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Caffeine blockade of the thermal antihyperalgesic effect of acute amitriptyline in a rat model of neuropathic pain

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Abstract

In the present study, we sought to determine whether administration of caffeine, a non-selective adenosine receptor antagonist, would affect the thermal antihyperalgesic efficacy of acute amitriptyline in a rat model of neuropathic pain. Rats were rendered neuropathic by unilateral tight ligation of the fifth and sixth lumbar spinal nerves, and tested for thermal hyperalgesia using a focused beam of light. *Systemic* administration of caffeine (1.5–7.5 mg/kg), at the same time as amitriptyline, blocked the thermal antihyperalgesic effect of 10 mg/kg amitriptyline. The greatest degree of block exerted by caffeine was observed with 3.75 mg/kg (100% block), a dose that had no observable intrinsic effect. *Spinal* administration of amitriptyline (60 μg) exhibited a mild antihyperalgesic effect that was unaffected by pretreatment with intrathecal caffeine (100 μg). *Peripheral* administration of amitriptyline into the neuropathic paw (under brief anesthesia) produced an antihyperalgesic effect at both 30 and 100 nmol, with a greater effect being observed at 100 nmol. Coadministration of caffeine (1500 nmol) partially antagonized the effects of both doses of amitriptyline. The results of this study suggest that the thermal antihyperalgesic effect of acute amitriptyline in this model may involve enhancement of an endogenous adenosine tone. This involvement is important in light of the widespread consumption of caffeine, which may potentially act to reduce the benefits of amitriptyline in the treatment of neuropathic pain.

Keywords: Amitriptyline; Caffeine; Thermal hyperalgesia; Neuropathic pain

1. Introduction

The pharmacotherapeutic treatment of neuropathic pain is constantly evolving in conjunction with an increased understanding of some of the underlying pathophysiological mechanisms causing neuropathic pain symptoms. While multiple therapeutic strategies are utilized in the treatment of neuropathic pain (reviewed in MacFarlane et al., 1997), front-line therapy still commonly involves use of antidepressants such as amitriptyline (McQuay et al., 1996). Amitriptyline inhibits the reuptake of noradrenaline and serotonin, acts as an antagonist at NMDA, α-adrenoceptor, muscarinic, histamine and substance P receptors, binds to opioid receptors, and has quinidine-like properties (reviewed in Eschalier et al., 1994, 1999). The pain alleviating properties of tricyclic antidepressants may therefore result from a number of these actions.

Other studies have suggested that amitriptyline may also interact with endogenous adenosine systems by inhibiting cellular reuptake (Phillis and Wu, 1982; Phillis, 1984). This possibility is intriguing since adenosine has been shown to have antinociceptive properties in animal studies of acute nociception, inflammation and neuropathic pain (reviewed in Sawynok, 1998). In humans, adenosine has been shown to be efficacious in the treatment of neuropathic pain after intravenous infusions (Belfrage et al., 1995; Sollevi et al., 1995). Furthermore, a recent clinical study has demonstrated that intrathecal adenosine prevents the development of allodynia in an experimental model of sensitization (Rane et al., 1998). It has also been reported that endogenous levels of adenosine are reduced in the plasma and cerebral spinal fluid of patients with neuropathic pain (Guieu et al., 1996). Such observations suggest that there may be a beneficial endogenous tone of adenosine that is diminished as part of the pathophysiology of neuropathic pain.

The multiple pharmacological actions of amitriptyline make it important to consider potential interactions with other agents that may influence its efficacy. Of particular

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interest in this study is the potential interaction with an endogenous adenosine mechanism. Caffeine is understood to exert its pharmacological effects through antagonism of adenosine receptors at low doses, but exhibits undesirable effects at higher doses through inhibition of phosphodiesterase enzymes (Fredholm, 1995). Caffeine, which is one of the most widely consumed drugs on a global scale (Gilbert, 1984), is a non-selective adenosine receptor antagonist being equally effective at both adenosine A_1 and A_2 receptors (Fredholm, 1995). Consumption of caffeine from various sources may therefore affect drugs acting either directly or indirectly on endogenous adenosine systems.

In the present study, we sought to determined whether an adenosine-related mechanism contributes to the thermal antihyperalgesic effect of acute amitriptyline in a neuropathic pain model. Previously, we demonstrated that amitriptyline was effective in reducing nerve injury-induced thermal hyperalgesia but not mechanical allodynia following spinal nerve injury, and that the extent of the amitriptyline effect was route-dependent (Esser and Sawynok, 1999). We have also shown that the local peripheral administration of amitriptyline produces antinociception in the rat formalin test, and that this effect is reduced by caffeine coadministration (Sawynok et al., 1999). We therefore examined the effect of caffeine on the actions of amitriptyline following multiple routes of administration in a rat-nerve-injury model. These results have been partially presented in abstract form (Esser and Sawynok, 1998).

2. Methods and materials

2.1. Animals

Experiments were conducted on male Sprague–Dawley rats (100–120 g) from Charles River, Quebec, Canada. Protocols were approved by the Dalhousie University Committee on Laboratory Animals. Animals were housed in pairs, maintained on a 12/12 h light/dark cycle at 22 ± 1°C and given ad libitum access to food and water.

2.2. Surgery

All surgery was performed using aseptic technique and with adherence to the guidelines of the International Association for the Study of Pain on animal experimentation in pain research (Zimmermann, 1983).

2.2.1. Spinal nerve ligation

Animals were rendered neuropathic through tight ligation of the fifth and sixth lumbar spinal nerves (L5 and L6), in accordance with the technique of Kim and Chung (1992). Animals were initially anaesthetized with halothane via an induction box and subsequently maintained at the appropriate anesthetic plane using 1.5–2.0% halothane. Rats were surgically clipped, administered Ringers lactate solution (5 ml; s.c.), atropine (0.6 ml/kg; i.m.), penicillin (Penlong; 1.0 ml/kg; s.c.) and topical eye ointment (Lacri-Lube; Allergen). The surgical area was aseptically scrubbed with alcohol and iodine, and a 3-cm midline dorsal incision made using the ischium as the midline. Using blunt dissection and partial removal of the articular facet and the L4 transverse process, the L5 and L6 spinal nerves were exposed. The nerves were then tightly ligated with sterilized 6-0 silk. After ensuring hemostasis, the wound was closed in layers through subcutaneous and cutaneous suturing (3.0 Novophil). Animals were then placed in a heated area for surgical recovery and monitoring.

2.2.2. Intrathecal cannulation

For animals used to study the effects of spinal administration of amitriptyline, intrathecal cannulas were implanted 7 days following nerve injury in accordance with the technique previously reported in Sawynok and Reid (1990). Under halothane anesthesia, the atlanto-occipital membrane was exposed by dermal incision and spreading of the nuchal muscles. The membrane was then cut, and a 7.5 cm length of saline filled PE-10 tubing was gently introduced into the subarachnoid space. The cannula was then held in place by a knot tied loosely to the nuchal muscles. The rostral end of the cannula was externalized through a small incision in the skin overlying the skull and capped with a stainless steel wire plug. Any animals showing signs of paralysis or motor impairment as a result of cannulation were excluded from the study.

2.3. Behavioral testing

All behavioral tests were conducted between at 0800 and 1400 h daily, and occurred at 7, 12, and 17 days following nerve injury. An exception to this was the cannulated animals, which were tested at 7 and 12 days after cannulation (14 and 19 days after nerve injury, respectively). Following a recovery period of 7 days, animals were moved from the vivarium, weighed, and acclimatized to the testing room for 40 min. After this initial period, the animals were placed in the testing apparatus for 30–40 min or until exploratory behavior ceased.

To test for thermal hyperalgesia, a paw thermal stimulator (UARDG, Dept. of Anesthesiology) was used to direct a focused beam of light at the paw, as described initially by Hargreaves et al. (1988). Rats were placed in pairs in a plexiglass box on top of the temperature maintained glass surface (30 ± 0.1°C) of the stimulator. After the initial acclimatization period, rats were tested for baseline withdrawal latencies (seconds) of both paws once every 20 min until three consistent baselines were achieved. The animals were then returned to their cages for 30–40 min prior to drug administration and given ad libitum access to food and water. Following drug administration, the animals

were returned to the testing boxes and tested for the time period as defined by the observation protocol.

2.4. Drug treatment

All drug treatments were blinded. Amitriptyline, caffeine (Sigma, St. Louis, MO) or saline was administered systemically by intraperitoneal (i.p.) injection, spinally through chronically implanted intrathecal cannulas (i.t.), or peripherally (subcutaneous; s.c.) in the dorsal aspect of the paw. Drugs were injected in a volume of 5 ml/kg for systemic injections, and a total volume of 20 μl (10 μl drug + 10 μl saline flush) for intrathecal injections. For local injections, the animals were briefly anaesthetized with halothane, and the solution administered s.c. to the dorsal aspect of the ipsilateral or contralateral paw in a volume of 50 μl. Caffeine was co-administered with amitriptyline in the systemic and local paradigms, but was given 10 min prior to amitriptyline in the spinal paradigm.

2.5. Data analysis

For each of the experiments, raw data for response thresholds of both paws in each animal were recorded and entered into a spreadsheet (Microsoft Excel 5.0). The data was then normalized for each animal as the Maximum Possible Effect (MPE) in terms of the change in the response threshold of the ipsilateral (neuropathic) paw

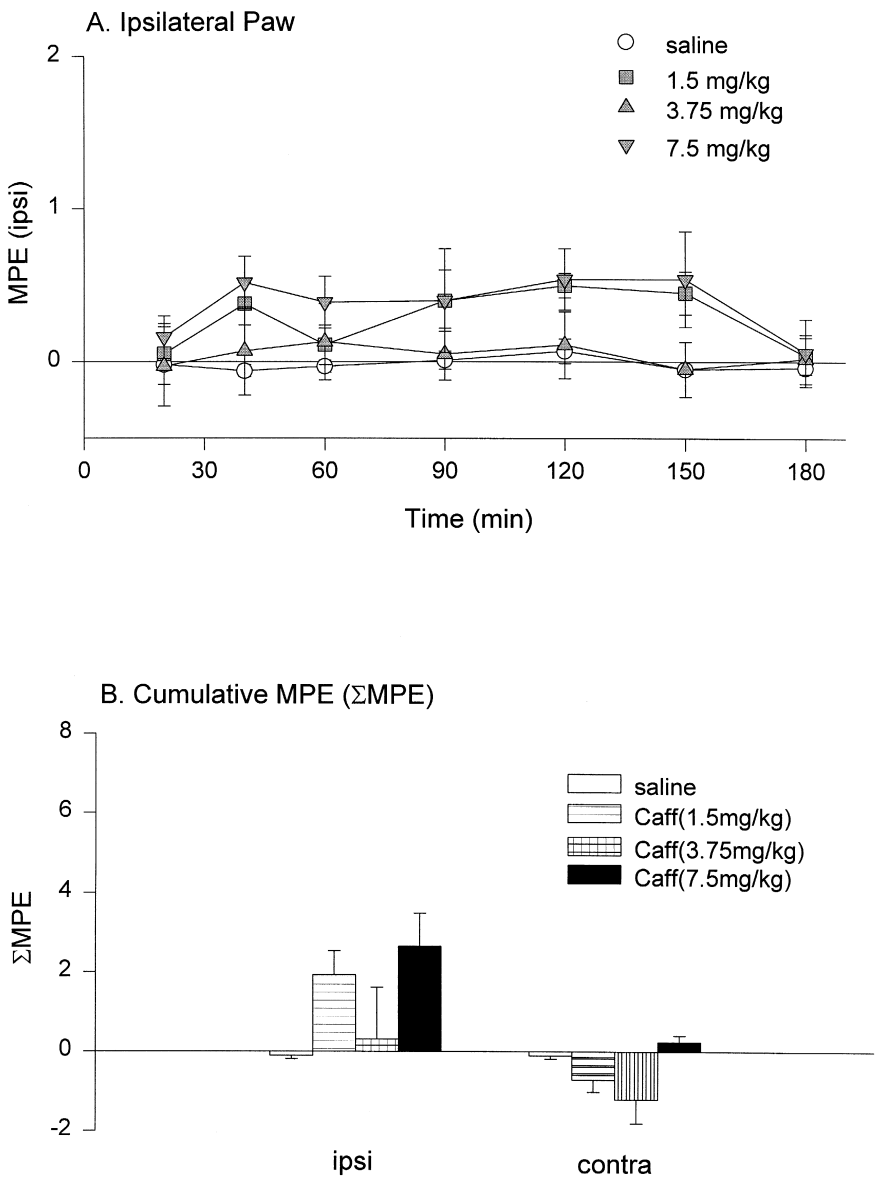

Fig. 1. Time course of the systemic effect of various doses of caffeine (Caff) on thermal hyperalgesia in the ipsilateral paw following nerve injury (A). The figure also depicts the cumulative MPE (ΣMPE) over the time course for both the ipsilateral (ipsi) and contralateral (contra) paws (B). Values depict group means ($n = 6–9$ per group) ± S.E.M. of the maximum possible effect.

[MPE (ipsi)] or in terms of the effect on the response threshold of the contralateral paw [MPE (cntra)]. These values were calculated as follows: MPE (ipsi) = (PDR − IBR)/(CBR − IBR), where PDR is the *p*ost *d*rug *r*esponse of the ipsilateral paw, IBR is the *i*psilateral paw *b*aseline *r*esponse, and CBR is the *c*ontralateral paw *b*aseline *r*esponse. Similarly, the MPE (cntra) = (PDR − CBR)/(cutoff − CBR), where cutoff was 20 s, and PDR is the post drug withdrawal threshold of the contralateral paw. Accordingly, the individual values reported or depicted are the MPE ± S.E.M., where a value of 1 represents a complete reversal of the nerve injury induced thermal hyperalgesia of the ipsilateral paw. The time course of the drug effect is also depicted as the cumulative MPE (∑MPE) for each treatment and route of administration. This value was calculated as the sum of the individual MPE values at each time point over the full time course, and was determined in an effort to obtain an indication of the overall effect of the treatment. Both individual time point and cumulative MPE data was statistically analyzed using an analysis of variance (ANOVA) followed by an all pairwise multiple comparison (Dunnet's method). In all cases a minimum of $P < 0.05$ was considered significant.

3. Results

We have previously demonstrated that acute amitriptyline is effective in alleviating thermal hyperalgesia when administered systemically (10 mg/kg), spinally (60 μg) and locally (100 nmol) (Esser and Sawynok, 1999). We therefore used the same doses of amitriptyline for analysis of potential caffeine interactions at each of these sites.

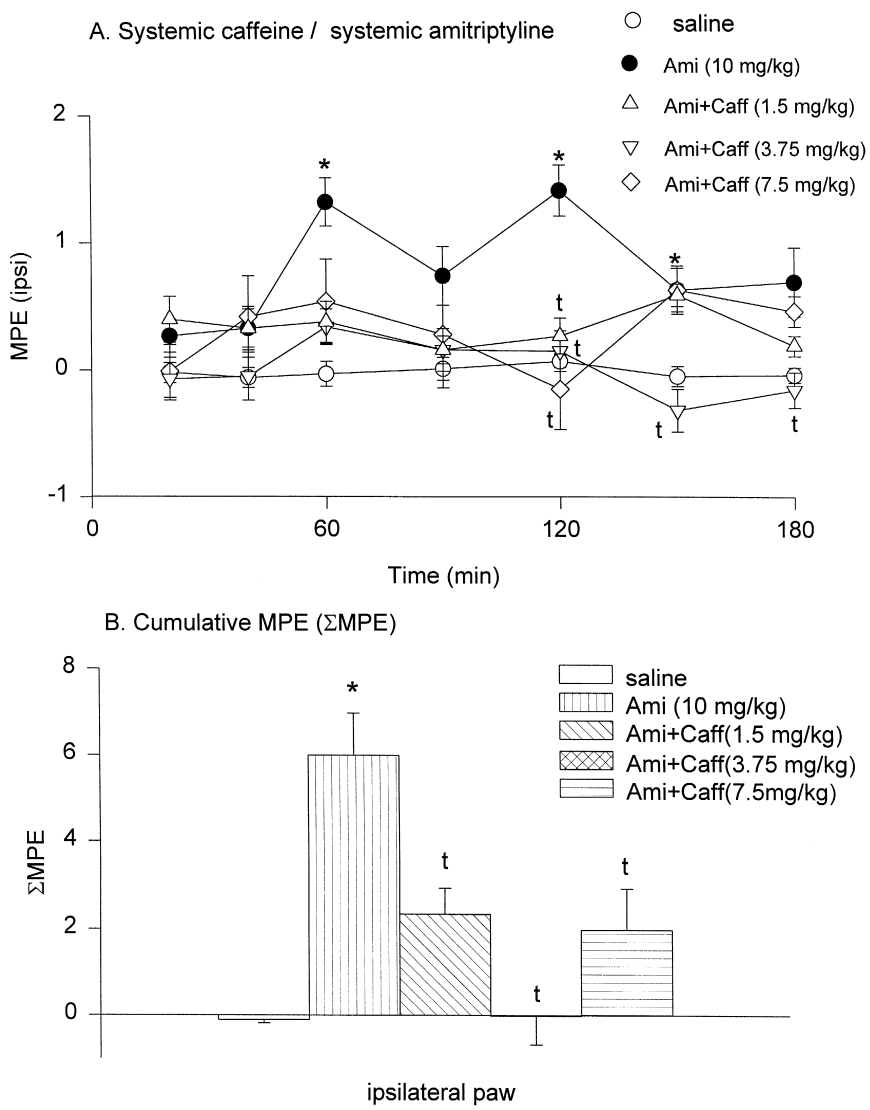

Fig. 2. Time course of the effect of systemic co-administration of increasing doses of caffeine (white symbols) on the systemic thermal antihyperalgesic effect of 10 mg/kg amitriptyline in the ipsilateral paw (Ami)(black symbols). The overall effect is shown as the cumulative MPE (∑MPE) (B). Values depict the mean ($n = 9$ per group) ± S.E.M. $^{*}P < 0.05$ compared to saline. $^{t}P < 0.05$ compared to Ami (10 mg/kg).

3.1. Intrinsic effect of systemic caffeine on thermal hyperalgesia following nerve injury

No statistically significant difference was observed at any of the individual time points or in the cumulative scores for 1.5, 3.75 or 7.5 mg/kg of caffeine, as compared to saline (Fig. 1A and B). A further increase in the dose of caffeine to 15 mg/kg produced an increase in the activity level of the rat, an effect that was not observed for the 1.5, 3.75, or 7.5 mg/kg doses (data not shown). This behavioral response had an effect on the withdrawal threshold of the ipsilateral paw, as accurate determination of the threshold requires the rat to remain still during presentation of the stimulus. We did not observe any significant effect on the thermal withdrawal threshold of the contralateral paw at any dose (Fig. 1B).

3.2. Effect of systemic caffeine on the thermal antihyperalgesic effect of systemically administered amitriptyline

Systemic amitriptyline (10 mg/kg) produced a significant reversal of nerve injury-induced thermal hyperalgesia as evidenced by the statistically significant difference in the MPE values for amitriptyline as compared to saline at both individual time points (Fig. 2A) and with respect to the overall cumulative effect (ΣMPE) (Fig. 2B). Coadministration of 1.5 mg/kg of caffeine with amitriptyline had a statistically significant effect on the thermal antihyperalgesic action of amitriptyline both at individual time points (Fig. 2A) and in the cumulative effect (Fig. 2B), producing a 60% block of the thermal antihyperalgesic effect of amitriptyline. Caffeine at 3.75 mg/kg completely blocked the thermal antihyperalgesic effect of amitriptyline (Fig.

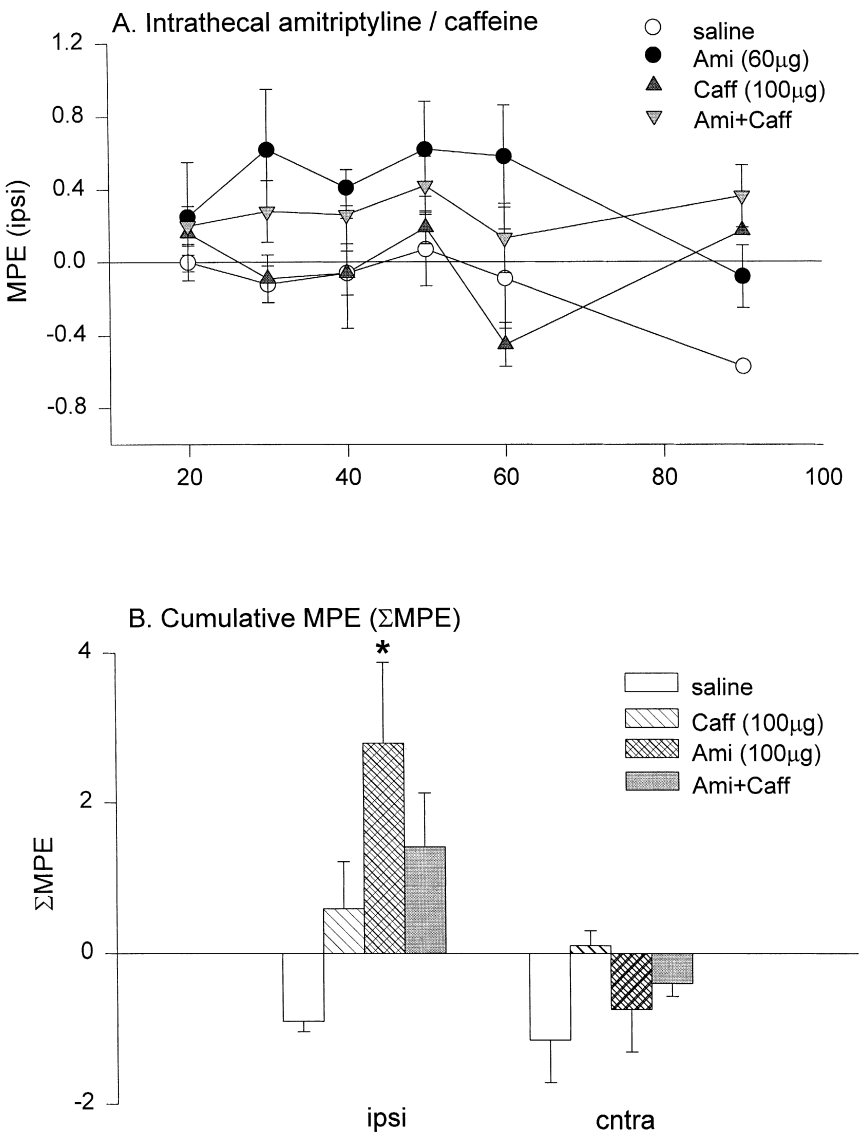

Fig. 3. Time course of the effect of spinal amitriptyline (Ami) and caffeine (Caff) alone, and in combination, on nerve-injury induced thermal hyperalgesia of the ipsilateral paw (A) for individual time points. The data is also depicted in terms of the maximum possible effect (ΣMPE) (B). Values depict the mean ($n = 6$ per group) ± S.E.M. $^{*}P < 0.05$ compared to saline.

2A and B) without exerting any observable psychomotor stimulatory effect, as mentioned above (Section 3.1). Similarly, while 7.5 mg/kg did not have any significant motor effect, it did partially block the thermal antihyperalgesic effect of amitriptyline at one individual time point (Fig. 2A) and in the cumulative effect (67% block) (Fig. 2B).

3.3. Effect of spinal caffeine on the thermal antihyperalgesic effect of spinally administered amitriptyline

The spinal administration of amitriptyline (60 μg) only produced a partial effect on nerve injury induced thermal hyperalgesia, and this effect was only significantly different from saline in the cumulative score (Fig. 3). Spinal pretreatment (10 min) with caffeine (100 μg) slightly reduced the cumulative thermal antihyperalgesic action of spinal amitriptyline (Fig. 3B), but this effect was not statistically significant. This dose of intrathecal caffeine was without intrinsic motor or antihyperalgesic activity (Fig. 3A). Higher spinal doses of amitriptyline were not investigated, as they produced pronounced sedative and/or motor effects severely compromising our ability to determine the withdrawal thresholds (Esser and Sawynok, 1999).

3.4. Effect of peripheral caffeine administration on the thermal antihyperalgesic effect of peripherally administered amitriptyline

The peripheral administration of amitriptyline (30 and 100 nmol) produced a significant thermal antihyperalgesic effect at individual time points (Fig. 4B and C) and in the cumulative data (Fig. 4D). This effect was previously determined to be due to a local effect since it was not observed following administration of amitriptyline in the contralateral paw (Esser and Sawynok, 1999). When coadministered peripherally with amitriptyline, caffeine (1500 nmol) significantly reduced the local antihyperalgesic effect of 30 nmol (66% block) (Fig. 4B and D) and 100 nmol (60% block) amitriptyline (Fig. 4C and D). Peripheral administration of caffeine alone however, failed to

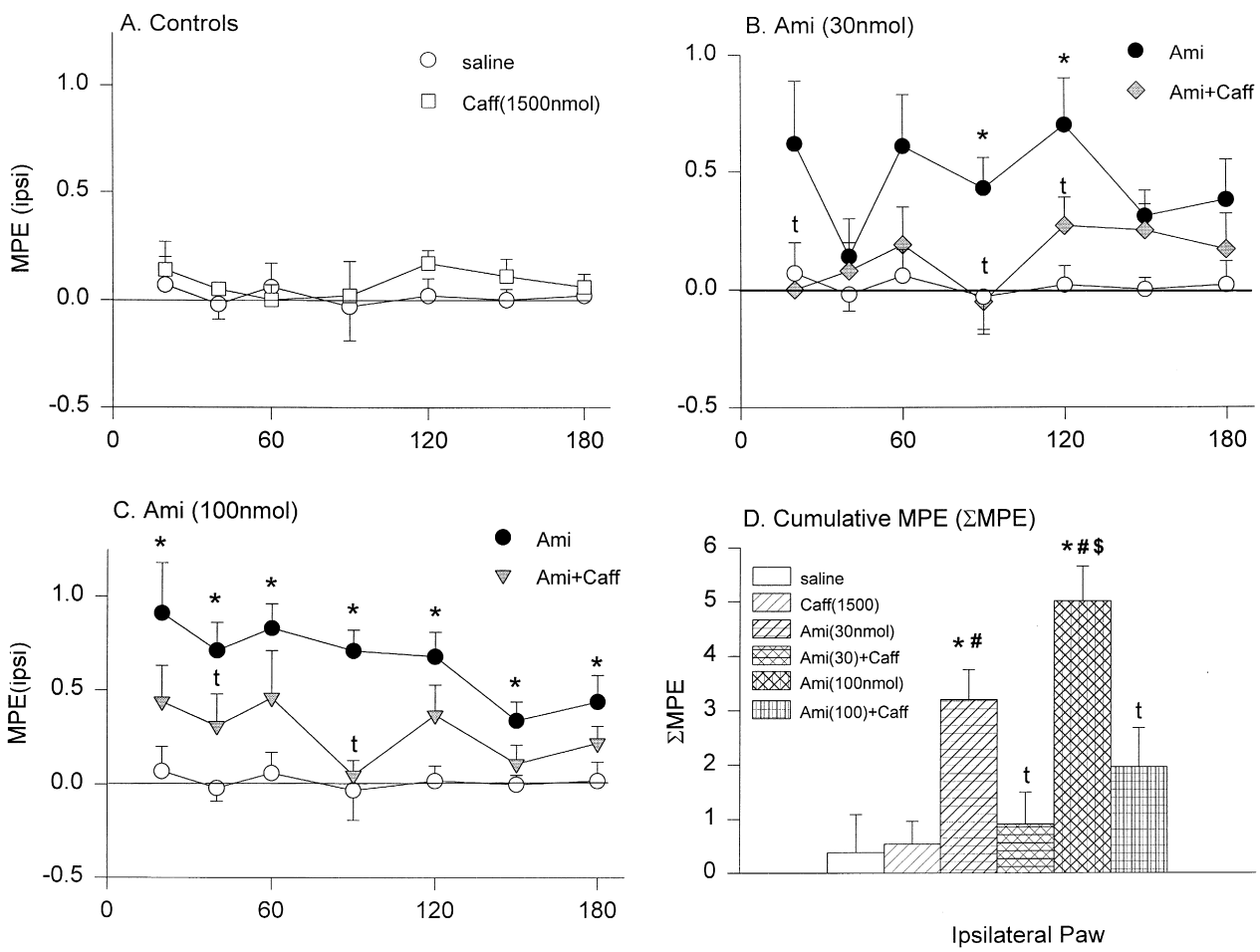

Fig. 4. Time course of the effect of locally administered caffeine (Caff; 1500 nmol), alone (A) and on the thermal antihyperalgesic effect of 30 nmol (B) and 100 nmol (C) amitriptyline (Ami). Amitriptyline alone is depicted by black symbols while combination of the respective doses with caffeine is depicted by shaded symbols. The cumulative effect of the drugs alone, and in combination, on the maximum possible effect (MPE) on the ipsilateral paw, is shown in (D). Values depict the mean ($n = 9$ per group) ± S.E.M. $^{*}P < 0.05$ compared to saline, $^{t}P < 0.05$, compared to the respective dose of amitriptyline, $^{\#}P < 0.05$ compared to caffeine, and $^{\$}P < 0.05$ for Ami (100 nmol) compared to Ami (30 nmol).

produce any significant effect (Fig. 4A). While there still remained a slight thermal antihyperalgesic effect following administration of the amitriptyline–caffeine combination, this effect was not significantly different from that of saline alone (Fig. 4B–D).

4. Discussion

The present study demonstrates a caffeine block of the thermal antihyperalgesic effect of amitriptyline in a rat model of neuropathic pain following both systemic and peripheral administration. As blockade of adenosine receptors is thought to underlie many of the pharmacological effects of caffeine, especially at moderate doses (reviewed in Fredholm, 1995), these observations suggest involvement of endogenous adenosine in the thermal antihyperalgesic effect of amitriptyline. Other studies have previously reported involvement of adenosine receptors in the antinociceptive effect of systemically administered antidepressants in the tail flick (Pareek et al., 1994) and writhing tests (Sierralta et al., 1995), and of peripheral amitriptyline in the formalin test (Sawynok et al., 1999). Collectively, these reports suggest that adenosine is an important endogenous mediator of the analgesic properties of tricyclic antidepressants in general, and of amitriptyline in particular.

The mechanism by which amitriptyline interacts with endogenous adenosine systems is likely through inhibition of adenosine uptake, as nortriptyline (the active metabolite of amitriptyline) was shown to be the most potent of a number of tricyclic antidepressants in inhibiting the neuronal uptake of adenosine (Phillis and Wu, 1982). Consistent with this interpretation is the observation that antidepressants enhance the inhibitory effects of adenosine on neuronal firing, but do not affect the actions of an adenosine analog which is not subject to uptake (Phillis, 1984). While neither an amitriptyline-facilitated release of adenosine nor a direct binding of amitriptyline to adenosine receptors have been reported, these actions remain mechanistic possibilities for an adenosine-linked action of amitriptyline. In this manner, amitriptyline is well known for its ability to bind to a diverse number of different receptor sites (reviewed in Eschalier et al., 1994, 1999). However, with the exception of opioid receptors, these actions are primarily inhibitory, which would tend to argue against a direct adenosine receptor-mediated action of amitriptyline.

Adenosine analogs have been shown to produce pain relieving effects in a variety of neuropathic pain models following both spinal (Sosnowski and Yaksh, 1989; Sjölund et al., 1996; Lee and Yaksh, 1996; Cui et al., 1997; Khandwala et al., 1998) and peripheral (Liu and Sawynok, 1998) administration. Considering the proposed adenosine-linked action of amitriptyline and the prominent efficacy of spinally administered adenosine analogs in neuropathic pain models, it is not clear why we only observed a modest thermal antihyperalgesic effect of spinally administered amitriptyline in this and a previous study (Esser and Sawynok, 1999). The spinal dose of amitriptyline used in the present study (60 μg) has been reported to produce complete reversal of thermal hyperalgesia following intraplantar carrageenan (Eisenach and Gebhart, 1995a), whilst having no affect on the response to radiant heat in a non-inflamed situation (Eisenach and Gebhart, 1995b). The apparent lack of block of the spinal action of amitriptyline by caffeine may reflect a low level of intrinsic spinal adenosine activity in these particular conditions. Interestingly, the greatest effect of spinal amitriptyline (Eisenach and Gebhart, 1995b) and other tricyclic antidepressants (Hwang and Wilcox, 1987) occurs in combination with other agents (e.g. neostigmine and morphine). It is therefore possible that a more robust effect of spinal amitriptyline in neuropathic pain would result from combination with other agents.

The manipulation of endogenous adenosine by amitriptyline, while important, is unlikely to be the sole mechanism underlying the antihyperalgesic effect since amitriptyline is known to produce a diverse number of biological actions. These include inhibition of monoamine reuptake, blockade of muscarinic, histamine, α-adrenoceptor, NMDA and substance P receptors, as well as blocking various ion channels (reviewed in Eschalier et al., 1994, 1999). Indeed, a number of these actions may contribute to the antinociceptive efficacy of amitriptyline as considered elsewhere (Esser and Sawynok, 1999). Additional lines of reasoning also support the premise that the pharmacology of amitriptyline does not solely result from an interaction with endogenous adenosine systems, or any other single mechanism. Thus, while spinal administration of adenosine analogs exert a prominent antiallodynic effect in the spinal nerve ligation model (Lee and Yaksh, 1996), we found amitriptyline to be without any antiallodynic effect in the same model (Esser and Sawynok, 1999). A similar argument can also be made with respect to potential NMDA receptor antagonism by amitriptyline. High potency NMDA receptor antagonists have been shown to produce both antiallodynic (Chaplan et al., 1997) and antihyperalgesic (Mao et al., 1992a,b, 1993; Yamamoto and Yaksh, 1992; Mao et al., 1993) effects following nerve injury, yet in a previous study we did not observe an antiallodynic effect of amitriptyline (Esser and Sawynok, 1999). It therefore appears that blockade of NMDA receptors can, at best, only partially account for the actions of amitriptyline. While such a mechanism for spinal amitriptyline was proposed recently in an inflammatory pain model (Eisenach and Gebhart, 1995a), the pharmacological profile of NMDA receptor antagonists at blocking inflammatory versus neuropathic pain can differ (Chaplan et al., 1997).

An important aspect of the present study is the modest doses of caffeine (1.5–7.5 mg/kg) that block the systemic antihyperalgesic effect of amitriptyline. These doses did

not produce overt behavioral stimulation, and are at the low end of the dose range for producing motor stimulant effects in rodents (Nehlig et al., 1992). Acute ingestion of two cups of strong coffee (2×100 mg; Barone and Roberts, 1996) would generate a human dose of approximately 2.8–3.6 mg/kg (70 kg male or 55 kg female). While direct extrapolation between rats and humans may not be entirely appropriate due to pharmacokinetic and other variables, the doses of caffeine which block the action of amitriptyline are clearly low enough to be relevant to human dietary intake levels. Therefore, the issue of whether caffeine consumption, in a dietary context, might interfere with the efficacy of amitriptyline in neuropathic pain needs to be addressed directly in human studies. This potential interaction could perhaps contribute to the limited efficacy of amitriptyline observed in a recent meta-analysis of antidepressants used for the treatment of clinical neuropathic pain (McQuay et al., 1996). The contribution of this mechanism to the efficacy of other antidepressants used clinically to treat neuropathic pain remains to be determined.

In summary, the results of this study suggest that the thermal antihyperalgesic effect of acute amitriptyline is mediated in part through manipulation of endogenous adenosine. This interaction occurs following both systemic and peripheral administration of amitriptyline, but is less apparent following spinal administration. This study raises the possibility that caffeine consumption in a dietary context might influence the efficacy of amitriptyline in alleviating neuropathic pain in humans.

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