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Adenosine A_3 receptor activation produces nociceptive behaviour and edema by release of histamine and 5-hydroxytryptamine

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Abstract

This study evaluated the pain enhancing properties of the adenosine A_3 receptor agonist N^6-benzyl-5′-*N*-ethylcarboxamidoadenosine (N^6-benzyl–NECA) by assessing behavioural effects following s.c. administration alone to the dorsal hindpaw of the rat, or in combination with a low concentration of formalin (0.5%). Edema formation was monitored by determining paw volume with plethysmometry. N^6-benzyl–NECA (0.005–10 nmol) produced a dose-related increase in intrinsic flinching behaviours, as well as an increase in phase 2A flinch responses in the presence of formalin. Intrinsic effects were blocked by the histamine H_1 receptor antagonist mepyramine and the 5-hydroxytryptamine$_2$ (5-HT_2) receptor antagonist ketanserin, but not by other 5-HT receptor antagonists or adenosine A_1 or A_2 receptor antagonists. N^6-benzyl–NECA also produced an increase in paw volume, both alone and in the presence of formalin, with higher doses being required to produce this effect than for the flinch response. The increase in paw volume was also blocked by mepyramine and ketanserin but not by other antagonists. These results indicate both a nociceptive response and a proinflammatory response resulting in edema formation following activation of adenosine A_3 receptors which is mediated by both 5-HT and histamine released most likely from mast cells. © 1997 Elsevier Science B.V.

Keywords: Adenosine A_3 receptor; Pain; Histamine; 5-HT (5-hydroxytryptamine, serotonin)

1. Introduction

A variety of chemical mediators are implicated in the peripheral activation of sensory nerve terminals under inflammatory conditions (reviewed by Levine and Taiwo, 1994; Dray, 1995). Adenosine has recently been proposed to be an endogenous anti-inflammatory agent and has the potential to modify inflammatory responses by actions on specific inflammatory cells such as neutrophils (reviewed by Cronstein, 1994) as well as to modify the pain signal by actions on sensory afferent nerve terminals (reviewed by Sawynok, 1997). The effect of adenosine on the pain signal can depend upon which receptor subtype is activated. Thus, in rodents, activation of adenosine A_1 receptors produces a local antinociceptive effect, while activation of adenosine A_2 receptors produces a hyperalgesic effect (Taiwo and Levine, 1990; Karlsten et al., 1992; Doak and Sawynok, 1995). Pronociceptive effects of adenosine have also been demonstrated in humans, but curiously, adenosine A_1 receptors have been implicated in this action (Pappagallo et al., 1993; Gaspardone et al., 1995).

A third receptor, the adenosine A_3 receptor, has recently been identified (reviewed by Linden, 1994). Perhaps the best characterized adenosine A_3 receptor mediated response is to stimulate mediator release from mast cells, an action which appears to contribute to allergic and inflammatory manifestations (Ramkumar et al., 1993; Linden, 1994) as well as to vascular responses (Hannon et al., 1995; Fozard et al., 1996). Mast cells are located in proximity to sensory nerve terminals and sensory nerve stimulation leads to mast cell degranulation and release of mediators (Dimitriadou et al., 1991). Adenosine A_3 receptor activation on mast cells can lead to release of both histamine (Fozard et al., 1996) and 5-hydroxytryptamine (5-HT) (Church et al., 1986). Histamine can produce itch and potentially pain by activation of sensory neurons (Keele and Armstrong, 1964; Simone et al., 1987) and induce edema (cf., Hill, 1990), with both actions being

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mediated by stimulation of histamine H_1 receptors (Ninkovic and Hunt, 1985; Saria et al., 1988). 5-HT also can enhance pain signalling by actions on sensory afferents and induce edema, with multiple 5-HT receptor subtypes at different sites being involved in these actions (Sufka et al., 1991; Doak and Sawynok, 1997). Adenosine A_3 receptor activation clearly has the potential to influence sensory neuron activation and to produce edema by its action on mast cells to promote histamine and 5-HT release.

In the present study, we have examined the effect of a selective adenosine A_3 receptor agonist on peripheral nerve terminals by determining intrinsic behavioural effects resulting from the local application of N^6-benzyl-5′-*N*-ethylcarboxamidoadenosine (N^6-benzyl–NECA) (Gallo-Rodriguez et al., 1994) into the dorsal hindpaw, and determining whether it can augment behaviours produced by a low concentration of formalin. The latter model reveals pronociceptive actions resulting from adenosine A_2 and 5-HT receptor activation (Karlsten et al., 1992; Doak and Sawynok, 1995, 1997). The ability of N^6-benzyl–NECA to produce edema was examined by determining paw volume by plethysmometry. The potential role of histamine in these responses was addressed by determining the effects of mepyramine (histamine H_1 receptor antagonist, Hill, 1990), on N^6-benzyl–NECA responses. The potential role of 5-HT in these actions was addressed by determining effects of propranolol, ketanserin, tropisetron and *N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide (GR113808A) (5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 antagonists respectively, see Doak and Sawynok, 1997) on N^6-benzyl–NECA responses. While N^6-benzyl–NECA is a selective agonist for adenosine A_3 receptors, it does have affinity for both adenosine A_1 and A_2 receptors at higher doses (Gallo-Rodriguez et al., 1994), so some studies also evaluated effects of the selective adenosine A_1 receptor antagonist 8-cyclopentyltheophylline (CPT) (Bruns et al., 1986) and the somewhat selective adenosine A_2 receptor antagonist 3,7-dimethyl-1-propargyl-xanthine (DMPX) (Ukena et al., 1986).

2. Materials and methods

2.1. Animals

Experiments were conducted using male Sprague Dawley rats (Charles River, Quebec, Canada) weighing between 100–150 g. Rats were housed in groups of 2–4, and maintained on a 12/12 h light/dark cycle at 22 ± 1°C. Food and water were freely available.

2.2. Behavioural testing

Rats were placed in the $28 \times 28 \times 28$ cm observation chamber for an initial 20 min accommodation interval to familiarize them with their surroundings. Individual drugs and drug combinations were injected s.c. in a volume of 50 μl into the dorsal side of the hindpaw of the rat. Following injections, rats were returned to the observation chamber and observed in 2 min bins for a 60 min interval for the expression of flinch behaviours. This includes lifting, shaking and overt flinching that manifests as a ripple over the haunch. These behaviours are discrete and easily quantifiable and exhibit good reproducibility between independent observers. Two rats were observed at one time, with observations taking place in alternating 2 min bins.

2.3. Plethysmometry

The volume of the hind paw was determined using a commercial plethysmometer (Ugo Basile, Milan, Italy). Paw swelling is assessed by volume displacement following immersion of the hindpaw to the junction of the hairy and non-hairy skin. Both the injected and contralateral non-injected hindpaws were determined in triplicate to establish a baseline value, then again in triplicate at 60 min following drug administration at the end of the behavioural observation period. A time-course was determined for paw volume changes in a separate group (Fig. 5 inset), and this revealed that the 60 min determination was an appropriate interval to reveal the degree of paw swelling.

2.4. Drugs

N^6-benzyl-5′-*N*-ethylcarboxamidoadenosine, 8-cyclopentyltheophylline, 3,7-dimethyl-1-propargyl-xanthine, *S*-propranolol, ketanserin and tropisetron were obtained from Research Biochemicals International (Natick, MA, USA), while formalin, mepyramine and dimethylsulfoxide (DMSO) were obtained from Sigma (St Louis, MO, USA). GR113808A was obtained from Glaxo (Greenford, UK). N^6-benzyl–NECA was dissolved in 10% DMSO/saline. Other drugs were dissolved in saline (most) or DMSO (CPT).

2.5. Data processing and statistics

Behavioural scores collected in any given bin were assumed to be similar to those in the adjacent bin, but no correction for this was made. Thus, cumulative values for any given interval represent about half of the real incidence of behaviours. Data is presented in 2-min bins to depict time-courses, or calculated cumulatively over specified time intervals. Data was analysed using analysis of variance followed by the Student–Newman–Keuls test.

3. Results

3.1. Behavioural effects of N^6-benzyl–NECA

Behavioural actions accepted as flinch responses were lifting, shaking and a rapid hindpaw shake that manifests

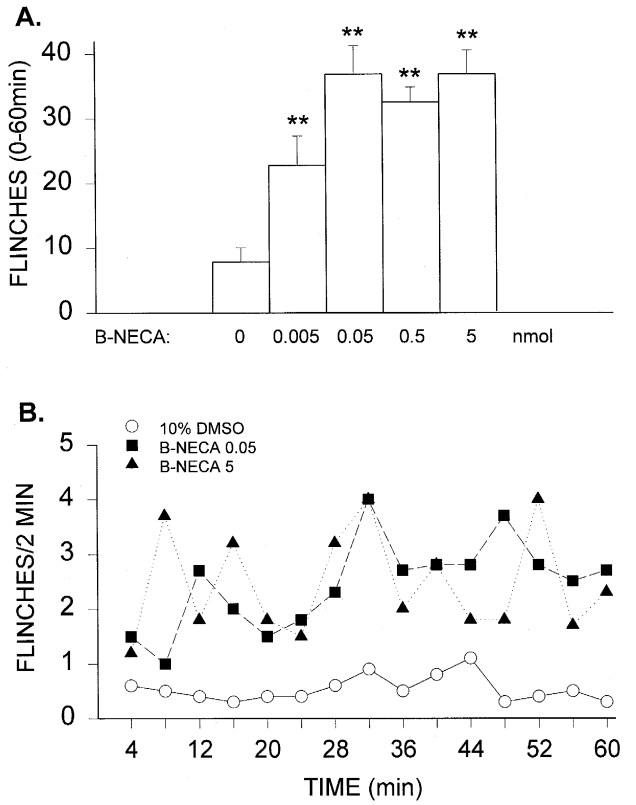

Fig. 1. Flinch responses induced by local administration of N^6-benzyl–NECA (B-NECA) into the dorsal surface of the hindpaw of the rat. (A) Cumulative incidence of flinches; (B) time-course of flinch responses. Values depict mean ± S.E.M. in this and subsequent figures. In (B), some doses and error bars omitted in the interest of clarity. $n = 6–8$ per group; ** $P < 0.01$ compared to vehicle (10% DMS0).

as a ripple in the haunch. The s.c. administration of N^6-benzyl–NECA into the dorsal hindpaw of the rat produces a dose-dependent monotonic increase in flinch responses (Fig. 1). The cumulative incidence of flinch responses over a 60 min interval reaches a plateau and is not further modified over a 100-fold increase in dose (Fig. 1A). While the actual incidence of behaviours in any given time bin exhibits some variations, there is no characteristic division into distinct phases as seen with formalin (cf., Fig. 2 inset). The coadministration of N^6-benzyl–NECA with formalin 0.5% significantly augments flinching responses produced by formalin (Fig. 2). This increase is seen primarily in the early part of the second phase (phase 2A), with less of an effect occurring in phase 1 and the latter part of the second phase (phase 2B) (Fig. 2). The increase in flinches observed in phase 2A is greater than additive, as intrinsic N^6-benzyl–NECA responses corresponding to this interval (16–36 min) for the 0.05 nmol dose (greatest degree of augmentation of the formalin response, about 50 flinches, Fig. 2A) were 14.3 ± 2.7 flinches (cf., 3.0 ± 0.6 for the vehicle).

3.2. Effects of histamine, 5-hydroxytryptamine and adenosine antagonists on flinching behaviours induced by N^6-benzyl–NECA

The coadministration of mepyramine produces a dose-dependent inhibition of flinching behaviours produced by N^6-benzyl–NECA (Fig. 3), but is without a significant intrinsic effect when given alone ($n = 4–6$ per group, data not shown). Of the 5-HT receptor antagonists evaluated (see Doak and Sawynok, 1997 for justification of doses), only ketanserin produced a significant inhibition of flinch responses (Fig. 4). Neither CPT nor DMPX (see Doak and Sawynok, 1995 for justification of doses) produced a significant effect on flinches produced by the higher dose of N^6-benzyl–NECA (Fig. 3).

3.3. Edema produced by N^6-benzyl–NECA and effects of histamine, 5-hydroxytryptamine and adenosine antagonists

N^6-benzyl–NECA produces a dose-dependent increase in paw volume (Fig. 5). The increase in paw volume develops within 30 min and is sustained for a two hour time interval (Fig. 5 inset). Formalin 0.5% alone does not

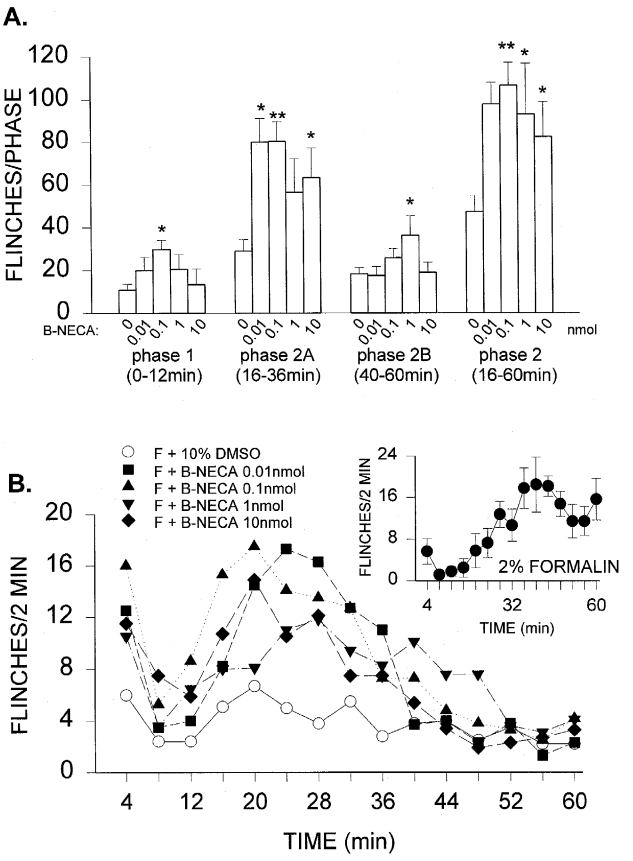

Fig. 2. Enhancement of flinch responses to formalin 0.5% produced by coadministration of N^6-benzyl–NECA (B-NECA) with the formalin (F) into the dorsal hindpaw. $n = 6–12$ per group; * $P < 0.05$, ** $P < 0.01$ compared to formalin + vehicle group.

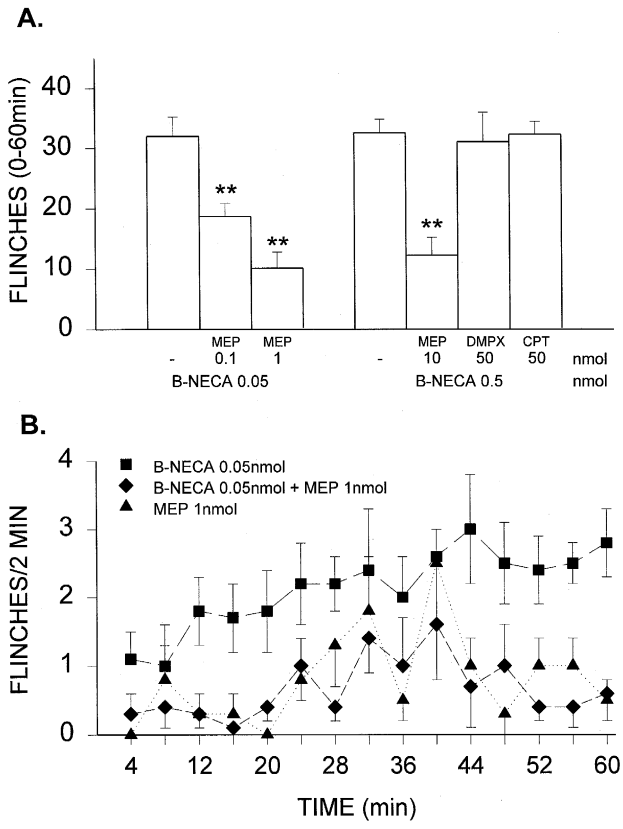

Fig. 3. Blockade of the flinch response induced by N^6-benzyl–NECA (B-NECA) with the histamine H_1 receptor antagonist mepyramine (MEP). (A) Cumulative responses; (B) time-course for 0.05 nmol N^6-benzyl–NECA. $n = 6–11$ per group; $^{**}P < 0.01$ compared to N^6-benzyl–NECA.

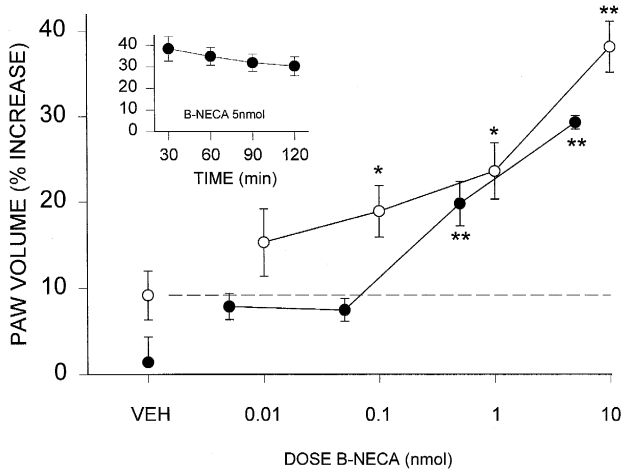

Fig. 5. Edema induced by N^6-benzyl–NECA (B-NECA) in the absence (●) and presence (○) of formalin 0.5%. Inset depicts time-course of paw volume measurements. $n = 6–12$ per group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to vehicle (VEH, 10% DMSO).

produce a significant effect on paw volume (Fig. 5). Coadministration of N^6-benzyl–NECA results in a significant increase in paw volume in the presence of formalin which is similar in magnitude to that seen in the absence of formalin (Fig. 5). The paw volume response to N^6-benzyl–NECA in the absence of formalin requires a higher dose (significant at 0.5–5 nmol) than does the behavioural response (significant at 0.005 and 0.05 nmol as well) (cf., Figs. 1 and 5). A similar enhanced dose requirement is also seen in the presence of formalin (cf., Figs. 2 and 5). Coadministration of mepyramine with N^6-benzyl–NECA does inhibit the paw volume response, but a higher dose of mepyramine is required (Fig. 6A). Coadministration of ketanserin, but not other 5-HT receptor antagonists, also inhibits the paw volume response at a higher concentration (Fig. 6B). Neither CPT nor DMPX had any significant effect on the increase in paw volume produced by the higher doses of N^6-benzyl–NECA (Fig. 6A). Mepyramine (10, 100 nmol) and ketanserin (500 nmol) given alone had

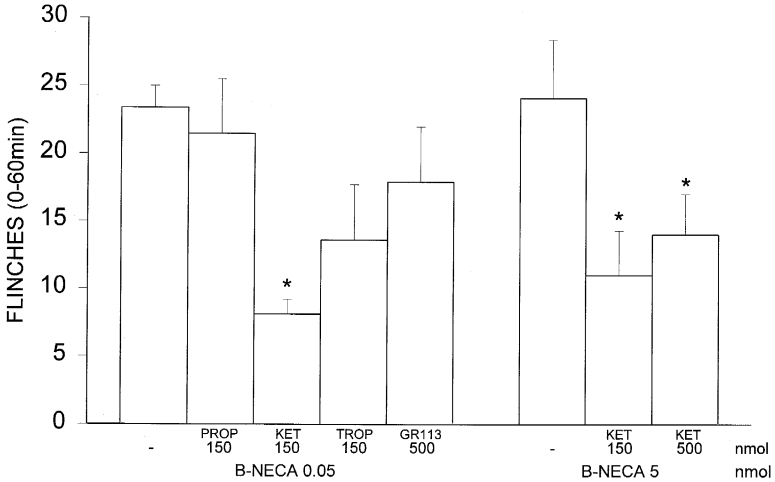

Fig. 4. Blockade of the flinch response induced by N^6-benzyl–NECA (B-NECA) with the 5-HT_2 receptor antagonist ketanserin (KET) but not by antagonists for 5-HT_1 (propranolol, PROP), 5-$HT_{3/4}$ (tropisetron, TROP) and 5-HT_4 (GR113808A, GR113) receptors. $n = 6–8$ per group; $^{*}P < 0.05$ compared to N^6-benzyl–NECA.

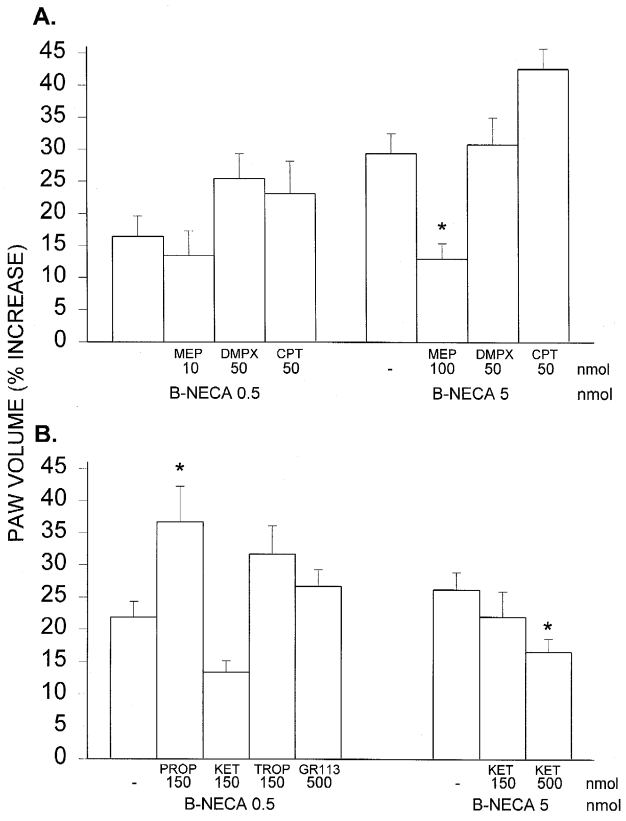

Fig. 6. Effects of (A) histamine (mepyramine, MEP) and adenosine receptor antagonists (CPT, DMPX) and (B) 5-HT receptor antagonists (propranolol PROP, ketanserin KET, tropisetron TROP, GR113808A GR113) on the increase in paw volume induced by N^6-benzyl–NECA (B-NECA). $n = 6$–12 per group; * $P < 0.05$ compared to N^6-benzyl–NECA.

no intrinsic effects on paw volume ($n = 4$ each, data not shown).

4. Discussion

The present study demonstrates an intrinsic flinch response following the local administration of the adenosine A_3 receptor agonist N^6-benzyl–NECA into the dorsal hindpaw of the rat, which is due to release of histamine and 5-HT. A previous study has observed scratching behaviours following i.p. administration of N^6-3-iodobenzyl-5′-*N*-methylcarboxamidoadenosine, IB-MECA, another adenosine A_3 receptor agonist, and this too was blocked by a histamine antagonist (Jacobson et al., 1993). The flinch response to N^6-benzyl–NECA is further enhanced in the presence of a low concentration of formalin. The intrinsic response is monotonic, being present through the entire 60 min observation period, but the enhancement of the formalin response is seen primarily in the early part of the second phase (phase 2A). The latter action is greater than additive as intrinsic responses account for only a small proportion of the increase during this interval. The expression of the enhanced response in phase 2A may be due to significant interactions between the histamine and 5-HT released by adenosine A_3 receptor activation and other preformed mediators released by the formalin, rather than with mediators that must be synthesized. Thus, formalin gives rise to a characteristic dose-dependent increase in nociceptive behaviours, with the second phase reflecting an inflammatory component in which a number of endogenous mediators play a role (reviewed by Tjølsen et al., 1992). Both histamine and 5-HT clearly contribute to the formalin response (Shibata et al., 1989; Abbott et al., 1996; Doak and Sawynok, 1997).

A number of electrophysiological and behavioural paradigms have been used to characterize nociceptive (nociceptor excitation) and pronociceptive (nociceptor sensitization) actions of inflammatory mediators at the sensory nerve terminal (reviewed by Treede et al., 1992). A paradigm for demonstrating such actions utilizing the behavioural rating scale developed for the formalin test has recently been described (Hong and Abbott, 1994). Thus, behaviours elicited by local injections of inflammatory mediators can reflect either hyperalgesia (favouring) or a nociceptive or pain response (lifting, licking). Within this scheme, the current behaviours seen with N^6-benzyl–NECA would be considered nociceptive. While histamine is normally considered to produce primarily itch rather than pain (Keele and Armstrong, 1964; Simone et al., 1987) and produces favouring as a predominant behavioural response when administered alone (Hong and Abbott, 1994), 5-HT produces behaviours considered to reflect nociception (Sufka et al., 1991; Hong and Abbott, 1994) and its co-presence changes the expression of histamine actions to a nociceptive behaviour (Hong and Abbott, 1994). It appears that adenosine A_3 receptor activation releases multiple mediators from mast cells, and that intrinsic effects of N^6-benzyl–NECA reflect the actions of multiple mediators. Thus, rat mast cells contain 5-HT as well as histamine, and adenosine releases both histamine and 5-HT from such cells (see below); these then act synergistically to activate the sensory nerve terminal (Hong and Abbott, 1994).

The observation that the behavioural response to N^6-benzyl–NECA is mediated by release of histamine and activation of histamine H_1 receptors is consistent with the localization of adenosine A_3 receptors on mast cells and a subsequent release of histamine following receptor activation (Ramkumar et al., 1993; Van Schaik et al., 1996). While in vitro data indicates the predominant effect of adenosine A_3 receptor activation is to augment mediator-induced release (Ramkumar et al., 1993), in vivo studies provide evidence that adenosine A_3 receptor activation per se is sufficient to promote mast cell histamine release (Hannon et al., 1995; Van Schaik et al., 1996). Histamine H_1 receptors are located directly on the afferent nerve terminal (Ninkovic et al., 1982; Ninkovic and Hunt, 1985), and activation of such receptors results in sensory neuron stimulation (Saria et al., 1988, Handwerker and Reeh,

1991). While the intracellular mediators of this action are not completely resolved, Ca^{2+} entry appears to contribute to the response (Tani et al., 1990). Adenosine also releases 5-HT from rodent mast cells in the presence of antigen (Church et al., 1986). The pharmacology of this response is consistent with adenosine A_3 receptor activation. Messenger RNA for multiple 5-HT receptor subtypes is present in sensory ganglia (Pierce et al., 1996), and multiple 5-HT receptors including 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 have been implicated in peripheral pronociceptive actions of 5-HT in different paradigms (see discussion in Doak and Sawynok, 1997). The ability of ketanserin to inhibit flinching behaviours by N^6-benzyl–NECA suggests the involvement of a 5-HT_2 receptor subtype in the nociceptive response generated by 5-HT in the presence of histamine following mast cell activation by adenosine A_3 receptors (cf., Abbott et al., 1996).

N^6-benzyl–NECA also produces an increase in paw volume, reflecting a local edema response. This action also appears due to histamine and 5-HT release, as it is reduced both by mepyramine and ketanserin. The local administration of histamine produces edema by an increase in vascular permeability leading to extravasation of plasma proteins as well as an action via a neurogenic component (Hill, 1990; Amann et al., 1995). The local administration of 5-HT also produces edema, and this may result from vascular, cellular and neuronal components of action (Jensen et al., 1990; Sufka et al., 1991; Doak and Sawynok, 1997). It should be noted that edema formation requires higher doses of N^6-benzyl–NECA than does the flinch response, that higher doses of mepyramine and ketanserin are required to block edema formation, and that this response is simply additive with that produced by the formalin. It may be that with the multiplicity of actions involved in edema formation, this response is less subject to manipulation by blockade of only a single component of the response. Alternatively, edema could reflect the concerted action of the 5-HT and histamine released from mast cells which is more effectively blocked by combinations of antagonists rather than individual antagonists (cf., responses to compound 48/80, Maling et al., 1974). The lack of interaction seen with formalin may indicate that maximal interactions between mediators already have been expressed at the higher concentrations required to see the effect.

In addition to activation of adenosine A_3 receptors, N^6-benzyl–NECA can also activate adenosine A_1 and A_2 receptors at higher doses (Gallo-Rodriguez et al., 1994). Activation of both of these receptor populations has the potential to modify flinch responses (Doak and Sawynok, 1995) as well as edema formation (Green et al., 1991), with A_1 agonists inhibiting and A_2 agonists augmenting responses in both cases. However, neither CPT nor DMPX produced a significant effect on either the flinch response or on the paw volume by the higher doses of N^6-benzyl–NECA that might be expected to activate these receptors. This indicates that the responses observed here are likely primarily mediated by adenosine A_3 receptors rather than other adenosine receptors, and that any potential modulation of these responses exerted by other adenosine receptor populations is not expressed under the conditions used here.

In summary, the present study demonstrates that local administration of the selective adenosine A_3 receptor agonist N^6-benzyl–NECA produces behaviours regarded as nociceptive (flinching) and that this action is augmented in the presence of a low concentration of formalin. At higher doses, N^6-benzyl–NECA produces an increase in paw volume (edema) but this is not further enhanced in the presence of formalin. Both the flinching response and the increase in paw volume involve release of histamine and 5-HT as they are blocked by mepyramine and ketanserin. The flinch response may reflect a direct activation of sensory neurons by H_1 and 5-HT_2 receptor stimulation acting together, and in concert with other agents, while the mechanism involved in edema formation appears to be more complex mechanistically.

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