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Short communication

Involvement of mast cells, sensory afferents and sympathetic mechanisms in paw oedema induced by adenosine A_1 and $A_{2B/3}$ receptor agonists

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Abstract

Both the adenosine A_1 receptor agonist N^6-cyclopentyladenosine and the adenosine $A_{2B/3}$ receptor agonist N^6-benzyl-5′-*N*-ethylcarboxamido adenosine (N^6-B-NECA) produce an acute paw oedema response following local s.c. injection into the rat hindpaw. This study characterized aspects of the mechanisms by which these responses occur by determining the effect of compound 48/80 (mast cell depleting agent), capsaicin (sensory neurotoxin) and 6-hydroxydopamine (sympathetic nervous system neurotoxin) on the paw oedema response produced by these agents. Compound 48/80 markedly reduced the increase in paw volume produced by both N^6-cyclopentyladenosine and N^6-B-NECA. Capsaicin significantly reduced paw oedema induced by N^6-cyclopentyladenosine but not N^6-B-NECA. In contrast, 6-hydroxydopamine reduced paw oedema induced by N^6-B-NECA but not N^6-cyclopentyladenosine. These results indicate an involvement of mast cells in paw oedema produced by both adenosine A_1 and $A_{2B/3}$ receptor agonists. For N^6-cyclopentyladenosine, this involvement may be a secondary involvement due to activation of a neurogenic mechanism, but for N^6-B-NECA, it may be a direct effect on mast cells. The nature of the involvement of the sympathetic nervous system in the action of N^6-B-NECA is not entirely clear.

Keywords: Oedema; Adenosine A_1 receptor; Adenosine A_{2B} receptor; Mast cell

1. Introduction

The adenosine A_1 receptor agonist N^6-cyclopentyladenosine and the adenosine $A_{2B/3}$ receptor agonist N^6-benzyl-5′-*N*-ethylcarboxamido adenosine (N^6-B-NECA) produce opposing effects on nociceptive signalling when administered locally into the hindpaw of the rat, producing suppression (Aley et al., 1995; Liu and Sawynok, 1998) and facilitation (Sawynok et al., 1997) of pain behaviours, respectively. Despite these opposing behavioural effects, they both produce an intrinsic paw oedema following local injection (Sawynok et al., 1999). Paw oedema by both agents is blocked by mepyramine and ketanserin, antagonists for histamine H_1 and 5-HT_2 receptors, respectively, indicating an involvement of mast cells, as well as by phentolamine, an α-adrenoceptor antagonist, indicating an involvement of the sympathetic nervous system in these responses (Sawynok et al., 1999). Rat mast cells express adenosine A_{2B} and A_3 receptors, with activation resulting in release of mediators (Linden, 1994; Feoktistov and Biaggioni, 1997), but do not express adenosine A_1 receptors (Ramkumar et al., 1993). Mast cells, however, also can contribute to oedema by a neurogenic mechanism. In this case, the mast cell is activated by the sensory afferent nerve as part of a local axon reflex that results in peripheral release of substance P and calcitonin gene-related peptide from the nerve terminal with a subsequent activation and degranulation of mast cells (reviewed by Holzer, 1988). Accordingly, plasma extravasation induced by capsaicin, which activates sensory nerves directly, is inhibited by pretreatment with both capsaicin acting as a sensory neurotoxin, as well as compound 48/80, which depletes mast cells (Arvier et al., 1977; Coderre et al., 1989). The nature of the involvement of the sympathetic nervous system in these paw oedema responses is not clear. Sympa-

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thetic mechanisms have been implicated in peripheral inflammatory responses in some (Nakamura and Ferreira, 1987), but not all (Donnerer et al., 1991; Perrot et al., 1994), studies with differing outcomes perhaps reflecting different phases or aspects of the inflammatory response (Woolf et al., 1996).

In the present study, we evaluated the involvement of mast cells (by using compound 48/80), sensory afferent nerve terminals (by using capsaicin) and sympathetic nerves (by using 6-hydroxydopamine) on paw oedema induced by N^6-cyclopentyladenosine and N^6-B-NECA in order to gain a clearer understanding of the mechanisms by which these two agents produce such actions.

2. Materials and methods

2.1. Animals

Experiments were performed on male Sprague–Dawley rats 120–160 g. Rats were housed in pairs and given ad libitum access to food and water. All procedures were approved by the University Committee on Laboratory Animals.

2.2. Paw volume determinations

Paw volumes were determined by volume displacement using a commercially available plethysmometer (Ugo Basile, Italy) as previously described (Sawynok et al., 1999). The hindpaw was immersed to the junction of the hairy skin, and volumes determined in triplicate prior to and at 30, 60, 90, 120 and 180 min following local drug administration.

2.3. Drug treatments

N^6-cyclopentyladenosine (dissolved in saline) and N^6-B-NECA (dissolved in 10% dimethylsulfoxide/saline) were administered s.c. in a volume of 50 μl. Compound 48/80 was dissolved in saline and administered at 1 mg/kg, i.p. four times at 3-h intervals on day 1, and at 1.5 mg/kg two times at a 7-h interval on day 2; rats were tested on day 3. Systemic pretreatments increased paw volumes by 20–30% within 2–3 h of the injection, but by the day of testing, paw volumes had returned to control values. Capsaicin was prepared as a 10 mg/ml solution in 20% ethanol, 10% Tween 80 and 70% saline, and administered s.c. under sodium pentobarbital anaesthesia (duration 1–2 h) at a dose of 30 mg/kg day 1, 50 mg/kg day 2 and 70 mg/kg day 3; rats were tested on day 4. 6-Hydroxydopamine was dissolved in 0.1% sodium metabisulfite in distilled water and administered at 75 mg/kg, i.p. on days 1, 2 and 3; rats were tested on day 4. The compound 48/80 protocol was adapted from Hannon et al. (1995), while the capsaicin and 6-hydroxydopamine protocols were those used by Zhou et al. (1998). Both of those studies verified that the treatments produced the anticipated effects.

2.4. Statistics

Time course experiments were analyzed by analysis of variance followed by the Student–Neuman–Keuls test,

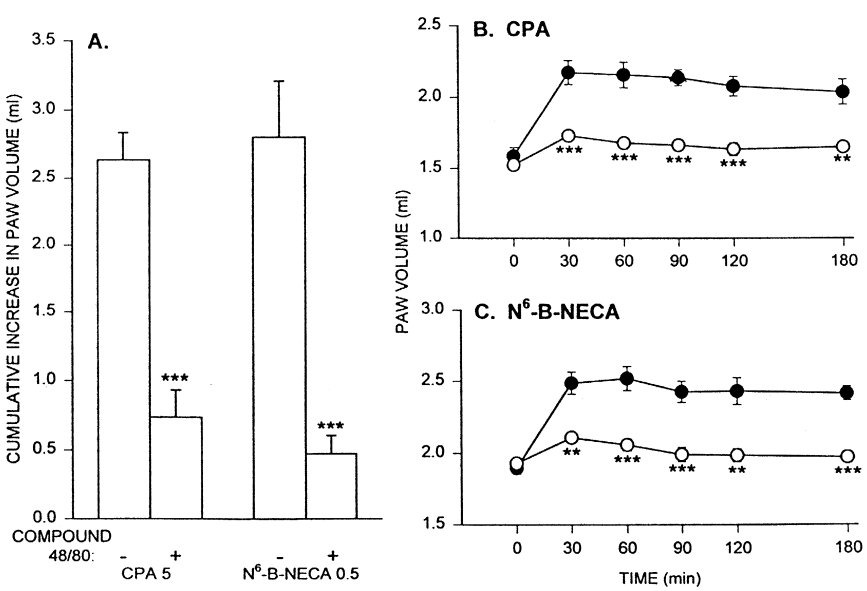

Fig. 1. Effect of pretreatment for 2 days with compound 48/80 on the paw oedema produced by the adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) 5 nmol and the adenosine $A_{2B/3}$ receptor agonist N^6-B-NECA 0.5 nmol. In (B) and (C), the time course following vehicle (●) or compound 48/80 pretreatment (○) is shown. Values represent mean ± S.E.M. for $n = 6$ per group. ** $P < 0.01$, *** $P < 0.001$ compared to saline-pretreated controls.

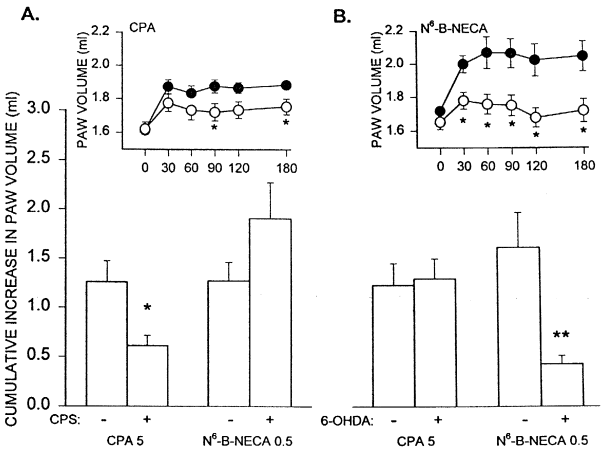

Fig. 2. Effects of (A) pretreatment with capsaicin (CPS) for 3 days and (B) pretreatment with 6-hydroxydopamine (6-OHDA) for 3 days on the paw oedema produced by N^6-cyclopentyladenosine (CPA) 5 nmol and N^6-B-NECA 0.5 nmol. Insets depict time course in vehicle (●) or respective toxin-pretreated rats (○). * $P < 0.05$, ** $P < 0.01$ compared to respective vehicle-pretreated controls ($n = 6$ per group).

while cumulative scores were compared by using the Student's *t*-test.

3. Results

Both N^6-cyclopentyladenosine and N^6-B-NECA produce a dose-related increase in paw volume when tested over a 3-h interval following s.c. injection into the rat hindpaw (Sawynok et al., 1999). Doses of N^6-cyclopentyladenosine and N^6-B-NECA producing equivalent increases in paw volume (5 and 0.5 nmol, respectively) were selected to determine the effects of the various pretreatment regimens. Pretreatment with compound 48/80 caused a marked reduction in the paw oedema produced by both N^6-cyclopentyladenosine and N^6-B-NECA (Fig. 1). This was clearly seen both in the cumulative score (Fig. 1A), as well as in the time courses (Fig. 1B, C). Pretreatment with capsaicin reduced the paw oedema produced by N^6-cyclopentyladenosine but not that produced by N^6-B-NECA (Fig. 2A). In contrast, pretreatment with 6-hydroxydopamine reduced the paw oedema produced by N^6-B-NECA but not that by N^6-cyclopentyladenosine (Fig. 2B). Pretreatment with the vehicle for capsaicin (Fig. 2A) and 6-hydroxydopamine (Fig. 2B) reduced control paw volume responses somewhat compared to pretreatment with the saline vehicle used for compound 48/80 (cf. Fig. 1) ($P < 0.05$ in each case), but the significance of this is not clear.

4. Discussion

The present study demonstrates that paw oedema produced by the adenosine A_1 receptor agonist N^6-cyclopentyladenosine is blocked by compound 48/80 and capsaicin but not 6-hydroxydopamine, indicating an involvement of mast cells and sensory afferents but not sympathetic nerve terminals in this action. It is proposed that N^6-cyclopentyladenosine activates sensory afferent nerve terminals, and this leads to a neurogenic involvement of mast cells to release histamine and 5-HT, which then produce paw oedema. This proposal is supported by the observations that (a) adenosine A_1 receptor agonists can enhance the firing of sensory afferent nerves in rats by a direct action of the sensory neurons (Dowd et al., 1998; Hong et al., 1998), (b) rat mast cells do not encode adenosine A_1 receptors (Ramkumar et al., 1993), and (c) involvement of a neurogenic mast cell degranulation is a well-recognized component of the inflammatory response (Holzer, 1988). Both histamine and 5-HT released from mast cells can produce paw oedema by subsequent actions on sensory afferents and the vasculature (Sufka et al., 1991; Amman et al., 1995). The sensitivity to compound 48/80 and capsaicin of paw oedema by N^6-cyclopentyladenosine is similar to plasma extravasation and/or paw oedema seen following capsaicin and formalin (Arvier et al., 1977; Coderre et al., 1989; Damas and Liégois, 1999), both of which are known to activate sensory afferents. Neurogenic inflammation produced by antidromic nerve stimulation has been shown to be relatively insensitive to chemical sympathectomy (Donnerer et al., 1991), and the lack of effect of 6-hydroxydopamine on the N^6-cyclopentyladenosine response would be consistent with such a mechanism (but see Coderre et al., 1989). It is not clear if the partial reduction in effect produced by capsaicin reflects an incomplete neurotoxic effect, or whether adenosine A_1 receptor activation can produce additional independent pro-inflammatory effects (e.g. Salmon and Cronstein, 1990).

This study also demonstrates that paw oedema produced by the adenosine $A_{2B/3}$ receptor agonist N^6-B-NECA is dependent on mast cells and sympathetic nerve terminals but not on primary afferents. It is likely that the N^6-B-NECA acts directly on the mast cells to cause release of histamine and 5-HT, and there is an additional involvement of sympathetic nerves in this action. Although N^6-B-NECA is an agonist at both adenosine A_{2B} and A_3 receptors and mast cells contain both receptors (Linden, 1994; Feokistov and Biaggioni, 1997), paw oedema by N^6-B-NECA is due to activation of adenosine A_{2B} receptors as it is blocked by a somewhat selective adenosine A_{2B} receptor antagonist but not a selective adenosine A_3 receptor antagonist (Sawynok et al., 1999). The N^6-B-NECA result is consistent with the observation that plasma extravasation induced by mast cell degranulation with an acute injection of compound 48/80 is dependent on sympathetic nerves but not on sensory afferents (Coderre et al., 1989). Both mast cell depletion and chemical sympathectomy reduce the early development of hyperalgesia following a non-neurogenic inflammation (Woolf et al., 1996).

There is thus a parallel between the N^6-B-NECA response and inflammatory responses in which there is a direct involvement of mast cells (see Coderre et al., 1989).

Acknowledgements

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