Modulation of paracetamol antinociception by caffeine and by selective adenosine A₂ receptor antagonists in mice.

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Abstract

This study aimed to investigate the involvement of adenosine receptors in the interaction between paracetamol and caffeine. Nociception was studied in mice, using paracetamol, caffeine, the A_{2A} antagonist, 5-amino-7-(β-phenylethyl)-2-(8-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolol[1,5-*c*]pyrimidine (SCH58261) or the A_{2B} receptor, 1-propyl-8-*p*-sulfophenylxanthine (PSB1115) in the tail immersion and hot plate tests. Paracetamol (10-200 mg/kg) was antinociceptive in both tests, but in contrast to previous studies caffeine (10 mg/kg) reduced this effect. SCH58261 (3 mg/kg) was antinociceptive in both tests and in its presence paracetamol (50 mg/kg) had no further effect. PSB1115 (10 mg/kg) had little effect alone but potentiated the effect of paracetamol (50 mg/kg) in the hot plate test and abolished it in the tail immersion test. A_{2B} receptors may therefore be involved in the action of paracetamol in a pathway-dependent manner. The results with SCH58261 support the existence of pronociceptive A_{2A} receptors, and suggest that A_{2A} antagonists may be effective analgesics.

Keywords: Adenosine antagonists; paracetamol; caffeine; antinociception

1. Introduction

The adenosine receptor family comprises four subtypes: A₁, A_{2A}, A_{2B} and A₃ (Fredholm et al., 2001). A₁ receptors are widely distributed in brain, spinal cord and periphery (Fredholm et al., 2001) while A_{2A} receptors are predominantly expressed in the periphery (Dunwiddle and Masino, 2001), with only a very restricted central expression in the striatum, nucleus accumbens and olfactory tubercle (Jarvis and Williams, 1989). A_{2B} and A₃ receptors are widely distributed, but are present at a low density in the central nervous system (Feoktistov and Biaggioni, 1997; Rivkees et al., 2000). Within the spinal cord adenosine acts to suppress nociceptive signalling, mediated via the A₁ receptor (DeLander and Hopkins, 1986; Holmgren et al., 1986), while in the periphery it has an algogenic activity which may be mediated via an A₂ receptor subtype (McQueen and Ribeiro, 1986; see Sawynok and Yaksh, 1993). In the mouse the location of these receptors are most probably on sensory nerves as there is no A_{2A} receptor binding in the spinal cord (Bailey et al., 2002b; Kelly et al., 2004).

Caffeine is a non-specific adenosine antagonist and binds A₁ and A₂ receptors with equal affinity, but does not bind A₃ receptors (see Fredholm and Lindstrom, 1999). It is used in combination with non-steroidal anti-inflammatory drugs as an analgesic adjuvant (Sawynok and Yaksh, 1993). In mice, rats and humans caffeine increases the antinociceptive effects of paracetamol (Laska et al., 1983; Granados-Soto et al., 1993; Engelhardt et al., 1997). When used alone caffeine has been shown to cause antinociception in rats and mice (Sawynok et al., 1995; Ghelardini et al., 1997; Abo-Salem et al., 2004).

It is unclear how caffeine produces its analgesic effect, but it is likely to be via adenosine receptor antagonism (Fredholm et al., 1996). The analgesic adjuvant effects of caffeine cannot be due to A₁ receptor blockade, as stimulation of A₁

receptors is known to be antinociceptive (Sawynok and Liu, 2003), genetically modified mice lacking the A₁ receptor are hyperalgesic (Johansson et al., 2001; Wu et al., 2005) and A₁ antagonists are pronociceptive (Bastia et al., 2002) or ineffective in animal models of pain (Abo-Salem et al., 2004). Abo-Salem et al (2004) demonstrated that novel selective A_{2B} adenosine receptor antagonists produced a similar antinociception to caffeine in the hot plate test in mice, raising the possibility that part of the action of caffeine might be mediated at the A_{2B} receptor. Although they also reported that adenosine A_{2A} antagonists were not antinociceptive (Abo-Salem et al., 2004), others have shown antinociceptive effects of A_{2A} antagonists in the writhing test (Bastia et al., 2002). Further, mice deficient in the adenosine A_{2A} receptor gene display hypoalgesia (Ledent et al., 1997; Bailey et al., 2002a), raising the possibility that both A₂ subtypes are involved in nociceptive modulation.

The aim of this study was to compare the effect of caffeine on paracetamol-induced antinociception with that of selective antagonists for A_{2A} and A_{2B} receptors. To address this we have studied the effects of paracetamol and caffeine alone and in combination in mice, using the hot plate and tail immersion nociceptive tests and also the effect of 5-amino-7-(β-phenylethyl)-2-(8-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (SCH58261) and 1-propyl-8-*p*-sulfophenylxanthine (PSB1115) as selective antagonists at A_{2A} and A_{2B} receptors respectively. In addition we have studied the effect of paracetamol and caffeine in mice lacking the adenosine A_{2A} receptor gene.

2. Materials and Methods

2.1. Wild type and adenosine A_{2A} receptor knockout mice

The mice used in this study were derived from a breeding colony of adenosine A_{2A} receptor knockout mice on a CD-1 background (Ledent et al., 1997) maintained at the University of Surrey. Matings between heterozygote (+/-) animals produced wildtype (+/+), heterozygote (+/-) and knockout (-/-) animals, and the genotype of the animals was established at 21 days by tail tipping and DNA amplification using PCR (Ledent et al., 1997). The majority of studies were carried out in the wildtype mice, but in some studies with paracetamol and caffeine the knockout mice were used. After weaning, all animals were housed three per cage in an air conditioned unit maintained at 20-22°C and 50-60 % humidity and were allowed free access to standard rodent chow and water. Lighting was controlled on a twelve-hour cycle, lights on at 07.00 hr. Animals were acclimatised to the procedure room for 2 hr prior to testing at 11.00 hr. All protocols were carried out in accordance with the Animals (Scientific Procedures) Act, 1984 and approved by a local ethical committee.

2.2. Drug treatment

Mice weighing 25-30 g received paracetamol, caffeine, the A_{2B} antagonist PSB1115 (Fredholm et al., 1998; Abo-Salem et al., 2004) or the A_{2A} antagonist SCH58261 (Zocchi et al., 1996; Fredholm et al., 1998; Abo-Salem et al., 2004) alone or in combination. All drugs were injected intraperitoneally (i.p.) in a volume of 8 ml/kg. Control groups were given drug vehicle (see Materials) in an equal volume.

2.3. Tail immersion test

Mice were restrained in a plastic tube with the tail exteriorised and a thermal stimulus was applied by immersion of approximately 3 cm of the tip of the tail in a thermostatically controlled water-bath kept at a temperature of 53 ± 0.1°C. Latencies for tail withdrawal were recorded for each animal before and after drug administration

using a hand-held stopwatch. A 10 s cut-off time was imposed to avoid tissue damage.

2.4. Hot plate

In a modification of the method of Chen (1951), a 3 mm aluminium plate was placed in a thermostatically controlled water-bath kept at a constant temperature of $55 \pm 0.1^{\circ}\text{C}$. The latency for nociceptive response (defined as paw licking or jumping) was recorded for each animal before and after drug administration using a hand-held stopwatch. A 30 s cut-off time was imposed to avoid tissue damage.

2.5. Statistical analysis

Statistical comparison of the drug-treated groups was performed using two-way ANOVA (for factors treatment and time) with repeated measures, followed by Scheffe's post hoc test. $P < 0.05$ was considered significant. Comparison of basal nociceptive latencies between wildtype and knockout animals were performed using unpaired Student's t-test.

2.6. Materials

Paracetamol and caffeine were purchased from Sigma-Aldrich (Dorset, UK) and dissolved in warmed PBS (Oxoid, Hampshire, UK). SCH58261 (Sigma-Aldrich, Dorset, UK) was dissolved in DMSO (5 mg/ml) which was further diluted in PBS to give a final concentration of 15 % DMSO (Fischer Scientific, Loughborough, UK) in the drug injection solution. PSB1115 was dissolved in PBS and was a kind gift from Prof. Christa Müller (Bonn University, DE).

3. Results

3.1. Effects of paracetamol and caffeine in the tail immersion test.

The basal response latency of all mice prior to drug administration was 2.23 ± 0.15 s. Paracetamol (50–200 mg/kg) administered alone produced significant antinociception ($P < 0.01$) at all time points, while 10 mg/kg paracetamol was without effect (Fig. 1A). Caffeine (10 mg/kg) administered alone produced a pronociceptive effect which was significant at 15 and 30 min (Fig. 1B). Caffeine (10 mg/kg) abolished the antinociceptive effect of paracetamol (50–200 mg/kg) (Fig. 1B,C).

In a further series of experiments caffeine and paracetamol were administered to A_{2A} receptor knockout mice, but the basal response latency of all A_{2A} knockout mice prior to drug administration was 3.40 ± 0.16 s, significantly higher than the wildtype control, ($P < 0.05$). In these mice paracetamol even at high doses (100-200 mg/kg) and caffeine (10 mg/kg) administered alone or in combination failed to increase nociceptive response latencies further.

3.2. The effect of SCH58261 and PSB1115 on the antinociceptive response to paracetamol in the tail immersion test.

Paracetamol (50 mg/kg) or the A_{2A} antagonist SCH58261 (3 mg/kg) administered alone produced significant antinociception, but in the presence of SCH58261 paracetamol was unable to induce a further increase in response latency (Fig. 2A). The A_{2B} antagonist PSB1115 (10 mg/kg) administered alone did not produce significant antinociception, but abolished the effect of paracetamol (50 mg/kg) (Fig. 2B).

3.3 Effects of paracetamol, caffeine and adenosine receptor antagonists in the hot plate test.

The basal response latency of all mice prior to drug administration was 8.03 ± 0.39 s. Paracetamol administered alone (10–200 mg/kg) produced significant antinociception ($P < 0.01$) at 30 min (Fig. 3A). Caffeine (10 mg/kg) administered alone had no effect on nociceptive response latencies (Fig. 3B) but reduced the antinociceptive effect of paracetamol (50–200 mg/kg). This effect was significant ($P < 0.05$) for the highest dose (200 mg/kg) of paracetamol (Fig. 3B,C).

In a further series of experiments caffeine and paracetamol were administered to A_{2A} receptor knockout mice, but the basal response latency of all knockout mice prior to drug administration was 11.35 ± 0.41 s, significantly higher than the wildtype control ($P < 0.05$). Paracetamol even at high doses (100–200 mg/kg) and caffeine (10 mg/kg) administered alone or in combination failed to increase nociceptive response latencies further.

3.4. The effect of SCH58261 and PSB1115 on the antinociceptive response to paracetamol in the hot plate test.

Paracetamol (50 mg/kg) or the A_{2A} antagonist SCH58261 (3 mg/kg) administered alone produced significant antinociception, but in the presence of SCH58261 (3 mg/kg) paracetamol (50 mg/kg) was unable to increase response latency further (Fig. 4A). The A_{2B} antagonist PSB1115 (10 mg/kg) administered alone induced a very small antinociceptive response that only achieved significance at 45 min ($P < 0.05$). The combination of paracetamol (50 mg/kg) and PSB1115 (10 mg/kg) produced antinociception that was significantly greater than paracetamol alone (Fig. 4B).

4. Discussion

Although other studies have reported the effect of high doses of paracetamol (400 mg/kg) (Pini et al., 1996; Srikiatkachorn et al., 1999; Sandrini et al., 2001; Bonnefont et al., 2003), we did not study doses above 200 mg/kg because of the known toxic effects (Tee et al., 1987; Gardner et al., 2002). Caffeine at a dose of 40 mg/kg has previously been shown to have an antinociceptive effect in the hot plate test when combined with paracetamol (Engelhardt et al., 1997). We also found an antinociceptive effect of 40 mg/kg caffeine in the tail immersion and hot plate test (data not shown) but doses at this level have questionable relevance to its use as an analgesic adjuvant in humans. As lower doses of caffeine have also been shown to exert an antinociceptive effect (Sawynok et al., 1995; Ghelardini et al., 1997; Abo-Salem et al., 2004), 10 mg/kg caffeine was chosen for the majority of the experiments.

Paracetamol produced an antinociceptive effect in both the tail immersion and the hot plate tests at doses between 10-200 mg/kg. Caffeine (10 mg/kg) administered alone in the tail immersion test produced a pronociceptive effect and abolished the antinociceptive effect of paracetamol. In the hot-plate test caffeine alone also did not produce an antinociceptive effect, in contrast to the results reported by Abo Salem et al (2004). When administered in combination with paracetamol, caffeine again inhibited the effects of paracetamol. In both nociceptive tests therefore caffeine at 10 mg/kg was not antinociceptive and inhibited rather than enhanced the effects of paracetamol. The studies described here differ from previous studies in both mouse strain and the temperature of the thermal stimulus (55°C here, 52°C in the experiments reported by Abo Salem et al (2004)), factors that could underlie the difference in the observed effects of caffeine (Wilson and Mogil, 2001). We used the higher temperature to ensure a supraspinal component to this antinociceptive test. This

emphasises the importance of the experimental model, and suggests that the effects of caffeine may be highly dependent on the nature of the nociceptive stimulus.

SCH58261 is a specific antagonist of the adenosine A_{2A} receptor (Zocchi et al., 1996) that has been shown to have antinociceptive effects in CD1 mice in the hot plate test (52°C), but only when administered intrathecally, and not when administered i.p. (Bastia et al., 2002). However in the experiments reported here SCH58261 (3 mg/kg) administered i.p. produced antinociception in the hot plate test (55°C), and this difference again may reflect the temperature or the nociceptive end point used. We chose paw licking rather than jumping, which has been suggested to be a more sensitive and reliable nociceptive response than jumping (Wilson and Mogil, 2001). We also observed antinociceptive effects of SCH58261 in the tail immersion test (53°C) when administered i.p. The antinociceptive effect of the A_{2A} antagonist, together with the hypoalgesia seen in the A_{2A} receptor knockout mice both in this study and previously (Ledent et al., 1997; Bailey et al., 2002a) confirms a pronociceptive role for adenosine A_{2A} receptors. We did not observe any further effect of paracetamol, caffeine or combinations of the two in either test in the knockout mice, probably because the increased nociceptive threshold in the knockouts masked any effects of the drugs. Similarly, paracetamol was not able to induce a further increase in response latency in the presence of SCH58261.

PSB1115 is a specific antagonist with 180-fold selectivity for human adenosine A_{2B} receptor compared to the rat adenosine A_{2A} receptor, that has been suggested to be unlikely to penetrate into the central nervous system due to its polar nature (Hayallah et al., 2002). Abo-Salem et al (2004) reported that PSB1115 at 10 mg/kg did not produce significant antinociception by itself in the hot plate test, but significantly potentiated the effects of morphine, presumably by an action at peripheral A_{2B} receptors. We also found little or no antinociceptive effect of PSB1115 alone at 10 mg/kg in either the hot plate or the tail immersion test. It significantly potentiated the

effect of paracetamol in the hot plate test, as was seen with morphine (Abo-Salem et al., 2004), but in contrast, it abolished the response to paracetamol in the tail immersion test. The tail immersion test is predominantly a spinal reflex while the hot plate test produces nociceptive responses predominantly mediated at higher centres, but the opposing effects of PSB1115 on paracetamol-induced antinociception in the two tests are unlikely to reflect opposing roles for A_{2B} receptors at the spinal and supraspinal levels if this compound does not enter the central nervous system. Instead, it is more likely to reflect opposing effects of peripheral A_{2B} receptors in the pain pathways activated by the two tests. Irrespective of the mechanism, the effect of PSB1115, like that of SCH58261, on paracetamol-induced antinociception did not mimic the effect of caffeine.

In conclusion, paracetamol is antinociceptive in both spinal and supraspinal tests and the co-administration of caffeine reduces the effect of paracetamol, suggesting that these thermal nociceptive tests in the mouse produce opposite effects for caffeine to what has been reported in man. Furthermore, antinociceptive responses to paracetamol and caffeine were dissimilar to responses of paracetamol combined with either an A_{2A} or an A_{2B} antagonist, suggesting that caffeine does not act at these receptors to oppose paracetamol antinociception. However, the opposing effects of the A_{2B} receptor antagonist, PSB1115, on paracetamol-induced antinociception alone in a spinal and supraspinal thermal test suggests that the A_{2B} receptor may be involved in the mechanisms of action of paracetamol and that this effect is dependent on the pain pathways activated. The hypoalgesic nature of the A_{2A} knockout mice, and the ability of the A_{2A} antagonist SCH58261 to induce an antinociceptive effect at least equal to that of paracetamol in both spinal and supraspinal tests, support the existence of pronociceptive A_{2A} receptors in pain pathways and suggest that an A_{2A} antagonist may be clinically effective as an analgesic drug.

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Figure Legends

Fig. 1. Antinociceptive effects of paracetamol and the effect of caffeine in the tail immersion test in mice. The mean nociceptive response latency ($\pm$ S.E.M; control $n=18$, treated groups $n=6$) in the tail immersion test ($53 \pm 1^\circ\text{C}$) prior and subsequent to the administration of (A) paracetamol (10-200 mg/kg), (B) paracetamol (10-200 mg/kg) + caffeine (10 mg/kg) and (C) dose response curve (at 30 min. time point) subsequent to the administration of paracetamol (10-200 mg/kg) $\pm$ caffeine (10 mg/kg). PBS=phosphate buffered saline. * Treatment vs PBS; # Paracetamol plus caffeine vs paracetamol alone ($P<0.05$, ANOVA, Scheffe's post hoc test).

Fig. 2. The effect of SCH58261 and PSB1115 alone and in the presence of paracetamol in the tail immersion test in mice. The mean nociceptive response latency ($\pm$ S.E.M; control $n= 6-9$, treated groups $n=6$) in the tail immersion test ($55 \pm 1^\circ\text{C}$) prior and subsequent to the administration of paracetamol (50 mg/kg) $\pm$ A2 antagonists; (A) SCH58261 (3 mg/kg) and (B) PSB1115 (10 mg/kg). PBS= phosphate buffered saline. * Treatment vs PBS. ($P<0.05$, ANOVA, Scheffe's or Fischer LSD post hoc tests).

Fig. 3. Antinociceptive effects of paracetamol and the effect of caffeine in the hot plate test in mice. The mean nociceptive response latency ($\pm$ S.E.M; control $n=18$, treated groups $n=6$) in the hotplate test ($55 \pm 1^\circ\text{C}$) prior and subsequent to the administration of (A) paracetamol (10-200 mg/kg), (B) paracetamol (50-200 mg/kg) + caffeine (10 mg/kg) and (C) dose response curve (at 30 min. time point) subsequent to the administration of paracetamol (10- 200 mg/kg) $\pm$ caffeine (10 mg/kg). PBS=phosphate buffered saline. * Treatment vs PBS; # Paracetamol plus caffeine vs

paracetamol alone ($P < 0.05$, ANOVA, Scheffe's post hoc test).

Fig.4. The effect of SCH58261 and PSB1115 alone and in the presence of paracetamol in the hotplate test in mice. The mean nociceptive response latency ($\pm$ S.E.M; control $n = 6-9$, treated groups $n = 6$) in the hotplate test ($55 \pm 1^\circ\text{C}$) prior and subsequent to the administration of paracetamol (50 mg/kg) $\pm$ A2 antagonists; (A) SCH58261 (3 mg/kg) and (B) PSB1115 (10 mg/kg). PBS= phosphate buffered saline.
* Treatment vs PBS; # PSB1115 + paracetamol vs PSB1115 or paracetamol alone ($P < 0.05$, ANOVA, Scheffe's or Fischer LSD post hoc tests).