30 min + 240 nmol/kg i.v., −5 min (open columns) are shown for each intradermal treatment. Rats received ^{125}I-bovine serum albumin i.v. at 0 min and this was followed at 5 min by i.d. injections into abdominal skin of Tyrode (TYR 0.1 ml); histamine (HIS 10 nmol); bradykinin (BRK 500 pmol) and GR73632 (30 pmol) each vasoactive agent given in the presence of a potentiating dose of the vasodilator CGRP (30 pmol). Accumulation at these sites was then allowed to occur for 5–300 min. Further i.d. injections of GR73632 + CGRP were then given at 60, 180, and 270 min. These latter injections allowed plasma accumulation to be measured over 60–300 min, 180–300 min and 270–300 min, respectively, after the administration of the tachykinin NK_1 receptor antagonist. Each pair of bars represents the means ± S.E.M. for $n = 3$ experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. solvent-treated corresponding controls; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Tyrode-treated control groups.

The effect of this pretreatment regime was investigated on neutrophil accumulation induced by thermal injury. Fig. 3 shows that SR140333 had no effect on neutrophil accumulation induced after thermal injury.

3.3. Effect of SR140333 pretreatment on mustard oil induced neurogenic inflammation

Topically applied 2% mustard oil induced pure neurogenic oedema in the abdominal skin for 30 min which was completely inhibited by SR140333 pretreatment (240 nmol/kg i.v; $n = 4$) as shown in Fig. 4.

Over a 5 h period mustard oil treatment did not induce neutrophil accumulation as compared to the paraffin-treated control contralateral skin. Assay of neutrophil myeloperoxidase showed: $0.120 \pm 0.04 \times 10^6$ neutrophils/100 mg tissue in mustard oil-treated skin, compared with

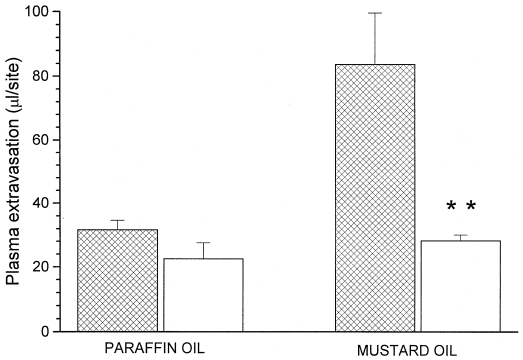

Fig. 4. Effect of SR140333 (240 nmol/kg i.v.) on 2% mustard oil induced neurogenic oedema formation. Cross-hatched columns represent plasma extravasation in the abdominal skin sites of solvent-treated, open colums show plasma extravasation in SR140333-treated animals. Plasma extravasation was measured 30 min after 2% mustard oil or paraffin oil application. Values are expressed as means ± S.E.M. of $n = 4$ experiments. $^{**}P < 0.01$ vs. solvent-treated control group; $^{\#\#}P < 0.01$ vs. paraffin oil-treated control.

$0.171 \pm 0.05 \times 10^6$ neutrophils/100 mg tissue in paraffin oil-treated skin (means ± S.E.M.; $n = 4$). Experiments were also carried out in the dorsal skin of the hind paw, which receives a dense innervation of sensory nerves. This yielded similar negative results as follows: $0.021 \pm 0.014 \times 10^6$ cells were measured after mustard oil, and $0.017 \pm 0.014 \times 10^6$ neutrophils/100 mg skin were detected in paraffin oil-treated leg skin (means ± S.E.M.; $n = 4$).

3.4. Effect of SR140333 pretreatment on neutrophil accumulation induced by intradermally injected drugs

High doses of intradermally injected substance P (1 nmol), and GR73632 (1 nmol) did not stimulate neutrophil accumulation over 5 h (Fig. 5), however interleukin-1β (0.03–3 pmol) elicited dose dependent neutrophil recruitment in the skin. Interleukin-1β induced neutrophil accumulation was not affected by SR140333 pretreatment (240 nmol/kg s.c. and 240 nmol/kg i.v.) (Fig. 6).

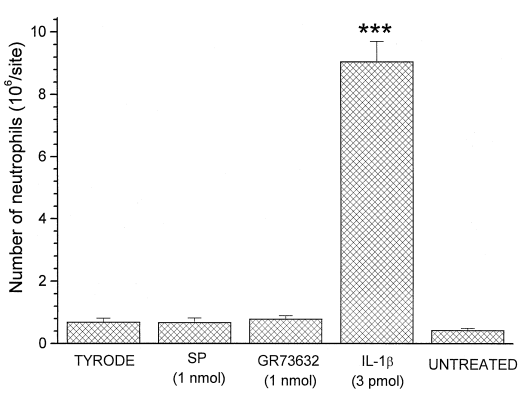

Fig. 5. Effect of intradermally injected substance P [1 nmol]; GR73632 [1 nmol] and interleukin-1β [3 pmol]) on neutrophil recruitment over 300-min accumulation period. Drugs were made-up in Tyrode solution and 100 μl volumes were injected i.d. into the abdominal skin of the rat. Results are expressed as means ± S.E.M. $n = 5$ experiments. $^{***}P < 0.001$ vs. Tyrode-treated controls.

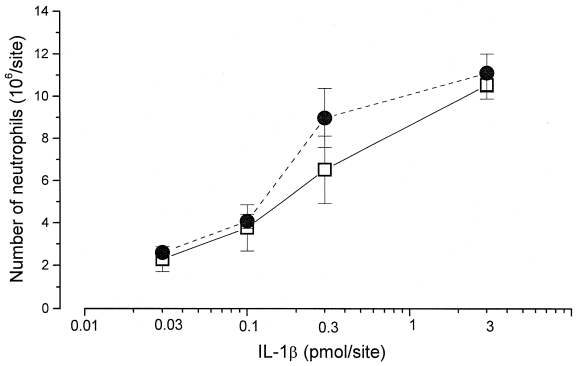

Fig. 6. Effect of SR140333 (240 nmol/kg i.v. and 240 nmol/kg s.c.) pretreatment on neutrophil accumulation induced by interleukin-1β (0.03–3 pmol/site) as (closed circles) compared to the solvent-treated controls (open squares). Results are expressed as means ± S.E.M., $n = 4$.

4. Discussion

Previous studies in our group have shown that treatment with tachykinin NK_1 receptor antagonist (SR140333) markedly suppressed early, but not ongoing oedema formation (Siney and Brain, 1996; Waller et al., 1997). The data established that substance P, in addition to CGRP, bradykinin and prostaglandins are major mediators involved in early plasma extravasation, while the mediators involved in the later ongoing oedema formation remained unidentified (Siney and Brain, 1996; Waller et al., 1997). In the present study, pretreatment with SR140333 (120 nmol/kg i.v.) (Emonds-Alt et al., 1993; Jung et al., 1994) significantly inhibited the immediate phase of thermal oedema (0–10 min), and totally blocked plasma extravasation induced by a hexapeptide substance P analogue GR73632. Higher doses of the antagonist (240 nmol/kg i.v.) abolished the thermal injury response indicating that the short immediate phase is predominantly neurogenic. It is generally assumed that the endogenous mediator of the thermally-induced neurogenic plasma leakage is substance P (Lundberg et al., 1984; Saria, 1984; Yonehara et al., 1987). Our results are in support of findings (Pradier et al., 1994; Hawcock et al., 1995) that indicate that selective agonists and antagonists of the tachykinin NK_1 receptors act at the same binding site on the NK_1 receptor, which is a distinct site from which substance P binds. Thus, higher doses of antagonist are required to antagonise substance P, compared with some other NK_1 agonists such as GR73632.

The present findings further our previous study and show that neutrophil accumulation in the thermal injury model is not mediated by NK_1 receptors. A combined pretreatment with SR140333 (240 nmol/kg s.c. + 240 nmol/kg i.v.) produced long-lasting (5 h) selective blockade of tachykinin NK_1 oedema-inducing receptors (Jung et

al., 1994; Amann et al., 1995a,b) but did not influence neutrophil accumulation. Neutrophils are the primary cells which accumulate following thermal injury to rats and have been suggested to play a significant role in the progression of thermal injury-induced microvascular damage (Bucky et al., 1994; Hansbrough et al., 1996). Our recent results suggest that depletion of circulating neutrophils by rat anti-neutrophil antiserum does not reduce oedema over 0–4 h (Waller et al., 1997). Thus, the combined results indicate a complete lack of link between the NK_1 receptor and neutrophil accumulation observed at the site of the wound.

It has been shown that substance P acting via tachykinin NK_1 receptors can stimulate the accumulation of neutrophils in vivo in the rat airways (Baluk et al., 1995). It has also been suggested that substance P causes leukocyte accumulation in vivo via a mast cell dependent (Matsuda et al., 1989; Yano et al., 1989), non-receptor-dependent mechanism. The mechanism may include interleukin-1 released from activated mast cells acting to recruit leukocytes (Iwamoto et al., 1992, 1993; Suzuki et al., 1995; Walsh et al., 1995). There is evidence to indicate that exogenous but not endogenous substance P evokes release of mediators from the mast cells (Tausk and Undem, 1995). The combined findings prompted us to investigate the ability of exogenous and endogenous neuropeptides to mediate neutrophil accumulation in rat skin. We investigated the ability of 2% mustard oil which can be applied topically to selectively activate capsaicin-sensitive nerve fibres to release neuropeptides which include substance P. Unlike capsaicin, repeated application of the mustard oil does not show the phenomenon of desensitisation (Lembeck et al., 1992; Inoue et al., 1997). Our studies show that mustard oil induced an acute NK_1 receptor-mediated oedema formation, which was not ongoing and which was not accompanied by neutrophil accumulation. This is clear evidence to indicate that the acute vascular phase of cutaneous neurogenic inflammation is not followed by neutrophil accumulation. Although early papers indicated that cutaneous mast cells play an essential role in the mediation of neurogenic vasodilatation and plasma extravasation (Kiernan, 1974; Arvier et al., 1977; Lembeck and Holzer, 1979; Couture and Cuello, 1984), this theory has been not confirmed. Our observations are in agreement with studies (Kowalski and Kaliner, 1988; Kowalski et al., 1990) which provided evidence that vascular effects of neurogenic inflammation are independent of mast cell activation in the skin. The data also support the hypothesis that induced neurogenic inflammation occurs in the superficial dermis of the rat skin and mast cells are predominantly located in deep dermis (Baraniuk et al., 1990). Thus, there is little contact between leaky vessels and mast cells. The findings are in keeping with Inoue et al. (1997) who demonstrated in the mouse, no significant differences in the ability of mustard oil to induce oedema formation in the ear in mast cell deficient and normal mice. We also considered that substance P released from cutaneous sensory fibres does not reach a sufficient local concentration in the skin to degranulate cutaneous mast cells and thus release mediators responsible for leukocyte accumulation. However, in contrast with neurogenic inflammation in the skin, there is evidence showing that mast cell-dependent neurogenic inflammation does occur in the rat airways and that this includes neutrophil accumulation (Umeno et al., 1989; Szolcsányi et al., 1991; Baluk et al., 1995).

High doses of exogenous substance P (10–30 times greater than the dose necessary to induce pronounced oedema formation) have been shown to directly influence neutrophil recruitment in a range of models which include the mouse air pouch (Perretti et al., 1993a,b) and guinea-pig skin (Walsh et al., 1995). However, in our hands substance P (1 nmol/site, i.d.,) was ineffective in inducing cutaneous neutrophil accumulation in rat skin. In a similar manner to substance P, a selective NK_1 receptor agonist, (GR73632) (1 nmol/site i.d.) had no effect on leukocyte recruitment. Thus, our evidence also suggests that exogenous substance P is unable to stimulate neutrophil accumulation in rat skin.

The results outlined above are of interest when put in the context of studies which demonstrate a clear role for NK_1 receptors in interleukin-1β induced polymorphonuclear leukocyte accumulation in the mouse air pouch (Perretti et al., 1993a,b; Ahluwalia and Perretti, 1996; Ahluwalia et al., 1998). Blockade of NK_1 receptors with NK_1 receptor antagonists, or in the latter study removal of the NK_1 receptor through the use of NK_1 receptor knockout mice, attenuate interleukin-1β-induced neutrophil accumulation. It is suggested that interleukin-1β releases tachykinins which stimulates neutrophil accumulation through the NK_1 receptors, although the precise mechanism has not been established. It is possible that NK_1 receptors act indirectly via a vasoactive effect. In our experiments we found that interleukin-1β induced dose-dependent neutrophil accumulation which was not affected by the blockade of tachykinin NK_1 receptors with SR140333 (240 nmol/kg s.c. + 240 nmol/kg i.v.) in the rat abdominal skin. Thus, our results suggest that tachykinin NK_1 receptor-dependent mechanisms cannot influence interleukin-1β-induced neutrophil accumulation directly. This may be due to different mechanisms operating in the rat and mouse. Alternatively, it is possible that cells or mediators systems, present in the air pouch, but not in normal skin, are important for influencing the tachykinin NK_1 receptor-dependent pathway.

In conclusion, despite an early pronounced tachykinin NK_1 receptor-dependent oedema response after thermal injury, our results suggest that neutrophil accumulation in response to thermal injury is not mediated by tachykinin NK_1 receptors. We also have, in keeping with the above finding, little evidence to suggest that either endogenous or exogenous tachykinins can directly induce neutrophil accumulation in the rat cutaneous microvasculature.

Acknowledgements

Erika Pintér was supported by a NATO/Royal Society Postdoctoral Fellowship.

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