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The plasma protein extravasation induced by adenosine and its analogues in the rat dorsal skin: evidence for the involvement of capsaicin sensitive primary afferent neurones and mast cells

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1 The contribution of sensory neurons and mast cells to the oedema evoked by adenosine A_1 (N_6-cyclopentyladenosine, CPA, 3–30 nmol site^{-1}), A_2 (5′N-ethylcarboxamidoadenosine, NECA, 1–10 nmol site^{-1}) and A_3 receptor agonists (N6-[3-iodobenzyl]-N-methyl-5′-carboxiamidoadenosine, IB-MECA, 0.01–3 nmol site^{-1}) was investigated in the rat skin microvasculature, by the extravascular accumulation of intravenously-injected (i.v.) ^{125}I-albumin.

2 Intradermal (i.d.) injection of adenosine and analogues induced increased microvascular permeability in a dose-dependent manner (IB-MECA > NECA > CPA > adenosine). The non-selective adenosine receptor antagonist theophylline (5–50 nmol site^{-1}) markedly inhibited adenosine, CPA or NECA but not IB-MECA-induced plasma extravasation. The A_1 receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 0.3–3 μmol kg^{-1}, i.v.) significantly reduced CPA-induced plasma extravasation whereas responses to adenosine, NECA or IB-MECA were unchanged. The A_2 receptor antagonist 3,7-dymethyl-1-proprargylxanthine (DMPX, 0.5–50 nmol site^{-1}) significantly reduced NECA-induced plasma extravasation without affecting responses to adenosine, CPA and IB-MECA.

3 The tachykinin NK_1 receptor antagonist (S)-1-[2-[3-(3,4-dichlorphenyl)-1 (3-isopropoxyphenylacetyl) piperidin-3-yl] ethyl]-4-phenyl-1 azaniabicyclo [2.2.2]octane chloride (SR140333), but not the NK_2 receptor antagonist (S)-N-methyl-N[4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)-butyl]-benzamide (SR48968), significantly inhibited the plasma extravasation evoked by higher doses of adenosine (100 nmol site^{-1}), CPA (100 nmol site^{-1}), NECA (1 nmol site^{-1}) and IB-MECA (0.1–1 nmol site^{-1}). In rats treated with capsaicin to destroy sensory neurons, the response to higher doses of adenosine, CPA and NECA, but not IB-MECA, was significantly inhibited.

4 The effects of adenosine and analogues were largely inhibited by histamine and 5-hydroxytryptamine (5-HT) antagonists and by compound 48/80 pretreatment.

5 In conclusion, our results provide evidence that adenosine A_1 and A_2, but not A_3, receptor agonists may function as cutaneous neurogenic pro-inflammatory mediators; acting *via* microvascular permeability-increasing mechanisms that can, depending on dose of agonist and purine receptor under study, involve the tachykinin NK_1 receptor and mast cell amines.

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Abbreviations: ADP, adenosine diphosphate; ATP, adenosine 5′-triphosphate; CPA, N_6-cyclopentyladenosine; DMPX, 3,7-dymethyl-1-proprargylxanthine; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; IB-MECA, N6-(3-iodobenzyl)-N-methyl-5′-cabamoiladenosine; NECA, 5′N-ethylcarboxamidoadenosine

Introduction

Adenosine and its metabolites are commonly found in the plasma and other extracellular fluids, especially after tissue injury where an increase in microvascular permeability and leakage is often observed. Adenosine has been shown to modulate a variety of physiological processes by binding to at least four cell surface receptors subtypes (A_1, A_{2a}, A_{2b} and A_3) in different cell types (i.e. monocytes, lymphocytes, neutrophils and mast cell; see reviews Feoktistov & Biaggioni, 1997; Ralevic & Burnstock, 1998; Dexter *et al*., 1999). Besides exerting their physiological role, ATP and its metabolites (ADP and adenosine) may well serve as mediators of pathological processes such as inflammatory pain (Bland-Ward & Humphrey, 1997; Hamilton *et al*., 1999) and in the regulation of the inflammatory process. Early studies revealed that suspensions of neutrophils release adenosine into the intracellular milieu and its removal leads to a significant increase in neutrophil responses to chemoattractants (Cronstein *et al*., 1983). This has led to the

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suggestion that adenosine may have an anti-inflammatory effect on neutrophil-endothelial interactions (Grisham *et al*., 1989; Rosengren *et al*., 1995), although the relevance of this effect has been shown to be minor in an ischaemia-reperfusion model (Kubes *et al*., 1998). One difficulty is that the effect of adenosine appears to be tissue specific and strain dependent (Church *et al*., 1983). For example, adenosine has been shown to enhance antigen-induced 5-HT and histamine release from rat and mouse mast cells (Reeves *et al*., 1997; Dexter *et al*., 1999). By comparison, adenosine did not degranulate human mast cells, but was able to inhibit immunologically-mediated histamine release from human basophils and lung mast cells (Church *et al*., 1983, Hughes *et al*., 1984). However, the general anti-inflammatory effect of adenosine is well established. Adenosine and its analogues have been shown to be potent inhibitors of inflammation in models including adjuvant-induced arthritis (Green *et al*., 1991) and carrageenan-induced pleural inflammatory response (Schrier *et al*., 1990). Furthermore, it has been demonstrated that adenosine A_1 receptor agonists are more potent inhibitors of pleural and peritoneal inflammation than A_2 receptor agonists (Lesch *et al*., 1991), even though *in vivo* studies show that adenosine modulates inflammation *via* A_2 receptors (Asako *et al*., 1993).

Interestingly, there is good evidence that the adenosine A_3 receptor agonist (IB-MECA) can mediate plasma protein extravasation in the rat dorsal skin, *via* a mast cell-dependent mechanism (Reeves *et al*., 1997; Tilley *et al*., 2000). Furthermore, it is known that adenosine can mediate increased microvascular leakage in the rat lung *via* the release of the neuropeptide substance P and hence stimulation of tachykinin NK_1 receptors, suggesting a neurogenic mechanism (Tamaoki *et al*., 1999).

We have designed experiments to investigate possible links between adenosine and inflammatory oedema formation. Initially we examined the ability of adenosine and selective A_1, A_2 and A_3 receptor agonists to increase microvascular permeability in the rat cutaneous microvasculature. Further experiments with selective A_1 and A_2 antagonists support the concept that adenosine receptors can participate in a selective manner in mediating oedema formation in skin. We also present data to demonstrate the involvement of mast cells and sensory nerves in the cutaneous response induced by adenosine and its analogues.

Methods

Experiments were performed in both male and female Wistar rats (200–300 g) bred in house. All experiments were carried in accordance with the guidelines for animal care of the State University of Campinas (UNICAMP).

Measurement of plasma protein extravasation in vivo

Local plasma protein extravasation was measured in the shaved dorsal rat skin, in response to intradermally injected (i.d.) adenosine and its analogues (100 μl site^{-1} in Tyrode solution). Agents were injected in a random order, according to a balanced site pattern. Plasma protein extravasation was measured by the accumulation of intravenously injected (i.v.) ^{125}I-human serum albumin (^{125}I-HSA; 2.5 μCi rat^{-1}) with Evan's blue dye (25 mg kg^{-1}; Brain & Williams, 1985) to act as a visual marker. Antagonists and other test agents were injected as required by specific protocols. At the end of the accumulation period (30 min), a cardiac blood sample (5 ml) was taken and the rats killed by anaesthetic overdose. The blood samples were centrifuged at 8000 × *g*

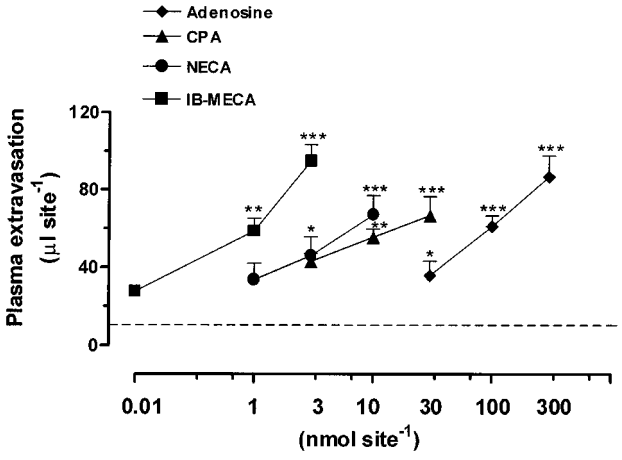

Figure 1 Concentration dose-dependent rat skin plasma extravasation induced by adenosine and adenosine A_1 (CPA), A_2 (NECA) and A_3 (IB-MECA) receptor agonists. Results are expressed as μl plasma extravasated per site and each point represents the mean ± s.e.mean of five animals. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to Tyrode (dotted line).

Table 1 Effect of the non-selective adenosine receptor antagonist, theophylline, on the plasma extravasation evoked by adenosine, CPA, NECA and IB-MECA

	Plasma extravasation (μl site^{-1}) *Theophylline* (nmol site^{-1})			
	Control	*5*	*15*	*50*
Tyrode	7 ± 0.4	13 ± 2.3	6.5 ± 0.7	8 ± 1.0
Adenosine (300 nmol site^{-1})	82 ± 0.6	88 ± 6.5	42 ± 6.3*	32 ± 5.3*
CPA (3 nmol site^{-1})	63 ± 3.3	88 ± 10.6	27 ± 13*	44 ± 4.0*
NECA (1 nmol site^{-1})	34 ± 2.7	36 ± 6.6	17 ± 2.5*	7.6 ± 0.4*
IB-MECA (1 nmol site^{-1})	32 ± 3.5	22 ± 2.9	27 ± 4.7	21 ± 2.3

Values are means ± s.e.mean for 4–5 rats. *Indicates $P<0.05$ compared to respective control values. CPA, N_6-cyclopentyladenosine; NECA, 5′N-ethylcarboxamidoadenosine; IB-MECA, N6-[3-iodobenzyl]-N-methyl-5′-carboxiamidoadenosine.

for 10 min to obtain a plasma sample. The injected sites were punched out and counted for radioactivity, with the plasma samples in a γ-counter. Plasma extravasation was expressed as the volume (μl) of plasma accumulated at each skin site compared to total counts in 1 ml of plasma.

Pretreatment of rats with capsaicin

Neuropeptides were depleted by neonatal capsaicin treatment. Rats (7–8 g) superficially anaesthetized with halothane (inhaled) were injected on the second day of life by a single subcutaneous (s.c.) injection of capsaicin (50 mg kg^{-1}; Jancsó *et al*., 1977). The corresponding volume (100 μl s.c.) of capsaicin-vehicle (1 : 1 : 8 ethanol:Tween 80:NaCl 0.9%, v v^{-1}) was injected in control rats. Animals were used 60–90 days later.

Systemic treatment with compound 48/80

Rats (180–250 g) under light halothane (inhaled) anaesthesia were pretreated with three consecutive daily s.c. injection of compound 48/80 (5 mg kg^{-1}) or the same volume (200 μl rat^{-1}) of NaCl (0.9%) solution. The skin oedema assay was performed on the third day (Ahluwalia *et al*., 1998).

Drugs

Capsaicin, compound 48/80, adenosine, 5′N-ethylcarboxamidoadenosine (NECA), N^6-cyclopentyladenosine (CPA), N^6-(3-iodoenzyl)-N-methyl-5′-carboxiamodoadenosine (IB-MECA), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 3,7-dymethyl-1-proprargylxanthine (DMPX), cyproheptadine, and theophylline were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). ^{125}I-human serum albumin was kindly radio-labelled by Dr Maria Tereza Ribella (IPEN/CENEN-USP). Sodium pentobarbitone (Sagatal) was purchased from Rhone Merieux (Dublin, Eire). GR73632 ((Ava[L-Pro^9,N-MeLeu10] substance P(7–11)) GR73632 was donated by Dr D. Beattie, Glaxo Group Research (Ware, U.K.). SR140333 ((S)1-{2-[3(3-4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl)piperidine-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]octone, chloride) and SR48968 ((S)-N-methyl-N-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-di-

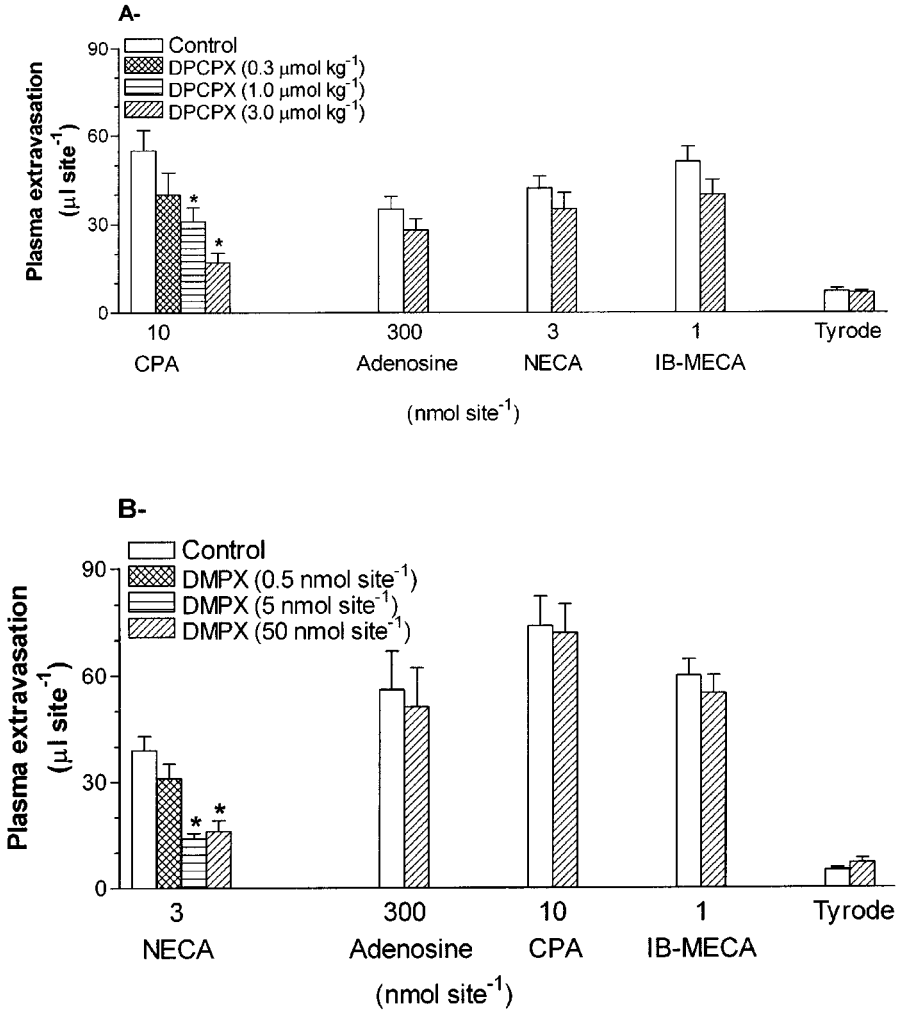

Figure 2 Effect of (A) the A_1 DPCPX (0.3–3 μmol kg^{-1}; i.v., $n=4-5$) and (B) A_2 DMPX (0.5–50 nmol site^{-1}; $n=5$) receptor antagonist on the plasma extravasation evoked by adenosine, CPA, NECA and IB-MECA. The dose-response curve for both antagonists is only shown with their respective agonists. Values are means ± s.e.mean. *Indicates significance at $P<0.05$.

chlorophenyl)buty]benzamide) were a gift from Dr Emonds-Alt, Sanofi Recherche (Montpellier, France). Test agents were stored at −20°C and diluted with a modified Tyrode solution composition (in mM): NaCl 137, KCl 2.7, $MgCl_2$ 0.5, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 5.6 prior to use.

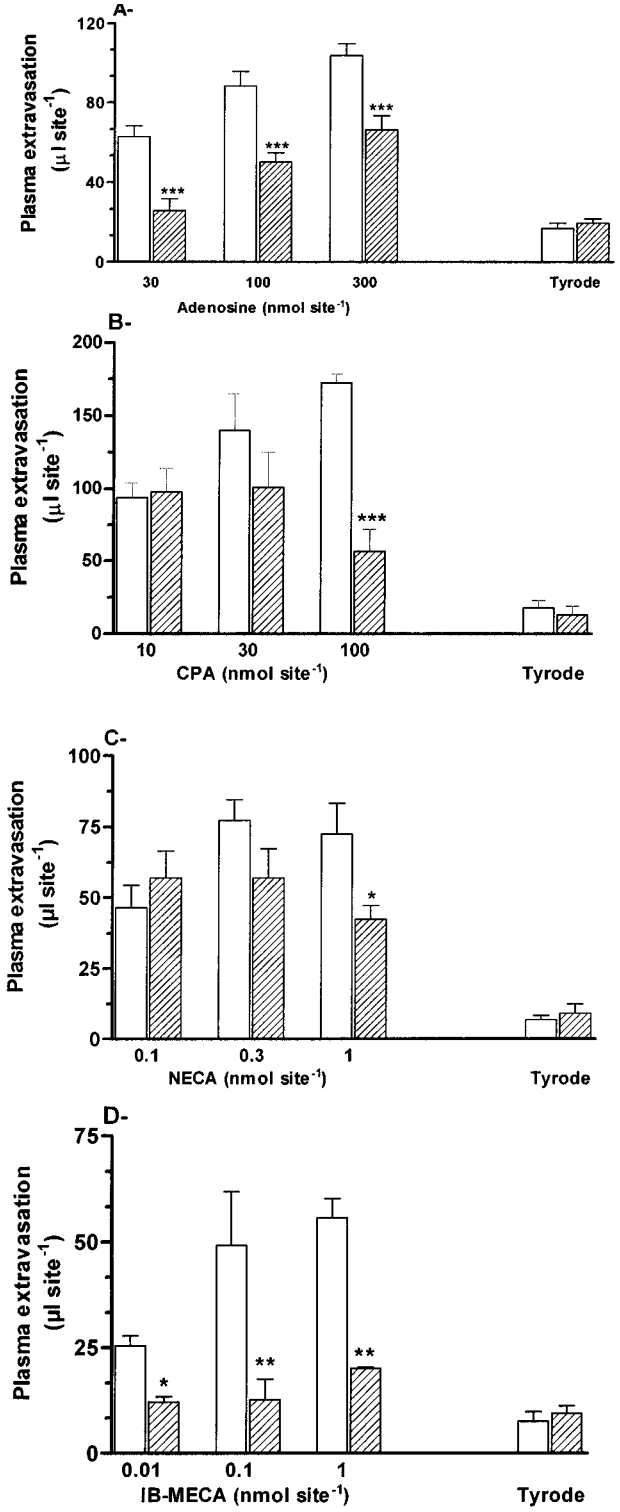

Figure 3 Effect of tachykinin NK_1 receptor antagonist SR140333 on adenosine and its analogues-induced plasma extravasation. Opens bars represent the response induced by adenosine (A), CPA (B), NECA (C) and IB-MECA (D) alone whereas stripped bars showed the effect mediated by these agents in the presence of SR140333 (1 nmol site^{-1}). Results are expressed as μl plasma extravasated per site. Mean ± s.e.mean, $n=4-5$. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared to control group.

Statistical analysis

Results are presented as mean values ± s.e.mean for n experiments. They were analysed by Student's unpaired t-test or Bonferroni's modified t-test, when appropriate. $P<0.05$ was taken as significant.

Results

Effect of intradermal injection of adenosine and its analogues in the rat dorsal skin (in vivo) *in the presence and absence of adenosine antagonists*

Intradermal injection of adenosine (30 – 300 nmol site^{-1}) and adenosine A_1 N_6-cyclopentyladenosine (CPA; 3 – 30 nmol site^{-1}), A_2 5′N-ethylcarboxamidoadenosine (NECA; 1 – 10 nmol site^{-1}) and A_3 receptor agonists N6-(3-iodobenzyl)-N-methyl-5′-cabamoiladenosine (IB-MECA; 0.01 – 3 nmol site^{-1}) caused a significant and concentration-dependent plasma protein extravasation in the rat dorsal skin, which was significantly different from that achieved by i.d. administration of Tyrode (Figure 1).

The co-injection of the non-selective adenosine receptor antagonist theophylline (5 – 50 nmol site^{-1}) significantly inhibited the plasma extravasation evoked by adenosine, CPA and NECA as compared to each substance injected alone (Table 1). Systemic treatment of rats with the adenosine A_1 receptor antagonist DPCPX (0.3 – 3 μmol kg^{-1}, i.v., 15 min before) caused a dose-dependent inhibition ($P<0.05$) of CPA-induced plasma extravasation compared to responses in control rats. At the highest dose, DPCPX did not significantly affect the response induced by adenosine, NECA, or IB-MECA (Figure 2A).

The co-injection of the A_2 adenosine receptor antagonist DMPX (0.5 – 50 nmol site^{-1}; $n=4$) significantly inhibited the

Table 2 The inhibitory effect of the 5-HT receptor antagonist cyproheptadine on the plasma extravasation induced by adenosine and its analogues

Agents	*Doses* (nmol site^{-1})	*% Reduction of plasma extravasation*
Adenosine	30	66 ± 5.7*
	100	79 ± 4.2*
	300	85 ± 4.8*
CPA	3	78 ± 3.2*
	10	74 ± 6.2*
	30	86 ± 3.1*
NECA	0.1	75 ± 6.1*
	1	71 ± 4.6*
	3	64 ± 9.5*
IB-MECA	0.1	76 ± 5.6*
	1	72 ± 3.7*
	3	71 ± 5.7*

Values are means ± s.e.mean for five rats. *Indicates significance at $P<0.05$. CPA, N_6-cyclopentyladenosine; NECA, 5′N-ethylcarboxamidoadenosine; IB-MECA, N6-[3-iodobenzyl]-N-methyl-5′-carboxiamidoadenosine.

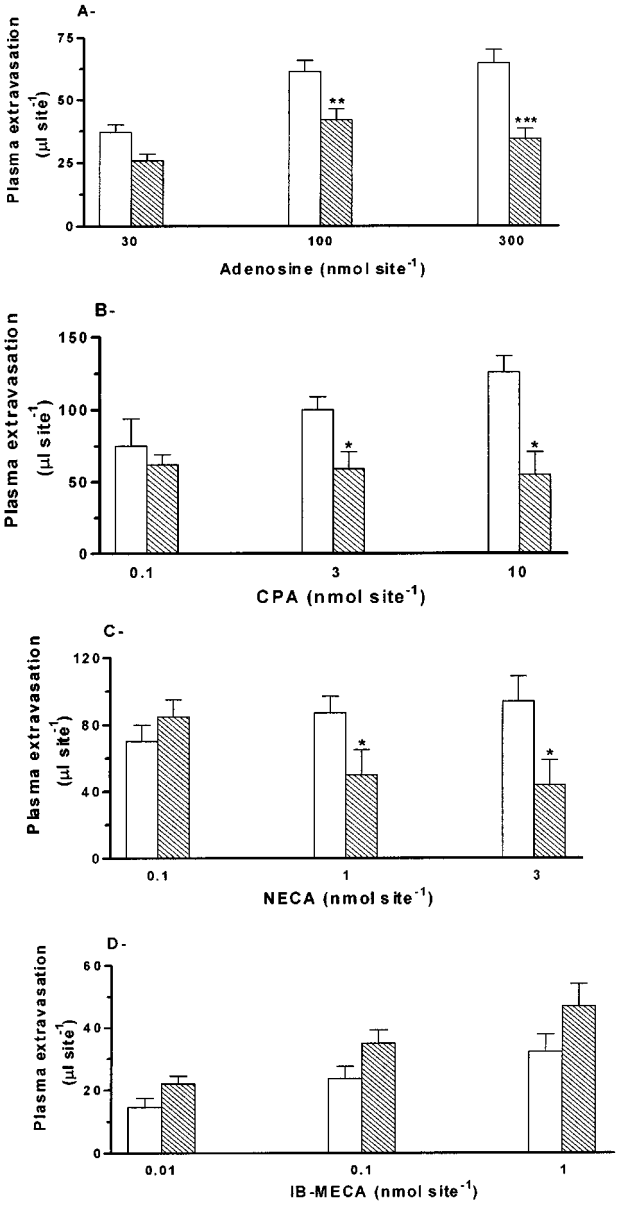

Figure 4 Effect of capsaicin-pretreatment on adenosine and its analogues-induced plasma extravasation. Responses to i.d. injection of adenosine (30–100 nmol site^{-1}, A), CPA (0.1–10 nmol site^{-1}, B), NECA (0.1–3 nmol site^{-1}, C), and IB-MECA (0.01–1 nmol site^{-1}, D) are shown by open bars (vehicle-pretreated rats) and striped bars (capsaicin-pretreated rats). Results are expressed as μl plasma extravasated per site. Mean$\pm$s.e.mean, $n=11$. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared to control group.

plasma extravasation evoked by the A_2 receptor antagonist NECA without significantly affecting that induced by adenosine, CPA or IB-MECA (Figure 2B).

Effects of a tachykinin NK_1 and NK_2 receptor antagonists

As previously described (Palframan *et al*., 1996), the plasma extravasation evoked by i.d. injection of the peptide tachykinin NK_1 receptor agonist (GR73632, 30 pmol site^{-1}; 75 ± 9 μl site^{-1}) in the rat dorsal skin can be completely inhibited by the co-injection with the tachykinin NK_1 receptor antagonist SR140333, at a dose of 1 nmol site^{-1} (13 ± 3 μl site^{-1}, $n=5$). At this dose of SR140333, plasma extravasation induced by all doses of adenosine (Figure 3A) and IB-MECA (Figure 3D) was significantly inhibited by this antagonist. In addition, SR140333 had no significant effect on plasma extravasation evoked by the lower doses of CPA and NECA but significantly reduced the responses induced by the higher doses of these compounds (Figure 3B,C).

The tachykinin NK_2 receptor antagonist SR48968 (0.3 μmol kg^{-1}, i.v.) failed to significantly affect the plasma extravasation induced by adenosine (100 nmol site^{-1}), CPA (10 nmol site^{-1}), NECA (3 nmol site^{-1}), IB-MECA and GR73632 (30 pmol site^{-1}) ($n=3-4$; not shown).

Effect of capsaicin pretreatment

The role of capsaicin sensitive primary afferent neurons in response to adenosine and its analogues (CPA, NECA, and IB-MECA) in dorsal skin was tested in rats treated at neonatal stage with capsaicin to deplete neuropeptides. Figure 4 shows that a significant inhibition of plasma extravasation in capsaicin-pretreated rats was observed in response to the highest and intermediate doses of adenosine (100 and 300 nmol site^{-1}, Figure 4A), CPA (3 and 10 nmol site^{-1}, Figure 4B) and NECA (1 and 3 nmol site^{-1}, Figure 4C) as compared to the response seen in control rats. The plasma extravasation evoked by IB-MECA (0.01–1 nmol site^{-1}) in capsaicin-pretreated rats was not statistically different from the responses obtained in control rats (Figure 4D). The GR73632 (30 pmol site^{-1})-induced plasma extravasation was unmodified in capsaicin-pretreated rats as compared to the values seen in control rats (26.4 ± 2 and 29.4 ± 4 μl site^{-1} for control and treated rats, respectively). Similarly, compound 48/80 (5 μg site^{-1})-induced plasma extravasation did not significantly differ among control and capsaicin-pretreated rats (not shown).

In capsaicin-pretreated rats a significant reduction ($P<0.05$) was observed in the total amount of dye leaked in response to topical administration of capsaicin solution (5%) within the area of treated hind paw skin (4.4 ± 0.3 μl per 100 mg paw skin^{-1}, $n=5$) as compared to the responses observed in vehicle-pretreated rats (26 ± 8 μl per 100 mg paw skin^{-1}, respectively, $n=5$). The topical administration of capsaicin-vehicle in the contralateral hind paw skin of control rats produced lower plasma extravasation (4.2 ± 0.14 μl per 100 mg paw skin^{-1}) than that induced by capsaicin solution in these animals and did not significantly differ from the response evoked by capsaicin (4.4 ± 0.3 μl per 100 mg paw skin^{-1}) in capsaicin-pretreated rats.

Role of mast cells

It is well established that compound 48/80 induces degranulation of mast cells leading to subsequent release of both histamine and 5-HT, which are able to produce plasma protein extravasation in the rat skin. In rats pretreated (30 min before) with histamine H_1 and 5-HT receptor antagonist, cyproheptadine (1 mg kg^{-1}, i.p.), the plasma extravasation evoked by compound 48/80 (500 ng site^{-1}) was markedly reduced (9 ± 2 μl site^{-1}) as compared to the response in control rats (45 ± 5.4 μl site^{-1}, $n=5$).

The plasma extravasation in response to adenosine (30–300 nmol site^{-1}), CPA (3–30 nmol site^{-1}), NECA (0.1–

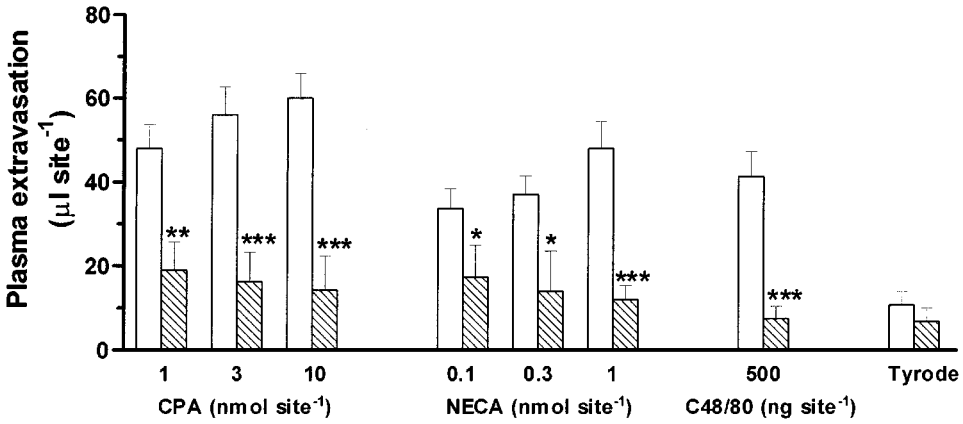

Figure 5 Effect of compound 48/80-pretreatment on plasma extravasation induced by CPA and NECA. Responses to CPA, NECA and compound 48/80 (C-48/80) are shown by open (control group, $n=5$) and striped bars (compound 48/80-pretreated group, $n=6$), respectively. Values are means$\pm$s.e.mean for n rats. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to control group.

3 nmol site^{-1}) and IB-MECA (0.1 – 3 nmol site^{-1}) was markedly inhibited by the cyproheptadine pretreatment (Table 2). Furthermore, a significant reduction in the plasma extravasation induced by both CPA (1 – 10 nmol site^{-1}) and NECA (0.1 – 1 nmol site^{-1}) was apparent in rats treated chronically with compound 48/80 (5 mg/kg; 3 days before, s.c.; Figure 5). The plasma extravasation induced by intradermal injection of compound 48/80 (500 ng site^{-1}) was abolished in those treated rats, as expected (Figure 5, $n=5$).

Discussion

The results from this study, where a range of adenosine agonists and antagonists have been used, provide information to suggest that adenosine A_1, A_2 and A_3 receptors are present in the rat cutaneous microvasculature where they can mediate increased microvascular permeability and, as a consequence, inflammatory oedema formation. The results further suggest that neurogenic mechanisms and mast cell-derived amines are involved in the responses of the adenosine analogues, although the respective contribution of these mechanisms at each receptor type appears dependent on the receptor type involved. Our finding that the adenosine A_3 receptor agonist (IB-MECA) induces plasma extravasation when injected into rat skin confirms previous results (Reeves *et al*., 1997). However, it is unclear why these same authors did not report a significant plasma extravasation in response to adenosine, the adenosine A_1 receptor agonist (CPA) or the A_2 agonist (NECA), at doses that we find here to be effective in producing plasma extravasation.

The plasma extravasation evoked by adenosine, CPA and NECA, but not by IB-MECA, was significantly inhibited by theophylline, a xanthine that is a non-selective adenosine receptor antagonist. The lack of effect of theophylline in the response evoked by the A_3 receptor agonist IB-MECA is striking but may be related to differences in affinity among species. It has been suggested that the difference in xanthine affinity among species and low degree of homology between the human and rat receptors can reflect the existence of two subtypes of A_3 receptors (see review by Jacobson, 1998).

The selective adenosine A_1 receptor antagonist (DPCPX) dose-dependently reduced the plasma extravasation evoked by CPA but did not modify the response induced by adenosine, NECA or IB-MECA. On the other hand, the selective adenosine A_2 receptor antagonist (DMPX) significantly reduced the plasma extravasation evoked by NECA without modifying the responses to adenosine, CPA and IB-MECA. This suggests that adenosine A_1 and A_2 receptors are directly involved in the nucleoside-mediated inflammatory response. Although a selective A_3 receptor antagonist has not been described, the results in this study confirm that IB-MECA-induced plasma extravasation is mediated by activation of adenosine A_3 receptors on rat (Reeves *et al*., 1997) and mouse (Tilley *et al*., 2000) mast cells.

The results with the tachykinin NK_1 receptor antagonist and capsaicin-depleted skin suggest that the A_1 and A_2 receptors are able to mediate substance P release that is important for the observed oedema formation. Substance P and NK_1 agonists act primarily *via* the tachykinin receptors to mediate vasoactive responses in post-capillaries venules. They are potent mediators of increased permeability and, as a consequence, oedema formation (see review Brain, 1996; Holzer, 1998). In this study, the responses to adenosine and analogues were partially inhibited by the tachykinin NK_1 antagonist SR140333, but not by the NK_2 SR48968 receptor antagonist, indicating that the tachykinin NK_1 (but not NK_2 receptors) are involved in the adenosine and related analogue-induced plasma extravasation in rat dorsal skin. Furthermore the repeated application of capsaicin leads to desensitization or degeneration of sensory neurons and consequently depletion of sensory neuropeptides (Jancsó *et al*., 1977; Gamse *et al*., 1980). The oedema formation induced by the highest doses of adenosine, CPA and NECA, but not IB-MECA, observed in capsaicin-pretreated rats was significantly reduced. These results suggest that A_1 and A_2 receptors, but not A_3 receptors, act directly to activate sensory neurons to release a tachykinin NK_1 receptor agonist. The responses induced by adenosine and analogues were only significantly reduced at their highest doses by SR140333 or capsaicin-pretreatment, which might suggest that only higher concentrations of adenosine, CPA and NECA are able to activate capsaicin sensitive primary afferent neurons. The

findings are in keeping with those of Tamaoki *et al*. (1999) where adenosine was shown to induce airway microvascular leakage in sensitized Brown Norway rat lung through stimulation of NK_1 receptors. By comparison, the results obtained with the NK_3 agonist IB-MECA are less clear in that IB-MECA responses were inhibited by NK_1 receptor antagonist treatment, but not by capsaicin treatment.

The present finding that adenosine and analogue-induced plasma extravasation was almost completely inhibited in the presence of the histamine and 5-HT receptors antagonist, cyproheptadine, or even by pretreatment of rats with compound 48/80, strongly indicate that mast cell-derived amines are also involved in these responses. Adenosine and its analogues have been shown to release histamine and 5-HT from mast cells but the exact pattern of receptor subtype expression depends on the source of mast cells (Gessi *et al*., 2000). It is known that mast cell-derived agents (i.e.: histamine, 5-HT and tryptase) are potentially able to stimulate sensory neurons to release neuropeptides that then amplify the inflammatory response (Gamse *et al*., 1981; Maggi & Melli, 1988; Steinhoff *et al*., 2000). Thus adenosine may act *via* mast cells to stimulate neuropeptides release. Alternatively, it has been reported that mast cell activation could occur secondary to stimulation of adenosine A_1 receptors on sensory neurons (Dowd *et al*., 1998; Hong *et al*., 1998). Therefore, afferent stimulation could lead to release of neuropeptides, which in turn could stimulate mast cells located in close proximity to sensory neuron endings (Newson *et al*., 1983; Ishida-Yamamoto *et al*., 1989).

In conclusion, our results provide direct evidence that adenosine A_1, A_2 and A_3 receptors can all mediate increased microvascular permeability in rat skin, leading to oedema formation. Furthermore, the results obtained suggest that the permeability increasing mechanisms induced by all three receptor subtypes involve, to differing degrees, the participation of the tachykinin NK_1 receptor, sensory nerves and mast cell amines.

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