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Attenuation of precipitated morphine withdrawal symptoms by acute i.c.v. administration of a group II mGluR agonist

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1 We previously showed that chronic i.c.v. antagonism of metabotropic glutamate receptors (mGluRs) concurrently with s.c. morphine significantly attenuated precipitated withdrawal symptoms. Conversely, acute i.c.v. injection of a selective group II mGluR antagonist just before the precipitation of withdrawal exacerbated abstinence symptoms.

2 In the present study, we showed that acute i.c.v. administration of the non-selective mGluR agonist 1-aminocyclopentane-1,3-dicarboxylic acid ((1**S**,3**R**)-ACPD), as well as the group II selective agonist (2**S**,1′**R**,2′**R**,3′**R**)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV), significantly attenuated the severity of precipitated withdrawal symptoms.

3 From these results we hypothesize that chronic opioid treatment may indirectly induce a desensitization of group II mGluRs, which contributes to the development of dependence.

Keywords: Morphine; dependence; (1**S**,3**R**)-ACPD; (1**S**,3**S**)-ACPD; DHPG; DCG-IV; L-AP4; metabotropic glutamate receptor; precipitated withdrawal

Introduction

Recently, we have shown that chronic i.c.v. antagonism of metabotropic glutamate receptors (mGluRs) concurrently with morphine treatment attenuates the severity of the precipitated withdrawal syndrome (Fundytus & Coderre, 1994; Fundytus *et al*., 1997). These results suggest that chronic opioid administration may elicit changes in glutamatergic, as well as opioidergic, neurones. Activation of μ-opioid receptors and mGluRs both affect adenosine 3′:5′-cyclic monophosphate (cyclic AMP) production and phosphoinositide (PI) hydrolysis. Acute activation of μ-opioid receptors decreases PI hydrolysis (Barg *et al*., 1992; 1993; 1994), while activation of group I mGluRs stimulates PI hydrolysis (Schoepp & Conn, 1993; Hayashi *et al*., 1994). Activation of μ-opioid receptors or group II (mGluR2 and 3) or group III (mGluR4, 6, 7 and 8) mGluRs decreases cyclic AMP production (Childers, 1991; Hayashi *et al*., 1994).

Both opioid receptors and mGluRs are directly coupled to these intracellular second messengers via guanine nucleotide (G) proteins, and opioid receptors and mGluRs are similarly distributed in the brain (Mansour *et al*., 1995; Masu *et al*., 1995), suggesting that they may be co-localized within the same cells. Thus, opioid receptors and mGluRs may share common pools of intracellular second messengers, and activity at one type of receptor may modulate activity at the other receptors via actions on second messengers. Whereas acute administration of μ-opioids decreases cyclic AMP production and PI hydrolysis, during chronic administration activity in both systems returns to near control levels (Dixon *et al*., 1990; Childers, 1991; Barg *et al*., 1992; 1993; 1994), suggesting that compensatory mechanisms are elicited. Given the similarities of the intracellular events triggered by opioid and mGluRs, and given that chronic antagonism of mGluRs reduces morphine withdrawal symptoms, we hypothesize that, via actions on second messengers, chronic opioid administration may induce a change in the sensitivity of mGluRs, which contributes to the development of opioid dependence.

In the present study, we examined the possibility that desensitization of mGluRs contributes to morphine dependence by assessing the ability of acute i.c.v. treatment, just before the precipitation of withdrawal, with both non-selective and subtype-selective mGluR agonists, to inhibit naloxone-precipitated morphine withdrawal. It is expected that if mGluR desensitization plays a role in opioid dependence, then acute administration of mGluR agonists just before the precipitation of withdrawal should reduce the severity of abstinence symptoms.

In the present study we examined the effect of 1-aminocyclopentane-1,3-dicarboxylic acid (1**S**,3**R**)-ACPD), which is a non-selective mGluR agonist that is ten times more potent as an agonist at mGluRs than ionotropic glutamate receptors (Palmer *et al*., 1989; Schoepp *et al*., 1991a, b; 1992; Watkins & Collingridge, 1994) on precipitated withdrawal symptoms in chronic morphine-treated rats. The effects of (1**S**,3**R**)-ACPD were then compared to a series of subtype-selective mGluR agonists. Group I mGluRs were selectively activated by (**RS**)-dihydroxyphenylglycine (DHPG) (Schoepp *et al*., 1994; Watkins & Collingridge, 1994). Group II mGluRs were selectively activated by (1**S**,3**S**)-ACPD and (2**S**,1′**R**,2′**R**,3′**R**)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV) (Ishida *et al*., 1993; Watkins & Collingridge, 1994; Pin & Duvoisin, 1995). To activate group III mGluRs, we used L-2-amino-4-phosphonobutyrate (L-AP4) (Nakanishi, 1992; Nakajima *et al*., 1993; Okamoto *et al*., 1994; Watkins & Collingridge, 1994).

In these studies, we found that acute administration of either the non-selective agonist (1**S**,3**R**)-ACPD, or the mGluR2/3 selective agonist DCG-IV, significantly reduced the severity of the precipitated morphine withdrawal syndrome.

Methods

Subjects and surgery

Subjects were male Long Evans rats (Charles River, PQ), housed 2–4 per cage, maintained on a 12:12 h light:dark cycle (lights on at 06h 00min), with food and water available *ad libitum*. Rats weighed 280–350 g at the time of surgery.

On Day 0, each rat was anaesthetized with sodium pentobarbitone (Somnotol, MTC Pharmaceuticals, 60 mg kg^{-1}), and a 23 gauge stainless steel guide cannula was implanted above the lateral ventricle (AP = −1.3 mm and L = −1.8 mm

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from bregma, and V = −3.0 mm from the top of the skull; Paxinos & Watson, 1986) so that the 30 gauge injection cannula extended 1 mm below this into the lateral ventricle. While the rat was still under pentobarbitone anaesthesia, one unprimed (i.e. not yet pumping) Alzet osmotic pump containing 50 mg ml^{-1} morphine sulphate was implanted subcutaneously (s.c.) on the back. On Day 1, rats were briefly anaesthetized with halothane and a second Alzet osmotic pump containing 70 mg ml^{-1} morphine sulphate was implanted s.c. on the back of each rat. This two day pump implantation procedure was used to prevent mortality from a lethal concentration of morphine before any tolerance had developed.

Drugs

Morphine sulphate (gift from Sabex, Quebec) was continuously infused subcutaneously (s.c.) at a rate of 10 μl h^{-1} for a total dose of 36.65 μmol day^{-1}. The mGluR agonists (1**S**,3**R**)-ACPD, (1**S**,3**S**)-ACPD, DHPG, L-AP4 and DCG-IV were all obtained from Tocris Cookson (Bristol, U.K.). (1**S**,3**R**)-ACPD (n = 18), (1**S**,3**S**)-ACPD (n = 18), DHPG (n = 16) and L-AP4 (n = 17) were given intracerebroventricularly (i.c.v.) as an acute injection in a volume of 4 μl at a dose of either 0 (vehicle) (n = 15), 0.12, 0.6 or 3 nmol, while DCG-IV (n = 11) was given i.c.v. in a dose of either 4.8 or 24 pmol, since higher doses would produce a non-selective activation of NMDA receptors (Ishida *et al.*, 1993).

Withdrawal measurement

The severity of abstinence symptoms was assessed on the seventh day of morphine treatment following an s.c. injection of 1 mg kg^{-1} naloxone. Behaviour was observed for 10 min before i.c.v. injection of either vehicle or one of the mGluR agonists, 10 min after i.c.v. injection but before naloxone, and for 40 min after the injection of naloxone. Teeth chattering and writhing were timed and combined into a time spent in withdrawal score. The severity of diarrhoea was assessed by the amount of weight lost during the test session. Severity of eye twitch and salivation were rated on a 4 point scale where 0 = absent and 3 = severe. To assess the effects of mGluR agonists on general behaviour, time spent in withdrawal and non-withdrawal (resting, ambulating, rearing, grooming) behaviours were compared between dependent (given morphine) and non-dependent rats given either i.c.v. vehicle or the highest dose of one of the agonists. Testing was performed throughout the 10 min period before the i.c.v. injection, the 10 min after i.c.v. but before naloxone injection, and the 10 min after naloxone injection.

Statistical analysis

Time spent in withdrawal and weight loss were analysed by a 1-way ANOVA performed on each mGluR agonist group with dose as the factor. Significant results were further analysed by *post-hoc* LSD t tests. Time spent in withdrawal and non-withdrawal behaviours were analysed with a mixed ANOVA with morphine treatment and i.c.v. treatment as between subject factors, and time block as a repeated measures factor, again followed by *post-hoc* LSD t tests on significant results. Severity of eye twitch and salivation were analysed with Kruskal-Wallis ANOVA for non-parametric data, followed by Mann-Whitney U-tests on significant results.

Results

Morphine sulphate 36.65 μmol day^{-1} produced an intense and reliable withdrawal syndrome as indicated by the presence of abstinence symptoms after naloxone injection. As shown in Figure 1a, the non-selective mGluR agonist (1**S**,3**R**)-ACPD ($F_{(3,29)} = 4.33$, $P < 0.05$) and the mGluR2/3 selective agonist DCG-IV ($F_{(2,23)} = 26.84$, $P < 0.01$) significantly decreased the amount of time spent in withdrawal, with DCG-IV producing the greatest decrease even at the very low doses used. Although DHPG ($F_{(3,27)} = 1.67$, $P > 0.05$) and L-AP4 ($F_{(3,28)} = 1.92$, $P > 0.05$) appeared to decrease time spent in withdrawal at the highest dose used, the results failed to reach statistical significance. The severity of diarrhoea was attenuated in DCG-IV-treated rats, as indicated by a reduction in weight loss ($F_{(2,23)} = 11.45$, $P < 0.01$) in Figure 1b. Figure 2a shows the severity of eye twitch, and Figure 2b shows the severity of salivation. DCG-IV decreased the severity of both eye twitch ($H_{(2,26)} = 11.03$, $P < 0.01$) and salivation ($H_{(2,26)} = 10.61$, $P < 0.01$), while the non-selective agonist (1**S**,3**R**)-ACPD decreased the severity of eye twitch ($H_{(3,33)} = 8.69$, $P < 0.05$). In contrast, the mGluR4 selective agonist L-AP4 significantly increased the severity of eye twitch ($H_{(3,32)} = 11.69$, $P < 0.05$).

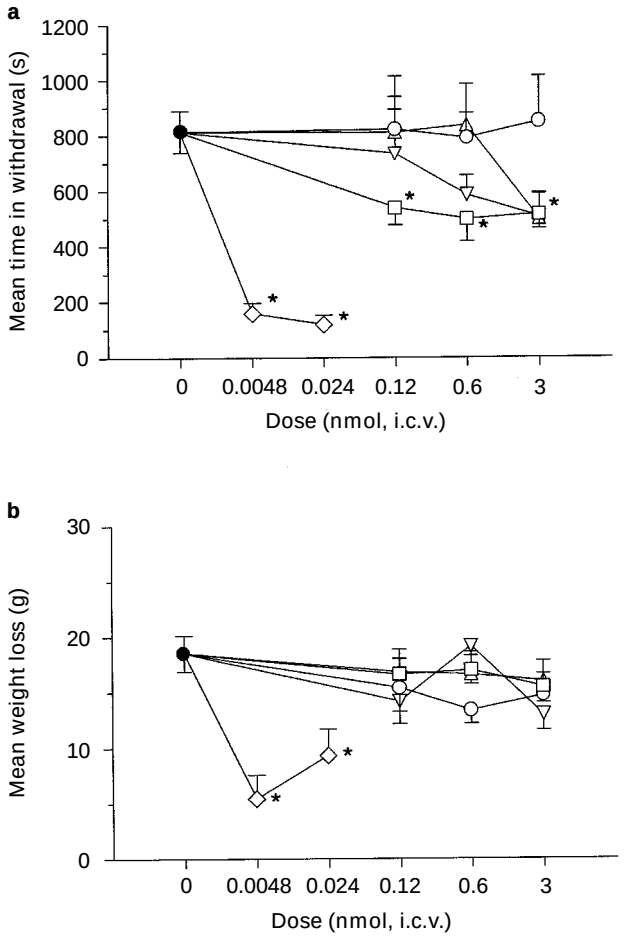

Figure 1 (a) Mean time spent in withdrawal (teeth chattering and writhing combined) during the 40 min withdrawal period in morphine-dependent rats given an acute i.c.v. injection of either vehicle (●; n = 15), or 0.12, 0.6 or 3 nmol of (1**S**,3**R**)-ACPD (□; n = 18), (1**S**,3**S**)-ACPD (○; n = 18), DHPG (△; n = 16) or L-AP4 (▽; n = 17), or 4.8 or 24 pmol of DCG-IV (◇; n = 11). (b) Mean weight loss during the 40 min withdrawal period in morphine-dependent rats given an acute i.c.v. injection of either vehicle (●), or 0.12, 0.6 or 3 nmol of (1**S**,3**R**)-ACPD (□), (1**S**,3**S**)-ACPD (○), DHPG (△) or L-AP4 (▽), or 4.8 or 24 pmol of DCG-IV (◇). *Significantly less than control ($P < 0.05$, LSD t test) in (a) and (b). Vertical lines show s.e.mean.

To assess the effects of acute i.c.v. injection of mGluR agonists on general behaviour, non-withdrawal and withdrawal behaviours were compared in non-dependent and morphine-dependent rats during the 10 min period before the i.c.v. injection, the 10 min after i.c.v. injection but before naloxone injection, and the 10 min following naloxone injection. Generally, rats were more active earlier in the test session, and by the third time block, after the injection of naloxone, non-

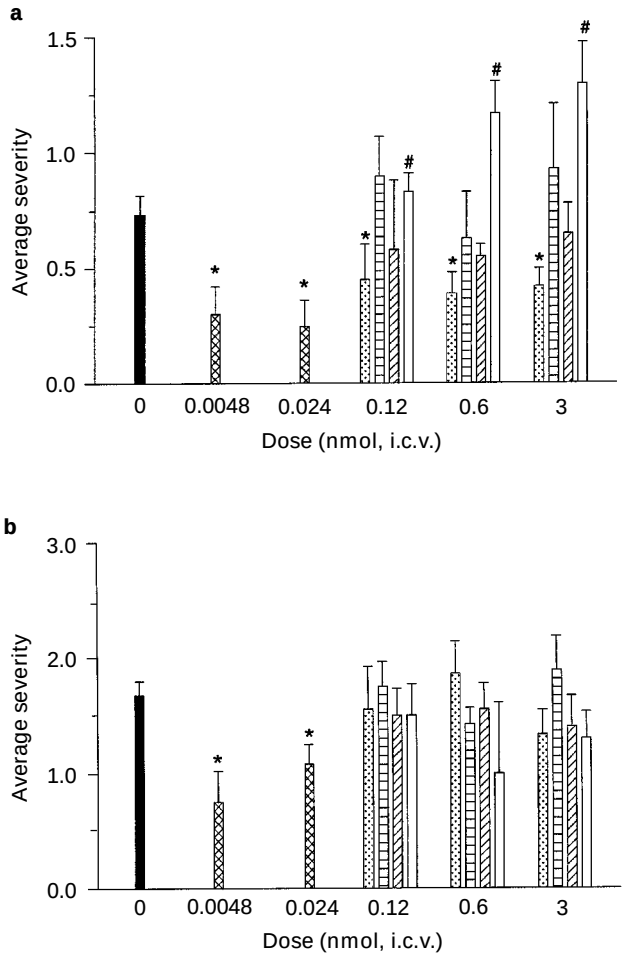

Figure 2 Average severity of (a) eye twitch and (b) salivation during the 40 min withdrawal period in morphine-dependent rats given an acute i.c.v. injection of either vehicle (solid columns), (1**S**,3**R**)-ACPD (stippled columns), (1**S**,3**S**)-ACPD (horizontally-hatched columns), DHPG (diagonally-hatched columns), L-AP4 (open columns) or DCG-IV (cross-hatched columns). *Significantly less than control ($P<0.05$, LSD t test); #significantly greater than control ($P<0.05$, LSD t test). Each column shows mean ± s.e.mean.

dependent rats spent most of their time resting. Morphine-dependent rats behaved very similarly to non-dependent rats until after the injection of naloxone, at which time they spent more time in withdrawal ($P<0.05$, LSD t test). There were no effects of i.c.v. treatment except that (1**S**,3**R**)-ACPD- and DCG-IV-treated rats spent significantly less time in withdrawal than vehicle-treated rats ($P<0.05$, LSD t test; data not shown).

Discussion

The present study shows that the severity of the precipitated morphine withdrawal syndrome was significantly decreased by acute i.c.v. administration of the non-selective mGluR agonist (1**S**,3**R**)-ACPD. Although the mGluR1/5 agonist DHPG and the mGluR4 agonist L-AP4 appeared to decrease withdrawal at the highest doses used, the results failed to reach statistical significance. In contrast, the selective mGluR2/3 agonist DCG-IV almost completely eliminated teeth chattering and writhing, as well as significantly attenuating the severity of diarrhoea (indicated by weight loss), despite the fact that DCG-IV, as well as the other agonists, produced negligible effects on non-withdrawal behaviours. The effect of i.c.v. administration of DCG-IV on diarrhoea was surprising since it is generally believed that opioids slow gastro-intestinal motility via local gut actions, with some spinal input. A decrease in withdrawal-induced diarrhoea is consistent with a decreased vagal output, perhaps via the actions of DCG-IV in peri-ventricular regions such as the thalamus and hypothalamus. Although DCG-IV effectively attenuated the severity of withdrawal, the mGluR2/3 agonist (1**S**,3**S**)-ACPD failed to do so. This may be due to the fact that (1**S**,3**S**)-ACPD is less selective, and stimulates PI hydrolysis and cyclic AMP production via actions at group I mGluRs, as well as decreasing cyclic AMP production via group II mGluRs (Schoepp & Conn, 1993).

Although there is some evidence that DCG-IV is an agonist at NMDA receptors at concentrations above 10 μM *in vitro* (Ishida *et al*., 1993), our highest dose was only 0.006 μM. Therefore, the doses we used were most likely selective for mGluR2/3 receptors. Thus, the efficacy of DCG-IV can be attributed to its agonist action at mGluR2/3. Moreover, we previously found that while chronic i.c.v. administration of a selective mGluR2/3 antagonist significantly decreased the severity of precipitated withdrawal, acute i.c.v. administration of this antagonist just before the precipitation of withdrawal significantly increased the severity of abstinence symptoms (Fundytus *et al*., 1997). Taken together, these results support the hypothesis that group II mGluRs may be desensitized during chronic morphine treatment. Chronic antagonism of a receptor is generally believed to induce up-regulation of the receptor. Thus, perhaps chronic antagonism of group II mGluRs induced an up-regulation of these receptors, which counterbalanced the desensitizing effects of morphine treatment, resulting in normally functioning receptors and thus a reduction of withdrawal symptoms. Also, acute administration of DCG-IV in morphine-treated rats may have restored function to group II mGluRs which were no longer sensitive to physiological concentrations of glutamate, enabling these receptors to inhibit cyclic AMP production, and thus decrease the severity of withdrawal.

Group II mGluRs may interact with other systems which have been postulated to be involved in morphine dependence. For example, it has been shown that whereas acute treatment with μ-opioids decreases phosphatidylinositol (PI) hydrolysis (Barg *et al*., 1992; 1994; Johnson *et al*., 1994), during chronic opioid treatment PI hydrolysis returns to near control levels (Pelligrini-Giampietro *et al*., 1988; Narita *et al*., 1994), and is greatly enhanced during withdrawal (Pelligrino-Giampietro *et al*., 1988; Narita *et al*., 1994; Busquets *et al*., 1995), suggesting that this system and the intracellular messengers produced may play a key role in the development of morphine dependence. Furthermore, we have previously shown that chronic antagonism of group I mGluRs, which are positively coupled to PI hydrolysis (Fundytus & Coderre, 1994), as well as chronic inhibition of protein kinase C (PKC) and intracellular Ca^{2+} (products of PI hydrolysis) (Fundytus & Coderre, 1996), attenuate the development of morphine-dependence. Thus, it is possible that chronic morphine treatment enhances the translocation and activation of protein kinase C (PKC), which may in turn phosphorylate group II mGluRs, leading to desensitization of these receptors.

There is also a large body of evidence suggesting that NMDA receptors are involved in the development of morphine dependence (Trujillo & Akil, 1991; 1994; Fundytus & Coderre, 1994; Mao *et al*., 1995). It has been suggested that during chronic morphine treatment, an increase in the translocation and activation of PKC induces phosphorylation of the NMDA ion channel, enhancing NMDA receptor activity, and related intracellular messengers such as nitric oxide (NO) (Mao *et al*., 1995). It has been shown that activation of group II mGluRs inhibits activity at NMDA receptors (Buisson & Choi, 1995). Perhaps a desensitization of group II mGluRs induced by chronic morphine treatment results in a disinhibition of NMDA receptor activity. Thus, acute administration of a group II mGluR agonist just be-

fore the precipitation of withdrawal may not only decrease excitability via a decrease in cyclic AMP production, and thus alleviate abstinence symptoms, but may also serve to decrease excitability by inhibiting NMDA receptor activity.

Our present results provide further support for the hypothesis that mGluR activity may contribute to the development of morphine dependence. Furthermore, our results are of clinical interest. If clinically safe group II mGluR agonists are developed, activation of mGluRs may aid in alleviating withdrawal symptoms of patients and thus make detoxification safer and easier.

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