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European Journal of Pharmacology 429 (2001) 147–155

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Involvement of primary sensory afferents, postganglionic sympathetic nerves and mast cells in the formalin-evoked peripheral release of adenosine

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Accepted 27 July 2001

Abstract

Injection of formalin into the rat hind paw produces a dose-dependent local peripheral release of adenosine. Low doses of formalin (0.5–2.5%) evoke release during the first 10 min following injection, while a high dose of formalin (5%) evokes release lasting for 60 min. The current study was designed to determine the possible origin of release produced by two doses of formalin (1.5% and 5%). Microdialysis probes were implanted into the subcutaneous tissue under the glabrous skin of the hind paw of anaesthetized rats, and adenosine was determined by high performance liquid chromatography. Pretreatment with capsaicin, a neurotoxin selective for unmyelinated small diameter primary afferent nerves, markedly reduced the adenosine released by 1.5% formalin and the early phase of release by 5% formalin. Acute injection of 1% capsaicin to the hind paw of untreated rats also induced adenosine release. Pretreatment with 6-hydroxydopamine, a neurotoxin selective for sympathetic postganglionic nerve terminals, had no effect on release evoked by 1.5% formalin, but significantly reduced adenosine release during the late phase of release induced by 5% formalin. Pretreatment with compound 48/80, which degranulates mast cells, had no effect on adenosine release evoked by either concentration of formalin. We conclude that the origin of the adenosine released peripherally by formalin depends on the formalin concentration. At the lower concentration (1.5%), release is predominately from unmyelinated sensory afferent nerve terminals, while at the higher concentration (5%), unmyelinated afferent nerve terminals are involved in the early phase, while sympathetic postganglionic nerve terminals are involved in the later phase. Mast cells do not contribute to release of adenosine evoked by either concentration of formalin.

Keywords: Adenosine; Formalin; Peripheral; Primary sensory afferent; Sympathetic nerve terminal; Mast cell

1. Introduction

The formalin test is widely used as a model of persistent pain involving tissue injury. Injection of formalin produces a biphasic response consisting of an initial phase lasting about 10 min, which is followed, after a short quiescent interphase, by a longer period of sustained activities lasting 45–60 min, and this is observed both electrophysiologically (Dickenson and Sullivan, 1987; Puig and Sorkin, 1995) and behaviourally (Dubuisson and Dennis, 1977; Tjølsen et al., 1992). The first phase results from direct activation of nociceptive nerve terminals, while the second phase is mediated by a combination of peripheral input and spinal cord sensitization (Tjølsen et al., 1992; Coderre et al., 1993b; Dallel et al., 1995). Formalin-evoked behaviours are dependent on the concentration of formalin used (Coderre et al., 1993a; Abbott et al., 1995). Formalin injection also induces a local inflammatory response involving paw edema, plasma extravasation, blood vessel dilation, and tissue ulceration (Rosland et al., 1990; Damas and Liégeois, 1999; Fu et al., 2000; Taylor et al., 2000). The inflammatory response in the early phase is neurogenic, resulting from neuropeptides released from nociceptive nerve terminals through a local axon reflex, while in the later phase, tissue injury and non-neurogenic inflammation are primarily involved (Hunskaar and Hole, 1987; Wheeler-Aceto and Cowan, 1991; Damas and Liégeois, 1999). Formalin-induced inflammation is also dependent on the formalin concentration; at low concentrations, in-

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01316-4

flammation is neurogenic, while at high concentrations, non-neurogenic inflammatory components are also involved (Damas and Liégeois, 1999).

Recently, we demonstrated that formalin injection into the rat hind paw induces a dose-dependent local peripheral release of adenosine (Liu et al., 2000). Adenosine is an endogenous neuromodulator producing complex effects on nociceptive signaling and inflammation (reviewed Sawynok, 1998; Sullivan and Linden, 1998). Peripherally, activation of adenosine A_1 receptors produces antinociceptive effects (Aley et al., 1995; Doak and Sawynok, 1995), while activation of adenosine A_{2A} receptors produces anti-inflammatory effects (Cronstein et al., 1995; Firestein 1996; Sullivan and Linden, 1998). Increasing endogenous levels of adenosine, by inhibiting enzymes involved in adenosine metabolism, produces both antinociceptive and anti-inflammatory effects in inflammatory pain models involving both peripheral and central sites (Sawynok et al., 1998; Poon and Sawynok, 1999; Kowaluk et al., 2000; McGaraughty et al., 2001).

Although behavioural (Doak and Sawynok, 1995; Sawynok et al., 1998) and neurochemical (Liu et al., 2000) studies demonstrate that formalin injection increases local extracellular adenosine levels, little is known about the origin of the released adenosine. The present study was undertaken to identify the possible origin of the peripheral release of adenosine induced by formalin. The involvement of sensory afferent nerve terminals, sympathetic postganglionic nerve terminals, and mast cells was examined by using capsaicin, 6-hydroxydopamine and compound 48/80, respectively (Coderre et al., 1989; Zhang et al., 1998). Two doses of formalin, 1.5% and 5%, were studied to represent different components of adenosine release. Thus, formalin at low doses (0.5–2.5%) induces a rapid spike of adenosine release, while at the higher dose (5%), it induces a more sustained release (Liu et al., 2000). As inflammation induced by a low dose of formalin is largely neurogenic, while at the high dose, non-neurogenic components also are involved (Damas and Liégeois, 1999), we hypothesized that different mechanisms may be involved in mediating adenosine release at different formalin concentrations.

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats (Charles River, Quebec, Canada) 200–250 g were used. Rats were housed in pairs and allowed free access to food and water on a 12/12 h light/dark cycle at 21 ± 1 °C. Procedures were approved by the University Committee on Laboratory Animals.

2.1.1. Capsaicin pretreatment

To reduce the function of unmyelinated C-fibre afferents, rats were pretreated with capsaicin (8-methyl-*N*-vanillyl-6-noneamide) (Sigma). Capsaicin was prepared as a 10 mg/ml solution in a solvent containing ethanol (10%), Tween 80 (10%) and saline (80%). Capsaicin and vehicle treatments were carried out under pentobarbital anaesthesia (45 mg/kg i.p.) and drugs were injected into the subcutaneous space under the loose neck skin for three consecutive days (30 mg/kg on day 1, 50 mg/kg on day 2, 70 mg/kg on day 3) (Zhang et al., 1998) and microdialysis was performed on day 4. This protocol substantially reduces 5% formalin-induced c-*Fos* expression in the superficial dorsal horn, indicating nociceptive sensory transmission is largely blocked (Zhang et al., 1998).

2.1.2. 6-Hydroxydopamine pretreatment

To reduce the function of sympathetic postganglionic nerves, 75 mg/kg i.p. 6-hydroxydopamine (Sigma) was injected for three consecutive days (Zhang et al., 1998) and microdialysis was performed on day 4. 6-Hydroxydopamine was dissolved in 0.1% sodium metabisulfite (Sigma) in distilled water. This protocol almost completely depletes catecholamines in subcutaneous paw tissues and in sciatic nerve sections (Zhang et al., 1998).

2.1.3. Compound 48/80 pretreatment

To degranulate mast cells, rats were pretreated with compound 48/80 over a 2-day period. Compound 48/80 (Sigma) 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 (Hannon et al., 1995). Microdialysis was performed on day 3. This protocol largely blocks mast cell degranulation and paw edema produced by adenosine A_3 receptor agonists in the rat hind paw (Hannon et al., 1995; Sawynok et al., 2000).

Four groups of experiments were conducted in the microdialysis studies. For the three drug pretreated groups (capsaicin, 6-hydroxydopamine, compound 48/80), 50 μl formalin (1.5% or 5%) was injected s.c. into the hind paw of both drug treated and vehicle treated rats. For acute capsaicin treatment, a 50-μl capsaicin (1%) or vehicle (ethanol/Tween 80/saline, 1:1:8) was injected into untreated rats. Behavioural studies and paw volume studies were conducted in awake rats pretreated with capsaicin, 6-hydroxydopamine and respective vehicles.

2.2. Peripheral microdialysis and adenosine measurement

Subcutaneous microdialysis was performed as described previously (Liu et al., 2000). Briefly, rats were anaesthetised throughout the procedure with sodium pentobarbital and body temperature was maintained. The microdialysis probe (LM-5 linear probe, 5 mm active membrane length, 320 μm OD, 35 kDa weight cut-off; Bioanalytical Systems, USA) was implanted into the subcutaneous area of the plantar surface of the rat hind paw. The probe was perfused with standard Krebs–Henseleit solution (mM:

NaCl, 111; $NaHCO_3$, 26.2; NaH_2PO_4, 1.2; KCl, 4.7; $CaCl_2$, 1.8; MgCl, 1.2, at pH 7.4) at a rate of 2 μl/min with a microsyringe pump (Harvard/22, USA) and dialysate was collected at 10 min intervals at room temperature. The probe was flushed for at least 2 h to achieve a steady basal level. After two baseline samples were collected, a 50-μl volume of formalin (1.5% or 5%) or capsaicin (1%) was injected using a 30-g needle into the plantar subcutaneous area approximately 3 mm parallel to the dialysis membrane. Post-injection dialysate collection was started with a 1.5-min delay because of the dead space of the outlet tubing.

Adenosine was measured as described previously (Liu et al., 2000). Samples were deproteinated with $Ba(OH)_2$ and $ZnSO_4$ immediately after collection and then derivatized with chloroacetaldehyde to form 1-N^6-ethenoadenosine to be assayed by high performance liquid chromatography (HPLC) with fluorescence detection. Adenosine (Sigma) standards were prepared in appropriate drug-containing Krebs-Henseleit solutions and adenosine content was quantitated by peak height compared with the standards. The in vitro recovery rate of adenosine from the microdialysis probe was 32.7%, and the recovery rate did not vary with the changes in adenosine concentration or in the presence of formalin (Liu et al., 2000).

2.3. Formalin test

The formalin test was performed as previously described (Sawynok et al., 1998). Rats were placed in a 28 × 28 × 28 cm plexiglass observation chamber for an initial 20 min to allow acclimatization to the testing environment. Formalin (1.5% and 5%) was injected subcutaneously in a volume of 50-μl into the dorsal aspect of the hind paw. Following injections, rats were returned to the observation chamber and monitored for flinching behaviours (lifting, shaking and overt flinching with a ripple over the haunch). Two rats in adjacent chambers were observed at one time, with observations occurring in alternate 2-min bins. Recorded episodes were not corrected, thus values represent about half of the total behaviours expressed.

2.4. Paw volume

Paw volumes were measured as previously described (Sawynok et al., 2000) using a commercially available plethysmometer (Ugo Basile). The hind paws were immersed to the junction of the hairy and non-hairy skin and volumes were read from a digital display. Measurements were performed at the end of the 60-min behavioural time course. Values were standardised by expression as a percentage of individual pre-injection volumes to accommodate the variation in body weights.

2.5. Data analysis and statistics

Microdialysis data were expressed as a time course of adenosine levels in individual dialysate (pmol/20 μl/10 min) or as cumulative release (pmol/120 μl/60 min). Significant changes over the time course were determined by two-way analysis of variance with repeated measurements (two-way RM ANOVA) followed by the Student–Neuman–Keuls test. The Student's *t*-test was used for comparing the cumulative release. Data were expressed as

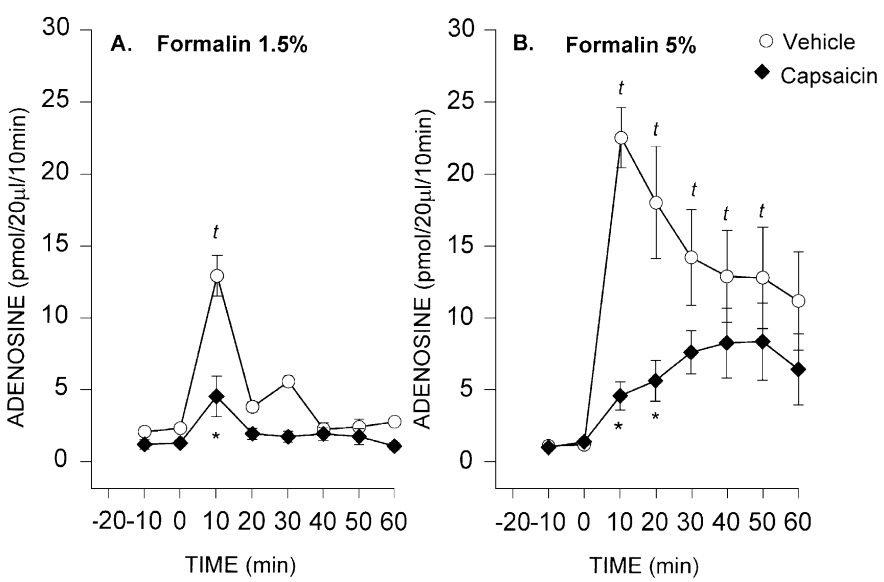

Fig. 1. The effect of pretreatment with capsaicin on (A) 1.5% and (B) 5% formalin-evoked release of adenosine. Formalin was injected at time 0. t—$p < 0.05$ compared to respective basal levels, * —$p < 0.05$ compared to vehicle-pretreated controls ($n = 7–8$ per group).

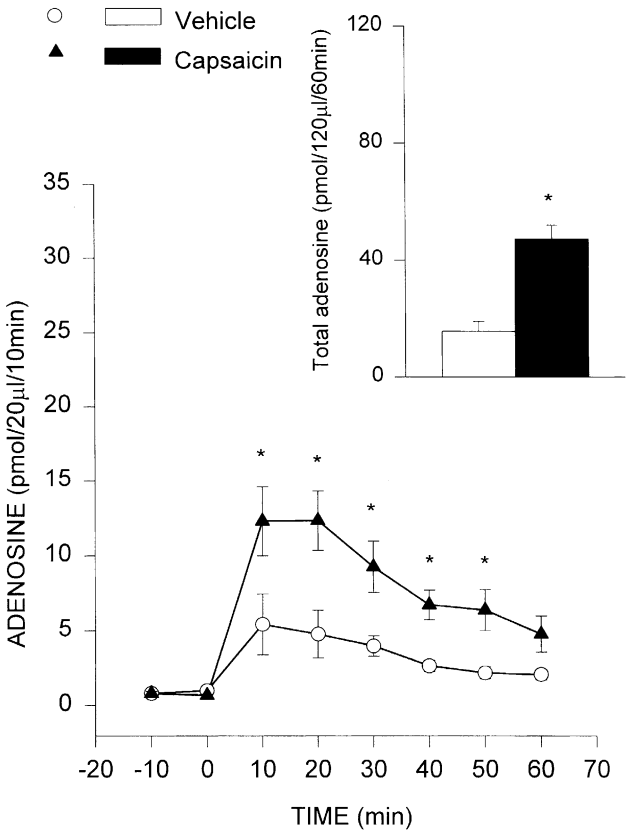

Fig. 2. The effect of acute injection of capsaicin (1%) into the rat hind paw on local adenosine levels, depicting time course and cumulative change (inset). Capsaicin and vehicle were injected at time 0. * — $p < 0.05$ compared to vehicle-pretreated controls ($n = 9$ per group).

means ± standard error of mean (SEM) and the level of significance was set at $p < 0.05$.

3. Results

3.1. Effect of capsaicin pretreatment on formalin-evoked adenosine release, flinches, and paw volume

Injection of formalin 1.5% induced a rapid increase in dialysate adenosine levels within the first 10 min following formalin injection in vehicle-pretreated rats (Fig. 1A). Adenosine levels returned to baseline by the second sample and there was no significant increase in adenosine levels over the remaining collection time. Pretreatment with capsaicin significantly decreased 1.5% formalin-evoked release of adenosine (Fig. 1A). Injection of 5% formalin induced a marked release of adenosine in vehicle-pretreated rats, and the increase was significantly higher than basal levels for 10–50 min following injection (Fig. 1B). Capsaicin pretreatment reduced 5% formalin-induced adenosine release, with the most pronounced decrease being observed within the first 20 min following formalin injection (Fig. 1B).

Pretreatment with capsaicin significantly reduced 1.5% formalin-evoked phase 2 flinches compared to the vehicle-pretreated rats, but did not alter phase 1 responses (Fig. 5A). Capsaicin pretreatment also significantly decreased

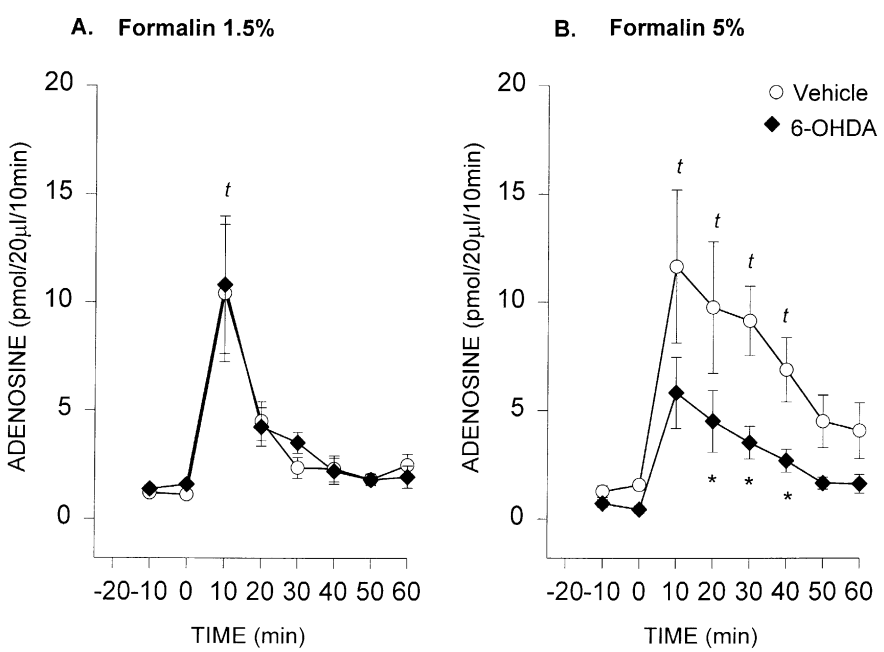

Fig. 3. The effect of pretreatment with 6-hydroxydopamine (6-OHDA) on (A) 1.5% and (B) 5% formalin-evoked release of adenosine. Formalin was injected at time 0. t—$p < 0.05$ compared to respective basal levels, * —$p < 0.05$ compared to vehicle-pretreated controls ($n = 7–8$ per group).

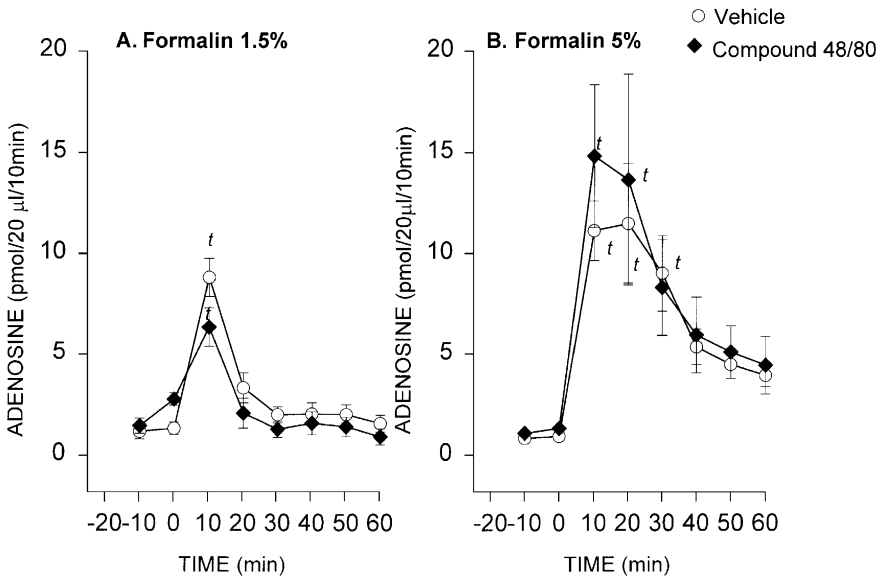

Fig. 4. The effect of pretreatment with compound 48/80 on (A) 1.5% and (B) 5% formalin-evoked adenosine release. Formalin was injected at time 0. t—$p < 0.05$ compared to respective basal levels ($n = 8$ per group).

the 1.5% formalin-evoked increase in paw volume (vehicle vs. capsaicin: $13.0 \pm 1.8\%$ vs. $3.5 \pm 0.9\%$, $p < 0.05$, *t*-test). However, capsaicin pretreatment did not affect either flinches (Fig. 5B) or paw volume (18.8 ± 3.0 vs. 13.0 ± 3.3, $p = 0.24$, *t*-test) evoked by 5% formalin.

3.2. Acute capsaicin injection and adenosine release

Capsaicin (1%, 50-μl) injected locally into the hind paw produced a sustained increase in dialysate adenosine levels. The increases were significantly elevated from baseline from the first sample until the fifth sample (at 50 min), with peak release at 10–20 min after injection (Fig. 2). Vehicle injection did not induce significant increases in adenosine levels over baseline (Fig. 2).

3.3. Effect of 6-hydroxydopamine pretreatment on formalin-evoked adenosine release, flinches and paw volume

At 1.5% formalin, both the vehicle and 6-hydroxydopamine pretreated rats exhibited rapid adenosine release within the first 10 min following formalin injection, and no difference was observed between groups (Fig. 3A). Injection of 5% formalin induced a sustained increase in adenosine levels in vehicle-pretreated rats (Fig. 3B). Pretreatment with 6-hydroxydopamine markedly reduced 5% formalin-evoked release of adenosine compared to vehicle-pretreated rats at 20–40 min following injection (Fig. 3B).

Pretreatment with 6-hydroxydopamine did not produce an effect on flinch behaviours evoked by 1.5% formalin (Fig. 5C), but significantly decreased phase 2 flinches by 5% formalin (Fig. 5D). There was no effect on paw volume produced by either dose of formalin following 6-hydroxydopamine pretreatment (data not shown).

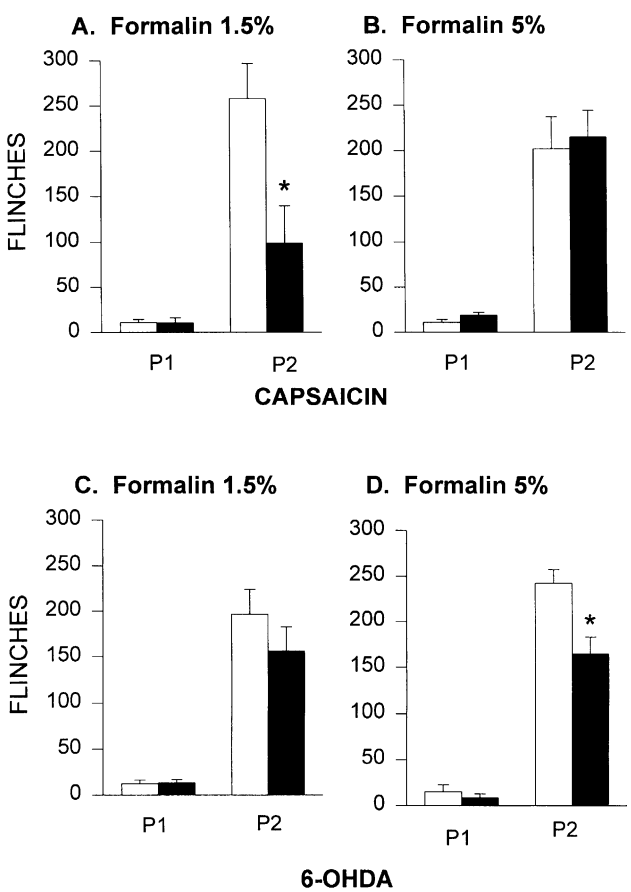

Fig. 5. Cumulative scores of formalin-evoked phase 1 (P1, 0–12 min) and phase 2 (P2, 16–60 min) flinches in (A, B) capsaicin pretreated and (C, D) 6-hydroxydopamine (6-OHDA) pretreated rats. Open column, vehicle pretreated group; filled column, drug-pretreated group. * — $p < 0.05$ compared to respective vehicle pretreated group ($n = 5$–6 per group).

3.4. Effect of compound 48/80 pretreatment on formalin-evoked adenosine release

Pretreatment with compound 48/80 did not produce a significant change in formalin-evoked adenosine release at either 1.5% or 5% over the entire time course (Fig. 4A and B).

4. Discussion

The present study demonstrates neuronal sources for the peripheral release of adenosine in a persistent inflammatory pain model, the formalin test. Following systemic pretreatment with capsaicin, 6-hydroxydopamine and compound 48/80, we demonstrated the involvement of capsaicin-sensitive primary sensory afferents and sympathetic postganglionic nerves, but not mast cells, in the formalin-evoked release of adenosine. We also demonstrated that the source of adenosine release is dependent on the formalin concentration. Thus, at 1.5% formalin, adenosine release is largely dependent on capsaicin-sensitive primary sensory afferents, while at 5%, capsaicin-sensitive primary sensory afferents contribute to the early phase, while sympathetic postganglionic nerve terminals contribute to the later, sustained phase of release.

4.1. Involvement of capsaicin sensitive sensory afferents in formalin-evoked adenosine release

Capsaicin, by activating non-selective cation channel receptors on small diameter unmyelinated C-fibers and thinly myelinated Aδ fibers, induces an acute release of pro-inflammatory neuropeptides such as substance P and calcitonin gene related peptide from both the central and the peripheral terminals of these afferents; large-dose repeated treatment of capsaicin selectively destroys these small diameter primary afferents (Holzer, 1991). In the present study, pretreatment with capsaicin almost eliminated the 1.5% formalin-evoked spike of adenosine release, and markedly reduced the adenosine release evoked by 5% formalin in the first 20 min, indicating the involvement of C-fibers and Aδ fibers in such release. This is consistent with electrophysiological studies that indicate formalin induces a predominant activation of Aβ, Aδ and C-fibers in phase 1, and that while the activity of Aδ and C-fibers persists into phase two, the magnitude is much lower (Puig and Sorkin, 1995; McCall et al., 1996).

Acute injection of 1% capsaicin into the untreated rat hind paw induced a significant release of adenosine, providing further evidence for the release of adenosine from the peripheral terminals of sensory afferents. In previous studies, capsaicin-sensitive release of adenosine and nucleotides from the central terminals of primary sensory afferents has been reported (Sweeney et al., 1989,1990; Cahill et al., 1997), and antidromic stimulation of sensory nerves induces 5′-adenosine triphosphate (ATP) release into the rabbit ear artery, indicating a peripheral release of purines (Holton, 1959; Holton and Holton, 1954). The present study extends those studies, identifying a capsaicin-sensitive peripheral release of adenosine from primary sensory afferent terminals upon chemical stimulation.

Consistent with microdialysis experiments, capsaicin pretreatment also significantly blocked flinch behaviours and paw volume changes evoked by 1.5% formalin. The block of all three features of 1.5% formalin responses by capsaicin pretreatment suggests a predominant small diameter sensory afferent activation following low dose formalin injection. This is consistent with previous studies that showed that block of capsaicin-sensitive sensory afferents reduces nociceptive behaviours (Shibata et al., 1989; Peterson et al., 1997) and inflammation (Damas and Liégeois, 1999). At 5% formalin, capsaicin pretreatment only blocked the early phase but not the late phase of adenosine release, indicating that nociceptive afferent activity is predominant in the early phase, while other inputs contribute to the later phase of adenosine release. Our capsaicin-pretreatment studies did not detect any changes in flinch behaviours and paw volume evoked by 5% formalin, suggesting the role of direct nociceptor excitation in modulating nociceptive behaviours and inflammation evoked by the high concentration formalin is less important compared with that evoked by lower concentrations of formalin. However, a previous study reported that neonatal pretreatment with capsaicin blocked nociceptive responses evoked by 5% formalin (Peterson et al., 1997). This difference may be due to the different capsaicin pretreatment protocols, as neonatal pretreatment with capsaicin produces a more complete depletion of nociceptive afferents compared to capsaicin pretreatment in adult rats (Holzer, 1991). Local capsaicin desensitization also produces a pronounced effect, on 5% formalin evoked responses, blocking nociceptive behaviours (Wheeler-Aceto and Cowan, 1991) and the 5% inflammatory response as revealed by plasma extravasation (Damas and Liégeois, 1999).

4.2. Involvement of sympathetic postganglionic neurons in formalin-evoked adenosine release

The inhibition of 5% formalin-evoked release of adenosine by 6-hydroxydopamine pretreatment indicates an involvement of sympathetic postganglionic nerve terminals in adenosine release in the late phase following a high dose of formalin. As 6-hydroxydopamine does not cross the blood brain barrier in adult rats, systemic administration selectively depletes sympathetic postganglionic nerve terminals rather than central aminergic nerves (Kostrzewa and Jacobowitz, 1974; Coderre et al., 1984a). ATP is a well-known neurotransmitter in sympathetic nerve terminals, and activation of such terminals can release ATP as a

cotransmitter with noradrenaline and neuropeptide Y (Racchi et al., 1999). Importantly, sympathetic stimulation also simultaneously releases nucleotidases, so that the released ATP is rapidly degraded into adenosine through both released nucleotidases and ecto-nucleotidase (Todorov et al., 1997).

Chemical sympathectomy with 6-hydroxydopamine pretreatment blocked late phase adenosine release and behavioural responses evoked by 5% formalin, but did not block such release or behaviours evoked by 1.5% formalin. Previous behavioural studies with pharmacological blockade of adrenoreceptors (Hong and Abbott, 1996) or surgical (Fuchs et al., 1999) and chemical sympathectomy (Coderre et al., 1984a, 1984b) showed a reduction in phase 2 formalin-induced nociceptive behaviours at lower formalin concentrations (1–2.5%) (Hong and Abbott, 1996; Fuchs et al., 1999; Coderre et al., 1984a,b). Different pretreatment procedures, strains of animals and behavioural recording methods may contribute to the discrepancy. However, consistent with our study, the involvement of sympathetic postganglionic neurons in the inflammatory response was only observed at a high dose of formalin (Coderre et al., 1984b; Lam and Ferrell, 1991). These results indicate sympathetic postganglionic neurons are selectively activated in circumstances where more pronounced inflammation and tissue injury are present (Yashpal and Coderre, 1998; Damas and Liégeois, 1999; Fu et al., 2000).

4.3. The lack of involvement of mast cells in formalin-evoked adenosine release

Pretreatment with compound 48/80 failed to demonstrate an involvement of mast cells in adenosine release evoked by both concentrations of formalin, even though it has been shown that acute challenge of mast cells in vitro with compound 48/80 releases adenosine (Marquardt et al., 1984). It is unlikely that the negative observation in the present study reflects an ineffective mast cell degranulation, as we used the same protocol to block inflammation induced by an adenosine A_3 receptor agonist which acts on mast cells (Sawynok et al., 2000). Although it has been suggested that C-fiber activation can release substance P, which consequently degranulates mast cells to release histamine and other substances (Coderre et al., 1989; Suzuki et al., 1999), there is little evidence implicating mast cell degranulation in the early phase of formalin-induced responses (Damas and Liégeois, 1999; Shibata et al., 1989). However, in the late phase, there is histological evidence for mast cell degranulation even at very low doses of formalin (Rosland et al., 1990), and mast cells appear to mediate late phase low dose (0.5%) formalin behavioural responses (Shibata et al., 1989) as well as high dose (5%) formalin inflammatory responses (Damas and Liégeois, 1999). The lack of effect of compound 48/80 in the present study suggests either that adenosine released from mast cells is not substantial enough to be detectable by the current method, or that mast cells are not as important as primary sensory neurons or sympathetic postganglionic nerves in formalin-induced responses. Consistent with our study, mast cell degranulation is much less effective than capsaicin pretreatment in blocking plasma extravasation induced by a wide range of concentrations of formalin (Arvier et al., 1977).

4.4. Qualitatively different mechanisms mediating low and high dose formalin-evoked neurochemical and nociceptive responses

Behavioural responses to formalin are clearly dose related (Rosland et al., 1990; Coderre et al., 1993a; Abbott et al., 1995). Many previous studies have considered the different concentrations of formalin to reflect only a quantitative difference in intensity of responses, rather than qualitative differences in the mediation of these responses. However, an emerging body of evidence indicates the responses at low and high concentrations of formalin are qualitatively quite distinct. (1) Yashpal et al. (1996) and Yashpal and Coderre (1998) have demonstrated a selective involvement of prostaglandins in nociceptive behaviours and inflammatory responses evoked by higher, but not lower, concentrations of formalin. (2) Damas and Liégeois (1999) have demonstrated that mechanisms mediating peripheral inflammation and neurochemical responses are quite different at low and high concentrations of formalin, as there is a predominant capsaicin-sensitive afferent activity at low doses, with the additional involvement of a more complex inflammatory component at the higher dose. (3) Fu et al. (1999, 2000) have demonstrated that only a high dose of formalin produces chronic effects such as spinal cord microglial activation, severe tissue ulceration, and long term hyperalgesia, but these are not observed at a low dose of formalin. The results in those studies, together with observations in the present studies, collectively demonstrate that distinct mechanisms are involved following low and high doses of formalin. Thus, when interpreting information obtained from the formalin model, it is important to consider the concentration used, and in order to obtain comprehensive information about drug actions, both low and high doses of formalin should be used.

4.5. Possible origins and functional aspects of adenosine released in inflammatory pain

Although it has been demonstrated that adenosine is released peripherally during inflammation, and that this released adenosine can be pharmacologically modulated to produce anti-inflammatory (Cronstein et al., 1995; Rosengren et al., 1995; Firestein 1996; Poon and Sawynok, 1999) and antinociceptive effects (Doak and Sawynok, 1995; Sawynok et al., 1998), little is known about the

origin of such release. Adenosine, as a metabolite of ATP, can potentially be released from all cell types, either by transport of adenosine per se by plasma membrane transporters, or by degradation of extracellular ATP or other nucleotides by 5′-ectonucleotidase (Geiger et al., 1997). Subcutaneous tissues of the rat hind paw exhibit marked activity of enzymes degrading ATP (Bland-Ward and Humphrey, 1997), so that released nucleotides can be rapidly broken down to adenosine at this site. It is generally believed that the non-specific release of adenosine or nucleotides from stressed cells, especially from neutrophils (Cronstein et al., 1983), endothelial cells (Ager and Gordon, 1984) and mast cells (Marquardt et al., 1984), is the major source of extracellular adenosine in inflamed tissues.

Although the present study does not exclude the involvement of non-specific adenosine release due to cell damage, the blockade by capsaicin and 6-hydroxydopamine pretreatment indicates a neuronal basis for substantial amounts of the adenosine released by formalin-induced inflammation. It should be noted that destroying nerve terminals might also reduce purine release secondarily from non-neuronal tissues that are innervated by these nerve terminals. For example, blocking primary sensory afferents (Taylor et al., 2000) and sympathetic nerve terminals (Fuchs et al., 1999) can decrease local blood flow and potentially decrease adenosine or ATP release from endothelial cells (Bodin and Burnstock, 1998). However, as ATP and adenosine can be released from the central terminals of sensory afferents in the spinal cord (Sweeney et al., 1989, 1990), as well as from peripheral terminals of sensory afferents following electrical stimulation (Holton, 1959; Holton and Holton, 1954), a direct purine release from peripheral terminals of nociceptive afferents following neurogenic inflammation may also occur. On the other hand, under circumstances where there is massive tissue damage and inflammation (Damas and Liégeois, 1999; Fu et al., 2000), sympathetic postganglionic neurons may be activated and become an important source of adenosine, likely originating as ATP. This adenosine may produce peripheral antinociceptive effects, by activating adenosine A_1 receptors (reviewed Sawynok, 1998) and anti-inflammatory effects by activating adenosine A_2 receptors (reviewed Sullivan and Linden, 1998).

5. Conclusion

The present study demonstrates that the peripheral origin of adenosine released by formalin in the rat hind paw depends on the formalin concentration. Only small diameter capsaicin-sensitive afferents are involved at a low dose of formalin, while both capsaicin-sensitive primary afferents (during early phase) and sympathetic postganglionic nerve terminals (during late phase) are involved in high dose formalin-evoked adenosine release. Mast cell degranulation is not involved in such release.

Acknowledgements

The present study was supported by the Canadian Institutes of Health Research. X.J. Liu is a recipient of an Izaak Walton Killam Memorial Scholarship. We thank A. Reid for technical assistance with behavioural and paw volume experiments.

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