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Amitriptyline produces multiple influences on the peripheral enhancement of nociception by P2X receptors

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Abstract

Peripherally administered amitriptyline exhibits potential to be a locally active analgesic, while ATP augments peripheral nociception by interacting with $P2X_3$ receptors on sensory afferents. The present study examined the effects of amitriptyline on flinching and biting/licking behaviours and thermal hyperalgesia produced by αβ-methylene-ATP (αβ-MeATP), a ligand for $P2X_3$ receptors, following intraplantar administration into the hindpaw of rats. Coadministration of low doses of amitriptyline (up to 100 nmol) with αβ-MeATP augmented thermal hyperalgesia and flinching behaviours. The most active dose of amitriptyline (100 nmol) had no intrinsic effect. Augmentation of αβ-MeATP actions appears to be due to increased tissue levels of biogenic amines resulting from inhibition of uptake, as phentolamine (α_1/α_2-adrenergic receptor antagonist) and methysergide (5-hydroxytryptamine or $5\text{-HT}_1/5\text{-HT}_2$ receptor antagonist) inhibit the augmented flinching produced by αβ-MeATP/amitriptyline. When noradrenaline and 5-HT were coadministered with αβ-MeATP (both increase the effect of αβ-MeATP), amitriptyline had no effect on flinching produced by αβ-MeATP/noradrenaline but inhibited flinching produced by αβ-MeATP/5-HT. In the presence of low concentrations of formalin (0.5%, 1%; which also increase the effect αβ-MeATP), amitriptyline inhibited augmented behaviours. Higher doses of amitriptyline (300–1000 nmol) increased thermal thresholds, suppressed thermal hyperalgesia produced by αβ-MeATP, and inhibited flinching produced by αβ-MeATP. Collectively, these results indicate that amitriptyline produces complex influences on peripheral pain signaling by P2X receptors. Lower doses *augment* nociception by αβ-MeATP (probably by inhibiting noradrenaline and 5-HT uptake) but *inhibit* αβ-MeATP responses in the presence of inflammatory mediators (perhaps reflecting receptor blocking properties); higher doses uniformly *inhibit* nociception by αβ-MeATP (perhaps reflecting local anesthetic properties).

Keywords: Amitriptylin; Nociception; P2X receptor

1. Introduction

Peripheral $P2X_3$ receptors, which are activated by ATP, may play a significant role in pain signaling following tissue injury (Burnstock, 2000; Jarvis and Kowaluk, 2001; Cook and McClesky, 2002). These receptors are selectively localized on (Chen et al., 1995; Bradbury et al., 1998; Llewellyn-Smith and Burnstock, 1998; Vulchanova et al., 1998) and highly expressed in (Xiang et al., 1998; Kim et al., 2003) small diameter sensory afferent neurons. αβ-Methylene-ATP (αβ-MeATP) has a low micromolar affinity for $P2X_3$ receptors (Dunn et al., 2001), and as its actions on sensory afferents are completely eliminated in $P2X_3$ receptor deficient animals (Cockayne et al., 2000; Souslova et al., 2000; Zhong et al., 2001), it is a useful agent for examining functional aspects of activation of this receptor. Local peripheral administration of αβ-MeATP to the hindpaw of adult rats produces a number of pain facilitatory actions including flinching and biting/licking behaviours (Bland-Ward and Humphrey, 1997; Hamilton et al., 1999; Jarvis et al., 2001; Wismer et al., 2003), thermal and chemical hyperalgesia (Bland-Ward and Humphrey, 1997; Sawynok and Reid, 1997; Hamilton et al., 1999; Tsuda et al., 2000) and mechanical allodynia (Tsuda et al., 2000).

We have recently demonstrated that both flinching behaviours and thermal hyperalgesia produced by local administration of αβ-MeATP to the hindpaw are augmented

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by the biogenic amines noradrenaline and 5-hydroxytryptamine (5-HT) (Waldron and Sawynok, 2004). Amitriptyline, a tricyclic antidepressant, is well known to block the reuptake of noradrenaline and 5-HT (Richelson and Pfenning, 1984), and potentially could enhance the pain facilitatory actions of αβ-MeATP by this mechanism. However, amitriptyline administered locally to the hindpaw produces a peripheral antinociception in a number of different paradigms, and this may reflect a number of its pharmacological actions (Esser and Sawynok, 1999; Sawynok et al., 1999, 2001; Gerner et al., 2001; Khan et al., 2002; Ulugol et al., 2002; Haderer et al., 2003). Amitriptyline can block ligand-gated $P2X_2$ receptors in a reconstituted in vitro system (Nakazawa et al., 1999), but effects on $P2X_3$ receptors have not been reported. In view of these observations, one cannot necessarily anticipate the influence of amitriptyline on the expression of effects mediated by peripheral $P2X_3$ receptors in vivo.

In the present study, we examined the effect of amitriptyline on flinching and biting/licking behaviours and thermal hyperalgesia produced by αβ-MeATP administered locally into the rat hindpaw by intraplantar injection. If endogenous ATP contributes significantly to peripheral pain signaling (Cockayne et al., 2000; Souslova et al., 2000; Jarvis et al., 2001), the effects of amitriptyline on such actions may be of relevance to the mechanisms by which amitriptyline produces a local antinociception. Initially, doses of amitriptyline that produce peripheral antinociception in persistent and neuropathic pain models (up to 100 nmol) were examined in combination with αβ-MeATP. Subsequently, the role of biogenic amines in these actions was examined (a) using amine receptor antagonists (phentolamine for α_1- and α_2-adrenoreceptors, methysergide for 5-HT_1 and 5-HT_2 receptors), and (b) by determining effects of amitriptyline in the presence of biogenic amines (noradrenaline, 5-HT). Effects of amitriptyline were also examined when αβ-MeATP was given in combination with low concentrations of formalin which are known to augment αβ-MeATP effects. Finally, the effects of higher doses of amitriptyline (300, 1000 nmol) were examined. The results indicate that amitriptyline produces complex effects on the expression of αβ-MeATP actions following peripheral administration of αβ-MeATP, with the direction of the effect depending on the context in which the αβ-MeATP is given (i.e. absence or presence of other agents or inflammation) and the dose.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Montreal), 125–300 g, were used in most experiments. In some experiments, where specifically indicated, male Wistar rats 125–300 g from the same source were used. A recent study has indicated a marked difference in the expression of flinching behaviours following local administration of αβ-MeATP to Sprague–Dawley and Wistar rats (Waldron and Sawynok, 2004); in the present study, Wistar rats were used when a strong expression of such behaviours was required. All procedures were approved by the University Committee on Laboratory Animals and conducted in accordance with Canadian Council on Animal Care guidelines.

Rats were housed in pairs, and maintained on a 12:12 h light/dark cycle at 22±2 °C with food and water freely available. Each hindpaw was tested once, with at least 2 days between successive trials. Control thermal hyperalgesia responses to αβ-MeATP exhibited a high degree of reproducibility between such trials. Rats receiving formalin were tested only in a second trial to avoid conditioning or sensitizing effects.

2.2. Drug injections

All drug injections were in a total volume of 50 μl saline at room temperature. Rats were accommodated in an open ended plastic container which allowed for the extrusion of the hindpaw, and injections were made s.c. into the plantar surface of the hindpaw between the centre and heel of the paw. All drugs were prepared on the day of injection, and 5-HT solutions were prepared immediately prior to each injection. All combination treatments involved a coadministration protocol in which drugs specified were contained in the single 50 μl injection.

2.3. Spontaneous pain behaviours

Rats were acclimatized for 15 min in a plexiglass observation chamber (28×28×28 cm), with two rats in adjacent chambers. Flinching (paw elevation or shaking of the hindpaw) behaviours were recorded as a cumulative number of episodes in alternating 2-min bins for each rat, and biting/licking behaviours were recorded as total time engaged in that behaviour. Behaviours were observed for 30 or 60 min as indicated in individual experiments. For formalin combination experiments, drugs were coadministered with formalin 0.5–1.5%, and behaviours observed for 60 min following injections. When biting/licking behaviours are not reported, these were not observed.

2.4. Thermal hyperalgesia

A thermal hyperalgesia test apparatus (University of California, San Diego, Department of Anesthesiology) was used. Rats were placed in plexiglass chambers on a glass surface maintained at 30 °C, and acclimatized for 30–45 min. Radiant heat (light beam) was directed at the plantar surface of the injected hindpaw, and three baseline responses (hindpaw withdrawal) were recorded at 5-min intervals prior to injection of drugs. Thermal withdrawal latencies were determined at 5, 15, 30, 45 and 60 min following drug injections.

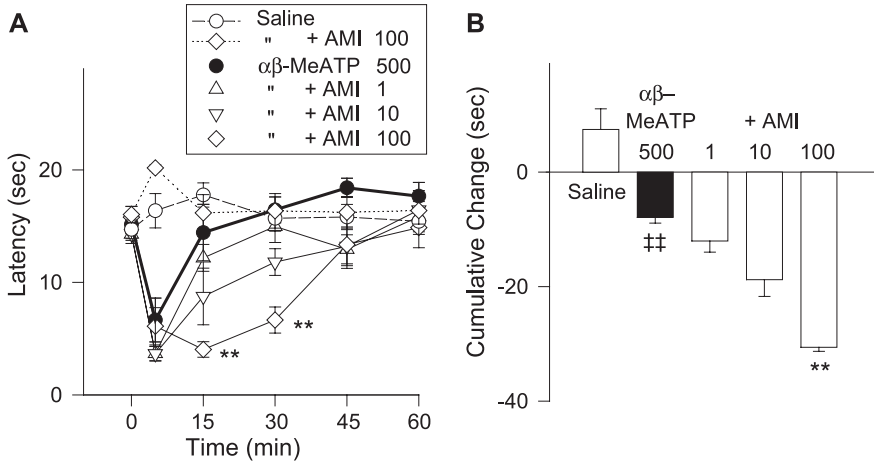

Fig. 1. Effect of coadministration of amitriptyline (AMI) with αβ-MeATP on thermal hyperalgesia. (A) Time course of response, and (B) cumulative change from baseline. Doses in nmol; n=6 per group. $^{**}P<0.01$ compared to αβ-MeATP; $^{‡‡}P<0.01$ compared to saline.

2.5. Data and statistics

Data are presented as mean and S.E.M. for the time course of hyperalgesia or spontaneous behaviours. Cumulative responses are depicted as a cumulative difference from the mean baseline value for the 60-min post-injection interval for thermal hyperalgesia, and this approximates an area under the time–response curve. Cumulative flinching behaviours are depicted as the total number of events over 30 or 60 min, or as total time spent biting or licking over that interval. Data were analyzed using analysis of variance followed by the Student–Newman–Keuls test, or in some instances where only two groups were compared, using the Student's t-test.

2.6. Drugs

αβ-MeATP, amitriptyline hydrochloride, noradrenaline bitartrate, 5-hydroxytryptamine hydrochloride, phentolamine hydrochloride, methysergide maleate and formalin (37% formaldehyde) were purchased from Sigma. Formalin concentrations refer to % solutions made from the formalin stock.

3. Results

3.1. Effects of lower doses of amitriptyline on pronociceptive αβ-MeATP actions

αβ-MeATP, administered into the hindpaw of Sprague–Dawley rats, produces thermal hyperalgesia which peaks at 5 min following injection (Fig. 1A). Coadministration of 100 nmol amitriptyline with αβ-MeATP led to a significant augmentation of thermal hyperalgesia, which manifested primarily as a prolongation of the effect (Fig. 1A). Amitriptyline alone had no intrinsic effect on thermal thresholds compared to saline (Fig. 1A). Lower doses of amitriptyline (10 and 1 nmol) produced a lesser or no effect

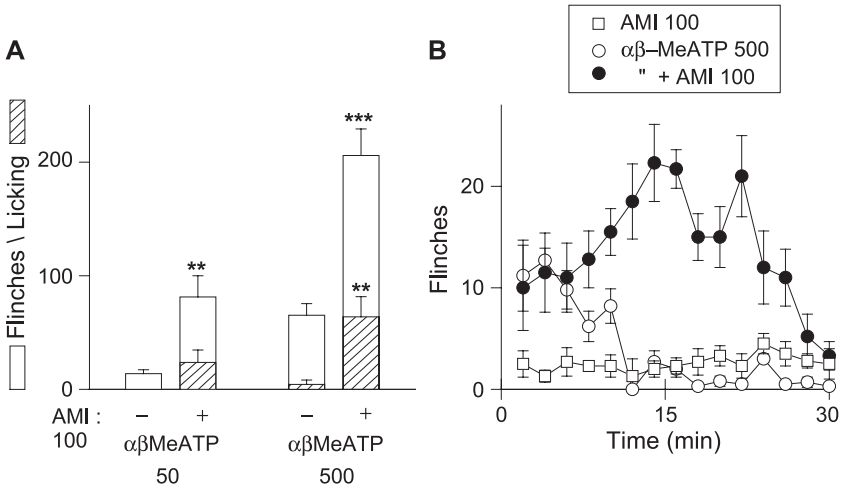

Fig. 2. Effect of coadministration of amitriptyline (AMI) with αβ-MeATP on flinching and biting/licking behaviours in Wistar rats. (A) Cumulative scores over 30 min for number of flinches (open columns) and seconds spent biting/licking (hatched column), and (B) time course of flinching behaviours. Doses in nmol; n=6 per group. $^{**}P<0.01$, $^{***}P<0.001$ compared to αβ-MeATP.

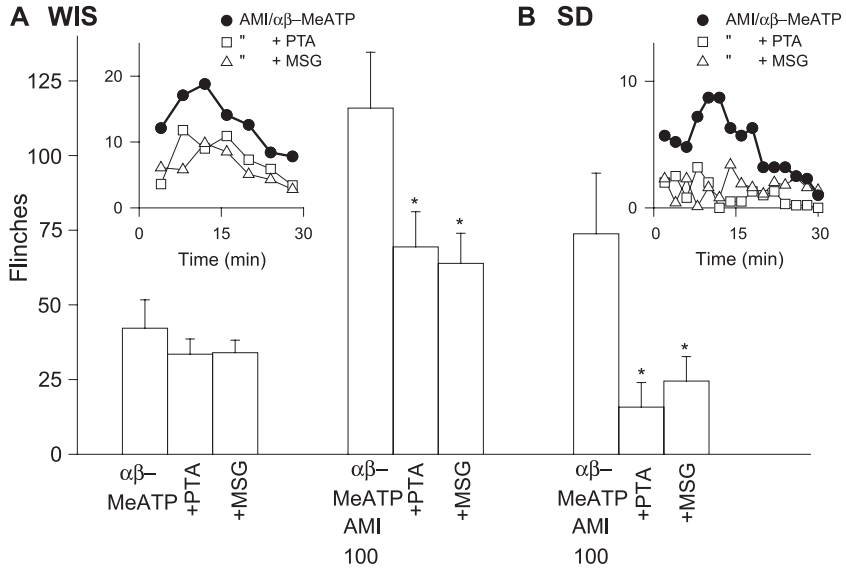

Fig. 3. Effects of phentolamine (PTA) and methysergide (MSG), 100 nmol each, on flinching produced by amitriptyline (AMI)/αβ-MeATP in (A) Wistar (WIS) and (B) Sprague–Dawley (SD) rats. Doses in nmol; n=6 per group. *P<0.05 compared to AMI/αβ-MeATP.

on αβ-MeATP, respectively (Fig. 1A, B); intrinsic effects of these doses on thermal thresholds were not determined.

αβ-MeATP generates few intrinsic flinching behaviours when given to Sprague–Dawley rats, but it does lead to such behaviours in Wistar rats (Waldron and Sawynok, 2004). When 100 nmol amitriptyline was coadministered with αβ-MeATP in Wistar rats, it led to a significant augmentation of both flinching and biting/licking behaviours (Fig. 2). The augmentation was apparent both in cumulative scores (Fig. 2A) and in individual time courses and lasted up to 30 min (Fig. 2B). Amitriptyline also

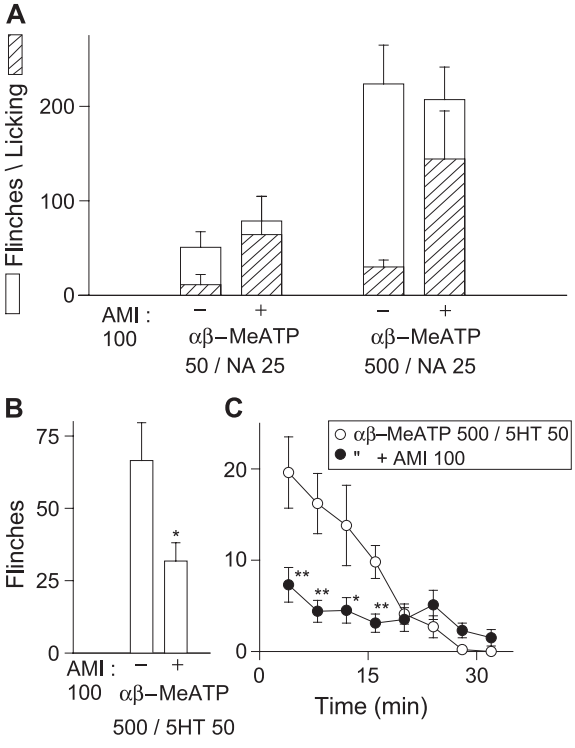

Fig. 4. Effect of amitriptyline (AMI) on spontaneous pain behaviours produced by αβ-MeATP in combination with (A) noradrenaline (NA) and (B, C) 5-HT. Open columns represent cumulative scores over 30 min for number of flinches, while hatched columns represent seconds spent biting/licking. Doses in nmol; n=6 (A) or n=12 (B, C) per group. *P<0.05, **P<0.01 compared to αβ-MeATP/5-HT.

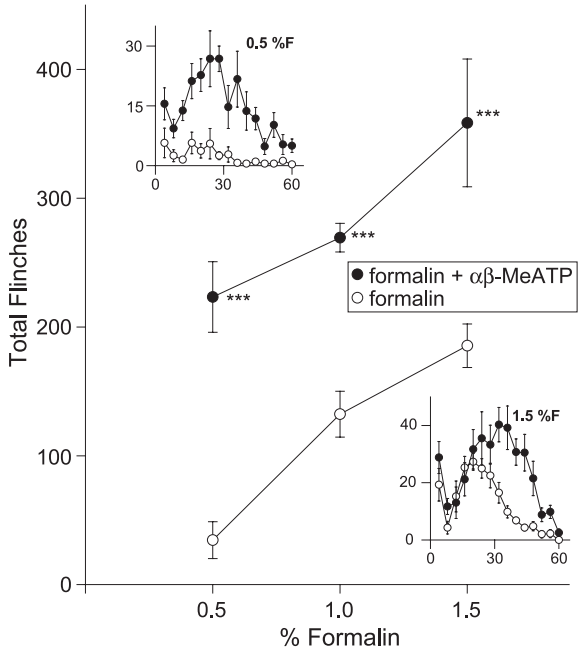

Fig. 5. αβ-MeATP (500 nmol) augmentation of flinches produced by different concentrations of formalin (F). Open symbols indicate responses to formalin alone, and solid symbols indicate responses in the presence of formalin. Insets depict time course of increase at the low (0.5%) and high (1.5%) concentration of formalin. n=6 per group. ***P<0.001 compared to formalin alone.

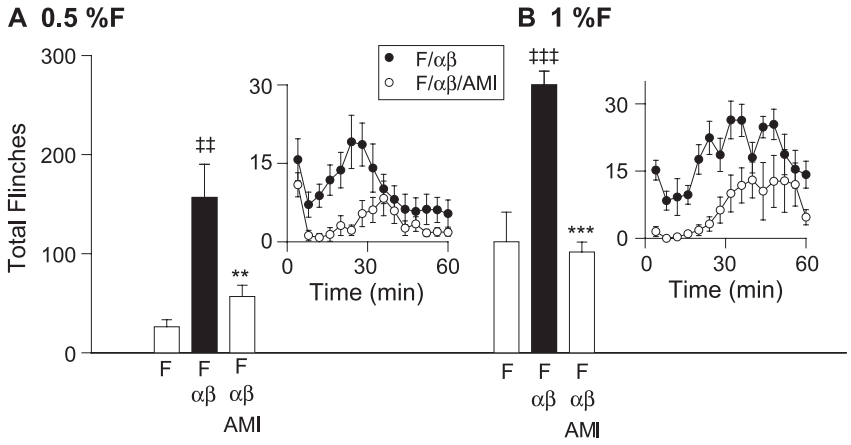

Fig. 6. Effect of amitriptyline (AMI) 100 nmol in combination with αβ-MeATP (αβ)/formalin at two concentrations of formalin. Solid symbols indicate responses in the presence of formalin/αβ-MeATP, and open symbols indicate such responses in the presence of amitriptyline. n=6 per group. ‡‡P<0.01, ‡‡‡P<0.001 compared to formalin; **P<0.01, ***P<0.001 compared to αβ-MeATP/formalin.

augmented flinching produced by αβ-MeATP in Sprague–Dawley rats, even though αβ-MeATP produced few intrinsic effects in this strain (Fig. 3B). In both Wistar and Sprague–Dawley rats, behaviours observed following injection of 100 nmol amitriptyline alone were indistinguishable from saline (data not shown).

3.2. Role of amines in amitriptyline/αβ-MeATP interactions

Both noradrenaline and 5-HT augment flinching behaviours and thermal hyperalgesia produced by αβ-MeATP (Waldron and Sawynok, 2004). As amitriptyline can inhibit the uptake of both noradrenaline and 5-HT, we determined whether endogenous amines might account for the action of amitriptyline by examining the effects of phentolamine (α_1- and α_2-adrenoreceptor antagonist) and methysergide (5-HT_1 and 5-HT_2 receptor antagonist) on the combination drug effect. Both phentolamine and methysergide inhibited flinching produced by amitriptyline/αβ-MeATP in both Wistar (Fig. 3A) and Sprague–Dawley rats (Fig. 3B). The residual effect in the presence of antagonists was greater in Wistar than in Sprague–Dawley rats, probably reflecting the greater relative intrinsic effect of the αβ-MeATP alone in this strain. Thus, the amine antagonists did not significantly alter flinching produced by αβ-MeATP alone (Fig. 3A).

Amitriptyline (100 nmol) was also administered in combination with αβ-MeATP/noradrenaline and αβ-MeATP/5-HT as an additional method of exploring the role of amines in the augmentation of flinching (and biting/licking) behaviours. Amitriptyline did not augment flinching produced by αβ-MeATP/noradrenaline (Fig. 4A), suggesting a ceiling effect was attained by adding noradrenaline and no further augmentation could then occur with amitriptyline. There appeared to be an increase with biting/licking behaviours, but this showed considerable variability, and was not statistically different. Curiously, amitriptyline clearly inhibited flinching behaviours produced by the αβ-MeATP/5-HT combination (Fig. 4B), which was an unexpected finding.

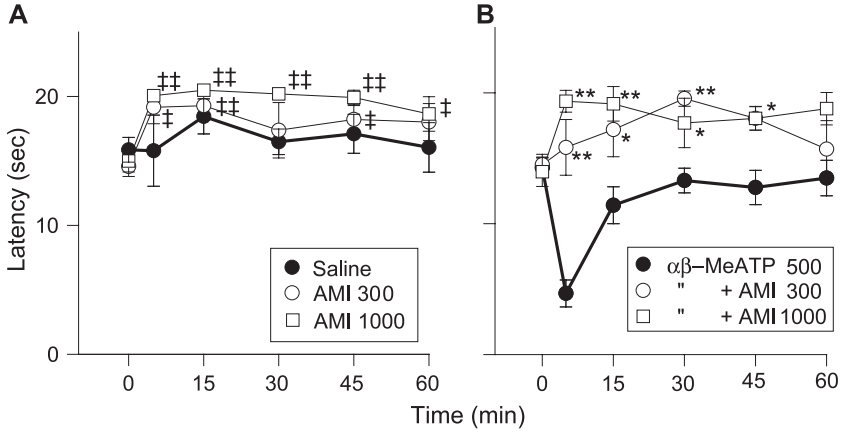

Fig. 7. Effect of higher doses of amitriptyline (AMI) on (A) thermal thresholds and (B) thermal hyperalgesia produced by αβ-MeATP. Doses in nmol; n=6 per group. ‡P<0.05, ‡‡P<0.01 compared to baseline; *P<0.05, **P<0.01 compared to αβ-MeATP.

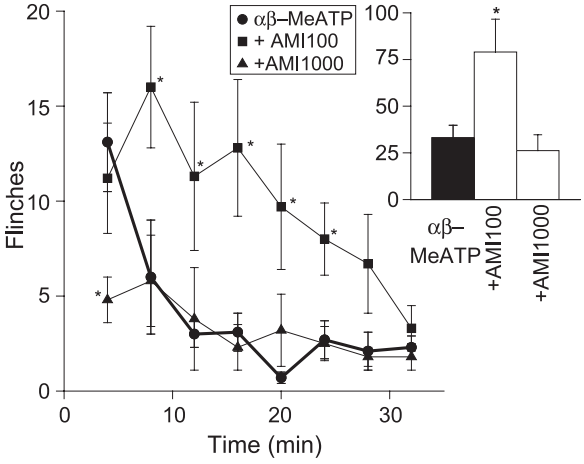

Fig. 8. Biphasic effects of 100 and 1000 nmol amitriptyline (AMI) on flinching behaviours produced by αβ-MeATP in Wistar rats. Doses in nmol; n=6 per group. *P<0.05 compared to αβ-MeATP alone.

3.3. Effects of amitriptyline on αβ-MeATP/formalin combinations

The ability of αβ-MeATP to generate flinching behaviours has been shown to be enhanced by a low concentration of formalin (Sawynok and Reid, 1997), and the present study extends the range of formalin concentrations examined in this regard. The αβ-MeATP-induced augmentation of formalin behaviours in Sprague–Dawley rats is strongly expressed over a range of concentrations; at 0.5% formalin, αβ-MeATP augments behaviours over 60 min (Fig. 5, upper inset), while at 1.5% formalin, augmentation is seen primarily later in the time course (30–60 min; Fig. 5, lower inset). The time course for αβ-MeATP in the presence of 1% formalin resembles that seen at 1.5% (data not shown). Amitriptyline given in combination with αβ-MeATP/formalin combinations significantly reduced flinching at both the low (0.5%, Fig. 6A) and a higher concentration of formalin (1%, Fig. 6B).

3.4. Effects of higher doses of amitriptyline on αβ-MeATP

At higher doses (300, 1000 nmol), amitriptyline produced an intrinsic increase in thermal thresholds (Fig. 7A), and completely suppressed the hyperalgesic effect of αβ-MeATP (Fig. 7B). The higher dose of amitriptyline (1000 nmol) also inhibited the initial peak of flinching produced by αβ-MeATP alone in Wistar rats (Fig. 8). A clear biphasic effect on αβ-MeATP responses was observed with 100 and 1000 nmol amitriptyline (Fig. 8).

4. Discussion

The present study demonstrates that low doses of amitriptyline (up to 100 nmol), administered peripherally to the hindpaw of the rat in combination with αβ-MeATP, produce complex effects on peripheral nociception as reflected in flinching (and biting/licking) behaviours and thermal hyperalgesia. At these doses, *augmentation of nociception* appears to involve biogenic amines (noradrenaline, 5-HT), likely reflecting inhibition of biogenic amine reuptake from the extracellular space. On the other hand, *inhibition of nociception* in the presence of inflammation (formalin) or certain inflammatory mediators (5-HT) may reflect inhibition of specific receptors (e.g. 5-HT). At high doses (1000 nmol), locally administered amitriptyline produces an intrinsic increase in thermal thresholds, suppresses thermal hyperalgesia and attenuates flinching behaviours produced by αβ-MeATP, probably reflecting local anesthetic actions of amitriptyline. The effects of amitriptyline, which exhibits potential as a peripheral analgesic (Gerner et al., 2003; Sawynok, 2003), are clearly complex, being condition- and dose-dependent in different paradigms. These complex functional effects indicate that while in vitro approaches may provide valuable insights into potential mechanisms of drug action, for a drug with multiple pharmacological effects such as amitriptyline, in vivo characterization over a wide dose range is essential for understanding the integrated expression of those actions.

This study used two main pharmacological agents. αβ-MeATP generates flinching and some biting/licking behaviours and thermal hyperalgesia when administered locally to the rat hindpaw, and these actions are mediated by activation of P2X receptors on capsaicin-sensitive afferent neurons (Bland-Ward and Humphrey, 1997; Tsuda et al., 2000). While αβ-MeATP has a low micromolar affinity for $P2X_1$, $P2X_3$ and $P2X_{2/3}$ receptors (Dunn et al., 2001), its activity is thought to reflect $P2X_3$ receptor activation because activation of sensory afferent nerves produced by αβ-MeATP is completely eliminated in $P2X_3$ receptor deficient animals (Cockayne et al., 2000; Souslova et al., 2000; Zhong et al., 2001), and flinching behaviours are inhibited by antisense oligonucleotides to $P2X_3$ receptors (Honoré et al., 2002). $P2X_1$ receptors are much less prominently expressed than $P2X_3$ receptors on sensory afferents (Xiang et al., 1998; Kim et al., 2003) and are, therefore, unlikely to be involved, while $P2X_{2/3}$ receptors mediate enhanced nociception (mechanical allodynia) by actions on capsaicin-insensitive sensory afferents (Tsuda et al., 2000). αβ-MeATP, therefore, appears to be a useful agent for examining the peripheral properties of $P2X_3$ receptors on capsaicin-sensitive afferents (C-fibres). The other agent used, amitriptyline, produces a number of acute pharmacological actions which may potentially contribute to peripheral antinociception, and these include (a) inhibition of the uptake of biogenic amines (noradrenaline, 5-HT) and adenosine, (b) inhibition of α-adrenergic, 5-HT, histamine, muscarinic and nicotinic cholinergic and *N*-methyl-D-aspartate receptors, (c) blockade of ion channels (Na^+, Ca^{2+}, K^+), and (d) interactions with opioid receptors (Sindrup, 1997; Eschalier et al., 1999; Sawynok et al., 2001). Applied peripherally to the rat hindpaw, low doses of amitriptyline (up to 100 nmol)

produce a local antinociception in models of persistent pain and nerve injury (Esser and Sawynok, 1999; Sawynok et al., 1999; Ulugol et al., 2002). Local injection of higher doses of amitriptyline (1000–2000 nmol, 0.2 ml of 5–10 mM solutions) adjacent to the sciatic nerve (Gerner et al., 2001; Sudoh et al., 2003) or into the dorsal skin (956–9557 nmol, 0.6 ml of 0.05–0.5% solutions; Khan et al., 2002) inhibits nociceptive, proprioceptive and motor input, and this reflects local anesthetic actions. At the highest doses (2000–16,000 nmol), amitriptyline produces neurotoxic effects when injected immediately adjacent to the nerve (Estebe and Myers, 2004).[1] Local anesthetics themselves have been recognized to produce neurotoxic actions following similar methods of administration (Myers et al., 1986).

In view of the above pharmacological and in vivo observations, we anticipated that amitriptyline would block peripheral pronociceptive effects of αβ-MeATP in the present study. The ability of low doses of amitriptyline (up to 100 nmol), which by themselves do not produce intrinsic effects in these paradigms, to augment flinching and thermal hyperalgesia produced by αβ-MeATP was, therefore, quite unexpected. This augmenting action appears to be mediated by inhibition of noradrenaline reuptake (noradrenaline likely arises from postganglionic sympathetic efferent neurons) as (a) noradrenaline strongly augments flinching and thermal hyperalgesia produced by αβ-MeATP (Waldron and Sawynok, 2004), (b) no further augmentation of flinches by amitriptyline was seen in the presence of noradrenaline, suggesting a ceiling effect, and (c) phentolamine inhibits flinching produced by the combination of amitriptyline/noradrenaline in two strains of rats. When epinephrine is added to high concentrations of amitriptyline, it both augments and increases the duration of nerve block (Khan et al., 2002), likely reflecting an effect on vascular α-adrenergic receptors to reduce clearance of the drug. There is, therefore, also a potential pharmacokinetic effect involved in the positive interaction observed between adrenergic agonists and αβ-MeATP. 5-HT (which is released from platelets and mast cells) may also play a role in the augmenting effect of amitriptyline, as 5-HT also augments flinching (although to a lesser extent than noradrenaline) and thermal hyperalgesia produced by αβ-MeATP (Waldron and Sawynok, 2004), and methysergide inhibits flinching produced by the combination of amitriptyline with αβ-MeATP in two strains of rats.

The present study further observed that at higher doses (300–1000 nmol) amitriptyline produces an intrinsic increase in thermal thresholds (see also Oatway et al., 2003), suppresses thermal hyperalgesia produced by αβ-MeATP, and reduces αβ-MeATP-induced flinches. These actions may reflect local anesthetic properties of amitriptyline, whereby all methods of activation of sensory nerves are suppressed. Thus, (a) amitriptyline blocks neuronal Na^+ currents in a voltage- and use-dependent manner similar to local anesthetics (Pancrazio et al., 1998; Song et al., 2000), (b) amitriptyline and local anesthetics bind to overlapping sites on the channel (Nau et al., 2000), and (c) high doses of amitriptyline produce effects similar to other local anesthetics in functional studies (Gerner et al., 2001; Khan et al., 2002; Sudoh et al., 2003). The local peripheral actions of amitriptyline in different dose ranges, both in this and other studies, appear to reflect different aspects of the pharmacology of this agent. The particular tissue and compartment concentrations attained after different local methods of delivery, and the extent to which particular mechanisms are involved after such delivery methods, remain to be determined.

The effects of low doses of amitriptyline are clearly dependent on the context in which they are administered. Inflammation and specific inflammatory mediators enhance the pronociceptive effect of αβ-MeATP in behavioural tests and the activation of sensory afferent nerves by $P2X_3$ receptor activation (Hu and Li, 1996; Hamilton et al., 1999, 2000; Paukert et al., 2001). In the presence of an acute form of inflammation (formalin), where multiple endogenous tissue mediators are released and contribute to nociception (Tjølsen et al., 1992), amitriptyline inhibits both intrinsic formalin-evoked behaviours (Sawynok et al., 1999) and the augmentation of such behaviours by αβ-MeATP (this study). At 1% formalin, one might expect inhibition of flinching produced by amitriptyline/formalin due to the intrinsic effect of amitriptyline on formalin-evoked behaviours, but a prominent suppression is also seen at 0.5% formalin where very little in the way of intrinsic behaviours occurs with formalin alone. Thus, while endogenous ATP and $P2X_3$ receptor activation can contribute to the expression of formalin-evoked behaviours (Cockayne et al., 2000; Souslova et al., 2000; Jarvis et al., 2001), the facilitatory interaction between amitriptyline and ATP, observed when these two agents are given alone, is obscured in the presence of multiple other tissue mediators that are involved in inflammation, and the net functional effect of amitriptyline is now inhibited. One potential inflammatory mediator that could contribute significantly to this outcome is 5-HT. Thus, 5-HT contributes to the expression of formalin-evoked behaviours as local administration of antagonists for multiple 5-HT receptors (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4) inhibits formalin-evoked responses (Abbott et al., 1996; Doak and Sawynok, 1997; Parada et al., 2001). Amitriptyline inhibits the effect of the combination of 5-HT with αβ-MeATP, likely reflecting the ability of amitriptyline to inhibit 5-HT_1, 5-HT_2 and 5-HT_3 receptors (Hall and Ögren, 1981; Fan, 1994; Honda et al., 2003; Sawynok and Reid, 2003). The effect of amitriptyline on other specific inflammatory mediators that increase $P2X_3$ receptor mediated responses,

[1] The Estebe and Myers (2004) report contains a significant error in calculating total doses. Thus, their injections of 0.625–5 mg or 0.2 ml of 3.125–25 mg/ml correspond to total doses of 2–16 μmol or 2000–16,000 nmol, and not 2–16 nmol as claimed. This computation error is maintained throughout much of the discussion.

such as substance P and bradykinin (Hu and Li, 1996; Paukert et al., 2001), remains to be determined.

If one were to extrapolate from the present preclinical observations, one might anticipate the following properties for peripherally administered amitriptyline in low doses in pain conditions. (1) Peripherally administered amitriptyline would produce analgesia under conditions involving inflammation where multiple tissue mediators are involved (cf. results in the presence of formalin). Chronic peripheral inflammation (with complete Freunds adjuvant) enhances ATP currents in dorsal root ganglion neurons and increases the expression of $P2X_2$ and $P2X_3$ receptors (Xu and Huang, 2002), and ATP influences might be even more prominent under such conditions. (2) The effects of amitriptyline might be less predictable in conditions in which there is a prominent involvement of the sympathetic nervous system. Thus, ATP is coreleased with noradrenaline from sympathetic nerves (Burnstock, 1995; Sneddon et al., 1996), and could influence responses in conditions of nerve injury and tissue trauma where the sympathetic nervous system interacts with sensory systems (Jänig et al., 1996; Baron et al., 1999; Michaelis, 2000). Considerable variability in the expression of $P2X_3$ and noradrenaline interactions following nerve injury have been reported (Park et al., 2000). Functional studies using particular neuropathic pain models in which a sympathetic influence is, or is not, clearly expressed would be helpful in further understanding the implications of this interaction. (3) Application of high concentrations of amitriptyline would produce a more complete analgesia, as it would potentially recruit a number of mechanisms that can suppress pain, including local anesthetic actions. However, the limitation of higher doses would be adverse effects. Thus, local administration of high doses of amitriptyline (1000 nmol) into the hindpaw produces paw edema (Oatway et al., 2003), and even higher doses (2000–16,000 nmol) produce neurotoxicity (Estebe and Myers, 2004). The development of novel application paradigms will require appropriate attention to the issue of tissue toxicity.

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