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Acute paw oedema induced by local injection of adenosine A_1, A_2 and A_3 receptor agonists

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Abstract

The present study used plethysmometry to examine oedema following local injection of selective adenosine A_1, A_2 and A_3 receptor agonists and inhibitors of adenosine metabolism into the hindpaw of the rat. N^6-Cyclopentyladenosine and L-N^6-phenylisopropyladenosine (A_1), 2-[*p*(2-carboxyethyl) phenethylamino]-5′-*N*-ethylcarboxamidoadenosine hydrochloride (CGS21680) (A_{2A}) and N^6-benzyl-5′-*N*-ethylcarboxamido adenosine (N^6-B-NECA) (A_3) all produced an increase in paw volume (N^6-B-NECA > N^6-cyclopentyladenosine, L-N^6-phenylisopropyladenosine > CGS21680). At the highest dose, each agent also produced a systemically mediated suppression of oedema. Oedema by N^6-cyclopentyladenosine was blocked by caffeine, 8-cyclopentyl-1,3-dimethylxanthine and enprofylline. Oedema by CGS21680 was blocked by caffeine and 8-cyclopentyl-1,3-dimethylxanthine. Oedema by N^6-B-NECA was blocked by enprofylline, but not by caffeine or 8-cyclopentyl-1,3-dimethylxanthine, or by systemic administration of MRS 1191. Oedema by both N^6-cyclopentyladenosine and N^6-B-NECA was blocked by mepyramine, ketanserin and phentolamine, but that by CGS21680 was not. The adenosine kinase inhibitor 5′-amino-5′-deoxyadenosine and the adenosine deaminase inhibitor 2′-deoxycoformycin produced only a limited increase in paw volume, and this was blocked by caffeine. This study demonstrates an acute paw oedema response following local administration of adenosine A_1, A_2 and A_3 receptor agonists, which likely results from different mechanisms of action in each case.

Keywords: Paw oedema; Adenosine A_1 receptor; Adenosine A_2 receptor; Adenosine A_3 receptor; Plethysmometry; (Rat)

1. Introduction

Nociceptive information is transmitted to the central nervous system via activation of small diameter C and Aδ fibres, which can be activated by mechanical, thermal and chemical stimulation. In inflammation, a wide range of chemical mediators can contribute to pain signalling both by direct actions on the sensory nerve terminal itself, as well as by actions on elements adjacent to the nerve terminal such as mast cells, sympathetic post-ganglionic neurons or neutrophils (reviewed by Dray, 1994; Levine and Taiwo, 1994). Inhibition of prostaglandin production by inhibition of cyclo-oxygenase represents one major class of therapeutic agents; other systems that are being, or potentially could be, explored for therapeutic development as analgesics include prostanoid receptor antagonists, kinin antagonists and nerve growth factor antagonists (Dray and Urban, 1996). With a prominent analgesic effect expressed at peripheral sites, there is the potential to develop some of these agents as local formulations that would produce higher local concentrations and lower systemic concentrations and thus minimize systemic side effects. Such local formulations have been prepared for cyclo-oxygenase inhibitors (Vaile and Davis, 1998), capsaicin and its analogs (Szallasi and Blumberg, 1996) and local anaesthetics (Rowbotham, 1994). However, with higher local concentrations, there may potentially be adverse effects that are locally expressed, which are not necessarily seen following systemic administration.

The peripheral adenosine receptor system represents another system that potentially could be targeted for therapeutic development of local or topical analgesics. Thus, the local administration of adenosine A_1 receptor agonists to the hindpaw produces analgesia or antihyperalgesic responses in nociceptive (Taiwo and Levine, 1990; Aley et al., 1995), inflammatory (Karlsten et al., 1992; Doak and Sawynok, 1995) and neuropathic (Liu and Sawynok, 1998) pain tests. This receptor exists as part of a tri-receptor

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complex on sensory afferents in association with α_2-adrenoceptors and μ-opioid receptors (Aley and Levine, 1997), and produces analgesia by inhibition of cyclic AMP production in the sensory nerve terminal (Taiwo and Levine, 1990; Khasar et al., 1995). This local receptor system also can be recruited indirectly by inhibition of adenosine kinase and adenosine deaminase (Sawynok et al., 1998).

The present study was initiated following the observation that local administration of selective adenosine A_1 receptor agonists into the hindpaw of rats with a spinal nerve ligation could produce an acute paw oedema in addition to producing a local antihyperalgesic action (Liu and Sawynok, 1998). We sought to investigate this action directly by measuring paw volume in normal rats. We determined (a) which adenosine receptor subtype mediates this response by comparing effects of adenosine A_1, A_2 and A_3 receptor agonists and determining effects of respective receptor antagonists, (b) determining whether peripheral biogenic amines potentially released from mast cells or sympathetic neurons could contribute to these responses, and (c) whether indirect manipulation of local adenosine levels by inhibition of adenosine kinase or adenosine deaminase would produce similar effects. These studies were considered necessary as it was not clear how adenosine A_1 receptor activation could produce this apparent pro-inflammatory response. Adenosine A_{2A} receptor agonists have well recognized anti-inflammatory actions by their effects on inflammatory and immune cells (Cronstein, 1994; Sullivan and Linden, 1998), while adenosine A_{2B} and A_3 receptor agonists produce pro-inflammatory effects by actions on mast cells to release mediators (Feoktistov and Biaggioni, 1997; Marquardt, 1998). Potential pro-inflammatory actions of adenosine A_1 receptor activation have been reported in in vitro systems (Cronstein, 1994; Sullivan and Linden, 1998), but there has been little in vivo data demonstrating or examining such actions.

2. Methods

2.1. Animals

Experiments were performed on male Sprague–Dawley rats 100–200 g. Rats were housed in pairs, maintained at 21 ± 1°C, and given ad libitum access to food and water. Rats were used three times during the course of 10 days, with drugs being injected into alternating hindpaws on sequential trials. No residual effects were seen 24 h following drug injections. The trial order had no significant effect on the expression of oedema to the various agents (Table 1). Groups of five to six rats were used in all experiments.

2.2. Paw volume determinations

Paw volumes were determined by volume displacement of an electrolyte solution in a commercially available plethysmometer (Ugo Basile, Italy). The hindpaw was immersed to the junction of the hairy skin, and volumes read from a liquid crystal display. Values were determined in triplicate prior to and at 30, 60, 90, 120 and 180 min following local drug injections. The mean of these values for each rat was converted to a percentage increase over the mean basal preinjection value to account for body weight changes between trials. Absolute paw volume values for rats in the 100 g range were 1.25 ± 0.11 ml ($n = 50$) and in the 200 g range, 1.84 ± 0.18 ml ($n = 50$).

Table 1
Reproducibility of paw volume changes induced by various agents in different trials. Values represent cumulative percentage increase in paw volume over the 3-h time course examined (see Figs. 1B, 2B, and 6B). No significant differences occur between trials
CPA, N^6-cyclopentyladenosine.

Treatment	Run 1	Run 2	Run 3
Saline	16.5 ± 2.0	10.6 ± 4.5	12.4 ± 5.2
CPA 5 nmol	123 ± 24	82 ± 15	95 ± 22
CGS21680 5 nmol	46.3 ± 8.0	39.7 ± 5.7	38.4 ± 9.1
N^6-B-NECA 0.5 nmol	91 ± 19	–	123 ± 18
N^6-B-NECA 5 nmol	181 ± 32	–	175 ± 27

2.3. Drugs

Drugs were injected s.c. into the dorsal surface of the hindpaw in a volume of 50 μl saline or vehicle. Agonists and antagonists were coinjected in the same final volume. N^6-cyclopentyladenosine, L-N^6-phenylisopropyladenosine, 2-[*p*(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine hydrochloride (CGS21680), 5′-amino-5′-deoxyadenosine, 2′-deoxycoformycin, caffeine, mepyramine maleate, ketanserin tartrate and phentolamine hydrochloride were dissolved in saline. 8-Cyclopentyl-1,3-dimethylxanthine and enprofylline were dissolved in 0.02 N NaOH, while N^6-benzyl-*N*-ethylcarboxamidoadenosine (N^6-B-NECA) was dissolved in 10% dimethylsulfoxide for dose–response determinations or 0.02 N NaOH for antagonist experiments. We attempted to use the selective adenosine A_3 receptor agonist IB-MECA (1-deoxy-1-[6-[[(3-iodophenyl)methyl]amino]-9*H*-purin-9-yl]-*N*-methyl-β-D-ribofuronamide) and the antagonist MRS1191 (3-ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(+/−)-dihydropyridine-3,5-dicarboxylate) (Table 2), but the concentrations of dimethylsulfoxide or ethanol required to dissolve these produced a pronounced paw oedema alone, so these agents could not be administered locally. Instead, MRS1191 was administered systemically (1–3 mg/kg) to determine if the N^6-B-NECA response was due to adenosine A_3 receptor activation. MRS1191 was dissolved in 10% dimethylsulfoxide/Emulphor (Rhone Poulenc).

2.4. Data expression and statistics

Data is expressed as a time course of the change in paw volume (percentage baseline values at each determination),

Table 2
Relative activity of agonists and antagonists used in this study for the various adenosine receptor subtypes
Values depict Ki values in nM for rat or human (h) binding studies. Numbers in square brackets represent EC_{50} values for adenosine agonists for cyclic AMP production in human erythroleukemia cells. CPA N^6-cyclopentyladenosine, L-PIA L-N^6-phenylisopropyladenosine, CPT 8-cyclopentyl-1,3-dimethylxanthine. Data from van Galen et al. (1994) and Feoktistov and Biaggioni (1997).

Agent	A_1	A_{2A}	A_{2B}	A_3
Agonists				
CPA	0.6	462	[203,000(h)]	240
L-PIA	1.2	124	[160,000(h)]	158
CGS21680	2600	15	[> 1 mM(h)]	584
N^6-B-NECA	87	95	–	6.8
IB-MECA	54	56	[200,000(h)]	1.1
Antagonists				
caffeine	29,000	48,000	–	30% at 100 μM
CPT	11	1400	–	39% at 100 μM
enprofylline	156,000(h)	32,000(h)	7000(h)	65,000(h)
MRS1191	40,100	> 100,000	–	31.4 (h)

as well as a cumulative percentage increase score, which is the sum of differences from the mean baseline. Time course experiments and comparisons of more than two groups were analyzed by analysis of variance followed by the Student–Neuman–Keuls test, while cumulative scores for two groups were compared by the Student's *t*-test.

3. Results

3.1. Effect of adenosine receptor agonists on paw volume

The local injection of saline produced a slight increase in paw volume at 30–60 min following injection, and generated cumulative percentage increase scores of between 10 and 20 units (Table 1, Figs. 1, 6 and 7). The local injection of the adenosine A_1 receptor agonists N^6-cyclopentyladenosine and L-N^6-phenylisopropyladenosine (Table 2) produced a significant increase in paw volume at 0.5–5 nmol N^6-cyclopentyladenosine and 1.5–15 nmol L-N^6-phenylisopropyladenosine (Fig. 1A). This effect was maximal within 30 min for N^6-cyclopentyladenosine and was maintained for much of the 180-min observation interval (Fig. 1B). L-N^6-phenylisopropyladenosine exhibited a similar time course of expression (data not shown). At 50 nmol, there was a reduction in paw volume compared to the 5 nmol dose for N^6-cyclopentyladenosine (Fig. 1A, B). This effect was likely due to a systemic anti-inflammatory effect, as the effect of administration of 5 nmol N^6-cyclopentyladenosine into the ipsilateral paw was suppressed by administration of 50 nmol N^6-cyclopentyladenosine into the contralateral paw (Fig. 1A). Local injection of the adenosine A_{2A} receptor agonist CGS21680 (Table 2) also produced a significant increase in paw volume, but the degree of increase was less than that seen with the adenosine A_1 receptor agonists, and the effect exhibited a plateau rather than being dose-related (Fig. 2A). A systemic effect was observed at the 50-nmol dose (Fig. 2A). The putative adenosine A_3 receptor agonist N^6-B-NECA (Table 2) produced a dose-related increase in paw volume between 0.05 and 5 nmol, with a systemic effect occurring at 50 nmol (Fig. 2A). The greatest effect with this agent, seen at 5 nmol, was greater than the peak effect seen with N^6-cyclopentyladenosine or L-N^6-phenylisopropyladenosine (cf. Figs. 1 and 2).

3.2. Effects of adenosine receptor antagonists on paw volume produced by adenosine agonists

Caffeine, a non-selective adenosine A_1 and A_2 receptor antagonist (Table 2), had no significant effect on the response produced by saline, but produced a dose-related inhibition of the response to N^6-cyclopentyladenosine (Fig. 3). The 1500 nmol dose also inhibited the response to CGS21680 (Fig. 3A). When 0.02 N NaOH was used as a

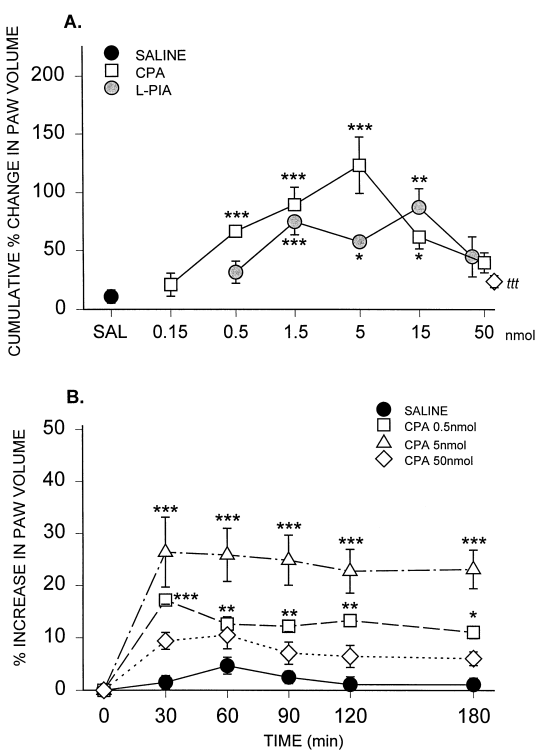

Fig. 1. Increase in paw volume induced by the local injection of the adenosine A_1 receptor agonists N^6-cyclopentyladenosine (CPA) and L-N^6-phenylisopropyladenosine (L-PIA). (A) Depicts the cumulative response over 3 h, while (B) depicts the time course of N^6-cyclopentyladenosine actions. The ◇ in (A) depicts the ipsilateral paw volume result for N^6-cyclopentyladenosine 5 nmol injected into the ipsilateral paw, and N^6-cyclopentyladenosine 50 nmol injected into the contralateral paw. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to saline, *ttt* $P < 0.001$ compared to N^6-cyclopentyladenosine 5 nmol.

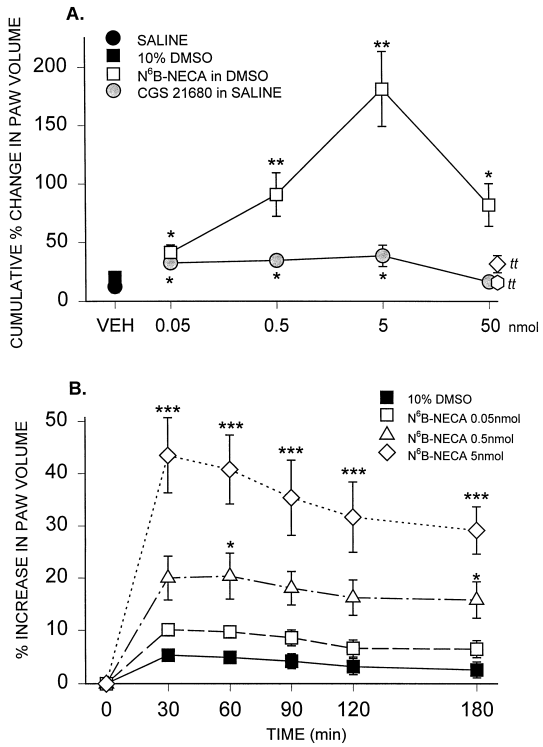

Fig. 2. Increase in paw volume induced by the local injection of the adenosine A_{2A} receptor agonist CGS21680 and the A_3 receptor agonist N^6-B-NECA. (A) Depicts the cumulative response over 3 h. The ipsilateral paw volume response to ⬠ CGS21680 and ◇ N^6-B-NECA (50 nmol each) injected into the contralateral paw with 5 nmol of the same agonist in the ipsilateral paw. (B) Time course of N^6-B-NECA actions at indicated doses. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to saline or vehicle, *tt* $P < 0.01$ compared to 5 nmol dose of corresponding agent.

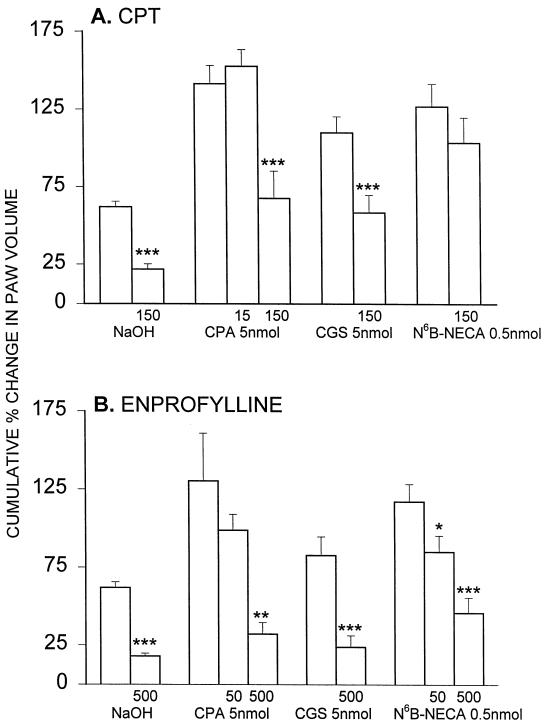

Fig. 4. Effects of (A) 8-cyclopentyl-1,3-dimethylxanthine (CPT) and (B) enprofylline (doses in nmol) on the increase in paw volume produced by the 0.02 N NaOH vehicle, N^6-cyclopentyladenosine (CPA), CGS21680 (CGS) and N^6-B-NECA. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to corresponding agonist.

vehicle (caffeine did not block the action of N^6-cyclopentyladenosine in dimethylsulfoxide, the vehicle used for N^6-B-NECA), it produced an intrinsic effect on paw volume and this was reduced by coadministration of caffeine (Fig. 3). Caffeine retained its ability to block the actions of N^6-cyclopentyladenosine and CGS21680 in the presence of this vehicle (data not shown), but had no significant effect on the increase in paw volume produced by N^6-B-NECA (Fig. 3). 8-Cyclopentyl-1,3-dimethylxanthine, a selective adenosine A_1 receptor antagonist (Table 2), inhibited the intrinsic effect of the 0.02 N NaOH vehicle and reduced the effects of both N^6-cyclopentyladenosine and CGS21680 (Fig. 4A). However, it did not alter the response to N^6-B-NECA (Fig. 4A). Enprofylline, an adenosine A_{2B} receptor antagonist with some selectivity (Table 2), reduced the effect of the vehicle and inhibited the

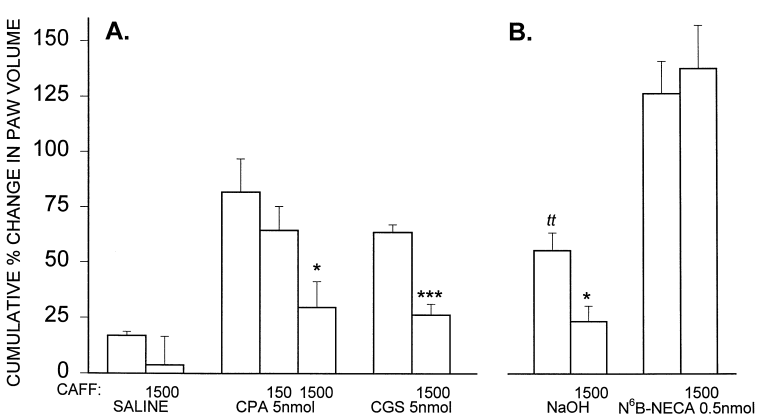

Fig. 3. Effects of caffeine (CAFF, doses in nmol) on the increase in paw volume produced by (A) saline, N^6-cyclopentyladenosine (CPA) and CGS21680 (CGS), and (B) the 0.02 N NaOH vehicle and N^6-B-NECA. $^*P < 0.05$, $^{***}P < 0.001$ compared to corresponding agonist, *tt* $P < 0.01$ compared to saline.

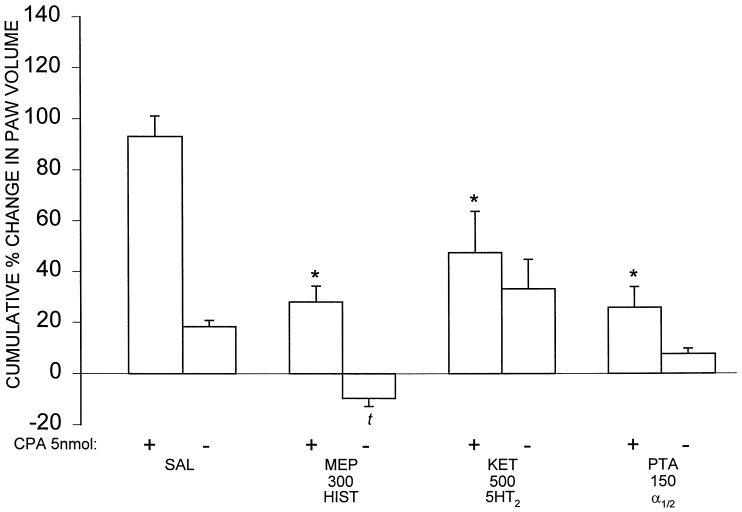

Fig. 5. Effects of histamine (HIST), 5-hydroxytryptamine (5-HT) and α-adrenoceptor antagonists (doses in nanomoles) on the increase in paw volume produced by saline or N^6-cyclopentyladenosine 5 nmol (open bars). MEP mepyramine, KET ketanserin, PTA phentolamine. $^*P < 0.05$ compared to N^6-cyclopentyladenosine (CPA); t $P < 0.05$ compared to saline.

actions of N^6-cyclopentyladenosine, CGS21680 and N^6-B-NECA (Fig. 4B). Systemic administration of MRS1191, a selective adenosine A_3 receptor antagonist (Table 2), at doses of 1 and 3 mg/kg given 15 min before the agonist, had no significant effect on the action of N^6-B-NECA (cumulative percentage increase scores over 3 h N^6-B-NECA 0.5 nmol 106 ± 14, N^6-B-NECA/MRS1191 1 mg/kg 127 ± 18, N^6-B-NECA/MRS1191 3 mg/kg 127 ± 10, $n = 8$ per group, $P > 0.05$).

3.3. Effects of biogenic amine receptor antagonists on the paw volume increase produced by adenosine agonists

Mepyramine (histamine H_1 receptor antagonist), ketanserin (5-hydroxytryptamine$_2$ or 5-HT_2 receptor antagonist) and phentolamine (α_1 and α_2-adrenoceptor antagonist) all inhibited the increase in paw volume produced by N^6-cyclopentyladenosine (Fig. 5). Curiously, mepyramine inhibited the small increase in paw volume seen with saline (Figs. 5 and 6) suggesting some form of involvement of histamine release in this response. Phentolamine and ketanserin were without intrinsic effects compared to saline (Fig. 5). None of the antagonists affected the cumulative paw volume response to CGS21680 (Fig. 6A), but there was some effect of mepyramine in the CGS21680 time course (Fig. 6B). Each of mepyramine, ketanserin and phentolamine significantly reduced the cumulative increase in paw volume produced by N^6-B-NECA (Fig. 6C and D).

3.4. Effects of inhibitors of adenosine kinase and adenosine deaminase on paw volume

Local injection of the adenosine kinase inhibitor 5′-amino-5′-deoxyadenosine produced a small but significant increase in paw volume (Fig. 7A and B). This was not dose-related as both 10 and 100 nmol produced a similar effect. The increase in paw volume was blocked by coadministration of caffeine (Fig. 7A). A similar pattern of effect with a modest expression and caffeine-reversibility

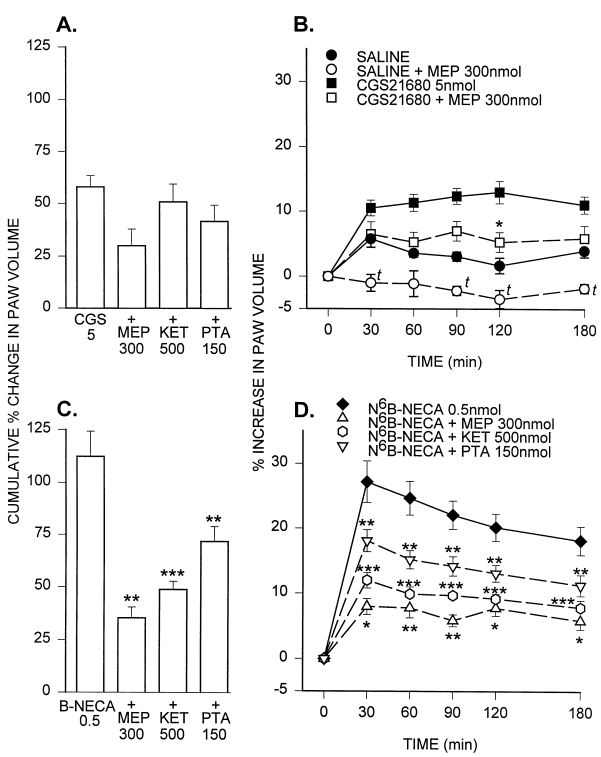

Fig. 6. Effects of histamine, α-adrenoceptor and 5-HT receptor antagonists (doses in nanomoles) on the increase in paw volume produced by (A, B) CGS21680 (CGS) and (C, D) N^6-B-NECA. The left panels depict cumulative responses, while the right panels depict time courses. MEP mepyramine, KET ketanserin, PTA phentolamine. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to CGS21680 or N^6-B-NECA, t $P < 0.05$ compared to saline.

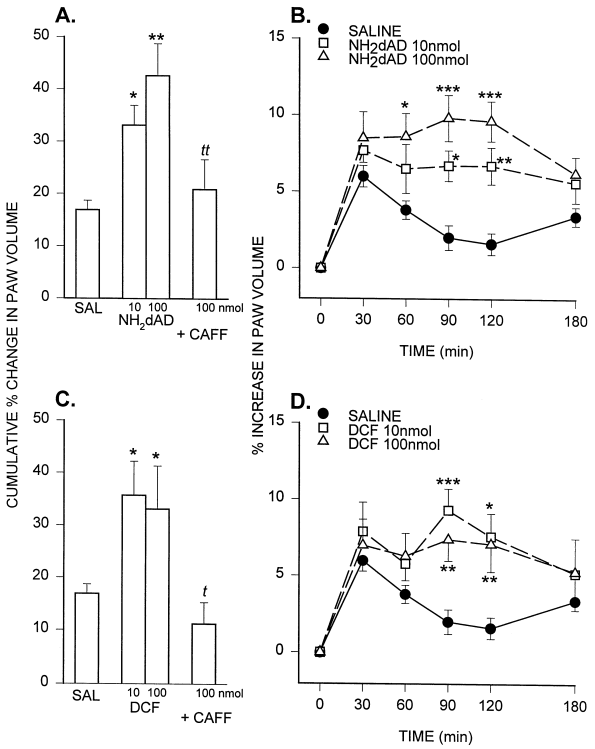

Fig. 7. Increase in paw volume produced by the local administration of (A, B) the adenosine kinase inhibitor 5′-amino-5′-deoxyadenosine, and (C, D) the adenosine deaminase inhibitor 2′-deoxycoformycin, and the block of this effect by caffeine (CAFF) 1500 nmol. Note difference in scale compared to (Figs. 1, 2, and 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to saline (SAL); t $P < 0.05$, tt $P < 0.01$ compared to corresponding dose of 5′-amino-5′-deoxyadenosine (NH_2dAD) (A, B) or 2′-deoxycoformycin (DCF) (C, D).

was observed with the adenosine deaminase inhibitor 2′-deoxycoformycin (Fig. 7C and D). When 1000 nmol of each agent was administered into the hindpaw, there was no further increase in paw volume compared to the 10 and 100 nmol doses (data not shown); these higher doses are known to exert systemic effects (see Discussion).

4. Discussion

The present study demonstrates that agonists selective for adenosine A_1, A_2 and A_3 receptors can produce oedema following local injection into the rat hindpaw. The responses produced by adenosine A_1 and A_3 receptor agonists are greater than those produced by the adenosine A_{2A} receptor agonist CGS21680, and occur by a different mechanism as revealed by amine antagonist studies. An earlier study had demonstrated paw oedema in response to the local injection of adenosine into the hindpaw of mice, but selective receptor agonists or antagonists were not examined (Zingashina et al., 1996). Other studies that have administered N^6-cyclopentyladenosine peripherally (1 μg or 3 nmol) did not report paw oedema (Taiwo and Levine, 1990; Aley and Levine, 1997; Aley et al., 1995), but it is not clear if this is lack of noting of an effect, or whether the use of a smaller injection volume (2.5 vs. 50 μl) minimizes the effect. Another study reported that intradermal N^6-cyclopentyladenosine produced an increase in plasma protein extravasation, but this action was attributed to activation of an adenosine A_3 receptor (Reeves et al., 1997). In our study, higher doses of all agonists produced a reduction in paw oedema, and this is a systemic effect as it was also seen following administration of the higher dose into the contralateral hindpaw. This may result from actions in the spinal cord, as spinal administration of adenosine analogs results in a peripherally expressed anti-inflammatory response, which is mediated by adenosine A_1 receptors (Bong et al., 1996). This accounts for the action of the high dose of N^6-cyclopentyladenosine, and likely N^6-B-NECA, as this agent has affinity for adenosine A_1 receptors at doses only somewhat higher (13-fold) than those which activate adenosine A_3 receptors (Table 2). CGS21680 exhibits a greater selectivity between adenosine A_{2A} and A_1 receptors (170-fold) (Table 2). However, the effects of CGS21680 are observed at quite low doses, such that selectivity may well be lost at the highest dose examined, which is 1000 times the lowest active dose.

4.1. Adenosine A_1 receptors

The increase in paw volume seen with selective adenosine A_1 receptor agonists involves adenosine A_1 receptors, as it is blocked by the selective adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine. Both caffeine and enprofylline, which exhibit limited selectivity for adenosine A_1 and A_2 receptors, also block this response. Some pro-inflammatory actions of adenosine A_1 receptor agonists on inflammatory cells have been reported in vitro (Cronstein et al., 1990, 1992), but the paw oedema observed in the present study occurs in the absence of an inflammatory stimulus and may not involve inflammatory cells. The inhibition of the response to N^6-cyclopentyladenosine by mepyramine and ketanserin indicate an involvement of histamine H_1 and 5-HT_2 receptors, respectively. Both histamine and 5-HT produce an acute paw oedema in rats when administered locally (Maling et al., 1974). This results from actions on the vasculature and on sensory afferents for histamine (Amman et al., 1995), and from actions on the vasculature for 5-HT (Sufka et al., 1991). Both amines could originate from mast cells (Maling et al., 1974; Scott et al., 1994). Rat mast cells do not encode adenosine A_1 receptors (Ramkumar et al., 1993), but mast cell activation could occur indirectly via a neurogenic mechanism. Thus, adenosine A_1 receptor activation has been reported to excite nociceptive afferents in rats (Dowd et al., 1998; Hong et al., 1998), and afferent stimulation could lead to stimulation of mast cells. Mast cells are located in close proximity to, or in direct contact with, peripheral sensory nerve endings (Weisner-Menzel et al., 1981; Newson et al., 1983; Marshall and Waserman,

1995), and sensory nerve stimulation leads to mast cell degranulation (Kiernan, 1971; Dimitriadou et al., 1991). The sympathetic nervous system also is involved in the adenosine A_1 receptor mediated effect, as phentolamine markedly reduced the paw volume response. It is unlikely that this is a direct effect, as adenosine A_1 receptors are known to inhibit, rather than promote, noradrenaline release from peripheral sympathetic nerve terminals (Fredholm and Hedqvist, 1980). It could represent a secondary effect due to local tissue inflammation (cf. involvement of histamine and 5-HT), as chemical sympathectomy reduces inflammatory responses (Coderre et al., 1989; Donnerer et al., 1991).

While the above scheme for the paw oedema response to adenosine A_1 agonists is plausible, it must be borne in mind that in contrast to the electrophysiological studies which indicate that adenosine A_1 receptors excite sensory afferents, behavioural studies in rodents uniformly demonstrate that peripherally administered adenosine A_1 agonists produce a reduction in pain signalling (Taiwo and Levine, 1990; Aley et al., 1995; Khasar et al., 1995; Liu and Sawynok, 1998), an action which is proposed to be due to a direct effect on the sensory nerve terminal (Aley and Levine, 1997). The behavioural data is thus in direct contradiction to the electrophysiological data. The fundamental observation of an oedema response to adenosine A_1 receptor agonists, which one would expect to be more consistent with a pronociceptive or pro-inflammatory response, appears paradoxical. However, the two effects occur by independent mechanisms, as the same doses of N^6-cyclopentyladenosine and L-N^6-phenylisopropyladenosine, which produce paw oedema also produce a local antinociception (Liu and Sawynok, 1998). If the antinociception results from suppression of afferent nerve activity, the involvement of a neurogenic mechanism must be questioned. This issue needs to be resolved by approaches which address the mechanism and the paradox directly.

4.2. Adenosine A_{2A} receptors

The paw oedema produced by CGS21680 likely results from activation of adenosine A_{2A} receptors due to (a) the selectivity of this agent for adenosine A_{2A} receptors (Table 2), (b) the different extent of the effect compared to other agents, and (c) the different profile of amine antagonists against CGS21680 compared to other agents. The latter two properties indicate a fundamental difference in mechanism from that recruited by N^6-cyclopentyladenosine or N^6-B-NECA. Mepyramine, histamine and phentolamine do not alter the cumulative effect of CGS21680, while markedly reducing that of N^6-cyclopentyladenosine and N^6-B-NECA. The modest effect seen with mepyramine in the time course likely reflects the intrinsic effect of mepyramine against the saline (which in itself indicates histamine involvement in the vehicle effect). The less selective antagonists used (caffeine, enprofylline) both block the response to CGS21680, and this is consistent with an adenosine A_{2A} receptor involvement. The block of CGS21680 action seen with 8-cyclopentyl-1,3-dimethylxanthine was surprising and may be an artefact. Thus, the vehicle used to dissolve 8-cyclopentyl-1,3-dimethylxanthine produced an intrinsic mild oedema, and this response was reduced by the 8-cyclopentyl-1,3-dimethylxanthine implying an involvement of adenosine A_1 receptors in the response. It is likely that the CGS21680/8-cyclopentyl-1,3-dimethylxanthine interaction reflects this vehicle component of action, particularly as the A_{2A} effect itself is so modest in extent.

It is likely that the adenosine A_{2A} receptor mediated effect results from local vascular effects of adenosine A_{2A} receptors producing vasodilation due to relaxation of vascular smooth muscle cells (Olsson and Pearson, 1990). This could result in plasma exudation via opening up of gaps between endothelial cells, with the limited extent of the effect reflecting the limited contribution that this mechanism can make to oedema. The lack of block by amine antagonists is consistent with such a mechanism. Adenosine A_{2A} receptors have well recognized anti-inflammatory effects (Cronstein, 1994; Sullivan and Linden, 1998), but the present responses were observed in the absence of an inflammatory stimulus making it unlikely that such actions are involved in the current responses. Interestingly, while lacking an intrinsic effect on plasma extravasation into the knee joint, CGS21680 augments plasma extravasation produced by bradykinin (Green et al., 1991). Thus, some of the CGS21680 effect observed here could be due to interactions with locally released tissue mediators in response to the injection itself.

4.3. Adenosine A_{2B} and A_3 receptors

The potential role of adenosine A_3 receptors in producing oedema was explored using N^6-B-NECA, which has some selectivity for adenosine A_3 receptors (Table 2). The more selective adenosine A_3 receptor antagonist IB-MECA could not be injected locally due to pronounced oedema produced by the vehicle required to dissolve it. N^6-B-NECA produced the greatest degree of oedema of all the adenosine agonists examined. This effect was not blocked by caffeine or 8-cyclopentyl-1,3-dimethylxanthine, indicating that it does not involve adenosine A_1 or A_{2A} receptors. The N^6-B-NECA response was blocked by enprofylline, a somewhat selective adenosine A_{2B} receptor antagonist (Table 2; Feoktistov and Biaggioni, 1997). However, it was not blocked by systemic doses of MRS1191, which are known to block adenosine A_3 receptor mediated responses (Von Lubitz et al., 1997). It thus appears that N^6-B-NECA activates adenosine A_{2B} rather than A_3 receptors to produce its paw oedema response in rats. While not directly evaluated for adenosine A_{2B} receptor activity in binding studies, the structure–activity profile of this receptor suggests that N^6-B-NECA would have a direct

affinity for this receptor (de Zwart et al., 1998). Adenosine A_{2B} receptor activity for N^6-B-NECA has been demonstrated in human mast cells, although it does not appear to be a full agonist in that system (Feoktistov and Biaggioni, 1998).

The N^6-B-NECA paw oedema response involves histamine, 5-HT and noradrenaline, as antagonists for each of these amines markedly reduces oedema (see also Sawynok et al., 1997). Rat mast cells contain both adenosine A_{2B} and A_3 receptors, and activation of both receptors can lead to release of histamine, 5-HT and other mediators from mast cells (Ramkumar et al., 1993; Linden, 1994; Marquardt et al., 1994; Feoktistov and Biaggioni, 1997). The nature of the involvement of noradrenaline in this response is less clear. Thus, as with the adenosine A_1 receptors, adenosine A_{2B} receptors also may inhibit noradrenaline release from sympathetic nerve terminals (Tamaoki et al., 1997). Again, the effect of block of sympathetic responses may be secondary to inflammatory processes activated by histamine and 5-HT (see above).

4.4. Indirectly acting agents

Adenosine mechanisms can also be activated indirectly by administering inhibitors of adenosine metabolism (Geiger et al., 1997), which cause an intracellular accumulation of adenosine and efflux into the extracellular space along a concentration gradient. The local administration of inhibitors of both adenosine kinase and adenosine deaminase can modify nociceptive responses (Liu and Sawynok, 1998; Sawynok et al., 1998) and inflammation (Poon and Sawynok, 1999). The present study demonstrates that 5′-amino-5′-deoxyadenosine and 2′-deoxycoformycin, respective inhibitors of these enzymes, produce a limited local paw oedema response, which appears to be a prolongation of the saline response (Fig. 7). Higher doses of 5′-amino-5′-deoxyadenosine and 2′-deoxycoformycin (1000 nmol) produce significant systemic effects following administration into the rat hindpaw (Poon and Sawynok, 1999), but produce no further increase in paw volume. The limited expression of the response to inhibition of adenosine metabolism may occur because in the absence of an inflammatory stimulus to promote local adenosine formation, inhibition of metabolism produces only modest tissue accumulations of adenosine. The actions of both 5′-amino-5′-deoxyadenosine and 2′-deoxycoformycin were blocked by caffeine indicating that the response is indeed due to activation of adenosine receptors resulting from accumulations of adenosine.

4.5. Summary and significance of observations

The present study demonstrates oedema following application of selective adenosine A_1, A_2 and A_3 receptor agonists into the rat hindpaw. Each of these receptors appears to act by different mechanisms. The adenosine A_1 receptor mediated response could reflect activation of a neurogenic mechanism and an indirect involvement of mast cells. However, this mechanism is at odds with the antinociception observed following local application of adenosine analogs into the rat hindpaw. Oedema and antinociception appear to occur by independent actions. If similar actions were observed following topical application, oedema may be a limitation for the development of local formulations (e.g., cream or gel) of adenosine A_1 agonists as analgesics. The adenosine A_{2A} receptor mediated response is quite limited in extent, and likely results from vasodilation. The adenosine A_{2B} receptor mediated response also involves mast cells, and likely results from a direct activation of stimulatory receptors on the mast cell to release amine mediators. The nature of the sympathetic nervous system involvement in the action of adenosine A_1 and A_{2B} receptors remains to be clarified. Inhibitors of adenosine kinase and adenosine deaminase produce only limited effects on paw volume.

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