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Caffeine antinociception: role of formalin concentration and adenosine A_1 and A_2 receptors

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Abstract

Caffeine produces antinociception in the formalin test at lower doses than in thermal threshold tests. In this study, we examined (a) the effect of formalin concentration on the action of caffeine, and (b) the relative involvement of adenosine A_1 and A_2 receptors in the action of caffeine. Formalin 1%, 2% and 5% produces a concentration-dependent increase in flinching behaviour in rats. At 5% formalin, the EC_{50} for caffeine in reducing phase 2 responses (16–60 min following formalin injection) is 40 mg/kg, but at 2%, this is reduced to 5 mg/kg. There is no further shift using 1% formalin. Caffeine has a more pronounced effect on phase 2A (16–36 min) than on phase 2, with significant effects being observed at lower doses. Both 8-cyclopentyltheophylline (A_1 selective) and 3,7-dimethyl-1-propargylxanthine (A_2 selective) produce a dose-related inhibition of 1.5% formalin flinching behaviours between 1–10 mg/kg. Both agents also produce locomotor stimulation over this dose range, but significant effects occur only at 10 mg/kg. These results indicate that antinociception by caffeine shows a high dependence on stimulus intensity. At 1–2% formalin, doses of caffeine that produce antinociception are now in the range that corresponds to normal human dietary and therapeutic intake levels. Both antinociception and locomotor stimulation appear to be dependent on activation of adenosine A_1 receptors, as effects of 3,7-dimethyl-1-propargylxanthine are observed only at a dose which blocks adenosine A_1 receptors in addition to A_2 receptors.

Keywords: Formalin test; Caffeine; Stimulus intensity; 8-Cyclopentyltheophylline; 3,7-Dimethyl-1-propargylxanthine

1. Introduction

Caffeine is widely used as an adjuvant analgesic in combination with non-steroidal anti-inflammatory drugs and/or acetaminophen (Laska et al., 1984; Forbes et al., 1991; Schachtel et al., 1991; Ward et al., 1991; reviewed: Sawynok and Yaksh, 1993). In animal studies, caffeine has intrinsic antinociceptive properties in threshold tests (Person et al., 1985; Malec and Michalska, 1988) and in inflammatory tests (Siegers, 1973; Seegers et al., 1981; Sawynok et al., 1995). Activity is generally seen at lower doses in the latter tests. In the formalin test, caffeine exhibits an even greater sensitivity in the early part of the second phase (Sawynok et al., 1995). This could be due to (a) a preferential role of adenosine (caffeine is understood to act as an adenosine receptor antagonist) during the initial phase of the inflammatory component of the formalin response, or (b) an effect of adenosine only at certain intensities of stimulation whereby the early rising phase can be considered a milder stimulus. In support of (a), endogenous amines, kinins and prostaglandins have been proposed to play a role only at certain stages of the inflammatory process (DiRosa et al., 1971; Neil et al., 1987); adenosine has recently been proposed to be a significant anti-inflammatory autocoid (Cronstein, 1994). In support of (b), the intrathecal administration of methylxanthines produces hyperalgesia only under low intensities of stimulation in the tail flick test (Jurna, 1984; Sawynok et al., 1986), while an adenosine kinase inhibitor produces antinociception only at a lesser intensity of stimulation in the formalin test (Poon and Sawynok, 1995). In the present study, we have evaluated the actions of caffeine at different formalin concentrations to determine the role of stimulus intensity in allowing for the expression of antinociceptive properties of this agent. The use of a lower formalin concentration has been considered previously in the context of ethical considerations (Tjølsen et al., 1992), and it has been argued that the milder stimulus intensity is useful

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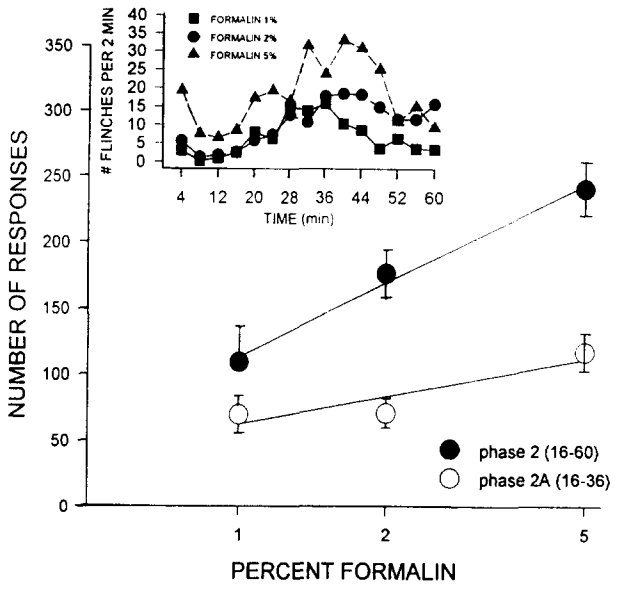

Fig. 1. Dose-related increases in flinching responses produced by increasing concentrations of formalin following s.c. injection into the dorsal hindpaw of the rat. Values are mean for $n = 6$ per group, with bars indicting S.E.M. Cumulative responses presented in the body of the figure, with the time course in the inset.

for detecting the activity of mild analgesics (Hunskaar et al., 1985; Shibata et al., 1989; Rosland et al., 1990).

Caffeine is a non-selective adenosine A_1 and A_2 receptor antagonist with comparable affinity at both receptors, but lacks activity at the recently described adenosine A_3 receptor (Daly, 1993; Fredholm et al., 1994). While adenosine can have complex effects on pain transmission, facilitating it at peripheral nerve endings and inhibiting it within the spinal cord and at supraspinal sites (reviewed: Sawynok, 1991), the analgesic properties of caffeine have been attributed to supraspinally mediated actions. This inference is based on the observations that neither the peripheral nor spinal administration of caffeine produce antinociception in the formalin test (Malmberg and Yaksh, 1993; Sawynok et al., 1995; Doak and Sawynok, 1995; Poon and Sawynok, 1995), while manipulation of central monoamine systems can alter the antinociceptive action of caffeine (Sawynok and Reid, 1995). The relative involvement of adenosine A_1 and A_2 receptor blockade in the antinociceptive action of caffeine is unknown. In this study, we have administered the selective adenosine A_1 receptor antagonist 8-cyclopentyltheophylline (CPT) (130-fold selectivity, Bruns et al., 1986) and the somewhat selective adenosine A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) (4- to 20-fold selectivity, Ukena et al., 1986) and determined their effects in the formalin test. Although more selective adenosine A_2 antagonists are available, their use in behavioural paradigms has been hampered by their limited solubility. Locomotor activity for CPT and DMPX was also monitored in an attempt to determine if changes in formalin behaviours might be contingent on changes in locomotor behaviour (Tjølsen et al., 1992).

2. Material and methods

2.1. Animals

Male Sprague-Dawley rats from Charles River Laboratories weighing 150–275 g were used. They were housed in groups of 2–3 in a room maintained at 21–23°C with free access to food and water on a 12/12 h light cycle. Each animal was used only once.

2.2. Formalin test

Rats were pretreated with different doses of caffeine, CPT or DMPX, and then habituated to the Plexiglas observation chamber (approximately 30 × 30 × 30 cm) for 20 min prior to the experiment. The experiment was commenced by the s.c. injection of 20 μl formalin 1%, 1.5%, 2% or 5% as indicated in individual experiments into the dorsal hindpaw of the rat. The rat was then

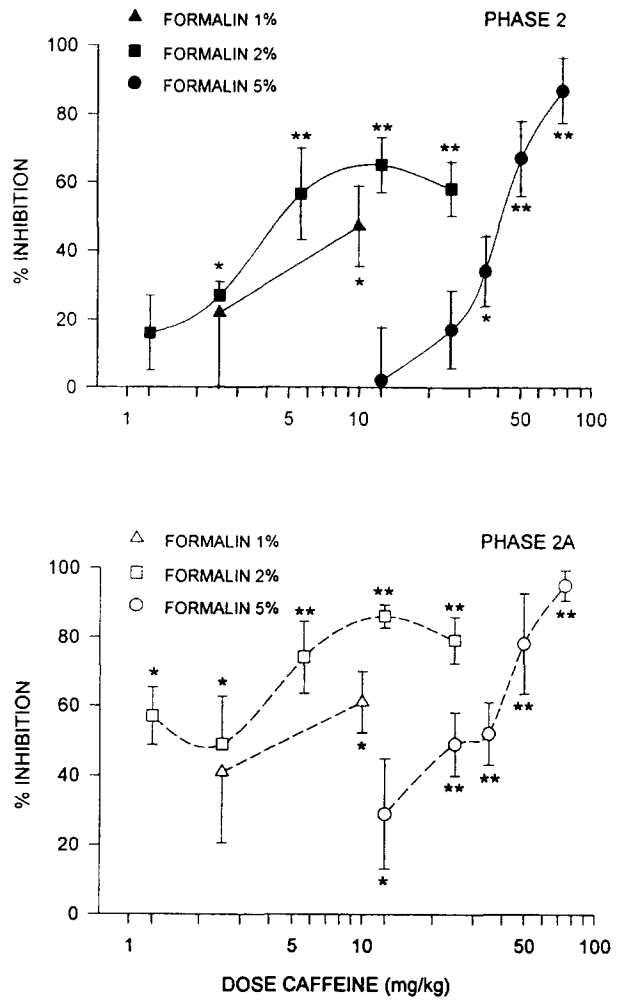

Fig. 2. Antinociception produced by caffeine in the 5%, 2% and 1% formalin test in rats. Values are mean ± S.E.M. for $n = 6$ per group. 5% data extracted from Sawynok et al. (1995). * $P < 0.05$, ** $P < 0.01$, calculated on raw data values.

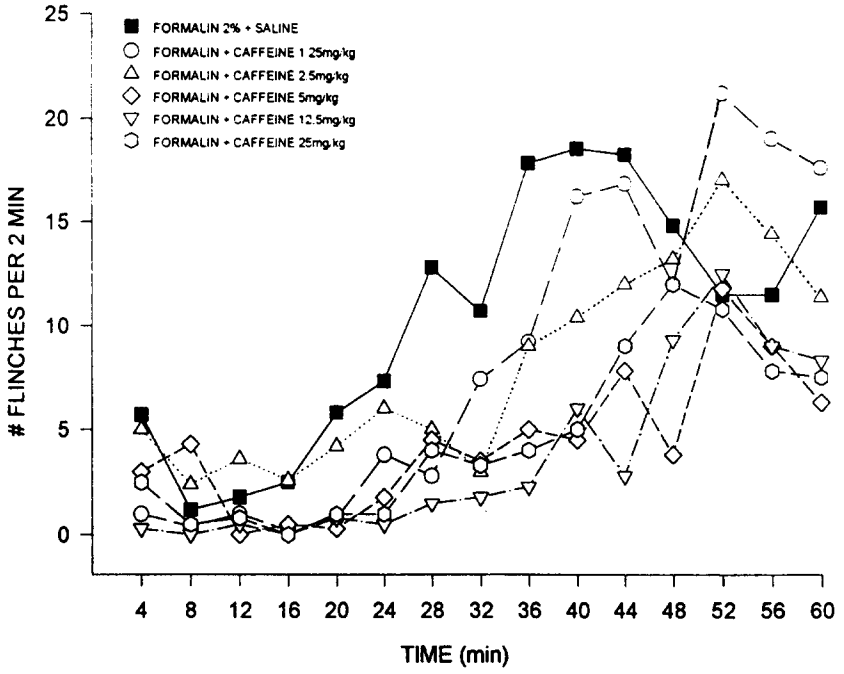

Fig. 3. Time-response curves indicating the selective phase 2A inhibition produced by caffeine in the 2% formalin test in rats. Values depict means ($n = 6$); error bars omitted in the interest of clarity.

returned to the observation chamber and the number of flinches (includes shakes, shivers, lifts) determined in 2 min bins over the 60 min observation period. Two animals were observed at a time in alternate bins. These interval values as well as a cumulative response over the 60 min interval are reported. Data for phase 2A (16–36 min following formalin injection) and phase 2 (16–60 min) are reported. While there are effects of caffeine on phase 1 flinching, these effects are not systematically dose-related (Sawynok et al., 1995; Fig. 3) and are not reported quantitatively in this study. The animals were killed by anaesthetic overdose at the end of the experiment.

2.3. Locomotor stimulation

Locomotor activity was assessed by determining the cumulative number of quadrant crossings of the square chamber during the 60 min observation period.

2.4. Plethysmometry

Paw volume was determined using a commercial plethysmometer (Ugo Basile). Paw swelling was assessed by volume displacement following immersion of the hind-paw to the junction of the hairy and non-hairy skin. Both the injected and contralateral non-injected paw volumes were determined in triplicate three times at 10 min intervals to establish a baseline, then again at 20, 40, 60, 120 and 180 min following formalin injection. These trials were conducted in a separate group of animals.

2.5. Drugs

Caffeine was dissolved in normal saline, while CPT and DMPX were dissolved in 0.02 N NaOH. Drugs were administered in a volume of 1 ml/200 g body weight. Appropriate vehicle controls were used for each treatment. Formalin was diluted in normal saline. Caffeine and formalin (37% formaldehyde) were obtained form the Sigma Chemical Co. and CPT and DMPX from Research Biochemicals.

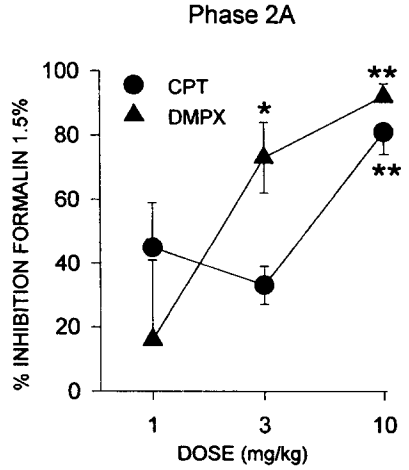

Fig. 4. Inhibition of phase 2A formalin responses in the rat by 8-cyclopentyltheophylline (CPT) and 3,7-dimethyl-1-propargylxanthine (DMPX). $n = 6$ per group. * $P < 0.05$, ** $P < 0.01$.

2.6. Statistics

Statistical significance was determined by analysis of variance followed by the Student-Newman-Keuls test. All statistical processing was performed on raw data values.

3. Results

3.1. Role of stimulus intensity in antinociception by caffeine

The s.c. injection of formalin 1%, 2% and 5% produces a dose-related increase in the number of flinches observed in phase 1 (0–12 min), phase 2A (16–36 min) and phase 2 (16–60 min) following the formalin injection (Fig. 1). Caffeine produces a dose-related inhibition of the 5% formalin response with an EC_{50} of approximately 40 mg/kg. It exhibits a greater sensitivity in phase 2A than in phase 2, producing significant effects at lower doses (Fig. 2). When 2% formalin is used, the dose-response curve is shifted considerably to the left (EC_{50} 5 mg/kg for phase 2), and the enhanced phase 2A versus phase 2 sensitivity is retained (Fig. 2). There is no further increase in sensitivity using 1% formalin (Fig. 2). Fig. 3 illustrates the time-response relationship for caffeine at 2% formalin. For subsequent experiments, a concentration of 1.5% formalin was selected.

A determination of changes in paw volume induced by 1.5% and 5% formalin by plethysmometry did not reveal any significant difference in the degree of paw swelling (increased to 115–125%, $P < 0.001$ at each post-injection interval) by the two concentrations of formalin over a 180 min observation interval ($n = 6$ per group, data not shown).

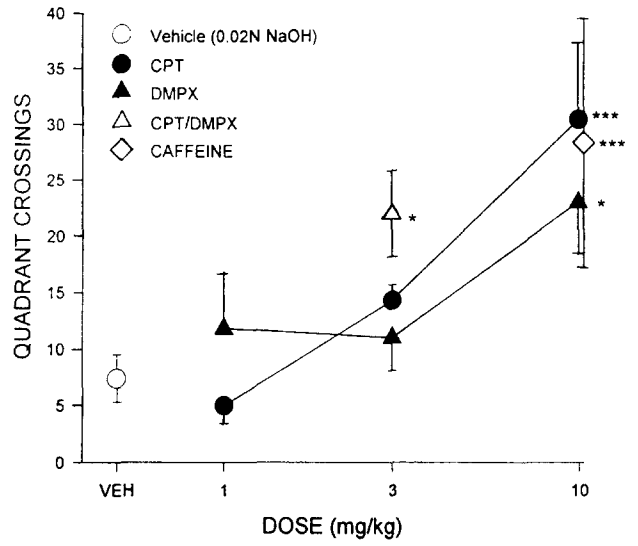

Fig. 6. Locomotor stimulation produced by CPT and DMPX. $n = 6$ per group except for the combination CPT/DMPX group where $n = 3$. * $P < 0.5$, *** $P < 0.001$ compared to vehicle value.

3.2. Role of adenosine A_1 and A_2 receptors in the antinociceptive action of caffeine

CPT produces antinociception at 10 mg/kg but not at 1–3 mg/kg (Fig. 4). In this case, phase 2A responses exhibit only a slightly greater degree of inhibition compared to phase 2 responses (cf. Fig. 5). With DMPX, antinociception is observed with 3 and 10 mg/kg, but

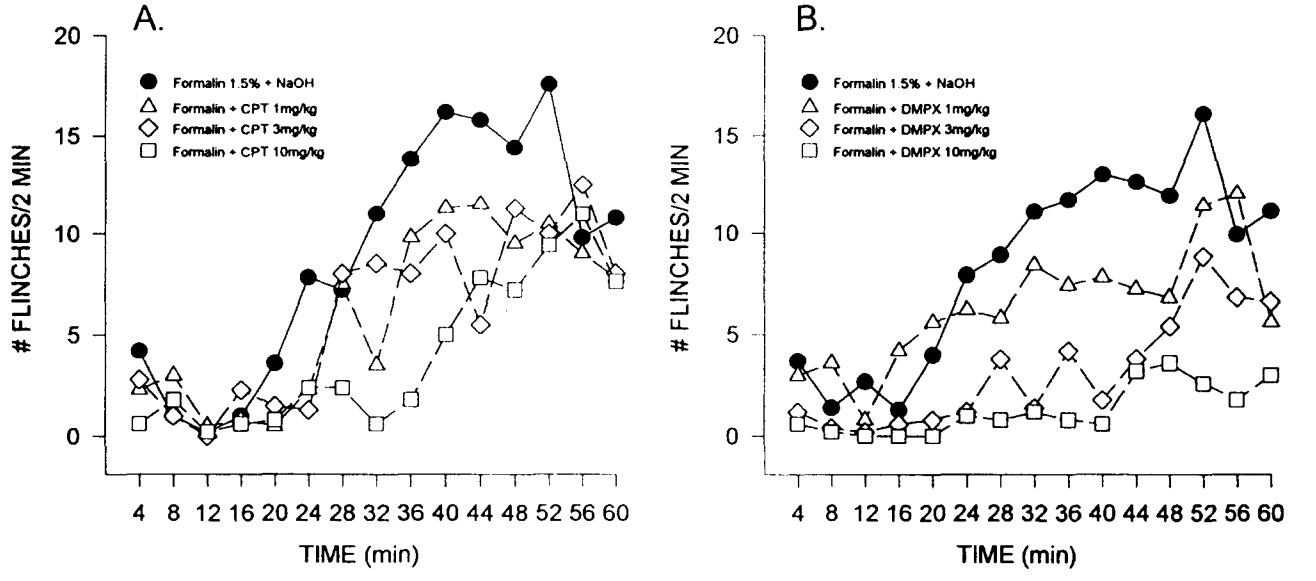

Fig. 5. Time-response curves for inhibition of the 1.5% formalin response by CPT and DMPX. Values depict means ($n = 6$); error bars omitted in the interest of clarity.

there is essentially no phase 2A versus phase 2 difference observed (Figs. 4 and 5).

3.3. Role of adenosine A_1 and A_2 receptors in locomotor stimulation

Locomotor activity was monitored simultaneously with formalin observations. Both CPT and DMPX produce dose-related increases in locomotor activity (Fig. 6). At 10 mg/kg, the degree of activation for each agent is similar to that observed with caffeine. Simultaneous administration of 3 mg/kg of each antagonist appears to produce an additive effect (Fig. 6).

4. Discussion

The use of a lower intensity of stimulation in the form of a lower concentration of formalin (2% vs. 5%) has revealed that caffeine is now effective in producing antinociception at considerably lower doses (EC_{50} 5 mg/kg compared to 40 mg/kg). Two possibilities were considered to account for the previously observed greater sensitivity of phase 2A compared to phase 2 of the formalin test (Sawynok et al., 1995): a significant role of adenosine only in the early stages of the response, or a selective effect on the rising phase at a lower intensity of stimulus. At the lower concentration of formalin (2%), a selective phase 2A effect is still observed even though the magnitude of the latter phase responses now corresponds to the 5% early phase responses. This suggests the former possibility is the more likely explanation for the effect of caffeine. Adenosine has been proposed to be an anti-inflammatory autocoid released peripherally under conditions of inflammation (Cronstein, 1994). The administration of selective adenosine A_1 and A_2 receptor antagonists can have opposing influences on the phase 2 pain signal in the formalin test (Doak and Sawynok, 1995), indicating that endogenously generated adenosine can modulate the pain signal under inflammatory conditions. There is little data on the disposition of adenosine under inflammatory conditions in central compartments. However, in chronic inflammation following administration of complete Freund's adjuvant, there is an increase in uric acid levels in the spinal cord (Weil-Fugazza et al., 1986), and this could reflect an upregulated spinal purine system under these conditions. The spinal administration of both adenosine analogs (Malmberg and Yaksh, 1993; Poon and Sawynok, 1995) and an adenosine kinase inhibitor (Poon and Sawynok, 1995) produces antinociception in the formalin test, indicating the adenosine sensitivity of this compartment in inflammation. Interestingly, the adenosine analog L-phenyl-isopropyladenosine also exhibits a greater inhibition of phase 2A activity (Malmberg and Yaksh, 1993) while the activity of the adenosine kinase inhibitor exhibits a marked dependence on stimulus intensity (Poon and Sawynok, 1995).

The dose of caffeine used in analgesic formulations in North America ranges from 16 to 250 mg (USPDI, 1993; CPS, 1994). This is similar to doses ingested following consumption of 1–2 cups of caffeinated beverages such as tea or coffee (30–85 mg/cup) (Graham, 1978; Barone and Roberts, 1984). The upper range of these doses correspond to human intake doses of up to 5 mg/kg. The observation that caffeine now produces significant antinociception at these doses may be highly relevant to normal human intake levels of caffeine, and provide a useful model for explaining just how caffeine does produce antinociception. In different dose ranges, caffeine appears to produce antinociception by different mechanisms (Sawynok et al., 1995).

The antinociceptive effect of caffeine has been ascribed to actions at supraspinal sites (see Introduction), but little is known about the details (specific sites or mechanisms) of such actions. In this study, we determined if the action of caffeine could be mimicked by a selective adenosine A_1 receptor antagonist (CPT) and a somewhat selective adenosine A_2 receptor antagonist (DMPX). CPT has 130-fold selectivity (Bruns et al., 1986) and is considered a suitable A_1-selective antagonist. Accordingly, up to 30 mg/kg, CPT selectively blocks locomotor depression produced by adenosine A_1 but not A_2 receptor agonists (Bruns et al., 1988). DMPX has 4- to 20-fold selectivity (Ukena et al., 1986), and has some utility as an A_2 adenosine receptor antagonist. Thus, DMPX blocks locomotor depression produced by a nonselective adenosine A_1 and A_2 receptor agonist (N^6-ethylcarboxamide adenosine) at doses 10 times lower that it blocks locomotor depression produced by a selective A_1 adenosine receptor agonist (cyclohexyladenosine) (Seale et al., 1988). It appears that at doses up to 1 mg/kg, this agent has A_2 adenosine receptor selectivity, but at 10 mg/kg it can block both receptors. Our results indicate that at a selective adenosine A_2 receptor blocking dose, DMPX lacks antinociceptive activity, while CPT in doses that have adenosine A_1 receptor selectivity produces antinociception. DMPX is only active at a dose that blocks adenosine A_1 receptors in addition to A_2 receptors. These results implicate adenosine A_1 receptors in the central antinociception produced by caffeine in the formalin test. Interestingly, the peripheral coadministration of CPT with formalin produces an enhancement of formalin responses (Doak and Sawynok, 1995) (probably due to blockade of a local antinociceptive action due to adenosine A_1 receptor activation; Taiwo and Levine, 1990; Karlsten et al., 1992), providing support for the notion that the effect of CPT observed following systemic administration is centrally mediated.

The conclusion that caffeine and CPT, via inhibition of adenosine A_1 receptors, produce a centrally mediated antinociception presents a paradox. Thus, both the supraspinal and spinal administration of adenosine analogs produces antinociception (Herrick-Davis et al., 1989; reviewed: Sawynok, 1991), and this has been attributed to

activation of adenosine A_1 receptors (Karlsten et al., 1991). This issue will need to be resolved by a more detailed examination of the effects of supraspinally administered adenosine agonists and antagonists into discrete brain sites.

Caffeine produces a biphasic effect of locomotor stimulation, stimulating it at doses of up to 30–40 mg/kg, but inhibiting it at higher doses (e.g. Finn and Holtzman, 1987; Seale et al., 1988; Kaplan et al., 1992). Stimulation is attributed to blockade of adenosine receptors, while inhibition results from phosphodiesterase inhibition (Snyder et al., 1981; Choi et al., 1988). There has been some debate over the relative involvement of adenosine A_1 and A_2 receptor blockade in locomotor stimulation (Bruns et al., 1988; Durcan and Morgan, 1989). Both receptor subtypes are now proposed to be involved in this effect, with a synergy expressed between the two classes of agonists (Nikodijević et al., 1991) and antagonists (Jacobson et al., 1993) in mice. In this study, we observed that both CPT and DMPX produced a significant increase in locomotor activity at 10 mg/kg in rats, as observed previously for these agents in mice (Nikodijević et al., 1990; Seale et al., 1988). However, DMPX at a dose that selectively blocks A_2 receptors (1 mg/kg) was inactive. This suggests a greater involvement of adenosine A_1 receptors in this action. In the present study with rats, the interaction between CPT and DMPX appears additive at the 3 mg/kg dose, and the effect of the non-selective dose of DMPX is no greater than that of caffeine (which is also non-selective) at doses that are maximally effective (cf. Seale et al., 1988). Of greatest importance for this study is that observations with locomotor stimulation do not suggest that inhibition of the behavioral expression of formalin responses is secondary to a non-specific motor stimulation which occludes flinching responses, as antinociception is not necessarily dependent on the copresence of a locomotor stimulation. Other studies have similarly reported that stimulants such as cocaine and amphetamine can produce antinociception in the formalin test (Lin et al., 1989; Morgan and Franklin, 1991) without implicating changes in motor activity in such actions.

Acknowledgements

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References

Barone, J.J. and H. Roberts, 1984, Human consumption of caffeine, in: Caffeine: Perspectives from Recent Research, ed. P.B. Dews (Springer Verlag, New York) p. 57.

Bruns, R.F., G.H. Lu and T.A. Pugsley, 1986, Characterization of the A_2 adenosine receptor labelled by [^{3}H]NECA in rat striatal membranes, Mol. Pharmacol. 29, 331.

Bruns, R.F., R.E. Davis, F.W. Ineteman, B.P.H. Poschel, J.N. Wiley and T.G. Heffner, 1988, Adenosine antagonists as pharmacological tools, in: Adenosine and Adenine Nucleotides, Physiology and Pharmacology, ed. D.M. Paton (Taylor and Francis, London) p. 39.

Choi, O.H., M.T. Shamim, W.L. Padgett and J.W. Daly, 1988, Caffeine and theophylline analogues: correlation of behavioral effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors, Life Sci. 43, 387.

Compendium of Pharmaceuticals and Specialties, 1994, 29th edn. (Canadian Manufacturers Association).

Cronstein, B.N., 1994, Adenosine, an endogenous anti-inflammatory agent, J. Appl. Physiol. 76, 5.

Daly, J.W., 1993, Mechanism of action of caffeine, in: Coffee and Health, ed. S. Garattini (Raven Press, New York) p. 97.

DiRosa, M., J.P. Giroud and D.A. Willoughby, 1971, Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine, J. Pathol. 104, 15.

Doak, G.J. and J. Sawynok, 1995, Complex role of peripheral adenosine in the genesis of the response to subcutaneous formalin in the rat, Eur. J. Pharmacol. 281, 311.

Durcan, M.J. and P.F. Morgan, 1989, Evidence for adenosine A_2 receptor involvement in the hypomobility effects of adenosine analogues in mice, Eur. J. Pharmacol. 168, 285.

Finn, I.B. and S.G. Holtzman, 1987, Pharmacologic specificity of tolerance to caffeine-induced stimulation of locomotor activity, Psychopharmacology 93, 428.

Forbes, J.A., W.T. Beaver, K.F. Jones, C.J. Kehm, W.K. Smith, C.M. Gongloff, J.R. Selenock and J.W. Smith, 1991, Effect of caffeine on ibuprofen analgesia in postoperative oral surgery pain, Clin. Pharmacol. Ther. 49, 674.

Fredholm, B.B., M.P. Abbracchio, G. Burnstock, J.W. Daly, T.K. Harden, K.A. Jacobson, P. Leff and M. Williams, 1994, Nomenclature and classification of purinoceptors, Pharmacol. Rev. 46, 143.

Graham, D.M., 1978, Caffeine – its identity, dietary sources, intake and biological effects, Nutr. Rev. 36, 97.

Herrick-Davis, K., S. Chippari, D. Luttinger and S.J. Ward, 1989, Evaluation of adenosine agonists as potential analgesics, Eur. J. Pharmacol. 162, 365.

Hunskaar, S., O.B. Fasmer and K. Hole, 1985, Formalin test in mice, a useful technique for evaluating mild analgesics, J. Neurosci. Methods 14, 69.

Jacobson, K.A., O. Nikodijević, W.L. Padgett, C. Gallo-Rodriguez, M. Maillard and J.W. Daly, 1993, 8-(3-Chlorostyrylcaffeine (CSC) is a selective A_2-adenosine antagonist in vitro and in vivo, FEBS Lett. 323, 141.

Jurna, I., 1984, Cyclic nucleotides and aminophylline produce different effects on nociceptive motor and sensory responses in the rat spinal cord, Naunyn-Schmied. Arch. Pharmacol. 327, 23.

Kaplan, G.B., D.J. Greenblat, M.A. Kent, M.M. Cotreat, G. Arcelin and R.I. Schrader, 1992, Caffeine-induced behavioral stimulation is dose-dependent and associated with A_1 adenosine receptor occupancy, Neuropsychopharmacology 6, 145.

Karlsten, R., C. Post, I. Hide and J.W. Daly, 1991, The antinociceptive effect of intrathecally administered adenosine analogs in mice correlates with the affinity for the A_1-adenosine receptor, Neurosci. Lett. 121, 267.

Karlsten, R., T. Gordh and C. Post, 1992, Local antinociceptive and hyperalgesic effects in the formalin test after peripheral administration of adenosine analogues in mice, Pharmacol. Toxicol. 70, 434.

Laska, E.M., A. Sunshine, F. Mueller, W.B. Elvers, C. Siegel and A. Rubin, 1984, Caffeine as an analgesic adjuvant, J. Am. Med. Assoc. 251, 1711.

Lin, Y., T.J. Morrow, J.A. Kiristy-Roy, L.C. Terry and K.L. Casey, 1989, Cocaine: evidence for supraspinal, dopamine-mediated, non-opiate analgesia, Brain Res. 479, 306.

Malec, D. and E. Michalska, 1988, The effect of methylxanthines on morphine analgesia in mice and rats, Pol. J. Physiol. Pharmacol. 40, 223.

Malmberg, A.B. and T.L. Yaksh, 1993, Pharmacology of the spinal action of ketolorac, morphine, ST-91, U50488H and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction, Anesthesiology 79, 270.

Morgan, M.J. and K.B. Franklin, 1991, Dopamine receptor subtypes and formalin test analgesia, Pharmacol. Biochem. Behav. 40, 317.

Neil, A., J.M. Benoist, V. Kayser and G. Guilbaud, 1987, Initial nociceptive sensitization in carrageenin-induced rat paw inflammation is dependent on amine autacoid mechanisms: electrophysiological and behavioural evidence obtained with a quaternary antihistamine, thiazinmium, Exp. Brain Res. 65, 343.

Nikodijević, O., J.W. Daly and K.A. Jacobson, 1990, Characterization of the locomotor depression produced by an A_2-selective adenosine agonist, FEBS Lett. 261, 67.

Nikodijević, O., R. Sarges, J.W. Daly and K.A. Jacobson, 1991, Behavioral effects of A_1- and A_2-selective adenosine agonists and antagonists: evidence for synergism and antagonism, J. Pharmacol. Exp. Ther. 259, 286.

Person, D.L., I. Kissin, P.T. Brown, A.V. Xavier, H.R. Vinik and E.L. Bradley, 1985, Anesth. Analg. 64, 851.

Poon, A. and J. Sawynok, 1995, Antinociception by adenosine analogs and an adenosine kinase inhibitor: dependence on formalin concentration, Eur. J. Pharmacol. 286, 177.

Rosland, J.H., A. Tjølsen, B. Maehle and K. Hole, 1990, The formalin test in mice: effect of formalin concentration, Pain 42, 235.

Sawynok, J., 1991, Adenosine and pain, in: Adenosine and Adenine Nucleotides as Regulators of Cellular Function, ed. J.W. Phillis (CRC Press, Boca Raton) p. 391.

Sawynok, J. and A.R. Reid, 1995, Neurotoxin-induced lesions to central serotonergic, noradrenergic and dopaminergic systems modify caffeine-induced antinociception in the formalin test and locomotor stimulation in rats, J. Pharmacol. Exp. Ther. (in press).

Sawynok, J. and T.L. Yaksh, 1993, Caffeine as an analgesic adjuvant: a review of pharmacology and mechanisms of action, Pharmacol. Rev. 45, 43.

Sawynok, J., M.I. Sweeney and T.D. White, 1986, Classification of adenosine receptors in mediating antinociception in the rat spinal cord, Br. J. Pharmacol. 88, 923.

Sawynok, J., A.R. Reid and G.J. Doak, 1995, Caffeine antinociception in the rat hot-plate and formalin tests and locomotor stimulation: involvement of noradrenergic mechanisms, Pain 61, 203.

Schachtel, B.P., J.M. Fillingim, A.C. Lane, W.R. Thoden and R.I. Baybutt, 1991, A double-blind study comparing aspirin with caffeine to aspirin and placebo in patients with sore throat, Arch. Int. Med. 151, 733.

Seale, T.W., K.A. Abla, M.T. Shamin, J.M. Carney and J.W. Daly, 1988, 3,7-Dimethyl-1-propargylxanthine: a potent and selective in vivo antagonist of adenosine analogs, Life Sci. 43, 1671.

Seegers, A.J.M., L.P. Jager, P. Zandberg and J. Van Noordwijk, 1981, The anti-inflammatory, analgesic and antipyretic activities of non-narcotic analgesic drug mixtures in rats, Arch. Int. Pharmacodyn. 251, 237.

Siegers, C.P., 1973, Effects of caffeine on the absorption and analgesic efficacy of paracetamol in rats, Pharmacology 10, 19.

Shibata, M., T. Ohkubo, H. Takahashi and R. Inoki, 1989, Modified formalin test: characteristic pain response, Pain 38, 347.

Snyder, S.H., J.J. Katims, Z. Annau, R.F. Bruns and J.W. Daly, 1981, Adenosine receptors and behavioral actions of methylxanthines, Proc. Natl. Acad. Sci. USA 78, 3260.

Taiwo, Y.O. and J.D. Levine, 1990, Direct cutaneous hyperalgesia by adenosine, Neuroscience 38, 757.

Tjølsen, A., O.G. Berge, S. Hunskaar, J.H. Rosland and K. Hole, 1992, The formalin test: an evaluation of the method, Pain 51, 5.

Ukena, D., M.T. Shamim, W. Padgett and J.W. Daly, 1986, Analogs of caffeine: antagonists with selectivity for A_2 adenosine receptors, Life Sci. 39, 743.

USPDI, 1993, Drug Information for the Health Care Professional, 13th edn., Vol. 1.

Ward, N., C. Whiteny, D. Avery and D. Dunner, 1991, the analgesic effects of caffeine in headache, Pain 44, 151.

Weil-Fugazza, J., F. Godefroy, V. Manceau and J.M. Besson, 1986, Increased norepinephrine and uric acid levels in the spinal cord of arthritis rats, Brain Research 374, 190.